IN VITRO PHARMACOLOGICAL PROPERTIES OF AN INDIGENOUS MEDICINAL PLANT, *ARTABOTRYS CRASSIFOLIUS* HOOK.F. & THOMSON (FAMILY: ANNONACEAE JUSS.)

TAN KOK KWAN

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ABSTRACT

The tropical rainforest of Malaysia is considered as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources. Exploitation of medicinal plants for bioactive compounds is of great potential and could be an imperative source of providing new vistas for novel drug discovery and development. The study was undertaken to evaluate the *in vitro* pharmacological properties of *Artabotrys crassifolius* including antibacterial, antifungal, anticancer and antioxidant activities of the plant. The leaves and bark of *Artabotrys crassifolius* were extracted sequentially using hexane, chloroform and ethanol. The prepared crude extracts were subjected to phytochemical screenings for the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins and terpenoids. Kirby-Bauer disc diffusion assay was conducted to examine the antibacterial and antifungal activities of crude extracts against ATCC and clinical strains. The anticancer effect of crude extracts was investigated against human breast and colorectal carcinoma cell lines using MTT assay whereas the antioxidant potential of crude extracts was assessed using TPC, TFC, ABTS, DPPH and FRAP assays. Among the crude extracts studied, hexane and chloroform extracts of bark exhibited pronounced antibacterial activities against ATCC and clinical strains with zones of inhibition ranging from 8.23±0.25 mm to 13.70±0.26 mm and 7.75±0.25 mm to 13.68±0.28 mm respectively. However, all the crude extracts were found to be devoid of antifungal activity except for hexane extract of bark which was able to inhibit the growth of the tested *Candida* species with zones of inhibition ranging from 7.81±0.27 mm to 9.77±0.25 mm. In addition, chloroform extract of bark was highly active against all of the tested carcinoma cell lines with GI_{50} values ranging from 4.23 µg/mL to 9.45 µg/mL, while hexane extract of bark potently inhibited the growth of MDA-468 breast and HCT-116 colorectal carcinoma cell lines with respective GI_{50} values of 6.10 µg/mL and 16.45 µg/mL. Furthermore, ethanol extract of bark that possessed the highest total phenolic and flavonoid contents (268.29±12.36 mg GAE/g and 179.54±4.98 mg CE/g) was shown to demonstrate prominent scavenging activities against ABTS cation and DPPH radicals with IC_{50} values of 16.50 µg/mL and 16.54 µg/mL respectively, as well as exceptionally high antioxidant power with FRAP value of 1884.35±83.78 µmol Fe(II)/g. The chromatographic separation of chloroform extract of bark led to the isolation of four alkaloids, namely artabotrine, liridine, atherospermidine and lysicamine. Among the compounds isolated, artabotrine displayed high antibacterial properties with respective MIC and MBC values ranging from 1.25 µg/mL to 5 µg/mL and 1.25 µg/mL to 20 µg/mL against all of the tested ATCC and clinical bacterial strains, with the exception of *Actinobacillus* sp. and *Klebsiella* sp.. Moreover, artabotrine was highly active in HCT-116 colorectal and MCF-7 breast carcinoma cell lines with GI_{50} values of 3.34 µM and 3.49 µM respectively. In conclusion, exploration of the *in vitro* pharmacological properties of *Artabotrys crassifolius* revealed that artabotrine with dual antibacterial and anticancer activities may represent a new generation of potential drug candidates for the treatment of bacterial infections and cancer. Hence, further *in vivo* studies and clinical trials are required to ascertain the efficacy, safety and mechanisms of action of artabotrine prior to application in the pharmaceutical industry as natural therapeutic agents.

LIST OF PUBLICATIONS

Tan, K.K., Khoo, T.J., and Wiart, C., 2013. Phytochemical screening of *Artabotrys crassifolius* Hook.f. & Thomson (Annonaceae Juss.). *Innovare Journal of Ayurvedic Sciences*, 1(2), 14–17.

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A

AAI	Antioxidant activity index
ALP	Alkaline phosphatase
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
$AlCl_3 \cdot 6H_2O$	Aluminium chloride hexahydrate

B

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene

C

°C	Degree Celsius
cm	Centimetre
CE	Catechin equivalents
CO_2	Carbon dioxide
CAR	Conditioned avoidance response
CFU	Colony forming unit
CNS	Central nervous system
CLSI	Clinical and Laboratory Standards Institute
$CDCl_3$	Deuterated chloroform

D

δ	Chemical shifts
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl

E

ER+	Estrogen receptor-positive
ER–	Estrogen receptor-negative
ESBL-EC	Extended-spectrum beta-lactamase-producing *Escherichia coli*
ESBL-KP	Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*

F

FC	Folin-Ciocalteu
FBS	Foetal bovine serum
FRAP	Ferric reducing antioxidant power
FRIM	Forest Research Institute Malaysia
$FeCl_3 \cdot 6H_2O$	Ferric chloride hexahydrate
$FeSO_4 \cdot 7H_2O$	Ferrous sulphate heptahydrate

G

g	Gram
GI_{50}	Half maximal growth inhibition
GAE	Gallic acid equivalents
GAS	Group A *Streptococcus*
GBS	Group B *Streptococcus*

H

h	Hour
^1H	Proton
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
H_2SO_4	Sulphuric acid

I

IC_{50}	Half maximal inhibitory concentration

K

K	Kelvin
kg	Kilogram
$K_2S_2O_8$	Potassium peroxodisulphate

L

L	Litre
LC_{50}	Half maximal lethal concentration
LPS	Lipopolysaccharides
LTA	Lipoteichoic acids

M

m	Metre
M	Molar
mg	Milligram
mm	Millimetre
mL	Millilitre
mM	Millimolar
min	Minute
MHz	Megahertz
MBC	Minimum bactericidal concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MH-GMB	Mueller-Hinton agar supplemented with glucose and methylene blue

N

nm	Nanometre
NA	Not available
NCI	American National Cancer Institute
NMR	Nuclear magnetic resonance
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCIM	National Collection of Industrial Microorganisms
$NaNO_2$	Sodium nitrite
Na_2CO_3	Sodium carbonate
NCCLS	National Committee for Clinical Laboratory Standards

O

ORCNS	Oxacillin-resistant coagulase-negative staphylococci
OSCNS	Oxacillin-sensitive coagulase-negative staphylococci

P

%	Percentage
pH	Potentiometric hydrogen ion concentration
ppm	Parts per million
PDA	Potato dextrose agar
PDB	Potato dextrose broth

R

RPMI	Roswell Park Memorial Institute

S

SMA	Spontaneous motor activity

T

TFC	Total flavonoid content
TGI	Total growth inhibition
TLC	Thin layer chromatography
TPC	Total phenolic content
TSA	Tryptic soy agar
TSB	Tryptic soy broth
TBHQ	tert-Butylhydroquinone
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine

U

µg	Microgram
µL	Microlitre
µM	Micromolar
UV	Ultraviolet
µmol	Micromole
UNMC	University of Nottingham Malaysia Campus
UKMMC	Universiti Kebangsaan Malaysia Medical Centre
UV/Vis	Ultraviolet–visible

V

v/v	Volume per volume

W

w/v	Weight per volume
WHO	World Health Organisation

CHAPTER I

INTRODUCTION

1.1 BACKGROUND

Since time immemorial, plants have been used extensively as a source of medicines for the treatment of various human ailments (Hussain and Khan 2010). According to the World Health Organisation (WHO), approximately 80% of the people in developing countries still rely on traditional medicines for their primary health care needs (Cheikhyoussef *et al.* 2011), and a major part of the traditional therapy involves the use of plant extracts or their active constituents (Murugan and Rajendran 2011). Furthermore, about 25% to 50% of current pharmaceuticals are plant-derived natural products, indicating the significance and efficacy of plants as an indispensable pharmacological tool (Cowan 1999).

Over the past few years, there has been a tremendous resurgence of interest in medicinal plants (Briskin 2000). This revival might be attributed to several driving factors such as rise in population, insufficient supply of drugs in certain parts of the world, prohibitive cost of treatments for common ailments, side effects of several allopathic drugs in current usage as well as development of resistance to currently used drugs for diseases (Panda and Ray 2012; Pathare and Wagh 2012). Consequently, exploitation of medicinal plants for bioactive compounds is of great potential and could be an imperative source of providing new vistas for novel drug discovery and development (Al-Zubairi *et al.* 2011; Kalaivani *et al.* 2011).

1.2 OBJECTIVES

The tropical rainforest of Malaysia is regarded as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources (Latiff 2011; Poh-Hwa *et al.* 2011). This unique natural heritage has brought renewed interest with the aim of screening indigenous medicinal plants for bioactive compounds. To the best of our knowledge, no detailed studies have been reported on the *in vitro* pharmacological properties of *Artabotrys crassifolius* Hook.f. & Thomson. Therefore, the specific objectives in this research were:

i. To determine the extraction yields and phytochemical constituents of *Artabotrys crassifolius*.

ii. To evaluate the *in vitro* antibacterial activity of *Artabotrys crassifolius*.

iii. To examine the *in vitro* antifungal activity of *Artabotrys crassifolius*.

iv. To investigate the *in vitro* anticancer effect of *Artabotrys crassifolius*.

v. To assess the *in vitro* antioxidant potential of *Artabotrys crassifolius*.

vi. To explore the *in vitro* pharmacological activity of isolated compounds from *Artabotrys crassifolius*.

CHAPTER II

LITERATURE REVIEW

2.1 THE GENUS *ARTABOTRYS*

2.1.1 Botanical descriptions

Artabotrys R.Br. (*Arta*-: to suspend; -*botrys*: a bunch of grapes) (Dalzell and Gibson 1861; Smith 1997; Schmidt *et al.* 2002) is one of the largest genera of the custard-apple family, Annonaceae Juss. (Murphy 2007; Triastinurmiatiningsih 2007; Murphy *et al.* 2008; Thongpairoj 2008). The genus *Artabotrys* comprises over 100 species of woody climbers and scandent shrubs (Cave *et al.* 1986; Posluszny and Fisher 2000; Brophy *et al.* 2004) distributed mainly in tropical and subtropical regions of the world (Chen *et al.* 2004; Li and Gilbert 2011), especially tropical Africa and Eastern Asia (Eloumi-Ropivia *et al.* 1985; Chan *et al.* 1987; Sagen *et al.* 2003; Lan *et al.* 2007; Gupta *et al.* 2010; Sichaem *et al.* 2011).

Generally, the leaves are simple, alternate (Aguilar 2001) or opposite (Kessler 1993), coriaceous (Riffle 1998), glabrous or glabrescent (Oliver 1868), glossy (Bentham 1861) and petiolate (Aguilar 2001). Accessory buds in the axils of leaves on the orthotropic shoots can either grow out vegetatively as plagiotropic shoots, form thorns especially in shady conditions, or develop into sympodial inflorescences, with each sympodial unit terminating in a hook (Posluszny and Fisher 2000; Bell and Bryan 2008; Mabberley 2008).

The flowers are white or yellow (Riffle 1998), highly fragrant (Llamas 2003), hermaphrodite (Aguilar 2001) or unisexual (Oliver 1868), solitary or in fascicles (Jayanthi 2011), and borne on woody, often stout (Bentham 1861), and almost invariably more or less sharply hooked peduncles, which are often leaf-opposed or opposite to lateral branches (Oliver 1868). The three sepals are valvate (Aguilar 2001), and free (Kessler 1993) or variably united at the base (Lindley and Moore 1866). The six petals are valvate (Bentham 1861), in two whorls of three each (Sambamurty 2005), subequal (Edwards *et al.* 1819), free, concave at the base (Oliver 1868), and connivent over the stamens and carpels (Keng and Keng 1990). The stamens are numerous (Chatrou *et al.* 2012), closely packed (Lindley and Moore 1866), quadrate-oblong (Oliver 1868) or cuneate (Jayanthi 2011), and have a truncate dilated connective apex (Kessler 1993). The carpels are numerous (Sharma 1993), oblong or oval (Bentham 1861), and contain two basal ovules in the ovary (Wight and Arnott 1834).

The fruits consist of monocarps that are cylindrical or ellipsoid, mostly sessile (Kessler 1993), indehiscent (Aguilar 2001), and one- or two-seeded (Oliver 1868). The seeds are oblong (Aguilar 2001), collateral (Wight and Arnott 1834), erect (Miller 1835), and without aril (Loudon *et al.* 1836). Detailed botanical descriptions of *Artabotrys* species regarding their morphological characters including habit, roots, stems, branches, leaves, inflorescences, flowers, fruits, seeds, as well as origin and distribution are presented in Table 2.1.

TABLE 2.1 Botanical descriptions of *Artabotrys* species.

Plant species	Botanical description		Reference
A. blumei	Habit	Woody climber	Bentham (1861)
	Leaf	Ovate-elliptic or oblong, 5.08–10.16 cm, coriaceous, both surfaces glabrous and glossy, vein slender but conspicuous, apex obtusely acuminate	
	Inflorescence	Solitary, peduncle hooked	
	Flower	Pedicel 0.85–1.1 cm; sepal short, broad; petal ovate-lanceolate, 1.27–1.9 cm, thick, pubescent; inner petal same as outer petal; carpel 6–8, pubescent	
A. brachypetalus	Habit	Woody climber to 2–10 m tall	Oliver (1868); Arnold and Gulumian (1984); Van Wyk and Van Wyk (1997); Schmidt *et al.* (2002); Sobiecki (2002); Sagen *et al.* (2003); Steenkamp (2003); Clarkson *et al.* (2004); Pillay *et al.* (2008); Stafford *et al.* (2008); Bruschi *et al.* (2011); Luo *et al.* (2011)
	Stem	Greyish brown, pubescent when young, glabrescent	
	Leaf	Elliptic to obovate-elliptic, ovate to oblong, 2.5–8.89 cm × 1.5–5 cm, coriaceous, abaxially pale green and densely pubescent, adaxially bright green and slightly pubescent, margin entire, base scarcely acute or obtuse, apex shortly acute to obtuse; petiole 2.11–8.46 mm	
	Inflorescence	Solitary, peduncle hooked	
	Flower	2 cm in diameter; pedicel 1.27–2.54 cm; sepal broadly oval or elliptic-oblanceolate, 8.46–12.7 mm, outside tomentose; petal creamy yellow, broadly ovate, base acute, apex shortly acute or rather obtuse; inner petal 3, slightly shorter, glabrous; torus pilose; stamen quadrate-oblong; connective apically truncate; carpel numerous	
	Fruit	Monocarp blackish purple, ellipsoid or obovoid, 1.27–2.2 cm, glabrous; stipitate 6.35–10.58 mm	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. brachypetalus	Seed	1 or 2	
	Distribution	Malawi and Mozambique, Southeastern Africa; Tropical Africa; Venda, Zambia and Zimbabwe, Southern Africa	
A. brevipes	Habit	Climber	Thongpairoj (2008)
	Branch	Slender, pubescent when young, glabrescent, minutely lenticellate	
	Leaf	Oblong-elliptic, 6.5–12 cm × 2.5–4.2 cm, slightly chartaceous, both surfaces glabrous except for abaxially sparsely golden pilose midrib, venation inconspicuously reticulate, secondary vein 10–12 on each side of midrib, anastomosing 3–4 mm before margin, margin ciliate, base cuneate to attenuate, apex caudate; petiole 5 mm	
	Inflorescence	Extra-axillary, fascicle 2–5-flowered, peduncle hooked	
	Flower	Pedicel 0.5–0.8 cm, pubescent; sepal ovate, 10 mm × 7–8 mm, erect, chartaceous, both surfaces puberulent, apex acute; petal green; outer petal oblong-elliptic, 3–3.3 cm × 1 cm, base shortly concave, apex obtuse; inner petal smaller than outer petal, both surfaces pubescent; stamen numerous, oblong, 1 mm; connective apically mucronate; carpel several; ovary glabrous; ovule 2, basal placentation; stigma cylindrical	
	Origin	Udon Thani, Northeastern Thailand	
	Distribution	Thailand; Vietnam	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. burmanicus	Habit	Climber	Thongpairoj (2008)
	Branch	Rusty or tawny pubescent when young	
	Leaf	Oblanceolate to narrowly obovate, 11–18 cm × 4.5–6 cm, chartaceous, both surfaces pubescent, midrib abaxially prominent, venation pinnate, secondary vein 9–15 on each side of midrib, anastomosing 3–4 mm before margin, margin entire, base acute or equal, apex acute to acuminate; petiole 2–3 mm	
	Inflorescence	Terminal or axillary, solitary, peduncle hooked	
	Flower	Pedicel 2–2.8 cm; sepal broadly ovate, 8–10 mm, deflexed, outside pubescent, apex acuminate; petal pale green to yellow, flatly ovate, 2.5–3.2 cm × 1.5–2 cm, very fleshy, both surfaces pubescent, petal vein conspicuous, margin slightly revolute, apex acuminate; inner petal 3, rhombic, 2.5–3 cm × 1.2–1.5 cm, pubescent, similar to outer petal; stamen 100, light yellow, oblong, 3 mm, free, glabrous; connective apically acute or triangular; carpel 29; ovary ovate-oblong or flask-shaped, 2–2.5 mm, grooved down the inner side, glabrous; stigma oblong, 2–2.5 mm, fascicle, woolly, mucilage with red smear	
	Origin	Kanchanaburi, Western Thailand; Phetchaburi and Prachuap Khiri Khan, Central Thailand	
	Distribution	Zibingyi, Mandalay, Burma	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. camptopetalus	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Black when dry, laterally straight, pubescent when young, lenticellate	
	Leaf	Pale brown when dry, ovate or elliptic, 3–8 cm × 2–4 cm, chartaceous, abaxially glabrous, adaxially glossy, midrib abaxially prominent and sparsely pubescent, secondary vein 6–8 on each side of midrib, anastomosing 3–4 mm before margin, base cuneate, apex shortly acuminate; petiole 2–4 mm, sparsely pubescent	
	Inflorescence	3–5-flowered; peduncle 3, terete, sparsely pubescent	
	Flower	Pedicel 0.2–0.3 cm, sparsely pubescent; sepal broadly ovate, 3–5 mm × 3 mm, erect, fleshy, valvate, sparsely pubescent, apex acute; outer petal lingulate, terete, 0.6–0.7 cm × 0.1–0.15 cm, fleshy, recurved, sparsely pubescent, apex obtuse; inner petal 0.5–0.6 cm × 0.1–0.15 cm, narrower, sparsely pubescent; torus flat; carpel 3, broadly ovoid, 2 mm × 1.5 mm; stigma ovoid	
	Fruit	Monocarp ellipsoid, 3–3.5 cm × 1.6 cm × 2 cm, glabrous	
	Distribution	New Guinea, Indonesia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. desmidantha	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Black when dry, laterally straight, pubescent when young, lenticellate	
	Leaf	Elliptic to oblong, 8–12(–13) cm × 3.5–6 cm, thinly coriaceous, both surfaces glabrous, abaxially dark brown when dry, adaxially pale brown, midrib abaxially prominent and sparsely pubescent, secondary vein 6–8 on each side of midrib, anastomosing 3–6 mm before margin, base cuneate, apex shortly acuminate; petiole 2–4 mm, pubescent	
	Inflorescence	3–5-flowered; peduncle 3, terete, sparsely pubescent; bract elliptic, 2 mm × 1.5 mm; flower bud ovoid, 4 mm × 3 mm, villous	
	Flower	Pedicel 0.2–0.3 cm, villous; sepal ovate, 3 mm × 2.5 mm, erect, fleshy, valvate, villous, apex acuminate; outer petal lanceolate, flat, 1.5 cm × 0.4 cm, fleshy, both surfaces villous, apex acute; inner petal flat, 1.3 cm × 0.35 cm, narrower, villous; torus flat; stamen 14, oblong; connective apically flat; carpel 5, broadly cylindrical, 1.5 mm × 0.5 mm; stigma ellipsoid, 1 mm × 1 mm, villous	
	Distribution	New Guinea, Indonesia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. fragrans	Habit	Liana to 20 m tall	Li and Gilbert (2011)
	Branch	Pubescent, glabrescent	
	Leaf	Oblong-lanceolate to oblong, 13–17 cm × 5–6 cm, abaxially densely pubescent when young and sparsely puberulent with age, adaxially glabrous and glossy, secondary vein 10–12 on each side of midrib, base cuneate to obtuse, apex obtuse to shortly acuminate; petiole 5–8 mm, pubescent	
	Inflorescence	1–3-flowered; peduncle 2 cm, glabrous	
	Flower	Pedicel 1–1.2 cm, pubescent; sepal triangular, golden pubescent; outer petal broadly triangular-ovate, 1.4 cm × 0.9 cm, densely villous except for base, base concave; inner petal triangular, 1–1.2 cm, pubescent except for base, base concave; connective apically semiorbicular; carpel 4–7, ovoid, glabrous	
	Fruit	Monocarp ellipsoid, 4 cm × 2 cm, glabrous; epicarp smooth	
	Origin	Vietnam	
	Distribution	Guangxi and Guizhou, Southern China; Yunnan, Southwestern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. gracilis	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Dark-coloured when dry, laterally straight, glabrous when young, lenticellate	
	Leaf	Brown when dry, elliptic to oblong, 6–12 cm × 5–7 cm, thinly coriaceous, both surfaces glabrous, adaxially glossy, midrib abaxially prominent and sparsely pubescent, secondary vein 7–9 on each side of midrib, anastomosing 4–6 mm before margin, base cuneate, apex acuminate; petiole 2–4 mm, pubescent	
	Inflorescence	5–7-flowered; peduncle 3, terete, sparsely pubescent; bract elliptic, 5–7 mm × 1–2 mm, pubescent; flower bud broadly ovoid, 1.5 mm × 2 mm, pubescent	
	Flower	Pedicel 0.2–0.25 cm, glabrous; sepal ovate, 2 mm × 2 mm, erect, valvate, outside sparsely pubescent, inside glabrous, apex acuminate; outer petal pale green, flat, 0.3–0.5 cm × 0.1 cm, linear, fleshy, tomentose, apex acute; inner petal terete, 0.2–0.3 cm × 0.1 cm, linear, slightly narrower, villous; torus flat; stamen 20–24, oblong, 1–2 mm × 0.5–1 mm; connective apically flat; carpel 5, broadly ovoid, 0.4–0.6 mm × 0.3–0.4 mm, glabrous; stigma cup-shaped, glabrous	
	Distribution	Borneo; New Guinea and Sumatra, Indonesia; Perak, Malaysia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. grandiflorus	Habit	Climber to 20–25 m tall	Chan *et al.* (1987); Chuakul and Soonthornchareonnon (2003); Chuakul *et al.* (2004); Thongpairoj (2008); Eswani *et al.* (2010)
	Branch	Pale, glabrous, well-marked striations	
	Leaf	Oblong-elliptic, 17–20 cm × 7–10 cm, thinly coriaceous, both surfaces glabrous, abaxially dull, adaxially glossy, midrib abaxially prominent, venation pinnate, secondary vein 10–12 on each side of midrib, anastomosing 3–5 mm before margin, margin entire, base cuneate to obtuse, apex abruptly acute or obtuse	
	Inflorescence	1–3-flowered; peduncle hooked	
	Flower	Pedicel 2–3 cm, hirsute; sepal ovate, 5–6 mm × 4–5 mm, outside tomentose, apex acuminate; petal pale green, coriaceous, rusty tomentose; outer petal broadly elliptic, 2–2.3 cm × 0.5–1 cm, apex acute; inner petal slightly shorter; stamen numerous, oblong, 2 mm; connective apically flat; carpel 20–25; ovary cylindrical, glabrous; stigma linear	
	Fruit	Monocarp obovoid-ellipsoid, glabrous, apex mamillate	
	Origin	Krabi, Narathiwat, Pattani, Satun, Songkhla and Surat Thani, Southern Thailand	
	Distribution	Dungun, Terengganu, Gopeng, Perak, and Jerantut, Pahang, Malaysia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. hainanensis	Habit	Climbing shrub to 4 m tall	Bi *et al.* (2004); Chen *et al.* (2004); Han *et al.* (2005); Li and Gilbert (2011)
	Branch	Glabrous	
	Leaf	Oblong-elliptic to oblong, 7–15 cm × 3–6 cm, chartaceous, both surfaces glabrous except for abaxially puberulent midrib, secondary vein 7–9 on each side of midrib, base broadly cuneate to obtuse, apex acute to acuminate; petiole 4–8 mm, glabrous	
	Inflorescence	Leaf-opposed, usually 1-flowered	
	Flower	Pedicel 1.2–1.5 cm; sepal ovate, 4–5 mm, sparsely pubescent; petal yellowish white, narrowly lanceolate, 2 cm × 0.2 cm, subequal, base slightly broad and concave; stamen oblong, 14 mm × 2 mm; connective apically obtuse to subtruncate; carpel 15, slightly longer than stamen; stigma shortly clavate	
	Fruit	Monocarp ellipsoid, 2.5 cm × 1.2 cm	
	Origin	Guangdong, Guangxi and Hainan, Southern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. harmandii	Habit	Woody climber	Chuakul and Soonthornchareonnon (2003); Chuakul *et al.* (2006); Thongpairoj (2008)
	Leaf	Oblanceolate, 9–15 cm × 3–7 cm, coriaceous, both surfaces glabrous, adaxially glossy, midrib abaxially prominent, base obtuse, apex acute or shortly acuminate; petiole 2–8 mm × 1 mm	
	Inflorescence	1–2-flowered; peduncle hooked, 1.8–2.5 cm	
	Flower	Pedicel trigonous, 1.5–2 cm, puberulent; sepal caudate, 6–7 mm, both surfaces pubescent, apex acute to acuminate; petal green, flat, slightly fleshy, glossy; outer petal ovate to oblong, 1.5–2 cm × 0.6–0.7 cm, both surfaces pubescent, apex acute; inner petal rather rhombic, 1.4–1.5 cm × 0.7–0.8 cm, pubescent, base concave, apex acute; stamen 89, oblong, 2 mm, glabrous; connective apically suborbicular with sharply apiculate; carpel 16, tomentose; ovary ovate to oblong, 1.5–2 mm, grooved down the inner side, glabrous; ovule 2, basal placentation; stigma cylindrical, 1–1.5 mm, slightly bent, tomentose	
	Fruit	Monocarp ellipsoid or obovoid, 3–3.5 cm × 2–2.5 cm, apex acute; stipitate 5–6 mm, glabrous	
	Seed	2, black, ellipsoid, transversely grooved, apex obtuse	
	Origin	Chachoengsao, Chonburi, Prachinburi and Saraburi, Central Thailand; Chaiyaphum, Nakhon Ratchasima, Sisaket, Surin and Yasothon, Northeastern Thailand; Chanthaburi and Trat, Eastern Thailand	
	Distribution	Laos; Pursat, Cambodia; Vietnam	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. hexapetalus	Habit	Liana or scandent shrub to 8–10 m tall	Hedberg *et al.* (1982); Li *et al.* (1997); Li and Yu (1998); Riffle (1998); Whistler (2000); Aguilar (2001); Yu *et al.* (2001); Wong and Brown (2002); Yu *et al.* (2002); Chuakul and Soonthornchareonnon (2003); Llamas (2003); Chen *et al.* (2004); Mahidol *et al.* (2005); Manner and Elevitch (2006); Triastinurmiatiningsih (2007); Khare (2008); Mishra *et al.* (2008); Thongpairoj (2008); Savadi (2009); Karthik (2010);
	Branch	Dark brown when dry, laterally straight, sparsely pubescent when young, glabrescent, lenticellate	
	Leaf	Dark green, pale green when dry, elliptic to oblong, lanceolate or oblanceolate, oblong to broadly lanceolate or oblanceolate, 5–25 cm × 2.5–10 cm, chartaceous to thinly coriaceous, abaxially glabrous or only midrib puberulent, adaxially glabrous, midrib abaxially prominent, secondary vein 8–16 on each side of midrib, anastomosing 3–4 mm before margin, margin entire, base acute to cuneate, apex acute to shortly acuminate; petiole 2–10 mm × 3 mm, stout, glabrous or sparsely pubescent	
	Inflorescence	1–4-flowered; peduncle 2, flat, hooked, sparsely pubescent; bract elliptic to oblong, 1–2 mm × 0.5–1 mm, pubescent; flower bud broadly ovoid	
	Flower	Creamy yellow to greenish yellow, 2.5–3.8 cm in diameter, banana-like fragrant; pedicel trigonous, 1.5–2.5 cm, sparsely pubescent or glabrescent; sepal green, ovate to triangular, 3–10 mm × 3–8 mm, erect, thinly fleshy or coriaceous, reflexed, both surfaces sparsely puberulent or softly pubescent, apex acute or acuminate; petal 6, green to bright yellow, 0.15–0.2 cm, free, very fleshy, fragrant; outer petal ovate-oblong to oblong-lanceolate, flat, 2–5 cm × 0.5–1.6 cm, outside basally densely pubescent, contracted nearly to	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. hexapetalus	Flower	base but basally expanded, vein adaxially conspicuous, margin ciliate and revolute, red or pink inside at base, apex acute; inner petal flat, 2–4.2 cm × 0.3–1.2 cm, smaller, shorter, narrower; torus convex; stamen 29–91, oblong to cuneate, 1–2.5 mm, glabrous; connective apically triangular, glabrescent; carpel 5–30, oblong or cylindrical, 2 mm × 0.5 mm; ovary ovate-oblong, 2.5–3 mm, grooved down the inner side, glabrous; stigma cylindrical or clavate, 3–3.5 mm, glabrous or densely tomentose	Jayanthi (2011); Li and Gilbert (2011); Manjula *et al.* (2011); Rajkumar and Rajanna (2011); Chatrou *et al.* (2012)
	Fruit	Monocarp yellow, ellipsoid to obovoid, globose to ovoid, 2.5–5 cm × 1.5–2.5 cm, fragrant, glabrous, apex conspicuously mucronate or apiculate; stipitate 2–5 mm × 1.5 mm, glabrous or sparsely pubescent, apex mamillate	
	Seed	2, pale or dark brown, ellipsoid or oblong, 1.7–1.9 cm × 1.2–1.4 cm, smooth, transversely grooved, apex obtuse to truncate	
	Origin	Southern India; Sri Lanka; Thailand	
	Distribution	Bangalore, Karnataka, Southwestern India; Bangladesh; Bhutan; Borneo; Burma; Fujian, Southeastern China; Guangdong, Guangxi, Guizhou, Hainan, Hong Kong and Jiangxi, Southern China; Java, Moluccas, Sulawesi and Sumatra, Indonesia; Korogwe, Tanzania, Eastern Africa; Malaysia; Philippines; Salem, Tamil Nadu, Southern India; Taiwan; Vietnam; Yunnan, Southwestern China; Zhejiang, Eastern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. hongkongensis	Habit	Climbing shrub to 8 m tall	Chen *et al.* (2004); Li and Gilbert (2011)
	Branch	Hispid	
	Leaf	Oblong-elliptic to oblong, 6–12 cm × 2.5–4 cm, coriaceous, abaxially glabrous or only midrib puberulent, adaxially glossy, secondary vein 8–10 on each side of midrib, base slightly oblique and obtuse; petiole 2–5 mm, puberulent	
	Inflorescence	1-flowered; peduncle hooked, puberulent	
	Flower	Pedicel slightly longer than peduncle; sepal triangular-ovate, 5 mm, glabrescent; outer petal ovate-lanceolate, 1–1.8 cm, thick, outside densely sericeous pubescent, base concave; inner petal basally concave; stamen cuneate; connective apically triangular, puberulent; carpel ovate-oblong, glabrous; ovule 2, basal placentation; stigma shortly clavate	
	Fruit	Monocarp black when dry, ellipsoid, 2–4 cm × 1.5–3 cm, apex subobtuse	
	Origin	Vietnam	
	Distribution	Guangdong, Guangxi, Guizhou, Hainan and Hunan, Southern China; Yunnan, Southwestern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. inodorus	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Brown to black when dry, laterally straight, sparsely pubescent when young, glabrescent, lenticellate	
	Leaf	Pale green when dry, lanceolate or obovate, 9–13 cm × 3–6 cm, coriaceous, both surfaces glabrous, midrib abaxially prominent and sparsely pubescent, secondary vein 8–12 on each side of midrib, anastomosing 4–5 mm before margin, base cuneate, apex acuminate; petiole 2–4 mm, sparsely pubescent	
	Inflorescence	3–5-flowered; peduncle 3, flat, sparsely pubescent; bract ovate, 2 mm × 1.5 mm, outside puberulent, inside glabrous; flower bud ellipsoid, 2 mm × 2 mm, puberulent	
	Flower	Pedicel 0.3–0.5 cm, glabrous; sepal lanceolate, 5 mm × 3 mm, thinly fleshy, deflexed, outside puberulent, inside glabrous, apex long acuminate; outer petal, terete, triquetrous, 1.5–2 cm × 0.1 cm, linear, fleshy, puberulent; inner petal terete, 1.5–1.8 cm × 0.1 cm, narrower; stamen oblong; connective apically flat; carpel 3, broadly oblong	
	Fruit	Monocarp obovoid, 3–4 cm × 1.5–2 cm; stipitate 0.5 cm	
	Distribution	New Guinea, Indonesia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. lowianus	Habit	Woody climber	Thongpairoj (2008)
	Branch	Dark-coloured when dry, glabrous	
	Leaf	Oblong-elliptic, 14–16 cm × 4–5.5 cm, slightly coriaceous, both surfaces glabrous, midrib abaxially prominent, venation reticulate, secondary vein 11–12 on each side of midrib, anastomosing 3 mm before margin, base acute or shortly attenuate, apex acuminate to caudate	
	Inflorescence	Extra-axillary, fascicle 1–2-flowered; peduncle hooked	
	Flower	Pedicel 0.5–1 cm; sepal triangular, 4–5 mm, small, coriaceous, apex acute; petal narrowly elliptic to oblanceolate, 3 cm × 0.8–1 cm, fleshy, base concave, apex acute to obtuse; inner petal narrower than outer petal, both surfaces puberulent; stamen broadly oblong, 2 mm; connective apically obtuse; ovary ovate-oblong, glabrous; stigma clavate	
	Origin	Sop Moei, Mae Hong Son, Northern Thailand	
	Distribution	Perak, Malaysia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. macranthus	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Pale brown when dry, laterally curved, red villous when young, lenticellate	
	Leaf	Whitish brown when dry, elliptic to oblong, obovate, 17.5–20 cm × 7–8 cm, coriaceous, villous when young, glabrous with age, midrib abaxially prominent and sparsely pubescent, secondary vein 10–11 on each side of midrib, anastomosing 2–3 mm before margin, base cuneate, apex shortly acuminate; petiole 4–6 mm, glabrous	
	Inflorescence	1–2-flowered; peduncle 2, terete, sparsely pubescent; flower bud ovoid, 3–4 mm × 2–3 mm, tomentose	
	Flower	Pedicel 1–1.2 cm, tomentose; sepal triangular, 10–20 mm × 5–10 mm, erect, fleshy, both surfaces tomentose, apex acute; outer petal greenish yellow, oblong, flat, 3.5–4.0 cm × 2.0–2.5 cm, fleshy, pubescent; inner petal flat, 3.5–3.8 cm × 1.5–2.0 cm, narrower; torus flat; stamen numerous, oblong, 1 mm × 0.5 mm; connective apically flat; carpel 8–12; stigma cylindrical bilobed, glabrous	
	Fruit	Monocarp oblong, 1.5 cm × 1 cm; stipitate 1.3 cm, pubescent	
	Distribution	Borneo; Moluccas, Sulawesi and Sumatra, Indonesia; Philippines	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. macrophyllus	Habit	Shrub	Oliver (1868)
	Leaf	Broadly elliptic, 17.78–25.4 cm × 12.7–15.24 cm, base obtuse, apex shortly acuminate; petiole 6.35–8.46 mm	
	Inflorescence	Many-flowered; peduncle 5.08 cm, hooked	
	Flower	Pedicel 0.42 cm; sepal broadly ovate, outside pilose-pubescent, apex shortly acuminate; petal 6, oblong-lanceolate, 0.85 cm, subequal	
A. monteiroae	Habit	Climbing shrub	Kato *et al.* (1993); Van Wyk and Van Wyk (1997); Nichols (2002); Schmidt *et al.* (2002); Clarkson *et al.* (2004); Pillay *et al.* (2008)
	Branch	Reddish brown to black, reddish brown pubescent	
	Leaf	Ovate to oblong-elliptic, both surfaces glabrous, abaxially pale green, adaxially dark bluish green, apex abruptly acuminate	
	Inflorescence	Peduncle hooked	
	Flower	Creamy yellow or white, 1 cm in diameter; petal linear, narrow	
	Fruit	Monocarp bright red or red, oval, 1.5 cm × 1 cm	
	Distribution	Kenya, Eastern Africa; Soutpansberg and Zululand, Southern Africa	
A. multiflorus	Habit	Liana to 20 m tall, 3 cm or less in diameter	Thongpairoj (2008); Li and Gilbert (2011)
	Branch	Grey to dark brown, minutely puberulent or sparsely pubescent when young, minutely lenticellate	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. multiflorus	Leaf	Elliptic to oblong-elliptic or oblong-oblanceolate, 10–26 cm × 4–9 cm, chartaceous to coriaceous, pubescent when young especially abaxially midrib, glabrescent, midrib abaxially prominent, venation pinnate, secondary vein 9–16 on each side of midrib, anastomosing 5–6 mm before margin, margin entire, base cuneate to obtuse, apex acuminate or apiculate to cuspidate; petiole 5–15 mm	
	Inflorescence	Terminal or axillary, fascicle 2–3, one apical and another slightly below it on outer side, 8–15-flowered; peduncle 1.5–2 cm, stout, sharply curved and hooked, bristly	
	Flower	Pedicel 0.8–1.5 cm, rufous hispid; sepal triangular-ovate, 2–4 mm, erect, coriaceous, outside rufous pubescent or puberulent, inside glabrous, apex acute; petal light green to yellow, narrowly oblong-lanceolate to oblong, flat, 1.8–3.5 cm × 0.6–0.7 cm, subequal, fragrant, both surfaces slightly pubescent, base deeply convex, apex obtuse; inner petal 3, slightly smaller, narrower and more deeply concave than outer petal, connivent over stamen and carpel, outside thinly pubescent but densely grey pubescent on basal concave part, inside glabrous; stamen 25, yellow, broadly oblong-cuneate, 1.5 mm, puberulent; connective apically truncate or slightly concave; carpel 10–21, oblong-lanceolate to narrowly oblong, 1.3–2 mm, glabrous; ovary ovate-oblong, grooved down the inner side, glabrous; style oblong to narrowly clavate, 1.3–1.5 mm; stigma oblong to ellipsoid, smaller than the ovary, woolly; boundary of carpel with stigma prominent	
	Fruit	Monocarp 4–6, green, ellipsoid, sessile, apex obtuse	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. multiflorus	Origin	Burma; Kanchanaburi, Western Thailand	
	Distribution	Burma; Guangxi and Guizhou, Southern China; Yunnan, Southwestern China	
A. oblanceolatus	Habit	Climber	Chuakul and Soonthornchareonnon (2003); Thongpairoj (2008)
	Branch	Dark brown, furrow, pubescent	
	Leaf	Narrowly elliptic, 10–11.4 cm × 3–4 cm, membranous, both surfaces glabrescent, midrib abaxially prominent, venation pinnate, secondary vein 10–11 on each side of midrib, anastomosing 3–4 mm before margin, margin entire, base obtuse, apex acuminate; petiole 2–3 mm, rugose	
	Inflorescence	Terminal or extra-axillary, solitary; peduncle 0.5–1 cm, hooked	
	Flower	Pedicel 0.4–0.6 cm; sepal broadly ovate, 5–6 mm, erect, membranous, outside minutely pubescent, apex acute; petal pale green to yellow, broadly ovate, flat, 0.8–1 cm × 0.6–0.7 cm, thick, both surfaces white pubescent, apex acute; inner petal 3, 0.6–0.7 cm × 0.5–0.6 cm, pubescent, similar to outer petal; stamen 65, oblong, 1.5–2 mm, free, glabrous; connective apically broadly flat; carpel 11; ovary flask-shaped, 2–3 mm, grooved down the inner side; stigma ovate-oblong, woolly	
	Origin	Chaiyaphum and Ubon Ratchathani, Northeastern Thailand; Pathum Thani and Saraburi, Central Thailand	
	Distribution	Vietnam	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. odoratissimus	Habit	Woody climber or scandent shrub	Wight and Arnott (1834); Miller (1835); Bentham (1861); Dalzell and Gibson (1861); Pardo De Tavera (1901); Kamboj and Dhawan (1982); Randhawa and Mukhopadhyay (1986); Hasan *et al.* (1987); Sharma (1993); Dey (1996); Groom (1997); Garg and Siddiqui (1998); Baker (1999); Dey (2001); Sharma *et al.* (2002); Singh *et al.* (2005); Hout *et al.* (2006); Bordoloi *et al.* (2009); Singh *et al.* (2009); Srivastava *et al.* (2009); Gupta *et al.* (2010); Kabir (2010)
	Root	Branched taproot	
	Stem	Aerial, branched, woody, fragrant	
	Leaf	Dark green, ovate-elliptic or oblong-lanceolate, 10.16–15.24 cm, scarcely coriaceous, both surfaces glabrous and glossy, venation reticulate, margin entire, base acute, apex acute; petiole short	
	Inflorescence	Axillary, 1- or 2-flowered; peduncle hooked, glabrous	
	Flower	Greenish yellow, ripe apple- or jackfruit-like fragrant; sepal 3, green, free, small, valvate; petal 6, greenish yellow, oblong-lanceolate to narrowly oblong, 2.54 cm, thick, fleshy, valvate, glabrous or rusty pubescent, base concave; inner petal similar to outer petal; stamen numerous; carpel many, pyriform, free, glabrous; ovule marginal placentation	
	Fruit	Monocarp yellow, oblong	
	Seed	2, large	
	Origin	China; Indonesia; Jorhat, Assam, Northeastern India; Malwa, Central India	
	Distribution	Bangladesh; Kandal, Cambodia; Malay Archipelago	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. oxycarpus	Habit	Scandent shrub	Thongpairoj (2008)
	Branch	Black and shiny when dry, slender	
	Leaf	Broadly elliptic, 8–11 cm × 3.8–5 cm, chartaceous, both surfaces glabrous, adaxially glossy, midrib abaxially prominent, venation pinnate, secondary vein 8–9 on each side of midrib, anastomosing before margin, base obtuse, apex acuminate; petiole 3–5 mm	
	Inflorescence	Solitary; peduncle slender, hooked, glabrous	
	Flower	Pedicel 0.7–1 cm; sepal ovate to triangular, 4–5 mm, coriaceous, glabrous, acute apex; petal yellow; outer petal oblong-elliptic, 4–5 cm × 1.3–1.4 cm, erect, coriacous, both surfaces pubescent, apex subacute to obtuse; inner petal smaller than outer petal; stamen oblong, 2 mm; connective apically obtuse or convex; carpel several; ovary glabrous	
	Fruit	Monocarp narrowly ellipsoid, 2.5–3 cm × 1 cm, sessile, glabrous, base and apex acuminate	
	Origin	Toh Moh, Narathiwat, Southern Thailand	
	Distribution	Perak, Malaysia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. pilosus	Habit	Climbing shrub to 5 m tall	Chen *et al.* (2004); Wang (2010); Li and Gilbert (2011)
	Branch	Densely tomentose when young	
	Leaf	Oblong-elliptic to oblong, 5–17 cm × 2–7.5 cm, chartaceous, abaxially densely tomentose, adaxially slightly glaucous and glabrous, secondary vein 8 on each side of midrib, base obtuse, apex obtuse to acuminate; petiole 2 mm, densely tomentose	
	Inflorescence	Leaf-opposed or extra-axillary, usually 1-flowered; peduncle longer than pedicel, flat, densely villous when young, glabrescent	
	Flower	Pedicel 0.6–1.2 cm, densely pubescent; sepal ovate, 4 mm, outside pubescent; petal green to yellow, narrowly oblong, 1.5–1.7 cm, pubescent; stamen cuneate; connective apically subtruncate; carpel 8, glabrous	
	Fruit	Monocarp dark brown, oblong-ellipsoid, 1.5–2.2 cm × 1.5 cm, glabrous	
	Origin	Guangdong and Hainan, Southern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. punctulatus	Habit	Climbing shrub to 4 m tall	Li and Gilbert (2011)
	Branch	Puberulent when young	
	Leaf	Oblong-elliptic, 7–13.5 cm × 3–5.5 cm, chartaceous, abaxially glabrous except for puberulent midrib, adaxially minutely punctate, secondary vein 12–14 on each side of midrib, base oblique to broadly cuneate, apex obtuse to acuminate; petiole 5–7 mm, puberulent	
	Inflorescence	Peduncle puberulent	
	Flower	3–4 cm in diameter; pedicel 1.5–2 cm; sepal broadly triangular-ovate, 5–7 mm, puberulent, base slightly attenuate; petal brownish green; outer petal ovate-oblong, 2.5 cm, base concave; inner petal 2 cm, concave and connivent, horizontally spreading from apical 1/3, base attenuate; stamen many, cuneate, 1.5 mm; connective apically subtruncate, glabrous; carpel 20, oblong, glabrous; stigma clavate, longer than ovary, pubescent	
	Fruit	Monocarp fusiform, slightly flattened, 3.5–4 cm × 1.5–1.7 cm	
	Origin	Yunnan, Southwestern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. rhynchocarpus	Habit	Climbing shrub to 10 m tall	Li and Gilbert (2011)
	Branch	Pilose when young, glabrescent	
	Leaf	Oblong-lanceolate to oblong, 8–13 cm × 3–4 cm, chartaceous, abaxially puberulent, adaxially glabrous, secondary vein 12–14 on each side of midrib, base cuneate, apex acuminate; petiole 3–5 mm, pilose	
	Inflorescence	Leaf-opposed, 2–5-flowered; peduncle 0.8–1.5 cm, pilose	
	Flower	Pedicel 1.2–1.5 cm, pubescent; sepal broadly ovate, 3–4 mm × 3–4 mm, both surfaces pilose; petal ovate-oblong, 1.5 cm × 0.6 cm, tawny pubescent; stamen cuneate, 1.5 mm; connective apically subtruncate, glabrous	
	Fruit	Monocarp oblong, 4.5–5 cm × 1.5–1.7 cm, apex conspicuously beaked; stipitate 5–7 mm	
	Seed	2, flat, 3 cm × 1.2 cm	
	Origin	Yunnan, Southwestern China	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. siamensis	Habit	Woody climber	Chuakul and Soonthornchareonnon (2003); Thongpairoj (2008)
	Leaf	Broadly elliptic or oblanceolate, 9–12 cm × 4–5 cm, chartaceous to coriaceous, abaxially tawny pubescent, adaxially glabrescent except for puberulent midrib, midrib abaxially prominent, secondary vein 8–9 on each side of midrib, anastomosing 3–4 mm before margin, margin ciliate, base obtuse, apex abruptly acute; petiole 3–6 mm × 1–1.5 mm	
	Inflorescence	Extra-axillary, solitary; peduncle flat, 1–1.5 cm, hooked, puberulent	
	Flower	Green to yellow; pedicel 0.8–1 cm; sepal broadly ovate to orbicular, 4–5 mm × 6–7 mm, coriaceous, reflexed, both surfaces pubescent, connate at base, apex acuminate; petal green to yellow, flat, 0.1 cm, fleshy, fragrant; outer petal broadly elliptic, 2.7–3 cm × 0.8–1 cm, both surfaces pubescent, apex acute; inner petal rather rhombic, 2.5–2.8 cm × 0.8–1 cm, puberulent; stamen 115, narrowly oblong, 2.5–3 mm, glabrous; connective apically triangular, shortly tomentose; carpel 25; ovary ovate-oblong, 2.5–3 mm, grooved down the inner side, glabrous; ovule 2, basal placentation; stigma cylindrical, 2.5–3 mm, slightly concave, woolly	
	Fruit	Monocarp broadly ellipsoid, 2–2.8 cm × 1.5–2 cm, puberulent, apex acute; stipitate 3–5 mm	
	Origin	Kanchanaburi, Western Thailand; Phetchaburi, Prachuap Khiri Khan and Ratchaburi, Central Thailand	
	Distribution	Burma; Thailand; Vietnam	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. speciosus	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Black when dry, laterally straight, pubescent when young, lenticellate	
	Leaf	Dark brown when dry, oblong, 13–16 cm × 3.5–6.5 cm, coriaceous, both surfaces glabrous, midrib abaxially prominent and sparsely pubescent, secondary vein 10–11 on each side of midrib, anastomosing 4–6 mm before margin, base obtuse, apex acuminate; petiole 3–5 mm, slightly pubescent	
	Inflorescence	5–6-flowered; peduncle 2, terete to subterete, sparsely pubescent; bract ovate, 2 mm × 1.5 mm, sparsely pubescent, apex acute; flower bud ovate, 2 mm × 2 mm, pubescent	
	Flower	Pedicel 0.4–0.6 cm, sparsely pubescent; sepal triangular, 2–3 mm × 2 mm, erect, fleshy, valvate, outside pubescent, inside glabrous, apex acuminate; outer petal lanceolate, flat, 2–2.5 cm × 0.1–0.3 cm, thinly fleshy, valvate, tomentose; inner petal terete, 2–2.5 cm × 0.1 cm, linear, tomentose; torus flat; stamen 20, oblong, 1 mm × 1 mm; connective apically acute; carpel 5, broadly ovoid, 1–1.5 mm × 1 mm	
	Fruit	Monocarp ellipsoid, 3–3.5 cm × 2–2.5 cm	
	Distribution	Malaysia; Moluccas and New Guinea, Indonesia	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. spinosus	Habit	Scandent shrub to 6 m tall	Chuakul and Soonthornchareonnon (2003); Thongpairoj (2008); Sichaem *et al.* (2011)
	Stem	Light brown, pubescent when young; spines in axillary pairs	
	Leaf	Narrowly elliptic or oblong-obovate, 6–8 cm × 2.5–4.5 cm, coriaceous, both surfaces glabrous, midrib abaxially prominent, secondary vein 7–8 on each side of midrib, anastomosing 1–2 mm before margin, base cuneate, apex retuse; petiole 2–3 mm × 1 mm	
	Inflorescence	Fascicle 1–3-flowered; peduncle 1.5–2 cm, hooked; bract triangular, 1.5 mm × 1.5 mm	
	Flower	Pedicel 1.3–1.5 cm × 0.1 cm; sepal broadly ovate, 5 mm × 5–7 mm, coriaceous, reflexed, both surfaces puberulent, apex acuminate; petal green, fleshy, abaxially densely red brown maculate, midrib adaxially prominent; outer petal ovate, 2–2.5 cm × 1–1.5 cm, both surfaces white puberulent except for tomentose base, base obcordate, apex acute; inner petal, obovate or broadly rhombic-elliptic, 1.5–2 cm × 0.6–0.8 cm, puberulent; torus sericeous; stamen light yellow, oblong, 2–2.5 mm, glabrous; connective apically apiculate, pubescent; carpel 12–13; ovary obclavate or narrowly ovate-oblong, 1.8–2 mm, grooved down the inner side, glabrous; stigma lingulate, 1–1.5 mm, elongated, woolly	
	Fruit	Monocarp glabrous; stipitate pale green, elliptic, white maculate	
	Origin	Loei, Maha Sarakham, Nakhon Ratchasima, Sisaket and Ubon Ratchatani, Northeastern Thailand	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. suaveolens	Habit	Woody climber or scandent scrub to 25 m tall	Pardo De Tavera (1901); Maranon (1929); Barger and Sargent (1939); Eloumi-Ropivia *et al.* (1985); Keng and Keng (1990); Aguilar (2001); Wiart (2006); Triastinurmiatiningsih (2007); Thongpairoj (2008)
	Branch	Black, laterally straight, glabrous or slightly pubescent when young, lenticellate, finely striated	
	Leaf	Dark green, brown when dry, elliptic to oblong or oval, 4.5–18 cm × 2–6 cm, chartaceous to thinly coriaceous, both surfaces glabrous and glossy except for abaxially midrib, midrib adaxially prominent, secondary vein 8–10 on each side of midrib, anastomosing 3–5 mm before margin, base acute to shortly attenuate or cuneate, apex shortly acuminate to cuspidate; petiole 2–10 mm, slender, sparsely pubescent	
	Inflorescence	1–5-flowered; peduncle 3, terete to subterete, hooked, pubescent; bract lanceolate, 1.5 mm × 0.5 mm, outside sparsely pubescent, inside glabrous; flower bud broadly ovoid, 2 mm × 1.5 mm, pubescent	
	Flower	Creamy white, fragrant; pedicel 0.4–1 cm, slender, sparsely pubescent; sepal broadly ovate, 1.5–2 mm × 1–1.5 mm, erect, fleshy, valvate, outside sparsely pubescent, inside glabrous, apex acute or acuminate; petal 6, creamy white or yellow, fleshy, fragrant; outer petal terete, linear, minutely tomentose or pubescent, claw orbicular, inside glabrous, limb terete, 0.7–1.5 cm × 0.05–0.1 cm, slender, slightly incurved, apex obtuse; inner petal terete, 0.7–1.5 cm × 0.05 cm, linear, slender, pubescent, apex obtuse; torus flat; stamen 20–25, broadly oblong, 0.2–1 mm × 0.5 mm, scarcely any filament; connective apically flat or discoid, minutely tomentose;	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. suaveolens	Flower	carpel 2–5, broadly ovoid, 0.4–0.5 mm, glabrous; ovary broadly ovoid, grooved down the inner side, glabrous; ovule 2, basal placentation; style linear, bent; stigma bilobed, rather broadly flattened or cup-shaped, woolly; boundary of carpel with stigma prominent	
	Fruit	Monocarp green or yellow, oblong to ellipsoid, 0.7–3.5 cm × 0.5–1.2 cm, sessile, glabrous, apex obtuse; stipitate 5–10 mm	
	Seed	2, brown, ellipsoid, plano-convex	
	Origin	Krabi, Phang Nga, Ranong, Songkhla, Surat Thani, Trang and Yala, Southern Thailand; Trat, Southeastern Thailand	
	Distribution	Borneo; Burma; India; Java, Moluccas, New Guinea, Sulawesi and Sumatra, Indonesia; Malacca, Malaysia; Philippines	
A. sumatranus	Habit	Climber	Triastinurmiatiningsih (2007)
	Branch	Dark brown or black when dry, laterally straight, sparsely pubescent when young, lenticellate	
	Leaf	Dark brown when dry, elliptic to oblong, obovate, 6–12 cm × 3–6 cm, thinly coriaceous, both surfaces glabrous except for abaxially midrib, midrib abaxially prominent, secondary vein 7–11 on each side of midrib, anastomosing 3–6 mm before margin, base cuneate to attenuate, apex shortly acuminate; petiole 3–5 mm, sparsely pubescent	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. sumatranus	Inflorescence	3–4-flowered; peduncle 3, flat, sparsely pubescent; bract ovate, 2–3 mm × 0.5–1 mm, outside pubescent, inside glabrous; flower bud broadly ovoid, 1.5–2 mm × 1–1.5 mm, pubescent	
	Flower	Pedicel 0.3–0.6 cm, sparsely pubescent; sepal ovate, 3–5 mm × 2–3 mm, erect, deflexed, valvate, outside densely pubescent, inside glabrous, connate at base, apex caudate; outer petal lanceolate, flat, 1.5–2 cm × 0.1–0.2 cm, thinly fleshy, densely pubescent, apex acute; inner petal terete, 0.8–1.7 cm, apex obtuse; torus flat; stamen 20–25, oblong; connective apically acute; carpel 3, ovoid, 1–2 mm × 0.5–1 mm, free, glabrous; stigma ovoid	
	Fruit	Monocarp 2–3, ellipsoid, 3–4 cm × 2–2.5 cm; stipitate 1–2 cm	
	Distribution	Borneo; Java, New Guinea and Sumatra, Indonesia	
A. thomsonii	Habit	Climber	Oliver (1868)
	Leaf	Oblong-elliptic, 10.16–20.32 cm × 5.08–7.62 cm, both surfaces glabrous or glabrescent except for rusty pubescent midrib, midrib abaxially prominent, base obtuse, apex shortly or scarcely obtusely acuminate; petiole 2.12–6.35 mm	
	Inflorescence	Extra-axillary; peduncle woody, hooked	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. thomsonii	Flower	Reddish brown, 1.27–1.9 cm; pedicle 1.27–1.9 cm; sepal broadly ovate, 4.23 mm, very small, apex acute; petal free, linear, narrow; outer petal 3, rather longer, dilated, outside rusty pilose, base concave; inner petal 3, trigonous, base concave; stamen minute, sessile or subsessile; connective apically dilated; ovary, slightly pilose; ovule 2, erect; stigma various, ovate-oblong or laterally dilated	
	Fruit	Monocarp numerous, ellipsoid, 1.27–1.7 cm, nearly glabrous, apex shortly acute; stipitate 6.67–12.7 mm	
	Seed	1	
A. uncinatus	Habit	Scandent shrub	Wu *et al.* (1989); Zhou and Xu (1994); Boukouvalas *et al.* (1995); Xu and Dong (1995); Hsieh *et al.* (1999); Hsieh *et al.* (2001); Sambamurty (2005); Szpilman *et al.* (2005); Li (2006); Vardhana (2006); Lan *et al.* (2007); Vardhana (2008); Zheng and Xing (2009); Gothandam *et al.* (2010)
	Leaf	Lanceolate or oblong, 8–15 cm, abaxially pale, adaxially glossy	
	Inflorescence	Peduncle hooked	
	Flower	Greenish yellow, fragrant; sepal ovate, reflexed, apex acute; petal 2–4 cm, pubescent	
	Fruit	Monocarp 6–10, green, obovoid	
	Seed	Oblong, deeply grooved on one side	
	Origin	Sri Lanka	
	Distribution	Guangdong and Hainan, Southern China; Kaohsiung, Southwestern Taiwan; Malay Archipelago; Pingtung, Southern Taiwan; Vellore, Tamil Nadu, Southern India	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. uniflorus	Habit	Woody climber	Thongpairoj (2008)
	Leaf	Oblong-elliptic, 12–16 cm × 2.5–4 cm, membranous to slightly chartaceous, abaxially pubescent, adaxially glabrous except for midrib, secondary vein 11–14 on each side of midrib, anastomosing 2–4 mm before margin, base acute or obtuse, apex acuminate to caudate; petiole 3–5 mm × 1 mm	
	Inflorescence	1-flowered; peduncle rather short compressed, woody, hooked	
	Flower	Pedicel trigonous, 0.6–0.7 cm, tawny tomentose; sepal triangular, 6–7 mm × 5–6 mm, coriaceous, reflexed, outside pubescent, inside glabrous, apex caudate; petal green, awl-shaped, very thick and hard; outer petal triquetrous, limb subulate, 2 cm × 0.4–0.5 cm, grey pubescent; inner petal similar to outer petal but slightly smaller; stamen 84, rather broadly oblong, 1–1.5 mm, glabrous; connective apically orbicular or convex, pubescent; carpel 8–9; ovary ellipsoid, 2–2.5 mm, golden pubescent; ovule 2, basal placentation; stigma dark colour, oblong, 1–2 mm, shorter but broader, puberulent	
	Fruit	Monocarp 5–12, ovoid, 2–2.4 cm × 1.3–1.5 cm, sessile, glabrous, apex acuminate	
	Seed	2, dark brown, oblong-ellipsoid, 1.4–1.5 cm × 0.7–0.8 cm, plano-convex	
	Origin	Phang Nga and Ranong, Southern Thailand	
	Distribution	Tenasserim, Southeastern Burma	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. vanprukii	Habit	Climber	Thongpairoj (2008)
	Branch	Dark-coloured, rusty pubescent when young	
	Leaf	Elliptic to obovate, 10–13 cm × 5.5–6 cm, coriaceous, abaxially rusty tomentose, adaxially brownish grey and glossy except for puberulent midrib, midrib abaxially prominent, venation pinnate, secondary vein 11–12 on each side of midrib, anastomosing 3–4 mm before margin, margin entire, base obtuse, apex abruptly acute; petiole 5–10 mm, inflated, pubescent	
	Inflorescence	Extra-axillary, 2-flowered; peduncle hooked	
	Flower	Sepal triangular, 6 mm × 7 mm; petal 6, green, flat, fleshy; outer petal, broadly elliptic, 1.4–2 cm × 1.5 cm, both surfaces pubescent, apex acute to obtuse; inner petal smaller than outer petal; stamen oblong to cuneate, 2 mm; connective apically triangular; carpel glabrous; ovary cylindrical, glabrous; ovule 2, basal placentation; stigma cylindrical, glabrous	
	Origin	Lampang, Northern Thailand; Prachuap Khiri Khan, Central Thailand	
	Distribution	Thailand; Vietnam	

TABLE 2.1 Botanical descriptions of *Artabotrys* species (continued).

Plant species	Botanical description		Reference
A. venustus	Habit	Climber to 25 m tall	Cave *et al.* (1986); Triastinurmiatiningsih (2007)
	Branch	Brown when dry, laterally straight, sparsely pubescent when young, lenticellate	
	Leaf	Yellowish brown when dry, elliptic to oblong, 9–15 cm × 3–6 cm, coriaceous, both surfaces glabrous, adaxially glossy, midrib abaxially and adaxially prominent, secondary vein 7–9 on each side of midrib, anastomosing 4–5 mm before margin, base cuneate, apex shortly acuminate; petiole 3–5 mm, sparsely pubescent	
	Inflorescence	3–5-flowered; peduncle 2, subterete, sparsely pubescent	
	Flower	Pedicel 2–2.5 cm, glabrous; sepal ovate, 3–4 mm × 2–3 mm, erect, deflexed, outside tomentose, apex caudate; outer petal greenish yellow, oblong, flat, 0.6–1 cm × 0.3–0.5 cm, fleshy, tomentose, apex acute; inner petal oblong, flat, 0.6–1 cm × 0.2–0.4 cm, narrower; carpel 6, ovoid, 1–2 mm × 0.5–1 mm; stigma axe-shaped	
	Distribution	Cameron Highlands, Pahang, and Larut, Perak, Malaysia; New Guinea and Sumatra, Indonesia	

2.1.2 Ethnomedicinal and non-medicinal uses

Artabotrys species have a long history of traditional use for a wide range of medical conditions, particularly malaria (Ranganathan *et al.* 2012a), scrofula (Lan *et al.* 2007) and cholera (Lal and Singh 2012). Table 2.2 lists the applications of *Artabotrys* species as folk medicines for the treatment of various ailments in different countries. According to the habitual remedies practised by local communities, roots are the most commonly used plant parts, followed by leaves, stems, flowers and fruits. Prior to administration, majority of the plant remedies are prepared as decoction and infusion by using single plant parts or in combination with different plant parts or species. The utilisation of more than one plant species in the preparation of remedies could be attributed to their synergistic effects that they could have during ailment treatment (Yineger and Yewhalaw 2007). With respect to the mode of administration, most of the preparations are taken orally whereas some are applied topically either as bath or massage.

In addition to their medicinal applications, *Artabotrys* species are employed in the manufacture of perfumes due to the fragrance of the flowers (George *et al.* 2011). These aromatic flowers are also used as flavouring agents (Seidemann 2005) as well as for making stimulating tea-like beverages (Mishra *et al.* 2008). Furthermore, both leaves and fruits of *Artabotrys* species are utilised as animal feeds, predominantly for cattle (Aguilar 2001), chimpanzees (Moore 1994) and goats (Marble 2012). Other non-medicinal uses of different plant parts of *Artabotrys* species are shown in Table 2.3.

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species.

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. aurantiacus	Not specified	Treatment of diabetes	Congo Basin Forest, DR Congo, Central Africa	Bruno *et al.* (2013)
A. brachypetalus	Roots	Remedy for abdominal pains during pregnancy (mush; orally)	Muda, Mozambique, Southeastern Africa	Bruschi *et al.* (2011)
		Remedy for abdominal troubles (mixed with roots of *Combretum erythrophyllum*, *Cyperus sexangularis* and *Salix mucronata*, stems of *Phragmites mauritianus*, and sedges)	Venda, Southern Africa	Mabogo (1990)
		Remedy for asthma and cough	Machava and Massingir, Mozambique, Southeastern Africa	Luo *et al.* (2011)
		Remedy for pelvic pains and stomach troubles (decoction with stem bark of *Parinari curatellifolia* subsp. *mobola* and *Rauvolfia caffra* to prepare soft porridge for older people)	Venda, Southern Africa	Mabogo (1990)
		Remedy for women with physical defects (mixed with roots of *Garcinia livingstonei* and *Heteropyxis natalensis*)		

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. brachypetalus	Roots	Treatment of convulsions (infusion; orally)	Malawi, Southeastern Africa	Sobiecki (2002); Stafford *et al.* (2008)
		Treatment of female infertility, food poisoning, general weakness, intestinal worms, snake bites, stomach ache and venereal diseases (maceration; orally)	Manica, Mozambique, Southeastern Africa	Bruschi *et al.* (2011)
		Treatment of impotence (powdered with roots of *Garcinia livingstonei* and *Securidaca longipedunculata*, and added to sorghum beer; orally)	Venda, Southern Africa	Arnold and Gulumian (1984); Mabogo (1990); Steenkamp (2003)
		Treatment of infertility (maceration with roots of *Antidesma venosum*, *Dichrostachys cinerea* subsp. *africana* and *Zantedeschia aethiopica*; orally twice daily for one week, or decoction with roots of *Berchemia discolor*, *Capparis tomentosa*, *Cassytha filiformis*, *Maerua cafra*, *Osyris lanceolata*, *Sphedamnocarpus galphimiifolius* subsp. *galphimiifolius* and *Sphedamnocarpus pruriens* to prepare soft porridge; orally for one week)		

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. brachypetalus	Roots	Used as aphrodisiac (mixed with root bark of *Acacia ataxacantha* and *Wrightia natalensis*, and bark of *Albizia versicolor*)	Venda, Southern Africa	Mabogo (1990)
		Used as aphrodisiac and stimulant (infusion)	Southern Africa	Abdillahi and Van Staden (2012)
		Used to improve health of babies (decoction with roots of *Cardiogyne africana*, *Celosia* spp., *Ficus platyphylla* and *Senna petersiana* to make tea)	Maputo, Mozambique, Southeastern Africa	Krog *et al.* (2006)
		Used to keep baby's stomach in good condition and cleanse the blood (infusion with roots of *Albizia brevifolia*, *Annona senegalensis*, *Bauhinia galpinii*, *Carissa edulis*, *Cassine* spp., *Crotalaria* spp., *Diospyros lycioides*, *Hippocratea* spp., *Maytenus senegalensis*, *Piliostigma thonningii*, *Rhoicissus tridentate*, *Sansevieria hyacinthoides* and *Terminalia sericea*, root bark of *Ficus sycomorus* and *Syzygium cordatum*, bark of *Acacia albida* and *Syzygium guineense*, and fruits of *Gardenia volkensii* to prepare soft porridge for baby)	Venda, Southern Africa	Mabogo (1990)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. brachypetalus	Root bark	Treatment of gonorrhoea (concoction)	Tanzania, Eastern Africa	Nyandoro *et al.* (2012)
A. grandifolius	Flowers	Used as cardiotonic (decoction)	Krabi and Pattani, Southern Thailand	Chuakul and Soonthornchareonnon (2003); Chuakul *et al.* (2004)
	Not specified	Treatment after childbirth	Jerantut, Pahang, Malaysia	Eswani *et al.* (2010)
A. hainanensis	Not specified	Treatment of malaria and scrofula	Hainan, Southern China	Han *et al.* (2005)
		Used as analgesic, antidotal, antiphlogistic and antipyretic		Bi *et al.* (2004); Chen *et al.* (2004)
A. harmandii	Roots and stems	Used as lactagogue	Chaiyaphum and Yasothon, Northeastern Thailand	Chuakul and Soonthornchareonnon (2003)
	Stems	Remedy for body pains (decoction; orally)	Chanthaburi, Eastern Thailand	Chuakul *et al.* (2006)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. harmandii	Not specified	Used to promote lactation in breastfeeding women (powdered with *Albizia myriophylla*, *Alyxia reinwardtii*, *Amaranthus spinosus*, *Amomum testaceum*, *Artemisia annua*, *Cinnamomum porrectum*, *Cinnamomum verum*, *Cyperus rotundus*, *Dryobalanops aromatica* (borneol), *Euphorbia hirta*, *Oenanthe stolonifera*, *Syzygium aromaticum*, *Tarenna hoaensis*, *Tinospora tomentosa*, *Xantolis cambodiana* and *Zingiber officinale*)	Thailand	Luecha and Umehara (2013)
A. hexapetalus	Roots and leaves	Remedy for abdominal and kidney pains (decoction with roots of *Uvaria leptocladon*)	Korogwe, Tanzania, Eastern Africa	Hedberg *et al.* (1982)
	Roots and fruits	Treatment of malaria and scrofula	Southern China	Li *et al.* (1997); Li and Yu (1998); Aguilar (2001); Yu *et al.* (2001); Wong and Brown (2002); Yu *et al.* (2002); Mahidol *et al.* (2005); Schobert and Schlenk (2008); Karthik (2010); Jayanthi (2011); Manjula *et al.* (2011)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. hexapetalus	Leaves	Treatment of cholera (decoction)	Hazaribagh, Jharkhand, Eastern India; Malay Archipelago; Philippines	Aguilar (2001); Brophy *et al.* (2004); Savadi (2009); Karthik (2010); Jayanthi (2011); Lal and Singh (2012)
		Treatment of itching	Southern India	Dhiman *et al.* (2012)
	Flowers	Treatment of bad breath, biliousness, bladder diseases, blood and heart diseases, headache, itching, leucoderma, sweating, thirst and vomiting	India	Aguilar (2001); Savadi (2009)
		Used as cardiotonic	Thailand	Chuakul and Soonthornchareonnon (2003)
	Not specified	Used as cardiac stimulant, muscle relaxant and uterine stimulant	Bangalore, Karnataka, Southwestern India	Khare (2008); Rajkumar and Rajanna (2011)
A. modestus	Roots	Remedy for diarrheoa and stomach ache (decoction)	Kenya and Tanzania, Eastern Africa	Kokwaro (2009); Nyandoro *et al.* (2012)
		Remedy for spiritual ailments (decoction)	Duruma, Kenya, Eastern Africa	Pakia (2000)
		Remedy for stomach ache	Tanzania, Eastern Africa	Burgess *et al.* (2000)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. modestus	Leaves	Treatment of nausea and vomiting (infusion)	Kenya and Tanzania, Eastern Africa	Kokwaro (2009); Nyandoro *et al.* (2012)
A. monteiroae	Roots	Remedy for backache, diarrhoea and stomach ache (decoction)	Kenya, Eastern Africa	Kato *et al.* (1993)
	Roots and bark	Treatment of malaria (decoction; orally)	Tanzania, Eastern Africa	Fowler (2011)
	Leaves	Treatment of malaria (decoction; bathing)		
A. oblanceolatus	Roots and stems	Used as lactagogue	Chaiyaphum, Northeastern Thailand	Chuakul and Soonthornchareonnon (2003)
A. odoratissimus	Roots	Treatment of malaria	Thiruvannamalai, Tamil Nadu, Southern India	Ranganathan *et al.* (2012a); Ranganathan *et al.* (2012b); Senthilkumar *et al.* (2014)
	Roots and fruits	Treatment of malaria and scrofula	China	Bordoloi *et al.* (2009); Gupta *et al.* (2010)
	Stems	Treatment of malaria	Kandal, Combodia	Hout *et al.* (2006)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. odoratissimus	Leaves	Treatment of cholera (decoction)	Malay Archipelago; Malwa, Central India; Thiruvannamalai, Tamil Nadu, Southern India	Pardo De Tavera (1901); Hasan *et al.* (1987); Garg and Siddiqui (1998); Sharma *et al.* (2002); Singh *et al.* (2005); Bourcet *et al.* (2008); Srivastava *et al.* (2009); Ranganathan *et al.* (2012a); Ranganathan *et al.* (2012b); Senthilkumar *et al.* (2014)
	Flowers	Treatment of bad breath, biliousness, bladder diseases, blood and heart diseases, headache, itching, leucoderma, sweating, thirst and vomiting	India	Garg and Siddiqui (1998); Sharma *et al.* (2002); Singh *et al.* (2009)
	Fruits	Treatment of topical fungal infection	Assam, Northeastern India	Bordoloi *et al.* (2009); Jayanthi (2011)
	Not specified	Used as emmenagogue and stimulant	Malay Archipelago	Pardo De Tavera (1901)
A. pallens	Stem woods	Treatment of gastritis (decoction with parasitic plant of *Buab lum* and whole plant of *Mak dai bai*; orally)	Bolikhamsai, Laos	Libman *et al.* (2006)
A. pilosus	Root, stems and leaves	Treatment of malaria and scrofula	Hainan, Southern China	Wang (2010)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. rhopalocarpus	Saps	Used as aphrodisiac (orally)	Mount Cameroon, Central Africa	Focho *et al.* (2010); Bele *et al.* (2011)
A. siamensis	Flowers	Used as cardiotonic	Thailand	Chuakul and Soonthornchareonnon (2003)
A. spinosus	Stem bark	Remedy for venereal diseases	Ubon Ratchathani, Northeastern Thailand	Chuakul and Soonthornchareonnon (2003)
A. stenopetalus	Saps	Used as aphrodisiac	Zaire, Central Africa	Fleischer *et al.* (1997)
	Twigs	Used as part of a prescription for the promotion of conception		
	Leaves	Treatment of enlarged spleen (orally)		
A. suaveolens	Roots and bark	Used as emmenagogue, and to relieve fatigue after childbirth (decoction; orally)	Philippines	Aguilar (2001); Wiart (2006)
	Leaves	Treatment of cholera (decoction or infusion; orally)	India; Java, Indonesia	Pardo De Tavera (1901); Maranon (1929); Aguilar (2001); Wiart (2006)
A. taynguyenensis	Not specified	Treatment of fever and inflammation	Vietnam	Thang *et al.* (2014)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
A. uncinatus	Roots	Treatment of malaria	Southern China; Taiwan	Wu *et al.* (1989); Zhou and Xu (1994); Boukouvalas *et al.* (1995); Xu and Dong (1995); Hsieh *et al.* (1999); Szpilman *et al.* (2005); Lan *et al.* (2007); Dewick (2011); Nyandoro *et al.* (2012)
	Bark	Remedy for gastrointestinal diseases (toasted; massaging)	Hainan, Southern China	Zheng and Xing (2009)
	Leaves	Treatment of cholera (decoction)	Malay Archipelago	Vardhana (2008)
	Fruits	Treatment of scrofula	Southern Taiwan	Hsieh *et al.* (1999); Lan *et al.* (2007)
	Whole plant	Treatment of hepatocarcinoma	Taiwan	Li (2006)
	Not specified	Treatment of glandular swellings	Taiwan	Nyandoro *et al.* (2012)
		Treatment of nasopharyngeal carcinoma		Hsieh *et al.* (2001)
A. zeylanicus	Flowers	Treatment of vomiting (decoction)	Kerala, Southwestern India	Yesodharan and Sujana (2007)

TABLE 2.2 Ethnomedicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Ethnomedicinal use	Region	Reference
Artabotrys sp.	Roots	Remedy for constipation and joint pains (decoction; orally)	Gombak, Selangor, Malaysia	Azliza *et al.* (2012)
	Not specified	Treatment of open sores	Chiang Rai, Northern Thailand	Anderson (1986)
		Used as stimulant	Madagascar	Sobiecki (2002)

TABLE 2.3 Non-medicinal uses of *Artabotrys* species.

Plant species	Part used	Non-medicinal use	Region	Reference
A. brachypetalus	Stems	Used for roof and courtyard wall construction	Venda, Southern Africa	Mabogo (1990)
	Fruits	Used for beverage making	Limpopo, Southern Africa	Rampedi (2010)
	Woods	Used to make household utensils and music instruments	Mutare, Zimbabwe, Southern Africa	Grundy *et al.* (1993)
	Not specified	Used as feeds for goats	Inhambane, Mozambique, Southeastern Africa	Marble (2012)
A. hexapetalus	Flowers	Used as flavouring in tea	Southern India; Sri Lanka	Seidemann (2005)
		Used in perfumery as the source of essential oils	Salem, Tamil Nadu, Southern India;	Aguilar (2001); Mishra *et al.* (2008)
		Used to prepare stimulating tea-like beverages		
	Whole plant	Used as ornamental plants	Jajpur, Odisha, Southeastern India	Mohanty *et al.* (2012)
		Used for screen planting in large gardens	Java, Indonesia; Philippines; Southern China	Aguilar (2001)

TABLE 2.3 Non-medicinal uses of *Artabotrys* species (continued).

Plant species	Part used	Non-medicinal use	Region	Reference
A. hexapetalus	Not specified	Used as hair lotion	Thoubal, Manipur, Northeastern India	Devi *et al.* (2014)
A. monteiroae	Fruits	Used as feeds for chimpanzees	Mahale Mountains and Ugalla, Tanzania, Eastern Africa	Nishida and Uehara (1983); Moore (1994)
A. odoratissimus	Flowers	Used in perfumery as the source of essential oils	Coimbatore, Tamil Nadu, Southern India	George *et al.* (2011)
A. scytophyllus	Flowers	Used as flavouring and spices for sauces	Madagascar; Southeastern Asia	Seidemann (2005)
A. speciosus	Fruits	Used to make head and neck garlands	Andaman Islands	Saxena *et al.* (2003)
A. suaveolens	Stems	Used as water substitutes	Kuala Pilah, Negeri Sembilan, Malaysia	Ong *et al.* (2011)
	Leaves	Used as feeds for cattle	Bali, Indonesia	Aguilar (2001)
	Whole plant	Used as living fences		
A. taynguyenensis	Not specified	Used as flavouring	Vietnam	Thang *et al.* (2014)
A. thomsonii	Stem saps	Used as water substitutes	Tshopo, DR Congo, Central Africa	Termote *et al.* (2011)
A. uncinatus	Flowers	Used to scent oil	Tonga	Weiner (1971)

2.1.3 Chemical constituents

To date, more than 200 chemical constituents have been identified and isolated from the genus *Artabotrys*, including 68 alkaloids (Hsieh *et al.* 2001), 28 phenolic compounds (Savadi 2009), 67 terpenoids (Fournier *et al.* 1997) and 40 other related compounds (Mahidol *et al.* 2005). The names and structures of these compounds, as well as their corresponding plant sources are given in Table 2.4–2.7.

Alkaloids are a group of natural organic compounds that contain mostly basic nitrogen atoms in a heterocyclic ring (Li *et al.* 2013). The majority of the alkaloids found in *Artabotrys* species are aporphinoids, with anonaine (Sagen *et al.* 2003), asimilobine (Han *et al.* 2005), atherospermidine (Lan *et al.* 2007) and norstephalagine (Sharma *et al.* 2002) being isolated as the predominant compounds.

Phenolic compounds are a class of chemical compounds that consist of one or more hydroxyl groups attached directly to an aromatic ring (Enache and Oliveira-Brett 2011). Flavonoids constitute the main group of phenolic compounds occurring in *Artabotrys* species, with catechin (Nyandoro *et al.* 2012) being the most abundant compound obtained.

Terpenoids are a large and diverse group of naturally occurring organic chemicals derived from five-carbon isoprene units assembled and modified in numerous ways (Meng *et al.* 2013). Most of the terpenoids present in *Artabotrys* species are sesquiterpenoids, with caryophyllene oxide (Phan *et al.* 2007) and spathulenol (Trang *et al.* 2014) being identified as the major components.

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species.

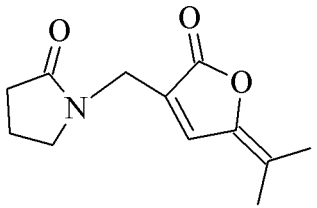
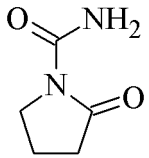
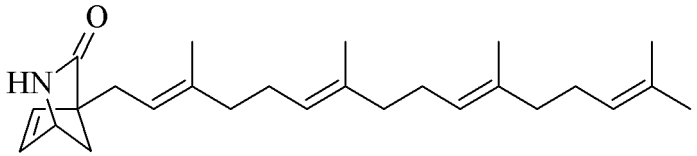
Compound	Structure	Plant species	Plant part	Reference
BUTENOLIDE ALKALOID				
Uncinine		*A. uncinatus*	Leaves	Hsieh *et al.* (2001)
			Whole plant	Li (2006)
PYRROLIDINE ALKALOID				
Squamolone		*A. uncinatus*	Stems	Hsieh *et al.* (2001)
			Whole plant	Li (2006)
TROPANE ALKALOID				
Artamodamide		*A. modestus*	Root bark	Nyandoro *et al.* (2012)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

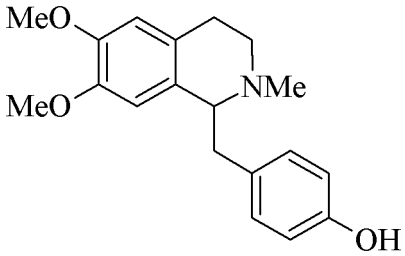
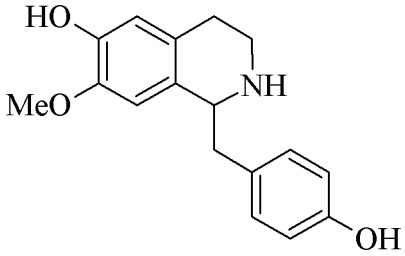
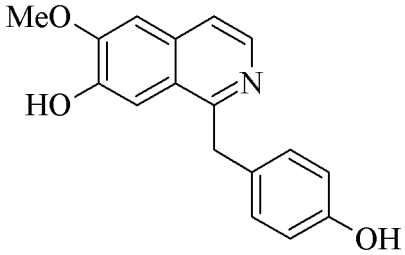
Compound	Structure	Plant species	Plant part	Reference
BENZYLTETRAHYDROISOQUINOLINE				
Armepavine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)
Isococlaurine		*A. hainanensis*	Stems	Han *et al.* (2005)
Juzirine		*A. hainanensis*	Stems	Han *et al.* (2005)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

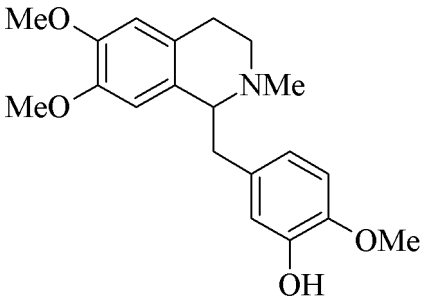
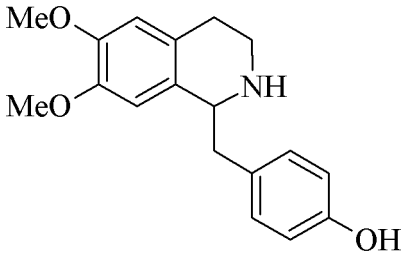
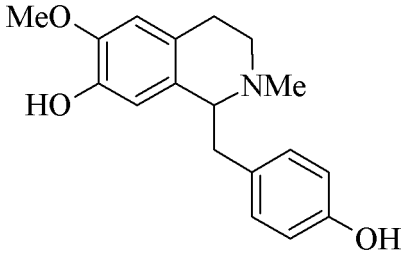
Compound	Structure	Plant species	Plant part	Reference
Laudanine		*A. modestus*	Root bark	Nyandoro *et al.* (2012)
N-demethylarmepavine		*A. hainanensis*	Stems	Han *et al.* (2005)
N-methylcoclaurine		*A. brachypetalus* *A. odoratissimus*	Stem bark Stem bark	Sagen *et al.* (2003) Sharma *et al.* (2002)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

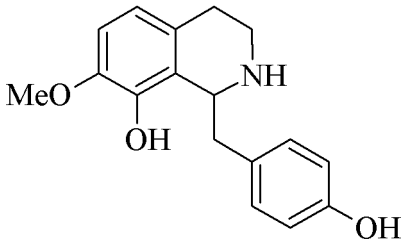
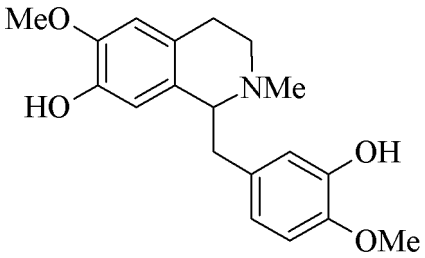
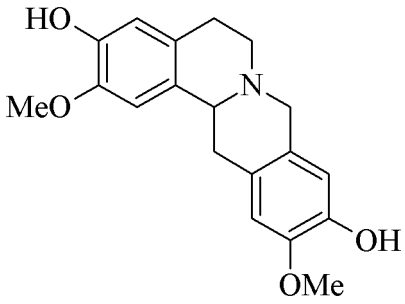
Compound	Structure	Plant species	Plant part	Reference
Norjuziphine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)
Reticuline		*A. monteiroae*	Roots	Kato *et al.* (1993)
		A. uncinatus	Leaves, roots and stems	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Kam (1999)
TETRAHYDROPROTOBERBERINE				
10-O-demethyldiscretine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)
		A. uncinatus	Roots	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

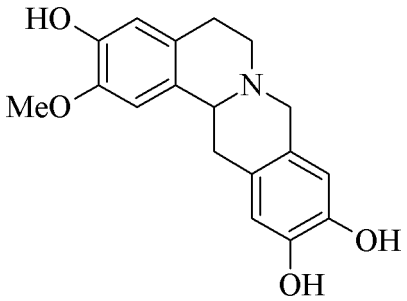
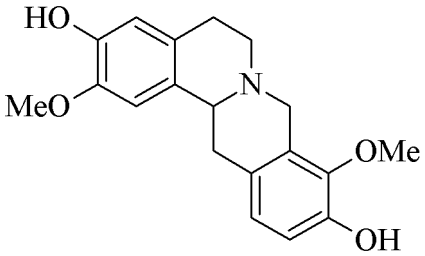
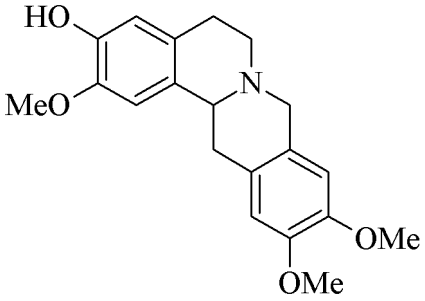
Compound	Structure	Plant species	Plant part	Reference
Artavenustine		*A. uncinatus*	Stems	Lan *et al.* (2007)
		A. venustus	Stem bark	Cave *et al.* (1986); Teo *et al.* (1990); Kam (1999)
Discretamine		*A. maingayi*	Bark	Cortes *et al.* (1990); Teo *et al.* (1990); Kam (1999)
		A. venustus	Stem bark	Cave *et al.* (1986); Kam (1999)
Discretine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

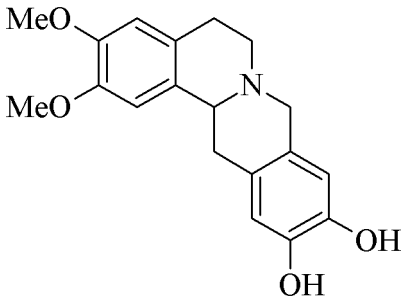
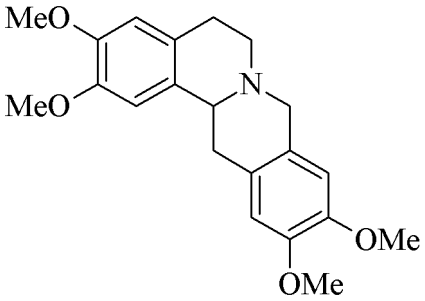
Compound	Structure	Plant species	Plant part	Reference
Spinosine		*A. hainanensis*	Stems	Han *et al.* (2005)
Xylopinine		*A. grandifolius*	Stems	Chan *et al.* (1987); Teo *et al.* (1990); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

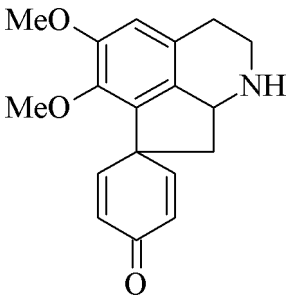
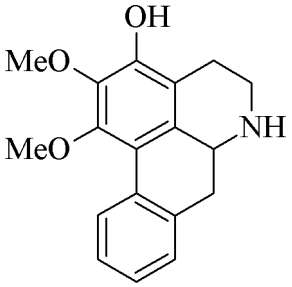
Compound	Structure	Plant species	Plant part	Reference
PROAPORPHINE				
Stepharine		*A. uncinatus*	Fruits	Hsieh *et al.* (1999)
			Leaves, roots and stems	Hsieh *et al.* (2001)
APORPHINOID				
Aporphine				
3-Hydroxynornuciferine		*A. hainanensis*	Stems	Han *et al.* (2005)
		A. maingayi	Bark	Cortes *et al.* (1990); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

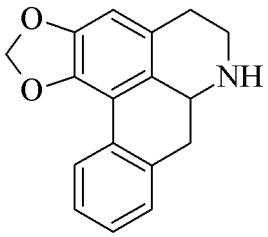
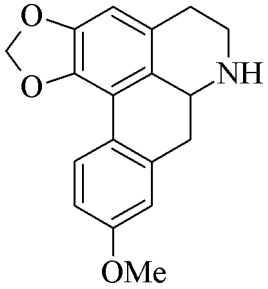
Compound	Structure	Plant species	Plant part	Reference
Anonaine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)
		A. madagascariensis	Not specified	Maranon (1929)
		A. maingayi	Bark	Cortes *et al.* (1990); Kam (1999)
		A. monteiroae	Roots	Kato *et al.* (1993)
		A. odoratissimus	Stem bark	Sharma *et al.* (2002)
		A. uncinatus	Fruits	Hsieh *et al.* (1999)
			Leaves, roots and stems	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
Artabotrinine		*A. suaveolens*	Bark	Barger and Sargent (1939); Leboeuf *et al.* (1982)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

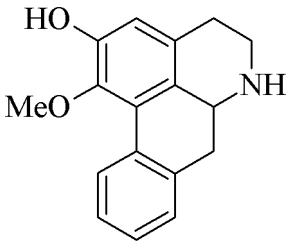
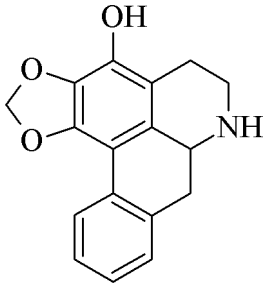
Compound	Structure	Plant species	Plant part	Reference
Asimilobine		*A. brachypetalus*	Stem bark	Sagen *et al.* (2003)
		A. hainanensis	Stems	Han *et al.* (2005)
		A. monteiroae	Roots	Kato *et al.* (1993)
		A. odoratissimus	Stem bark	Sharma *et al.* (2002); Savadi (2009)
		A. uncinatus	Fruits	Hsieh *et al.* (1999)
			Leaves, roots and stems	Hsieh *et al.* (2001)
			Stems	Lan *et al.* (2007)
		A. venustus	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
Cissaglaberrimine		*A. uncinatus*	Leaves and stems	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

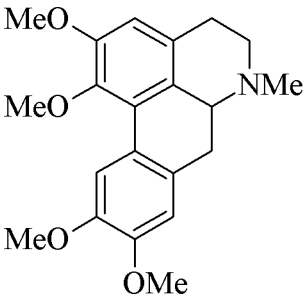
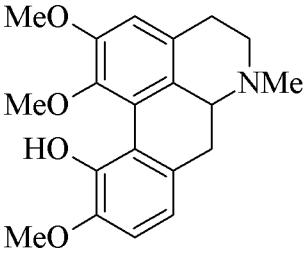
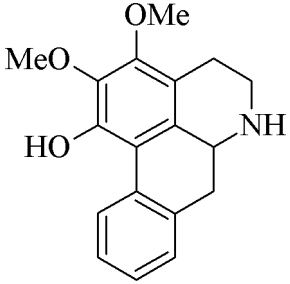
Compound	Structure	Plant species	Plant part	Reference
Glaucine		*A. lastourvillensis*	Bark	Eloumi-Ropivia *et al.* (1984); Eloumi-Ropivia *et al.* (1985); Cave *et al.* (1986); Guinaudeau *et al.* (1988)
Isocorydine		*A. uncinatus*	Roots	Hsieh *et al.* (2001)
Isopiline		*A. odoratissimus*	Stem bark	Sharma *et al.* (2002)
		A. uncinatus	Roots and stems	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

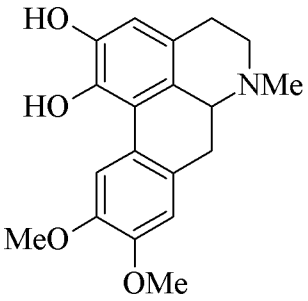
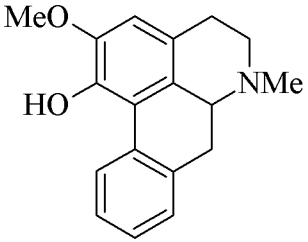
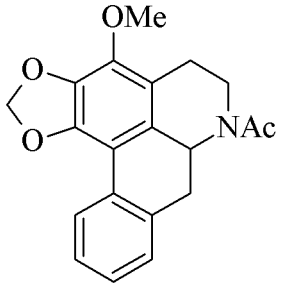
Compound	Structure	Plant species	Plant part	Reference
Lastourvilline		*A. lastourvillensis*	Bark	Eloumi-Ropivia *et al.* (1985); Cave *et al.* (1986); Guinaudeau *et al.* (1988)
Lirinidine		*A. venustus*	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
N-acetylnorstephalagine		*A. uncinatus*	Roots	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

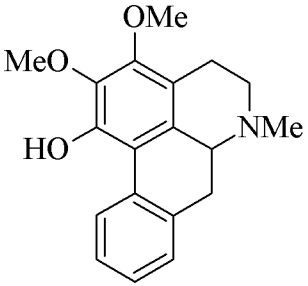
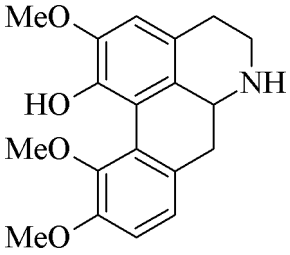
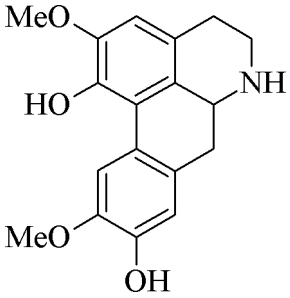
Compound	Structure	Plant species	Plant part	Reference
N-methylisopiline		*A. uncinatus*	Stems	Hsieh *et al.* (2001)
Norcorydine		*A. venustus*	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
Norisoboldine		*A. monteiroae*	Roots	Kato *et al.* (1993)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

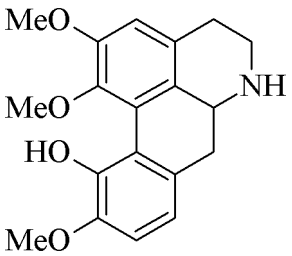
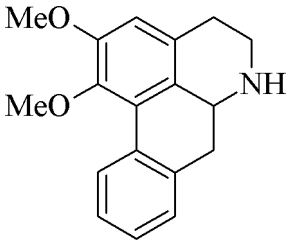
Compound	Structure	Plant species	Plant part	Reference
Norisocorydine		*A. uncinatus*	Roots and stems	Hsieh *et al.* (2001)
Nornuciferine		*A. maingayi*	Bark	Cortes *et al.* (1990); Kam (1999)
		A. uncinatus	Leaves and roots	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

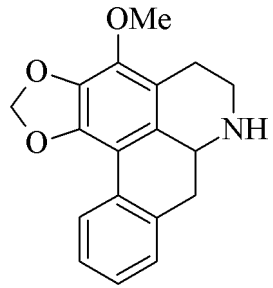
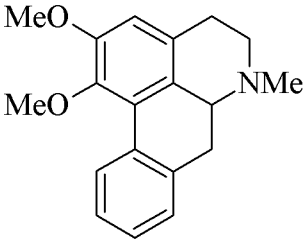
Compound	Structure	Plant species	Plant part	Reference
Norstephalagine		*A. grandifolius*	Stems	Chan *et al.* (1987); Teo *et al.* (1990); Kam (1999)
		A. maingayi	Bark	Cortes *et al.* (1990); Teo *et al.* (1990); Kam (1999)
		A. odoratissimus	Stem bark	Sharma *et al.* (2002)
		A. uncinatus	Roots and stems	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
Nuciferine		*A. venustus*	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

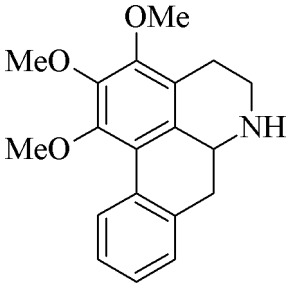
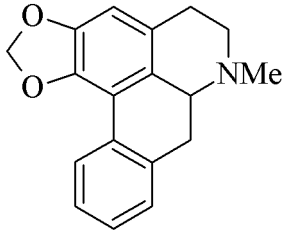
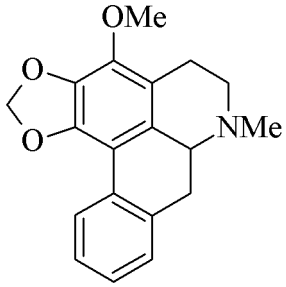
Compound	Structure	Plant species	Plant part	Reference
O-methyl-N-norlirinine		*A. uncinatus*	Stems	Hsieh *et al.* (2001)
Roemerine		*A. uncinatus*	Stems	Hsieh *et al.* (2001)
Stephalagine		*A. uncinatus*	Stems	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

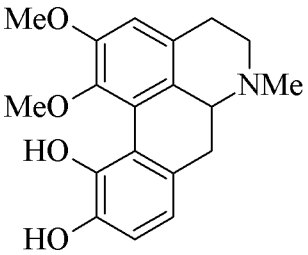
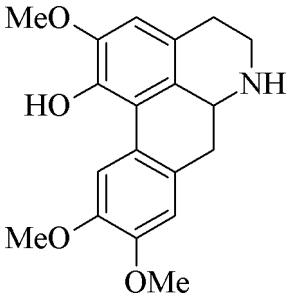
Compound	Structure	Plant species	Plant part	Reference
Suaveoline		*A. suaveolens*	Bark	Barger and Sargent (1939); Leboeuf *et al.* (1982); Teo *et al.* (1990)
Wilsonirine		*A. monteiroae*	Roots	Kato *et al.* (1993)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

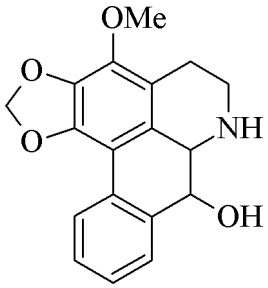
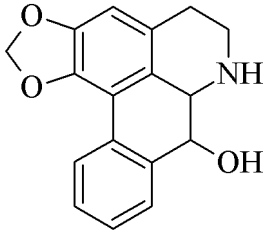
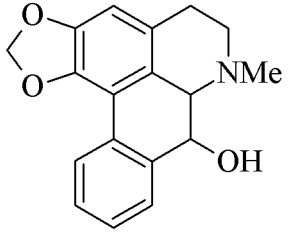
Compound	Structure	Plant species	Plant part	Reference
7-Hydroxyaporphine				
Artabonatine B		*A. uncinatus*	Fruits	Hsieh *et al.* (1999)
Norushinsunine		*A. uncinatus*	Fruits	Hsieh *et al.* (1999)
			Roots	Hsieh *et al.* (2001)
		A. venustus	Stem bark	Cave *et al.* (1986); Guinaudeau *et al.* (1988); Kam (1999)
Ushinsunine		*A. maingayi*	Bark	Cortes *et al.* (1990); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

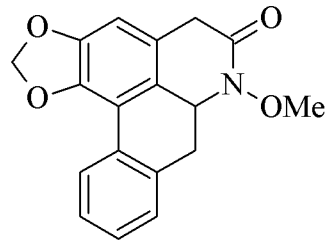
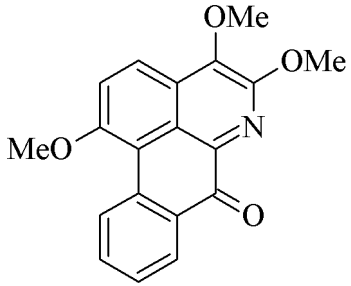
Compound	Structure	Plant species	Plant part	Reference
5-Oxoaporphine				
Artamonteirine		*A. monteiroae*	Stem bark	Nyandoro *et al.* (2012)
7-Oxoaporphine				
Artabonatine C		*A. spinosus*	Roots	Sichaem *et al.* (2011)
		A. uncinatus	Stems	Hsieh *et al.* (2001)
			Whole plant	Li (2006)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

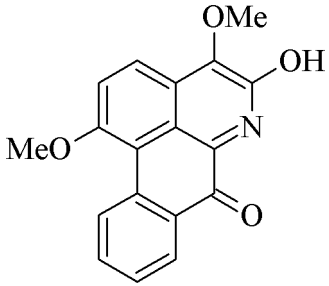
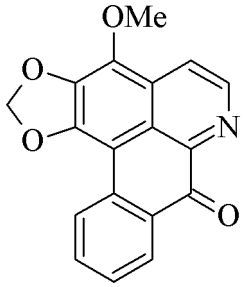
Compound	Structure	Plant species	Plant part	Reference
Artabonatine D		*A. uncinatus*	Stems	Hsieh *et al.* (2001)
			Whole plant	Li (2006)
Atherospermidine		*A. grandifolius*	Stems	Chan *et al.* (1987); Teo *et al.* (1990); Kam (1999)
		A. maingayi	Bark	Cortes *et al.* (1990); Teo *et al.* (1990); Kam (1999)
		A. odoratissimus	Stem bark	Sharma *et al.* (2002)
		A. uncinatus	Roots and stems	Hsieh *et al.* (2001)
			Stems	Lan *et al.* (2007)
			Stems and stem bark	Wu *et al.* (1989); Wiart (2006)
			Whole plant	Li (2006)
		A. zeylanicus	Stem bark	Wijeratne *et al.* (1995); Wijeratne *et al.* (1996)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

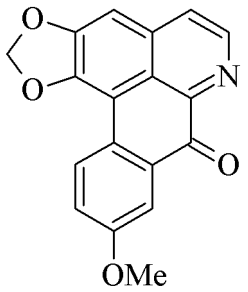
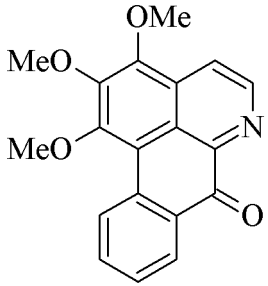
Compound	Structure	Plant species	Plant part	Reference
Lanuginosine		*A. zeylanicus*	Stem bark	Wijeratne *et al.* (1996)
Liridine		*A. hainanensis*	Stems	Han *et al.* (2005)
		A. odoratissimus	Stem bark	Sharma *et al.* (2002)
		A. spinosus	Roots	Sichaem *et al.* (2011)
		A. uncinatus	Roots and stems	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

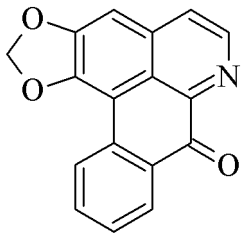
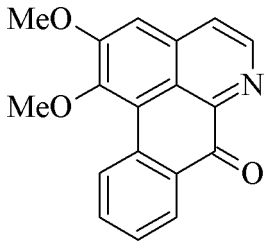
Compound	Structure	Plant species	Plant part	Reference
Liriodenine		*A. grandifolius*	Stems	Chan *et al.* (1987); Teo *et al.* (1990); Kam (1999)
		A. maingayi	Bark	Cortes *et al.* (1990); Kam (1999)
		A. uncinatus	Fruits	Hsieh *et al.* (1999)
			Leaves, roots and stems	Hsieh *et al.* (2001)
			Stems	Lan *et al.* (2007)
			Stems and stem bark	Wu *et al.* (1989); Wiart (2006)
		A. zeylanicus	Stem bark	Wijeratne *et al.* (1996)
Lysicamine		*A. maingayi*	Bark	Cortes *et al.* (1990); Kam (1999)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

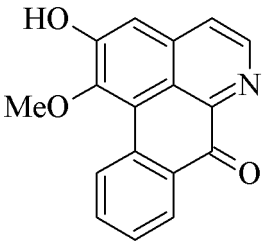
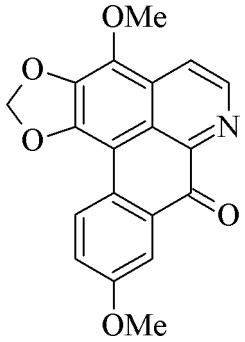
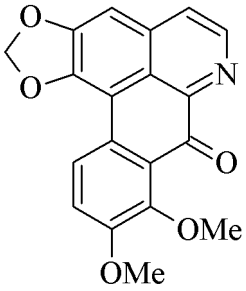
Compound	Structure	Plant species	Plant part	Reference
Oxoasimilobine		*A. uncinatus*	Leaves	Hsieh *et al.* (2001)
Oxobuxifoline		*A. zeylanicus*	Stem bark	Wijeratne *et al.* (1996)
Oxocrebanine		*A. zeylanicus*	Stem bark	Wijeratne *et al.* (1996)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

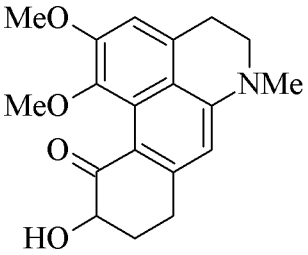
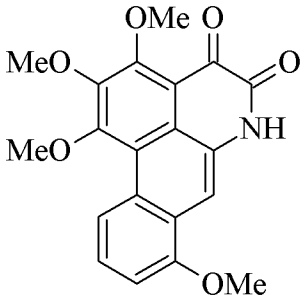
Compound	Structure	Plant species	Plant part	Reference
11-Oxoaporphine				
Artacinatine		*A. spinosus*	Roots	Sichaem *et al.* (2011)
		A. uncinatus	Stems	Hsieh *et al.* (2001); Lan *et al.* (2007)
			Stems and stem bark	Wu *et al.* (1989)
4,5-Dioxoaporphine				
8-Methoxyouregidione		*A. zeylanicus*	Stem bark	Wijeratne *et al.* (1996)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

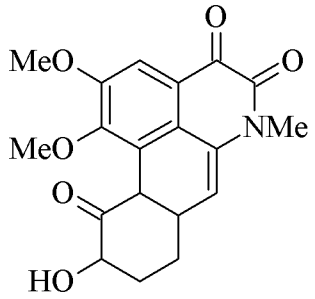
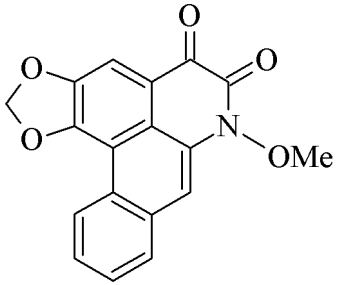
Compound	Structure	Plant species	Plant part	Reference
4,5-Dioxoartacinatine		*A. uncinatus*	Stems	Lan *et al.* (2007)
Artabotrine		*A. hainanensis*	Stems	Han *et al.* (2005)
		A. stenopetalus	Stem bark	Fleischer *et al.* (1997)
		A. suaveolens	Roots and stem bark	Maranon (1929); Barger and Sargent (1939); Leboeuf *et al.* (1982)
		A. zeylanicus	Stem bark	Wijeratne *et al.* (1995); Wijeratne *et al.* (1996); Ding *et al.* (2006); Wiart (2006)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

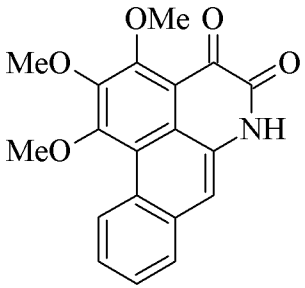
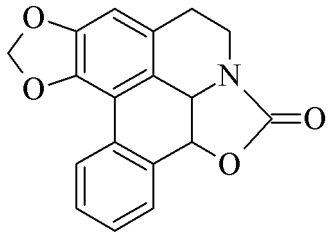
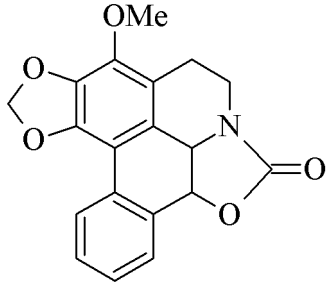
Compound	Structure	Plant species	Plant part	Reference
Ouregidione		*A. zeylanicus*	Stem bark	Wijeratne *et al.* (1996)
Oxazoloaporphine				
Artabonatine A		*A. uncinatus*	Fruits	Hsieh *et al.* (1999)
Artabonatine E		*A. uncinatus*	Roots Whole plant	Hsieh *et al.* (2001) Li (2006)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

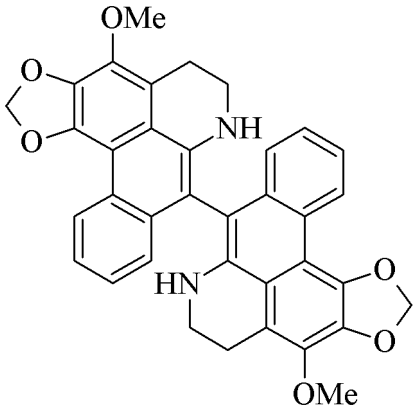
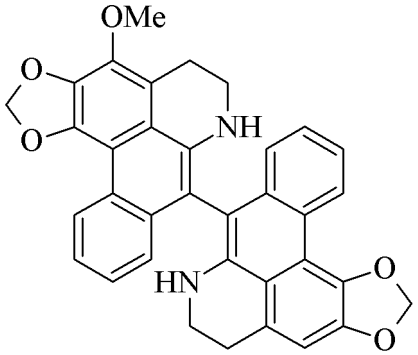
Compound	Structure	Plant species	Plant part	Reference
7,7'-Bisdehydroaporphine				
Artabonatine F		*A. uncinatus*	Roots	Hsieh *et al.* (2001)
			Whole plant	Li (2006)
Artabotrysine		*A. spinosus*	Roots	Sichaem *et al.* (2011)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

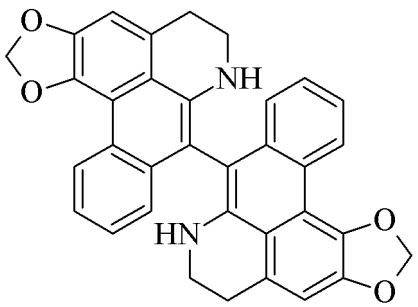
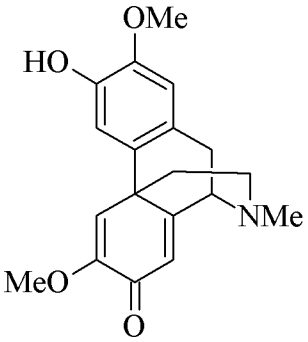
Compound	Structure	Plant species	Plant part	Reference
Bidebiline A		*A. spinosus*	Roots	Sichaem *et al.* (2011)
MORPHINANDIENONE ALKALOID				
Flavinantine		*A. uncinatus*	Roots and stems	Hsieh *et al.* (2001)

TABLE 2.4 Occurrence of alkaloids in *Artabotrys* species (continued).

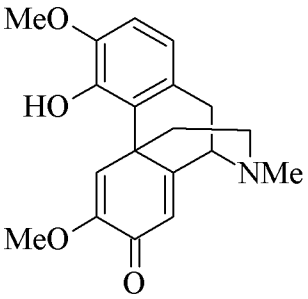
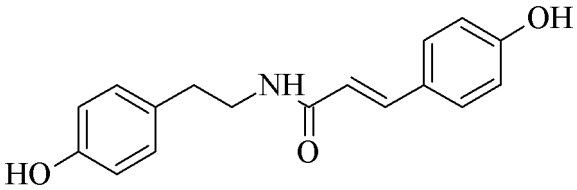
Compound	Structure	Plant species	Plant part	Reference
Salutaridine		*A. uncinatus*	Leaves, roots and stems	Hsieh *et al.* (2001)
PROTOALKALOID				
Paprazine		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species.

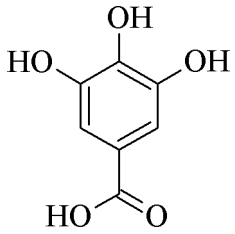
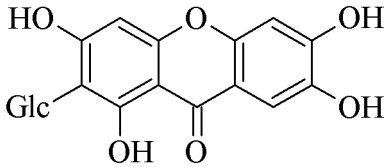
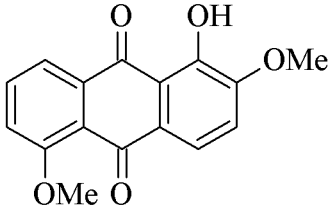
Compound	Structure	Plant species	Plant part	Reference
PHENOLIC ACID				
Gallic acid		*A. hexapetalus*	Leaves	Savadi (2009)
XANTHONOID				
Mangiferin		*A. hainanensis*	Leaves	Chen *et al.* (2004)
ANTHRAQUINONE				
1-Hydroxy-2,5-dimethoxy-9,10-anthraquinone		*A. odoratissimus*	Leaves	Singh *et al.* (2005)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

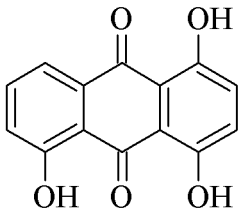
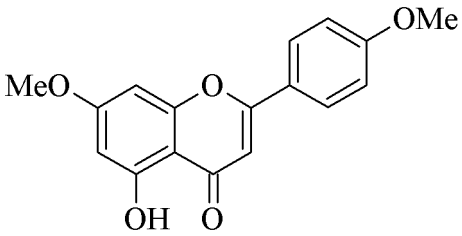
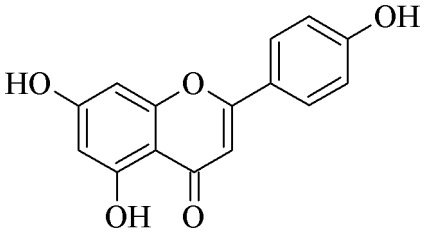
Compound	Structure	Plant species	Plant part	Reference
1,4,5-Trihydroxy-9,10-anthraquinone		*A. odoratissimus*	Leaves	Singh *et al.* (2005)
FLAVONOID				
Flavone				
5-Hydroxy-7,4'-dimethoxyflavone		*A. uncinatus*	Stems	Lan *et al.* (2007)
Apigenin		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

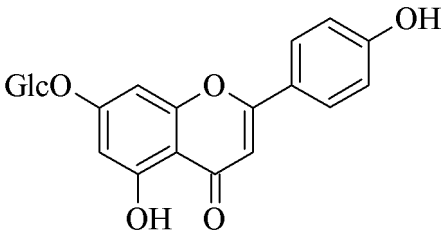
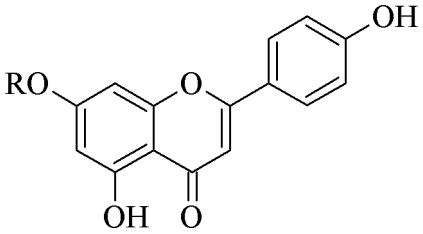
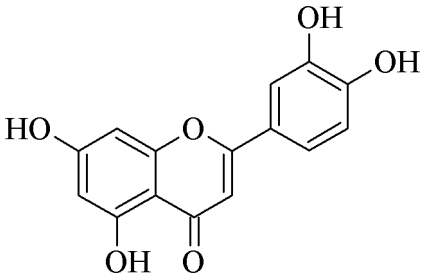
Compound	Structure	Plant species	Plant part	Reference
Apigetrin (Apigenin 7-O-β-D-glucopyranoside)		*A. hexapetalus*	Leaves	Somanawat *et al.* (2012)
Apiin [Apigenin 7-O-β-D-apiosyl-(1→2)-β-D-glucopyranoside]	 R = Api-(1→2)-Glc	*A. hexapetalus*	Leaves	Li *et al.* (1997); Li and Yu (1998); Yu *et al.* (2002); Savadi (2009)
Luteolin		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

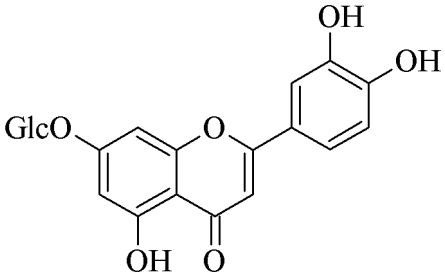
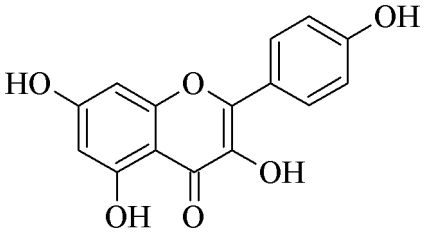
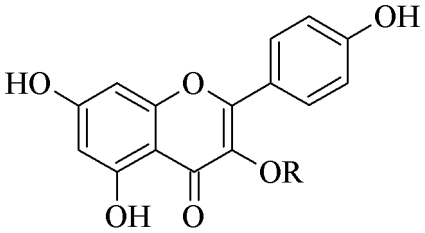
Compound	Structure	Plant species	Plant part	Reference
Cynaroside (Luteolin 7-O-β-D-glucopyranoside)		*A. hexapetalus*	Leaves	Li *et al.* (1997); Li and Yu (1998); Yu *et al.* (2002); Savadi (2009)
Flavonol				
Kaempferol		*A. hexapetalus*	Leaves	Savadi (2009)
Artabotryside B [Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-α -L-arabinofuranoside]	 R = α-L-rham-(1➔2)-α-L-ara	*A. hexapetalus*	Leaves	Li *et al.* (1997); Li and Yu (1998); Yu *et al.* (2002); Savadi (2009)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

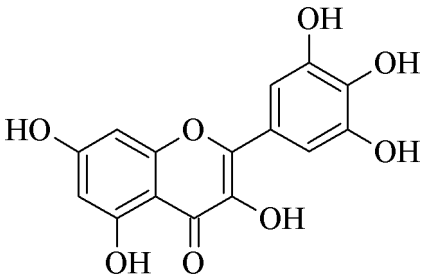
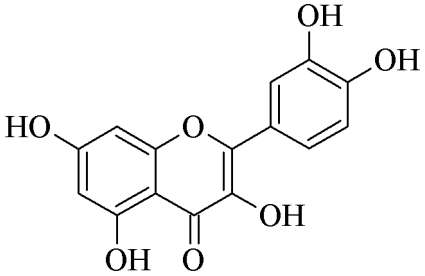
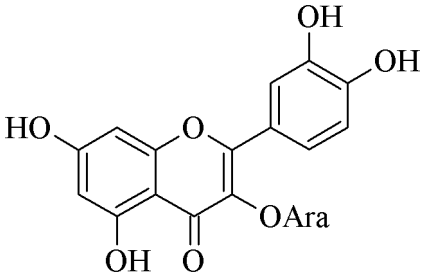
Compound	Structure	Plant species	Plant part	Reference
Myricetin		*A. hexapetalus*	Leaves	Savadi (2009)
Quercetin		*A. hexapetalus*	Leaves	Savadi (2009)
Guaijaverin (Quercetin 3-O-α-L-arabinopyranoside)		*A. hexapetalus*	Leaves	Savadi (2009)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

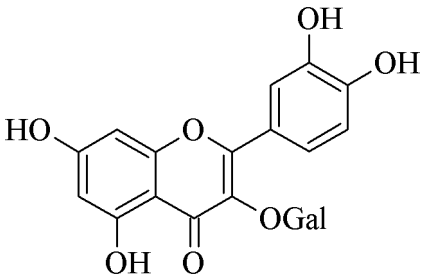
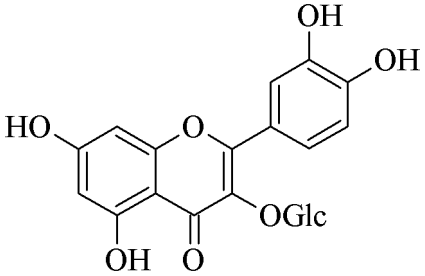
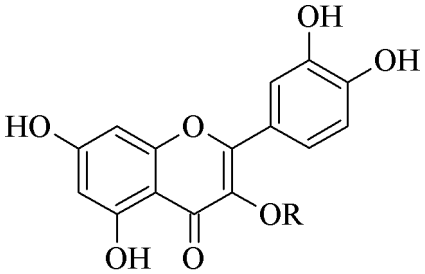
Compound	Structure	Plant species	Plant part	Reference
Hyperoside (Quercetin 3-O-β-D-galactopyranoside)		*A. hexapetalus*	Leaves	Savadi (2009)
Isoquercetin (Quercetin 3-O-β-D-glucopyranoside)		*A. hexapetalus*	Leaves	Savadi (2009)
Artabotryside A [Quercetin 3-O-α-L-rhamnopyranosyl-(1→2)-α -L-arabinofuranoside]	 R = α-L-rham-(1→2)-α-L-ara	*A. hexapetalus*	Leaves	Li *et al.* (1997); Li and Yu (1998); Yu *et al.* (2002); Savadi (2009); Somanawat *et al.* (2012)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

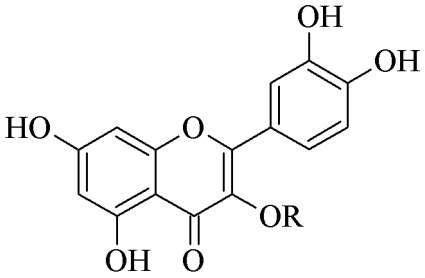
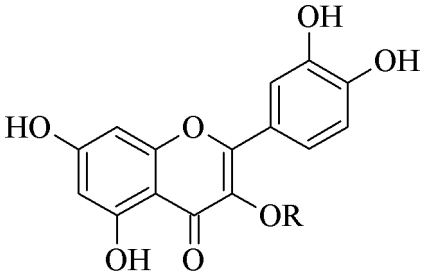
Compound	Structure	Plant species	Plant part	Reference
Rutin [Quercetin 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	 R = α-L-rham-(1➔6)-β-D-glc	*A. hexapetalus*	Leaves	Somanawat *et al.* (2012)
Quercetin 3-O-α-L-rhampyranosyl rutinoside [Quercetin 3-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	 R = α-L-rham-(1➔3)-α-L-rham-(1➔6)-β-D-glc	*A. hexapetalus*	Leaves	Somanawat *et al.* (2012)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

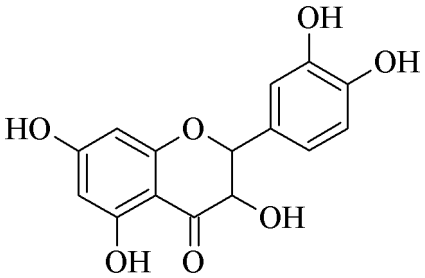
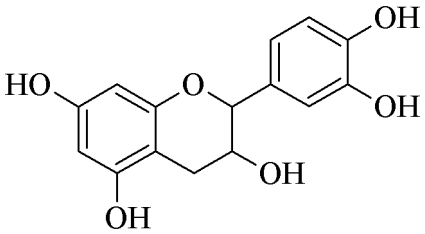
Compound	Structure	Plant species	Plant part	Reference
Flavanonol				
Taxifolin		*A. hexapetalus*	Leaves	Li *et al.* (1997); Li and Yu (1998); Yu *et al.* (2002); Savadi (2009)
Flavanol				
Catechin		*A. hainanensis*	Leaves	Chen *et al.* (2004)
		A. modestus	Stem bark	Nyandoro *et al.* (2012)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

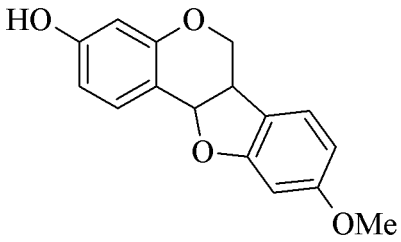
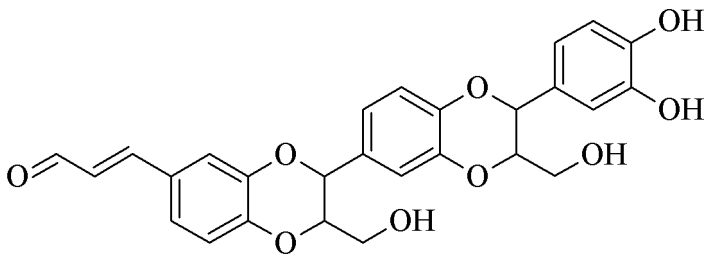
Compound	Structure	Plant species	Plant part	Reference
ISOFLAVONOID				
Medicarpin		*A. odoratissimus*	Seeds	Singh *et al.* (2009)
LIGNAN				
Americanin B		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

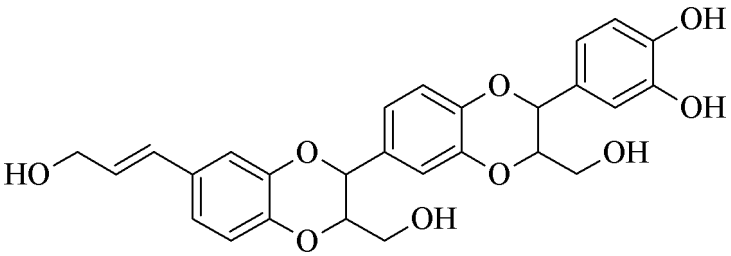
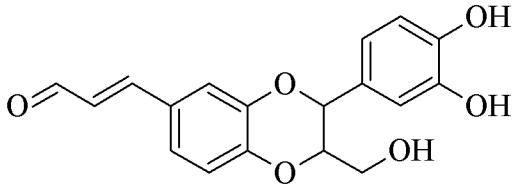
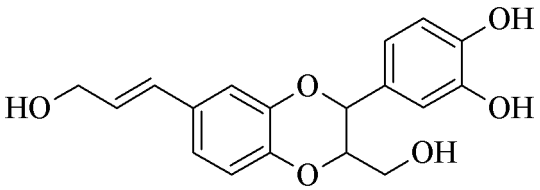
Compound	Structure	Plant species	Plant part	Reference
Artabotrycinol		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
Isoamericanin A		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
Isoamericanol A		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)

TABLE 2.5 Occurrence of phenolic compounds in *Artabotrys* species (continued).

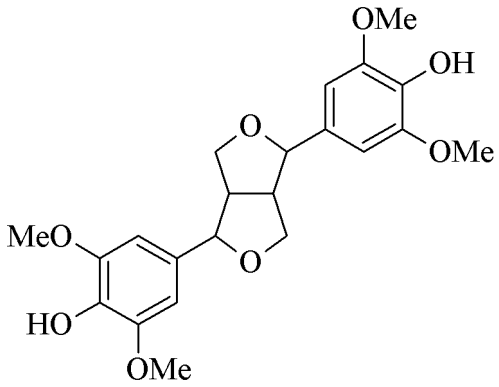
Compound	Structure	Plant species	Plant part	Reference
Syringaresinol		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species.

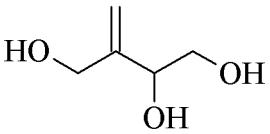
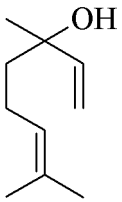
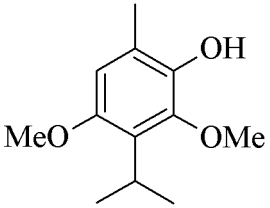
Compound	Structure	Plant species	Plant part	Reference
HEMITERPENOID				
Artabotriol		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
		A. modestus	Stem bark	Nyandoro *et al.* (2012)
MONOTERPENOID				
Acyclic				
Linalool		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
		A. odoratissimus	Leaves	Garg and Siddiqui (1999)
Monocyclic				
3,5-Dimethoxy carvacrol		*A. brachypetalus*	Stem bark	Odebode *et al.* (2006)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

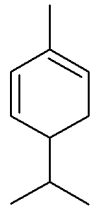
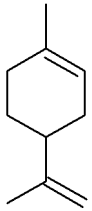
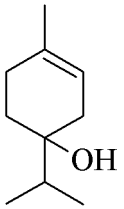
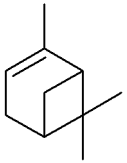
Compound	Structure	Plant species	Plant part	Reference
α-Phellandrene		*A. pallens*	Leaves	Trang *et al.* (2014)
Limonene		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Terpinen-4-ol		*A. odoratissimus*	Leaves	Garg and Siddiqui (1999)
Bicyclic				
α-Pinene		*A. taynguyenensis*	Leaves	Trang *et al.* (2014)
		A. vinhensis	Leaves	Trang *et al.* (2014)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

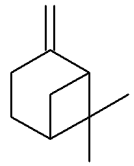
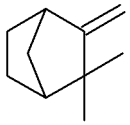
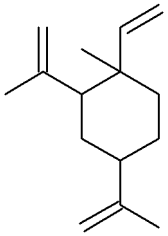
Compound	Structure	Plant species	Plant part	Reference
β-Pinene		*A. vinhensis*	Leaves	Trang *et al.* (2014)
Camphene		*A. insignis*	Root bark and stem bark	Fournier *et al.* (1997)
		A. thomsonii	Stem bark	Fournier *et al.* (1997)
		A. venustus	Stem bark	Fournier *et al.* (1997)
SESQUITERPENOID				
Monocyclic				
β-Elemene		*A. lastourvillensis*	Bark	Fournier *et al.* (1997)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

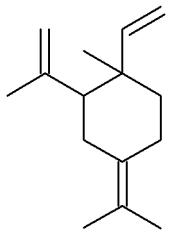
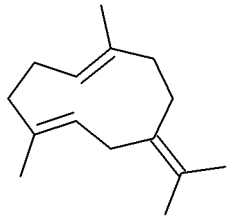
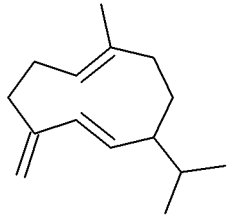
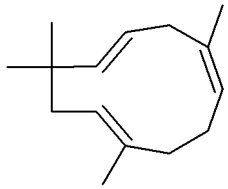
Compound	Structure	Plant species	Plant part	Reference
γ-Elemene		*A. hongkongensis*	Leaves	Trang *et al.* (2014)
Germacrene B		*A. vinhensis*	Leaves	Trang *et al.* (2014)
Germacrene D		*A. vinhensis*	Leaves	Trang *et al.* (2014)
Humulene (α-Caryophyllene)		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

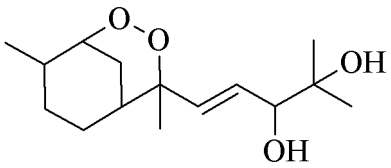
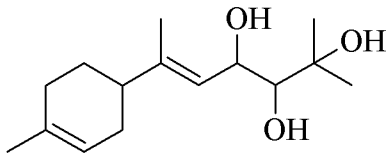
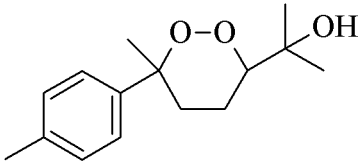
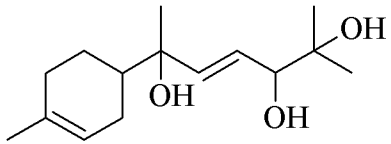
Compound	Structure	Plant species	Plant part	Reference
Yingzhaosu A		*A. uncinatus*	Roots	Hedberg *et al.* (1982); Leboeuf *et al.* (1982); Lee and Hufford (1990); Zhou and Xu (1994); Boukouvalas *et al.* (1995); Xu and Dong (1995); Szpilman *et al.* (2005); Dewick (2011)
Yingzhaosu B		*A. uncinatus*	Roots	Hedberg *et al.* (1982); Leboeuf *et al.* (1982); Xu and Dong (1995)
Yingzhaosu C		*A. uncinatus*	Not specified	Boukouvalas *et al.* (1995); Xu and Dong (1995); Dewick (2011)
Yingzhaosu D		*A. uncinatus*	Not specified	Xu and Dong (1995)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

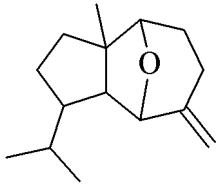
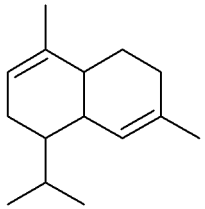
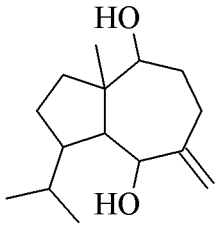
Compound	Structure	Plant species	Plant part	Reference
Bicyclic				
1,5-Epoxysalvial-4(14)-ene		*A. insignis*	Root bark and stem bark	Fournier *et al.* (1997)
		A. rufus	Root bark	Fournier *et al.* (1997)
		A. thomsonii	Stem bark	Fournier *et al.* (1997)
		A. venustus	Stem bark	Fournier *et al.* (1997)
α-Muurolene		*A. taynguyenensis*	Leaves	Trang *et al.* (2014)
Artabotrol		*A. stenopetalus*	Stem bark	Fleischer *et al.* (1997)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

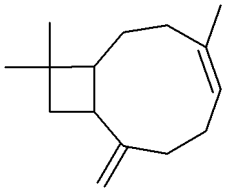
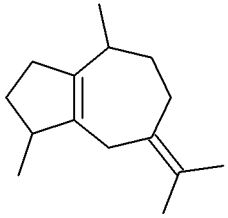
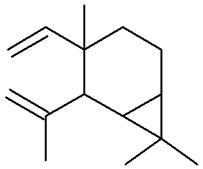
Compound	Structure	Plant species	Plant part	Reference
β-Caryophyllene		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)
			Flowers	Mahidol *et al.* (2005); Phan *et al.* (2007); Trang *et al.* (2014)
		A. hongkongensis	Leaves	Trang *et al.* (2014)
		A. rufus	Root bark	Fournier *et al.* (1997)
		A. vinhensis	Leaves	Trang *et al.* (2014)
β-Guaiene		*A. lastourvillensis*	Bark	Fournier *et al.* (1997)
Bicycloelemene		*A. pallens*	Leaves	Trang *et al.* (2014)
		A. taynguyenensis	Stems	Trang *et al.* (2014)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

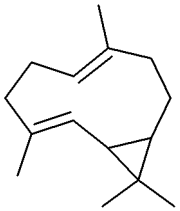
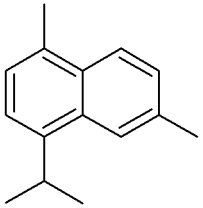
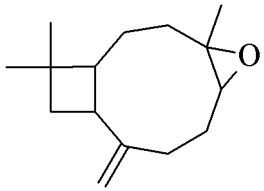
Compound	Structure	Plant species	Plant part	Reference
Bicyclogermacrene		*A. taynguyenensis*	Stems	Trang *et al.* (2014)
Cadalene		*A. lastourvillensis*	Bark	Fournier *et al.* (1997)
		A. pierreanus	Stem bark	Fournier *et al.* (1997)
Caryophyllene oxide		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)
			Flowers	Phan *et al.* (2007)
		A. insignis	Root bark	Fournier *et al.* (1997)
		A. lastourvillensis	Bark	Fournier *et al.* (1997)
		A. odoratissimus	Leaves	Garg and Siddiqui (1999)
		A. pierreanus	Stem bark	Fournier *et al.* (1997)
		A. rufus	Root bark	Fournier *et al.* (1997)
		A. stenopetalus	Stem bark	Fleischer *et al.* (1997)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

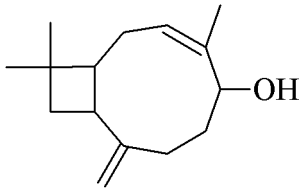
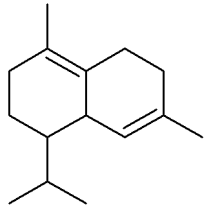
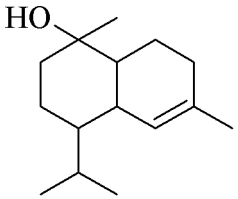
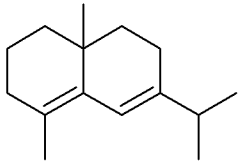
Compound	Structure	Plant species	Plant part	Reference
Caryophyllenol		*A. insignis*	Root bark	Fournier *et al.* (1997)
		A. rufus	Root bark	Fournier *et al.* (1997)
δ-Cadinene		*A. hongkongensis*	Leaves	Trang *et al.* (2014)
δ-Cadinol		*A. insignis*	Root bark	Fournier *et al.* (1997)
δ-Selinene		*A. taynguyenensis*	Leaves	Trang *et al.* (2014)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

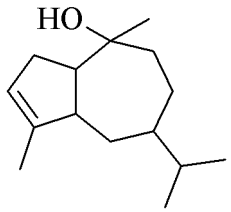
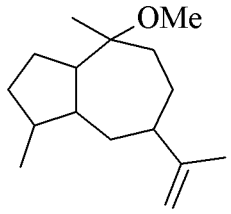
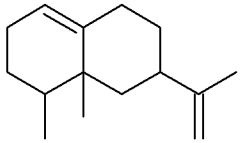
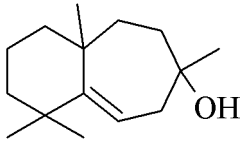
Compound	Structure	Plant species	Plant part	Reference
Karatavin		*A. modestus*	Stem bark	Nyandoro *et al.* (2012)
Pogostol O-methyl ether		*A. stenopetalus*	Stem bark	Fleischer *et al.* (1997); Booker-Milburn *et al.* (2003)
Valencene		*A. taynguyenensis*	Leaves	Trang *et al.* (2014)
Widdrol		*A. insignis*	Stem bark	Fournier *et al.* (1997)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

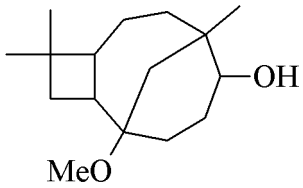
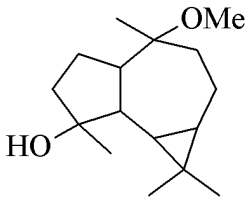
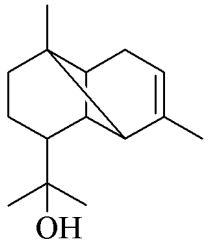
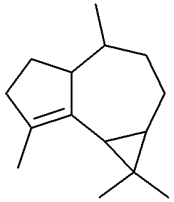
Compound	Structure	Plant species	Plant part	Reference
Tricyclic				
1-Methoxy-9-caryolanol		*A. uncinatus*	Stems	Lan *et al.* (2007)
4β-hydroxy-10α-methoxyaromadendrane		*A. uncinatus*	Stems	Lan *et al.* (2007)
α-Copaen-11-ol		*A. rufus*	Root bark	Fournier *et al.* (1997)
α-Gurjunene		*A. pallens*	Leaves	Trang *et al.* (2014)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

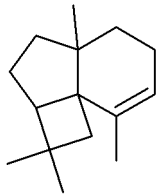
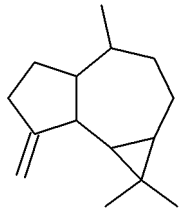
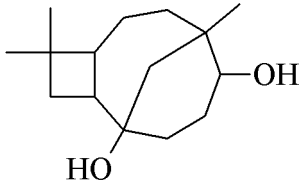
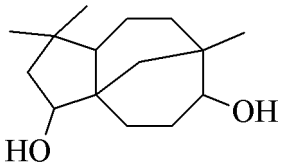
Compound	Structure	Plant species	Plant part	Reference
α-Panasinsene		*A. taynguyenensis*	Leaves	Trang *et al.* (2014)
β-Gurjunene		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Caryolane-1,9β-diol		*A. uncinatus*	Stems	Lan *et al.* (2007)
Cloven-2β,9α-diol		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

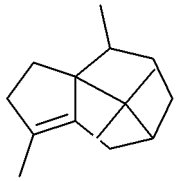
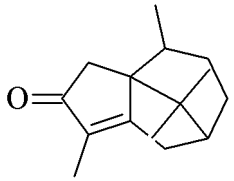
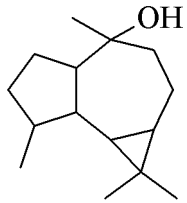
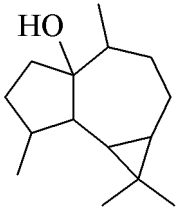
Compound	Structure	Plant species	Plant part	Reference
Cyperene		*A. lastourvillensis*	Bark	Fournier *et al.* (1997)
		A. pierreanus	Stem bark	Fournier *et al.* (1997)
Cyperenone		*A. lastourvillensis*	Bark	Fournier *et al.* (1997)
		A. pierreanus	Stem bark	Fournier *et al.* (1997)
		A. thomsonii	Stem bark	Fournier *et al.* (1997)
Globulol		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
		A. venustus	Stem bark	Fournier *et al.* (1997)
Palustrol		*A. thomsonii*	Stem bark	Fournier *et al.* (1997)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

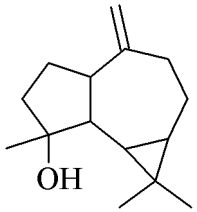
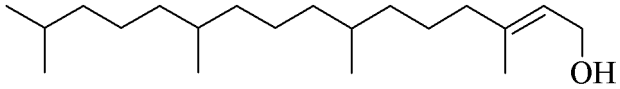
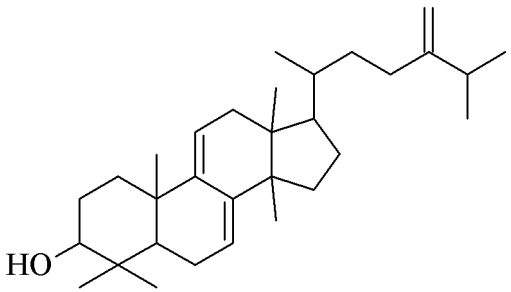
Compound	Structure	Plant species	Plant part	Reference
Spathulenol		*A. odoratissimus*	Stem bark	Sharma *et al.* (2002)
		A. hongkongensis	Leaves	Trang *et al.* (2014)
		A. taynguyenensis	Stems	Trang *et al.* (2014)
		A. thomsonii	Stem bark	Fournier *et al.* (1997)
		A. uncinatus	Stems	Lan *et al.* (2007)
DITERPENOID				
Phytol		*A. uncinatus*	Stems	Lan *et al.* (2007)
TRITERPENOID				
24-Methylene-lanosta-7,9(11)-dien-3β-ol		*A. modestus*	Root bark and stem bark	Nyandoro *et al.* (2012)
		A. odoratissimus	Stem bark	Hasan *et al.* (1987); Singh *et al.* (2005); Savadi (2009); Kaisar *et al.* (2011)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

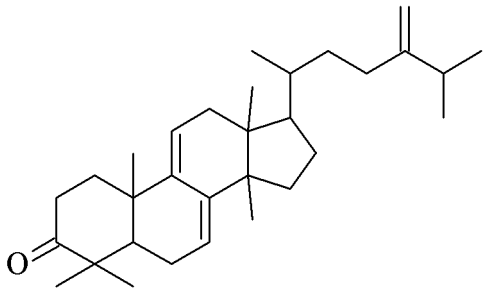
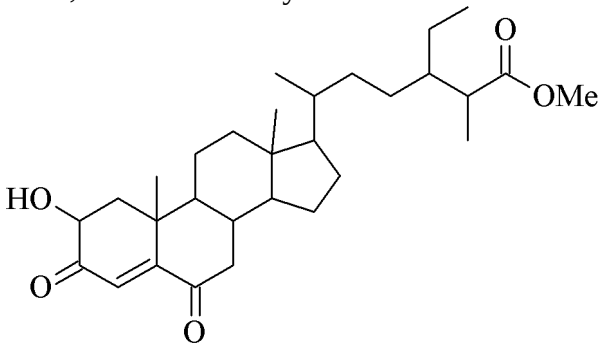
Compound	Structure	Plant species	Plant part	Reference
24-Methylene-lanosta-7,9(11)-diene-3-one		*A. uncinatus*	Stems	Lan *et al.* (2007)
2β-Hydroxy-stigmasta-4-en-3,6-dione-methoxy ester		*A. odoratissimus*	Leaves	Khaleel *et al.* (2014)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

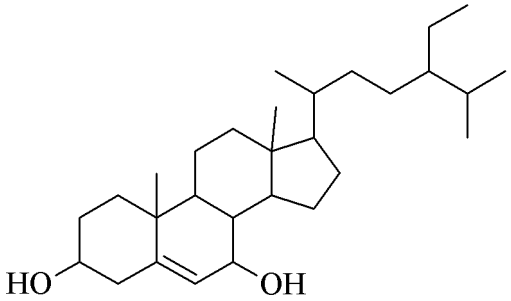
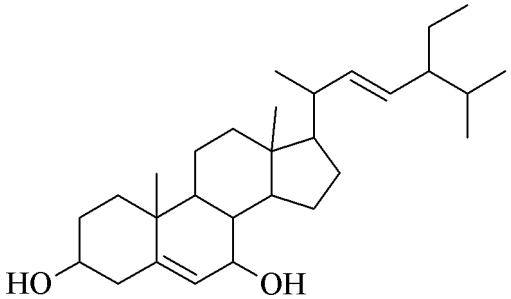
Compound	Structure	Plant species	Plant part	Reference
7α-Hydroxysitosterol [(24R)-stigmasta-5-en-3β,7α-diol]		*A. uncinatus*	Stems	Lan *et al.* (2007)
7α-Hydroxystigmasterol [(22E,24S)-stigmasta-5,22-dien-3β,7α-diol]		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

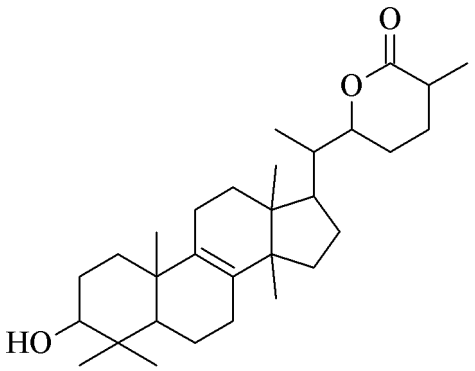
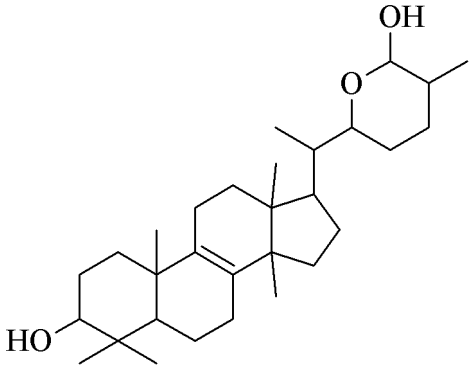
Compound	Structure	Plant species	Plant part	Reference
Artabotryol A		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)
Artabotryol B		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

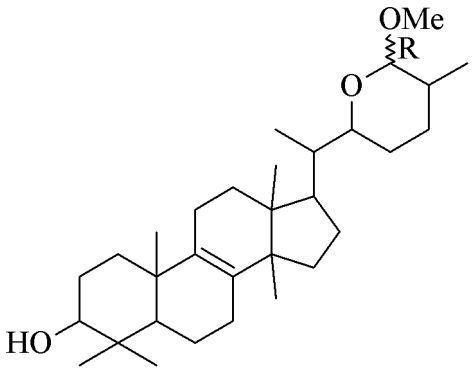
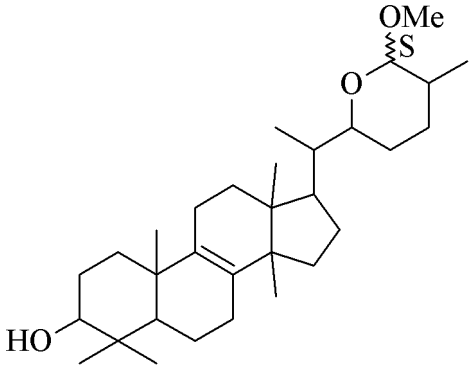
Compound	Structure	Plant species	Plant part	Reference
Artabotryol C1		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)
Artabotryol C2		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

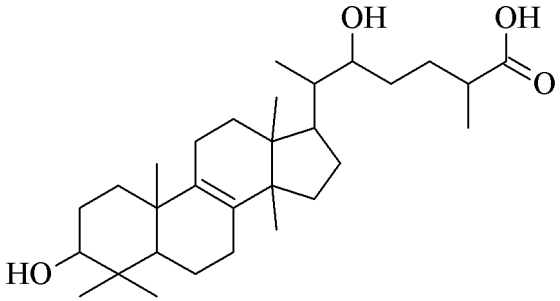
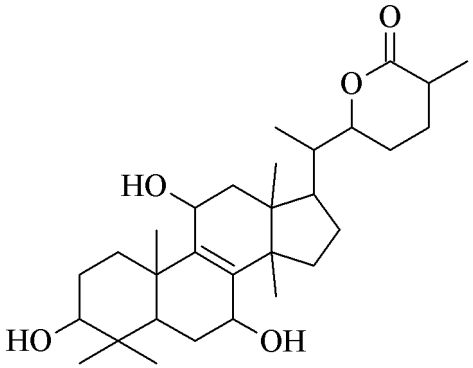
Compound	Structure	Plant species	Plant part	Reference
Artabotryol D		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)
Artabotryol E		*A. odoratissimus*	Seeds	Gupta *et al.* (2010)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

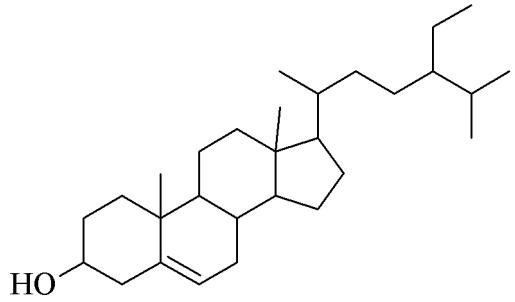
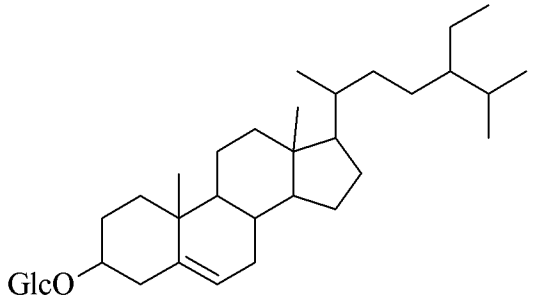
Compound	Structure	Plant species	Plant part	Reference
β-Sitosterol (Stigmasta-5-en-3β-ol)		*A. hainanensis*	Leaves	Chen *et al.* (2004)
		A. hexapetalus	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
		A. odoratissimus	Fruits	Singh *et al.* (2005)
			Stem bark	Sharma *et al.* (2002); Savadi (2009)
		A. suaveolens	Not specified	Teo *et al.* (1990)
		A. uncinatus	Stems	Lan *et al.* (2007)
Daucosterol (β-Sitosterol 3-O-β-D-glucopyranoside)		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
		A. uncinatus	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

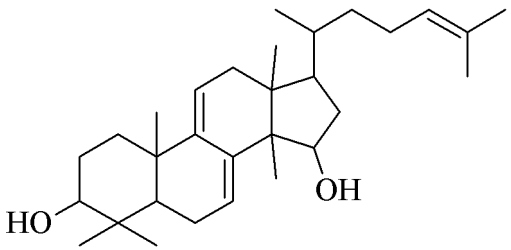
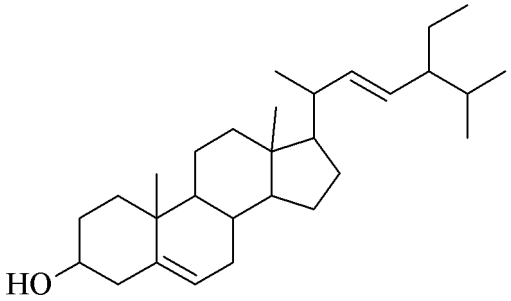
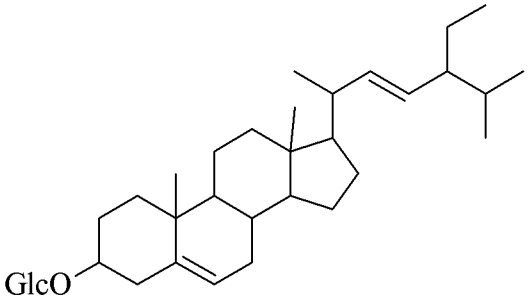
Compound	Structure	Plant species	Plant part	Reference
Polycarpol		*A. madagascariensis*	Fruits and leaves	Murphy (2007); Murphy *et al.* (2008)
		A. modestus	Stem bark	Nyandoro *et al.* (2012)
		A. monteiroae	Stem bark	Nyandoro *et al.* (2012)
		A. spinosus	Roots	Sichaem *et al.* (2011)
		A. suaveolens	Not specified	Teo *et al.* (1990)
Stigmasterol (Stigmasta-5,22-dien-3β-ol)		*A. odoratissimus*	Stem bark	Sharma *et al.* (2002); Savadi (2009)
		A. uncinatus	Stems	Lan *et al.* (2007)
Stigmasterol 3-O-β-D-glucopyranoside		*A. uncinatus*	Stems	Lan *et al.* (2007)

TABLE 2.6 Occurrence of terpenoids in *Artabotrys* species (continued).

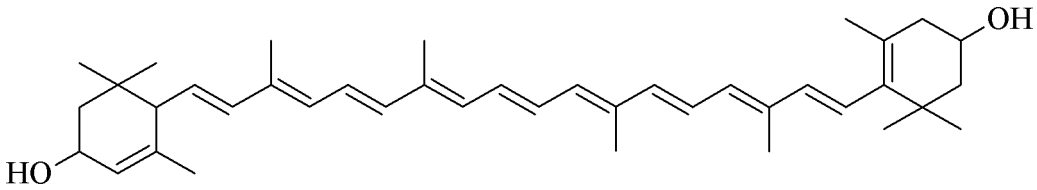
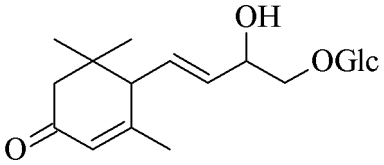
Compound	Structure	Plant species	Plant part	Reference
TETRATERPENOID				
Lutein		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)
NORTERPENOID				
7E-9-hydroxy-4,7-megastigmane-3-one-10-O-β-D-glucopyranoside		*A. hexapetalus*	Leaves	Somanawat *et al.* (2012)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species.

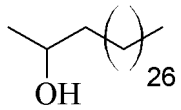
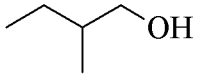
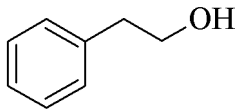
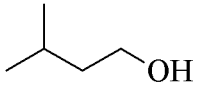
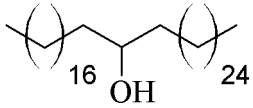
Compound	Structure	Plant species	Plant part	Reference
ALCOHOL				
Monohydric				
2-Hydroxytricontane		*A. odoratissimus*	Leaves	Mehta *et al.* (1999)
2-Methyl-1-butanol		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
2-Phenylethanol		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
3-Methyl-1-butanol		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Pentatetracontan-19-ol		*A. odoratissimus*	Leaves	Mehta *et al.* (1999)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

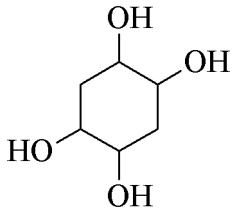
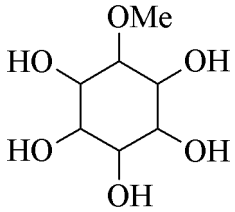
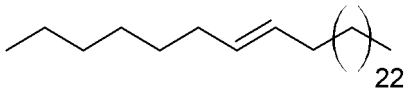
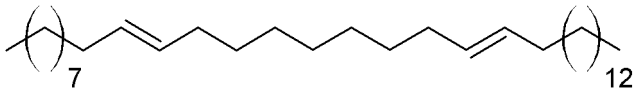
Compound	Structure	Plant species	Plant part	Reference
Polyhydric				
Cyclohexane-1,2,4,5-tetrol		*A. modestus*	Root bark	Nyandoro *et al.* (2012)
Quebrachitol		*A. modestus*	Stem bark	Nyandoro *et al.* (2012)
ALKENE				
Dotriacont-7-ene		*A. odoratissimus*	Leaves	Sharma *et al.* (2002)
Tetratriacont-10,19-diene		*A. odoratissimus*	Leaves	Sharma *et al.* (2002)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

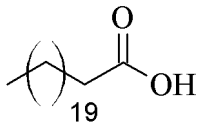
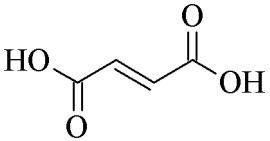
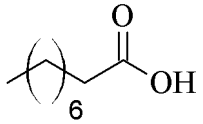
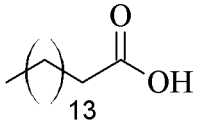
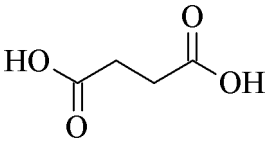
Compound	Structure	Plant species	Plant part	Reference
CARBOXYLIC ACID				
Docosanoic acid		*A. odoratissimus*	Seeds	Singh *et al.* (2009)
Fumaric acid		*A. hexapetalus*	Leaves	Li and Yu (1998)
Nonanoic acid		*A. odoratissimus*	Leaves	Sharma *et al.* (2002); Srivastava *et al.* (2009)
Palmitic acid		*A. hexapetalus*	Seeds	Yu *et al.* (2001); Yu *et al.* (2002); Savadi (2009)
Succinic acid		*A. hexapetalus*	Leaves	Li and Yu (1998)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

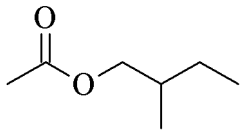
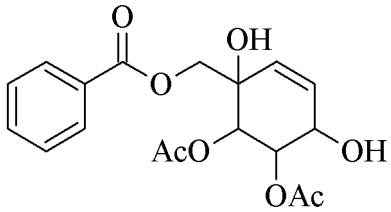
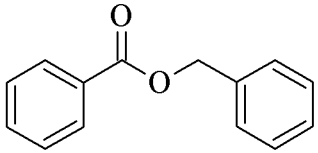
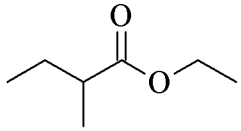
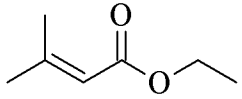
Compound	Structure	Plant species	Plant part	Reference
ESTER				
2-Methylbutyl acetate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
		A. vinhensis	Leaves	Trang *et al.* (2014)
Artabotrol A		*A. madagascariensis*	Fruits and leaves	Murphy (2007); Murphy *et al.* (2008)
Benzyl benzoate		*A. odoratissimus*	Stem bark	Sharma *et al.* (2002); Savadi (2009)
		A. vinhensis	Leaves	Trang *et al.* (2014)
Ethyl 2-methylbutanoate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Ethyl 3-methyl-2-butenoate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

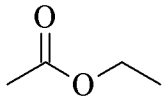
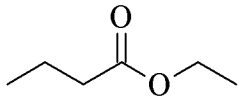
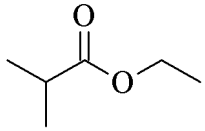
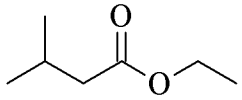
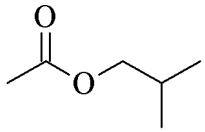
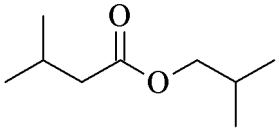
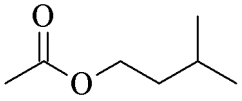
Compound	Structure	Plant species	Plant part	Reference
Ethyl acetate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Ethyl butanoate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Ethyl isobutanoate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Ethyl isovalerate (Ethyl 3-methylbutanoate)		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Isobutyl acetate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Isobutyl isovalerate (Isobutyl 3-methylbutanoate)		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)
Isopentyl acetate		*A. hexapetalus*	Flowers	Mahidol *et al.* (2005)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

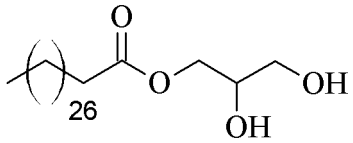
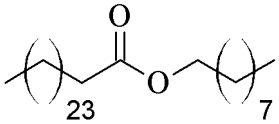
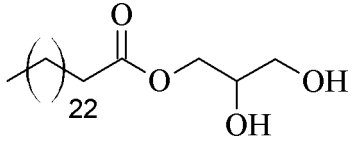
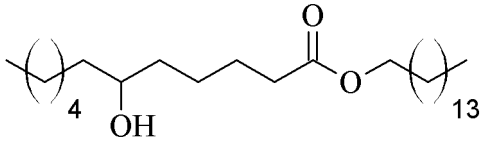
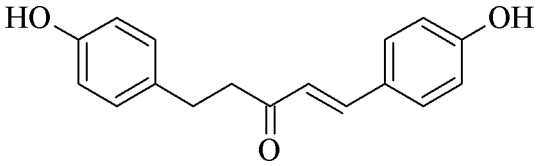
Compound	Structure	Plant species	Plant part	Reference
Nonacosanoic acid 2',3'-dihydroxypropyl ester		*A. odoratissimus*	Seeds	Singh *et al.* (2009)
Nonacosanyl hexacosanoate		*A. odoratissimus*	Leaves	Mehta *et al.* (1999)
Pentacosanoic acid 2,-3'-dihydroxypropyl ester		*A. odoratissimus*	Seeds	Singh *et al.* (2009)
Pentadecyl 6-hydroxydodecanoate		*A. odoratissimus*	Leaves	Bourcet *et al.* (2008)
KETONE				
Artamenone		*A. modestus*	Stem bark	Nyandoro *et al.* (2012)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

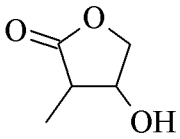
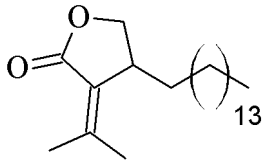
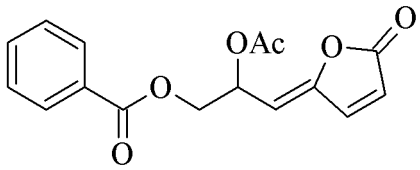
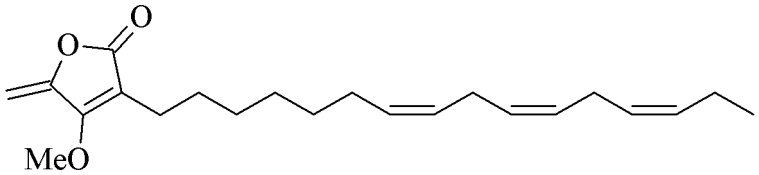
Compound	Structure	Plant species	Plant part	Reference
LACTONE				
(2R,3R)-3-hydroxy-2-methylbutyrolactone		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)
		A. uncinatus	Stems	Lan *et al.* (2007)
3-Methylene-4-pentadecyldihydrofuran-2-one		*A. odoratissimus*	Fruits	Bordoloi *et al.* (2009)
Acetylmelodorinol		*A. madagascariensis*	Fruits and leaves	Murphy (2007); Murphy *et al.* (2008)
Artapetalin A		*A. hexapetalus*	Aerial parts	Wong and Brown (2002); Schobert and Schlenk (2008); Savadi (2009)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

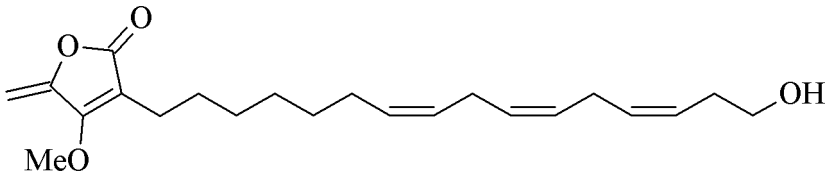
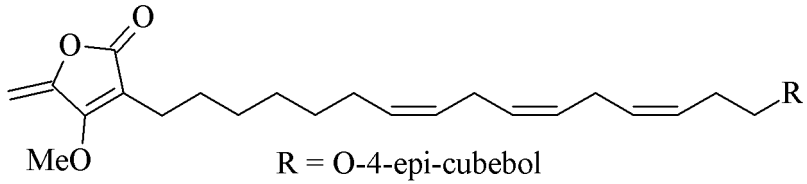
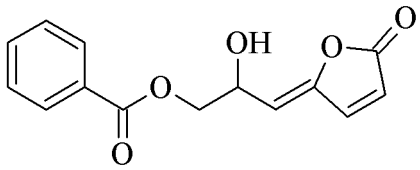
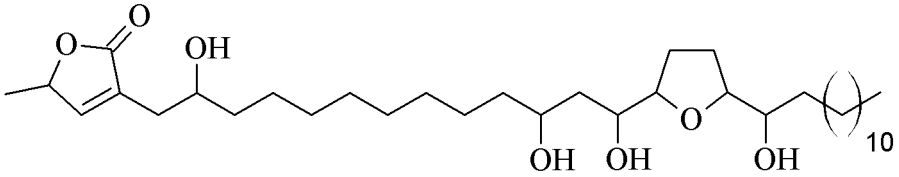
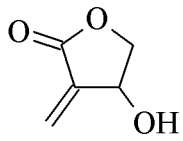
Compound	Structure	Plant species	Plant part	Reference
Artapetalin B		*A. hexapetalus*	Aerial parts	Wong and Brown (2002); Savadi (2009)
		A. modestus	Stem bark	Nyandoro *et al.* (2012)
Artapetalin C	 R = O-4-epi-cubebol	*A. hexapetalus*	Aerial parts	Wong and Brown (2002); Savadi (2009)
Melodorinol		*A. madagascariensis*	Fruits and leaves	Murphy (2007); Murphy *et al.* (2008)
Tetrahydroxylated monohydrofuranyl acetogenin		*A. brachypetalus*	Root bark	Nyandoro *et al.* (2012)

TABLE 2.7 Occurrence of other chemical constituents in *Artabotrys* species (continued).

Compound	Structure	Plant species	Plant part	Reference
Tulipalin B		*A. hexapetalus*	Aerial parts	Wong and Brown (2002)

2.1.4 Pharmacological properties

As mentioned previously, the genus *Artabotrys* has been widely employed as folk medicines in different parts of the world. The extensive usages coupled with their vast beneficial properties have attracted considerable interest of researchers in giving scientific credence and validity to the ethnomedicinal uses of *Artabotrys* species. These species could eventually serve as potential therapeutic agents for the treatment of various diseases.

Although there are over 100 species in the genus *Artabotrys*, only 10 species have been examined so far. The crude extracts and pure compounds derived from *Artabotrys* species were reported to possess a wide spectrum of *in vitro* pharmacological effects, especially antimicrobial activities (Table 2.8). In an earlier study, Srivastava *et al.* (2009) investigated the antifungal and antiaflatoxigenic activities of essential oil from leaves of *A. odoratissimus*. Through poisoned food technique, MIC value of the essential oil against *Aspergillus flavus* Navjot 4NSt was found to be 750 μL/L, at which it demonstrated superiority over different prevalent synthetic fungicides. With respect to the nature of toxicity, the essential oil exhibited fungistatic nature up to 750 μL/L and turned fungicidal nature at increased concentrations. Besides inhibiting the growth of the toxigenic strain at all levels of inoculum density, the essential oil also showed significant efficacy in arresting aflatoxin B_1 secretion at 750 μL/L. The study therefore recommended the essential oil as postharvest antimicrobial food additive in safe preservation of food commodities from storage fungi as well as renewable alternative to imported synthetic pesticides in postharvest pest management programmes.

Apart from being assessed *in vitro*, *Artabotrys* species have also been evaluated for their *in vivo* pharmacological properties including antifertility (rat) (Karthik *et al.* 2012), cardiovascular (dog, frog and rabbit) (Trivedi *et al.* 1971), central nervous system (CNS) depressant (rat) (Garg and Siddiqui 1998) and muscle contractile activities (rabbit and rat) (Cortes *et al.* 1990) (Table 2.9). Considering that many species belonging to the genus *Artabotrys* are still poorly investigated, more phytochemical and pharmacological studies are necessary in order to elucidate the active principles as well as their mechanisms of action.

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species.

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
ANTIBACTERIAL ACTIVITY				
Methanol extract	*A. hexapetalus*	Leaves	Remarkable inhibition against *Bacillus megaterium*, *Enterococcus faecalis*, *Lactobacillus casei*, *Staphylococcus aureus*, *Streptococcus entericus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Xanthomonas campestris*, with zones of inhibition ranging from 11 mm to 18 mm	Sowjanya *et al.* (2013)
Methanol extract	*A. uncinatus*	Leaves	High antibacterial activity against *Bacillus megaterium* NCIM 2032, *Micrococcus luteus* NCIM 2704, *Staphylococcus aureus* NCIM 2672, *Streptococcus lactis* NCIM 2606, *Enterobacter aerogenes* NCIM 5139, *Escherichia coli* NCIM 2810, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501, with zones of inhibition ranging from 7 mm to 16 mm at the concentration of 50 mg/disc	Gothandam *et al.* (2010)
Water extract	*A. hexapetalus*	Flowers	Strong antibacterial activity against *Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC 14028, with zones of inhibition ranging from 17±0.11 mm to 19±0.03 mm	Manjula *et al.* (2011)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
Water extract	*A. uncinatus*	Leaves	Superior efficacy against *Xanthomonas campestris* pv. *oryzae*	Yenjerappa (2009)
Artamonteirine	*A. monteiroae*	Stem bark	Selective activity against *Staphylococcus aureus* ATCC 25923 with MIC value of 2.5 µg/mL	Nyandoro *et al.* (2012)
Not specified	*A. hexapetalus*	Leaves	High inhibitory effect on *Xanthomonas oryzae* pv. *oryzae*	Kagale *et al.* (2004)
Not specified	*A. uncinatus*	Leaves	Significant inhibitory effect on *Pseudomonas fluorescens* isolates (Cla_1B_8, Cla_1B_{10}, Cla_1B_{18}, Cla_2B_7, P_2F_2, P_2F_4, PFK_{13}, PFN_3 and $PuKL_2$)	Foysal *et al.* (2011)
ANTIFUNGAL ACTIVITY				
Chloroform extract	*A. hexapetalus*	Flowers	Strong antifungal activity against *Candida albicans* and *Aspergillus niger* with respective zones of inhibition of 10±0.98 mm and 9±0.67 mm	Manjula *et al.* (2011)
Ethanol extract	*A. hexapetalus*	Leaves	Absolute fungitoxicity against *Colletotrichum oxysporum*	Jayanthi (2011)
Methanol extract	*A. hexapetalus*	Leaves	Remarkable inhibition against *Candida albicans*, *Candida rugosa*, *Aspergillus niger* and *Rhizopus oryzae*, with zones of inhibition ranging from 9 mm to 14 mm	Sowjanya *et al.* (2013)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
Methanol : water (2:1) extract	*A. odoratissimus*	Leaves	Moderate fungitoxicity against aflatoxin B_1-producing strain of *Aspergillus flavus* NKD-235 with 72.4±1.0% inhibition of mycelial growth	Shukla *et al.* (2012)
Water extract	*A. odoratissimus*	Leaves	Absolute fungitoxicity against aflatoxigenic strain of *Aspergillus flavus* Navjot 4NSt	Srivastava *et al.* (2009)
3-Methylene-4-pentadecyldihydrofuran-2-one	*A. odoratissimus*	Fruits	Good inhibitory effect against *Alternaria tenuissima* with respective MIC and IC_{50} values of 300 µg/mL and 51.37 µg/mL	Bordoloi *et al.* (2009)
8-Methoxyouregidione Ouregidione Oxocrebanine	*A. zeylanicus*	Stem bark	Preferential toxicity towards DNA repair-deficient RS 321N and RS 322YK (RAD 52Y) strains of *Saccharomyces cerevisiae*	Wijeratne *et al.* (1996)
Acetogenin	*A. brachypetalus*	Stem bark	Strong antifungal effect on *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae* and *Fusarium solani*, with MIC values of 250 ppm and 200 ppm in radial growth and mycelial dry weight measurements respectively	Odebode *et al.* (2006)
Artabotrine Atherospermidine	*A. zeylanicus*	Stem bark	Significant selective DNA-damaging activity against DNA repair-deficient RS 321N and RS 322YK (RAD 52Y) strains of *Saccharomyces cerevisiae*, with IC_{12} values ranging from 1.20 µg/mL to 27 µg/mL	Wijeratne *et al.* (1995)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
Artabotrol Artapetalin B	*A. modestus*	Stem bark	Active against *Candida albicans* DSM 1665 and *Cryptococcus neoformans* ATCC 90112	Nyandoro *et al.* (2012)
Essential oil	*A. hexapetalus*	Leaves	Moderate fungitoxicity against *Aspergillus flavus* and *Aspergillus niger* with 54.2% and 46.7% inhibition of mycelial growth respectively	Tripathi and Kumar (2007)
Essential oil	*A. odoratissimus*	Leaves	Absolute fungitoxicity against *Aspergillus flavus* (750 µL/L), *Aspergillus fumigates* (1000 µL/L), *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Microsporum gypseum*, *Mucor racemosus*, *Penicillium italicum*, *Pythium debaryanum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Trichoderma viride* (500 µL/L)	Srivastava *et al.* (2009)
Volatile compound	*A. uncinatus*	Leaves	Absolute fungitoxicity against *Alternaria alternata*, *Curvularia lunata*, *Fusarium nivale* and *Helminthosporium gramineum*	Kumari (2009); Jayanthi (2011)
Not specified	*A. uncinatus*	Leaves	Absolute fungitoxicity against *Ustilago maydis* and *Ustilago nuda*	Aguilar (2001)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
ANTIPARASITIC ACTIVITY				
Dichloromethane : methanol (1:1) extract	*A. monteiroae*	Leaves	Good antiprotozoal activity against *Plasmodium falciparum* K1 and *Trypanosoma brucei rhodesiense* STIB 900 with respective IC_{50} values of 8.79 μg/mL and 10.3 μg/mL	Mokoka *et al.* (2011)
Dichloromethane : methanol (1:1) extract	*A. monteiroae*	Twigs	Promising antiplasmodial activity against chloroquine-sensitive D10 strain of *Plasmodium falciparum* with IC_{50} value of 8.7 μg/mL	Clarkson *et al.* (2004); Pillay *et al.* (2008)
Methanol extract	*A. hexapetalus*	Bark	Significant anthelmintic activity against *Pheretima posthuma* (earthworm)	Morshed *et al.* (2012)
Water extract	*A. odoratissimus*	Leaves	Strong nematicidal activity against *Meloidogyne incognita* (roundworm)	Aguilar (2001); Jayanthi (2011)
Essential oil	*A. odoratissimus*	Leaves	Strong anthelmintic activity against *Ascaris lumbricoides* (roundworm), *Pheretima posthuma* (earthworm) and *Taenia solium* (tapeworm)	Akhtar *et al.* (2000); Iqbal *et al.* (2005); Hussain (2008); Badar (2011); Tandon *et al.* (2011)
Yingzhaosu A	*A. uncinatus*	Roots	Moderate antimalarial activity against chloroquine-resistant K1 strain of *Plasmodium falciparum* with IC_{50} value of 115 nM	Szpilman *et al.* (2005)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
ANTICANCER ACTIVITY				
Acetylmelodorinol Melodorinol	*A. madagascariensis*	Leaves and fruits	Moderate antiproliferative activity against MDA-MB-435 breast, HT-29 colon, U937 leukemia, H522-T1 non-small cell lung and A2780 ovarian carcinoma cell lines, with GI_{50} values ranging from 2.4 μM to 12 μM	Murphy *et al.* (2008)
Artabotrine	*A. zeylanicus*	Stem bark	Strong inhibitory effect on both camptothecin-resistance and wild-type P388 leukemia cell lines with respective GI_{50} values of 1.12 μM and 1.59 μM	Wijeratne *et al.* (1995); Ding *et al.* (2006)
Atherospermidine	*A. uncinatus*	Stems and stem bark	Significant cytotoxicity against KB oral carcinoma cell lines with GI_{50} value of 2.5 μg/mL	Wu *et al.* (1989)
Atherospermidine Squamolone	*A. uncinatus*	Stems	Significant cytotoxicity against Hep 2,2,15 and Hep G2 hepatocellular carcinoma cell lines with GI_{50} values ranging from 0.8 μg/mL to 2.8 μg/mL	Hsieh *et al.* (2001)
Liriodenine	*A. uncinatus*	Stems and stem bark	Potent cytotoxicity against HCT-8 colorectal, L1210 and P388 leukemia, A549 lung, as well as KB oral carcinoma cell lines, with GI_{50} values ranging from 0.57 μg/mL to 2.33 μg/mL	Wu *et al.* (1989)

TABLE 2.8 *In vitro* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
ANTI-INSECT ACTIVITY				
Alkaloidal fraction	*A. odoratissimus*	Stem bark	Significant larvicidal effect against *Culex quinquefasciatus* (mosquito) with LC_{50} value of 42.03 ppm	Kabir (2010)
Artamonteirine	*A. monteiroae*	Stem bark	Strong larvicidal potency against *Anopheles gambiae* (mosquito) with LC_{50} value of less than 1 µg/mL	Nyandoro *et al.* (2012)
Essential oil	*A. hexapetalus*	Leaves	Moderate repellency (50%) against *Bruchus pisorum* (beetle) with a dose of 0.02 mL	Kumar (2014)
Essential oil	*A. hexapetalus*	Leaves	Moderate repellency (70%) against *Trogoderma granarium* (beetle) with a dose of 0.02 mL	Tripathi and Kumar (2007)
CYTOTOXIC ACTIVITY				
Methanol extract	*A. hexapetalus*	Bark	Significant cytotoxicity against *Artemia salina* (brine shrimp) with LC_{50} value of 7.688 µg/mL	Morshed *et al.* (2012)
RECEPTOR BINDING ACTIVITY				
Methanol extract	*A. roseus*	Bark	Active against 5-HT1A (5-hydroxytryptamine) receptor with 72±1% inhibition of specific binding	Chung *et al.* (2005)

TABLE 2.9 ***In vivo*** **pharmacological properties of** ***Artabotrys*** **species.**

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
ANTIFERTILITY ACTIVITY				
Ethanol : water (70:30) extract	*A. hexapetalus*	Leaves	Significant decrease in total sperm count ($22.67\pm0.88 \times 10^5$), weights of testis (1.165±0.02 g), epididymis (0.246±0.004 g) and seminal vesicle (0.443±0.08 g), serum testosterone and testicular cholesterol levels (5.097±0.05 ng/mL and 4.752±0.701), as well as increase in testicular alkaline phosphatase (ALP) level (844.1±6.34) at a dose of 600 mg/kg on days 1-45 in male albino rat (Wistar)	Karthik *et al.* (2012)
Ethanol : water (90:10) extract Water extract	*A. odoratissimus*	Leaves	Potent anti-implantation activity (60% and 67%) at a dose of 150 mg/kg on days 1-7 post-coitum in female albino rat	Kamboj and Dhawan (1982)
Ethanol : water (95:5) extract Water extract	*A. odoratissimus*	Leaves	Significant prolongation of dioestrus stage (9.0±0.45 days and 8.6±0.23 days) and anti-implantation activity (66.6% and 83.3%) at a dose of 250 mg/kg on days 1-10 post-coitum in female albino rat (Wistar)	Geetha *et al.* (2005); Tran and Hinds (2012)
Petroleum ether extract	*A. hexapetalus*	Leaves	High anti-implantation activity (72%) at a dose of 250 mg/kg on days 1-7 post-coitum in female albino rat	Johri *et al.* (2009)

TABLE 2.9 *In vivo* pharmacological properties of *Artabotrys* species (continued).

Extract / Compound	Plant species	Plant part	Pharmacological property	Reference
CARDIOVASCULAR ACTIVITY				
Ethanol extract	*A. odoratissimus*	Fruits	Cardiac depressant activity in isolated heart of frog, cardiac stimulant activity in hearts of dog (*in situ*), frog and rabbit (isolated), dilatation of perfused hind-limb blood vessels of dog, and hypotensive effect in anaesthetised dog	Trivedi *et al.* (1971)
CENTRAL NERVOUS SYSTEM (CNS) DEPRESSANT ACTIVITY				
Essential oil	*A. odoratissimus*	Leaves	Significant reduction of spontaneous motor activity (SMA) (32%), potentiation of pentobarbitone sodium-induced hypnosis (204.6±4.827 min), manifestation of neurological deficit (45%), and conditioned avoidance response (CAR) (40.6%) at a dose of 250 mg/kg in albino rat	Garg and Siddiqui (1998)
MUSCLE CONTRACTILE ACTIVITY				
Ethanol extract	*A. odoratissimus*	Fruits	Relaxant action on isolated ileum of rabbit, and spasmogenic effect on uterine smooth muscle of rat	Trivedi *et al.* (1971)
Atherospermidine	*A. maingayi*	Bark	Relaxant activity on oxytocin- or vanadate-induced rat uterine contractions in the absence of calcium	Cortes *et al.* (1990)
Atherospermidine Norstephalagine	*A. maingayi*	Bark	Relaxant activity on rat uterine contractions induced by potassium chloride, or rhythmic contractions induced by oxytocin in the presence of calcium	Cortes *et al.* (1990)

CHAPTER III

SEQUENTIAL EXTRACTION AND PHYTOCHEMICAL SCREENING OF *ARTABOTRYS CRASSIFOLIUS*

3.1 INTRODUCTION

Extraction as a pharmaceutically used term can be defined as the method of separating medicinally active portions of plant tissues from the inactive or inert components using selective solvents (Das *et al.* 2010), which will diffuse into the solid plant material and dissolve compounds with similar polarity (Tiwari *et al.* 2011). The quality of an extract is dependent on several parameters including the plant part used as starting material, the choice of solvent, and the extraction method (Ncube *et al.* 2008). Moreover, extraction of plant tissues with solvents of different polarity ranging from hexane to water could provide a comprehensive study for a variety of bioactive compounds (Green 2004).

Phytochemical screening is an effective technique for the detection of different classes of compounds such as alkaloids, flavonoids and terpenoids (Attard and Pacioni 2012). These qualitative chemical tests are useful in establishing profile of the extracts for their nature of chemical composition (Ghannadi *et al.* 2012). Besides generating hypotheses regarding the types of secondary metabolites present in an extract, the preliminary chemical evaluation also helps in monitoring the presence of compounds of interest (Jones and Kinghorn 2005). Hence, phytochemical analysis is of paramount importance in identifying new sources of therapeutically and industrially valuable compounds (Mungole *et al.* 2010).

3.2 METHODOLOGY

3.2.1 Collection and identification of plant material

The leaves and bark of *Artabotrys crassifolius* Hook.f. & Thomson (Figure 3.1), with the local name of *akar mempisang*, were collected from Kuala Kangsar, Perak, Malaysia (4°46′N, 100°56′E) in March 2011. The plant was identified and authenticated by Mr. Kamarudin Saleh, Forest Biodiversity Division, Forest Research Institute Malaysia (FRIM). Voucher specimens were prepared and deposited in the Kepong Herbarium (KEP) of FRIM (PID 080311–05), and the School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus (UNMC 65) for future reference.

FIGURE 3.1 ***Artabotrys crassifolius*** **Hook.f. & Thomson.**

3.2.2 Preparation of plant material

(a) Drying and grinding of plant material

After removal of extraneous matter, the freshly collected leaves and bark were air-dried in the shade at room temperature for at least 2 weeks. The dried leaves and bark were then finely pulverised by grinding using aluminium collection blender (Philips, China), followed by weighing with top loading balance (Sartorius AG, Germany) prior to extraction.

(b) Sequential extraction of plant material

The pulverised leaves (1.30 kg) and bark (4.79 kg) were extracted sequentially with solvents of increasing polarity starting from hexane (Friendemann Schmidt, Australia), chloroform (Friendemann Schmidt, Australia) and 95% (v/v) of ethanol (John Kollin Chemicals, India). Each extraction was performed in triplicate at a solid-to-solvent ratio of 1:5 (w/v) in a 40°C water bath (Julabo, Germany) for three days. The respective extract was subsequently filtered through qualitative filter papers No. 1 (Whatman International Ltd., England) and the collected filtrate was concentrated to dryness under reduced pressure at 40°C using rotary evaporator (Buchi Labortechnik AG, Switzerland). Eventually, the dried extract obtained was weighed with analytical balance (Sartorius AG, Germany) and stored in glass scintillation vials (Kimble, USA) at –20°C until further use. For stock solutions, each crude extract was dissolved in dimethyl sulphoxide (DMSO) (R & M Chemicals, UK) at a concentration of 100 mg/mL and stored at 4°C.

3.2.3 Determination of extraction yield

For each extraction, the extraction yields of crude extracts were calculated. The extraction yield was expressed as the weight percentage of the dried plant extract obtained with respect to the dried plant material used (Pin *et al.* 2010; Kosma *et al.* 2011), which was given as follows:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried plant extract (g)}}{\text{Weight of dried plant material (g)}} \times 100\%$$

3.2.4 Evaluation of organoleptic properties

The organoleptic properties of crude extracts were assessed by their colour, texture and odour (Arya *et al.* 2010). These organoleptic characters were determined using the senses of sight (eyes), touch (skin) and smell (nose) (Ma 2006).

(a) Colour

For colour determination, each crude extract was properly examined under diffuse daylight. If necessary, an artificial light source with wavelengths similar to those of daylight might be used (WHO 2011).

(b) Texture

For texture determination, small quantity of each crude extract was taken and examined by rubbing it between the thumb and index finger (Chaturvedi *et al.* 2011).

(c) Odour

For odour determination, if the crude extract was expected to be innocuous, small portion of the respective extract was placed in the palm of the hand or in a beaker of suitable size, and examined by slow and repeated inhalation of the air over the extract. If no distinct odour was perceptible, the crude extract was rubbed between the thumb and index finger or between the palms of the hands using gentle pressure. If the crude extract was known to be dangerous, small quantity of boiling water was poured onto the respective extract placed in a beaker (Chandel *et al.* 2011).

3.2.5 Phytochemical screening

The phytochemical screenings of crude extracts were carried out using standard procedures. Each crude extract (final concentration of 1 mg/mL) was assayed for the presence of phytochemical constituents such as alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins and terpenoids.

(a) Test for alkaloids (Dragendorff's test)

Prior to detection of alkaloids, solution A was prepared by dissolving 1.7 g of bismuth subnitrate (Mallinckrodt, USA) in 100 mL of 4:1 (v/v) of distilled water and acetic acid (Systerm, Malaysia), whereas 40 g of potassium iodide (R & M Chemicals, UK) was dissolved in 100 mL of distilled water as solution B. To prepare Dragendorff's reagent, 5 mL of solution A and B was added in 20 mL of acetic acid and topped up with distilled water to 100 mL (Mehrotra *et al.* 2011).

Approximately 1 mL of each crude extract was mixed with 4 mL of methanol (Friendemann Schmidt, Australia). The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with 1 mL of 1% (v/v) of hydrochloric acid (HCl) (Systerm, Malaysia) and warmed on steam bath, followed by addition of a few drops of Dragendorff's reagent. Reddish orange precipitation indicated the presence of alkaloids (Magadula and Tewtrakul 2010).

(b) Test for cardiac glycosides (Keller-Kiliani test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with 2 mL of acetic acid containing one drop of 5% (w/v) of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Systerm, Malaysia). This was underlayed with 1 mL of concentrated sulphuric acid (H_2SO_4, 98%) (Fisher Scientific, UK). A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring, while in the acetic acid layer, a greenish ring might form just above the brown ring and gradually spread throughout this layer (Hussain *et al.* 2011).

(c) Test for flavonoids (Shinoda test)

Approximately 1 mL of each crude extract was mixed with 4 mL of ethanol. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with few fragments of magnesium ribbon, followed by dropwise addition of concentrated HCl (fuming 37%). Pink scarlet, crimson red or occasionally green to blue colour appeared after few minutes indicated the presence of flavonoids (Itoria *et al.* 2011).

(d) Test for phenolic compounds (Ferric chloride test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$. Bluish-green or bluish-black colour indicated the presence of phenolic compounds (Kripa *et al.* 2011).

(e) Test for saponins (Frothing test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was shaken vigorously for 1 min and allowed to stand for 15 min. Frothing persistence indicated the presence of saponins (Vesoul and Cock 2011).

(f) Test for tannins (Gelatine-salt test)

Approximately 1 mL of each crude extract was mixed with 4 mL of hot distilled water. The mixture was filtered and the filtrate was divided into three test tubes. To the first portion of the filtrate, 1 mL of 1% (w/v) of sodium chloride (NaCl) (R & M Chemicals, UK) was added as the control. Second portion of the filtrate was treated with 1 mL of 1% (w/v) of NaCl and 1 mL of 5% (w/v) of gelatine (R & M Chemicals, UK), whereas a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$ were added to the third portion of the filtrate. Formation of a precipitate in the second treatment suggested the presence of tannins, and a positive response after addition of $FeCl_3 \cdot 6H_2O$ to the third portion supported this inference (Jones and Kinghorn 2005).

(g) Test for terpenoids (Salkowski test)

Approximately 1 mL of each crude extract was mixed with 4 mL of chloroform. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was carefully treated with 3 mL of concentrated H_2SO_4 to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids (Khan *et al.* 2011).

3.3 RESULTS AND DISCUSSION

3.3.1 Extraction yields of crude extracts of *Artabotrys crassifolius*

Extraction yield is a measure of the solvent efficiency to extract specific components from the original material (Aspe and Fernandez 2011; Tsai *et al.* 2012). The extraction yield in percentage for each crude extract is shown in Figure 3.2 (Appendix A). Among the different solvents used for extraction, ethanol provided the highest yield of crude extracts from both leaves and bark with extraction yields of 5.00% and 4.02% respectively. In contrast, the lowest extraction yield was recorded for hexane extract of bark with 0.53%. This indicates that ethanol is a superior extraction solvent to hexane or chloroform in terms of providing a better yield due to its high polarity.

3.3.2 Organoleptic properties of crude extracts of *Artabotrys crassifolius*

Organoleptic evaluation refers to the evaluation of individual drugs and formulations by colour, texture and odour (Satheesh *et al.* 2011; Vishvnath and Jain 2011). The colour, texture and odour of crude extracts in different solvents were characterised (Table 3.1). As compared to hexane and chloroform extracts, ethanol extracts were found to be better in retaining the natural fragrances of the plants. This may be attributed to the preservative ability of ethanol (reducing breakdown of organic compounds by microorganisms), its enhanced extraction capability (more fragrant components extracted) or a combination of both (Chan *et al.* 2008; Arya *et al.* 2010).

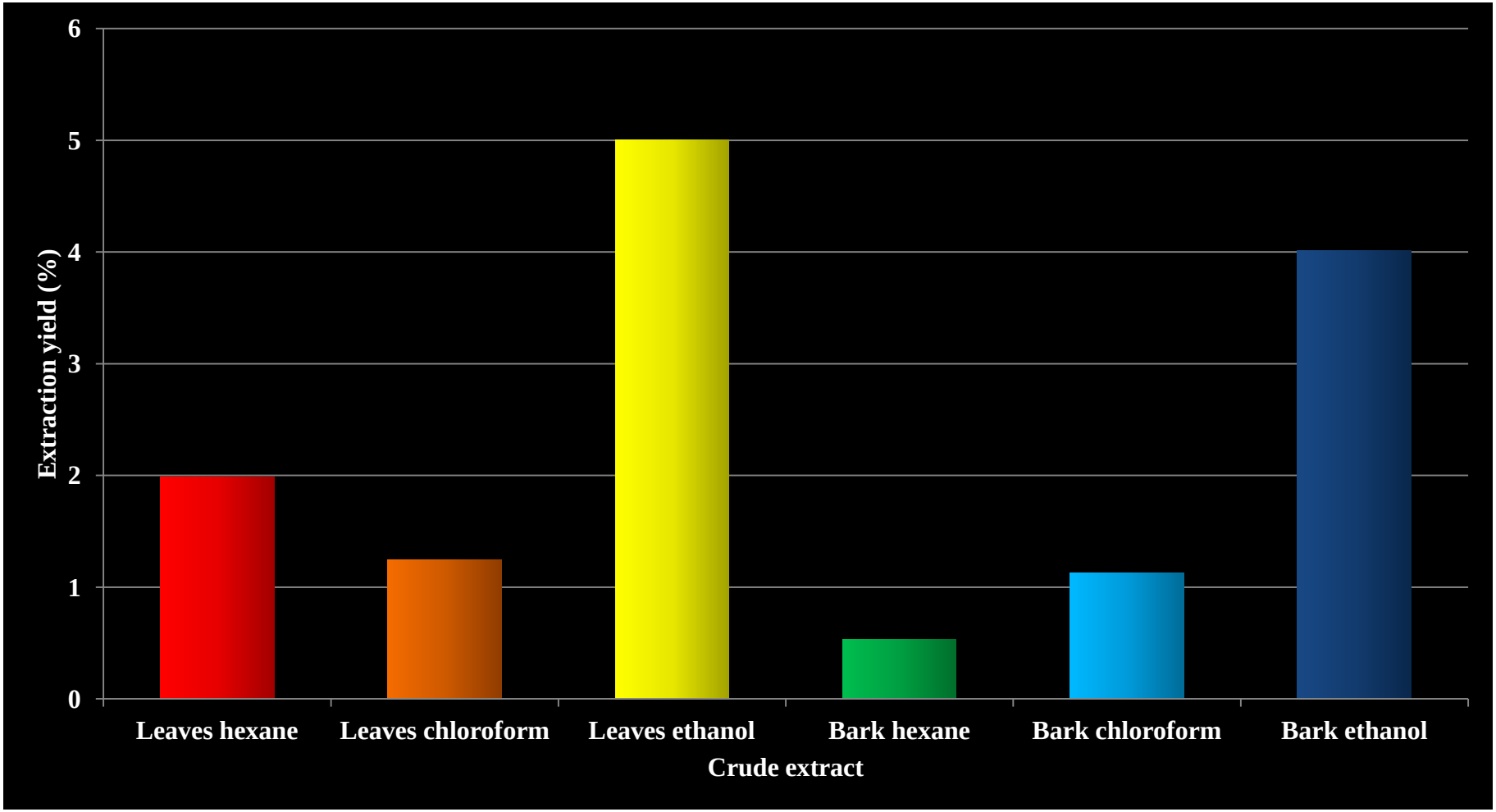
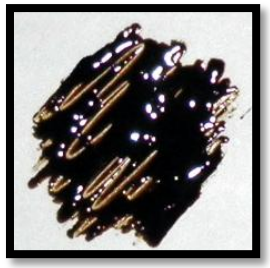


FIGURE 3.2 Extraction yields of crude extracts of *Artabotrys crassfolius*.

TABLE 3.1 Organoleptic properties of crude extracts of *Artabotrys crassifolius*.

Organoleptic property	Crude extract					
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol
						
Colour	Greenish black	Greenish black	Brownish black	Golden brown	Greenish black	Brownish black
Texture	Waxy	Waxy	Sticky	Waxy	Powdery	Flaky
Odour	Leafy smell	Pungent smell	Sweet smell	Pungent smell	Fishy smell	Sweet smell

3.3.3 Phytochemical screenings of crude extracts of *Artabotrys crassifolius*

Prior to pharmacological evaluation of plant extracts, phytochemical screening is the initial and essential step towards understanding the nature of active principles in medicinal plants (Kakpure and Rothe 2012). Based on the preliminary phytochemical analysis of crude extracts presented in Table 3.2–3.9, bark extracts were found to have more secondary metabolites than leaves extracts. Both chloroform and ethanol extracted the widest range of phytochemical constituents from bark including cardiac glycosides, flavonoids, phenolic compounds and terpenoids. The only difference detected between these extracts was the presence of alkaloids and saponins in chloroform and ethanol extracts of bark respectively. Additionally, hexane extract of bark showed positive results for alkaloids, cardiac glycosides and terpenoids.

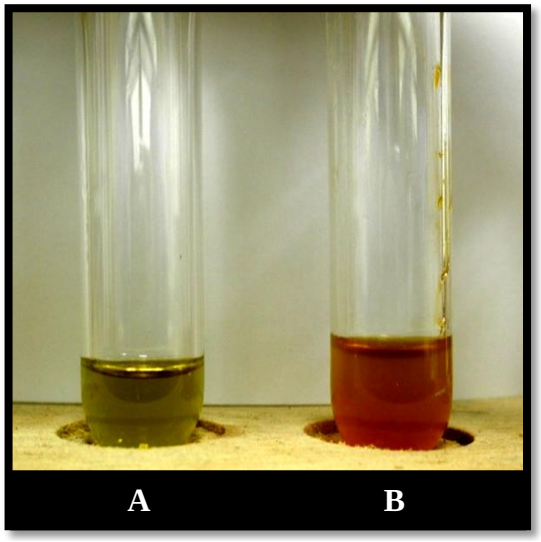
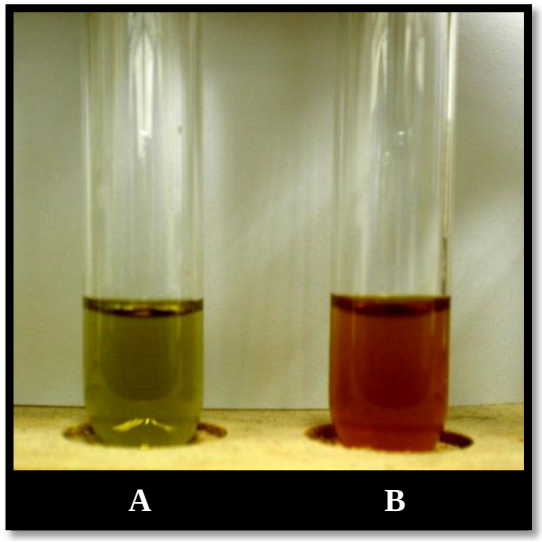
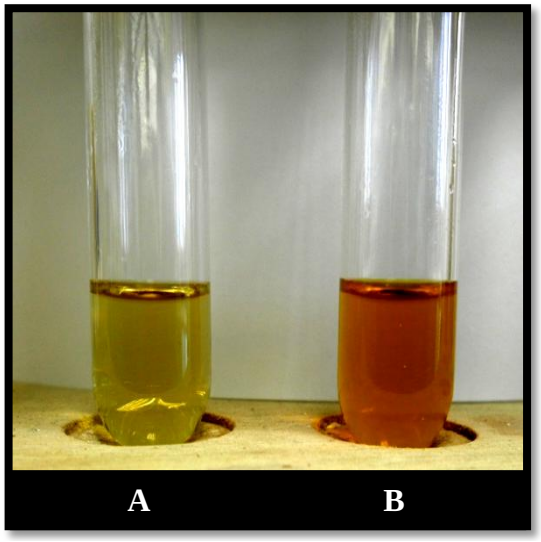
On the other hand, ethanol extract of leaves possessed similar phytochemical constituents to ethanol extract of bark. However, hexane and chloroform extracts of leaves exhibited positive reaction only to Keller-Kiliani test for cardiac glycosides. Among the phytochemical constituents analysed, tannins were absent in all of the tested extracts. This implies that the extracts from leaves and bark may constitute a different source of secondary metabolites that can serve as a constructive reference for further detailed studies on the pharmacological activities of *Artabotrys crassifolius*.

TABLE 3.2 Phytochemical screenings of crude extracts of *Artabotrys crassifolius*.

Phytochemical constituent	Test used	Crude extract					
		Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol
Alkaloids	Dragendorff's test	–	–	–	+	+	–
Cardiac glycosides	Keller-Kiliani test	+	+	+	+	+	+
Flavonoids	Shinoda test	–	–	+	–	+	+
Phenolic compounds	Ferric chloride test	–	–	+	–	+	+
Saponins	Frothing test	–	–	+	–	–	+
Tannins	Gelatin-salt test	–	–	–	–	–	–
Terpenoids	Salkowski test	–	–	+	+	+	+

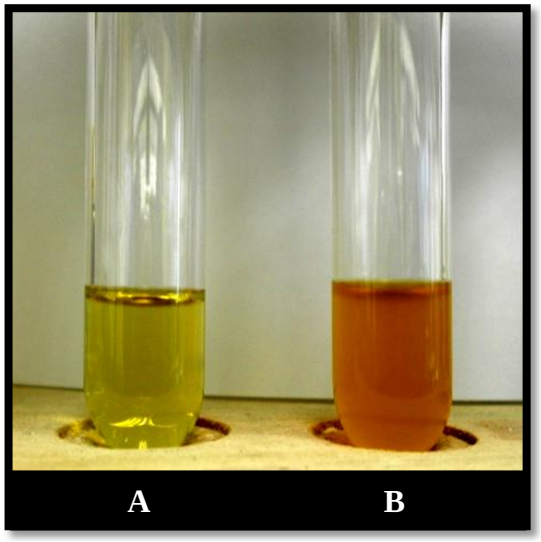
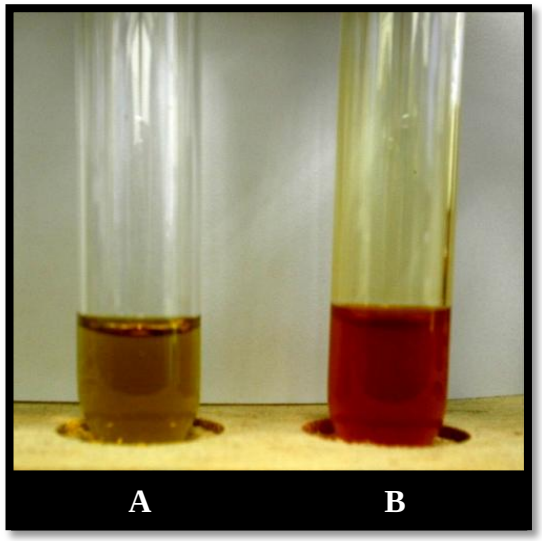
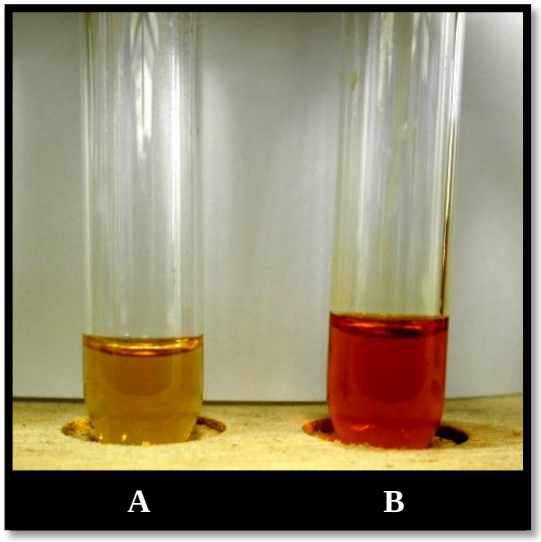
Note: (+) indicates the presence of phytochemical constituents, (–) indicates the absence of phytochemical constituents.

TABLE 3.3 Phytochemical analyses for the presence of alkaloids from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	Absence of reddish orange precipitate	Absence of reddish orange precipitate	Absence of reddish orange precipitate
Inference	Negative result for alkaloids	Negative result for alkaloids	Negative result for alkaloids

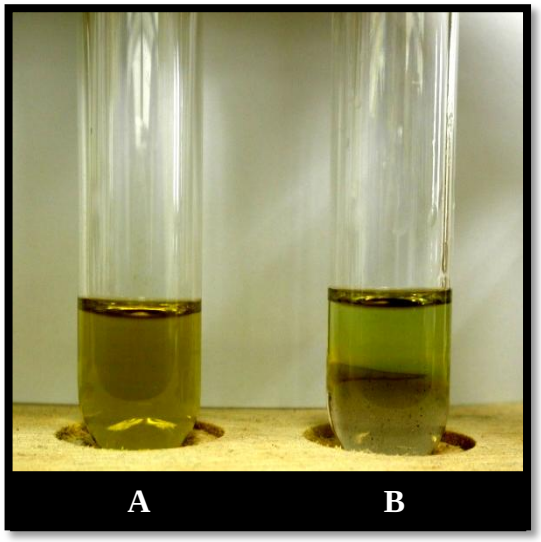
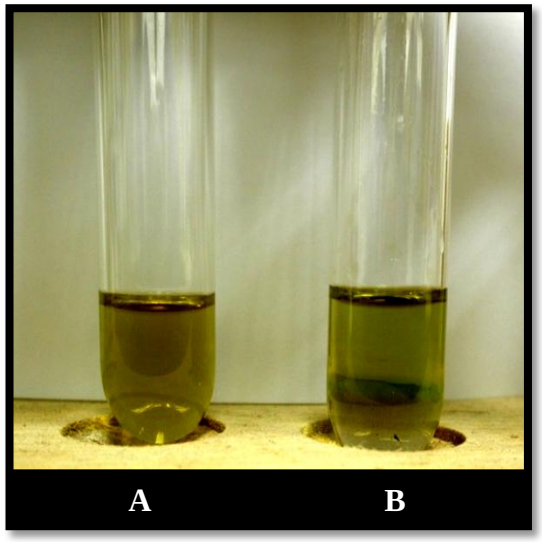
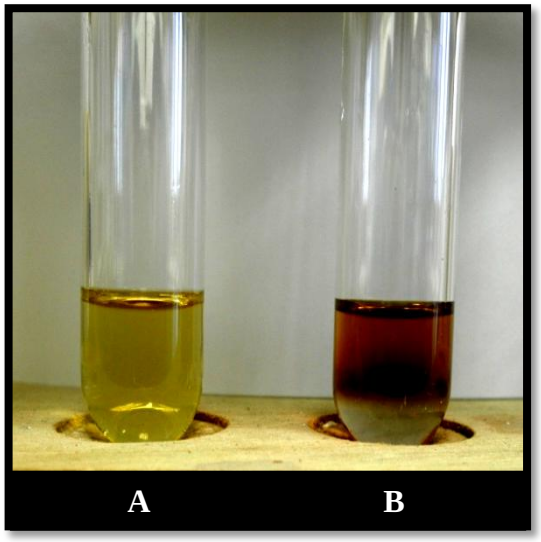
Note: Test tube A was served as the control whereas test tube B was treated with 1% (v/v) of HCl and warmed on steam bath, followed by addition of a few drops of Dragendorff's reagent.

TABLE 3.3 Phytochemical analyses for the presence of alkaloids from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	Reddish orange precipitate was observed	Reddish orange precipitate was observed	Absence of reddish orange precipitate
Inference	Positive result for alkaloids	Positive result for alkaloids	Negative result for alkaloids

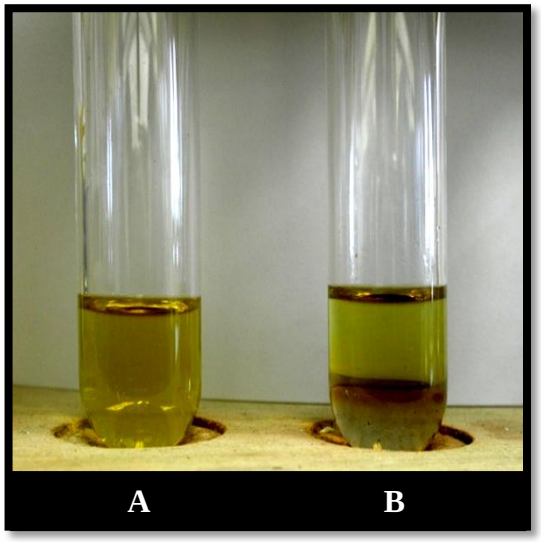
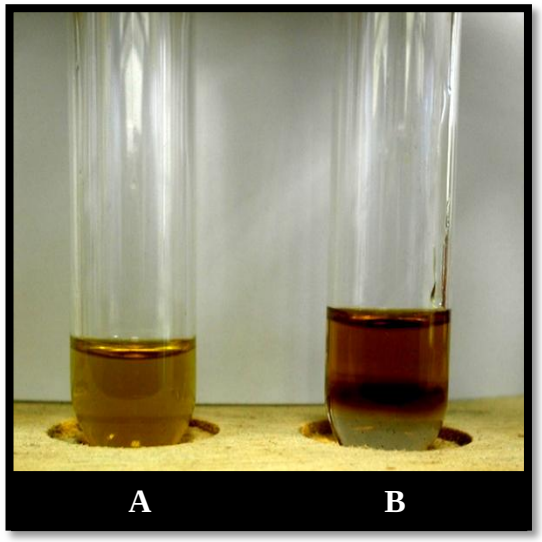
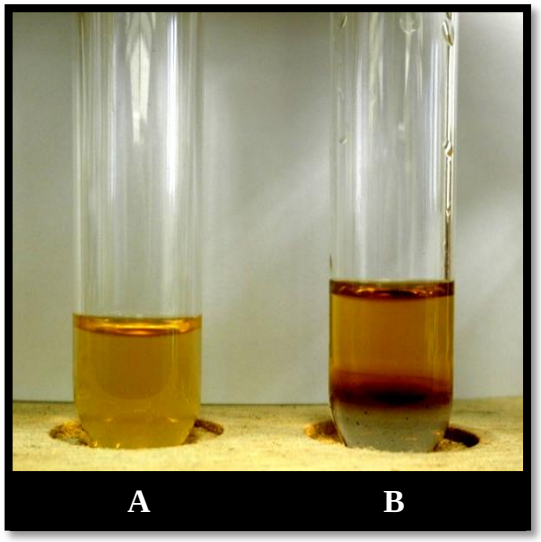
Note: Test tube A was served as the control whereas test tube B was treated with 1% (v/v) of HCl and warmed on steam bath, followed by addition of a few drops of Dragendorff's reagent.

TABLE 3.4 Phytochemical analyses for the presence of cardiac glycosides from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	Brown ring at the interface was observed	Brown ring at the interface was observed	Brown ring at the interface was observed
Inference	Positive result for cardiac glycosides	Positive result for cardiac glycosides	Positive result for cardiac glycosides

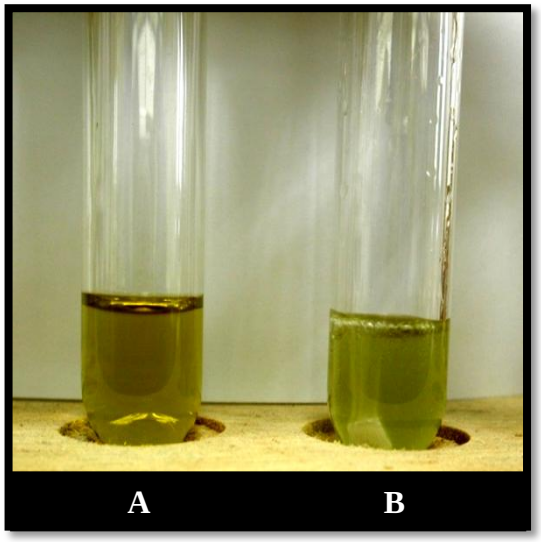
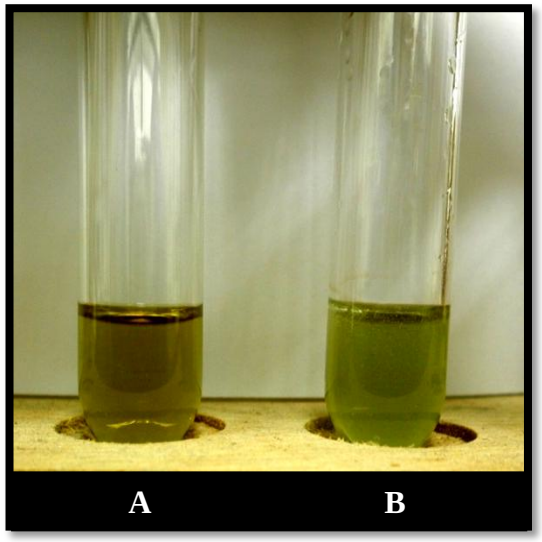
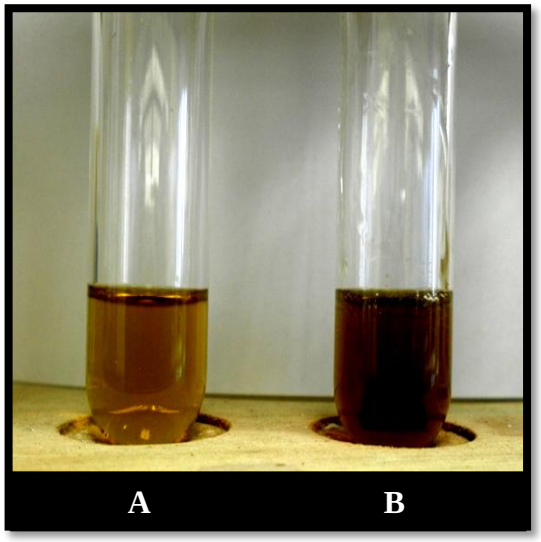
Note: Test tube A was served as the control whereas test tube B was treated with acetic acid containing one drop of 5% (w/v) of $FeCl_3 \cdot 6H_2O$, and underlayed with concentrated H_2SO_4.

TABLE 3.4 Phytochemical analyses for the presence of cardiac glycosides from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	Brown ring at the interface was observed	Brown ring at the interface was observed	Brown ring at the interface was observed
Inference	Positive result for cardiac glycosides	Positive result for cardiac glycosides	Positive result for cardiac glycosides

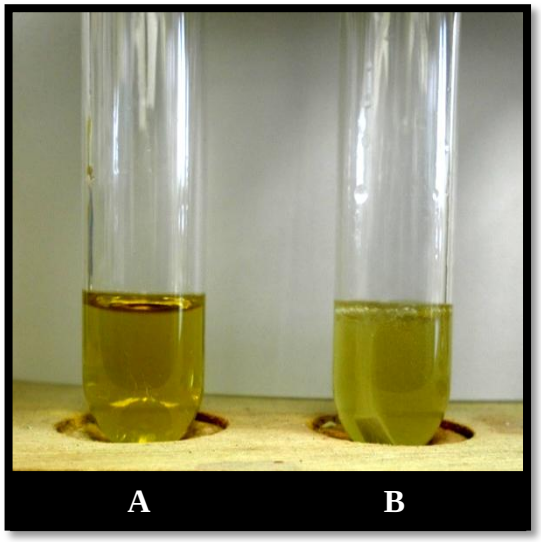
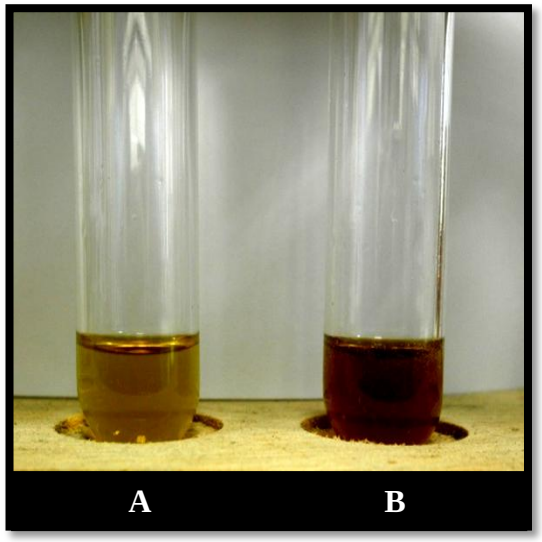
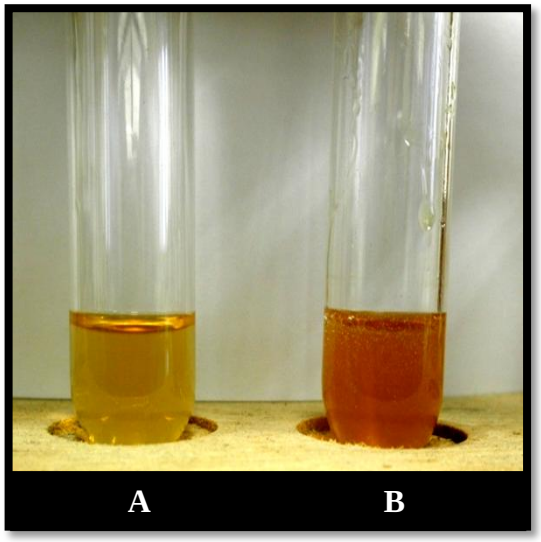
Note: Test tube A was served as the control whereas test tube B was treated with acetic acid containing one drop of 5% (w/v) of $FeCl_3 \cdot 6H_2O$, and underlayed with concentrated H_2SO_4.

TABLE 3.5 Phytochemical analyses for the presence of flavonoids from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	No colour change was observed	No colour change was observed	Crimson red colouration was observed
Inference	Negative result for flavonoids	Negative result for flavonoids	Positive result for flavonoids

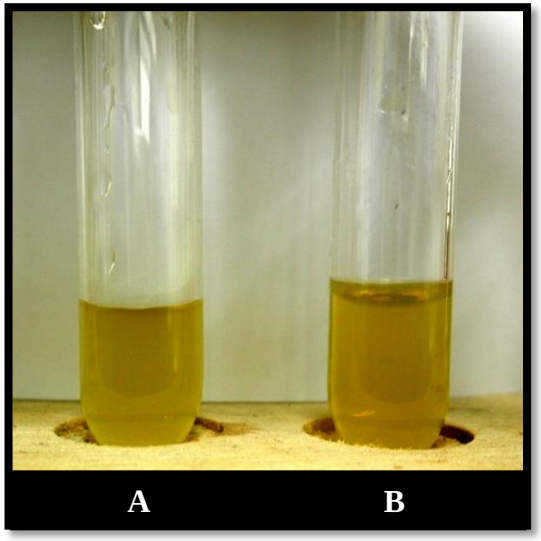
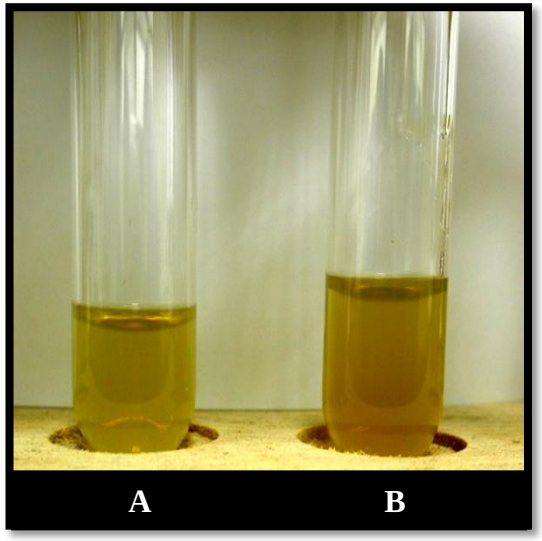
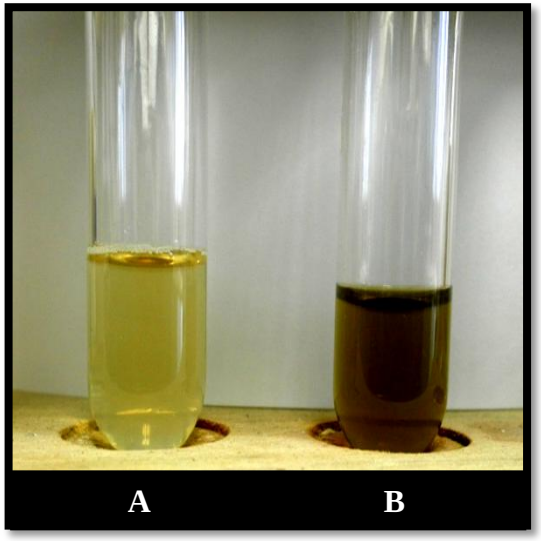
Note: Test tube A was served as the control whereas test tube B was treated with few fragments of magnesium ribbon, followed by dropwise addition of concentrated HCl.

TABLE 3.5 Phytochemical analyses for the presence of flavonoids from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	No colour change was observed	Crimson red colouration was observed	Pink scarlet colouration was observed
Inference	Negative result for flavonoids	Positive result for flavonoids	Positive result for flavonoids

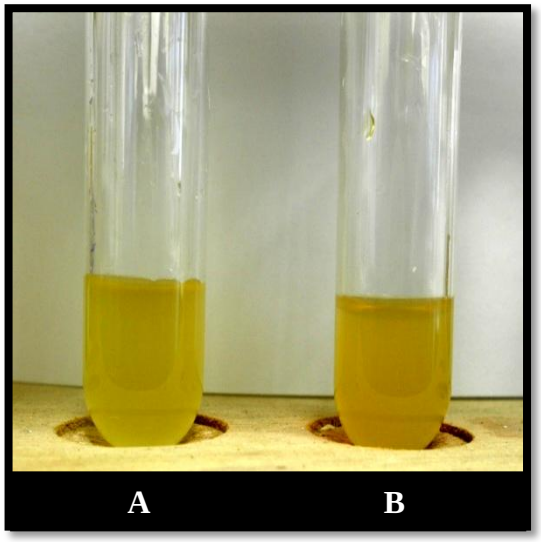
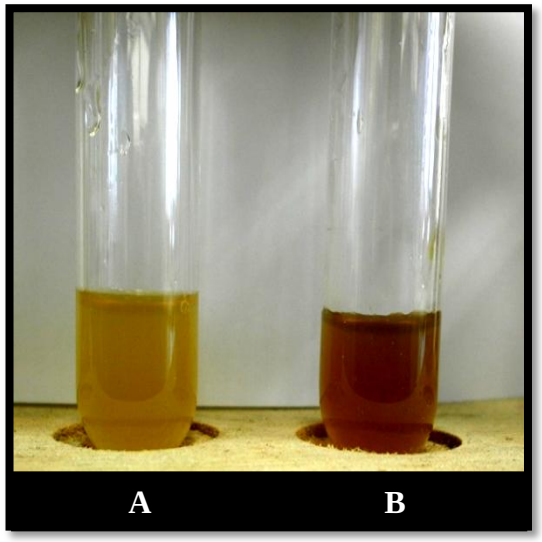
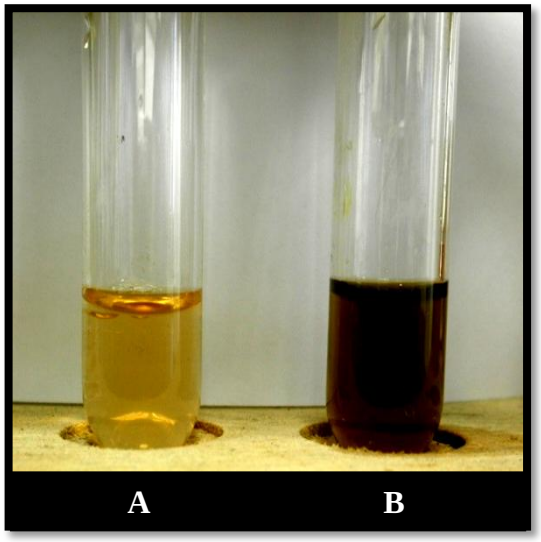
Note: Test tube A was served as the control whereas test tube B was treated with few fragments of magnesium ribbon, followed by dropwise addition of concentrated HCl.

TABLE 3.6 Phytochemical analyses for the presence of phenolic compounds from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	No colour change was observed	No colour change was observed	Bluish-black colouration was observed
Inference	Negative result for phenolic compounds	Negative result for phenolic compounds	Positive result for phenolic compounds

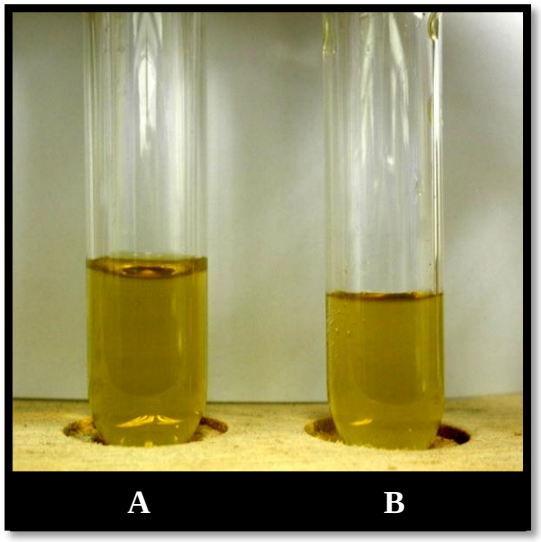
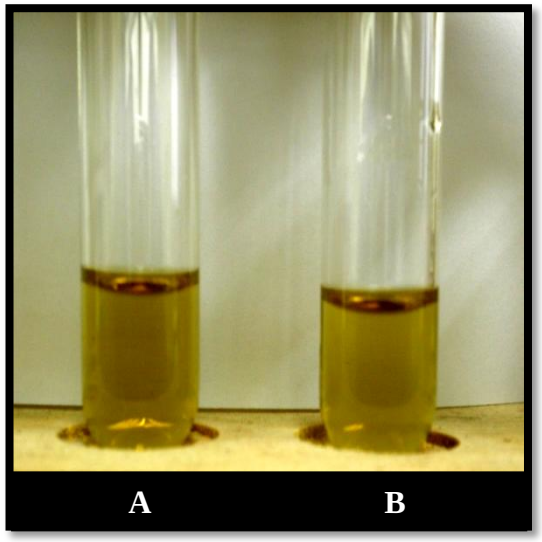
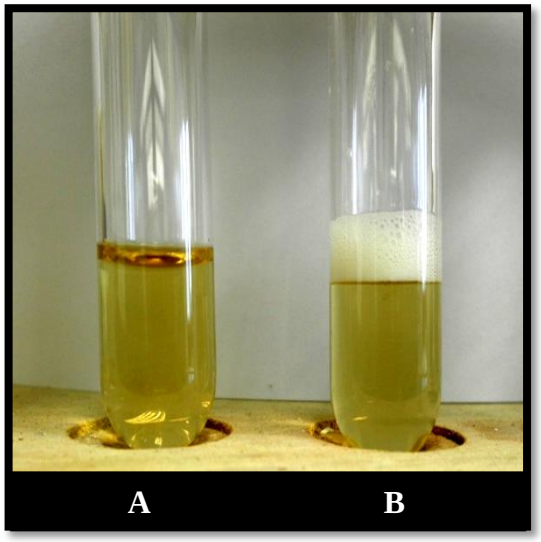
Note: Test tube A was served as the control whereas test tube B was treated with a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$.

TABLE 3.6 Phytochemical analyses for the presence of phenolic compounds from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	No colour change was observed	Bluish-black colouration was observed	Bluish-black colouration was observed
Inference	Negative result for phenolic compounds	Positive result for phenolic compounds	Positive result for phenolic compounds

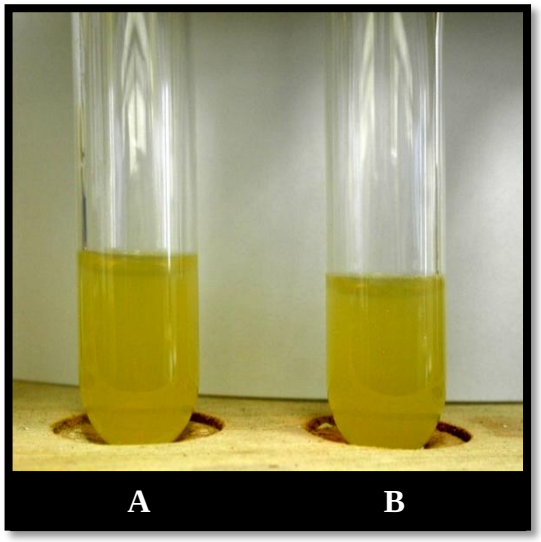
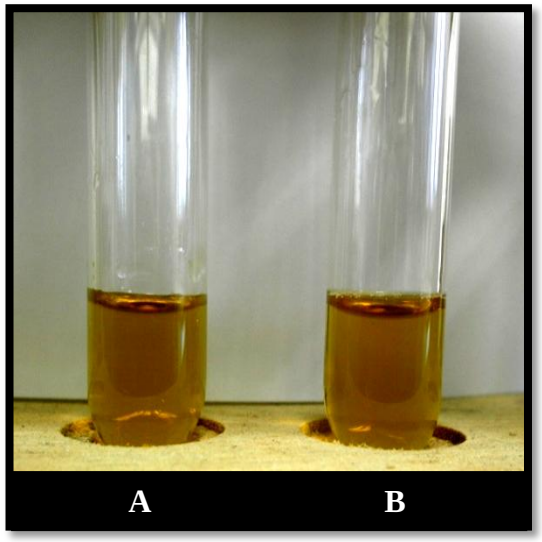
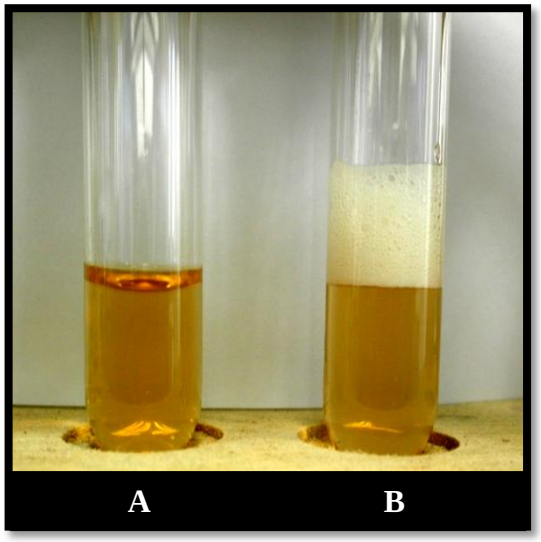
Note: Test tube A was served as the control whereas test tube B was treated with a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$.

TABLE 3.7 Phytochemical analyses for the presence of saponins from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	No frothing was observed	No frothing was observed	Frothing persistence was observed
Inference	Negative result for saponins	Negative result for saponins	Positive result for saponins

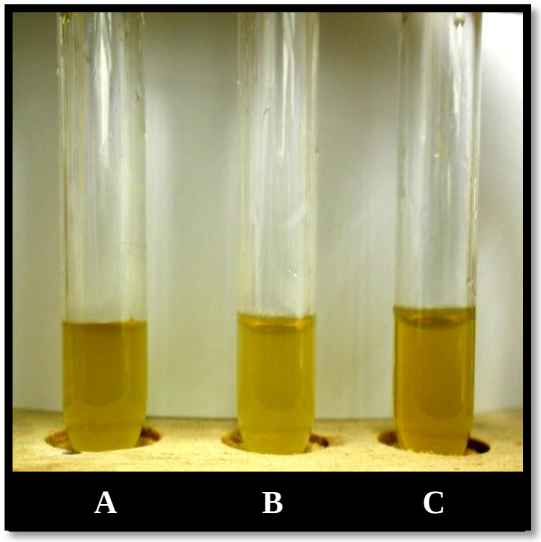
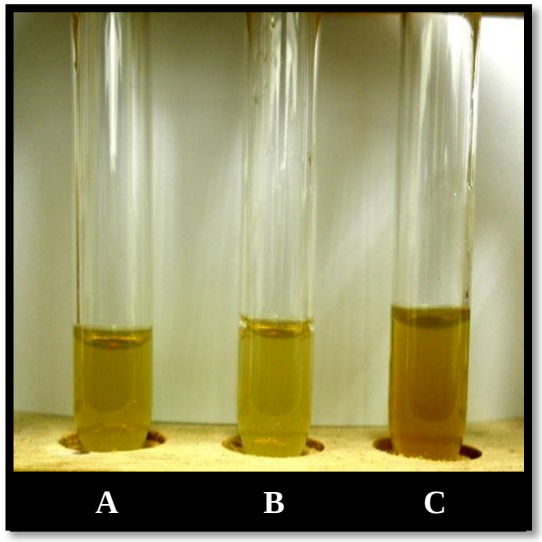
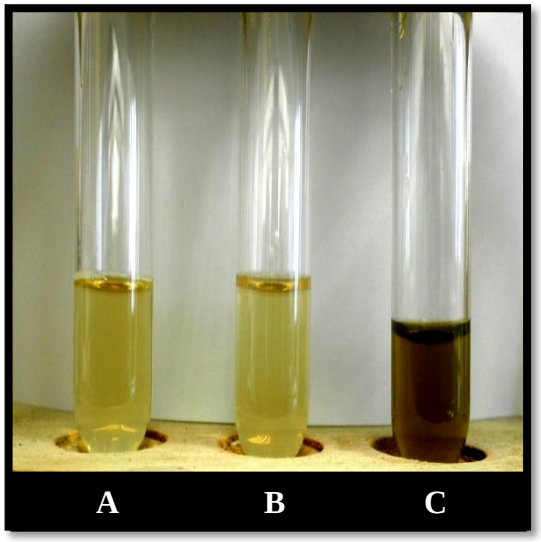
Note: Test tube A was served as the control whereas test tube B was shaken vigorously and allowed to stand.

TABLE 3.7 Phytochemical analyses for the presence of saponins from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	No frothing was observed	No frothing was observed	Frothing persistence was observed
Inference	Negative result for saponins	Negative result for saponins	Positive result for saponins

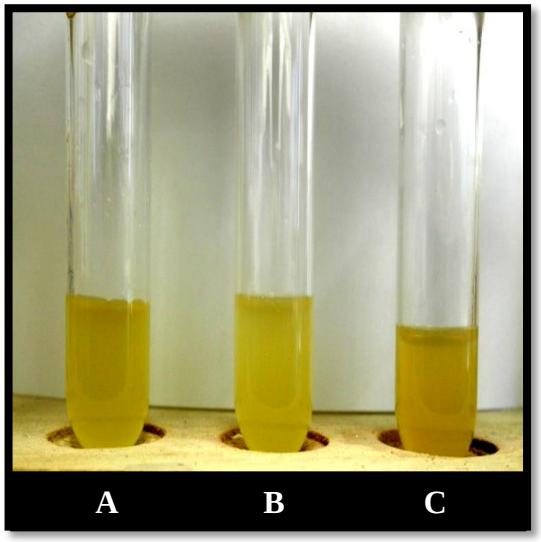
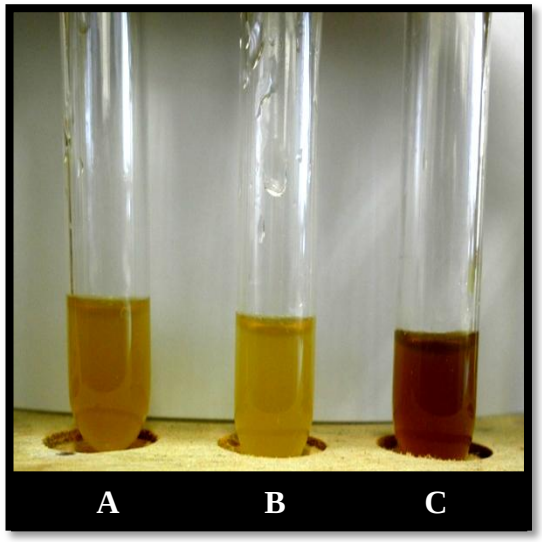
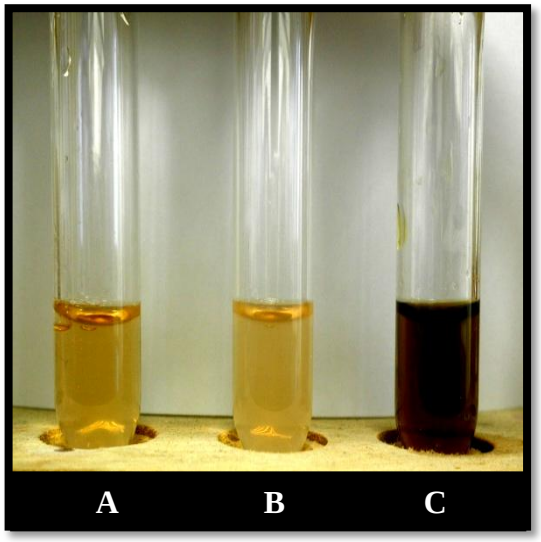
Note: Test tube A was served as the control whereas test tube B was shaken vigorously and allowed to stand.

TABLE 3.8 Phytochemical analyses for the presence of tannins from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	No appearance of precipitate in test tube B while no colour change was observed in test tube C	No appearance of precipitate in test tube B while no colour change was observed in test tube C	No appearance of precipitate in test tube B while bluish-black colouration was observed in test tube C
Inference	Negative result for tannins	Negative result for tannins	Negative result for tannins

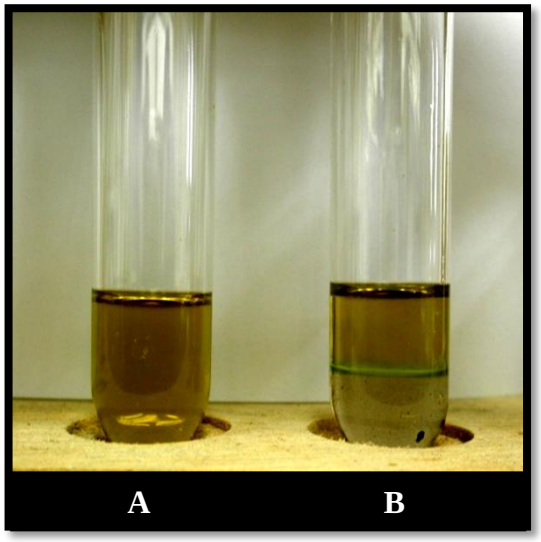
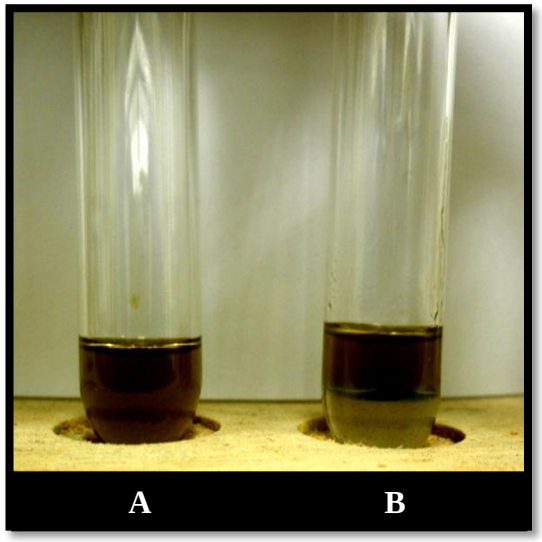
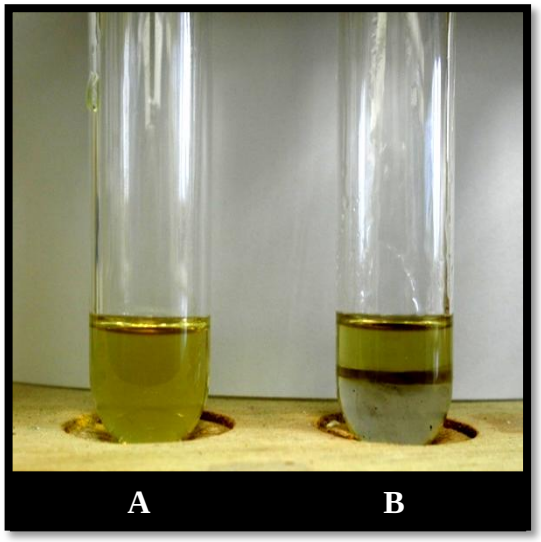
Note: Test tube A was served as the control whereas test tubes B and C were treated with 1% (w/v) of NaCl and 5% (w/v) of gelatine, and a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$ respectively.

TABLE 3.8 Phytochemical analyses for the presence of tannins from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	No appearance of precipitate in test tube B while no colour change was observed in test tube C	No appearance of precipitate in test tube B while bluish-black colouration was observed in test test tube C	No appearance of precipitate in test tube B while bluish-black colouration was observed in test test tube C
Inference	Negative result for tannins	Negative result for tannins	Negative result for tannins

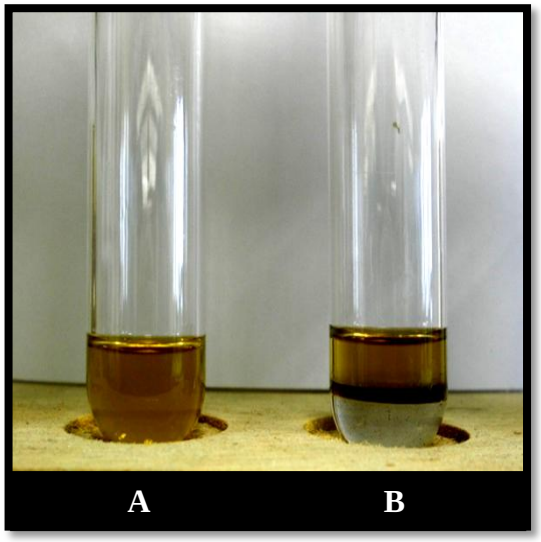
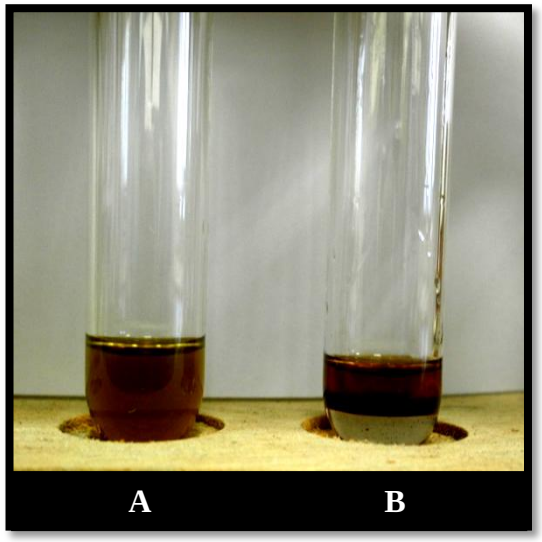
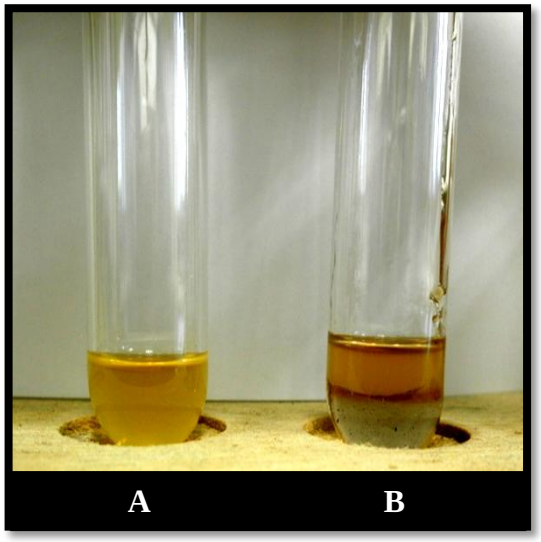
Note: Test tube A was served as the control whereas test tubes B and C were treated with 1% (w/v) of NaCl and 5% (w/v) of gelatine, and a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$ respectively.

TABLE 3.9 Phytochemical analyses for the presence of terpenoids from crude extracts of *Artabotrys crassifolius*.

Phytochemical analysis	Crude extract		
	Leaves hexane	Leaves chloroform	Leaves ethanol
			
Observation	Green colouration of the interface was observed	Green colouration of the interface was observed	Reddish brown colouration of the interface was observed
Inference	Negative result for terpenoids	Negative result for terpenoids	Positive result for terpenoids

Note: Test tube A was served as the control whereas test tube B was treated with concentrated H_2SO_4.

TABLE 3.9 Phytochemical analyses for the presence of terpenoids from crude extracts of *Artabotrys crassifolius* (continued).

Phytochemical analysis	Crude extract		
	Bark hexane	Bark chloroform	Bark ethanol
			
Observation	Reddish brown colouration of the interface was observed	Reddish brown colouration of the interface was observed	Reddish brown colouration of the interface was observed
Inference	Positive result for terpenoids	Positive result for terpenoids	Positive result for terpenoids

Note: Test tube A was served as the control whereas test tube B was treated with concentrated H_2SO_4.

3.4 CONCLUSION

The preliminary qualitative phytochemical analysis of crude extracts of *Artabotrys crassifolius* revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins and terpenoids. Consequently, the chemical profile of crude extracts can help to provide guidance for further investigations in pharmacological properties of *Artabotrys crassifolius*.

CHAPTER IV

IN VITRO ANTIBACTERIAL ACTIVITY OF *ARTABOTRYS CRASSIFOLIUS*

4.1 INTRODUCTION

Bacteria are unicellular prokaryotic microorganisms that exhibit different cellular sizes and shapes ranging from spheres to rods and spirals (Charalampopoulos and Rastall 2009). In the human body, most of the bacteria are rendered harmless or beneficial by the protective effects of the immune system (Cioffi and Rai 2012). Nevertheless, some species of bacteria are pathogenic and capable of causing infectious diseases such as anthrax, bubonic plague, cholera, leprosy, syphilis and tuberculosis (Liu 2011).

In spite of the widespread availability of antibacterial therapies, bacterial infections continue to pose a significant threat to public health worldwide (Bow 2013; Gaca *et al.* 2013). More importantly, the clinical efficacy of many existing antibacterial drugs is declining precipitously due to the emergence and dissemination of multiple drug resistant pathogens (Jahan *et al.* 2013). These bacteria are endowed with the ability to become resistant to antibiotics through mutation or gene transfer (Aggarwal *et al.* 2013; Omar *et al.* 2013), which further leads to higher morbidity, prolonged length of stay, increased mortality, and costly healthcare as compared to antibiotic-susceptible microorganisms (Morales *et al.* 2012). Therefore, alternative antibacterial agents with diverse chemical structures as well as novel mechanisms of actions are urgently required to combat the new and re-emerging bacterial infections.

4.2 METHODOLOGY

4.2.1 Microorganisms and culture media

The microorganisms used in the current study can be categorised into two main groups, namely Gram-positive and Gram-negative bacteria as shown in Table 4.1. Thirty bacterial strains including American Type Culture Collection (ATCC) and clinical strains were procured from the Biosciences Laboratory, Faculty of Science, University of Nottingham Malaysia Campus, and the Bacteriology Unit, Department of Medical Microbiology and Immunology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) respectively (Appendix B1–B2). Table 4.1 also includes the types of culture media required for the growth of the respective bacteria.

TABLE 4.1 Types of microorganisms and culture media.

Microorganism	Bacterial strain	Culture medium
	ATCC strain	
Gram-positive bacteria	*Bacillus cereus* ATCC 10876	Tryptic soy broth (Difco Laboratories, USA)
	Bacillus subtilis ATCC 21332	
	Listeria monocytogenes ATCC 15313	Tryptic soy agar (HiMedia, India)
	Micrococcus luteus ATCC 10240	
	Proteus vulgaris ATCC 13315	
	Rhodococcus equi ATCC 33701	
	Staphylococcus aureus ATCC 11632	
	Staphylococcus epidermidis ATCC 12228	
	Streptococcus pyogenes ATCC 19615 (Group A *Streptococcus*, GAS)	
Gram-negative bacteria	*Citrobacter freundii* ATCC 22636	
	Escherichia coli ATCC 10536	
	Klebsiella pneumoniae ATCC 13883	
	Pseudomonas aeruginosa ATCC 10145	
	Salmonella enteritidis ATCC 13076	
	Salmonella typhimurium ATCC 14028	

TABLE 4.1 Types of microorganisms and culture media (continued).

Microorganism	Bacterial strain	Culture medium
	Clinical isolate	
Gram-positive bacteria	*Enterococcus faecalis*	Tryptic soy broth
	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Tryptic soy agar
	Methicillin-sensitive *Staphylococcus aureus* (MSSA)	
	Oxacillin-resistant coagulase-negative staphylococci (ORCNS)	
	Oxacillin-sensitive coagulase-negative staphylococci (OSCNS)	
	Streptococcus agalactiae (Group B *Streptococcus*, GBS)	
	Streptococcus pneumoniae	
Gram-negative bacteria	*Actinobacillus* sp.	
	Enterobacter sp.	
	Escherichia coli	
	Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-EC)	
	Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP)	
	Klebsiella sp.	
	Moraxella sp.	
	Serratia sp.	

4.2.2 Preparation of culture media

(a) Preparation of broth medium

Tryptic soy broth (TSB) was prepared by suspending 30 g of TSB powder in 1 L of sterile distilled water. The solution was mixed thoroughly and warmed slightly to completely dissolve the powder before dispensing into universal bottles. After autoclaving at 121°C for 15 min, the broth was allowed to cool down to room temperature before storing at 4°C until further use.

(b) Preparation of agar medium

Tryptic soy agar (TSA) was prepared by suspending 40 g of TSA powder in 1 L of sterile distilled water. The solution was mixed thoroughly and heated to boiling to dissolve the powder completely, followed by autoclaving at 121°C for 15 min. The autoclaved medium was allowed to cool down by immersing into a 45°C to 50°C water bath (Julabo, Germany) before pouring into sterile Petri dishes (Favorit, Malaysia) in laminar flow cabinet (Esco Micro, Malaysia). After pouring, the molten agar was allowed to solidify and dried for 30 minutes before covering the plates to prevent formation of water on the agar surface. The prepared agar medium was stored in a 4°C chiller until further use.

4.2.3 Maintenance and storage of stock cultures

(a) Preparation of plate cultures

The streak plate method was employed to obtain pure bacterial cultures. A sterile inoculating loop was dipped into the culture of bacteria and streaked in a pattern over the surface of the TSA plate. The inoculating loop was sterilised following each streak series. As the pattern was traced, bacteria were rubbed off the loop onto the medium. The last cells to be rubbed off the loop were far enough apart to grow into isolated colonies. Streaked plates were incubated at 35°C for 24 h.

(b) Preparation of broth cultures

The bacterial cell suspension was prepared by picking a single isolated colony from freshly streaked plate with a sterile inoculating loop and transferring into universal bottles containing sterile TSB. The prepared cell suspension was vortexed thoroughly and incubated at 35°C for 24 h.

(c) Preparation of glycerol stocks

The glycerol stock was prepared by transferring the bacterial cell suspension into cryovials containing a final concentration of 20% (v/v) of sterile glycerol (R & M Chemicals, UK). The prepared glycerol stock of bacteria was well-mixed before storing at –20°C for 24 h and subsequently at –80°C for long-term storage.

4.2.4 Kirby-Bauer disc diffusion assay

The antibacterial activities of crude extracts were evaluated against 30 ATCC and clinical strains using Kirby-Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2012), formerly known as National Committee for Clinical Laboratory Standards (NCCLS).

(a) Preparation of Mueller-Hinton agar

Mueller-Hinton agar (MHA) (Difco Laboratories, USA) was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, the agar medium was allowed to cool in a 45°C to 50°C water bath. The freshly prepared and cooled medium was poured into plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm, which corresponded to 25 mL to 30 mL of medium for plates with a diameter of 100 mm. The agar medium was allowed to cool further to room temperature, and unless the plates were used the same day, stored in a 2°C to 8°C refrigerator.

(b) Preparation of impregnated filter paper discs

Qualitative filter paper No. 1 (Whatman International Ltd., England) was used to prepare discs approximately 6 mm in diameter, which were sterilised by autoclaving at 121°C for 15 min. Sterile filter paper discs were impregnated with 10 µL of each crude extract (100 mg/mL) to give a final concentration of 1 mg/disc. Streptomycin sulphate (5 µg/disc) (Fisher BioReagents, China) and DMSO (R & M Chemicals, UK) were served as positive and negative controls respectively. Impregnated discs were left to dry under laminar flow cabinet overnight.

(c) Preparation of inoculum

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a universal bottle containing sterile TSB. The broth culture was incubated at 35°C for 2 h to 6 h until it achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard using a UV/Vis spectrophotometer (Biochrom Libra, UK) at 625 nm. The absorbance at 625 nm should be in the range of 0.08 to 0.13 for the 0.5 McFarland standard. This resulted in a suspension containing approximately 1×10^8 CFU/mL to 2×10^8 CFU/mL.

(d) Inoculation of test plates

Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the universal bottle above the fluid level to remove excess inoculum from the swab. The dried surface of a MHA plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 min to 5 min, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the impregnated discs.

(e) Application of discs to inoculated agar plates

The impregnated disc was placed individually using sterile forceps onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. Eight discs were placed in each plate. The plates were inverted and placed in an incubator (Binder, Germany) set to 35°C within 15 min after the discs were applied.

(f) Reading plates and interpreting results

After 16 h to 18 h of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimetre, using sliding callipers (American Scientific LLC, USA) or a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background and illuminated with reflected light. Eventually, the sizes of the zones of inhibition were interpreted.

4.2.5 Statistical analysis

Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

4.3 RESULTS AND DISCUSSION

Plants constitute a vast untapped source of medicines with great therapeutic values (Nakhuru *et al.* 2013). The prospects for the development of antibacterial drugs from medicinal plants appear to be rewarding as they can mitigate the adverse effects that are often associated with synthetic antibiotics (Majumdar and Parihar 2012; Sharma *et al.* 2014). In the present study, Kirby-Bauer disc diffusion assay was conducted to evaluate the antibacterial activities of crude extracts against ATCC and clinical strains. This qualitative method is extensively used for antibiotic susceptibility testing in which filter paper discs impregnated with antibacterial agents are applied on the inoculated agar plate (Hakonen *et al.* 2014). The efficacy of these agents can subsequently be determined by measuring the diameter of the zones of inhibition that resulting from their diffusion into the agar medium around the discs (Johnson *et al.* 2012).

The inhibitory effects of crude extracts on the growth of ATCC and clinical bacterial strains are depicted in Figure 4.1–4.4 (Appendix B3–B4). Among the crude extracts investigated, hexane and chloroform extracts of bark demonstrated potent antibacterial activities against ATCC and clinical strains with zones of inhibition ranging from 8.23±0.25 mm to 13.70±0.26 mm and 7.75±0.25 mm to 13.68±0.28 mm respectively. However, all extracts of leaves were found to be less effective in inhibiting the growth of the tested bacteria. This was in contrary to the studies of Gothandam *et al.* (2010) and Sowjanya *et al.* (2013), in which methanol extract of leaves (*Artabotrys uncinatus* and *Artabotrys hexapetalus*) displayed remarkable antibacterial activity with zones of inhibition ranging from 7 mm to 18 mm.

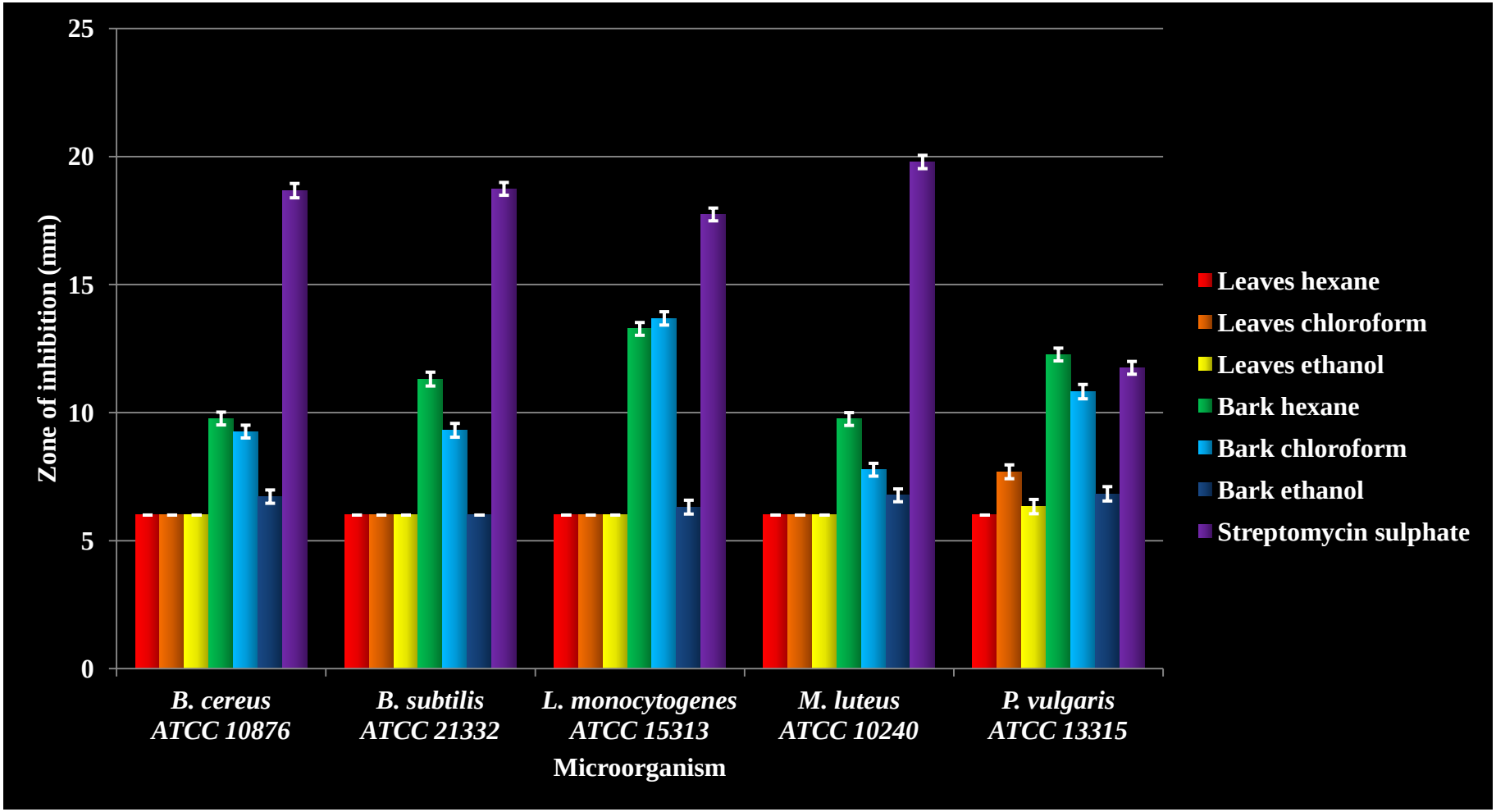


FIGURE 4.1 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against ATCC strains. Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

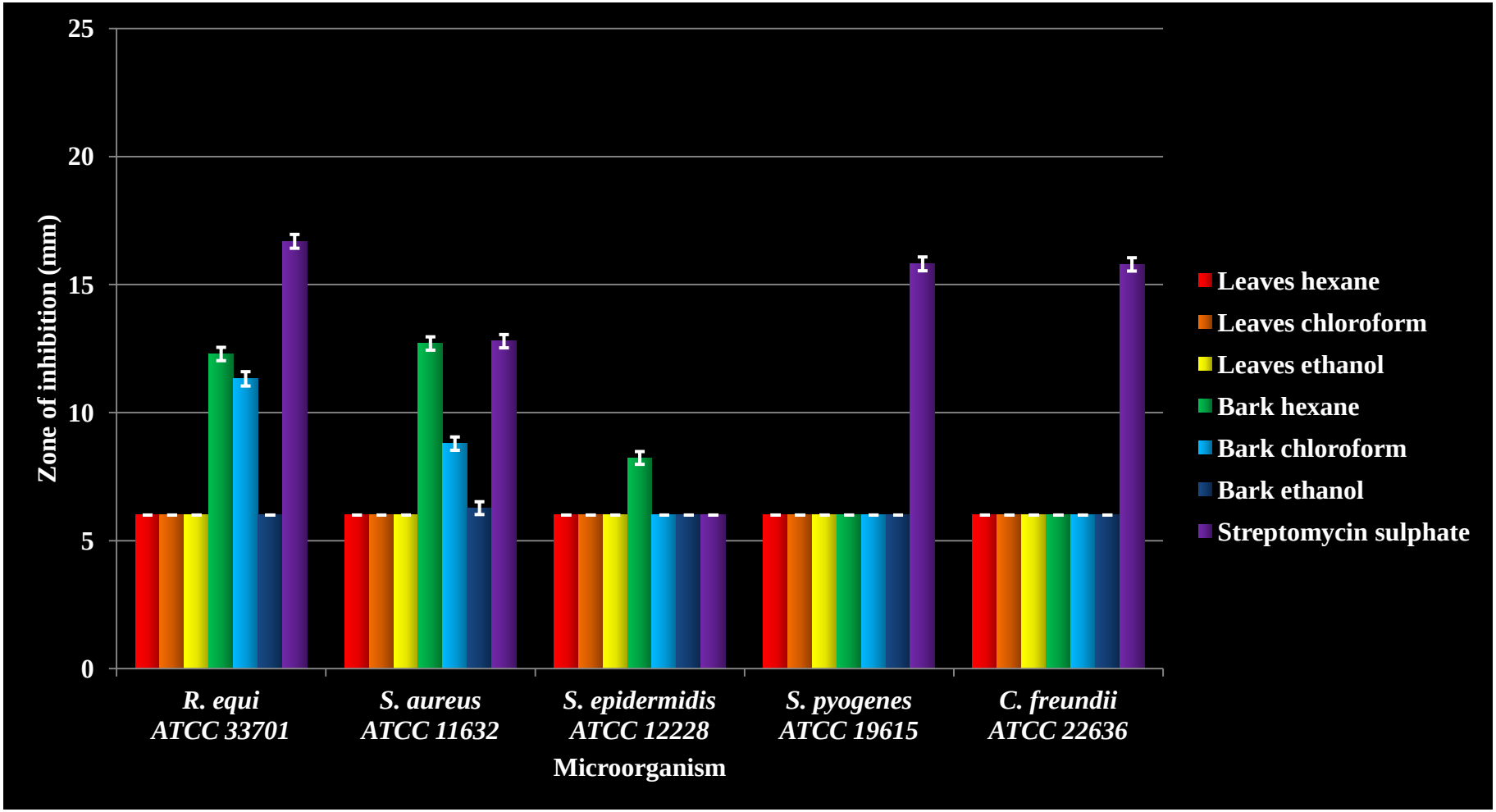


FIGURE 4.1 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against ATCC strains (continued). Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

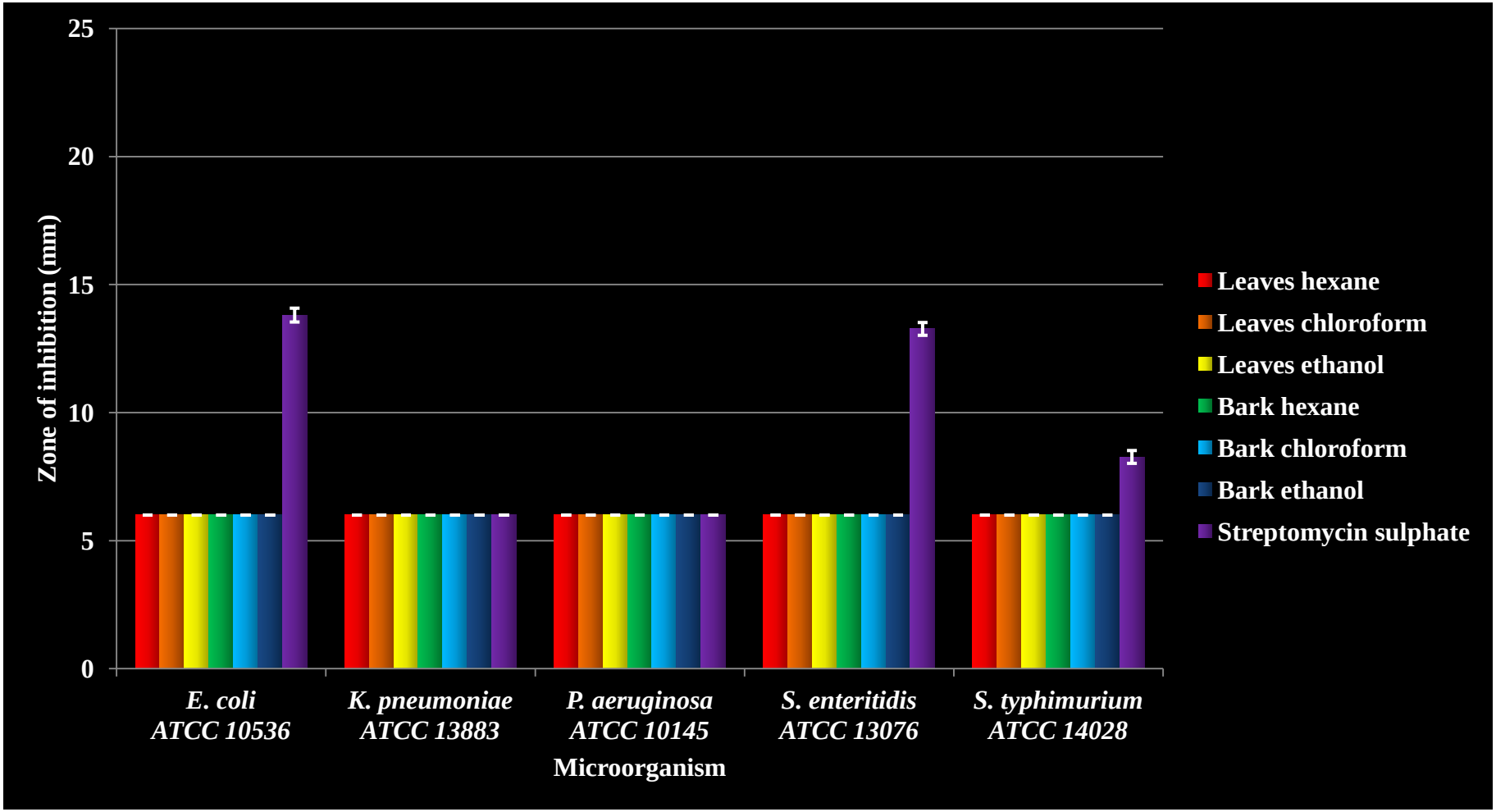


FIGURE 4.1 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against ATCC strains (continued). Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

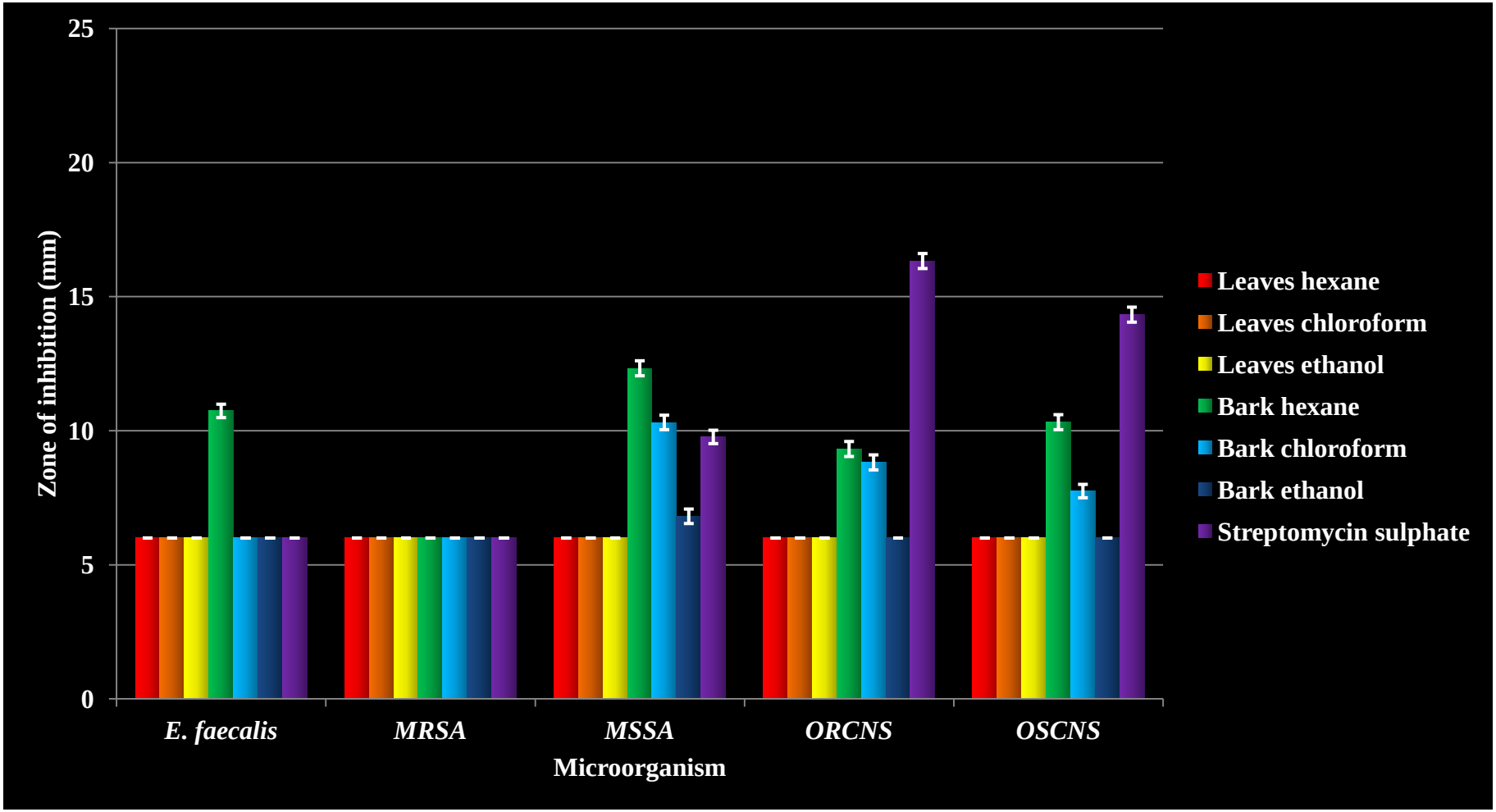


FIGURE 4.2 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against clinical isolates. Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

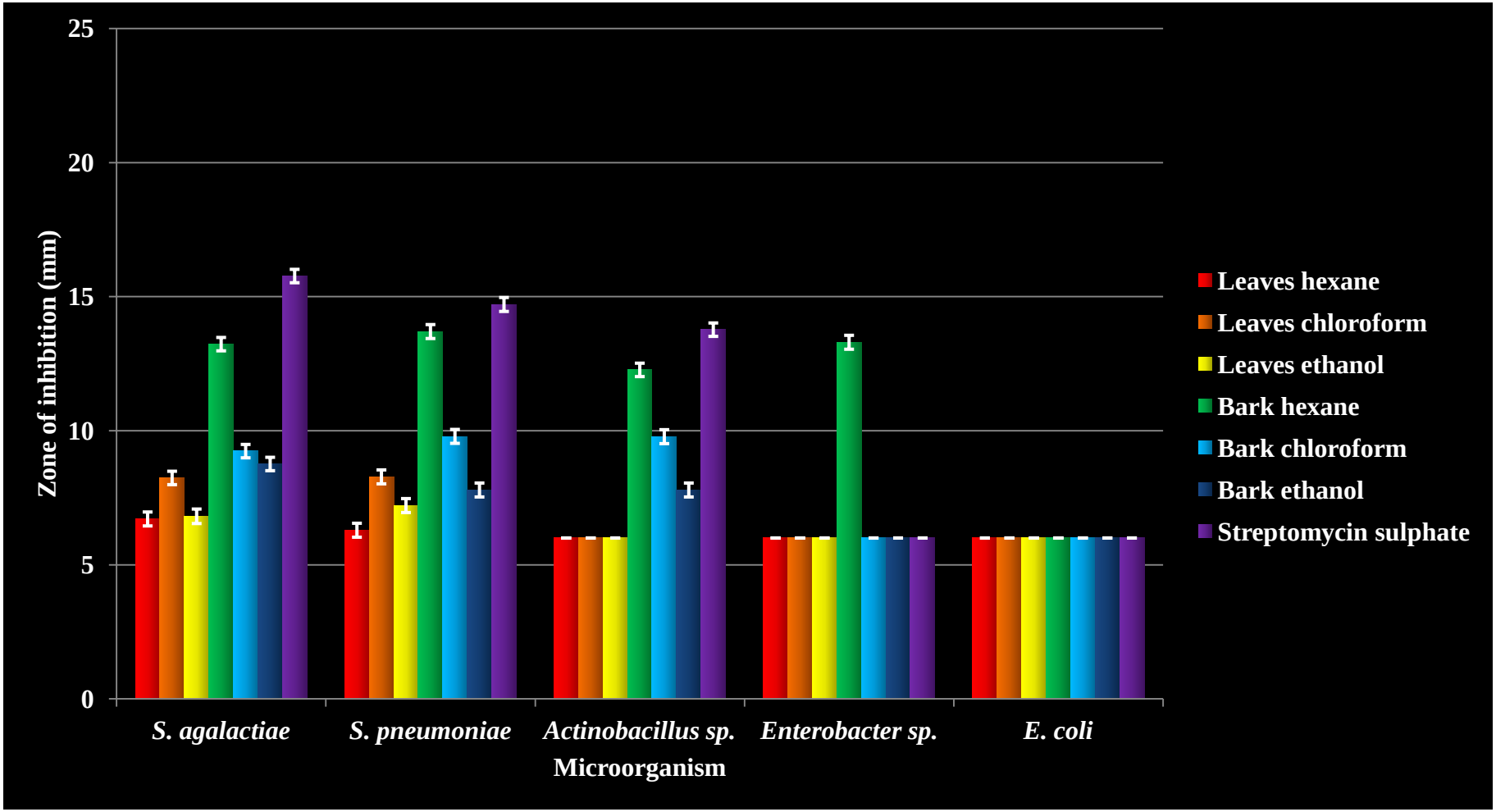


FIGURE 4.2 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against clinical isolates (continued). Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

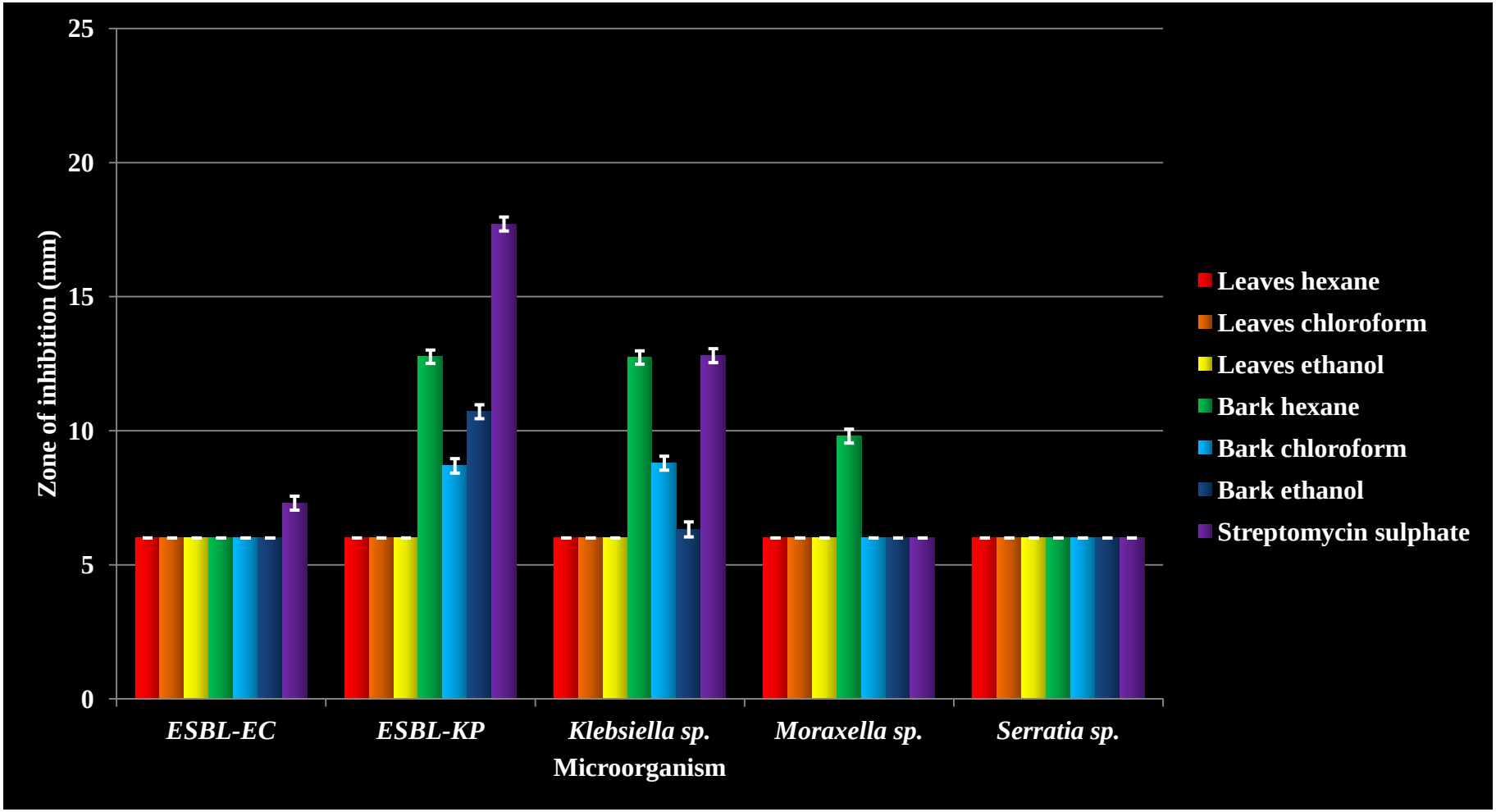


FIGURE 4.2 Antibacterial activities of crude extracts of *Artabotrys crassifolius* against clinical isolates (continued). Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

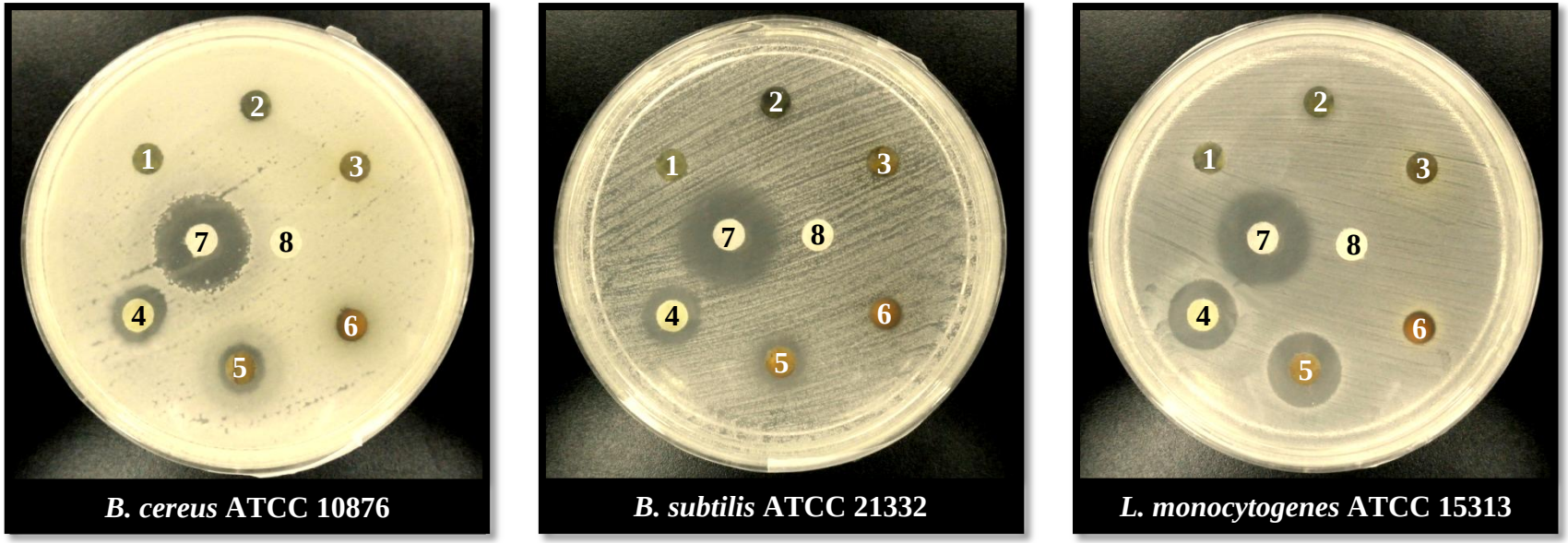


FIGURE 4.3 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against ATCC bacterial strains using Kirby-Bauer disc diffusion assay. The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

FIGURE 4.3 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against ATCC bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

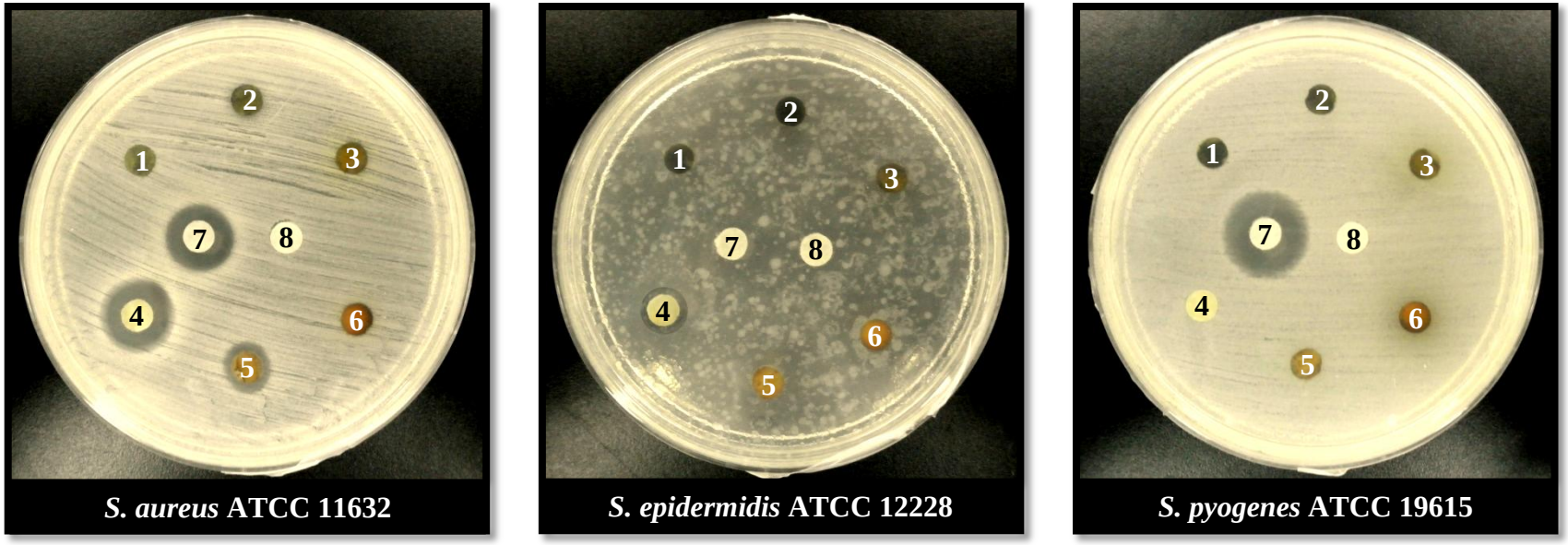


FIGURE 4.3 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against ATCC bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

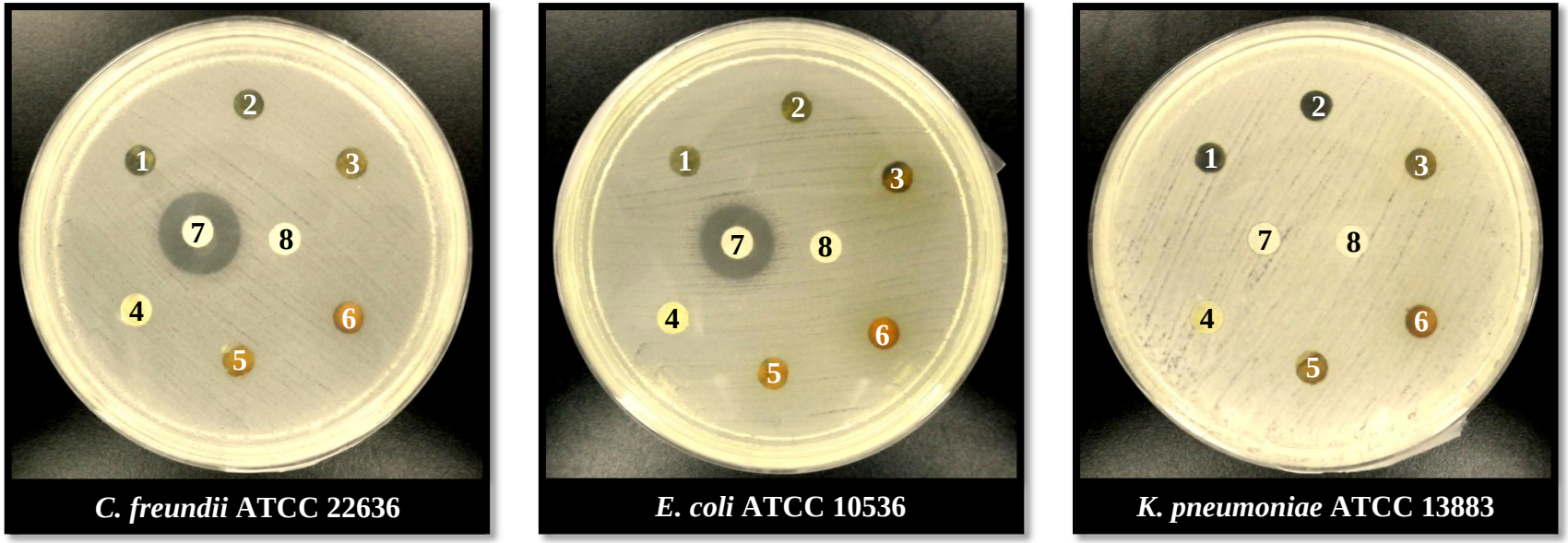


FIGURE 4.3 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against ATCC bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

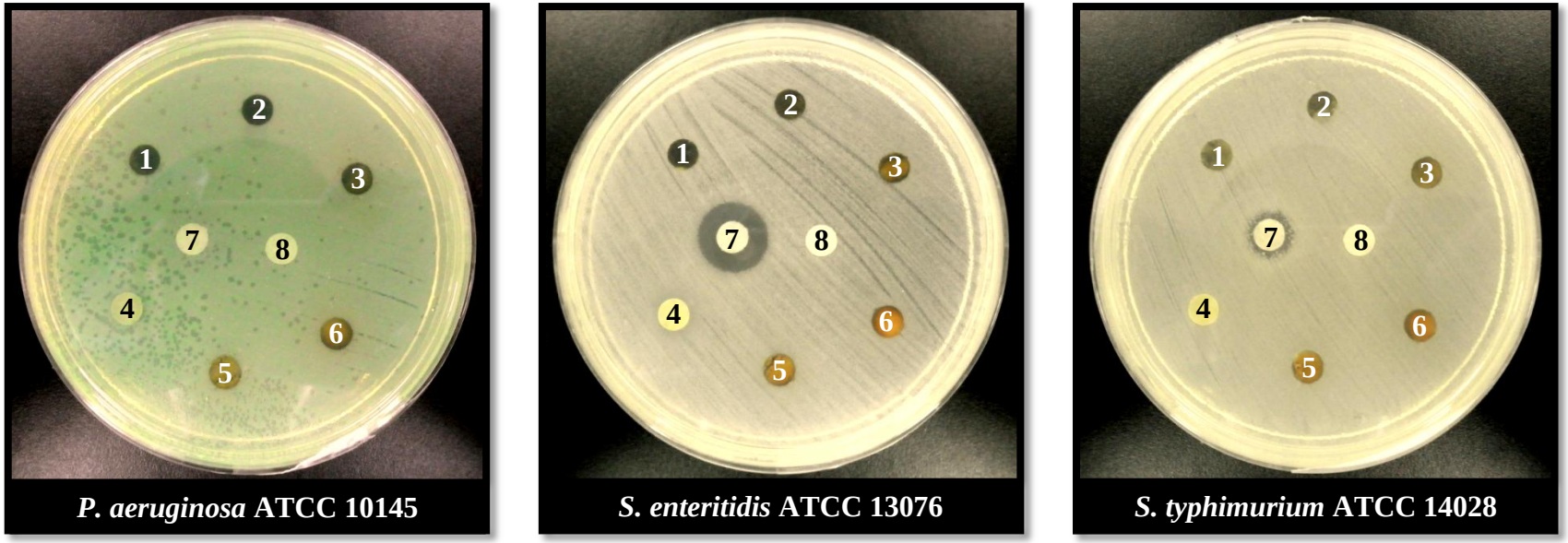


FIGURE 4.3 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against ATCC bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

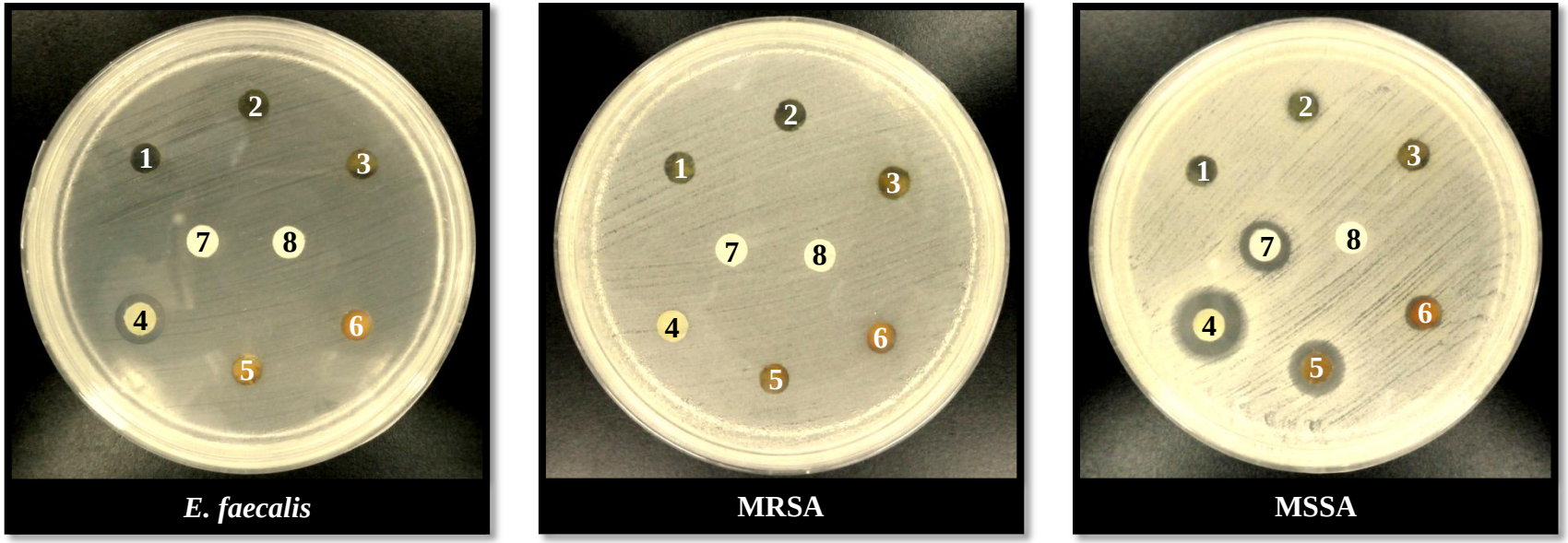


FIGURE 4.4 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical bacterial strains using Kirby-Bauer disc diffusion assay. The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

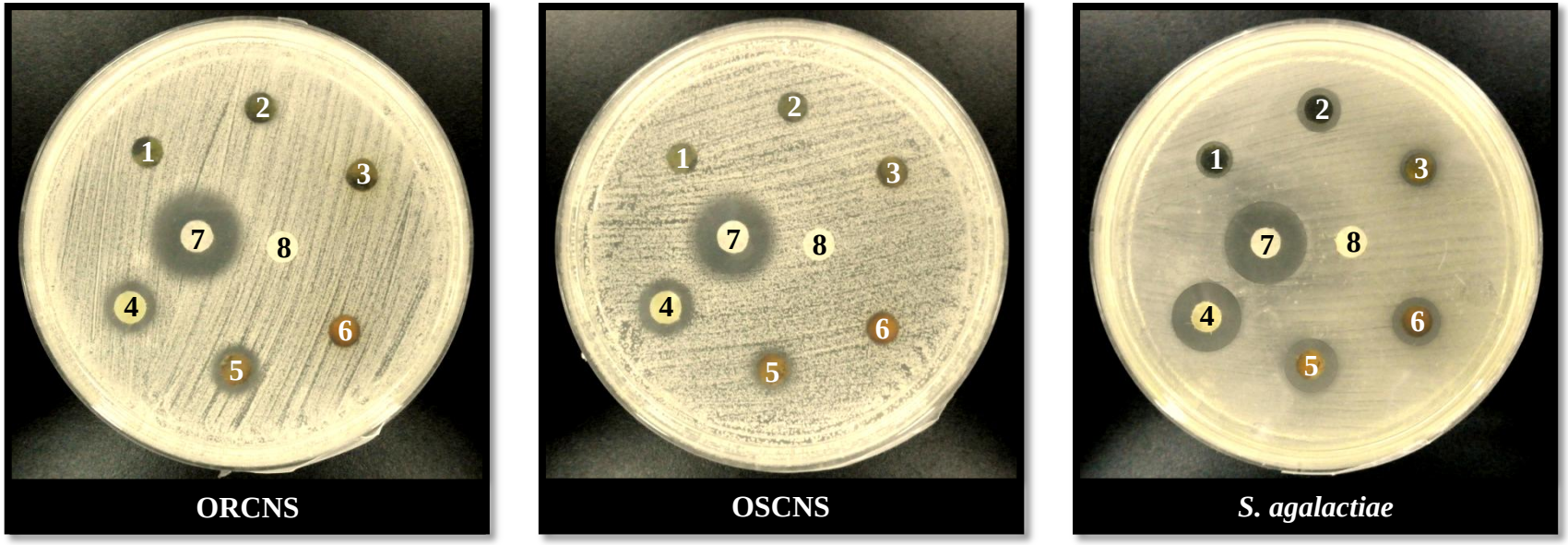


FIGURE 4.4 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

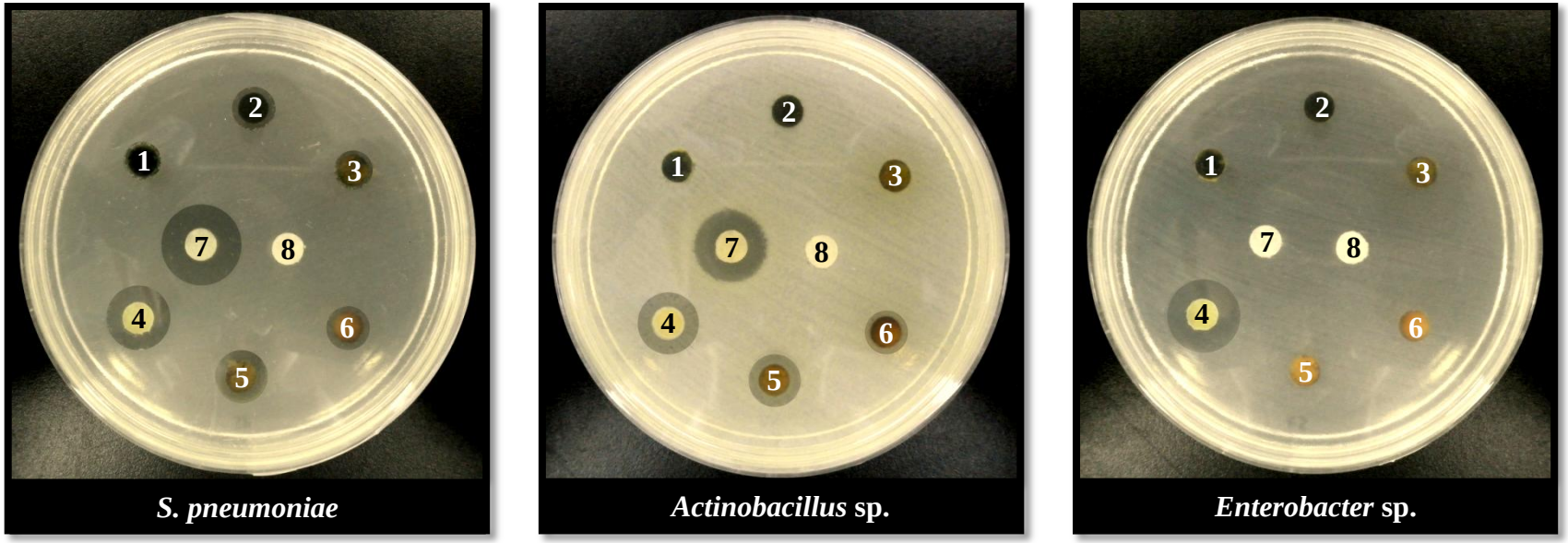


FIGURE 4.4 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

FIGURE 4.4 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

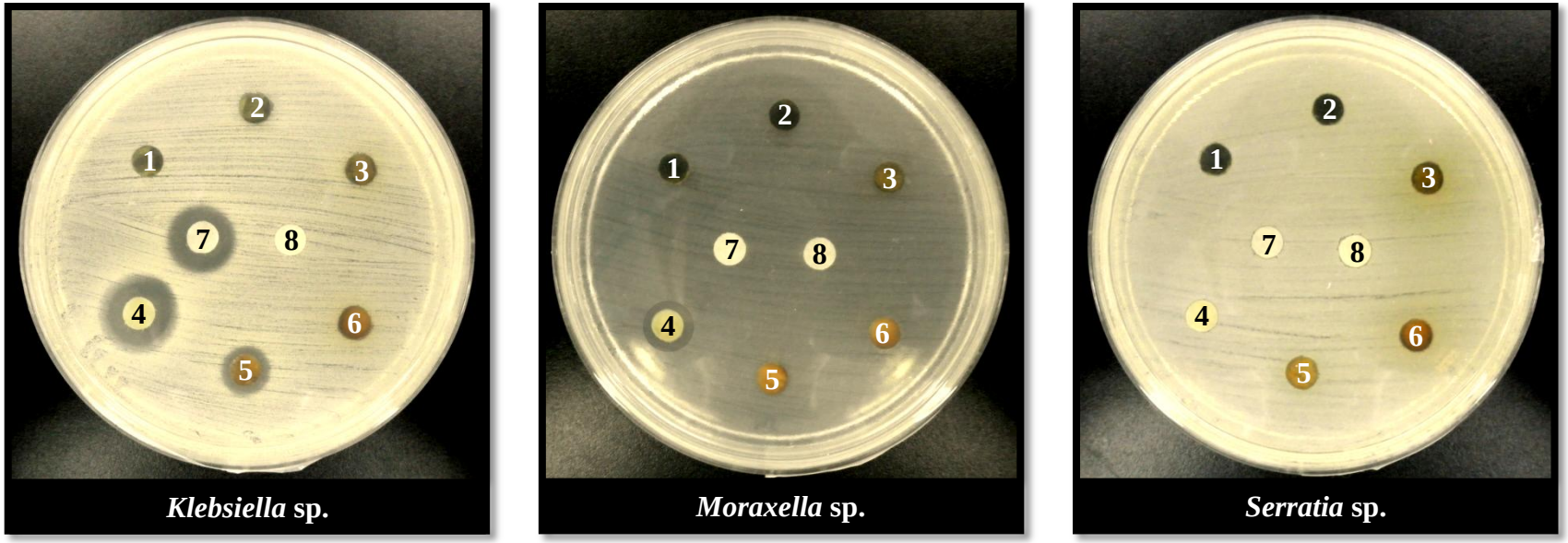


FIGURE 4.4 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical bacterial strains using Kirby-Bauer disc diffusion assay (continued). The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) streptomycin sulphate, and (8) DMSO.

Moreover, the positive control, streptomycin sulphate, created zones of inhibition ranging from 7.30±0.26 mm to 19.79±0.26 mm against all of the tested ATCC and clinical bacterial strains, with the exception of *S. epidermidis* ATCC 12228, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 10145, *E. faecalis*, MRSA, *Enterobacter* sp., *E. coli*, *Moraxella* sp. and *Serratia* sp.. In contrast, no inhibitory activity was observed in the negative control, DMSO. This implies that DMSO, the solvent used for the reconstitution of crude extracts, does not influence the susceptibility of the ATCC and clinical bacterial strains to the corresponding extracts.

Considering the zones of inhibition produced by crude extracts, *S. pneumoniae* and *S. agalactiae* were found to be the most sensitive bacteria, followed by *P. vulgaris* ATCC 13315, *L. monocytogenes* ATCC 15313, ESBL-KP, *Actinobacillus* sp., MSSA, *Klebsiella* sp., *S. aureus* ATCC 11632, *B. cereus* ATCC 10876, *M. luteus* ATCC 10240, *R. equi* ATCC 33701, *B. subtilis* ATCC 21332, ORCNS, OSCNS, *Enterobacter* sp., *E. faecalis*, *Moraxella* sp. and *S. epidermidis* ATCC 12228, with *S. pyogenes* ATCC 19615, *C. freundii* ATCC 22636, *E. coli* ATCC 10536, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 10145, *S. enteritidis* ATCC 13076, *S. typhimurium* ATCC 14028, MRSA, *E. coli*, ESBL-EC and *Serratia* sp. being the least susceptible to crude extracts. This suggests that the respective extracts may be more effective in inhibiting the growth of Gram-positive bacteria.

In general, Gram-negative bacteria are more resistant to plant-based antibacterial agents in comparison to Gram-positive bacteria (Biswas *et al.* 2013). The susceptibility differences between these two groups of bacteria can be attributed to their distinct cell wall structures (Singariya *et al.* 2012).

Gram-negative bacteria are characterised by an outer membrane that encloses a comparatively thin layer of peptidoglycan (Silhavy *et al.* 2010). The outer membrane has a phospholipid-rich inner leaflet of similar composition to the cytoplasmic membrane, while the outer leaflet facing the extracellular environment is composed primarily of lipopolysaccharides (LPS) (Clifton *et al.* 2013), which provide an effective permeability barrier against hydrophobic compounds (Touw *et al.* 2010). In addition to these structural components, the asymmetric lipid bilayer also contains porins, which form water-filled channels that selectively facilitate the passage of hydrophilic compounds based on their molecular weight and ionic charge (Raghavendra 2011; Van Dam *et al.* 2014).

On the other hand, Gram-positive bacteria possess a relatively thick peptidoglycan layer with lipoteichoic acids (LTA) anchored to the cytoplasmic membrane (Karlsson *et al.* 2002). Nonetheless, they are devoid of a highly impermeable outer membrane, making them more susceptible to antibacterial compounds (Ipharraguerre and Clark 2003). These chemical composition and organisation of bacterial cell wall may rationalise the variations in the sensitivity of the ATCC and clinical bacterial strains to the crude extracts.

With regard to the phytochemical screening of crude extracts (Tan *et al.* 2013), the occurrence of alkaloids, cardiac glycosides and terpenoids in hexane and chloroform extracts of bark may explain their superior activity as compared to the other crude extracts studied. This warrants further isolation and characterisation of the potentially active principles from the respective crude extracts.

4.4 CONCLUSION

Assessment of the *in vitro* antibacterial activity of *Artabotrys crassifolius* revealed that hexane and chloroform extracts of bark may be an important source of novel antibacterial compounds in view of their prominent inhibitory activities particularly against Gram-positive bacteria. Hence, further studies are required to isolate and characterise the bioactive compounds responsible for the observed antibacterial properties of *Artabotrys crassifolius*.

CHAPTER V

IN VITRO ANTIFUNGAL ACTIVITY OF *ARTABOTRYS CRASSIFOLIUS*

5.1 INTRODUCTION

Candida is a genus of yeast-like fungi that reproduce by budding or fission (Vazquez and Sobel 2011). Many species of this genus are harmless commensals that exist as part of the normal human microflora of the skin, oral cavity, respiratory, gastrointestinal and genitourinary tracts (Shamim *et al.* 2004; Herrera *et al.* 2010). Nonetheless, certain *Candida* species are responsible for causing opportunistic mycoses ranging from superficial mucosal infections to life-threatening systemic diseases, predominantly in immunocompromised patients with cancer, human immunodeficiency virus (HIV) infection or organ transplantation (Shin *et al.* 2005).

In spite of the availability of wide array of antifungal agents, candidiasis remains as the fourth leading cause of nosocomial infections with an unacceptably high mortality rate (Santos *et al.* 2008; Papon *et al.* 2013). More crucially, majority of the clinically used antifungal drugs are associated with various drawbacks including high toxicity (Omran and Esmailzadeh 2009), limited efficacy (Supreetha *et al.* 2011), narrow spectrum of activity (Moussa *et al.* 2011) as well as poor tolerability (Ishida *et al.* 2011). Their widespread usage has also led to the rapid development of drug resistant strains during the course of therapy (Nayak *et al.* 2010). Consequently, it is imperative to search for alternative strategies for the effective management of *Candida* infections.

5.2 METHODOLOGY

5.2.1 Microorganisms and culture media

The microorganisms used in the present study were clinical isolates as shown in Table 5.1. Four fungal strains were procured from the Mycology Unit, Department of Medical Microbiology and Immunology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) (Appendix C1). Table 5.1 also includes the types of culture media required for the growth of the respective fungi.

TABLE 5.1 Types of microorganisms and culture media.

Microorganism	Fungal strain	Culture medium
	Clinical isolate	
Yeasts	*Candida albicans*	Potato dextrose broth (EMD Chemicals Inc., Germany)
	Candida glabrata	
	Candida parapsilosis	Potato dextrose agar (Merck, Germany)
	Candida tropicalis	

5.2.2 Preparation of culture media

(a) Preparation of broth medium

Potato dextrose broth (PDB) was prepared by suspending 24 g of PDB powder in 1 L of sterile distilled water. The solution was mixed thoroughly and heated to boiling with frequent agitation to dissolve the powder completely before dispensing into universal bottles. After autoclaving at 121°C for 15 min, the broth was allowed to cool down to room temperature before storing at 4°C until further use.

(b) Preparation of agar medium

Potato dextrose agar (PDA) was prepared by suspending 39 g of PDA powder in 1 L of sterile distilled water. The solution was mixed thoroughly and heated in a boiling water bath (Julabo, Germany) or in a current of steam to dissolve the powder completely, followed by autoclaving at 121°C for 15 min. The autoclaved medium was allowed to cool down by immersing into a 45°C to 50°C water bath before pouring into sterile Petri dishes (Favorit, Malaysia) in laminar flow cabinet (Esco Micro, Malaysia). After pouring, the molten agar was allowed to solidify and dried for 30 minutes before covering the plates to prevent formation of water on the agar surface. The prepared agar medium was stored in a 4°C chiller until further use.

5.2.3 Maintenance and storage of stock cultures

(a) Preparation of plate cultures

The streak plate method was employed to obtain pure yeast cultures. A sterile inoculating loop was dipped into the culture of yeasts and streaked in a pattern over the surface of the PDA plate. The inoculating loop was sterilised following each streak series. As the pattern was traced, yeasts were rubbed off the loop onto the medium. The last cells to be rubbed off the loop were far enough apart to grow into isolated colonies. Streaked plates were incubated at 35°C for 24 h.

(b) Preparation of broth cultures

The yeast cell suspension was prepared by picking a single isolated colony from freshly streaked plate with a sterile inoculating loop and transferring into universal bottles containing sterile PDB. The prepared cell suspension was vortexed thoroughly and incubated at 35°C for 24 h.

(c) Preparation of glycerol stocks

The glycerol stock was prepared by transferring the yeast cell suspension into cryovials containing a final concentration of 20% (v/v) of sterile glycerol (R & M Chemicals, UK). The prepared glycerol stock of yeasts was well-mixed before storing at –20°C for 24 h and subsequently at –80°C for long-term storage.

5.2.4 Kirby-Bauer disc diffusion assay

The antifungal activities of crude extracts were examined against 4 clinical isolates using Kirby-Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2004), formerly known as National Committee for Clinical Laboratory Standards (NCCLS).

(a) Preparation of supplemented Mueller-Hinton agar

Mueller-Hinton agar (Difco Laboratories, USA) was prepared from a commercially available dehydrated base according to the manufacturer's instructions, and supplemented with 2% (w/v) of D(+)-glucose anhydrous (Systerm, Malaysia) and 0.5 µg/mL of methylene blue (R & M Chemicals, UK) (MH-GMB). Immediately after autoclaving, the agar medium was allowed to cool in a 45°C to 50°C water bath. The freshly prepared and cooled medium was poured into plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm, which corresponded to 25 mL to 30 mL of medium for plates with a diameter of 100 mm. The agar medium was allowed to cool further to room temperature, and unless the plates were used the same day, stored in a 2°C to 8°C refrigerator.

(b) Preparation of impregnated filter paper discs

Qualitative filter paper No. 1 (Whatman International Ltd., England) was used to prepare discs approximately 6 mm in diameter, which were sterilised by autoclaving at 121°C for 15 min. Sterile filter paper discs were impregnated with 10 µL of each crude extract (100 mg/mL) to give a final concentration of 1 mg/disc. Amphotericin B (1 µg/disc) (Sigma-Aldrich, USA) and DMSO (R & M Chemicals, UK) were served as positive and negative controls respectively. Impregnated discs were left to dry under laminar flow cabinet overnight.

(c) Preparation of inoculum

Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24 h-old culture grown on PDA. Colonies were suspended in 5 mL of sterile saline. The resulting suspension was vortexed for 15 s and the turbidity was adjusted either visually or with a UV/Vis spectrophotometer (Biochrom Libra, UK) by adding sufficient sterile saline or more colonies to adjust the transmittance to that produced by a 0.5 McFarland standard at 530 nm wavelength. The absorbance at 530 nm should be in the range of 0.12 to 0.15 for the 0.5 McFarland standard. This yielded a yeast stock suspension of 1×10^6 cells/mL to 5×10^6 cells/mL.

(d) Inoculation of test plates

Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly against the inside wall of the universal bottle above the fluid level to remove excess inoculum from the swab. The dried surface of a MH-GMB agar plate was inoculated by evenly streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 min to 5 min, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the impregnated discs.

(e) Application of discs to inoculated agar plates

The impregnated disc was placed individually using sterile forceps onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. Eight discs were placed in each plate. The plates were inverted and placed in an incubator (Binder, Germany) set to 35°C within 15 min after the discs were applied.

(f) Reading plates and interpreting results

After 20 h to 24 h of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimetre at the point at which there was a prominent reduction in growth, using sliding callipers (American Scientific LLC, USA) or a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background and illuminated with reflected light. Eventually, the sizes of the zones of inhibition were interpreted.

5.2.5 Statistical analysis

Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

5.3 RESULTS AND DISCUSSION

Plants have received considerable research attention from the scientific community as potential candidates for the development of antifungal drugs (Ferreira *et al.* 2013). The utilisation of medicinal plants appears to be an alternative to synthetic antibiotics in the prevention and treatment of *Candida* infections because they are relatively safe, easily accessible as well as inexpensive (Maharajan *et al.* 2012; Doddanna *et al.* 2013). In the current study, Kirby-Bauer disc diffusion assay was performed to examine the antifungal activities of crude extracts against clinical isolates. This qualitative method is widely employed for antibiotic susceptibility testing in which filter paper discs impregnated with antifungal agents are applied on the inoculated agar plate (Hakonen *et al.* 2014). The efficacy of these agents can subsequently be determined by measuring the diameter of the zones of inhibition that resulting from their diffusion into the agar medium around the discs (Johnson *et al.* 2012).

The inhibitory effects of crude extracts on the growth of clinical fungal strains are illustrated in Figure 5.1–5.2 (Appendix C2). All the crude extracts were found to be devoid of antifungal activity except for hexane extract of bark which was able to inhibit the growth of the tested *Candida* species with zones of inhibition ranging from 7.81±0.27 mm to 9.77±0.25 mm. Different observation was reported by Sowjanya *et al.* (2013), in which methanol extract of leaves (*Artabotrys hexapetalus*) demonstrated strong antifungal activity against *C. albicans* and *C. rugosa* with zones of inhibition of 14 mm and 13 mm respectively.

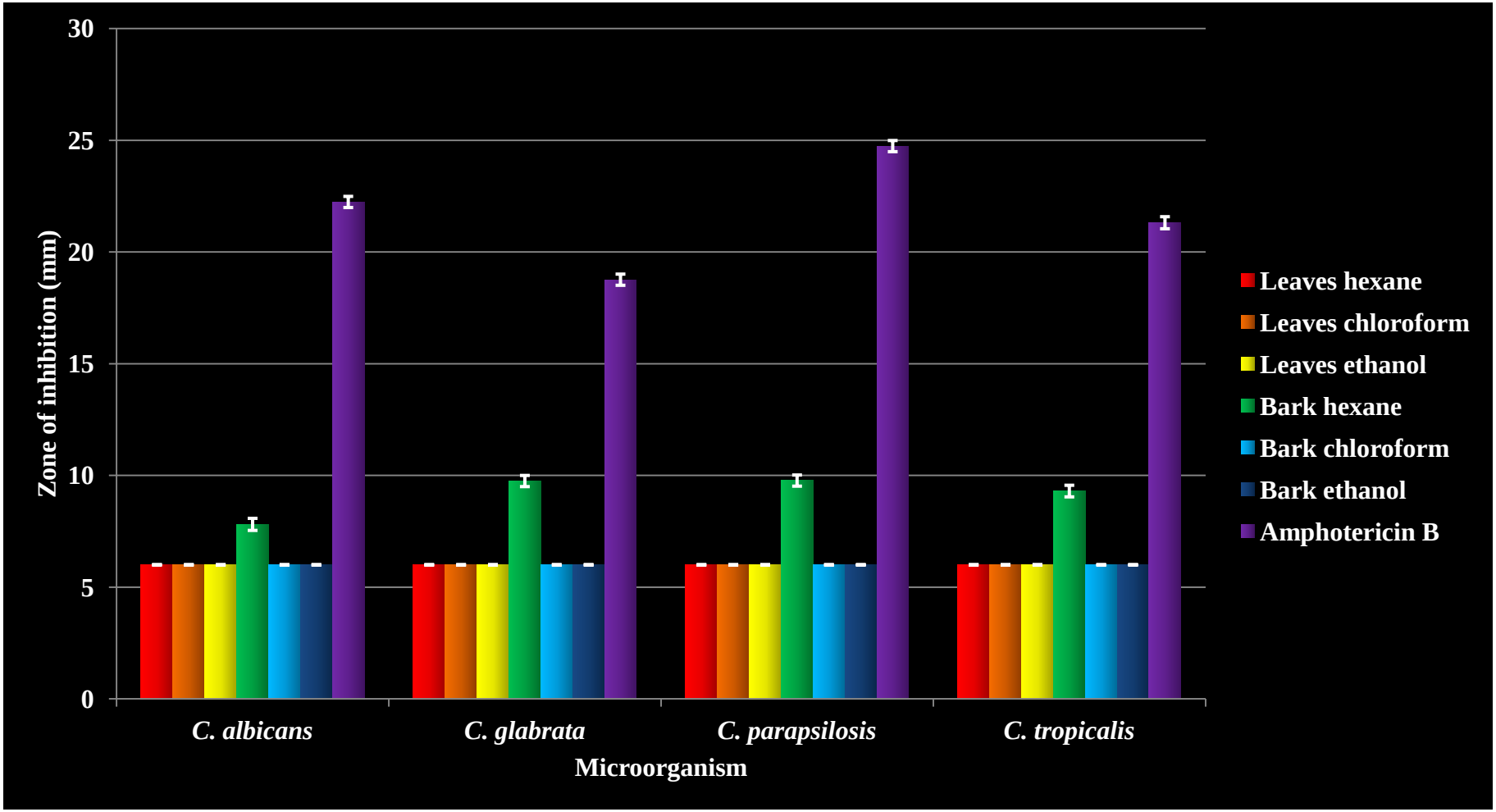


FIGURE 5.1 Antifungal activities of crude extracts of *Artabotrys crassifolius* against clinical isolates. Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

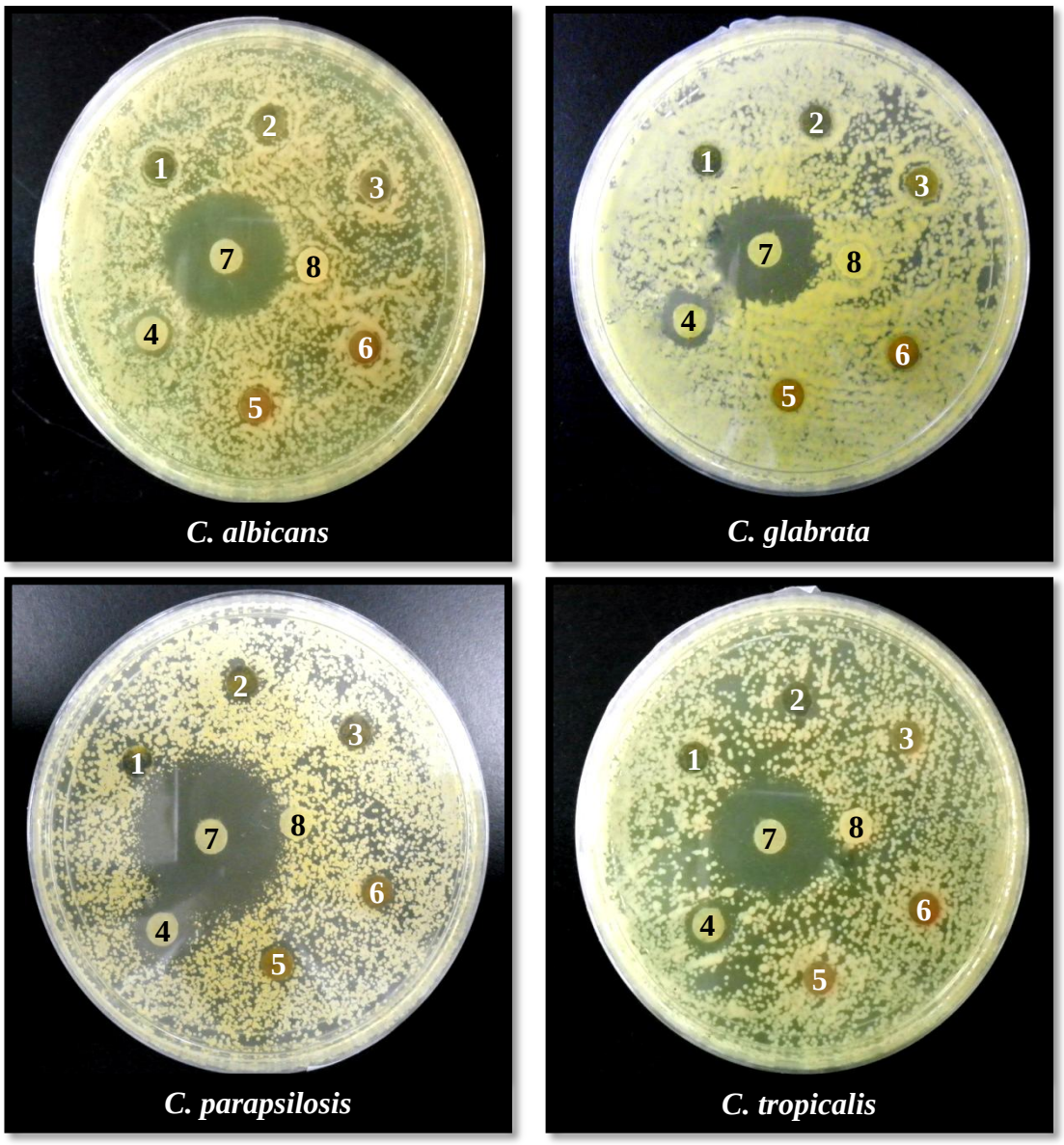


FIGURE 5.2 Zone of inhibition of crude extracts of *Artabotrys crassifolius* against clinical fungal strains using Kirby-Bauer disc diffusion assay. The numbers indicate: (1) leaves hexane, (2) leaves chloroform, (3) leaves ethanol, (4) bark hexane, (5) bark chloroform, (6) bark ethanol, (7) amphotericin B, and (8) DMSO.

Furthermore, the positive control, amphotericin B, produced zones of inhibition ranging from 18.76±0.25 mm to 24.74±0.25 mm against the tested *Candida* species. On the contrary, no inhibitory activity was detected in the negative control, DMSO. This implies that DMSO, the solvent used for the reconstitution of crude extracts, does not affect the susceptibility of the clinical fungal strains to the respective extracts.

Candida albicans is the most commonly isolated etiologic agent of candidiasis (Jabra-Rizk *et al.* 2004). Nevertheless, there has been a significant epidemiological shift in the species of *Candida* causing nosocomial candidemia, with the emergence of non-*albicans Candida* species, particularly those exhibiting reduced susceptibility or intrinsic resistance to antifungal drugs (Bruder-Nascimento *et al.* 2010; Eggimann *et al.* 2012). The emerging species of clinical importance include *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* (Kemoi *et al.* 2013). Among the *Candida* species evaluated in the present study, *C. parapsilosis* was shown to be the most sensitive species, followed by *C. glabrata* and *C. tropicalis*, with *C. albicans* being the least susceptible to hexane extract of bark. This suggests that the corresponding extract may be more effective in inhibiting the growth of non-*albicans Candida* species.

With regard to the phytochemical analysis of crude extracts (Tan *et al.* 2013), the occurrence of alkaloids, cardiac glycosides and terpenoids in hexane extract of bark may explain its superior activity as compared to the other crude extracts tested. This warrants further isolation and characterisation of the potentially active principles from the respective crude extract.

5.4 CONCLUSION

Investigation of the *in vitro* antifungal activity of *Artabotrys crassifolius* revealed that hexane extract of bark may be an important source of novel antifungal compounds in consideration of its prominent inhibitory activity predominantly against non-*albicans Candida* species. Therefore, further studies are required to isolate and characterise the bioactive compounds responsible for the observed antifungal properties of *Artabotrys crassifolius*.

CHAPTER VI

IN VITRO ANTICANCER EFFECT OF *ARTABOTRYS CRASSIFOLIUS*

6.1 INTRODUCTION

Cancer is a group of diseases characterised by uncontrolled growth and spread of abnormal cells, which can lead to death if left untreated (Goyal 2012). The etiology of cancer can be associated with both external factors, including tobacco, infectious organisms, chemicals and radiation, as well as internal factors, such as inherited mutations, hormones, immune conditions and mutations occurring from metabolism (Sandeep *et al.* 2012). These causal factors may act synergistically or sequentially to initiate or promote carcinogenesis (Madan and Esmaeili 2012).

Despite the considerable progress made over the last few decades in oncology research and treatment, cancer remains as one of the foremost causes of morbidity and mortality worldwide, with 12.7 million new cases and 7.6 million deaths in 2008 (Msyamboza *et al.* 2012). More significantly, the most common cancer treatments are restricted to surgery, radiation and chemotherapy (Topcul and Cetin 2013), which are severely fraught with challenges concerned with adverse side effects of drugs (Jiang *et al.* 2010) due to their non-specific systemic distribution (Drabu *et al.* 2010), inadequate drug concentrations reaching the tumour (Wang *et al.* 2009), intolerable toxicity (Jeyaraj *et al.* 2013), and development of multiple drug resistance acquired upon repeated chemotherapeutic cycles (Shahbazi *et al.* 2012). Hence, there is clearly a need for novel chemotherapeutic agents with enhanced potency and specificity.

6.2 METHODOLOGY

6.2.1 Cell lines and culture media

The cell lines used in the current study were derived from human carcinoma as shown in Table 6.1. Three human carcinoma cell lines were procured from the Centre for Biomolecular Sciences, University of Nottingham UK Campus.

TABLE 6.1 Types of human cell lines.

Human cell line	
Origin	Designation
Breast carcinoma	MCF-7 (estrogen receptor-positive, ER+)
Breast carcinoma	MDA-468 (estrogen receptor-negative, ER–)
Colorectal carcinoma	HCT-116

Each cell line was routinely maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich, USA) supplemented with 2 mM of L-glutamine (Sigma-Aldrich, USA) and 10% (v/v) of foetal bovine serum (FBS) (Sigma-Aldrich, USA) at 37°C in a humidified 5% (v/v) of CO_2 incubator (Binder, Germany), and subcultured twice weekly to maintain continuous logarithmic growth.

6.2.2 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The anticancer effects of crude extracts were investigated against human breast and colorectal carcinoma cell lines using MTT assay according to the methods of Vasselin *et al.* (2006) and Bradshaw *et al.* (2008). Each cell line was seeded in 96-well microtiter plates (Jet Biofil, China) at a density of 5×10^3 cells/well, and allowed to adhere for 24 h before crude extracts were introduced. Serial dilutions of crude extracts (final concentrations ranging from 6.25 µg/mL to 200 µg/mL) were prepared in medium immediately prior to assay. Viable cell masses at the time of crude extract addition (time zero) and following 72 h exposure were determined by cell-mediated reduction of MTT. A final concentration of 400 µg/mL of MTT (Sigma-Aldrich, USA) was added to each well, and plates were incubated at 37°C for 4 h to allow reduction of MTT by viable cells to an insoluble formazan product. The well supernatant was subsequently aspirated and the cellular formazan was solubilised by addition of DMSO (R & M Chemicals, UK) and glycine buffer (pH 10.5) (Sigma-Aldrich, USA) in a ratio of 4:1 (v/v). Quercetin (Sigma-Aldrich, Germany) was used as positive control. Eventually, the absorbance was read at 550 nm using an Anthos Labtec systems plate reader as a measure of cell viability.

Using the eight absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of crude extracts at the six concentration levels (Ti)], the percentage growth was calculated at each of the crude extract concentration levels (Noolvi *et al.* 2011). Percentage growth inhibition was calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$

$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz$$

Three dose response parameters were calculated for each crude extract. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which was the crude extract concentration resulting in a 50% reduction in the net cell growth during the incubation. The crude extract concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of crude extract resulting in a 50% reduction of initial cells at the end of the treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz)] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested (Mayer and Bracher 2011).

6.2.3 Statistical analysis

Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

6.3 RESULTS AND DISCUSSION

Plants have an almost unlimited capacity to synthesize diverse secondary metabolites that attract researchers and scientists in the quest for new chemotherapeutics (Talib and Mahasneh 2010). The continuing exploration for novel chemical classes of anticancer agents in medicinal plants is one of the realistic and promising approaches for cancer prevention (Vijayarathna and Sasidharan 2012).

In the present study, MTT assay was conducted to investigate the anticancer effects of crude extracts on the growth of human breast and colorectal carcinoma cell lines. This colourimetric method is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate (MTT) into an insoluble, purple coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the cell viability (Mattana *et al.* 2012).

According to the American National Cancer Institute (NCI), crude extracts could be considered as active for a GI_{50} value of less than 20 µg/mL (Hashim *et al.* 2012). Based on the NCI criterion, chloroform extract of bark was highly active against all of the tested carcinoma cell lines with GI_{50} values ranging from 4.23 µg/mL to 9.45 µg/mL (Figure 6.1–6.3; Appendix D). Furthermore, hexane extract of bark potently inhibited the growth of MDA-468 breast and HCT-116 colorectal carcinoma cell lines with respective GI_{50} values of 6.10 µg/mL and 16.45 µg/mL. This indicates that the non-polar active principles present in the bark may be responsible for the anticancer activity of this plant.

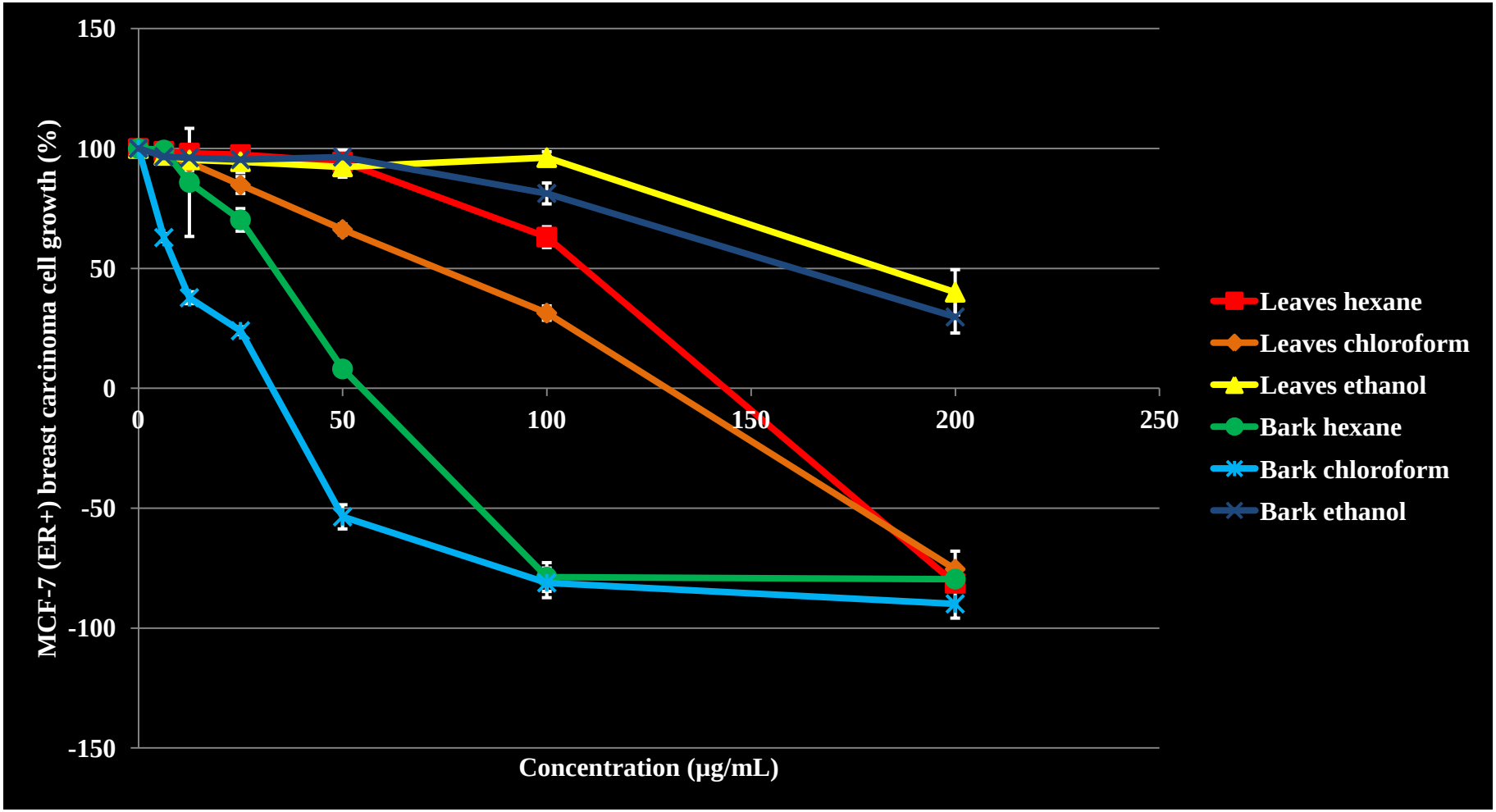


FIGURE 6.1 Anticancer effects of crude extracts of *Artabotrys crassifolius* against MCF-7 (ER+) breast carcinoma cell line. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

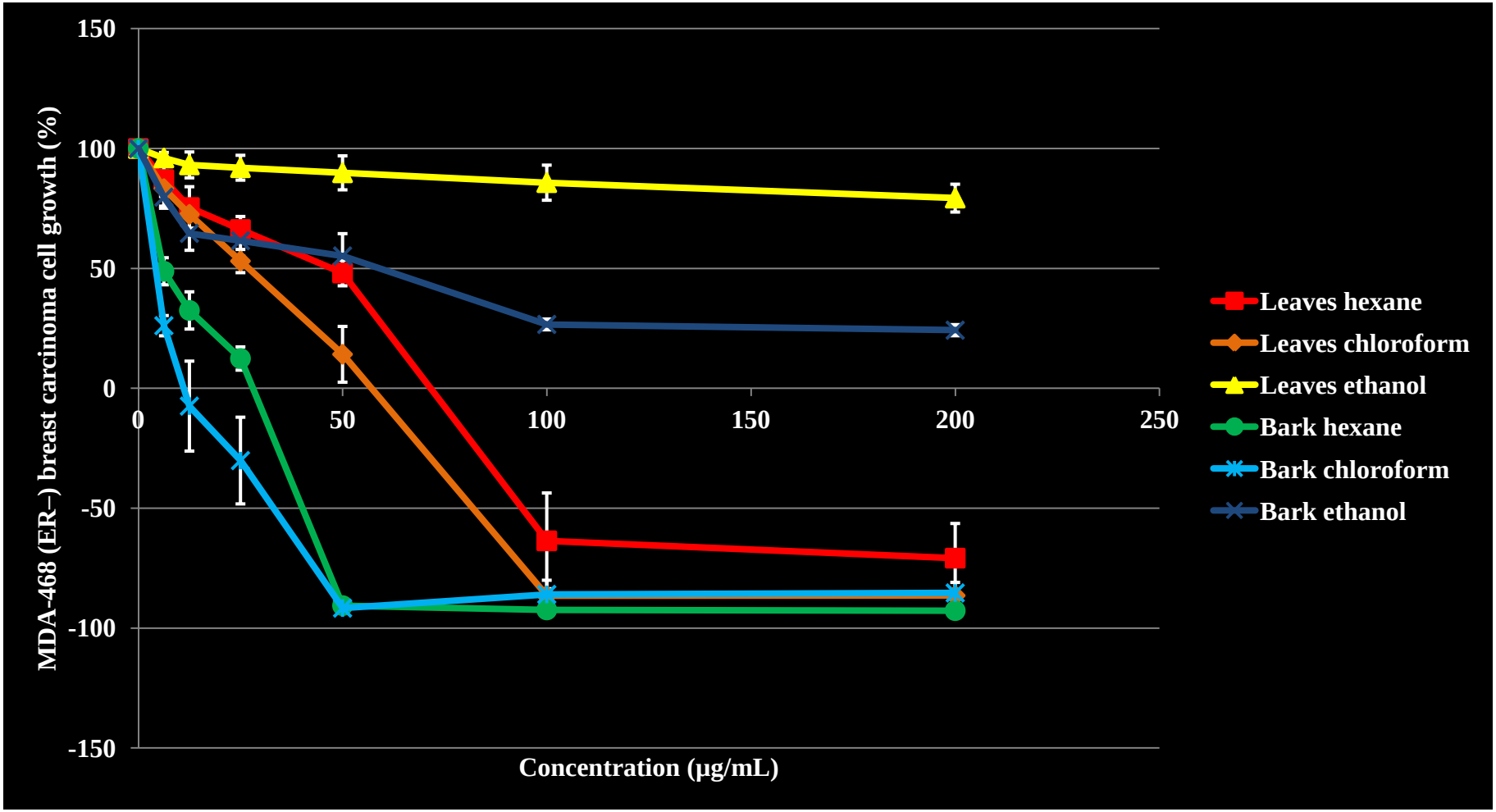


FIGURE 6.2 Anticancer effects of crude extracts of *Artabotrys crassifolius* against MDA-468 (ER–) breast carcinoma cell line. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

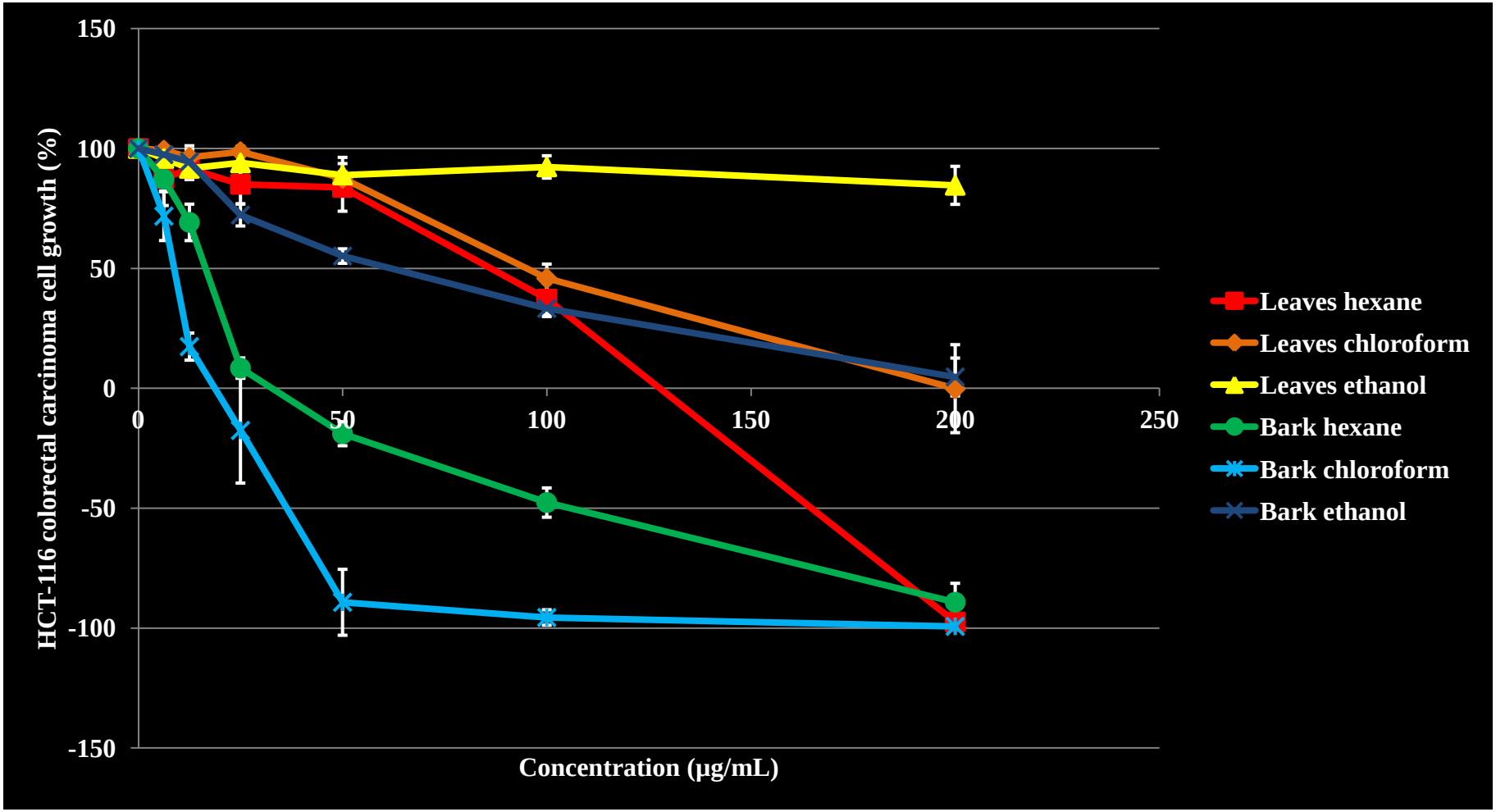


FIGURE 6.3 Anticancer effects of crude extracts of *Artabotrys crassifolius* against HCT-116 colorectal carcinoma cell line. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

For better visualisation of the differences in achieving complete cell growth inhibition for all the crude extracts, TGI concentrations were expressed. In MCF-7 breast carcinoma cell line, chloroform and hexane extracts of bark were less potent as shown by their respective TGI values of 32.73 µg/mL and 54.63 µg/mL. In MDA-468 breast carcinoma cell line, chloroform extract of bark induced total growth inhibition at the lowest concentration of 11.11 µg/mL, whereas hexane extract of bark displayed a comparable TGI value of 28.01 µg/mL. In HCT-116 colorectal carcinoma cell line, chloroform extract of bark exhibited a 1.75-fold lower TGI concentration than that of hexane extract of bark. However, no total growth inhibition was obtained upon treatment with ethanol extract of leaves and bark.

At doses higher than the TGIs, net cell killing was observed in all of the tested carcinoma cell lines. Chloroform extract of bark demonstrated the highest cytotoxic action with LC_{50} values ranging from 33.06 µg/mL to 48.84 µg/mL, while hexane extract of bark showed less pronounced cytotoxicity with LC_{50} values ranging from 40.14 µg/mL to 105.61 µg/mL. Considering the TGI concentrations and the net cell killing achieved by crude extracts, MDA-468 breast carcinoma cell line was found to be the most sensitive cell line, followed by HCT-116 colorectal carcinoma cell line, with MCF-7 breast carcinoma cell line being the least susceptible to crude extracts.

With respect to the phytochemical screening of crude extracts (Tan *et al.* 2013), the presence of alkaloids, cardiac glycosides and terpenoids in chloroform and hexane extracts of bark may explain their superior activity in comparison to the other crude extracts studied. This necessitates further isolation and characterisation of the potentially active principles from the respective crude extracts.

Although anticancer activities of isolated compounds from *Artabotrys* species have been previously published, no detailed anticancer studies have been reported on the crude extracts from which they were derived. This study could be useful prior to the selection of active extracts for isolation and characterisation.

6.4 CONCLUSION

Examination of the *in vitro* anticancer effect of *Artabotrys crassifolius* revealed that chloroform and hexane extracts of bark may be a significant source of novel anticancer compounds in view of their promising inhibitory activities particularly against MDA-468 breast carcinoma cell line. Therefore, further studies are needed to isolate and characterise the bioactive compounds responsible for the observed anticancer properties of *Artabotrys crassifolius*.

CHAPTER VII

IN VITRO ANTIOXIDANT POTENTIAL OF *ARTABOTRYS CRASSIFOLIUS*

7.1 INTRODUCTION

Free radicals are atomic or molecular species that can exist independently with one or more unpaired electrons in their outermost shell (Craft *et al.* 2012). They are generated as by-products during normal cellular metabolism (Barrera 2012). Due to their highly reactive and unstable properties in nature (Kamboj *et al.* 2014), they are capable of inducing oxidative damage to all the major classes of biomolecules including carbohydrates, lipids, proteins, and nucleic acids (Khasawneh *et al.* 2011). These damages are further implicated in the pathogenesis of atherosclerosis, cancer, diabetes mellitus, ischemia and reperfusion injury, neurodegenerative diseases, obstructive sleep apnea, rheumatoid arthritis as well as senescence (Droge 2002).

Although the human body possesses the comprehensive network of antioxidant defence and repair systems, these endogenous protective mechanisms are inadequate to counteract the damaging effects of free radicals completely (Lima-Saraiva *et al.* 2012). More importantly, the application of currently available synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallic acid esters and tert-butylhydroquinone (TBHQ) is often restricted because of their low solubility, moderate antioxidant activity and possible toxicity (Kiran *et al.* 2012). Therefore, the exploration of alternative antioxidants from natural sources is highly desirable.

7.2 METHODOLOGY

7.2.1 Determination of total phenolic content (TPC)

The total phenolic contents of crude extracts were assessed using Folin-Ciocalteu (FC) assay according to the methods of Zongo *et al.* (2010) and Lee and Vairappan (2011). In a 96-well microtiter plate (Jet Biofil, China), 100 µL of 10% (v/v) of FC reagent (R & M Chemicals, UK) was added to 5 µL of each crude extract (final concentration of 50 µg/mL). After 5 min incubation at room temperature, 80 µL of 7.5% (w/v) of sodium carbonate (Na_2CO_3) (R & M Chemicals, UK) was added to each well containing the previous mixture. The plate was shaken gently and incubated for 30 min at room temperature in the dark. Gallic acid (final concentrations ranging from 5 µg/mL to 25 µg/mL) (R & M Chemicals, UK) was used to establish the standard curve. Eventually, the absorbance was measured at 765 nm against the corresponding blank solution using Varioskan Flash microplate reader with SkanIt Software 2.4.3 RE (Thermo Scientific, Malaysia). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of extract (mg GAE/g extract) (Iqbal *et al.* 2012), which was calculated by the following equation:

$$T_1 = C_1 \times \frac{V}{M},$$

where T_1 is the total phenolic content, mg/g of extract, in GAE; C_1 is the concentration of gallic acid established from the calibration curve, mg/mL; V is the volume of crude extract, mL; M is the weight of crude extract, g.

7.2.2 Determination of total flavonoid content (TFC)

The total flavonoid contents of crude extracts were assessed using aluminium chloride colourimetric assay according to the methods of Tavares *et al.* (2010) and Yang *et al.* (2012). In a 96-well microtiter plate, 15 µL of 5% (w/v) of sodium nitrite ($NaNO_2$) (Merck, Germany) was added to 5 µL of each crude extract (final concentration of 50 µg/mL). The plate was allowed to stand for 6 min at room temperature and subsequently 30 µL of 10% (w/v) of aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) (R & M Chemicals, UK) was added to the mixture. After a further 5 min incubation at room temperature, 100 µL of 1 M of sodium hydroxide (NaOH) (Merck, Germany) was added to the mixture and immediately diluted by the addition of 55 µL of distilled water. (+)-Catechin hydrate (final concentrations ranging from 5 µg/mL to 25 µg/mL) (Sigma-Aldrich, Indonesia) was used to establish the standard curve. Eventually, the absorbance was measured at 510 nm against the corresponding blank solution using Varioskan Flash microplate reader with SkanIt Software 2.4.3 RE. The total flavonoid content was expressed as catechin equivalents (CE) in milligram per gram of extract (mg CE/g extract) (Vyas 2010), which was calculated by the following equation:

$$T_2 = C_2 \times \frac{V}{M} ,$$

where T_2 is the total flavonoid content, mg/g of extract, in CE; C_2 is the concentration of catechin established from the calibration curve, mg/mL; V is the volume of crude extract, mL; M is the weight of crude extract, g.

7.2.3 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation radical scavenging assay

The antioxidant potentials of crude extracts were assessed using ABTS cation radical scavenging assay according to the methods of Bunea *et al.* (2011) and Sampath and Vasanthi (2013). The ABTS cation radical was produced by reacting 7 mM of ABTS diammonium salt solution (Sigma-Aldrich, Canada) with 2.45 mM of potassium peroxodisulphate solution ($K_2S_2O_8$) (Fluka, Germany) in equal volume. The mixture was allowed to stand in the dark at room temperature for 12 h to 16 h. Prior to assay, the ABTS working solution was prepared by diluting the stock solution with methanol (Friendemann Schmidt, Australia) to an absorbance of 0.70±0.02 at 734 nm. In a 96-well microtiter plate, 195 µL of the diluted ABTS solution was added to 5 µL of each crude extract (final concentrations ranging from 3.125 µg/mL to 100 µg/mL). The plate was shaken gently and incubated for 6 min at room temperature in the dark. Trolox (final concentrations ranging from 3.125 µg/mL to 100 µg/mL) (Acros Organics, Belgium) was used as positive control. Eventually, the absorbance was measured at 734 nm against the corresponding blank solution using Varioskan Flash microplate reader with SkanIt Software 2.4.3 RE. The percentage of ABTS cation radical scavenging activity was calculated by the following equation:

$$\text{ABTS cation radical scavenging activity (\%)} = \frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100\% \quad ,$$

where A_0 is the absorbance of negative control; A_1 is the absorbance of reaction mixture; A_2 is the absorbance of crude extract or positive control. The IC_{50} value was determined from the plotted graph of scavenging activity against the concentration of crude extracts or positive control (Yang *et al.* 2011).

7.2.4 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant potentials of crude extracts were assessed using DPPH radical scavenging assay according to the methods of Yang *et al.* (2009) and Mutee *et al.* (2010). In a 96-well microtiter plate, 195 µL of 0.1 mM of DPPH (Sigma-Aldrich, USA) was added to 5 µL of each crude extract (final concentrations ranging from 3.125 µg/mL to 100 µg/mL). The plate was shaken gently and incubated for 30 min at room temperature in the dark. L(+)-ascorbic acid (final concentrations ranging from 3.125 µg/mL to 100 µg/mL) (Systerm, Malaysia) was used as positive control. Eventually, the absorbance was measured at 517 nm against the corresponding blank solution using Varioskan Flash microplate reader with SkanIt Software 2.4.3 RE. The percentage of DPPH radical scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100\% \quad ,$$

where A_0 is the absorbance of negative control; A_1 is the absorbance of reaction mixture; A_2 is the absorbance of crude extract or positive control. The IC_{50} value was determined from the plotted graph of scavenging activity against the concentration of crude extracts or positive control (Tibuhwa 2012). The antioxidant activity index (AAI) (Zongo *et al.* 2011) was calculated as follows:

$$\text{AAI} = \frac{\text{Final concentration of DPPH (µg/mL)}}{IC_{50} \text{ value of crude extract (µg/mL)}}$$

7.2.5 Ferric reducing antioxidant power (FRAP) assay

The antioxidant potentials of crude extracts were assessed using FRAP assay according to the methods of Gan *et al.* (2010) and Song *et al.* (2010). The FRAP reagent was freshly prepared by mixing 300 mM of acetate buffer (pH 3.6) [mixture of sodium acetate trihydrate (Merck, Germany) and glacial acetic acid (Systerm, Malaysia)], 10 mM of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, Switzerland) in 40 mM of hydrochloric acid (HCl) (Systerm, Malaysia), and 20 mM of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Systerm, Malaysia) in a volume ratio of 10:1:1 respectively. Prior to assay, the FRAP reagent was warmed to 37°C in a water bath (Julabo, Germany). In a 96-well microtiter plate, 195 µL of the FRAP reagent was added to 5 µL of each crude extract (final concentrations ranging from 3.125 µg/mL to 100 µg/mL). The plate was shaken gently and incubated at 37°C for 4 min. Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) (final concentrations ranging from 50 µM to 250 µM) (Systerm, Malaysia) was used to establish the standard curve. Eventually, the absorbance was measured at 593 nm against the corresponding blank solution using Varioskan Flash microplate reader with SkanIt Software 2.4.3 RE. The FRAP value was expressed as $FeSO_4 \cdot 7H_2O$ equivalents, Fe(II) in micromole per gram of extract [µmol Fe(II)/g extract], which was calculated by the following equation:

$$T_3 = C_3 \times \frac{V}{M} ,$$

where T_3 is the FRAP value, µmol/g of extract, in Fe(II); C_3 is the concentration of $FeSO_4 \cdot 7H_2O$ established from the calibration curve, µmol/L; V is the volume of crude extract, L; M is the weight of crude extract, g.

7.2.6 Statistical analysis

Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

7.3 RESULTS AND DISCUSSION

Plants represent an invaluable source of raw materials for the development of natural antioxidants (Ghasemzadeh *et al.* 2012). Nevertheless, due to the diverse and complex nature of the phytochemical constituents, there is no single universal method that can accurately evaluate the antioxidant activities of plant extracts (Ksiksi and Hamza 2012). In the current study, TPC, TFC, ABTS, DPPH and FRAP assays were performed to assess the antioxidant potentials of crude extracts.

7.3.1 Total phenolic contents of crude extracts of *Artabotrys crassifolius*

Folin-Ciocalteu assay is extensively used for the quantification of total phenolic content (Prasain *et al.* 2008). This method is based on the reduction of phosphomolybdic and phosphotungstic acid complexes to blue chromogens in the presence of phenolic compounds under alkaline conditions (Mehran *et al.* 2014).

The total phenolic contents of crude extracts were calculated from the regression equation of the calibration curve of gallic acid ($y = 0.0384x$, $R^2 = 0.9996$) (Figure 7.1; Appendix E1). According to Rufino *et al.* (2010), the content of total phenolics of crude extracts could be categorised into three classes: low (less than 10 mg GAE/g), medium (ranging from 10 mg GAE/g to 50 mg GAE/g) and high (more than 50 mg GAE/g). Under this classification, ethanol extract of bark and leaves demonstrated remarkably high total phenolic contents of 268.29±12.36 mg GAE/g and 154.91±4.26 mg GAE/g respectively (Figure 7.2; Appendix E2). This may be due to the greater solubility of phenolic compounds in ethanol.

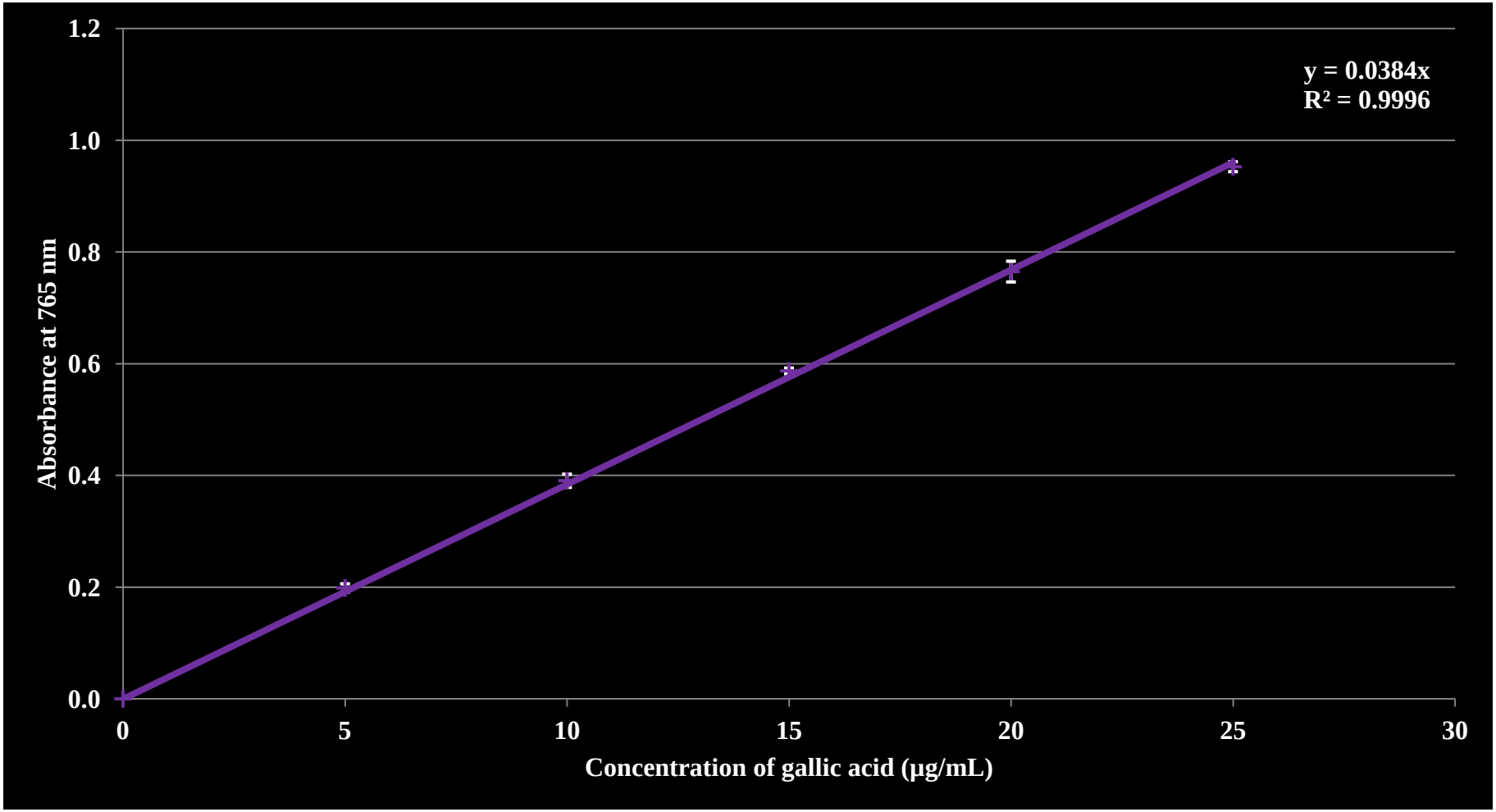


FIGURE 7.1 Standard curve of gallic acid for the determination of total phenolic contents of crude extracts of *Artabotrys crassifolius*. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

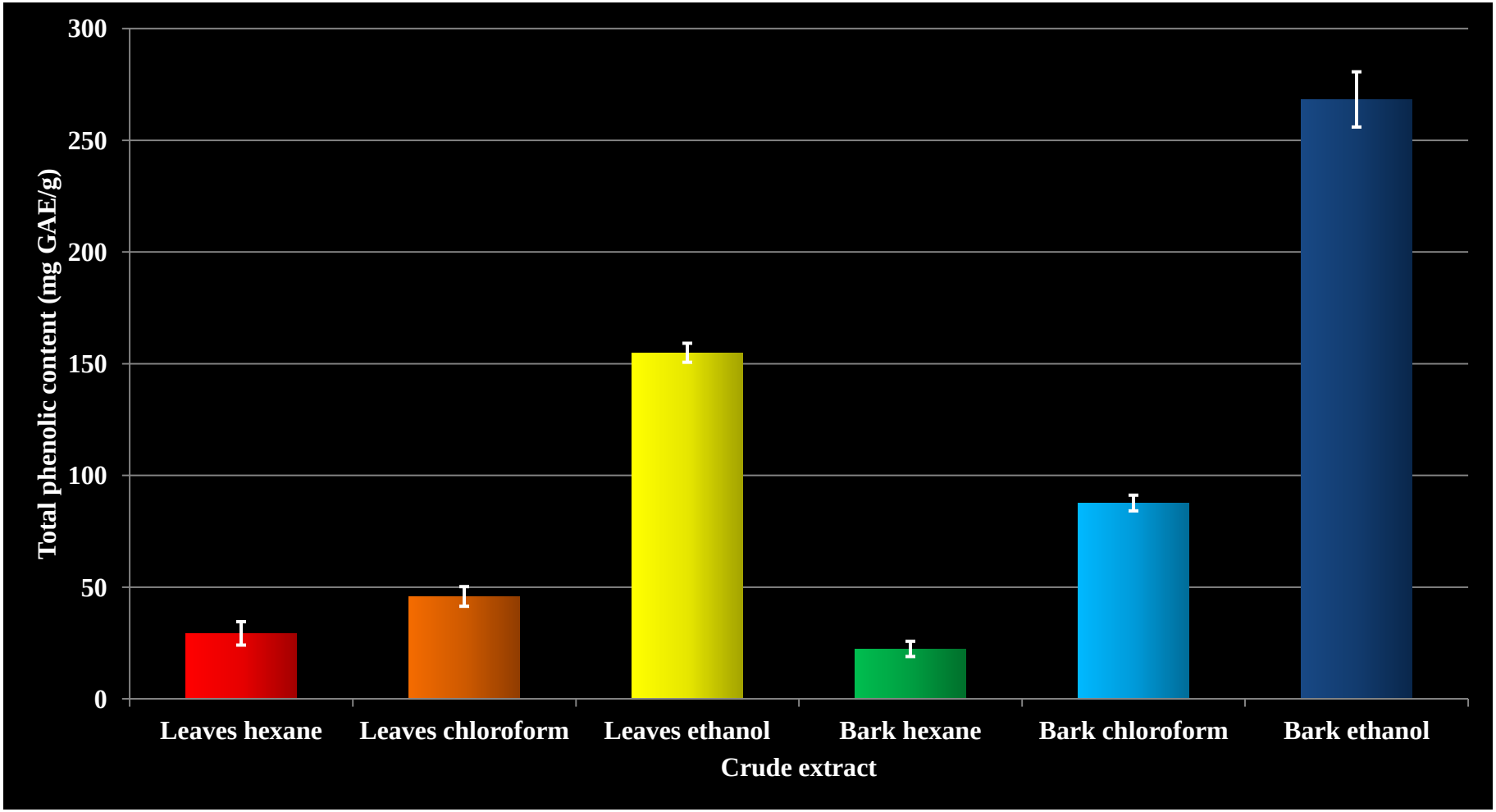


FIGURE 7.2 Total phenolic contents of crude extracts of *Artabotrys crassifolius*. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

7.3.2 Total flavonoid contents of crude extracts of *Artabotrys crassifolius*

Aluminium chloride assay is widely employed for the estimation of total flavonoid content (Corpuz *et al.* 2013). This method is based on the development of acid-stable complexes between aluminium chloride and the C-4 keto group along with either the C-3 or C-5 hydroxyl group of flavones and flavonols. Additionally, aluminium chloride also forms acid-labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Rajanandh and Kavitha 2010). These complexes subsequently produce pink chromogens upon reacting with sodium nitrite under alkaline conditions (Kaur 2010).

The total flavonoid contents of crude extracts were determined from the regression equation of the calibration curve of catechin ($y = 0.0184x$, $R^2 = 0.9964$) (Figure 7.3; Appendix E1). Their content of total flavonoids expressed as catechin equivalents was recorded in the range from 6.29±4.27 mg/g to 179.54±4.98 mg/g (Figure 7.4; Appendix E2).

Among the crude extracts examined, ethanol extract of bark displayed the highest total flavonoid content (179.54±4.98 mg CE/g), which was approximately 2.13-fold greater than that of ethanol extract of leaves (84.47±6.61 mg CE/g). On the contrary, the content of total flavonoids was found to be comparatively low in both hexane extracts of leaves (9.48±4.53 mg CE/g) and bark (6.29±4.27 mg CE/g). This indicates that ethanol has superior extraction capacity as well as selectivity for flavonoids in comparison to hexane.

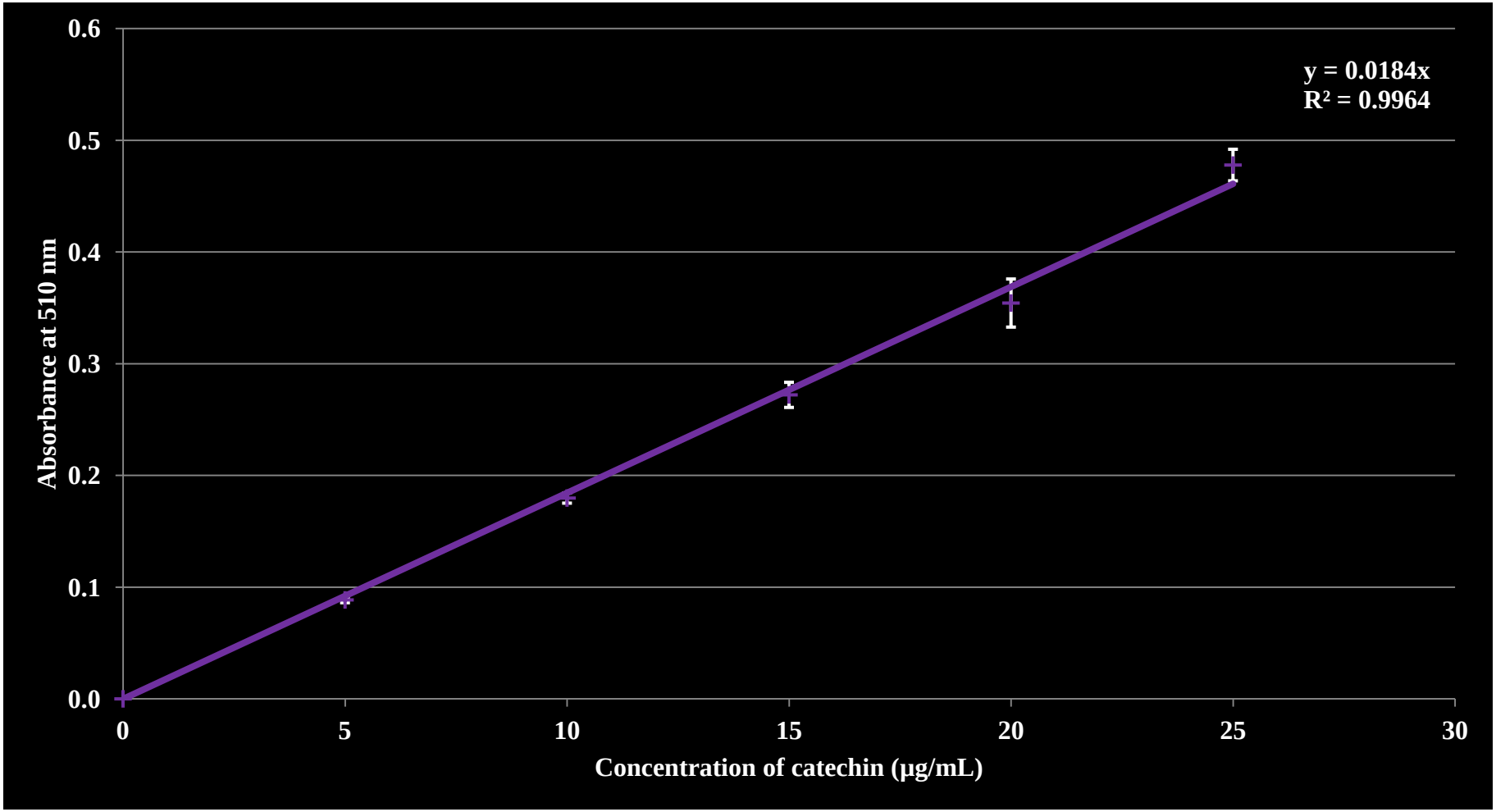


FIGURE 7.3 Standard curve of catechin for the determination of total flavonoid contents of crude extracts of *Artabotrys crassifolius*. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

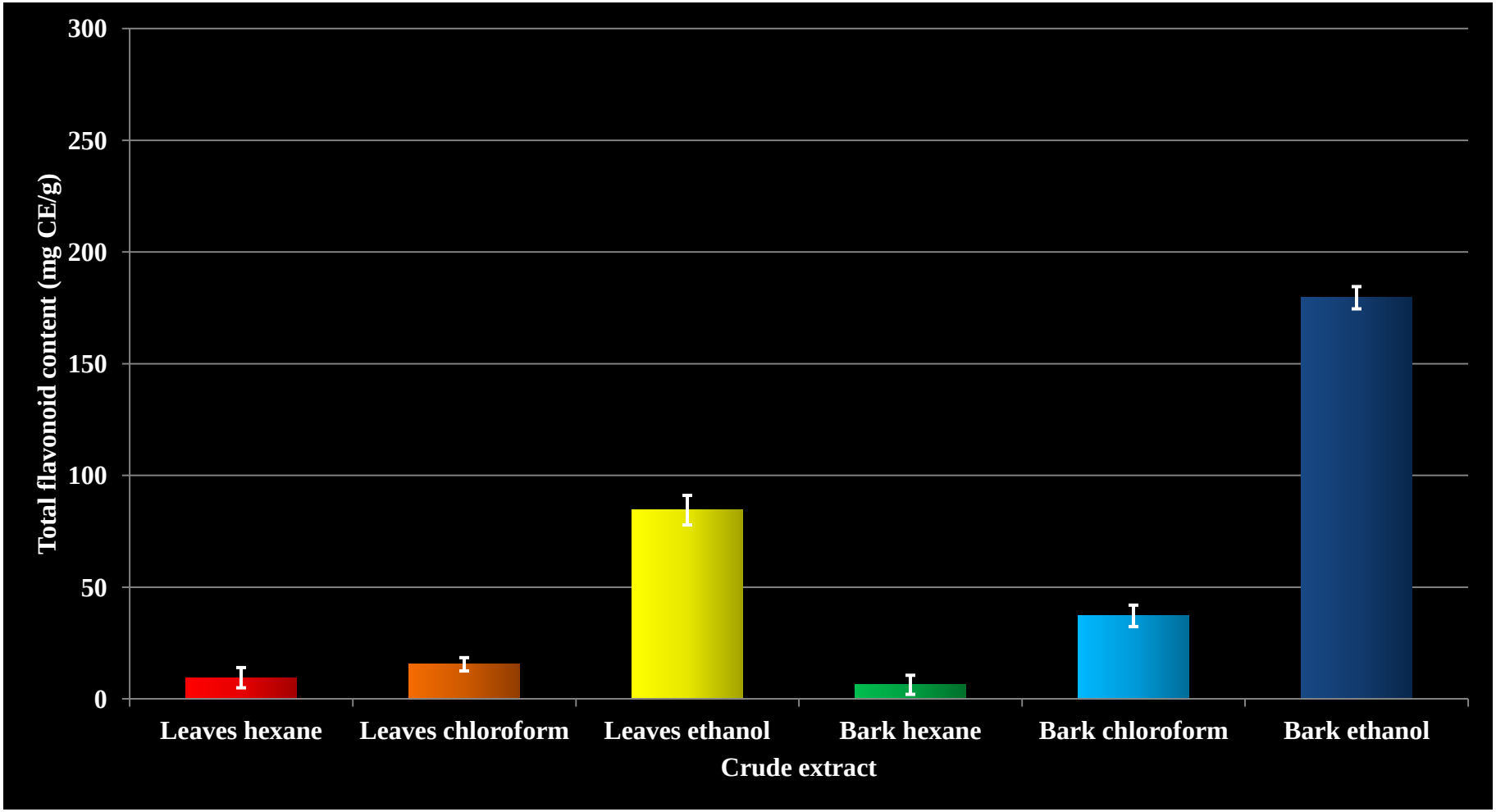


FIGURE 7.4 Total flavonoid contents of crude extracts of *Artabotrys crassifolius*. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

7.3.3 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using ABTS cation radical scavenging assay

ABTS assay is commonly employed for the determination of antioxidant capacity of plant extracts (Contreras-Calderon *et al.* 2011). This method is based on the reduction of blue-green chromogens produced from the reaction between ABTS and potassium peroxodisulphate in the presence of electron-donating antioxidants (Ahmed 2012).

Figure 7.5 depicts the ABTS cation radical scavenging activities of crude extracts (Appendix E3). At 100 µg/mL concentration, ethanol extract of bark and leaves exhibited the maximum scavenging effects of 99.86±0.06% and 99.76±0.26% against ABTS cation radical respectively, which were similar to that of the standard, trolox (99.72±0.15%), a water-soluble vitamin E analogue.

The cut-off point for antioxidant potentials of crude extracts was suggested to be 50 µg/mL (Omisore *et al.* 2005). According to Kuete and Efferth (2010) and Chew *et al.* (2011), the radical scavenging ability of crude extracts could be classified based on their IC_{50} values as follows: high antioxidant capacity (IC_{50} value less than 50 µg/mL), moderate antioxidant capacity (IC_{50} value ranging from 50 µg/mL to 100 µg/mL) and low antioxidant capacity (IC_{50} value more than 100 µg/mL). Under this categorisation, ethanol extract of bark and leaves demonstrated high antioxidant capacity with respective IC_{50} values of 16.50 µg/mL and 30.77 µg/mL whereas trolox gave an IC_{50} value of 6.88 µg/mL. This implies that the corresponding extracts may function as effective scavengers of ABTS cation radical.

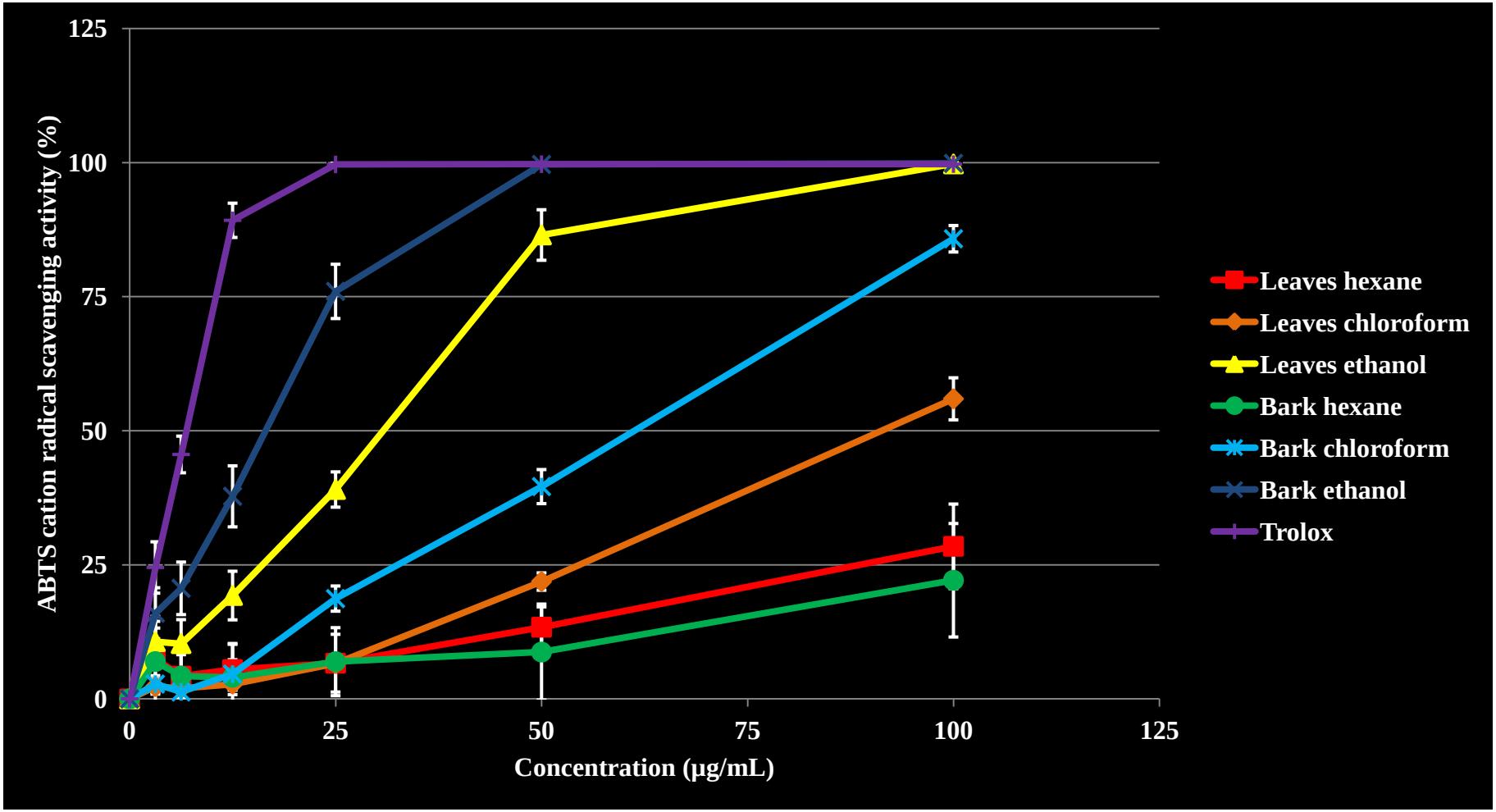


FIGURE 7.5 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using ABTS cation radical scavenging assay. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

7.3.4 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using DPPH radical scavenging assay

DPPH assay is widely used for the evaluation of radical scavenging activity of plant extracts (Ndhlala *et al.* 2010). This method is based on the reduction of purple-coloured DPPH radical (2,2-diphenyl-1-picrylhydrazyl) to yellow-coloured non-radical form of DPPH (2,2-diphenyl-1-picrylhydrazine) in the presence of hydrogen-donating antioxidants (Murali *et al.* 2011).

The scavenging effects of crude extracts on DPPH radical are illustrated in Figure 7.6 (Appendix E4). All the crude extracts showed a concentration-dependent increase in scavenging DPPH radical. At a concentration of 100 µg/mL, the highest DPPH radical scavenging activity was observed in ethanol extract of bark (95.47±2.37%; IC_{50} value of 16.54 µg/mL), which had comparable scavenging effect to that of the positive control, ascorbic acid (95.34±0.64%; IC_{50} value of 7.59 µg/mL), a water-soluble form of vitamin C.

According to the scale proposed by Scherer and Godoy (2009), crude extracts could be considered to show poor antioxidant activity when AAI less than 0.5, moderate antioxidant activity when AAI ranging from 0.5 to 1.0, strong antioxidant activity when AAI ranging from 1.0 to 2.0, and very strong antioxidant activity when AAI more than 2.0. Based on this classification, ethanol extract of bark displayed very strong antioxidant activity with an AAI value of 2.32, while the AAI value of ascorbic acid was found to be 5.07. This suggests that the respective extract may possess compounds with hydrogen-donating ability that can efficiently scavenge DPPH radical.

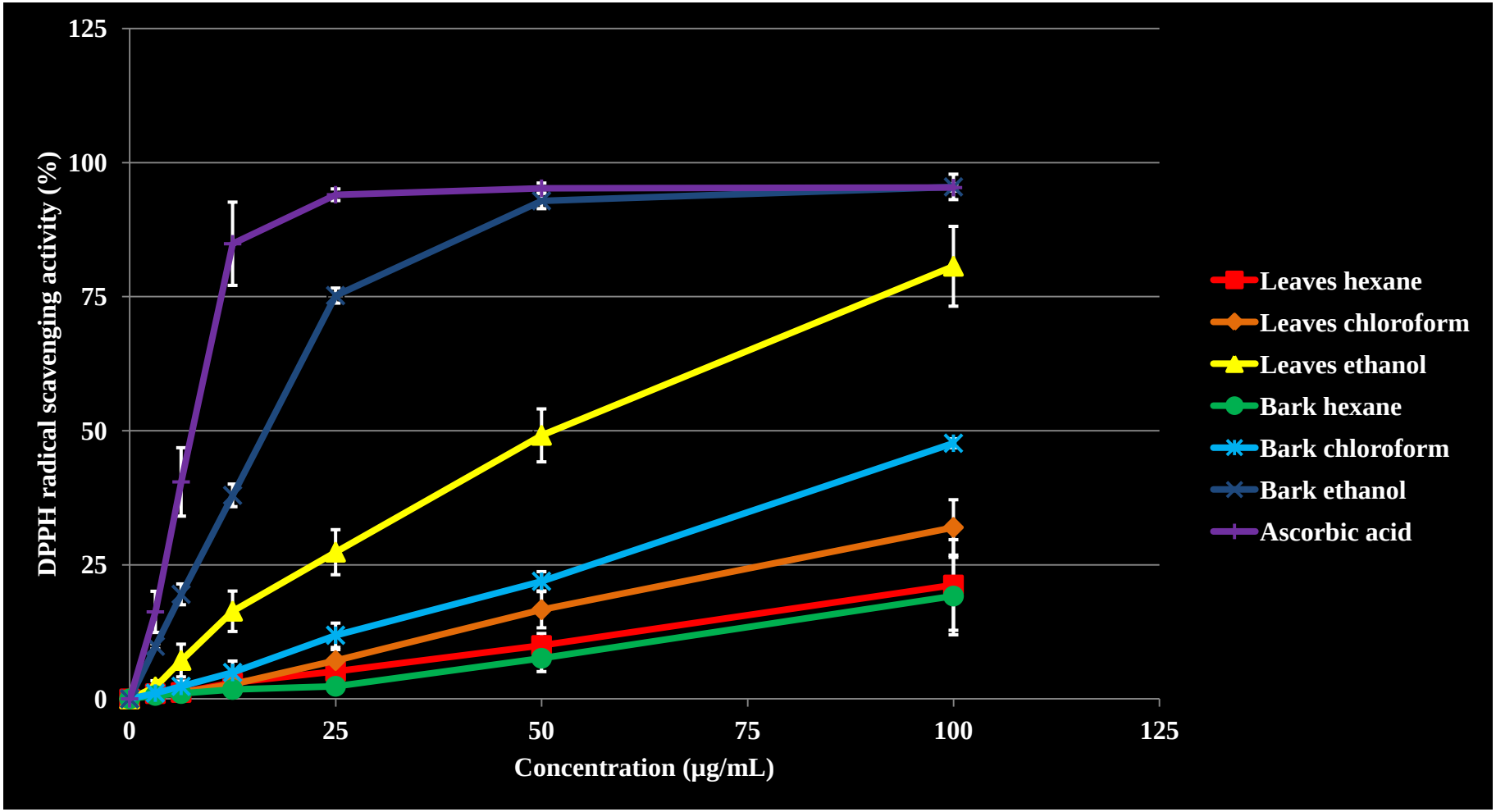


FIGURE 7.6 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using DPPH radical scavenging assay. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

7.3.5 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using FRAP assay

FRAP assay is extensively applied for the measurement of reducing power of plant extracts (Magalhaes 2007). This method is based on the reduction of colourless ferric complex (Fe^{3+}-tripyridyltriazine) to blue-coloured ferrous complex (Fe^{2+}-tripyridyltriazine) in the presence of electron-donating antioxidants under acidic conditions (Irshad *et al.* 2012).

The FRAP values of crude extracts were obtained from the regression equation of the calibration curve of $FeSO_4 \cdot 7H_2O$ ($y = 0.0111x$, $R^2 = 0.9963$) (Figure 7.7; Appendix E5). Their FRAP values expressed as $FeSO_4 \cdot 7H_2O$ equivalents, Fe(II) were varied from 67.64±23.40 µmol/g to 1884.35±83.78 µmol/g (Figure 7.8; Appendix E6).

According to Wong *et al.* (2006) and Oonsivilai *et al.* (2008), crude extracts could be classified into four categories based on their antioxidant power: low [less than 10 µmol Fe(II)/g], medium [ranging from 10 µmol Fe(II)/g to 100 µmol Fe(II)/g], high [ranging from 100 µmol Fe(II)/g to 500 µmol Fe(II)/g], and extremely high [more than 500 µmol Fe(II)/g]. Based on this categorisation, ethanol extract of bark and leaves exhibited exceptionally high antioxidant power with respective FRAP values of 1884.35±83.78 µmol Fe(II)/g and 979.57±57.17 µmol Fe(II)/g. In contrast, medium antioxidant power was detected in both hexane extracts of leaves [92.26±5.99 µmol Fe(II)/g] and bark [67.64±23.40 µmol Fe(II)/g]. This may be attributed to the better electron-donating capabilities of ethanol extracts as compared to hexane extracts.

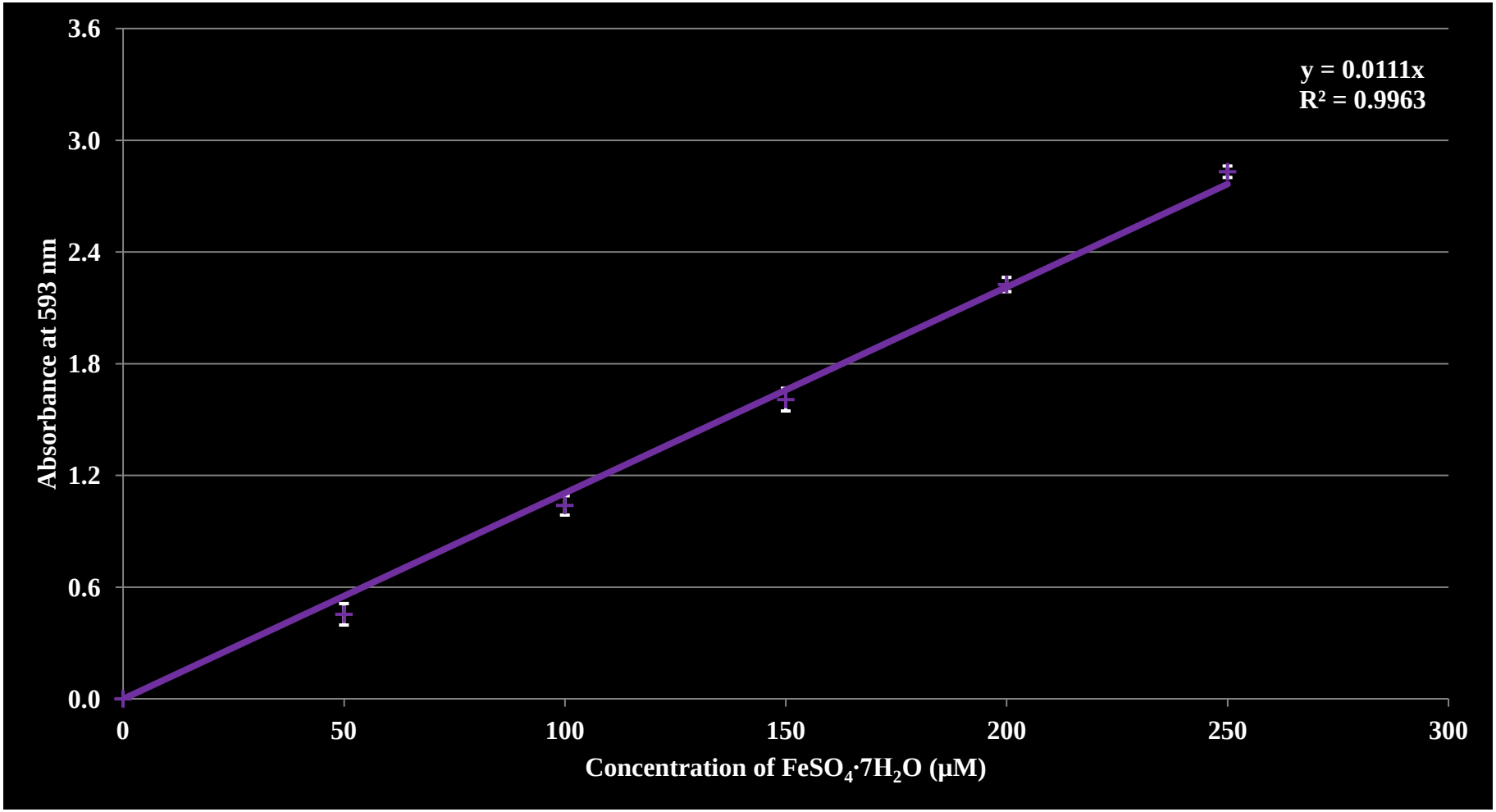


FIGURE 7.7 Standard curve of $FeSO_4 \cdot 7H_2O$ for the antioxidant potentials of crude extracts of *Artabotrys crassifolius* using FRAP assay. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

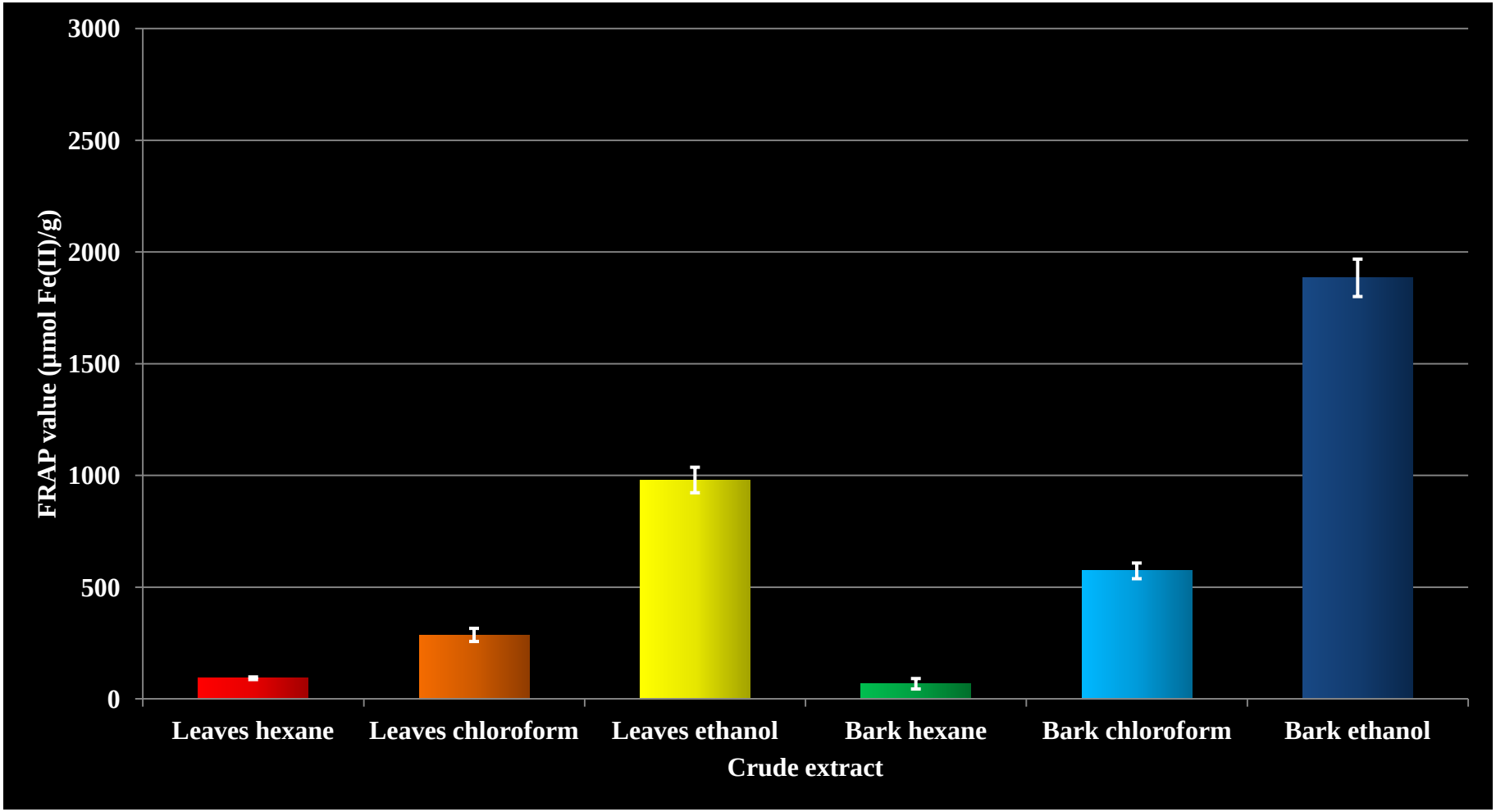


FIGURE 7.8 Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using FRAP assay. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

With respect to the phytochemical analysis of crude extracts (Tan *et al.* 2013), the presence of cardiac glycosides, flavonoids, phenolic compounds, saponins and terpenoids in ethanol extract of bark may explain its superior activity in comparison to the other crude extracts tested. This necessitates further isolation and characterisation of the potentially active principles from the respective crude extract. Considering that no literature review has been assembled to comprehensively address the antioxidant properties of *Artabotrys* species, this study could be the first report providing new insights into the antioxidant activity in the genus *Artabotrys*.

7.4 CONCLUSION

Evaluation of the *in vitro* antioxidant potential of *Artabotrys crassifolius* revealed that ethanol extract of bark may be a significant source of novel antioxidant compounds in consideration of its promising scavenging activity predominantly against ABTS cation radical. Consequently, further studies are needed to isolate and characterise the bioactive compounds responsible for the observed antioxidant properties of *Artabotrys crassifolius*.

CHAPTER VIII

IN VITRO PHARMACOLOGICAL ACTIVITY OF ISOLATED COMPOUNDS FROM *ARTABOTRYS CRASSIFOLIUS*

8.1 INTRODUCTION

Over the last century, natural products have provided considerable value to the pharmaceutical industry in the discovery of novel chemical structures and bioactive lead molecules for drug development (Baker *et al.* 2007). Numerous natural products and their synthetically modified derivatives such as morphine and aspirin have been developed clinically to treat human diseases in all therapeutic areas, particularly infectious diseases and oncology (Brahmachari 2012).

Despite the availability of various approaches in drug discovery and development including synthetic and combinatorial chemistry, as well as computer-based molecular modeling design, none of them can substitute the central role of natural products as the majority of core structures or scaffolds for synthetic compounds are based upon natural products (Jesus 2003; Chinyama 2009; Veeresham and Chitti 2013). More significantly, not all natural products can be prepared by total synthesis, and many of them possess highly complex structures that are too difficult and economically infeasible to synthesize on an industrial scale (Ige *et al.* 2012). Consequently, isolation and characterisation of pharmacologically active compounds from natural products remain to be the only viable option.

8.2 METHODOLOGY

8.2.1 Isolation and characterisation

The chloroform extract of bark (24.79 g), which exerted pronounced antibacterial and anticancer activities, was subjected to silica gel 60 (0.063–0.200 mm, 70–230 mesh ASTM) (Merck, Germany) column chromatography eluted with hexane–chloroform (100:0 to 10:90, v/v) (Friendemann Schmidt, Australia) and chloroform–methanol (100:0 to 80:20, v/v) (Friendemann Schmidt, Australia) to afford 141 fractions. Each collected fraction was monitored by analytical thin layer chromatography (TLC) on silica gel 60 F_{254} aluminium sheets (0.2 mm thickness) (Merck, Germany) using chloroform–methanol (95:5, v/v) as eluent. The spots were visualised under ultraviolet (UV) light at 254 nm and 365 nm, followed by spraying with Dragendorff's reagent for alkaloid detection. Fractions with similar TLC profiles were combined to give 9 major fractions (A–I).

Fraction C was purified by Sephadex LH-20 (GE Healthcare, Sweden) column chromatography eluted with chloroform to furnish 7 subfractions (C1–7). Subfractions C2–4 were washed with diethyl ether (RCI Labscan, Thailand) and recrystallised from chloroform to afford compound **1** (10.9 mg). Fraction D was repeatedly chromatographed over silica gel column using chloroform–methanol (100:0 to 80:20, v/v) as eluent to yield 10 subfractions (D1–10). Subfractions D2–4 were purified by preparative TLC on silica gel 60 F_{254} glass plates (2 mm thickness) (Merck, Germany) eluted with chloroform–methanol (98:2, v/v) to furnish compound **2** (3.0 mg).

Fractions E–G were separately chromatographed over Sephadex LH-20 column using chloroform as eluent, followed by purification on silica gel column eluted with chloroform–methanol (100:0 to 80:20, v/v) to give 9 subfractions (EG1–9). Subfractions EG4–7 were further purified on preparative TLC plates using chloroform–methanol (98:2, v/v) as eluent to yield compounds **3** (2.9 mg) and **4** (10.3 mg).

The structures of the isolated compounds were elucidated on the basis of spectroscopic analysis including single-crystal X-ray diffraction (compound **1**) and one-dimensional nuclear magnetic resonance (NMR) (compounds **2**, **3** and **4**), as well as comparison with data reported in the literature.

A suitable crystal of compound **1** was selected and measured on a SuperNova, single source at offset, Atlas diffractometer. The crystal was kept at 120(2) K during data collection. Using Olex2 (Dolomanov *et al.* 2009), the structure was solved with the olex2.solve structure solution program using Charge Flipping and refined with the XL (Sheldrick 2008) refinement package using Least Squares minimisation.

The proton NMR (^{1}H NMR) spectra of compounds **2**, **3** and **4** were recorded on a Bruker Avance 3400 spectrometer at 400 MHz using deuterated chloroform ($CDCl_3$) (Sigma-Aldrich, USA) as solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent peak.

8.2.2 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of isolated compounds were evaluated against ATCC and clinical strains using broth microdilution method according to the guidelines of the CLSI (CLSI 2012), formerly known as National Committee for Clinical Laboratory Standards (NCCLS).

(a) Preparation of Mueller-Hinton broth

Mueller-Hinton broth (MHB) (Difco Laboratories, USA) was prepared from a commercially available dehydrated base according to the manufacturer's instructions. After autoclaving at 121°C for 15 min, the broth was allowed to cool down to room temperature before storing at 4°C until further use.

(b) Preparation of microdilution plates

In a 96-well microtiter plate (Jet Biofil, China), 175 µL of MHB was added to 5 µL of each isolated compound (final concentrations ranging from 0.3125 µg/mL to 20 µg/mL) prior to inoculation. Streptomycin sulphate (final concentrations ranging from 0.3125 µg/mL to 20 µg/mL) (Fisher BioReagents, China) and DMSO (R & M Chemicals, UK) were served as positive and negative controls respectively.

(c) Preparation of inoculum

A standardised inoculum was prepared using the growth method as described in Chapter 4.2.4(c). Optimally within 15 min of preparation, the adjusted inoculum suspension was diluted in water, saline, or broth to obtain a final test concentration of bacteria of approximately 5×10^5 CFU/mL in each well.

(d) Inoculation of microdilution plates

Within 15 min after the inoculum was standardised, each well of a microdilution plate was inoculated with 20 µL of the prepared inoculum using a multichannel pipette. To prevent drying, each plate was sealed in a plastic bag, with plastic tape, or with a tight-fitting plastic cover before incubating.

(e) Incubation of microdilution plates

The inoculated microdilution plates were incubated at 35°C for 16 h to 20 h in an ambient air incubator (Binder, Germany) within 15 min of adding the inoculum. To maintain the same incubation temperature for all cultures, microdilution plates should not be stacked more than four high.

(f) Reading MIC results

The amount of growth in the wells containing isolated compounds was compared with that in the positive growth control wells (without isolated compounds) used in each set of tests when determining the growth endpoints. Eventually, the MIC value was recorded as the lowest concentration of each isolated compound that completely inhibited the growth of bacteria in the microdilution wells as detected by the unaided eye.

8.2.3 Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentrations (MBC) of isolated compounds were evaluated according to the guidelines of the CLSI (CLSI 1999), formerly known as NCCLS. After MIC determination, an aliquot of 10 μL was removed from each well showing inhibition of growth and subcultured on Mueller-Hinton agar (MHA) (Difco Laboratories, USA) plates. The plates were incubated at 35°C for 24 h. Eventually, the MBC value was taken as the lowest concentration of each isolated compound that resulted in 99.9% killing of the final inoculum.

8.2.4 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The anticancer effects of isolated compounds were investigated against human breast and colorectal carcinoma cell lines using MTT assay as described in Chapter 6.2.2.

8.2.5 Statistical analysis

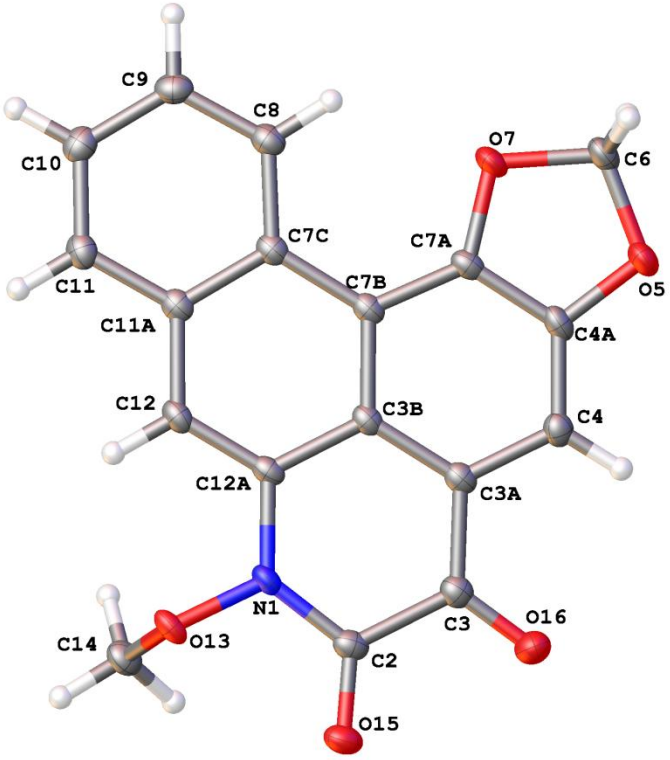
Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

8.3 RESULTS AND DISCUSSION

Plants provide a rich and intriguing source of secondary metabolites that differ widely in terms of their chemical structures, pharmacological properties and mechanisms of actions (Shukla *et al.* 2012; Amari *et al.* 2014). This considerable untapped potential has resulted in the search for natural alternatives, with the objective of discovering promising active lead compounds which can serve as novel therapeutic agents or templates for the design and synthesis of new drug entities (Nino *et al.* 2012; Daniels and Malomo 2014). In the present study, MIC, MBC and MTT assays were conducted to explore the pharmacological activities of isolated compounds.

8.3.1 Isolation and characterisation of bioactive compounds from *Artabotrys crassifolius*

The chromatographic separation of chloroform extract of bark of *Artabotrys crassifolius* led to the isolation of four alkaloids. The structures of the compounds were characterised as artabotrine (**1**) (Figure 8.1), liridine (**2**), atherospermidine (**3**) and lysicamine (**4**) (Figure 8.2). All these compounds were isolated for the first time from this plant. Interestingly, the 4,5-dioxoaporphine alkaloid, artabotrine, exists only in the genus *Artabotrys* (Wijeratne *et al.* 1996; Fleischer *et al.* 1997; Han *et al.* 2005) whereas liridine, atherospermidine and lysicamine are 7-oxoaporphine alkaloids commonly found in almost all the genera of the family Annonaceae (Leboeuf *et al.* 1982; Torres *et al.* 2007; Ortiz *et al.* 2007; Malebo *et al.* 2013).



(**1**)

FIGURE 8.1 X-ray structure of artabotrine.

Artabotrine (**1**) was obtained as orange-red crystals. The crystal data and structure refinement, fractional atomic coordinates and equivalent isotropic displacement parameters, anisotropic displacement parameters, bond lengths, bond angles, torsion angles, as well as hydrogen atom coordinates and isotropic displacement parameters are shown in Table 8.1–8.7.

TABLE 8.1 Crystal data and structure refinement for artabotrine.

Crystal data and structure refinement	
Empirical formula	$C_{18}H_{11}NO_5$
Formula weight	321.28
Temperature (K)	120(2)
Crystal system	monoclinic
Space group	$P2_1/n$
a (Å)	8.2597(4)
b (Å)	9.9728(5)
c (Å)	16.4041(8)
α (°)	90.00
β (°)	93.833(5)
γ (°)	90.00
Volume (Å^3)	1348.21(12)
Z	4
ρ_{calc} (mg/mm^3)	1.583
μ (mm^{-1})	0.985
F(000)	664.0
Crystal size (mm^3)	0.6056 × 0.5003 × 0.399
2Θ range for data collection (°)	10.82 to 150.22°
Index ranges	$-10 \leq h \leq 8, -12 \leq k \leq 12, -20 \leq l \leq 20$
Reflections collected	10238
Independent reflections	2727[R_{int} = 0.0169]
Data/restraints/parameters	2727/0/218
Goodness-of-fit on F^2	1.069
Final R indexes [$I>=2\sigma(I)$]	R_1 = 0.0330, wR_2 = 0.0919
Final R indexes [all data]	R_1 = 0.0349, wR_2 = 0.0938
Largest diff. peak/hole (e Å^{-3})	0.26/–0.20

TABLE 8.2 Fractional atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for artabotrine. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
N1	1652.3(10)	–819.0(9)	3276.2(5)	17.4(2)
C2	719.4(12)	–1948.2(11)	3287.0(6)	18.5(2)
C3B	1971.1(12)	–562.6(10)	4754.0(6)	15.3(2)
C3	406.9(12)	–2485.1(11)	4137.4(6)	19.0(2)
C3A	1016.7(12)	–1719.9(11)	4851.3(6)	16.9(2)
C4A	1298.7(12)	–1464.6(11)	6282.3(6)	18.4(2)
C4	668.1(12)	–2188.4(11)	5628.1(6)	18.7(2)
O5	1135.6(10)	–1722.0(9)	7091.9(4)	24.9(2)
C6	1976.0(13)	–662.8(12)	7538.4(6)	21.2(2)
C7B	2633.0(12)	183.6(10)	5442.0(6)	15.6(2)
C7C	3647.0(12)	1352.3(10)	5335.5(6)	16.1(2)
C7A	2246.3(12)	–328.5(11)	6197.2(6)	16.8(2)
O7	2723.6(9)	166.3(8)	6946.9(4)	22.42(19)
C8	4340.5(13)	2125.3(11)	5989.0(6)	18.9(2)
C9	5363.6(13)	3181.2(11)	5848.1(7)	20.7(2)
C10	5723.8(13)	3519.0(11)	5049.1(7)	20.5(2)
C11	5019.8(13)	2810.7(11)	4399.2(7)	19.3(2)
C11A	3979.3(12)	1720.1(10)	4529.7(6)	16.8(2)
C12A	2329.9(12)	–103.1(10)	3955.3(6)	15.7(2)
C12	3291.7(12)	976.8(10)	3847.0(6)	17.3(2)
O13	1872.3(9)	–270.7(8)	2509.5(4)	19.80(19)
C14	3258.0(13)	–870.0(12)	2154.6(6)	22.4(2)
O15	166.9(9)	–2504.0(9)	2667.9(5)	24.4(2)
O16	–350.3(10)	–3529.1(9)	4168.4(5)	28.2(2)

TABLE 8.3 Anisotropic displacement parameters ($Å^2 \times 10^3$) for artabotrine. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11} + ... + 2hka \times b \times U_{12}]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N1	19.6(4)	22.8(5)	10.0(4)	1.4(3)	1.7(3)	1.3(3)
C2	15.9(5)	22.6(5)	16.8(5)	–2.9(4)	0.5(4)	3.1(4)
C3B	14.3(5)	18.1(5)	13.7(5)	0.6(4)	1.9(4)	4.3(4)
C3	17.2(5)	22.0(5)	17.9(5)	–0.4(4)	1.5(4)	1.8(4)
C3A	14.7(5)	20.5(5)	15.4(5)	0.2(4)	1.0(4)	3.6(4)
C4A	17.3(5)	24.6(5)	13.5(5)	3.6(4)	2.9(4)	3.6(4)
C4	16.4(5)	21.5(5)	18.3(5)	2.3(4)	2.1(4)	0.3(4)
O5	30.0(4)	32.2(5)	12.6(4)	2.9(3)	2.5(3)	–6.3(3)
C6	21.4(5)	29.1(6)	13.3(5)	2.4(4)	2.4(4)	–0.3(4)
C7B	14.7(5)	18.5(5)	13.7(5)	0.3(4)	1.1(4)	5.2(4)
C7C	15.8(5)	17.3(5)	15.2(5)	0.3(4)	1.1(4)	4.9(4)
C7A	15.9(5)	21.3(5)	13.0(5)	–0.7(4)	0.2(4)	4.4(4)
O7	27.0(4)	29.8(4)	10.5(3)	0.0(3)	1.6(3)	–4.2(3)
C8	20.5(5)	19.9(5)	16.5(5)	–0.7(4)	1.1(4)	4.4(4)
C9	21.7(5)	19.0(5)	21.2(5)	–4.2(4)	–1.0(4)	3.1(4)
C10	19.1(5)	16.2(5)	26.3(6)	0.0(4)	2.4(4)	2.5(4)
C11	20.8(5)	17.9(5)	19.6(5)	3.1(4)	3.3(4)	4.0(4)
C11A	16.4(5)	17.5(5)	16.4(5)	1.1(4)	0.9(4)	5.4(4)
C12A	15.9(5)	19.0(5)	12.3(5)	–0.7(4)	0.1(4)	5.2(4)
C12	19.1(5)	19.6(5)	13.4(4)	2.6(4)	2.4(4)	4.8(4)
O13	21.7(4)	27.6(4)	10.3(3)	2.8(3)	2.3(3)	4.7(3)
C14	21.5(5)	28.2(6)	18.0(5)	–1.8(4)	5.3(4)	1.8(4)
O15	24.9(4)	31.5(4)	16.9(4)	–6.2(3)	1.2(3)	–3.0(3)
O16	34.1(5)	27.5(4)	22.8(4)	–1.6(3)	–0.1(3)	–10.1(3)

TABLE 8.4 Bond lengths for artabotrine.

Atom	Atom	Length (Å)
N1	C2	1.3654(14)
N1	C12A	1.4074(13)
N1	O13	1.3944(11)
C2	C3	1.5320(14)
C2	O15	1.2185(13)
C3B	C3A	1.4127(15)
C3B	C7B	1.4297(14)
C3B	C12A	1.4374(14)
C3	C3A	1.4591(14)
C3	O16	1.2174(14)
C3A	C4	1.4049(14)
C4A	C4	1.3672(15)
C4A	O5	1.3681(12)
C4A	C7A	1.3893(15)
O5	C6	1.4374(14)
C6	O7	1.4445(13)
C7B	C7C	1.4526(15)
C7B	C7A	1.3967(14)
C7C	C8	1.4105(14)
C7C	C11A	1.4162(14)
C7A	O7	1.3587(12)
C8	C9	1.3793(16)
C9	C10	1.4043(16)
C10	C11	1.3750(15)
C11	C11A	1.4113(15)
C11A	C12	1.4287(14)
C12A	C12	1.3570(15)
O13	C14	1.4475(13)

TABLE 8.5 Bond angles for artabotrine.

Atom	Atom	Atom	Angle (˚)
C2	N1	C12A	127.08(9)
C2	N1	O13	116.29(8)
O13	N1	C12A	116.50(8)
N1	C2	C3	115.45(9)
O15	C2	N1	122.99(10)
O15	C2	C3	121.56(10)
C3A	C3B	C7B	121.52(9)
C3A	C3B	C12A	120.94(9)
C7B	C3B	C12A	117.53(9)
C3A	C3	C2	118.55(9)
O16	C3	C2	117.08(9)
O16	C3	C3A	124.36(10)
C3B	C3A	C3	120.12(9)
C4	C3A	C3B	121.60(10)
C4	C3A	C3	118.26(10)
C4	C4A	O5	127.29(10)
C4	C4A	C7A	122.64(9)
O5	C4A	C7A	110.07(9)
C4A	C4	C3A	116.48(10)
C4A	O5	C6	106.34(8)
O5	C6	O7	106.97(8)
C3B	C7B	C7C	121.06(9)

TABLE 8.5 Bond angles for artabotrine (continued).

Atom	Atom	Atom	Angle (˚)
C7A	C7B	C3B	114.32(9)
C7A	C7B	C7C	124.61(10)
C8	C7C	C7B	123.73(9)
C8	C7C	C11A	118.27(10)
C11A	C7C	C7B	117.99(9)
C4A	C7A	C7B	123.44(10)
O7	C7A	C4A	109.67(9)
O7	C7A	C7B	126.88(10)
C7A	O7	C6	106.79(8)
C9	C8	C7C	120.80(10)
C8	C9	C10	120.66(10)
C11	C10	C9	119.69(10)
C10	C11	C11A	120.57(10)
C7C	C11A	C12	120.37(10)
C11	C11A	C7C	119.94(10)
C11	C11A	C12	119.68(9)
N1	C12A	C3B	117.68(9)
C12	C12A	N1	120.28(9)
C12	C12A	C3B	122.04(9)
C12A	C12	C11A	120.98(9)
N1	O13	C14	110.79(7)

TABLE 8.6 Torsion angles for artabotrine.

A	B	C	D	Angle (˚)
N1	C2	C3	C3A	4.09(14)
N1	C2	C3	O16	–176.32(9)
N1	C12A	C12	C11A	179.54(9)
C2	N1	C12A	C3B	–2.42(15)
C2	N1	C12A	C12	177.27(9)
C2	N1	O13	C14	–87.92(10)
C2	C3	C3A	C3B	–4.42(14)
C2	C3	C3A	C4	177.41(9)
C3B	C3A	C4	C4A	0.09(15)
C3B	C7B	C7C	C8	–179.70(9)
C3B	C7B	C7C	C11A	–0.86(14)
C3B	C7B	C7A	C4A	–0.07(15)
C3B	C7B	C7A	O7	179.21(9)
C3B	C12A	C12	C11A	–0.78(15)
C3	C3A	C4	C4A	178.24(9)
C3A	C3B	C7B	C7C	178.41(9)
C3A	C3B	C7B	C7A	–0.26(14)
C3A	C3B	C12A	N1	2.12(14)
C3A	C3B	C12A	C12	–177.57(9)
C4A	O5	C6	O7	–3.80(11)
C4A	C7A	O7	C6	–2.80(11)
C4	C4A	O5	C6	–178.51(10)
C4	C4A	C7A	C7B	0.44(17)
C4	C4A	C7A	O7	–178.95(9)
O5	C4A	C4	C3A	–179.67(10)
O5	C4A	C7A	C7B	179.79(9)
O5	C4A	C7A	O7	0.41(12)
O5	C6	O7	C7A	4.07(11)

TABLE 8.6 Torsion angles for artabotrine (continued).

A	B	C	D	Angle (˚)
C7B	C3B	C3A	C3	–177.85(9)
C7B	C3B	C3A	C4	0.26(15)
C7B	C3B	C12A	N1	–178.70(8)
C7B	C3B	C12A	C12	1.61(15)
C7B	C7C	C8	C9	176.49(9)
C7B	C7C	C11A	C11	–177.05(9)
C7B	C7C	C11A	C12	1.73(14)
C7B	C7A	O7	C6	177.84(10)
C7C	C7B	C7A	C4A	–178.69(9)
C7C	C7B	C7A	O7	0.59(17)
C7C	C8	C9	C10	0.68(16)
C7C	C11A	C12	C12A	–0.95(15)
C7A	C4A	C4	C3A	–0.43(16)
C7A	C4A	O5	C6	2.18(11)
C7A	C7B	C7C	C8	–1.17(16)
C7A	C7B	C7C	C11A	177.67(9)
C8	C7C	C11A	C11	1.85(14)
C8	C7C	C11A	C12	–179.36(9)
C8	C9	C10	C11	1.55(16)
C9	C10	C11	C11A	–2.04(15)
C10	C11	C11A	C7C	0.32(15)
C10	C11	C11A	C12	–178.47(9)
C11	C11A	C12	C12A	177.83(9)
C11A	C7C	C8	C9	–2.35(15)
C12A	N1	C2	C3	–0.70(15)
C12A	N1	C2	O15	179.43(9)

TABLE 8.6 Torsion angles for artabotrine (continued).

A	B	C	D	Angle (˚)
C12A	N1	O13	C14	95.91(10)
C12A	C3B	C3A	C3	1.29(15)
C12A	C3B	C3A	C4	179.40(9)
C12A	C3B	C7B	C7C	–0.76(14)
C12A	C3B	C7B	C7A	–179.43(9)
O13	N1	C2	C3	–176.41(8)
O13	N1	C2	O15	3.73(15)
O13	N1	C12A	C3B	173.27(8)
O13	N1	C12A	C12	–7.03(13)
O15	C2	C3	C3A	–176.04(9)
O15	C2	C3	O16	3.54(16)
O16	C3	C3A	C3B	176.03(10)
O16	C3	C3A	C4	–2.14(16)

TABLE 8.7 Hydrogen atom coordinates (Å × 10^4) and isotropic displacement parameters (Å^2 × 10^3) for artabotrine.

Atom	*x*	*y*	*z*	U(eq)
H4	28	–2967	5695	22
H6A	2812	–1040	7934	25
H6B	1205	–128	7842	25
H8	4100	1916	6533	23
H9	5829	3685	6296	25
H10	6451	4234	4958	25
H11	5236	3058	3858	23
H12	3511	1242	3309	21
H14A	3322	–543	1594	34
H14B	3139	–1848	2148	34
H14C	4251	–625	2481	34

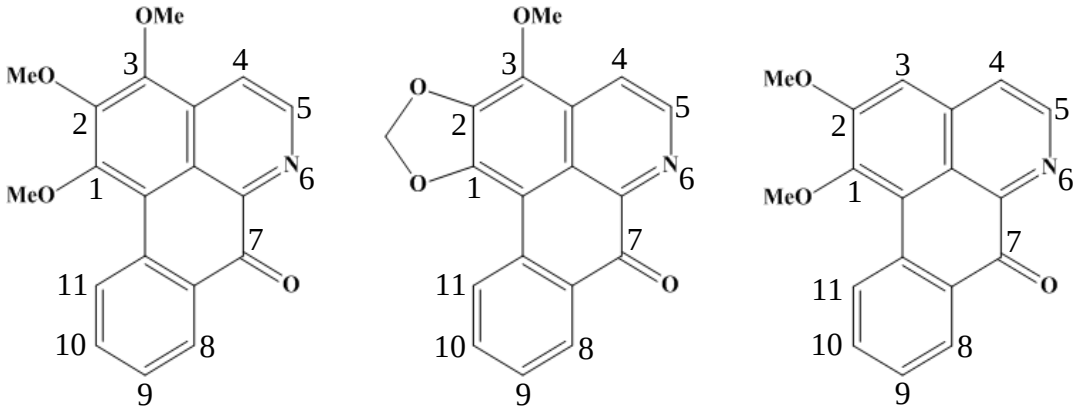


FIGURE 8.2 Chemical structures of liridine, atherospermidine and lysicamine.

Liridine (**2**): $C_{19}H_{15}NO_4$, Orange amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (1H, d, H-11), 9.01 (1H, d, H-5), 8.62 (1H, dd, H-8), 8.26 (1H, d, H-4), 7.78 (1H, ddd, H-10), 7.58 (1H, ddd, H-9), 4.23 (3H, s, 3-OMe), 4.14 (3H, s, 2-OMe), 4.11 (3H, s, 1-OMe) (Figure 8.3) (Li *et al.* 2009; Costa *et al.* 2011b).

Atherospermidine (**3**): $C_{18}H_{11}NO_4$, Orange amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (1H, d, H-5), 8.62 (1H, dd, H-8), 8.62 (1H, d, H-11), 8.22 (1H, d, H-4), 7.77 (1H, ddd, H-10), 7.56 (1H, ddd, H-9), 6.37 (2H, s, 1-OCH_2O-2), 4.34 (3H, s, 3-OMe) (Figure 8.4) (Ortiz *et al.* 2007; Costa *et al.* 2011b).

Lysicamine (**4**): $C_{18}H_{13}NO_3$, Yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (1H, d, H-11), 8.96 (1H, d, H-5), 8.63 (1H, dd, H-8), 7.85 (1H, d, H-4), 7.81 (1H, ddd, H-10), 7.62 (1H, ddd, H-9), 7.28 (1H, s, H-3), 4.15 (3H, s, 2-OMe), 4.06 (3H, s, 1-OMe) (Figure 8.5) (Husain *et al.* 2012; Lin *et al.* 2014).

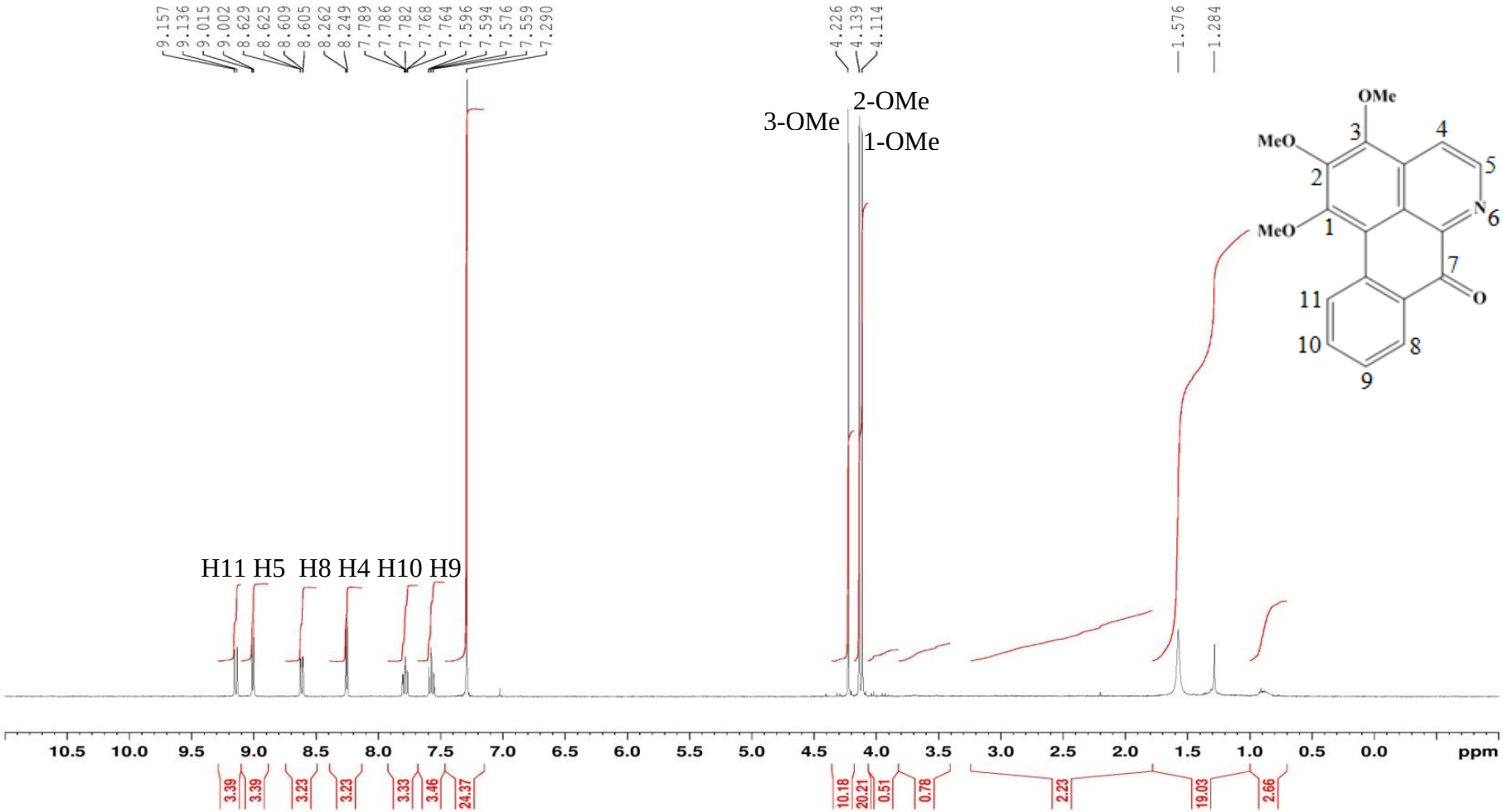


FIGURE 8.3 ^{1}H NMR spectrum of liridine.

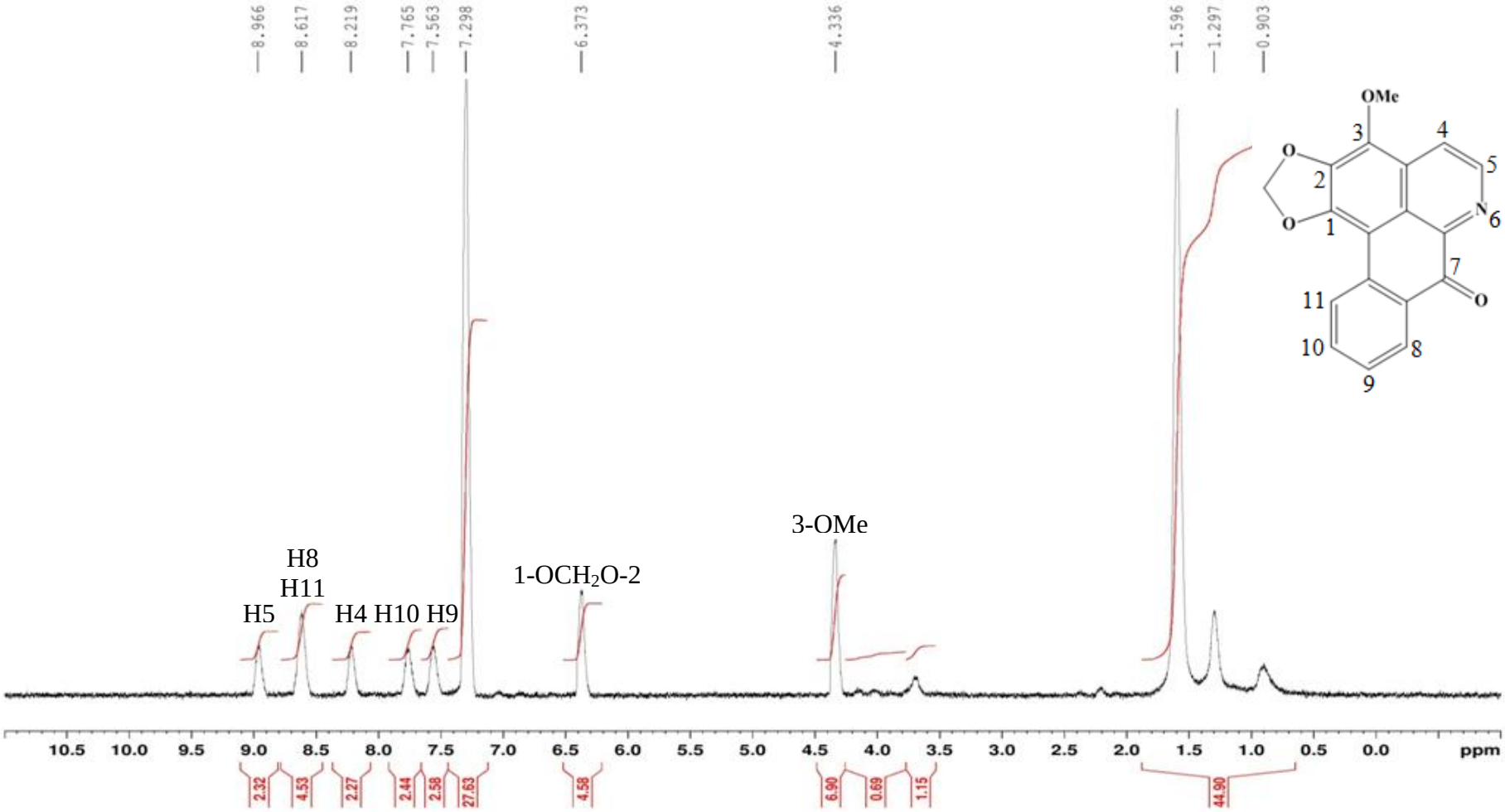


FIGURE 8.4 ^{1}H NMR spectrum of atherospermidine.

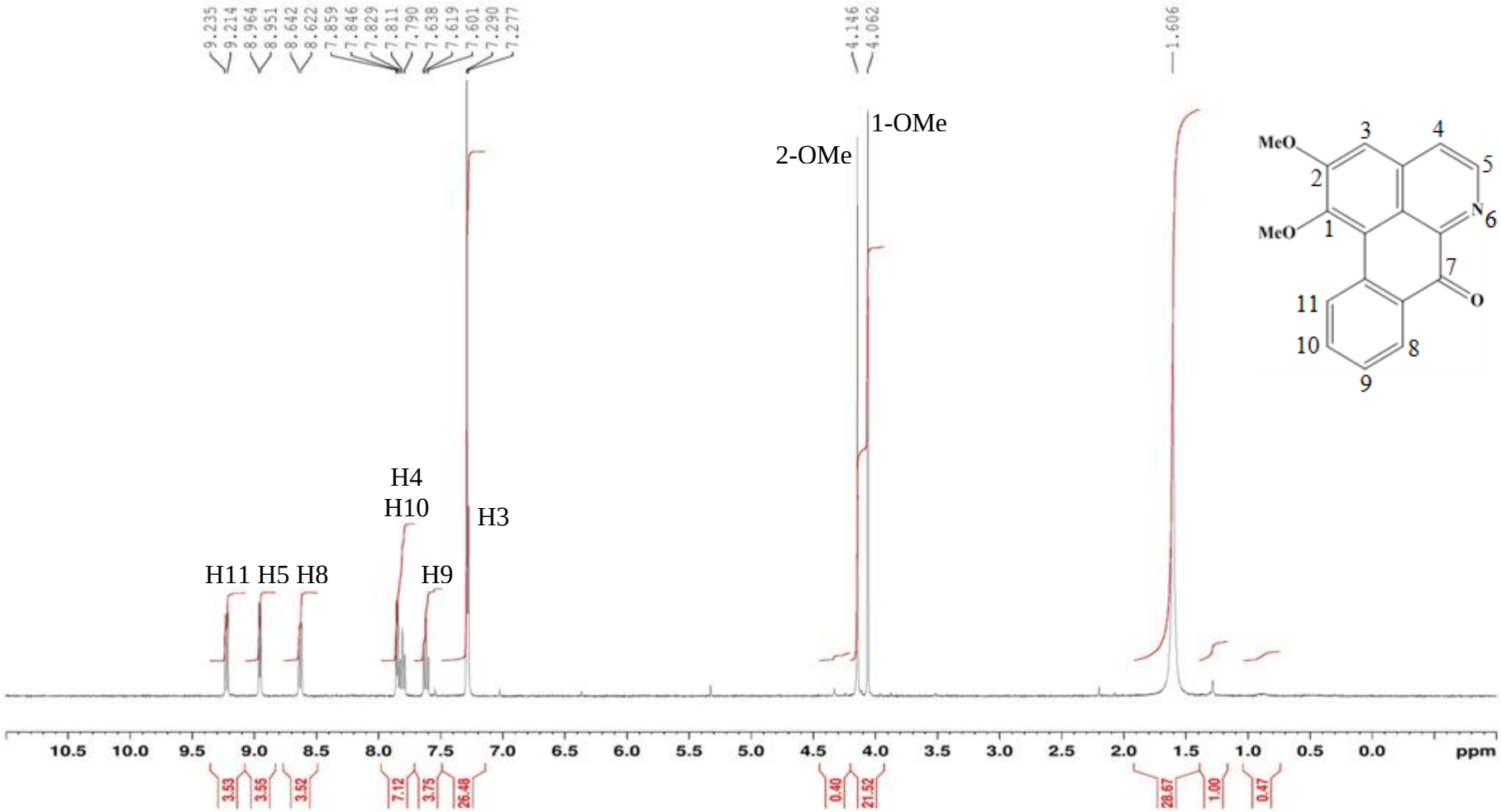


FIGURE 8.5 ^{1}H NMR spectrum of lysicamine.

8.3.2 Minimum inhibitory concentrations of isolated compounds from *Artabotrys crassifolius*

The cut-off point for antibacterial compounds was suggested to be 25 µM (Cos *et al.* 2006). According to Kuete (2010), the antibacterial activity of pure compounds could be classified into three categories: high (MIC value less than 10 µg/mL), moderate (MIC value ranging from 10 µg/mL to 100 µg/mL) and low (MIC value more than 100 µg/mL). Based on this categorisation, artabotrine (**1**) demonstrated high antibacterial properties with MIC values ranging from 1.25 µg/mL to 5 µg/mL against all of the tested ATCC and clinical bacterial strains except for *Actinobacillus* sp. and *Klebsiella* sp. (Table 8.8–8.9).

Furthermore, liridine (**2**) and lysicamine (**4**) strongly inhibited the growth of *B. subtilis* ATCC 21332, *M. luteus* ATCC 10240 and *R. equi* ATCC 33701 with MIC values ranging from 0.625 µg/mL to 2.5 µg/mL and 5 µg/mL to 10 µg/mL respectively. Different observation was reported by Costa *et al.* (2011a), in which liridine and lysicamine (*Guatteria blepharophylla*) exhibited no significant activity against *B. subtilis* ATCC 5061, *M. luteus* ATCC 4698 and *R. equi* ATCC 6939. This implies that the susceptibility of the tested bacteria to the corresponding compounds may be strain-specific.

Nonetheless, atherospermidine (**3**) showed a MIC value of greater than 20 µg/mL when tested on ATCC and clinical bacterial strains, which was less active as compared to that of the positive control, streptomycin sulphate (MIC values ranging from 0.3125 µg/mL to 20 µg/mL).

TABLE 8.8 Minimum inhibitory concentrations of isolated compounds from *Artabotrys crassifolius* against ATCC strains.

Microorganism	Minimum inhibitory concentration (MIC) (µg/mL)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
B. cereus ATCC 10876	1.25	5	>20	10	2.5
B. subtilis ATCC 21332	2.5	0.625	>20	10	0.625
L. monocytogenes ATCC 15313	1.25	1.25	>20	2.5	0.3125
M. luteus ATCC 10240	5	1.25	>20	10	1.25
P. vulgaris ATCC 13315	1.25	>20	>20	10	5
R. equi ATCC 33701	1.25	2.5	>20	5	1.25
S. aureus ATCC 11632	2.5	>20	>20	>20	10

Note: Results are from three independent experiments performed in triplicate, n = 9.

TABLE 8.9 Minimum inhibitory concentrations of isolated compounds from *Artabotrys crassifolius* against clinical strains.

Microorganism	Minimum inhibitory concentration (MIC) (µg/mL)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
MSSA	5	>20	>20	20	10
ORCNS	1.25	>20	>20	20	2.5
OSCNS	2.5	1.25	>20	10	0.3125
S. agalactiae	5	1.25	>20	5	1.25
S. pneumoniae	2.5	10	>20	2.5	0.3125
GRAM-NEGATIVE BACTERIA					
Actinobacillus sp.	>20	>20	>20	>20	20
ESBL-KP	2.5	2.5	>20	10	0.3125
Klebsiella sp.	>20	>20	>20	>20	>20

Note: Results are from three independent experiments performed in triplicate, n = 9.

8.3.3 Minimum bactericidal concentrations of isolated compounds from *Artabotrys crassifolius*

The minimum bactericidal concentrations of isolated compounds are given in Table 8.10–8.11. All the isolated compounds, with the exception of atherospermidine (**3**), displayed MBC values ranging from 0.625 µg/mL to 20 µg/mL against most of the tested ATCC and clinical bacterial strains, which were comparable to that of the positive control, streptomycin sulphate (MBC values ranging from 0.3125 µg/mL to 20 µg/mL).

According to Krishnan *et al.* (2010), antibacterial compounds could be categorised into two classes: bacteriostatic (MBC/MIC ratio more than 4) and bactericidal (MBC/MIC ratio less than or equal to 4). Under this classification, artabotrine (**1**) exerted bactericidal activity against *B. cereus* ATCC 10876, *B. subtilis* ATCC 21332, *L. monocytogenes* ATCC 15313, *M. luteus* ATCC 10240, *P. vulgaris* ATCC 13315, *R. equi* ATCC 33701, MSSA, OSCNS, *S. agalactiae, S. pneumoniae* and ESBL-KP with MBC/MIC ratios ranging from 1 to 4 (Table 8.12–8.13). However, the respective compound was bacteriostatic for both *S. aureus* ATCC 11632 and ORCNS with MBC/MIC ratio of 8.

In addition, liridine (**2**) and lysicamine (**4**) presented equivalent MIC and MBC values (MBC/MIC ratio of 1) against *B. subtilis* ATCC 21332, *L. monocytogenes* ATCC 15313, *S. agalactiae* and *S. pneumoniae*. This suggests that the corresponding compounds may possess absolute bactericidal effects on the tested bacteria.

TABLE 8.10 Minimum bactericidal concentrations of isolated compounds from *Artabotrys crassifolius* against ATCC strains.

Microorganism	Minimum bactericidal concentration (MBC) (µg/mL)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
B. cereus ATCC 10876	1.25	10	>20	10	2.5
B. subtilis ATCC 21332	2.5	0.625	>20	10	0.625
L. monocytogenes ATCC 15313	2.5	1.25	>20	2.5	0.3125
M. luteus ATCC 10240	5	2.5	>20	10	1.25
P. vulgaris ATCC 13315	5	>20	>20	10	5
R. equi ATCC 33701	2.5	5	>20	10	1.25
S. aureus ATCC 11632	20	>20	>20	>20	20

Note: Results are from three independent experiments performed in triplicate, n = 9.

TABLE 8.11 Minimum bactericidal concentrations of isolated compounds from *Artabotrys crassifolius* against clinical strains.

Microorganism	Minimum bactericidal concentration (MBC) (µg/mL)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
MSSA	20	>20	>20	20	10
ORCNS	10	>20	>20	>20	2.5
OSCNS	5	2.5	>20	20	0.625
S. agalactiae	5	1.25	>20	5	1.25
S. pneumoniae	2.5	10	>20	2.5	0.3125
GRAM-NEGATIVE BACTERIA					
Actinobacillus sp.	>20	>20	>20	>20	>20
ESBL-KP	2.5	5	>20	20	0.625
Klebsiella sp.	>20	>20	>20	>20	>20

Note: Results are from three independent experiments performed in triplicate, n = 9.

TABLE 8.12 MBC/MIC ratios of isolated compounds from *Artabotrys crassifolius* against ATCC strains.

Microorganism	MBC/MIC ratio				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
B. cereus ATCC 10876	1	2	NA	1	1
B. subtilis ATCC 21332	1	1	NA	1	1
L. monocytogenes ATCC 15313	2	1	NA	1	1
M. luteus ATCC 10240	1	2	NA	1	1
P. vulgaris ATCC 13315	4	NA	NA	1	1
R. equi ATCC 33701	2	2	NA	2	1
S. aureus ATCC 11632	8	NA	NA	NA	2

Note: NA indicates not available.

TABLE 8.13 MBC/MIC ratios of isolated compounds from *Artabotrys crassifolius* against clinical strains.

Microorganism	MBC/MIC ratio				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Streptomycin sulphate
GRAM-POSITIVE BACTERIA					
MSSA	4	NA	NA	1	1
ORCNS	8	NA	NA	NA	1
OSCNS	2	2	NA	2	2
S. agalactiae	1	1	NA	1	1
S. pneumoniae	1	1	NA	1	1
GRAM-NEGATIVE BACTERIA					
Actinobacillus sp.	NA	NA	NA	NA	NA
ESBL-KP	1	2	NA	2	2
Klebsiella sp.	NA	NA	NA	NA	NA

Note: NA indicates not available.

8.3.4 Anticancer effects of isolated compounds from *Artabotrys crassifolius*

The cut-off point for anticancer compounds was suggested to be 10 µM (Brahemi *et al.* 2010). According to the American National Cancer Institute (NCI), pure compounds could be considered as active for a GI_{50} value of less than 4 µg/mL (Abu-Dahab and Afifi 2007).

Based on the NCI criterion, artabotrine (**1**) was highly active in HCT-116 colorectal and MCF-7 breast carcinoma cell lines with GI_{50} values of 3.34 µM (1.07 µg/mL) and 3.49 µM (1.12 µg/mL) respectively (Figure 8.6–8.7; Appendix F). This was in accordance with the study of Wijeratne *et al.* (1995), where artabotrine (*Artabotrys zeylanicus*) exhibited strong inhibitory effects on both camptothecin-resistance and wild-type P388 leukemia cell lines with respective GI_{50} values of 1.12 µM and 1.59 µM. However, Ding *et al.* (2006) showed that artabotrine (synthetic compound) was less effective against HeLa cervical, BEL-7404 hepatocellular, A549 lung, CNE nasopharyngeal, KB oral and PC-3 prostate carcinoma cell lines.

Moreover, lysicamine (**4**) potently inhibited the growth of HCT-116 colorectal and MCF-7 breast carcinoma cell lines with GI_{50} values of 3.44 µM (1 µg/mL) and 3.93 µM (1.14 µg/mL) respectively. Different results were obtained by Silva *et al.* (2007), Nakano *et al.* (2013), Omar *et al.* (2013) and Kang *et al.* (2014), who reported moderate activity of lysicamine (*Unonopsis lindmanii*, *Annona reticulata*, *Annona squamosa*, *Phoebe grandis* and *Liriodendron tulipifera*) against HEp-2 laryngeal, MT-1 and MT-2 leukaemia, MCF-7 breast, Hep G2 hepatocellular carcinoma and A375 melanoma cell lines with GI_{50} values ranging from 16.25 µM to 103.6 µM.

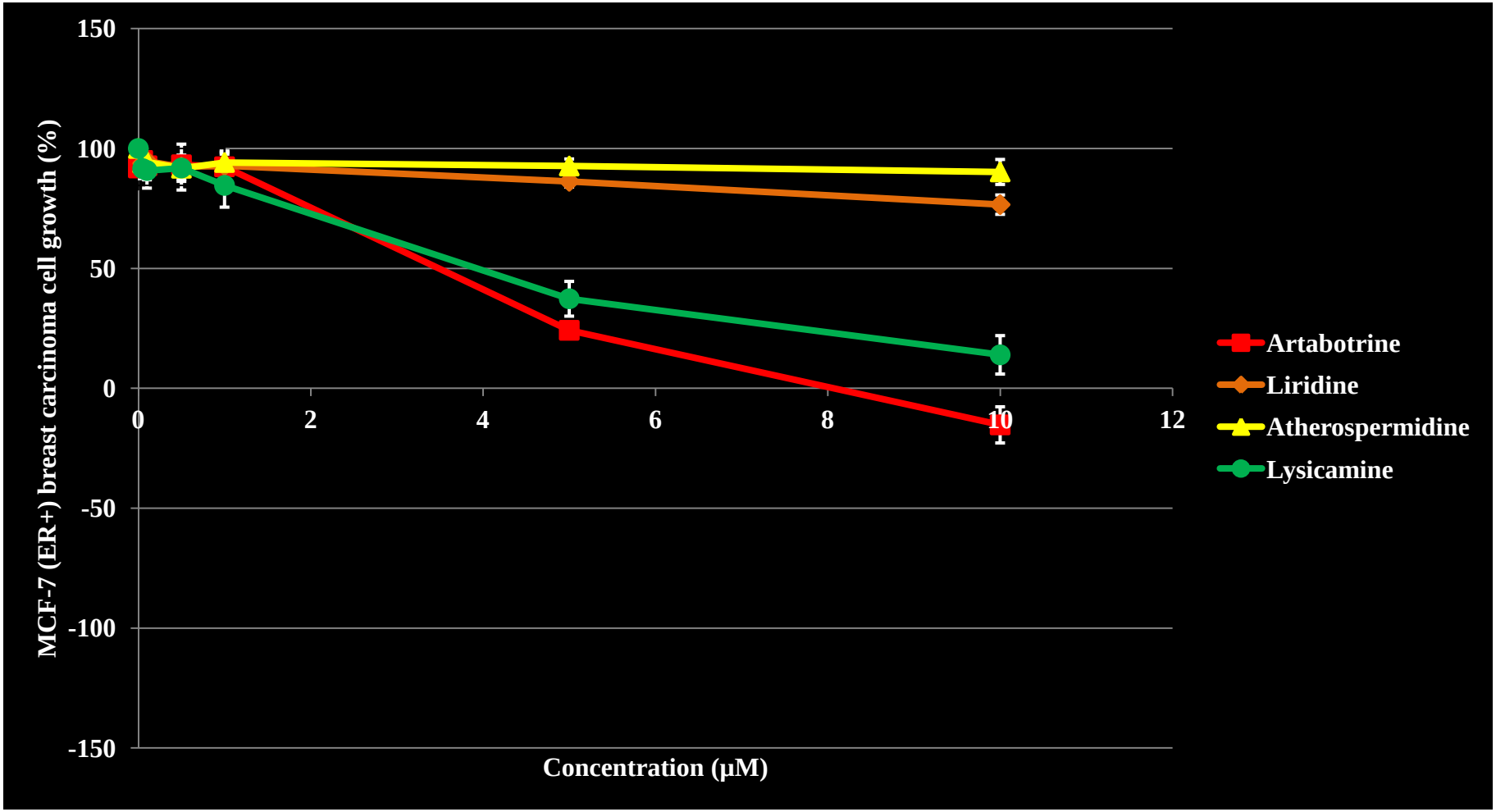


FIGURE 8.6 Anticancer effects of isolated compounds from *Artabotrys crassifolius* against MCF-7 (ER+) breast carcinoma cell line. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

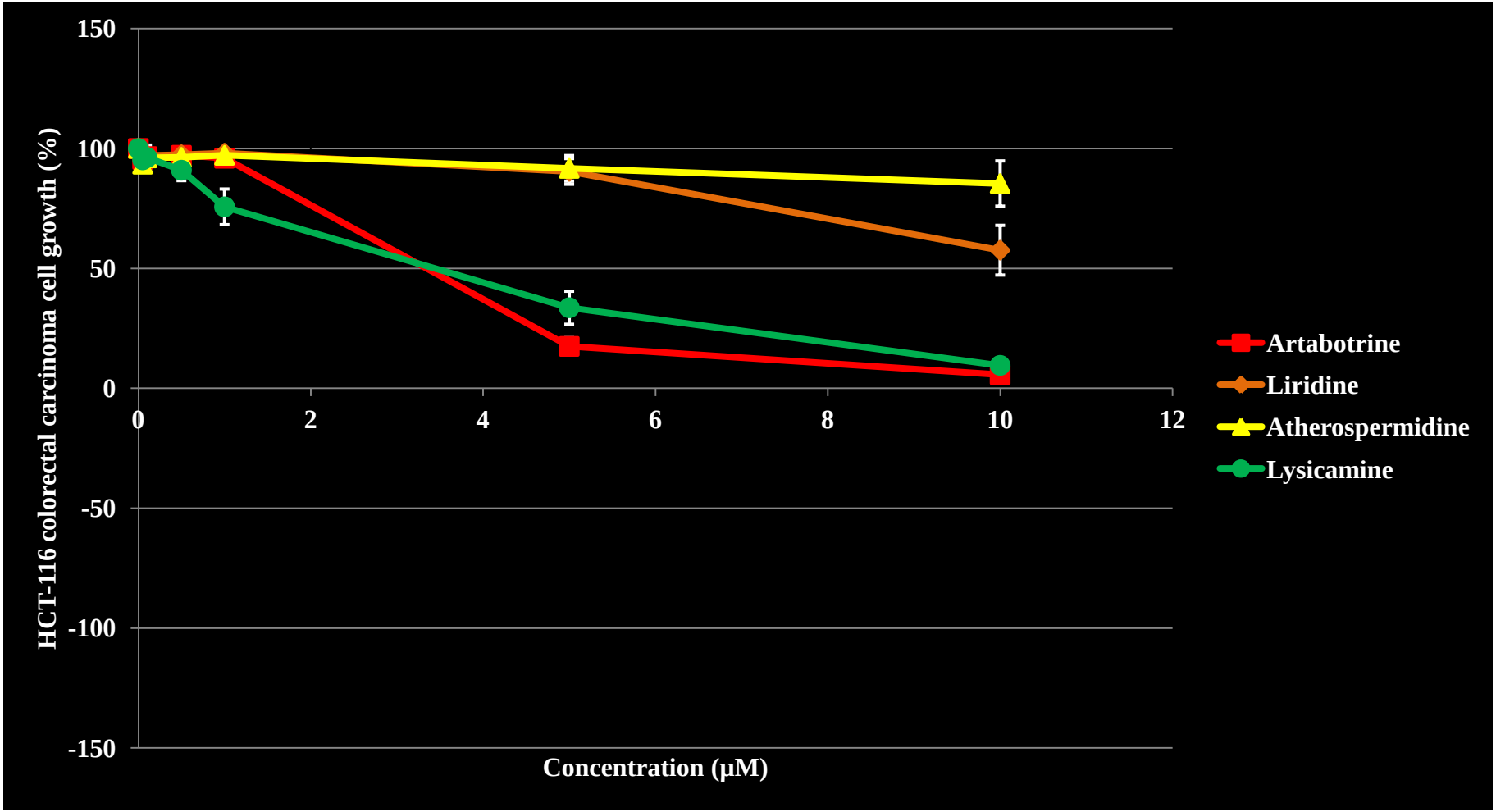


FIGURE 8.7 Anticancer effects of isolated compounds from *Artabotrys crassifolius* against HCT-116 colorectal carcinoma cell line. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

Nevertheless, the GI_{50} value of liridine (**2**) was found to be higher than 10 μM when tested on MCF-7 breast and HCT-116 colorectal carcinoma cell lines. Similar works were done by Osorio *et al.* (2006), Silva *et al.* (2007), Sichaem *et al.* (2011) and Liu *et al.* (2014), in which liridine (*Rollinia pittieri*, *Duguetia glabriuscula*, *Artabotrys spinosus* and *Polyalthia plagioneura*) displayed moderate activity against U937 leukaemia, HEp-2 laryngeal, HeLa cervical, KB oral and SGC-7901 gastric carcinoma cell lines with GI_{50} values ranging from 12.4 μM to 121.78 μM.

Similarly, atherospermidine (**3**) gave a GI_{50} value of more than 10 μM for both MCF-7 breast and HCT-116 colorectal carcinoma cell lines. This was in contrary to the findings of Wu *et al.* (1989) and Hsieh *et al.* (2001), who observed significant inhibitory effects of atherospermidine (*Artabotrys uncinatus*) on KB oral, Hep 2,2,15 and Hep G2 hepatocellular carcinoma cell lines with respective GI_{50} values of 2.5 μg/mL, 2.2 μg/mL and 0.8 μg/mL. However, Osorio *et al.* (2006) demonstrated that atherospermidine (*Pseudomalmea boyacana*) was less potent against U937 leukaemia cell line with a GI_{50} value of 10 μg/mL.

Considering the structures of the isolated compounds, the presence of N-methoxy group may play an important role in the pharmacological properties of artabotrine (**1**) as most of the reported 4,5-dioxoaporphines were N-methylated or unsubstituted. Additionally, liridine (**2**), atherospermidine (**3**) and lysicamine (**4**) possessed the same basic skeleton with different substitution patterns, where methoxy substituent at the C-3 position may reduce the inhibitory effect of liridine (**2**) or even result in an inactive compound as atherospermidine (**3**), while hydrogen substituent at the same position may enhance the pharmacological action of lysicamine (**4**).

8.4 CONCLUSION

Exploration of the *in vitro* pharmacological activity of isolated compounds from *Artabotrys crassifolius* revealed that artabotrine may be a potential therapeutic agent in view of its dual-acting antibacterial and anticancer properties. Hence, further studies are required to elucidate the mechanisms underlying the observed inhibitory effect of artabotrine.

CHAPTER IX

CONCLUSION AND FUTURE PERSPECTIVES

Being as one of the most evolved and complex ecosystems in the world, the tropical rainforest of Malaysia serves a vast untapped biodiversity of natural resources. This has led to the current investigation on the *in vitro* antibacterial, antifungal, anticancer and antioxidant activities of *Artabotrys crassifolius*.

Among the crude extracts evaluated, hexane and chloroform extracts of bark displayed promising antibacterial activities against ATCC and clinical strains with zones of inhibition ranging from 8.23±0.25 mm to 13.70±0.26 mm and 7.75±0.25 mm to 13.68±0.28 mm respectively. However, all the crude extracts were shown to be devoid of antifungal activity except for hexane extract of bark which was able to inhibit the growth of the tested *Candida* species with zones of inhibition ranging from 7.81±0.27 mm to 9.77±0.25 mm. Additionally, chloroform extract of bark was highly active against all of the tested carcinoma cell lines with GI_{50} values ranging from 4.23 µg/mL to 9.45 µg/mL, whereas hexane extract of bark strongly inhibited the growth of MDA-468 breast and HCT-116 colorectal carcinoma cell lines with respective GI_{50} values of 6.10 µg/mL and 16.45 µg/mL. Moreover, ethanol extract of bark that possessed the highest total phenolic and flavonoid contents (268.29±12.36 mg GAE/g and 179.54±4.98 mg CE/g) was found to demonstrate pronounced scavenging activities against ABTS cation and DPPH radicals with IC_{50} values of 16.50 µg/mL and 16.54 µg/mL respectively, as well as extremely high antioxidant power with FRAP value of 1884.35±83.78 µmol Fe(II)/g.

The chromatographic separation of chloroform extract of bark led to the isolation of four alkaloids, namely artabotrine, liridine, atherospermidine and lysicamine. Among the compounds isolated, artabotrine exhibited high antibacterial properties with respective MIC and MBC values ranging from 1.25 µg/mL to 5 µg/mL and 1.25 µg/mL to 20 µg/mL against all of the tested ATCC and clinical bacterial strains, with the exception of *Actinobacillus* sp. and *Klebsiella* sp.. Furthermore, artabotrine was highly active in HCT-116 colorectal and MCF-7 breast carcinoma cell lines with GI_{50} values of 3.34 µM and 3.49 µM respectively.

As a conclusion, exploration of the *in vitro* pharmacological properties of *Artabotrys crassifolius* revealed that artabotrine with dual antibacterial and anticancer activities may represent a new generation of potential drug candidates for the treatment of bacterial infections and cancer. Therefore, further *in vivo* studies and clinical trials are needed to ascertain the efficacy, safety and mechanisms of action of artabotrine prior to application in the pharmaceutical industry as natural therapeutic agents.

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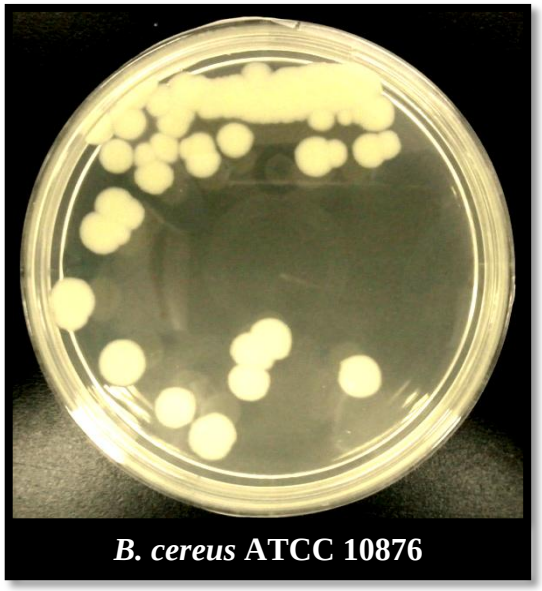
APPENDIX A

Extraction yields of crude extracts of *Artabotrys crassifolius*.

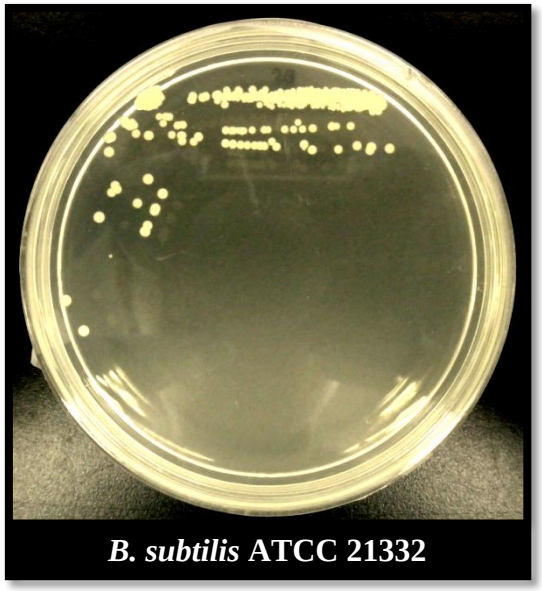
Extraction yield	Crude extract					
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol
Extraction yield (g)	25.87	16.18	65.03	25.45	53.92	192.38
Extraction yield (%)	1.99	1.24	5.00	0.53	1.13	4.02

APPENDIX B1

Streak plates of ATCC bacterial strains.



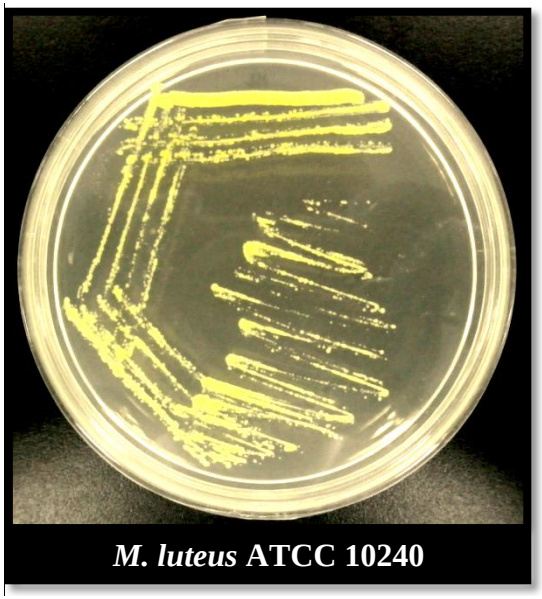
***B. cereus* ATCC 10876**



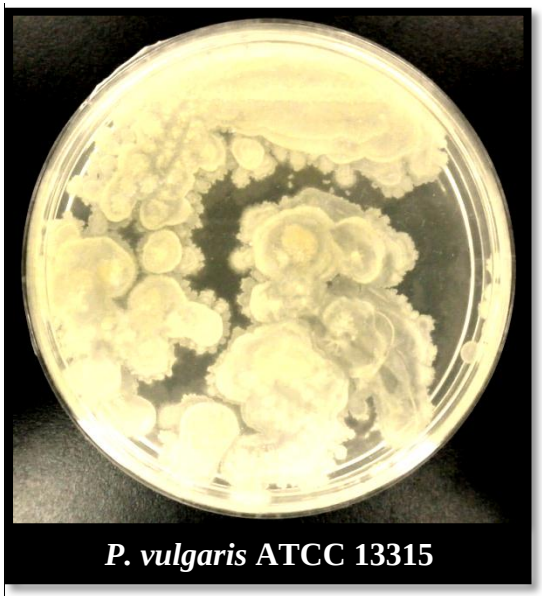
***B. subtilis* ATCC 21332**

***L. monocytogenes* ATCC 15313**

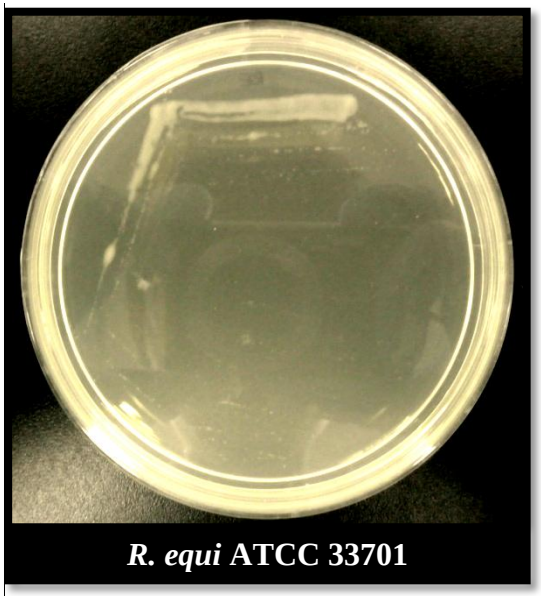
Streak plates of ATCC bacterial strains (continued).



***M. luteus* ATCC 10240**

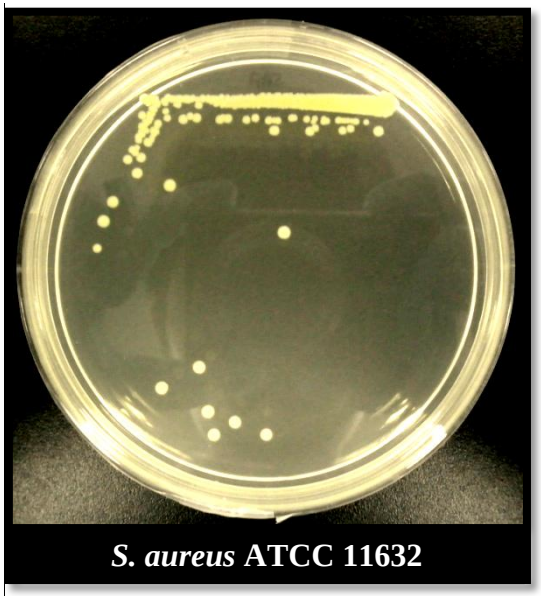


***P. vulgaris* ATCC 13315**



***R. equi* ATCC 33701**

Streak plates of ATCC bacterial strains (continued).

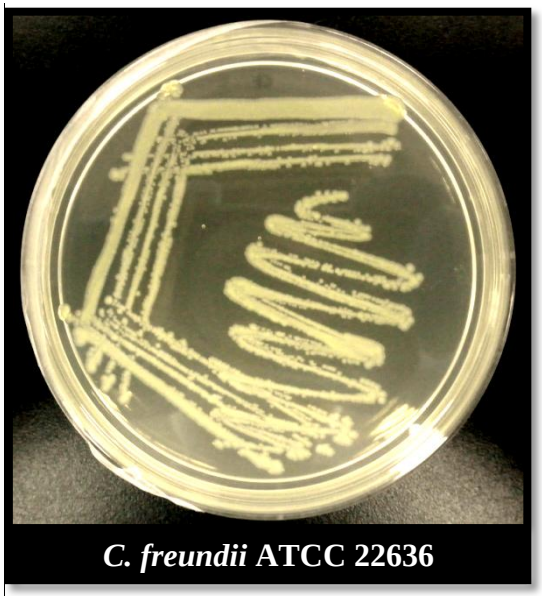


***S. aureus* ATCC 11632**

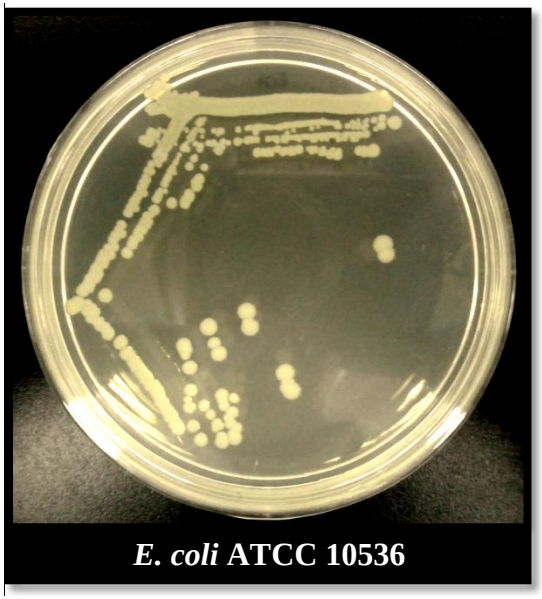
***S. epidermidis* ATCC 12228**

***S. pyogenes* ATCC 19615**

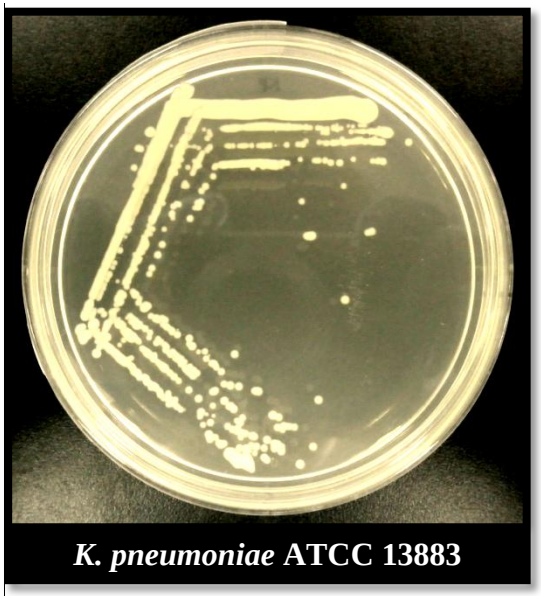
Streak plates of ATCC bacterial strains (continued).



***C. freundii* ATCC 22636**

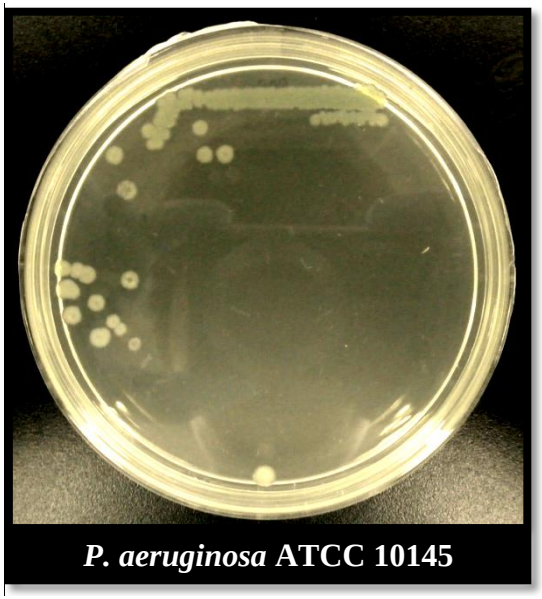


***E. coli* ATCC 10536**

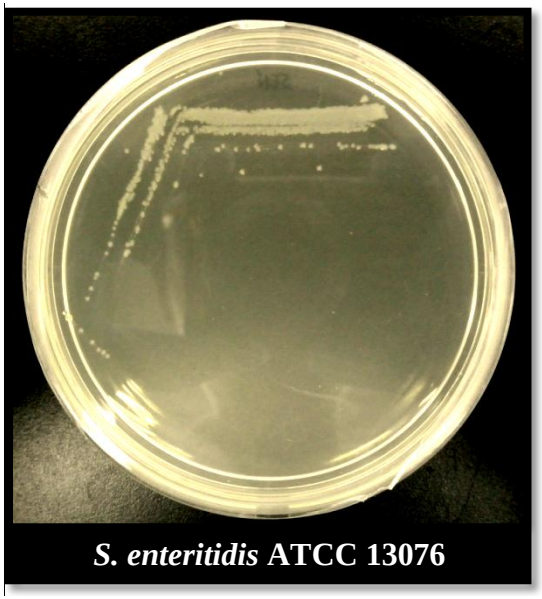


***K. pneumoniae* ATCC 13883**

Streak plates of ATCC bacterial strains (continued).



***P. aeruginosa* ATCC 10145**

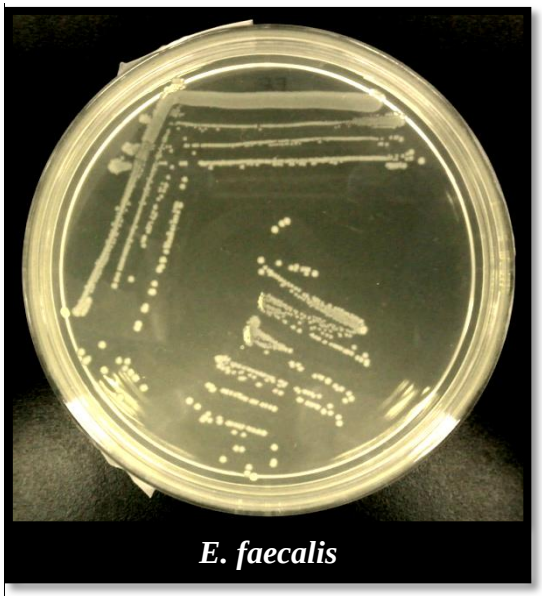


***S. enteritidis* ATCC 13076**

***S. typhimurium* ATCC 14028**

APPENDIX B2

Streak plates of clinical bacterial strains.

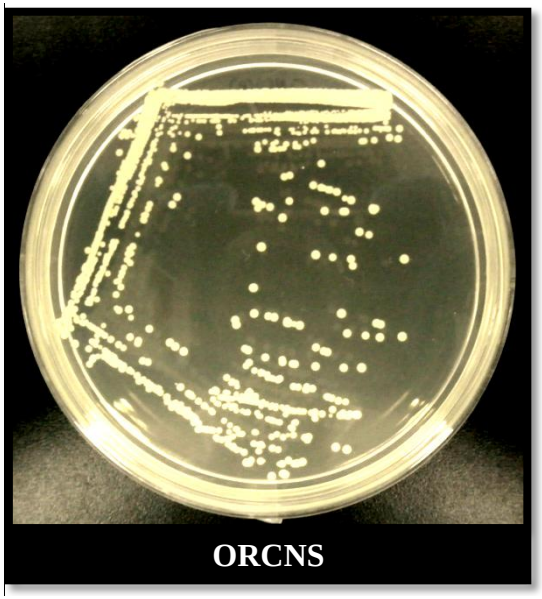


E. faecalis

MRSA

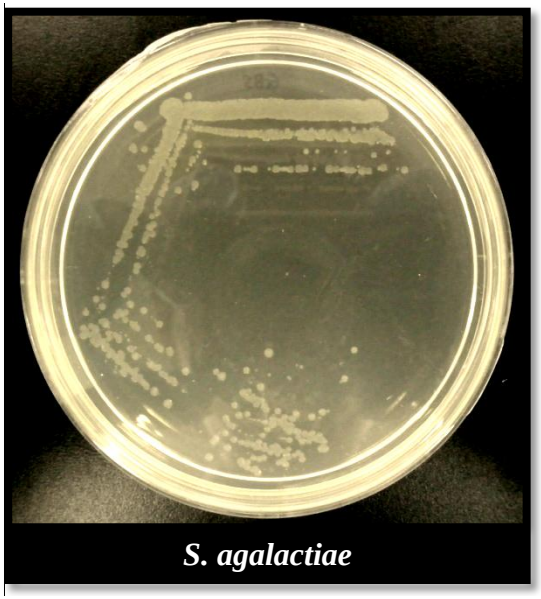
MSSA

Streak plates of clinical bacterial strains (continued).



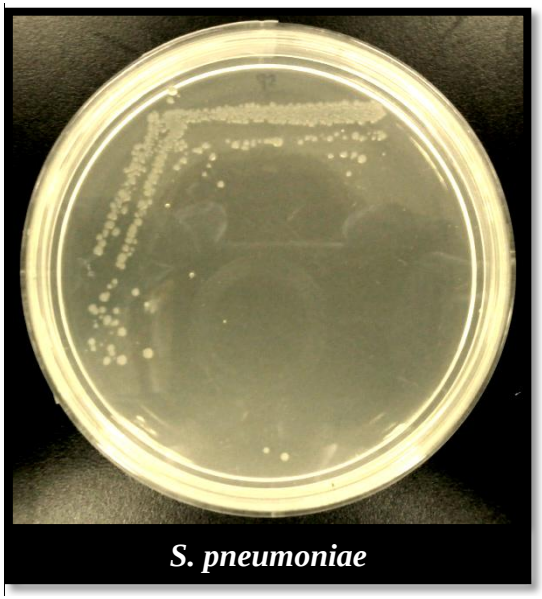
ORCNS

OSCNS

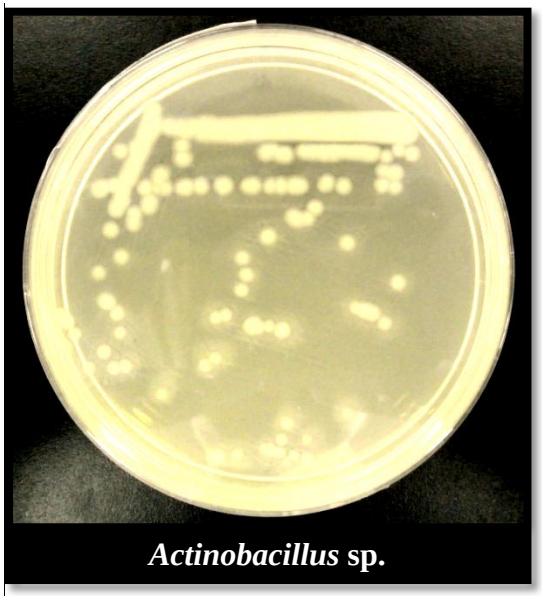


S. agalactiae

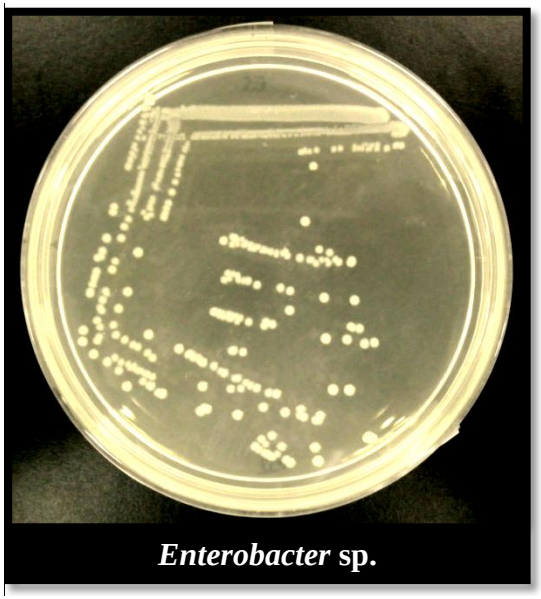
Streak plates of clinical bacterial strains (continued).



S. pneumoniae

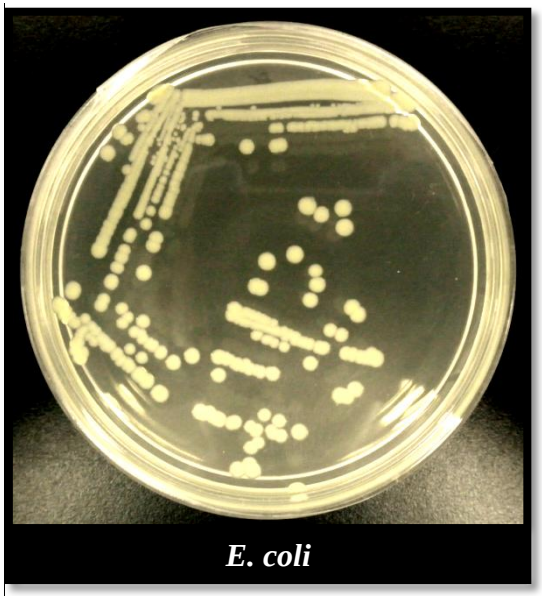


***Actinobacillus* sp.**



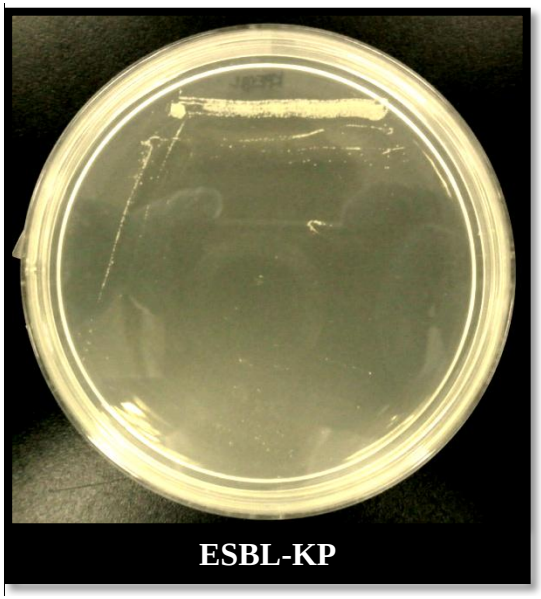
***Enterobacter* sp.**

Streak plates of clinical bacterial strains (continued).



E. coli

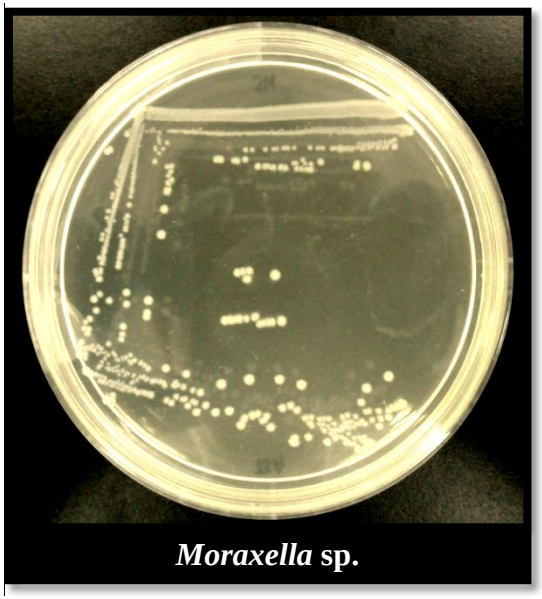
ESBL-EC



ESBL-KP

Streak plates of clinical bacterial strains (continued).

***Klebsiella* sp.**



***Moraxella* sp.**

***Serratia* sp.**

APPENDIX B3

Antibacterial activities of crude extracts of *Artabotrys crassifolius* against ATCC strains.

Microorganism	Zone of inhibition (mm)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Streptomycin sulphate
GRAM-POSITIVE BACTERIA							
B. cereus ATCC 10876	6.00±0.00	6.00±0.00	6.00±0.00	9.77±0.25	9.26±0.25	6.72±0.26	18.67±0.28
B. subtilis ATCC 21332	6.00±0.00	6.00±0.00	6.00±0.00	11.31±0.27	9.31±0.27	6.00±0.00	18.74±0.25
L. monocytogenes ATCC 15313	6.00±0.00	6.00±0.00	6.00±0.00	13.27±0.25	13.68±0.28	6.31±0.27	17.74±0.25
M. luteus ATCC 10240	6.00±0.00	6.00±0.00	6.00±0.00	9.75±0.25	7.77±0.25	6.77±0.25	19.79±0.26
P. vulgaris ATCC 13315	6.00±0.00	7.69±0.27	6.33±0.28	12.27±0.25	10.82±0.28	6.83±0.28	11.75±0.25
R. equi ATCC 33701	6.00±0.00	6.00±0.00	6.00±0.00	12.29±0.26	11.32±0.28	6.00±0.00	16.69±0.27
S. aureus ATCC 11632	6.00±0.00	6.00±0.00	6.00±0.00	12.70±0.26	8.79±0.26	6.27±0.25	12.79±0.26
S. epidermidis ATCC 12228	6.00±0.00	6.00±0.00	6.00±0.00	8.23±0.25	6.00±0.00	6.00±0.00	6.00±0.00
S. pyogenes ATCC 19615	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	15.81±0.27

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. A zone of inhibition of 6 mm corresponds to the diameter of the disc and indicates no zone of inhibition observed.

Antibacterial activities of crude extracts of *Artabotrys crassifolius* against ATCC strains (continued).

Microorganism	Zone of inhibition (mm)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Streptomycin sulphate
GRAM-NEGATIVE BACTERIA							
C. freundii ATCC 22636	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	15.79±0.26
E. coli ATCC 10536	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	13.81±0.27
K. pneumoniae ATCC 13883	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
P. aeruginosa ATCC 10145	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
S. enteritidis ATCC 13076	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	13.27±0.25
S. typhimurium ATCC 14028	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	8.27±0.25

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. A zone of inhibition of 6 mm corresponds to the diameter of the disc and indicates no zone of inhibition observed.

APPENDIX B4

Antibacterial activities of crude extracts of *Artabotrys crassifolius* against clinical isolates.

Microorganism	Zone of inhibition (mm)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Streptomycin sulphate
GRAM-POSITIVE BACTERIA							
E. faecalis	6.00±0.00	6.00±0.00	6.00±0.00	10.74±0.25	6.00±0.00	6.00±0.00	6.00±0.00
MRSA	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
MSSA	6.00±0.00	6.00±0.00	6.00±0.00	12.33±0.28	10.31±0.27	6.81±0.27	9.77±0.25
ORCNS	6.00±0.00	6.00±0.00	6.00±0.00	9.32±0.28	8.82±0.28	6.00±0.00	16.33±0.28
OSCNS	6.00±0.00	6.00±0.00	6.00±0.00	10.32±0.28	7.75±0.25	6.00±0.00	14.33±0.28
S. agalactiae	6.71±0.26	8.24±0.25	6.81±0.27	13.23±0.25	9.24±0.25	8.76±0.25	15.77±0.25
S. pneumoniae	6.29±0.26	8.28±0.26	7.21±0.26	13.70±0.26	9.79±0.26	7.79±0.26	14.71±0.26

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. A zone of inhibition of 6 mm corresponds to the diameter of the disc and indicates no zone of inhibition observed.

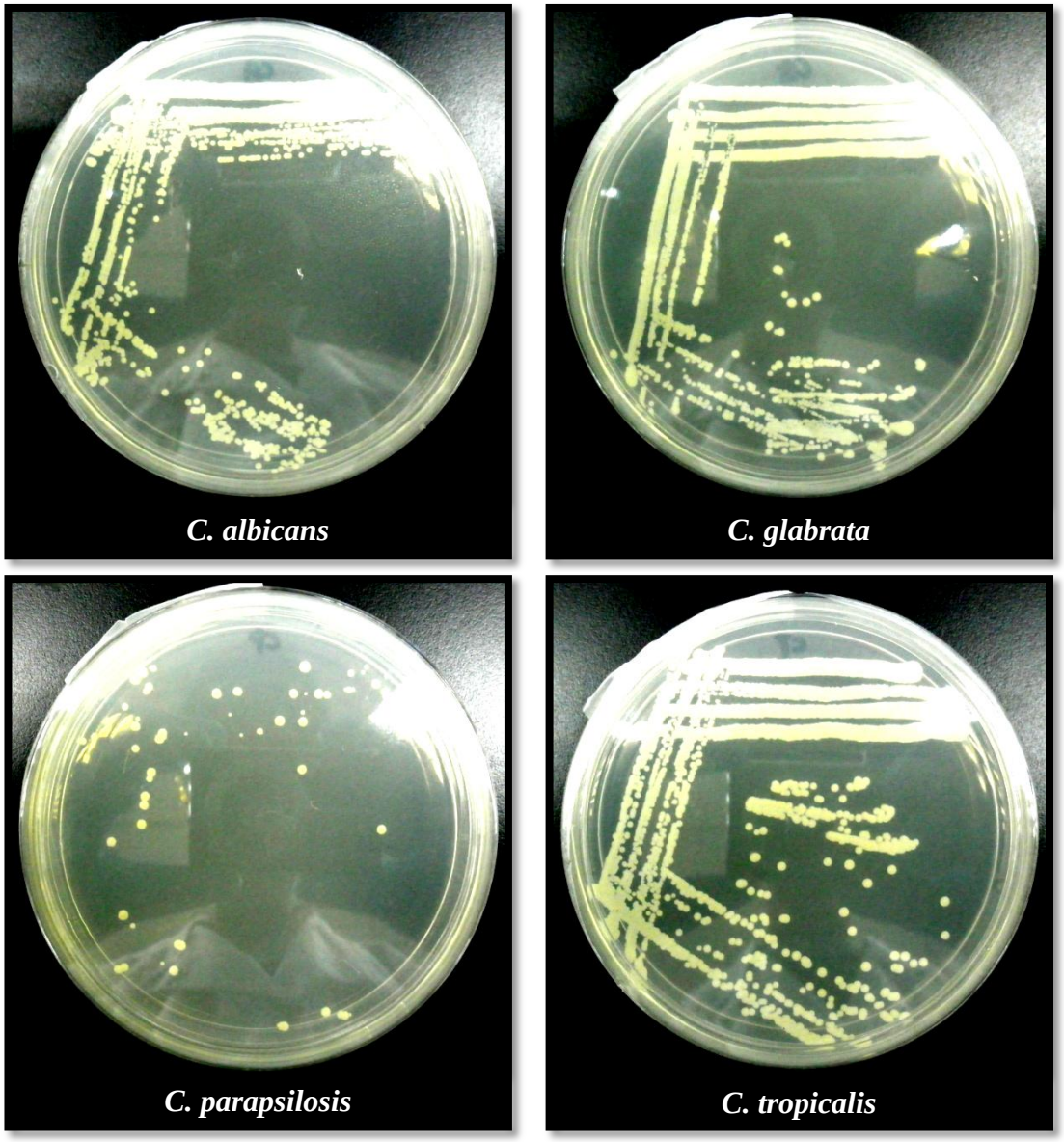
Antibacterial activities of crude extracts of *Artabotrys crassifolius* against clinical isolates (continued).

Microorganism	Zone of inhibition (mm)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Streptomycin sulphate
GRAM-NEGATIVE BACTERIA							
Actinobacillus sp.	6.00±0.00	6.00±0.00	6.00±0.00	12.27±0.25	9.78±0.26	7.79±0.26	13.77±0.25
Enterobacter sp.	6.00±0.00	6.00±0.00	6.00±0.00	13.30±0.26	6.00±0.00	6.00±0.00	6.00±0.00
E. coli	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
ESBL-EC	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.30±0.26
ESBL-KP	6.00±0.00	6.00±0.00	6.00±0.00	12.76±0.25	8.69±0.27	10.71±0.26	17.71±0.26
Klebsiella sp.	6.00±0.00	6.00±0.00	6.00±0.00	12.73±0.25	8.79±0.26	6.32±0.28	12.80±0.26
Moraxella sp.	6.00±0.00	6.00±0.00	6.00±0.00	9.80±0.26	6.00±0.00	6.00±0.00	6.00±0.00
Serratia sp.	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. A zone of inhibition of 6 mm corresponds to the diameter of the disc and indicates no zone of inhibition observed.

APPENDIX C1

Streak plates of clinical fungal strains.



APPENDIX C2

Antifungal activities of crude extracts of *Artabotrys crassifolius* against clinical isolates.

Microorganism	Zone of inhibition (mm)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Amphotericin B
YEASTS							
C. albicans	6.00±0.00	6.00±0.00	6.00±0.00	7.81±0.27	6.00±0.00	6.00±0.00	22.24±0.25
C. glabrata	6.00±0.00	6.00±0.00	6.00±0.00	9.75±0.25	6.00±0.00	6.00±0.00	18.76±0.25
C. parapsilosis	6.00±0.00	6.00±0.00	6.00±0.00	9.77±0.25	6.00±0.00	6.00±0.00	24.74±0.25
C. tropicalis	6.00±0.00	6.00±0.00	6.00±0.00	9.30±0.26	6.00±0.00	6.00±0.00	21.31±0.27

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. A zone of inhibition of 6 mm corresponds to the diameter of the disc and indicates no zone of inhibition observed.

APPENDIX D

Anticancer effects of crude extracts of *Artabotrys crassifolius* against human carcinoma cell lines.

Concentration (µg/mL)	MCF-7 (ER+) breast carcinoma cell growth (%)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Quercetin
6.25	98.80±1.95	97.25±2.92	97.10±2.68	99.34±0.19	62.82±1.66	96.60±2.04	NA
12.5	98.05±3.18	94.08±4.92	95.23±4.38	85.85±22.54	37.76±2.48	96.14±3.40	NA
25	97.46±3.11	84.77±3.50	94.48±4.51	70.22±4.70	23.98±1.63	95.34±3.18	NA
50	94.40±4.86	66.20±2.28	92.31±4.30	8.04±0.71	–53.62±5.04	96.43±2.85	NA
100	63.02±4.34	31.35±2.99	96.16±2.47	–78.73±6.01	–81.14±6.18	81.24±4.36	NA
200	–81.36±2.42	–75.44±7.49	40.04±9.41	–79.61±2.91	–89.91±5.95	29.72±6.69	NA
GI_{50} (µg/mL)	109.01	73.25	182.25	33.13	9.45	160.64	5.33
TGI (µg/mL)	143.64	129.37	>200	54.63	32.73	>200	NA
LC_{50} (µg/mL)	178.27	176.19	>200	83.45	48.84	>200	NA

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. NA indicates not available.

Anticancer effects of crude extracts of *Artabotrys crassifolius* against human carcinoma cell lines (continued).

Concentration (µg/mL)	MDA-468 (ER–) breast carcinoma cell growth (%)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Quercetin
6.25	87.02±7.03	83.26±7.31	96.02±2.32	48.79±5.65	26.09±4.23	79.69±4.63	NA
12.5	75.37±8.69	72.52±5.82	93.15±5.40	32.45±7.77	–7.44±18.75	64.60±7.06	NA
25	66.21±5.42	53.05±4.84	91.97±5.18	12.40±4.85	–30.15±18.08	61.54±6.45	NA
50	48.07±5.35	14.16±11.63	89.84±7.09	–90.65±1.42	–91.69±0.40	55.19±9.29	NA
100	–63.63±19.96	–86.55±6.48	85.73±7.32	–92.44±0.87	–85.97±1.31	26.62±2.25	NA
200	–70.85±14.47	–86.44±5.48	79.29±5.80	–92.74±0.73	–85.36±0.85	24.22±2.32	NA
GI_{50} (µg/mL)	47.34	26.96	>200	6.10	4.23	59.09	22.88
TGI (µg/mL)	71.52	57.03	>200	28.01	11.11	>200	NA
LC_{50} (µg/mL)	93.90	81.85	>200	40.14	33.06	>200	NA

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. NA indicates not available.

Anticancer effects of crude extracts of *Artabotrys crassifolius* against human carcinoma cell lines (continued).

Concentration (µg/mL)	HCT-116 colorectal carcinoma cell growth (%)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Quercetin
6.25	87.39±5.22	99.28±0.50	95.82±1.33	87.17±10.95	71.78±10.18	97.45±3.41	NA
12.5	91.61±4.22	96.32±3.36	91.71±4.66	69.18±7.60	17.37±5.65	94.48±6.65	NA
25	85.05±8.13	98.64±1.90	93.98±6.86	8.40±4.25	–17.59±21.93	72.24±4.57	NA
50	83.76±9.94	87.58±2.68	88.91±7.37	–18.97±5.01	–89.26±13.75	55.13±3.02	NA
100	37.19±6.61	45.81±5.95	92.30±4.67	–47.67±6.11	–95.60±3.22	33.27±3.38	NA
200	–97.45±2.50	–0.18±18.35	84.62±7.93	–89.22±7.89	–99.30±0.45	4.70±7.90	NA
GI_{50} (µg/mL)	86.25	94.98	>200	16.45	8.75	61.73	21.47
TGI (µg/mL)	127.62	199.61	>200	32.67	18.71	>200	NA
LC_{50} (µg/mL)	164.76	>200	>200	105.61	36.30	>200	NA

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. NA indicates not available.

APPENDIX E1

Absorbance of gallic acid and catechin used for the preparation of standard curve for the total phenolic and flavonoid contents of crude extracts of *Artabotrys crassifolius*.

Concentration (µg/mL)	Absorbance	
	Gallic acid (765 nm)	Catechin (510 nm)
5	0.20±0.01	0.09±0.00
10	0.39±0.01	0.18±0.00
15	0.59±0.01	0.27±0.01
20	0.76±0.02	0.35±0.02
25	0.95±0.01	0.48±0.01

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX E2

Total phenolic and flavonoid contents of crude extracts of *Artabotrys crassifolius*.

Total phenolic and flavonoid content	Crude extract					
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol
Total phenolic content (mg GAE/g)	29.30±5.23	45.84±4.40	154.91±4.26	22.37±3.41	87.65±3.51	268.29±12.36
Total flavonoid content (mg CE/g)	9.48±4.53	15.47±2.97	84.47±6.61	6.29±4.27	37.15±4.79	179.54±4.98

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX E3

Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using ABTS cation radical scavenging assay.

Concentration (µg/mL)	ABTS cation radical scavenging activity (%)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Trolox
3.125	7.46±5.75	2.28±1.39	10.66±3.80	6.95±8.43	2.76±1.58	15.97±4.76	24.50±4.79
6.25	4.24±3.99	1.94±1.59	10.29±4.49	4.25±5.54	1.30±0.92	20.61±4.90	45.58±3.42
12.5	5.48±4.69	2.76±1.14	19.27±4.53	4.02±6.31	4.62±2.69	37.77±5.69	89.24±3.19
25	6.66±5.38	6.59±0.61	39.04±3.29	6.94±6.34	18.70±2.35	75.99±5.06	99.66±0.21
50	13.37±3.79	21.88±1.62	86.50±4.71	8.73±8.95	39.59±3.18	99.66±0.20	99.71±0.12
100	28.45±7.88	55.96±3.92	99.76±0.26	22.12±10.58	85.80±2.47	99.86±0.06	99.72±0.15
IC_{50} (µg/mL)	>100	91.26	30.77	>100	61.27	16.50	6.88

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX E4

Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using DPPH radical scavenging assay.

Concentration (µg/mL)	DPPH radical scavenging activity (%)						
	Crude extract						Positive control
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol	Ascorbic acid
3.125	0.98±1.46	1.02±0.67	2.23±1.16	0.64±0.61	1.08±1.13	9.81±0.19	16.22±3.84
6.25	1.20±0.18	1.23±0.83	7.15±3.05	1.00±1.06	2.33±0.98	19.49±1.94	40.46±6.38
12.5	3.01±0.25	2.71±1.70	16.34±3.77	1.75±0.64	4.92±2.13	37.95±2.11	84.87±7.77
25	5.13±0.99	7.14±2.05	27.35±4.20	2.35±0.96	11.86±2.27	75.21±1.40	94.01±1.09
50	9.96±2.22	16.63±3.38	49.14±4.94	7.57±2.49	21.93±1.80	92.88±1.46	95.22±0.97
100	21.23±8.45	31.97±5.16	80.67±7.44	19.17±7.22	47.67±0.83	95.47±2.37	95.34±0.64
IC_{50} (µg/mL)	>100	>100	51.37	>100	>100	16.54	7.59
AAI	<0.38	<0.38	0.75	<0.38	<0.38	2.32	5.07

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX E5

Absorbance of $FeSO_4 \cdot 7H_2O$ used for the preparation of standard curve for the antioxidant potentials of crude extracts of *Artabotrys crassifolius* using FRAP assay.

Concentration (µM)	Absorbance
	$FeSO_4 \cdot 7H_2O$ (593 nm)
50	0.45±0.06
100	1.04±0.05
150	1.61±0.06
200	2.23±0.04
250	2.83±0.03

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX E6

Antioxidant potentials of crude extracts of *Artabotrys crassifolius* using FRAP assay.

FRAP value	Crude extract					
	Leaves hexane	Leaves chloroform	Leaves ethanol	Bark hexane	Bark chloroform	Bark ethanol
FRAP value (µmol Fe(II)/g)	92.26±5.99	286.22±29.32	979.57±57.17	67.64±23.40	572.90±35.10	1884.35±83.78

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9.

APPENDIX F

Anticancer effects of isolated compounds from *Artabotrys crassifolius* against human carcinoma cell lines.

Concentration (µM)	MCF-7 (ER+) breast carcinoma cell growth (%)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Quercetin
0.05	94.99±4.66	93.79±6.36	93.97±3.24	91.59±2.94	NA
0.1	92.78±5.85	94.71±2.27	94.33±3.77	90.78±7.31	NA
0.5	93.20±3.33	92.24±9.56	91.75±5.47	91.85±4.69	NA
1	92.37±6.56	92.80±5.62	94.14±3.51	84.59±9.07	NA
5	24.17±1.84	86.29±2.39	92.68±2.93	37.32±7.24	NA
10	–15.27±7.54	76.57±4.04	90.21±5.21	13.94±8.02	NA
GI_{50} (µM)	3.49	>10	>10	3.93	5.33
TGI (µM)	8.06	>10	>10	>10	NA
LC_{50} (µM)	>10	>10	>10	>10	NA

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. The GI_{50} value for positive control is in µg/mL. NA indicates not available.

APPENDIX F

Anticancer effects of isolated compounds from *Artabotrys crassifolius* against human carcinoma cell lines (continued).

Concentration (µM)	HCT-116 colorectal carcinoma cell growth (%)				
	Isolated compound				Positive control
	Artabotrine	Liridine	Atherospermidine	Lysicamine	Quercetin
0.05	95.44±2.95	96.19±2.87	93.46±1.65	95.26±2.34	NA
0.1	96.61±1.56	97.04±2.69	96.09±1.14	96.24±5.16	NA
0.5	97.16±1.56	97.39±1.42	96.32±2.15	90.93±4.34	NA
1	95.94±2.11	98.05±1.41	97.16±2.38	75.66±7.44	NA
5	17.46±3.62	90.45±5.36	91.71±5.34	33.57±6.90	NA
10	5.64±2.31	57.58±10.37	85.38±9.43	9.54±1.81	NA
GI_{50} (µM)	3.34	>10	>10	3.44	21.47
TGI (µM)	>10	>10	>10	>10	NA
LC_{50} (µM)	>10	>10	>10	>10	NA

Note: Results are expressed as mean ± standard deviation of three independent experiments performed in triplicate, n = 9. The GI_{50} value for positive control is in µg/mL. NA indicates not available.

APPENDIX G

List of conferences, seminars and trainings attended.

Title	Organizer	Venue	Date
CONFERENCE			
11th International Conference on Natural Products (ICNP) 2011 (Poster Presentation)	Institute of Bioscience and Faculty of Science, Universiti Putra Malaysia; Malaysian Natural Products Society (MNPS)	Palm Garden Hotel, IOI Resort, Putrajaya	14–16 Nov 2011
Exhibition Showcase of UNMC Global Research Workshop 2012 (Poster Presentation)	Graduate School, The University of Nottingham Malaysia Campus	Foyer, Block A, UNMC	24 Apr 2012
Graduate School Research Showcase 2012 (Poster Presentation)	Graduate School, The University of Nottingham Malaysia Campus	Foyer, Block B, UNMC	18 May 2012
Graduate School Research Showcase 2013 (Poster Presentation – Best Visual Flair)	Graduate School, The University of Nottingham Malaysia Campus	Foyer, Block B, UNMC	10 May 2013
1st European Conference on Natural Products (ECNP) 2013 (Poster Presentation)	DECHEMA Biotechnologie	DECHEMA-Haus, Frankfurt am Main, Germany	22–25 Sep 2013
5th Global Summit on Medicinal and Aromatic Plants (GOSMAP) 2013 (Oral Presentation)	V. Sivaram Research Foundation (VSRF) and Century Foundation Bangalore; Universiti Teknologi MARA	Miri Marriott Resort & Spa, Miri, Sarawak	8–12 Dec 2013

List of conferences, seminars and trainings attended (continued).

Title	Organizer	Venue	Date
SEMINAR			
Merck Thin Layer Chromatography Seminar	Faculty of Medicine and Health Sciences, Universiti Putra Malaysia	Main Lecture Hall, FMHS, UPM	30 Jun 2011
Fisher Seminar	Fisher Scientific Sdn Bhd	CB01, Block C, UNMC	8 Sep 2011
Biacore and Microcal Seminar	GE Healthcare; Interscience Sdn Bhd	BA06, Block B, UNMC	12 Sep 2011
Horizon Technology Seminar	Orbiting Scientific and Technology Sdn Bhd	Holiday Villa Subang, Subang Jaya	23 Sep 2011
Proteomics and Metabolomics Seminar	Alpha Analytical Sdn Bhd	Grand Dorsett Subang Hotel, Subang Jaya	26 Mar 2012
Faculty of Science Research Seminar 2012 (Oral Presentation)	Faculty of Science, The University of Nottingham Malaysia Campus	F1A10, Block F1, UNMC	18 Apr 2012
Sample Preparation and LC Columns Seminar	Agilent Technologies Sdn Bhd; IT Tech Research Sdn Bhd	Carlton Holiday Hotel and Suites, Shah Alam	23 Apr 2012
National Laboratory Productivity and Technology Seminar	Lesoshoppe Sdn Bhd; Evergreen Engineering and Resources	BA05, Block B, UNMC	17 Jan 2013

List of conferences, seminars and trainings attended (continued).

Title	Organizer	Venue	Date
TRAINING			
Demonstrating Skills in Laboratory Practicals	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	17 Jan 2011
Getting Going on Your Thesis	Graduate School, The University of Nottingham Malaysia Campus	BA05, Block B, UNMC	18 Feb 2011
Planning Research and Time Management	Graduate School, The University of Nottingham Malaysia Campus	BA05, Block B, UNMC	2 Mar 2011
Creative Thinking	Graduate School, The University of Nottingham Malaysia Campus	BA05 Block B, UNMC	13 May 2011
Working Effectively in Research	Graduate School, The University of Nottingham Malaysia Campus	BA05, Block B, UNMC	24 Jun 2011
What Do I Want to Get Out of A Conference?	Graduate School, The University of Nottingham Malaysia Campus	BA05, Block B, UNMC	12 Aug 2011
Nature of PhD	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	10 Oct 2011
Critical Thinking	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	21 Oct 2011
Library Information Skills and Endnote Briefing	Library Services, The University of Nottingham Malaysia Campus	GD14, Block G, UNMC	17 Nov 2011

List of conferences, seminars and trainings attended (continued).

Title	Organizer	Venue	Date
TRAINING			
Using Posters to Communicate Research	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	5 Dec 2011
Understanding What Interviewers Are Looking For	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	13 Feb 2012
Communicating Your Accomplishments and Goals Effectively	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	20 Feb 2012
Creating A Poster in PowerPoint	Graduate School, The University of Nottingham Malaysia Campus	EA21, Block E, UNMC	22 Feb 2012
Presentation Skills	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	5 Mar 2012
Communicating Your Research	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	7 Mar 2012
Academic Writing and Getting Published	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	14 Mar 2012
Meet The Editors	Graduate School, The University of Nottingham Malaysia Campus	BA05, Block B, UNMC	21 Mar 2012
Further Presentation Skills	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	2 May 2012

List of conferences, seminars and trainings attended (continued).

Title	Organizer	Venue	Date
TRAINING			
How to Use Mendeley in Research	Library Services, The University of Nottingham Malaysia Campus	TCR 3, Block F2, UNMC	4 May 2012
Word Essentials for Researchers	Graduate School, The University of Nottingham Malaysia Campus	GD14, Block G, UNMC	6 Mar 2013
How to Write A Press Release	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	7 Mar 2013
Preparing For Your Annual Review	Graduate School, The University of Nottingham Malaysia Campus	BA64, Block B, UNMC	31 May 2013

APPENDIX H1

Abstract for 11th International Conference on Natural Products (ICNP) 2011.

PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ARTABOTRYS CRASSIFOLIUS*

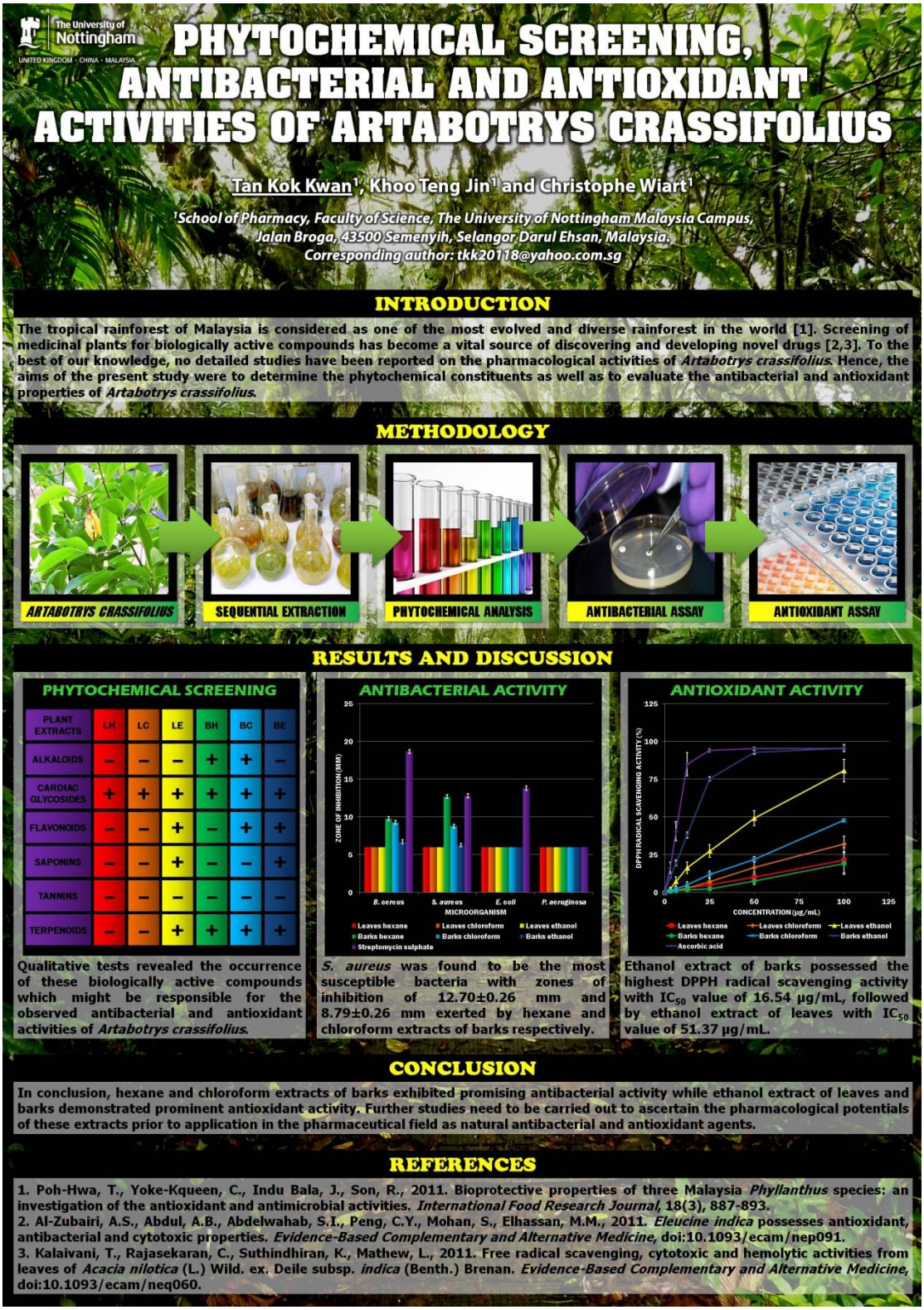
Tan Kok Kwan[1], Khoo Teng Jin[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
Corresponding author: tkk20118@yahoo.com.sg

The tropical rainforest of Malaysia is considered as one of the most evolved and diverse rainforest in the world. Screening of medicinal plants for biologically active compounds has become an important source of discovering and developing novel drugs. The aims of the present study were to determine the phytochemical constituents, as well as to evaluate the antibacterial and antioxidant properties of *Artabotrys crassifolius*. The leaves and bark of *Artabotrys crassifolius* were extracted sequentially using hexane, chloroform and ethanol to obtain the respective extracts. The prepared extracts were then subjected to phytochemical analysis for the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. The antibacterial activity of the extracts was tested against both Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) using Kirby-Bauer disc diffusion assay whereas DPPH radical scavenging assay was employed to investigate the antioxidant activity of the extracts. Among the bacteria examined, *Staphylococcus aureus* was found to be the most susceptible bacteria with zones of inhibition of 12.70±0.26 mm and 8.79±0.26 mm exerted by hexane and chloroform extracts of bark respectively. In addition, ethanol extract of bark demonstrated the highest DPPH radical scavenging activity of 95.47±2.37% with IC_{50} value of 16.54 µg/mL, followed by ethanol extract of leaves displaying DPPH radical scavenging activity of 80.67±7.44% with IC_{50} value of 51.37 µg/mL. Current findings suggested that the observed antibacterial and antioxidant activities may be attributed to the presence of alkaloids and flavonoids. In conclusion, hexane and chloroform extracts of bark, as well as ethanol extract of leaves and bark could be the potential sources of natural antibacterial and antioxidant agents respectively.

APPENDIX H2

Poster for 11th International Conference on Natural Products (ICNP) 2011.



APPENDIX H3

Abstract for Faculty of Science Research Seminar 2012.

EVALUATION OF THE ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF *ARTABOTRYS CRASSIFOLIUS*

Tan Kok Kwan[1], Khoo Teng Jin[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
Corresponding author: tkk20118@yahoo.com.sg

The tropical rainforest of Malaysia is considered as one of the most evolved and diverse rainforest in the world. Screening of medicinal plants for biologically active compounds has become a vital source of discovering and developing novel drugs. The aims of the present study were to determine the phytochemical constituents, as well as to evaluate the antibacterial, antioxidant and anticancer properties of *Artabotrys crassifolius*. The leaves and bark of *Artabotrys crassifolius* were extracted sequentially with hexane, chloroform and ethanol to obtain the respective extracts. The prepared crude extracts were then subjected to phytochemical screenings for the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins and terpenoids. The antibacterial activity of the extracts was tested against both Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) using Kirby-Bauer disc diffusion assay. The DPPH radical scavenging assay was conducted to investigate the antioxidant activity of the extracts whereas MTT assay was performed to assess the cytotoxicity of the extracts against HCT-116 colorectal carcinoma cell line. Among the bacteria examined, *Staphylococcus aureus* was found to be the most susceptible bacteria with zones of inhibition of 12.70±0.26 mm and 8.79±0.26 mm exerted by hexane and chloroform extracts of bark respectively. In addition, ethanol extract of bark possessed the highest DPPH radical scavenging activity with IC_{50} value of 16.54 µg/mL, followed by ethanol extract of leaves with IC_{50} value of 51.37 µg/mL. Among the extracts studied, chloroform and hexane extracts of bark displayed potent cytotoxicity with GI_{50} values of 8.75 µg/mL and 16.45 µg/mL respectively. Current findings suggested that the observed antibacterial, antioxidant and anticancer activities may be attributed to the occurrence of the phytochemical constituents analysed. In conclusion, hexane and chloroform extracts of bark exhibited promising antibacterial and anticancer activities while ethanol extract of leaves and bark demonstrated prominent antioxidant activity. Further studies need to be carried out to ascertain the pharmacological potentials of these extracts prior to application in the pharmaceutical field as natural antibacterial, antioxidant and anticancer agents.

APPENDIX H4

Press release for Graduate School Research Showcase 2012.

TREASURES OF RAINFOREST: THE SOURCE OF NEW MEDICINES

Tan Kok Kwan[1], Khoo Teng Jin[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
Corresponding author: tkk20118@yahoo.com.sg

Throughout the history of mankind, plants have been used extensively as a source of medicines for a wide variety of human ailments. According to the World Health Organisation, approximately 80% of the people in developing countries still rely on traditional medicines for their primary health care needs. Having a wide array of therapeutic properties, plants have become an indispensable pharmacological tool in which many modern drugs today have been isolated from, especially plant-based products for therapeutic purposes in health care.

Nevertheless, the research and development in the area of medicinal plants has been continuously expanding due to several driving factors such as rise in population, insufficient supply of drugs in certain parts of the world, prohibitive cost of treatments for common ailments, side effects of several allopathic drugs in current usage as well as development of resistance to currently used drugs for diseases. Therefore, exploitation of medicinal plants for biologically active compounds is of great potential and could be an excellent source of providing new vistas for novel drug discovery and development.

The tropical rainforest of Malaysia is considered as one of the most evolved and diverse ecosystems in the world. It is a unique natural heritage that serves a vast untapped biodiversity of natural resources. This has led to the current investigation on the pharmacological potentials of an indigenous medicinal plant named *Artabotrys crassifolius* in order to explore the antibacterial, antioxidant and anticancer properties of the plant prior to application in pharmaceutical industry as a potentially promising therapeutic agent.

APPENDIX H5

Poster for Exhibition Showcase of UNMC Global Research Workshop 2012.

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

TREASURES OF RAINFOREST: THE SOURCE OF NEW MEDICINES

Tan Kok Kwan[1], Khoo Teng Jin[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
Corresponding author: tkk20118@yahoo.com.sg

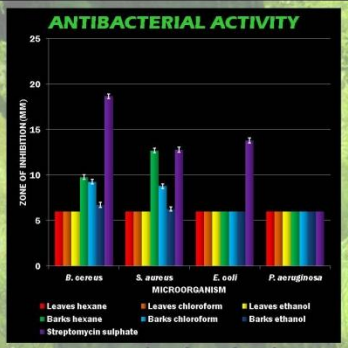
INTRODUCTION

The tropical rainforest of Malaysia is considered as one of the most evolved and diverse ecosystems in the world [1]. Exploitation of medicinal plants for biologically active compounds is of great potential and could be an excellent source of providing new vistas for novel drug discovery and development [2,3]. To the best of our knowledge, no detailed studies have been reported on the pharmacological properties of *Artabotrys crassifolius*. Therefore, the objective of the current study was to evaluate the antibacterial, antioxidant and anticancer activities of *Artabotrys crassifolius*.

METHODOLOGY

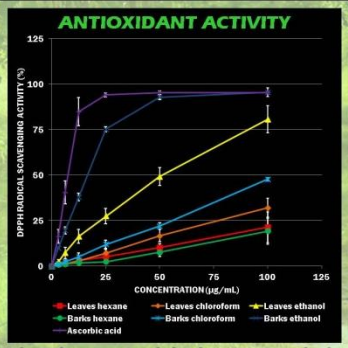
RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY



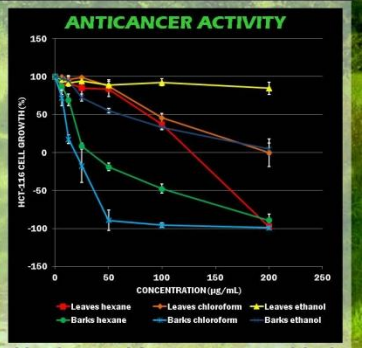
S. aureus was found to be the most susceptible bacteria with zones of inhibition of 12.70±0.26 mm and 8.79±0.26 mm exerted by hexane and chloroform extracts of barks respectively.

ANTIOXIDANT ACTIVITY



Ethanol extract of barks possessed the highest DPPH radical scavenging activity with IC_{50} value of 16.54 µg/mL, followed by ethanol extract of leaves with IC_{50} value of 51.37 µg/mL.

ANTICANCER ACTIVITY



Chloroform and hexane extracts of barks displayed the most potent cytotoxicity against HCT-116 colorectal carcinoma cell line with respective GI_{50} values of 8.75 µg/mL and 16.45 µg/mL.

CONCLUSION

In conclusion, hexane and chloroform extracts of barks exhibited promising antibacterial and anticancer activities while ethanol extract of leaves and barks demonstrated prominent antioxidant activity. Further studies need to be carried out to ascertain the pharmacological potentials of these extracts prior to application in the pharmaceutical industry as natural therapeutic agents.

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APPENDIX H6

Poster for Graduate School Research Showcase 2012.

APPENDIX H7

Press release for Graduate School Research Showcase 2013.

RAINFORESTS: OUR NATURE'S MEDICINE CABINET

Tan Kok Kwan[1], Khoo Teng Jin[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
Corresponding author: tkk20118@yahoo.com.sg

According to the World Health Organisation, approximately 80% of the people in developing countries still rely on traditional medicines for their primary health care needs. Plants have been used extensively as a source of medicines for a wide variety of human ailments throughout the history of mankind due to several driving factors such as rise in population, insufficient supply of drugs in certain parts of the world, prohibitive cost of treatments for common ailments, side effects of several allopathic drugs in current usage as well as development of resistance to currently used drugs for diseases.

Having a wide array of therapeutic properties, plants have become an indispensable pharmacological tool in which many modern drugs today have been isolated from, especially plant-based products for therapeutic purposes in health care. Nevertheless, the research and development in the area of medicinal plants has been continuously expanding as exploitation of medicinal plants for bioactive compounds is of great potential and could be an excellent source of providing new vistas for novel drug discovery and development.

The tropical rainforest of Malaysia is regarded as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources. This unique natural heritage has brought renewed interest in the screening of indigenous medicinal plants for bioactive compounds and could be the plus factor that makes natural products excellent candidates for screening programme prior to application in pharmaceutical industry as potentially promising therapeutic agents.

APPENDIX H8

Poster for Graduate School Research Showcase 2013.

APPENDIX H9

Abstract for 1st European Conference on Natural Products (ECNP) 2013.

PHARMACOLOGICAL PROPERTIES OF AN INDIGENOUS MEDICINAL PLANT, *ARTABOTRYS CRASSIFOLIUS* HOOK.F. & THOMSON (ANNONACEAE)

Kok Kwan Tan[1], Teng Jin Khoo[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.

Introduction

The tropical rainforest of Malaysia is regarded as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources. Exploitation of indigenous medicinal plants for bioactive compounds is of great potential and could be an excellent source of providing new vistas for novel drug discovery and development.

Objectives

The study was undertaken to evaluate the pharmacological properties of *Artabotrys crassifolius* including antibacterial, antioxidant and anticancer activities of the plant.

Methodology

The leaves and bark of *Artabotrys crassifolius* were extracted sequentially using hexane, chloroform and ethanol. The prepared crude extracts were subjected to antibacterial activity against ATCC and clinical strains using Kirby-Bauer disc diffusion method. The ABTS cation and DPPH radical scavenging assays were conducted to assess the antioxidant potential of the extracts whereas MTT assay was performed to investigate the anticancer effect of the extracts against breast and colorectal carcinoma cell lines.

Results

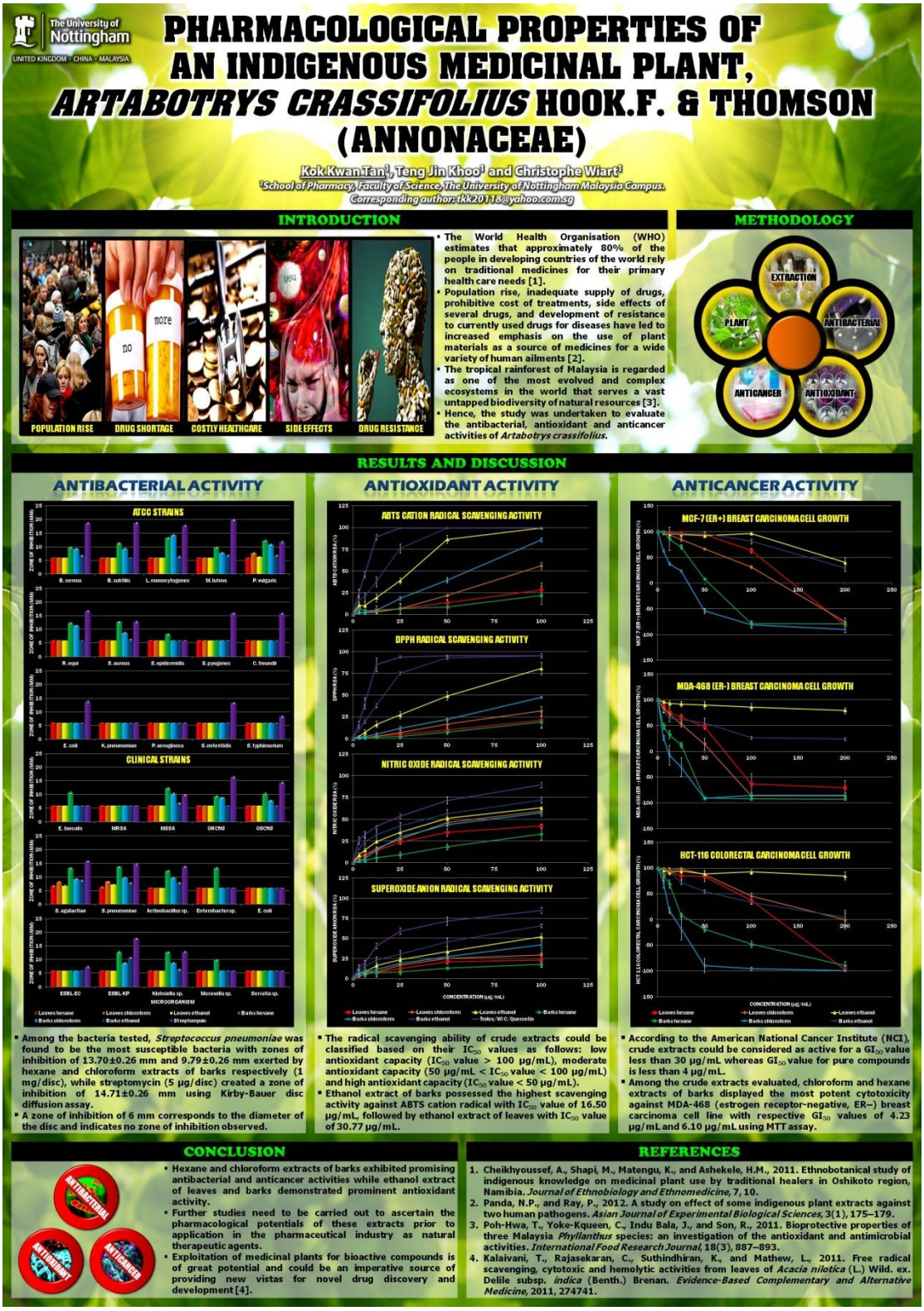
Among the bacteria examined, *Streptococcus pneumoniae* was found to be the most susceptible bacteria with zones of inhibition of 13.70±0.26 mm and 9.79±0.26 mm exerted by hexane and chloroform extracts of bark respectively. As for the antioxidant potential, ethanol extract of bark possessed the highest scavenging activity against ABTS cation radical with IC_{50} value of 16.50 µg/mL, followed by ethanol extract of leaves with IC_{50} value of 30.77 µg/mL. Among the extracts studied, chloroform and hexane extracts of bark displayed the most potent cytotoxicity against MDA-468 breast carcinoma cell line with respective GI_{50} values of 4.23 µg/mL and 6.10 µg/mL.

Conclusion

Hexane and chloroform extracts of bark exhibited promising antibacterial and anticancer activities while ethanol extract of leaves and bark demonstrated prominent antioxidant activity. Further studies need to be carried out to ascertain the pharmacological potentials of these extracts prior to application in the pharmaceutical industry as natural therapeutic agents.

APPENDIX H10

Poster for 1st European Conference on Natural Products (ECNP) 2013.



APPENDIX H11

Abstract for 5th Global Summit on Medicinal and Aromatic Plants (GOSMAP) 2013.

ARTABOTRYS CRASSIFOLIUS HOOK.F. & THOMSON: A POTENTIAL SOURCE OF NATURAL THERAPEUTIC AGENTS?

Kok Kwan Tan[1], Teng Jin Khoo[1] and Christophe Wiart[1]

[1]School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.

The tropical rainforest of Malaysia is regarded as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources. Exploitation of indigenous medicinal plants for bioactive compounds is of great potential and could be an excellent source of providing new vistas for novel drug discovery and development. The study was undertaken to evaluate the *in vitro* pharmacological properties of *Artabotrys crassifolius* including antibacterial, antifungal and anticancer activities of the plant. The leaves and bark of *Artabotrys crassifolius* were extracted sequentially using hexane, chloroform and ethanol. The prepared crude extracts were subjected to antibacterial and antifungal activities against clinical strains using Kirby-Bauer disc diffusion method whereas MTT assay was performed to investigate the anticancer effect of the extracts against human breast and colorectal carcinoma cell lines. Among the bacteria examined, *Streptococcus pneumoniae* was found to be the most susceptible bacteria with zones of inhibition of 13.70±0.26 mm and 9.79±0.26 mm exerted by hexane and chloroform extracts of bark respectively. Nevertheless, among the extracts studied, only hexane extract of bark demonstrated antifungal activity against *Candida* species with zones of inhibition ranging from 7.81±0.27 mm to 9.77±0.25 mm. As for the anticancer effect, chloroform and hexane extracts of bark displayed the most potent cytotoxicity against MDA-468 breast carcinoma cell line with respective GI_{50} values of 4.23 µg/mL and 6.10 µg/mL. Examination of the *in vitro* pharmacological properties of *Artabotrys crassifolius* revealed that hexane and chloroform extracts of bark may be a significant source of novel bioactive compounds in view of their promising antibacterial, antifungal and anticancer activities. Further studies need to be carried out to ascertain the pharmacological potentials of these extracts prior to application in the pharmaceutical industry as natural therapeutic agents.

Keywords: *Artabotrys crassifolius*, antibacterial, antifungal, anticancer