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Full Length Research Paper

Antimicrobial, antioxidant and cytotoxic activites and chemical profile of species of *Miconia* Ruiz & Pav., *Clidemia* D. Don and *Tibouchina* Aubl. (Melastomataceae)

Ellen Matos Silva Bomfim[1,3*], Tamires Gomes dos Santos[1], Adriele Santana de Oliveira Carneiro[1], Marcos da Costa Silva[2,3], Edson de Jesus Marques[1] and Vera Lúcia Costa Vale[1,3]

[1]Department of Exact and Earth Sciences, Bahia State University, *Campus* II. Alagoinhas / Salvador -BR 110 Highway, Km 03, 48040-210, Alagoinhas, Bahia, Brazil.
[2]Department of Life Sciences, Bahia State University, *Campus* I. Silveira Martins Street, 2555 - Cabula, 41000-150, Salvador, Bahia, Brazil.
[3]Postgraduate Program in Vegetable Biodiversity – PPGBVeg, Bahia State University, Salvador, Bahia, Brazil.

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The resistance of microorganisms to current antimicrobials, and the deleterious effects caused by the excessive free radical manufacturing in the human body and their relationship with increasing global incidence of cancer, has led to a continuous search for new chemical agents that can contribute to the fight against these ills. The objective of the present work was to evaluate the antimicrobial, antioxidant and cytotoxic activities and determine the chemical profile of ethyl acetate extract of ten species of the family Melastomataceae. Antimicrobial activity was assessed by the methods of disk diffusion in agar and microdilution in broth (MIC-µg/mL). Antioxidant activity was measured by DPPH free radical capture assay while toxicity was evaluated with *Artemia salina* Leach. Cytoxicity was evaluated by *in vitro* tests with THP-1-cells. Identification of the classes of metabolites was performed using chemical reagents, while quantification of total phenols (EGA/g) and total flavonoids (EQ/g) was done by spectrophotometry. The extract of *Clidemia capitellata* exhibited activity against *Micrococcus luteus* with MIC = 62.5-µg/mL. The extract of *C. hirta* had the highest sequestering activity of DPPH free radicals (63.54-%). The toxicological assay revealed high toxicity for *Miconia alborufescens* extract (CL_{50} 61.6-µg/mL). Cytotoxic activity of extracts for THP-1-cells was observed through visualization of apoptotic bodies and cell death. Phytochemical analysis detected the presence of condensed tannins, terpenes, steroids and polyphenols, and the absence of alkaloids. The assays performed provided promising results, suggesting the continuation of new chemical-pharmacological evaluations and the isolation of the active principle of the extracts.

Key words: Bacteria, *Artemia*, total phenols, THP-1 cells, toxicity.

INTRODUCTION

Excessive and indiscriminate use of antibiotics has generated increased bacterial resistance to conventional antibiotics. Increased estimates for cancer incidence and excessive free radical manufacturing by the human body have become concerns of health authorities throughout the world. Due to their diverse metabolic capacities,

plants represent a potential alternative for the isolation of new drugs (Hosseinzadeh et al., 2015). Of the drugs on the market for the treatment for infectious diseases, 75% are of natural origin or analogous derivatives (Newman and Cragg, 2016).

There are approximately 374,000 species of plants on the planet (Christenhusz and Byng, 2016), of which 46,403 are distributed among the several biomes and ecosystems of Brazil (Flora do Brasil 2020, 2017). The Atlantic Forest is the most diversified phytogeographical domain in Brazil, with more than 15,001 plant species. Melastomataceae is the fifth largest family of angiosperms in Brazil with 66 genera and 1,367 species, and is one of the most represented in the Atlantic Forest, which is home to 582 species (BFG, 2015).

The Melastomataceae family presents a variety of secondary metabolite classes, including flavonoid derivatives, tannins, terpenes, fatty acids and anthocyanidins (Bonfim-Patricio et al., 2001; Yoshida et al., 2005; Grayer et al., 2008). Some studies with representatives of this family have already demonstrated that they possess analgesic, antipyretic, neuroprotective, anti-parasitic, immunological, antioxidant, anti-tuberculotic, anti-inflammatory, antitumor, antimicrobial and anticancer potentials (Pavan et al., 2009; Tan et al., 2012; Balamurugan et al., 2013; Nono et al., 2014; Nguta et al., 2016).

The objective of the present work was to investigate the antimicrobial, antioxidant and cytotoxic activity and to determine the chemical profile of crude extracts obtained with ethyl acetate from the leaves of *Miconia albicans*, *M. alborufescens*, *M. amoena*, *M. ciliata, M. fallax*, *Clidemia capitellata*, *C. hirta*, *C. sericea, Tibouchina francavillana* and *T. lhotzkyana*, belonging to the family Melastomataceae.

MATERIAL AND METHODS

Aerial parts of ten plant species of the family Melastomataceae [*Miconia albicans* (Sw.) Triana (14025); *Miconia ciliata* (Rich.) DC. (14036); *Miconia fallax* DC. (14022); *Miconia amoena* Triana (14024); *Miconia alborufescens* Naudin (14023); *Clidemia hirta* (L.) D.Don (13982); *Clidemia sericea* D.Don (13517); *Clidemia capitellata* (Bonpl.) D.Don (7685); *Tibouchina lhotzkyana* (C.Presl) Cogn. (13981) and *Tibouchina francavillana* Cogn.] (13984) were collected in an Atlantic Forest remnant in the municipality of Alagoinhas, state of Bahia, Brazil (12°10'42.62"S, 38°24'39.52'W). The plants were identified and exsiccates deposited in the Alagoinhas, Bahia, collection of herbarium da Bahia State University. The leaves were dried in an drying oven at 50°C and pulverized manually. Extraction was performed by percolation in ethyl acetate (PA), with three successive extractions at intervals of 72 h. The extract was obtained after filtration and evaporation of the solvent, and then preserved at 4°C until use.

In vitro evaluation of antimicrobial activity

Antimicrobial activity was evaluated by the method of disk diffusion in agar according to the recommendations of Clinical and Laboratory Standards Institute (CLSI; 2003), with fungal bacterial lineages belonging to American Type Culture Collection (ATCC) — *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 94863), *Micrococus luteus* (ATCC 10240) and *Bacillus subtilis* (ATCC 6633) — and the fungus *Aspergillus niger* (16404). The bacteria cultures were cultivated in Müeller-Hinton Agar at 37ºC for 24 hours while the fungus was cultivated in Sabouraud Dextrose Agar at 37°C for 48 h.

Aliquots of 10 µl of plant extract (100.0 mg/ml) were applied to sterile filter-paper disks (6.0 mm in diameter). The microorganisms were then cultured in Muller-Hinton Agar and, as a control, DMSO with chloramphenicol (0.1%), for bacteria and with Cyclopiroxolamine (0.1%) for the fungus. The bacterial and fungal plates were incubated at 37°C for 24 and 48 h, respectively. Antimicrobial activity was evaluated by inhibition halo measurement.

Minimal inhibitory concentration (MIC)

The Minimal Inhibitory Concentration (MIC) was determined based on CLSI document M7-A6 (CLSI, 2003), with some modifications. The microorganisms were cultured on plates with 96 wells and evaluated in the presence of different concentrations of extract (500 to 3.90 µg/ml) for 24 h at 37°C, with chloramphenicol (0.1%) and DMSO (4%) as controls. Wells without turbidity were considered to contain active extracts.

In vitro analysis of antioxidant activity

The antioxidant capacity of the extracts was determined by photocolorimetric assay of the free radicle 2,2-diphenyl-1-picrylhydrazyl (DPPH), using plates with 96 wells, with adaptations (Brand-Willians et al., 1995). The extracts were diluted in ethanol (6.0 mg/ml), and then 50 µl of this solution was added to microplate wells containing 150 µl of ethanol. Serial dilutions were performed to produce extract concentrations ranging from 3.0 to 0.045 mg/ml. Next, 100 µL DPPH solution (0.5 mM) was added to each well. After 1 h of reaction at room temperature, and in the absence of light, absorbance readings were made using a UV-Vis spectrophotometer at 492 nm. All tests were performed in triplicate. The percentage of free radical sequestration (% FRS) was determined using the equation:

$$\%FRS = \{[(Abs_{control} - (Abs_{sample} - Abs_{white})] \times 100\} / Abs_{control}\}$$

Where $Abs_{control}$ is the absorbance of DPPH in the presence of ethanol; Abs_{sample} is the absorbance of extract after the reaction with DPPH; and Abs_{white} is the absorbance of ethanol (Moreira et al., 2005).

Phytochemical screening

The phytochemical profile of the extracts was determined using chemical reactions to identify alkaloids (Petruczynik, 2012) and terpenes and steroids (Harbone, 1998) by means of thin layer chromatography (TLC) using a 0.2 mm silica gel (F_{254}) in aluminum (MERCK). Total phenols were determined by spectrophotometry using the Folin-Ciocalteu reaction, and read with a UV-Vis spectrophotometer at 620 nm, with adaptations (Singleton et al., 1999). The calibration curve for gallic acid was obtained using the same conditions for the preparation of the extracts (y = 3.8883x +

*Corresponding author. E-mail: ellen.matos1@gmail.com. Tel:+55 (75) 98808-0648.

Table 1. Minimum inhibitory concentration (µg/mL) of ethyl acetate extracts of leaves of *Clidemia hirta* and *Clidemia capitellata* (Melastomataceae).

Extract	Sample	*M. luteus*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Ethyl acetate	*C. hirta*	125	500	500	NT
	C. capitellata	62.5	125	125	125
Control	Chloramphenicol	15.62	31.25	31.25	15.62

NT: Not tested.

0.1141. $R^2 = 0.9954$). The results were determined by the interpolation of the absorbance of the samples with the standard curve of gallic acid expressed in milligrams of gallic acid per gram of crude extract (mg EGA/g of extract). Total falvonoids was determined by reaction with aluminum chloride ($AlCl_3$) (Arvouet-Grand et al., 1994), which was measured by absorbance using a UV-Vis spectrophotometer at 492 nm. The results were expressed in milligrams of quercetin per gram of crude extract (mg EQ/g of extract). The calibration curve for quercetin was obtained under the same preparation conditions ($y = 1.2078x – 0.1175$. $R^2 = 0.9889$). The tests were performed in triplicate.

Toxicity assays with *Artemia salina* leach

In vitro evaluation of the toxicity of extracts was performed according to the methodology proposed by Meyer et al. (1982). The extracts were categorized according to Clarkson et al. (2004) as: CL_{50} > 1000 µg/ml are nontoxic; CL_{50} > 500 µg/ml are of low toxicity; CL_{50} between 500 and 100 µg/ml are moderately toxic; and CL_{50} < 100-µg/ml are highly toxic.

Ten *Artemia* nauplii extracts were used in each well in three different conditions - 10, 100 and 1000 µg/ml - with 10 other nauplii exposed to marine water and another 10 exposed to DMSO (1.0%) as positive controls. All wells were submitted to artificial lighting and the tests were performed in triplicate. The nauplii were counted after 24 and 48 h of exposure. The mean lethal concentration (CL_{50}) was determined using the program *GraphPadPrism* 5.0.

Lineages and cell culture

The acute monocytic leukemic cell line (THP-1) was donated by the Immunology Laboratory of the Institute of Health Sciences (ICS) of the Federal University of Bahia. The cells were cultured in 5.0 mL of RPMI medium supplemented with 10% v/v fetal bovine serum (PBS) and 20 µg/ml of penicillin/streptomycin (1%) obtained from Cultilab. Cells were seeded and cultured to confluency in 25 cm² culture bottle at 37°C in an atmosphere of 5% CO_2 and controlled humidity.

Preliminary determination of *in vitro* cytotoxicity to THP-1 cells

Cytotoxicity of plant extracts to the THP-1 cell lineage was evaluated using an inverted microscope (20x). Cells ($1×10^6$ /-well) were pated in 1.0 ml RPMI complete medium per well in 24 well plates. The cells were then incubated in the presence of crude plant extract (100 µg/ml), diluted in DMSO for 24 h at 37°C, and then evaluated morphologically. Wells used for control contained RPMI cells and wells containing cells with RPMI and DMSO (0.5%).

Statistical analysis

Analyses were based on the mean in triplicate ± standard deviation of the mean, (one-way ANOVA; $p<0.05$), using *GraphPadPrism* 5.0, Minitab 18 and Excel 2013.

RESULTS

For the diffusion disk tests, *Clidemia hirta* exhibited activity against *M. luteus*, *S. aureus* and *P. aeruginosa*, with halos of 13.0 ± 1.02, 9.0 ± 0.57 and 10.0 ± 1.52 mm, respectively, while *Clidemia capitellata* exhibited activity against *M. luteus*, *S. aureus*, *P. aeruginosa* and *B. subtili*, with halos of 12.0 ± 0.57, 10.0 ± 0.57, 9.0 ± 0.57 and 11.0 ± 1.73 mm, respectively. For MIC (Table 1), the greatest activity was for extract of *C. capitellata* against the bacteria strain *M. luteus* (MIC 62.5 µg/ml).

For antioxidant activity (DPPH sequestration capacity), the extract of *C. hirta* was most effective (63.54 ± 1.46%), followed by *M. amoena* (51.66 ± 1.29%) and *M. fallax* (51.24 ± 1.43%). All extracts gave positive reactions in tests for total phenols, total flavonoids and tannins, but were negative for alkaloids. Half of the extracts were positive for the presence of terpenes or steroids (Table 2). The extracts of *Miconia alborufescens* (CL_{50} 61.6 µg/ml) and *C. hirta* (CL_{50} 75.8 µg/ml) exhibited high toxicity for *Artemia salina*. The extracts of *M. albicans* (CL_{50} 512.7 µg/ml), *C. sericea* (CL_{50} 831.7 µg/ml) and *Tibouchina lhotzkyana* (CL_{50} 537.0 µg/ml) exhibited low toxicity. All other extracts were inactive. Tests against the monocytic cell lineage THP-1 revealed that 90% of the extracts were capable of stimulating the formation of apoptotic bodies and induce cell death, except for *C. sericea.*

DISCUSSION

The phytochemical data for *C. hirta*, *C. capitellata* and the other extracts were consistent with other studies (Gordon et al., 2011; Abdellaoui et al., 2014; Tracanna et al., 2015; Scalco and Munhoz, 2016). The absence of alkaloids in the extracts confirms this as a chemotaxonomic characteristic of the family Melastomataceae (Silva et al., 2010). Differences between the present study and previous studies in the content of phenols and flavonoids may be a reflection of differing factors, including the extraction solvent, seasonality and the collection site.

The absence of growth inhibition halos in the agar

Table 2. Preliminary phytochemical screening and percentage free radical sequestration (% FRS) *in vitro* of extracts in ethyl acetate from leaves of species of the family Melastomataceae.

Sample	Total phenols (mg EGA/g)	Total flavonoids (mg EQ/g)	Al	Ta	Ter/Ste	%FRS
Miconia albicans	172.19 ±1.95	72.69 ± 0.90	-	+	-	39. 68 ± 0.87
Miconia ciliata	146.40 ± 1.15	60.41 ±3.96	-	+	-	8.30 ± 1.30
Miconia fallax	199.76 ± 5.67	100.52 ± 0.97	-	+	-	51.24 ± 1.43
Miconia amoena	254.09 ± 4.24	73.67 ± 0.90	-	+	-	51.66 ± 1.29
Miconia alborufescens	47.28 ± 0.59	3.94 ± 1.073	-	+	+	4.83 ± 1.95
Clidemia hirta	197.18 ± 1.35	123.52 ± 2.27	-	+	+	63.54 ± 1.46
Clidemia sericea	105.46 ± 4.55	76.69 ± 0.90	-	+	+	37.36 ± 1.17
Clidemia capitellata	127.04 ± 2.01	53.41 ± 1.69	-	+	+	28.60 ± 1.54
Tibouchina francavillana	122.51 ± 0.77	73.25 ± 1.30	-	+	+	47.90 ± 0.77
Tibouchina lhotzkyana	42.12 ± 0.17	31.55 ± 0.62	-	+	+	3.72 ± 1.54

Al: alkaloids; Ta: condensed tannins; Ter/Ste**:** terpenes/steroids; (-): absence; (+): presence.

diffusion tests was observed for most of the extracts in ethyl acetate, with the exception of *C. hirta* and *C. capitellata*. In concordance with the results of the present study, Meléndez and Capriles (2006) found antimicrobial activity for methanolic extract of leaves of *C. hirta* against fifteen bacterial strains, among them *S. aureus* and *M. luteus*, but no such activity for *C. capitellata*.

Dianita et al. (2011) demonstrated bactericidal activity of the ethyl acetate extract of the leaves of *C. hirta* against *P. aeruginosa* and bacteriostatic activity for *E. faecalis.*

For antioxidant activity, the data show that extracts with significant free radical scavenging activity also had a high content of polyphenols and total flavonoids, suggesting a direct connection between antioxidant action and the presence of these compounds. The phenolic constituents, among them flavonoids and phenolic acids, are known for their antioxidant effect in biological systems due the arrangement of their function groups, in particular hydroxyl groups, capable of neutralizing or sequestering reactive species (Fabri et al., 2009). Santos et al. (2017) reported the presence of compounds of this nature in different extracts of *Miconia*, and Lopez et al. (2016) for *C. hirta*, and Gordon et al. (2011) for *C. rubra*. Plant extracts that demonstrated toxicity for *Artemia salina*, according to the literature, possess cytotoxic compounds in concentrations sufficient to potentiate antitumor (Mclaughlin et al., 1991), antiplasmodic (Amarante et al., 2011) and actions against bacterial (Zuque et al., 2004) and fungal lineages (Niño et al*.,* 2006). The evaluation of the THP-1 lineage revealed that the extracts stimulated the formation of apoptotic bodies and cell death.

Antiproliferative effects- induction for the formation of apoptotic bodies and cell death- are an interesting approach in the treatment of leukemia (Kapoor and Kakkar, 2012). Research shows that most antitumor drugs cause cancer cells to die (Park et al., 2013) through induction of the apoptotic process (Onrubia et al., 2012). This, thus, suggests that the preliminary data of this evaluation of leaf extracts of species of the family Melastomataceae are promising in the search for new antitumor agents.

The presence of non-toxic extracts for *A. salina* and, concomitantly, cytotoxicity against THP-1 cells, may be indicative of the chemical constituents of the plant acting against antitumor cells without affecting the health cells in the body. However, toxicity in *A. salina* cannot be directly translated into human toxicity since the physiological media are different, and thus further analysis is needed to determine the effects on different human cell lines. Similar results for *M. fallax* ethyl acetate extract in THP-1 cells were observed by Cunha et al. (2008), for the ethanol extract and for two triterpenes isolated, ursolic acid and oleanolic acid; Aristizabal et al. (2013) for the butanolic extract of *C. hirta* in Vero cells and by Narasimham et al. (2017), using Dalton's lymphoma ascites cells (DLA), extracts of *C. hirta* leaves in petroleum ether and in chloroform.

Conclusion

The extracts from the species of Melastomataceae evaluated in the present work can be considered promising as potential sources of chemical agents with antimicrobial, antitumor and antioxidant properties.
Furthermore, *C. hirta* should be emphasized for exhibiting activity in all the tests performed. New chemical-pharmacological evaluations and the isolation of the active principle of the extracts should be performed to demonstrate the relationships and mechanisms involved in their antibacterial, antioxidant and cytotoxic activity

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Dominant lethal mutations in rats fed extracts of *Mucuna urens* (Linn.)

Etta Hannah Edim*, Udoh Samnuel and Eneobong Effiom Eneobong

Biological Science Department, Cross River University of Technology, Calabar, Cross River State, Nigeria.

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Dominant lethal mutation assay was carried out on rats after being treated with graded doses of ethanol extract of the seeds of *Mucuna urens.* Male albino rats (Wistar strain) were caged in three groups labeled, groups II, III and IV and treated with three different dosages of the ethanol extract of the seeds of *M. urens*; 70, 140 and 210 mg/kg body weight (BW), respectively, for 14 days. The positive control animals (group I), were treated with distilled water for the entire period. At the end of the feeding period of two weeks, they were co-habited with virgin female albino rats at a ratio of 1:1 for 3 days. 14 days after mating, the females were sacrificed for the dominant lethal mutation assay. The results of the dominant lethal mutation assay showed that only female rats in group II had implants on the uterine horn, of all the treated groups. The rats in groups III and IV did not have any implants at all. Biological evaluations (pre-implantation losses) carried out showed 0, 76, 100 and 100% lethal mutations in groups I, II, III and IV, respectively. The statistical evaluations obtained showed $8.6^{b} \pm 0.47$, $6.6^{a} \pm 0.94$, 0 ± 0.0 and 0 ± 0.0 in groups I, II, III and IV, respectively. Photographs of the *corpora lutea* were obtained using a digital camera (DCR-HC48E, KODAK). The results obtained can be attributed to the induction of dominant lethal mutations in spermatocytes and early spermatids in the male Albino rats, showing the mutagenic effect of the seeds of the plant *M. urens* and its potential as a male contraceptive.

Key words: *Mucuna urens* seeds, dominant lethal mutations assay, mutagenesis, male Albino rats.

INTRODUCTION

The seeds of the herb, *Mucuna urens*, are commonly found in home gardens in the south eastern parts of Nigeria, West Africa, where the Efiks, Ibibios and Igbos use it as a major soup condiment for thickening. It is called “Ibaba” by the Efiks/Ibibios and “Ukpor” by the Igbos and is usually sold in the local markets during its harvest season which is in the month of January.

In Calabar and in its immediate localities, *Mucuna* seeds are prepared in various ways. The seeds are used in sws (soup) preparations, where they are briefly dry-roasted (5-10 min) in a pot on fire until the seed becomes spotty brown. The roasted seeds are then pounded, sieved (to separate the seed coats) and added to a soup consisting of greens, vegetables, oil and sometimes meat. In that soup, they are allowed to cook for at least 30 min (Elittä and Carsky, 2003). It was estimated that

*Corresponding author. E-mail: sarahrhoda@yahoo.co.uk. Tel: +234-080-35835981.

a cup containing about 200 seeds feeds approximately 10 people. It is therefore used in the same way as the seeds of “egusi” (melon), an extremely popular ingredient in Nigerian cooking, and sometimes the seeds of *Mucuna* and “egusi” are mixed to prepare the soup (Ukachukwu and Obioha, 2000). Another recipe developed for preparing it involves cracking the seeds, two overnight soakings, various water changes, and boiling for at least 20 min (Elittä and Carsky, 2003).

Mucuna is typically planted near trees and climbs around the trees as support producing high amount of seed per plant. It is also planted by the fence or allowed to climb yam stakes together with yam. Both white- and black-seeded *Mucunas* (scientists in the region refer to these types typically as var. *cochinchinensis* and *urens*, respectively) are used but the white-seeded type is preferred (Sridhar and Bhat, 2007). In other localities where *M. urens* is found, it is known as velvet bean, *pica-pica*, bengal bean, *nescafé*, *ojo de venado*, *pois mascate, kara benguk*, *olhos de burro* (Esonu et al., 2001). One of its Sanskrit names, *atmagupta*, (“having hidden properties”), seemingly denotes its importance as a medicinal plant while another, *kapikachchhu* (“monkey’s itch”), refers to the unpleasant characteristic of its many accessions. Horse eye bean, ox-eye bean and devil bean are all common English names for *Mucuna*. The seeds are also known as "sea beans," because they are commonly carried by rivers into the ocean (Armstrong, 1998).

Taxonomists classify *Mucuna* as a vascular seed plant belonging to the subclass Rosidae and order Fabales. A seeming consensus exists among the food scientists familiar with *Mucuna*, as well as with the recent developers of *Mucuna* recipes, that the intoxication associated with eating *Mucuna* seed is mainly related to the high L-Dopa content of the raw seeds. *M. urens* seed was chosen for this investigation because it is believed in our immediate localities, where it is consumed, that they lower the sperm integrity in men, thereby reducing erection and invariably, fertility. Among the various procedures proposed for use in assessing the mutagenic potential of drugs, the dominant-lethal assay stands currently as one of the few tests for measuring mutagenic effects on germ cells (Ray et al., 1974).

In general, the animal treated is a male because chemicals acting systemically on females may interfere with hormonal status, possibly interfering with the development of normal foetuses, or the chemical may act directly on the maturing oocyte, causing death other than by a dominant lethal mutation (Takagi et al., 2000). Several dominant lethal indices can be employed when examining dominant lethals in mammals; these include pre-implantation losses estimated on the basis of *corpora lutea* counts, dead implants per female, dead implants/total implants, females with one or more post-implantation losses, and females with two or more post implantation losses (Chellman et al., 1986; www.setonresourcecenter.com, 2002).

The aim of this study, therefore, is to investigate the mutagenic effect(s) of the ethanol seed extracts of *M. urens* on the sperm integrity in male albino rats using the dominant lethal mutation assay (Plates I and II).

MATERIALS AND METHODS

The 24 albino rats (12 males and 12 females) used as mammalian models for this study were obtained from and housed in the animal house of the Pharmacology Department of the University of Calabar, Calabar. Preparation of the extract and sacrificing of treated and non-treated animals were carried out in the research laboratory of the same department.

Collection and preparation of seeds

The dry seeds of *M. urens* (Linn.) were bought from the local market (Watt market) and identified by the chief technologist in the Pharmacology Department of the University of Calabar, Calabar. The seeds were further air-dried for 3 days and then pulverized using a blender, Laprivia 3000, China.

Animal treatment

The rats, weighing between 100 and 150 g were housed in groups of three each in rubber cages and kept under optimum laboratory conditions (ambient temperature of 25±3°C; relative humidity: 50-55%; 12:12 dark:light cycle) and given food (commercial poultry growers’ mash from the Top Feeds LTD., Calabar) and water *ad libitum.*

Plant extraction

The plant extract was prepared by Soxhlet extraction following standard procedures. Ethanol was used as the extracting solvent since the powder was not soluble in water. The powdered sample (100 g) was wrapped in filter paper (Whatmann’s No. 40) and placed in the thimble in the main chamber of the Soxhlet apparatus. The Soxhlet extractor was placed on a flask containing 400 ml 80% ethanol and then equipped with a condenser. The ethanol was heated to reflux and the vapor travelled up a distillation arm to the chamber housing the thimble of *M. urens* powder. As the Soxhlet chamber filled up the solvent automatically emptied into the distillation flask through a siphon. This cycle was allowed to repeat for 72 h at 80°C. The ethanol extract was evaporated using a hot air oven (STUARC scientific, England) for 24 h at 50°C. 1 g of the paste extract was also dissolved in 10 ml Corn oil (vehicle) to make up 100 mg/ml concentration which was the stock solution. This was kept refrigerated at 4°C for later use.

Administration of extract

After an acclimatization period of two weeks, administration of the herb extracts commenced and continued every day for 14 days. Three concentrations (doses) of the plant extract were fed to the male rats while the female rats were left untreated. These doses were 70 mg/kg Body Weight (group I), 140 mg/kg Body Weight

Plate I. *Mucuna urens* seeds.

Plate II. Pulverized *Mucuna urens* seeds.

(group II) and 210 mg/kg Body Weight (group III) (Udoh and Ekpeyong, 2001). The control animals (group I) were not fed any extract (0 mg/kg BW). The doses were computed based on the initial body weights of the experimental animals. The experimental animals received the extract before their normal chow every day. Administration was done by feeding with oral gavage tube. At the end of the extract administration, the male rats were co-habited with untreated virgin females for three days at a ratio of 1: 1 to examine the dominant lethal parameters.

Experimental

Mating was confirmed by examination of the vaginal plug. The rats were then separated again and the female rats observed closely for 14 days at the end of which period, they were sacrificed by cervical dislocation and thereafter, dissected. The uterine contents were examined to observe the number of implants and life and dead embryos in the *corpora lutea* for determining pre-implantation losses and pictures were obtained using a digital camera (DCR-

Table 1. Dominant lethal mutation assay of rats treated with ethanol extracts of *Mucuna urens.*

Treatment group (mg/kg BW)	No. of males	No. of pregnant females	No. of non-pregnant females	Biological evaluations
I	3	3	-	0
II	3	3	-	76
III	3	-	3	100
IV	3	-	3	100

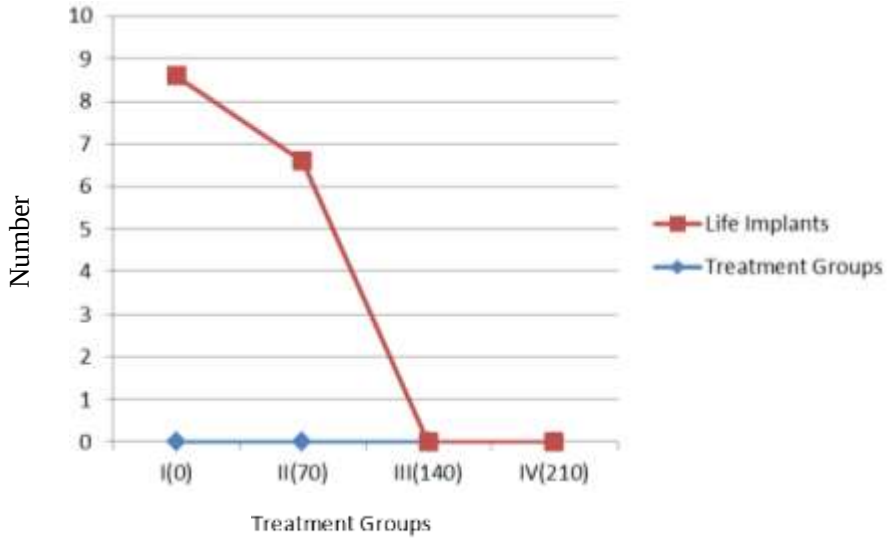

Figure 1. Life implants in the uterine horn of female albino rats.

HC48E, KODAK).

Data analyses

Biological evaluation of dominant lethal mutation was analyzed using the formula (http://www.setonresourcecenter.com, 2002):

1 - (Mean life implant in each treatment group / Mean life implant in control) × 100%

Results were reported in accordance with the CFR P798-024 Rodent Dominant Lethal Mutation Assay guidelines (1978). The statistical evaluation of the means and standard deviations of the observed implants were also carried out. All evaluations were done with the aid of the Genstat (7.2) statistical package.

RESULTS AND DISCUSSION

The results of the Dominant Lethal Mutation Assay of male rats fed with ethanol extract of seeds of *M. urens* for 14 days are presented in Table 1 and Figure 1. Photos of the *corpora lutea* (Plate IIIA to D) of untreated female rats mated with treated males, at mid-gestation, have also been presented. The biological evaluation of the mutations was observed as 0, 76, 100 and 100% lethal mutations in groups I, II, III and IV, respectively. This data which was obtained from the biological evaluations shows that the ethanol extract of the herb, *M. urens*, had lethal effects on the spermatocytes and early spermatids of the male albino rat (Udoh and Ekpeyong, 2001).

Pre-implantation losses were recorded in all the treatment groups, with rats in groups III and IV scoring no implants. On the other hand, the percentage implantation in the control rats was 100%. Means and standard deviations of life implants observed were $8.6^{b} \pm 0.47$, $6.6^{a} \pm 0.94$, 0 ± 0.0, and 0 ± 0.0 for the treatment groups I, II, III, and IV, respectively. These results are similar to those obtained by several independent workers on the dominant lethal effects of Acrylamide in male mouse germ cells. It was observed to induce dominant lethal mutations, heritable translocations and specific locus mutations in the mouse.

Shelby et al. (1986), Ehling and Neuhauser-Klaus (1992), Adler et al. (1994) Udoh and Ekpeyong (2001) showed the antifertility potentials of *M. urens* extract in

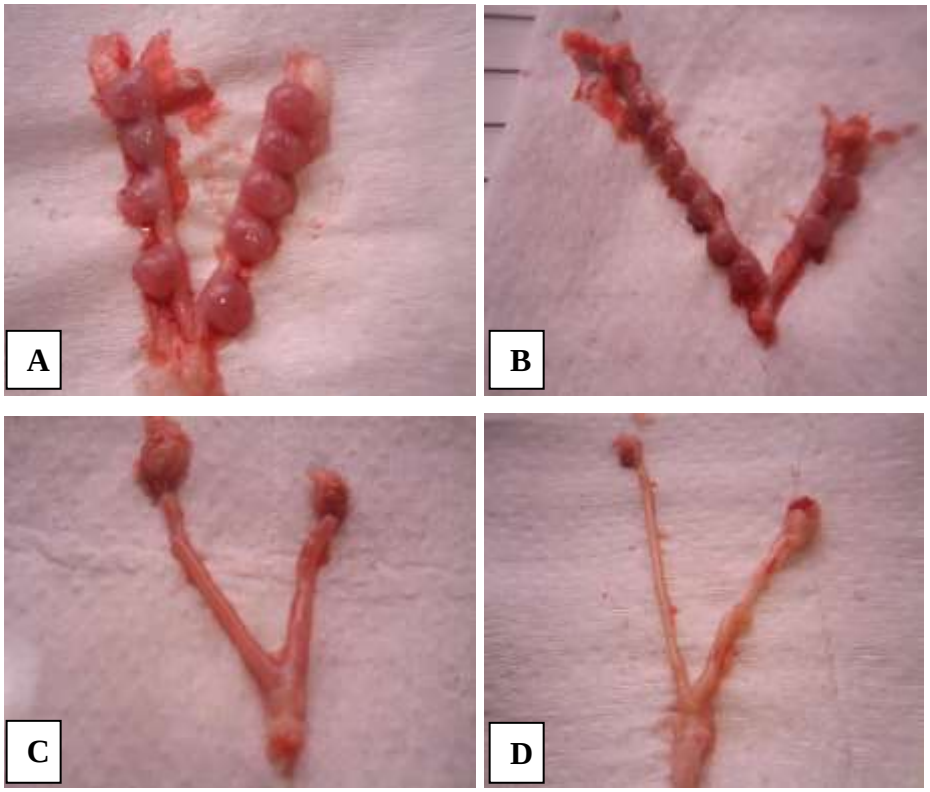

Plate III. A-Healthy implantation (group I); Plate B-Poor implantation (group II); Plate C- No implantation (group III); Plate D- No implantation (group IV).

male guinea pigs. From their findings, histological observations at high dose (140 mg/kg BW) showed complete degeneration of sperm in the testicular tubules. This will suggest that under the test conditions, the ethanol extract of the seeds of *M. urens* maybe genotoxic in the germplasm of the treated male rats (CFR, 2002).

Similar results were obtained in investigations on Neem plant (*Azadirachta indica*) when the Neem oil was shown to inhibit sperm motility *in vitro* (Riar et al., 1990; Upadhyay et al., 1990). This genotoxicity is also implicated as the cause of partial sterility in the lower doses and total sterility in the male rats at higher doses (Generoso et al., 2003). The *corpora lutea graviditas* counted also showed a dose-related decrease in number. Since the *corpora lutea* is critical in hormone production at the early stages of conception, its reduction in number will also reduce hormone production and may, therefore be implicated in the inability of the conceptus to stay alive (WHO, 1985).

Conclusion

The mutation observed in the treated rats at the different doses of treatment may be attributed to the effects of a component or some components of the seeds of *M. urens* acting either alone or in synergy. It is recommended that further studies on the effects of *M. urens* seed extracts in male animals with emphasis on mutagenesis be carried out. Characterization and further fragmentation to determine the active antifertility ingredient(s) is also suggested.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

The effect of *Thymus vulgaris* L. on renal and liver toxicity in wistar rats exposed to aluminum

Nawel Mokrane[1*], Omar Kharoubi[1], Fatima Zohra Tahari[1], Akila Guenzet[2] and Abdelkader Aoues[1]

[1]Laboratory of Experimental Biotoxicology, University of Ahmed Ben Bella "1", Oran, Algeria.
[2]Laboratory of Clinic and Metabolic Nutrition, University of Ahmed Ben Bella "1", Oran, Algeria.

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The main sources of human exposure to aluminum are found in the extensive presence of the metal in the environment and its growing industrial applications. The present study was carried out to determine the effectiveness of the *Thymus vulgaris* L. extract in alleviating aluminum chloride ($AlCl_3$) toxicity on biochemical and antioxidant parameters. The experiment's rats were divided into five groups: a control group; an intoxicated group (300 mg $AlCl_3$/kg bw); and three other groups which were given $AlCl_3$ (300mg/kg/bw) then *T. vulgaris.* L extract *(T.v)*, Malic Acid (MA) and Vitamin E (Vit E) at a concentration of 150mg/kg/bw. Each group was given its respective dosage, daily for 90 days. The results showed a significant decrease in the body/liver/kidney weights, the plasma total protein (T. Protein) and albumin (Alb) levels (p≤0.05) in the intoxicated rats group. However, a significant increase in the plasma uric acid (Uac), alkaline (AlP) and acid phosphatase (AcP) levels was noted (p≤0.05) in the same group. The amount of aluminum, TBARS and nitrate/nitrite (NO) in the liver and kidney tissues of the rats treated with $AlCl_3$ was also found to have increased (p≤0.05), while the levels of glutathione peroxidase (GSH-Px) and glutathione transferase (GSH-St) have decreased significantly (p≤0.05). These altered parameters were restored in the rats treated with the *T. vulgaris* L. extract. Therefore, due to these beneficial effects, *T. vulgaris* L. could potentially be used to antagonize $AlCl_3$ toxicity.

Key words: *Thymus vulgaris* L., aluminum chloride, oxidative stress, malic acid, vitamin E.

INTRODUCTION

Aluminum is a metal used more and more in our daily routine in spite of its toxic impact its biological functions remain unknown. Al has been considered as a toxic agent in many pathological processes such as neurodegenerative diseases (Exley, 2016), mycrocytic anaemia and osteoporosis (Chappard et al., 2016). Once absorbed, Al gathers necessarily in the bones, brain, liver and kidneys (Kinawy, 2019). This metal was considered hurtless since its presence in the trivalent form meant that it would easily attach to ions and forms colloidal polymeric particles. This form of particles is insoluble as a result it was believed that the absorption of the metal would be restricted (Bondy, 2010). In addition, toxic impacts of Al have led to histopathological alterations in

*Corresponding author. E-mail: monawel@hotmail.fr. Tel: +213 (0) 69.73.28.32.

the kidney and liver (Ghorbel et al., 2015). The molecular mechanisms include membrane function disruption, oxidative stress induction and disruption of several metals' metabolism (Hasona and Ahmed, 2017). Therefore, the estimation of antioxidant defense has become an important aspect of investigation of Al toxicity.

There is a growing tendency towards using phytotherapy owing to the general belief that it has no side effects compared to chemotherapy (Geleta et al., 2016). Many natural product extracts have been found to have a variety of pharmacological and antioxidant effects. *Thymus vulgaris* (common thyme), locally known in Algeria as "Zaatar", belonging to the Lamiaceae family, is a perennial herb indigenous in North Africa, Central and Southern Europe. Common Thyme's stems are branching and of a silver-grey color. Its leaves are small, oval and coiled at the edges and very fragrant while its flowers appear from May to September and are of a white or pale pink color. *T. vulgaris* L. has been commonly used since ancient times in the treatment of burns and poisoning caused by snakes and scorpions. It is an aromatic medicinal herb which is widely used in traditional folk medicine for its antimicrobial effects (Hosseinzadeh et al., 2015), anti-inflammatory and antalgic effects, antioxidant benefits (Tural and Turhan, 2017) and its antifungal properties (Benabed et al., 2018). It is also recognized for its antispasmodic, antiseptic, anthelmintic, diuretic and sedative properties (Hosseinzadeh et al., 2015). It is these various benefits of *T. vulgaris* L. that form the motivation behind the current study which aims to evaluate the protective effects of *T. vulgaris* L. against $AlCl_3$ toxicity in rats.

Various acute and chronic metal intoxications treatments are available. These include chelating agents which have been used clinically as antidotes that bind and enhance the excretion of toxic elements. The chelating agent most commonly used in Al disorders is desferrioxamine (DFO), which has a great ability to decrease the Al body toxicity by increasing its excretion (Kruck et al., 2004). However, DFO therapy is associated with toxic side effects, is very expensive and is only efficient intravenously or subcutaneously. Therefore, alternative molecules with the same efficiency as DFO, but ones which can be orally administered were needed. One such alternative is the Malic acid chelator which is a non-toxic and natural compound containing dicarboxylic acid, and magnesium. The chelation abilities of MA-mg against $AlCl_3$ toxicity were assessed when it was administered to mice exposed to Al at about one-fourth of the LD_{50} level. Compared to other chelators, the MA-mg product showed a better therapeutic effectiveness.

Vitamin E (Vit E) (alpha-tocopherol) is a powerful naturel antioxidant. It acts in synergy with other molecules like vitamin C, selenium and zinc. A good intake of vitamin E can neutralize the excess of free radicals and therefore prevent cellular activity disruption which is normally caused by exposure to various stressors. The antioxidant function of this micronutrient could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells (El-Demerdash, 2004; Kutlbay et al., 2007).

The present study was carried out to examine the effects of $AlCl_3$ poisoning on the biochemical and antioxidant parameters of the liver and kidney tissues of rats. Due to the health problems caused by $AlCl_3$ and many other environmental pollutants, various investigations have been undertaken in this study to evaluate the chelator effects offered by the natural Thyme plant and the Malic acid as well as the relative antioxidant potential offered by Vit E. The present study however, focuses on determining the efficiency of these treatments especially that of *T. vulgaris* L. aqueous extract in antagonizing the biochemical alterations and oxidative stress leading to liver and renal dysfunction in male rats.

MATERIALS AND METHODS

Collection of plant material

T. vulgaris L. (Lamiaceae) was collected in June 2014 from the Mostaganem region in the north west of Algeria. The plant's identification was confirmed at the department of Botany of Ahmed Ben Bella University 1 (Oran, Algeria) where a specimen (voucher No. LB 2368) was kept.

Preparation of *T. vulgaris* L. extracts

The aerial parts of the plant (leaves, flowers and stems) were air-dried in the dark away from humidity at room temperature and then ground to fine powder. The powdered aerial parts of *T. vulgaris* L. (5 g) were then extracted with boiled distilled water (500 ml) for 30 min. This water extract was then filtered, lyophilized and stored at -20°C until use. In order to extract the adequate amount of extract for experimental animal, the procedure of preparation was repeated multiple times. The yield of extraction is 20.22%.

Animals and experimental design

Male Wistar rats of four weeks old weighing (70 ± 10 g) were housed in standard cages in groups of six rats each. All groups were kept in a 12 h light/12 h dark cycle, at a room temperature of (22 ± 2°C) and were fed *ad libitum*. All experiments reported in this study were carried out in accordance with current guidelines for the care and use of laboratory animals (8th edition, 2011). All animals within each treatment group were given their respective diet for 90 days as follows:

(i) Group 1 (C): Untreated control group.
(ii) Group 2 ($AlCl_3$): The rats were orally given a solution composed of $AlCl_3 6H_2O$ which was dissolved in distilled water. The daily dose given was 300 mg/kg bw.
(iii) Group 3 ($AlCl_3$ + *T. vulgaris* L.): During the first 45 days, the rats were given to drink by feeding bottle the same $AlCl_3 6H_2O$ solution as Group 2 at a daily dose of 300 mg/kg bw. This was then followed by giving the rats a treatment solution of *T. vulgaris* L. aqueous extract at a daily dose of 150 mg/kg bw for the following 45 days for a total duration of 90 days.
(iv) Group 4 ($AlCl_3$ + MA): The rats in this group were given to drink

the same $AlCl_3 6H_2O$ solution for 45 days which was then followed by a Malate Magnesium chelator solution for 45 days at a concentration of 150 mg/Kg bw.
(v) Group 5 ($AlCl_3$ + Vit E): The rats in this group were given to drink the same $AlCl_3 6H_2O$ solution for 45 days followed by a Vitamin E solution for 45 days at a dose of 150 mg/Kg bw.

T. vulgaris L. (150 mg/Kg) is a tolerated dose and without toxic effect because it represents the (1/30) of LD_{50} (lethal dose of 50% of population) of alcoholic extract which is 5 g/kg of body weight used in the study of El-Newary et al., 2017. In another study (Geleta et al., 2016) researchers tested the dose (10.000 mg/kg) of aqueous extract of *T. shimperi* on acute intoxication also tested the dose of (600 mg/kg) in subchronic toxicity and observed their non-toxicity. Therefore the dose chosen in this study is lower than the LD_{50} previously noted. The animals were observed daily for any signs of toxicity and their body weight was recorded on a weekly basis throughout the experimental period.

Blood collection and tissue sample preparation

At the end of the experimental period and after the administration of the last dose, the rats were left to rest overnight and were sacrificed under pentobarbital anesthesia the following day. The rats' blood samples were then collected, and the liver and kidney organs were harvested, rinsed with a saline solution (0.9% NaCl) and then weighed. The organs' weight ratios were estimated and their relative weight calculated as g/100 g BW. To evaluate the oxidative status, the liver and kidney were homogenized in suitable buffers**:** in 1.15% KCl for thiobarbituric acid reactive substances (TBARS), in a 0.1 M phosphate buffer (pH 7.2) for glutathione transferase (GSH-ST) and glutathione peroxidase (GSH-PX), in a PBS (Phosphate buffered saline, PH 7.4) solution for nitrite estimation In addition, some portions of the liver and kidney were digested with nitric acid to determine the aluminum concentration.

Biochemical parameters

Alkaline phosphatase (AlP), acid phosphatase (AcP), uric acid (Uac) and albumin (Alb), were estimated in serum using Chrono-Lab kits (Spain). Total protein was measured by using bovine serum albumin as a standard (Lowry et al., 1951) Folin and Ciocalteus Phenol reagents were used to develop the blue color that was measured spectrophotometrically at 750 nm.

Lipid peroxidation (TBARS)

Lipid peroxidation in the liver and kidney tissues was estimated by measuring the formed malondialdehyde (MDA) using thiobarbituric acid reactive substances, according to the spectrophotometric method of (Okhawa et al., 1979). Aliquots (0.2 ml) of hepatic or renal homogenates were added to 0.2 ml of 8.1% (w/v) sodium dodecyl sulfate, 1.5 ml of 20% (w/v) acetic acid buffer (pH 3.5), and 1.5 ml of 0.8% (w/v) thiobarbituric acid. After completing the volume with 4 ml of distilled water, the mixture was heated for 1h at 90°C then cooled and centrifuged at 4000 r/min for 10 min. TBARS were measured at 532 nm and expressed as MDA equivalents (nmol/g of Protein) using a molar extinction co-efficient: 1.56×10^5mol/L/cm.

Glutathione transferase (GSH-ST)

Glutathione transferase (GSH-ST) activity in the liver and kidney tissues was assessed using an assay kit provided by Cayman (Chemical, USA).

Glutathione peroxidase (GSH-Px)

Glutathione peroxidase (GSH-Px) activity in the liver and kidney tissues was assessed by the method of Rotruck et al. (1973). Briefly, the reaction mixture contained 0.2 ml of Tris–HCl buffer (0.4 mol/L, pH 7.0), 0.2 ml of reduced GSH (1 mmol/L), 0.1 mL of Sodium Azide (10 mmol/L), 0.1 ml of H_2O_2 (1 mmol/L) and 0.2 ml of tissue homogenates. After incubation of the mixture at 37°C for 10 minutes, the reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid, and tubes were subjected to centrifugation at 2400 r/min for 10 min. The supernatant (0.2 ml) was then added with 0.1 ml of Ellman's reagent (0.0198 g of DTNB prepared in 0.1% sodium citrate). The Absorbance was recorded at 340 nm.

Nitrite (NO)

Nitrate Nitrite activity in the liver and kidney tissues was assessed using an assay kit provided by Cayman (Chemical, USA).

Aluminum Measurement

The concentration of $AlCl_3$ was estimated in the liver and kidney organs by using an atomic absorption spectrometer (Shimadzu, AA6200) following a wet acid digestion method as modified for dry-weight samples (Van Ginkel et al., 1990). A fraction of each organ (approximately 100 mg) was heated at 60°C for 24 h to obtain a constant dry weight. This was then placed in weighed flasks and digested with a nitric acid solution (65°) for 8 h. To get a final volume of 4 ml, a nitric acid solution (1% concentration) was then added to determine the aluminum concentration in the sample. The atomic absorption signal was measured by integrating the total absorption profile at 309.3 nm with a spectral bandwidth of 0.5 nm. All the analyses were performed in triplicate, the limit detection to Al is of 0.02 mg/L and the results were expressed in µg/g tissue wet.

Statistical analysis

The results are expressed as a mean ± standard error of the mean (SEM). Data comparison was determined by one-way ANOVA followed by Tukey *post hoc* analysis and the results were considered statistically significant when $p < 0.05$.

RESULTS

Throughout the study, some clinical signs of toxicity were observed in the $AlCl_3$-treated rats at the dose previously indicated. These included; fur loss, urine coloration, as well as a lack of activity (dullness, spirit depression) when compared to the rats in the control group. However, no rat deaths were recorded during the experimental period.

Effect of treatment on body, liver and kidney weights

At the end of the experiment, $AlCl_3$ administration in rats revealed a significant decrease in body weight levels by 19.13% when compared to the control group. A significant decrease ($p \leq 0.05$) is also observed in the absolute liver and kidney tissue weights when compared to the control group as shown in Table 1. However, the group of rats treated with *T. vulgaris* L. showed

Table 1. The Effect of Treatment on Body Weight, Absolute and Relative Liver and Kidney Weights.

Groups	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
Body weight BW (g)	335.95 ± 15.11	271.65 ± 11.27*	320.53 ± 7.14$^{\$}$	325.95 ± 12.84$^{\$}$	291.18 ±11.48*
Liver Absolute weight (g)	9.690 ± 0.131	7.376 ± 0.244*	8.525 ± 0.399$^{*\$}$	7.668 ± 0.392*	7.616 ± 0.135*
Relative weight (g)	3.007 ± 0.270	2.737 ± 0.155	2.759 ± 0.151	2.573 ± 0.112*	2.659 ± 0.053*
Kidney Absolute weight (g)	2.315 ± 0.054	1.930 ± 0.102*	2.287 ± 0.138$^{\$}$	2.098 ± 0.021*	2.005 ± 0.071*
Relative weight (g)	0.787 ± 0.022	0.716 ± 0.056	0.753 ± 0.041	0.677 ± 0.042*	0.627 ± 0.073$^{*\$}$

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

Table 2. The Effect of Treatment on Serum Biochemical Parameters.

Parameters	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
AlP (U/l)	31.81 ± 1.44	47.02 ± 2.3*	31.54 ± 1.73$^{\$}$	36.23 ± 0.83$^{*\$}$	44.56 ± 1.53*
AcP (U/l)	4.94 ± 0.38	13.62 ± 0.44*	5.76 ± 0.87$^{\$}$	8.10 ± 1.06$^{*\$}$	12.97 ± 0.48*
T.Prot (g/l)	54.02 ± 3.64	26.37 ± 3.09*	48.16 ± 1.32$^{*\$}$	32.17 ± 2.36*	29.5 ±2.43*
Alb (g/l)	43.42 ± 4.26	18.81 ± 1.25*	35.83 ± 3.91$^{*\$}$	23.2 ± 2.67*	24.22 ± 3.45*
Uac (g/l)	4.98 ± 0.68	11.55 ± 1.39*	5.89 ± 0.65$^{\$}$	8.98 ± 1.11*	8.3 ± 1.29$^{*\$}$

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

considerably less weight loss at the end of the experiment ($p \leq 0.05$) when compared to the intoxicated group. A similar improvement, albeit to a lesser extent than the one recorded in the *T. vulgaris* L-treated group, was also observed in the MA and Vit E treated groups.

Effect of treatment on serum biochemical parameters in aluminum-induced hepatic and renal toxicity in rats

Treatment with $AlCl_3$ increased significantly the plasma AlP level by 32.34% when compared to the control group (Table 2). In addition, it was found that the concentration of plasma AcP and Uac has significantly increased by 3-fold in the rats treated with $AlCl_3$. Plasma Alb and T. Protein, however, decreased significantly by (56.68 and 51.18% respectively) in the intoxicated rats group.

Examining the groups treated with either *T. vulgaris* L. or MA, a significant reduction in the plasma AlP (*T. vulgaris* L. 32.92%, MA: 22.95%) and AcP (*T. vulgaris* L. 57.70%, MA: 40.53%) was observed when compared to the intoxicated group. In addition, the Vit E treated group showed a low decrease in the same parameters that soared in the intoxicated group. The concentration of Uac was also significantly reduced by the *T. vulgaris* L. and Vit E treatments (49%, 28.14% respectively). The effect of the MA treatment on this parameter was found to be smaller (22.25%) in comparison. These results were accompanied by an increase in Alb and T. Protein concentrations in the *T. vulgaris* L. treated group (47.50 and 45.24% respectively) when compared to those of the intoxicated group. MA and Vit E treatments only slightly improve these parameters.

Effect of treatment on lipid peroxidation on aluminum-induced hepatic and renal toxicity in rats

As shown in Figure 1, a significant increase in the TBARS level (55.67% in the liver tissue and 62.64% in the kidney tissue) was observed in the intoxicated rats when compared to the control groups. The administration of *T. vulgaris* L. decreased the TBARS production significantly by a rate of 50.64% in the liver and 56.3% in the kidney when compared to the intoxicated rats. Malate and Vitamin E treatments showed less efficient results in term of restoring normal values of TBARS.

Effect of treatment on antioxidant parameters on aluminum-induced hepatic and renal toxicity in rats

The results obtained showed that GST and GPX levels (Figures 2 and 3) were significantly decreased in the liver (62.87 and 57.23% respectively) and kidney (73.23 and 75.18% respectively) of the rats treated with $AlCl_3$. The concentration of NO was also significantly increased ($p \leq 0.05$) as shown in Figure 4. However, liver and kidney GST and GPX levels increased by 2 and 3-fold respectively in the groups treated with *T. vulgaris* L. A significant increase ($p \leq 0.05$) in liver GST and GPX levels

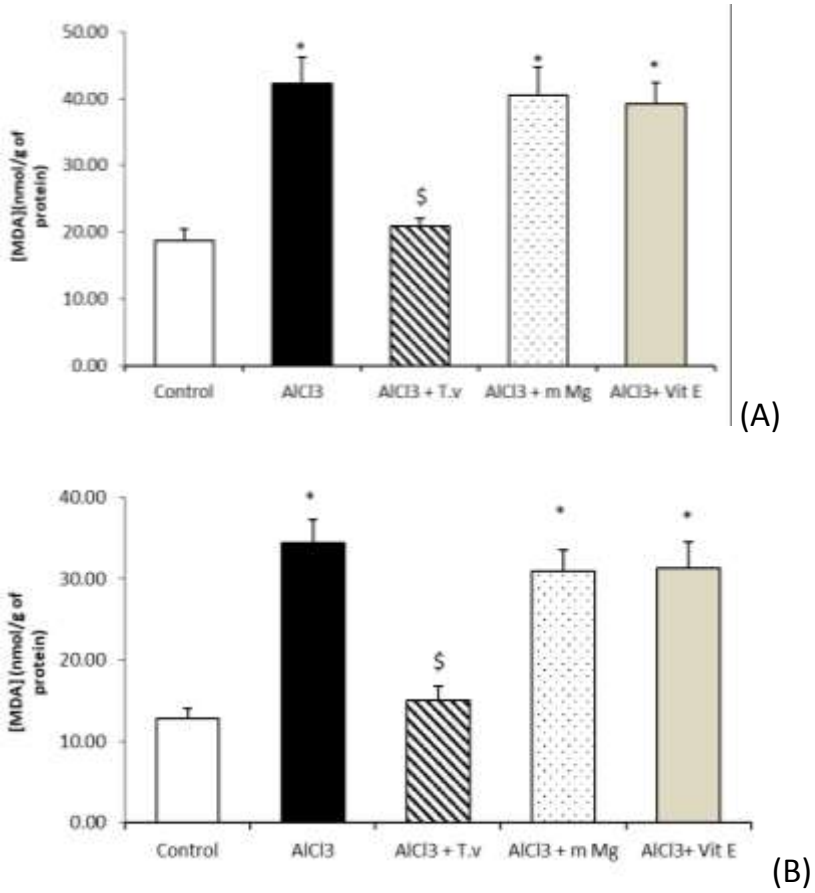

Figure 1. The Effect of Treatment on TBARS level (nmol/g of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

was also respectively noted in the groups treated with MA and Vit E when compared to the $AlCl_3$-intoxicated group. In addition, treatment with *T. vulgaris* L. caused a significant decrease in NO concentration (67.46 and 41.96%) in the liver and kidney tissues respectively. Only a slight reduction in liver and kidney ($p \leq 0.05$) NO concentration resulted from the Vit E treatment when compared to the intoxicated group.

Effect of treatment on liver and kidney aluminum content

The $AlCl_3$ concentration in the liver and kidney of rats was measured after 3 months of oral $AlCl_3$ exposure and the results are presented in Table 3. The concentration of $AlCl_3$ in the liver and kidney of the intoxicated group was higher by 3 and 5 fold respectively when compared to the control group. However, this concentration was significantly decreased in liver and kidney of the group treated with *T. vulgaris* L. (32.42 and 59.28%) when compared to the intoxicated group.

DISCUSSION

Aluminum absorbed by the human body via the gastro-intestinal and the respiratory tracts, is known to disrupt the pro-oxidant and antioxidant balance of tissues, leading to various biochemical and physiological dysfunctions (Exley, 2004; Nehru and Bhalla, 2006). In this study, Aluminum Chloride was chosen over other Aluminum compounds because the stomach already contains and utilizes Chloride. Therefore, this form of Aluminum can be introduced with minimal change to gastric fluid composition. The focus of the present study is on the liver and kidney organs where metals are usually accumulated and where toxic effects can be expected.

The results of the study have indicated that body, liver and kidney weights gain in the rats treated with $AlCl_3$ was markedly less ($p \leq 0.05$) when compared to the normal control group. This demonstrates that Al administration has a detrimental effect on the rats' body weight. This finding agrees with the results of other studies where balgoon (2019) noticed a significant reduction of about

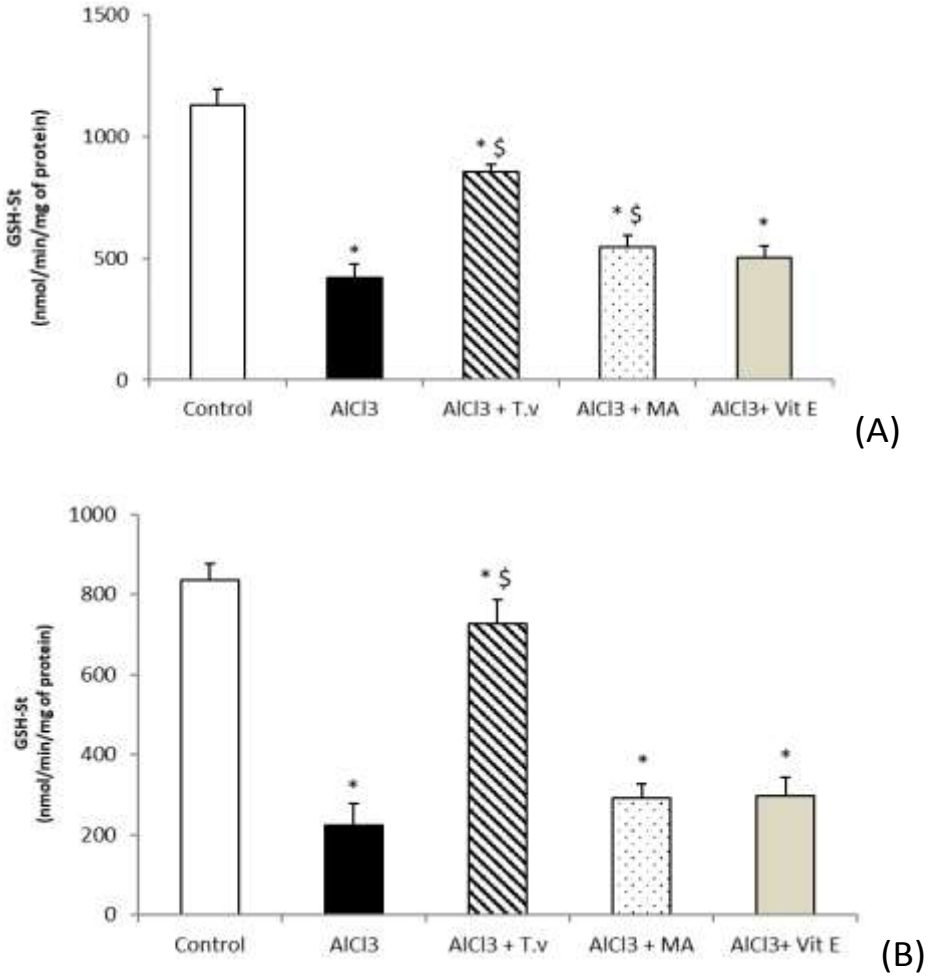

Figure 2. The Effect of Treatment on GSH-St Activity (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

3.51% in weight gain in $AlCl_3$-treated rats. Similar results were also obtained by (Singla and Dhawan, 2013). A significant decrease in absolute and relative liver and kidney weights was observed in $AlCl_3$-treated rats which is in agreement with previous reports by (Yeh et al., 2009; Paz et al., 2017). However, the current study has found that rats treated with *T. vulgaris* L. showed a significant improvement in body, liver and kidney weights gain. In the study of Manafi et al. (2014) broilers intoxicated with aflatoxin and treated with Thyme essence showed a significant gain in body weight revealing the same beneficial impact.

Several studies have reported that Al accumulates in mammalian tissues such as brain, bone, liver and kidney (Belaïd-Nouira et al., 2013). As a result, Al causes alterations in the biliary secretory function and an increase in oxidative stress in hepatic tissues (Gonzalez et al., 2004). Such accumulation might be the result of the higher affinity of Al for transferrin, which, in turn, might also explain the interference it causes with iron metabolism (Crichton et al., 2002). The present study results are also consistent with recent findings that showed that chronic Al consumption causes significant plasmatic increases in the activities of AlP and AcP enzymes which could be due to severe damage in the tissue membranes (Esmaeili et al., 2000; Al-Qhtani and Farran, 2017). Moreover, Rahman et al. (2000) suggested that the decrease in the activities of AlP and AcP in different tissues might be due to the increased permeability of the plasma membrane or the cellular necrosis of the liver, kidneys and lungs. In addition, Ochmanski and Barabasz (2000) proposed that the salts of Al could potentially enter inside the cells and might directly bind to the DNA and RNA helices. This prevents their replication and then inhibits the activities of AlP and AcP. In this study, it was shown that an administration of the *Thymus vulgaris* L. aqueous extract has led to a marked reduction in the elevated activities of AlP and AcP in $AlCl_3$ treated rats. In the study of Abd El Kader and Mohamed (2012) rats intoxicated with paracetamol

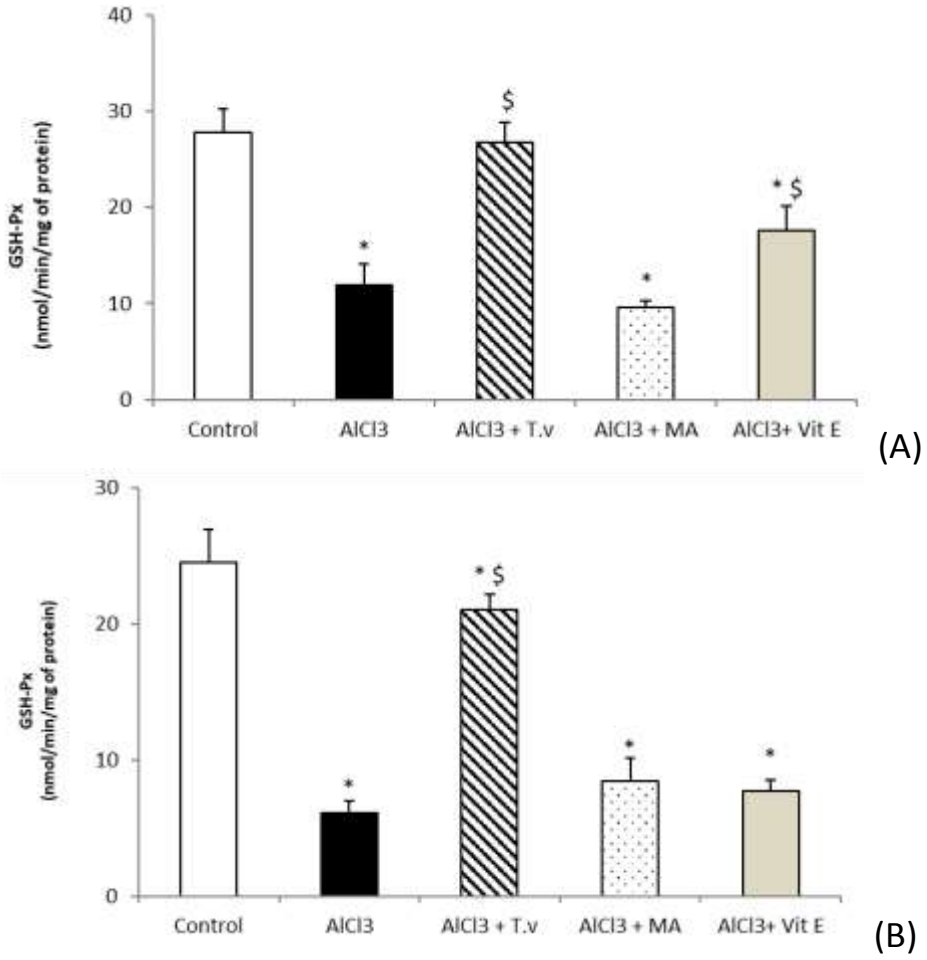

Figure 3. The Effect of Treatment on GSH-Px Activity (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

and treated with thyme showed the same effect.

The observed decline in the level of protein in $AlCl_3$ treated rats in this study agrees with the findings of Kinawy (2019) and might be due to changes in the metabolism of proteins, particularly albumin. On one hand, this could be attributed to malnutrition but on the other hand, it could be due to a reduction in the protein synthesis in the liver (Tripathi et al., 2009). The same results also revealed that $AlCl_3$ intoxication caused a significant decrease ($p< 0.05$) in plasma T. Protein and Alb (Massoud et al., 2016), while an increase in AlP parameter was observed. Furthermore, aluminum may promote proteinuria by causing a nephritic syndrome or chronic glomerulonephritis.

The accumulation of Al in the kidney is usually accompanied by renal failure (Ecelbarger et al., 1994). Moreover, Al accumulation in the kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity (Somova et al., 1997; Mohamed et al., 2017). In addition, renal functions can also be altered in the early stages of hepatic damage before the formation of ascites (Monasterolo et al., 1993). Uric acid is the major product of purine bases, adenine and guanine. It is partially considered as a significant marker of renal function. The Plasma Uac (Uric acid) elevation recorded in this study after $AlCl_3$ exposure is in agreement with the majority of researches (Sivakumar et al., 2014; Al-Qhtani and Farran, 2017), and is also supported by the findings of Rudenko et al. (1998) who reported that $AlCl_3$ intensifies the acid–secretion function of the kidney and changes the transport of sodium.

It has been suggested that the toxic effects associated with aluminum are due to the generation of reactive oxygen species, which results in the oxidative deterioration of cellular lipids, proteins and DNA. In the present experiment, we observed a significant increase in liver and kidney lipid peroxidation (TBARS) after $AlCl_3$ exposure. These results are similar to previously published findings demonstrating that aluminum salts intake promoted lipid peroxidation in liver and kidney

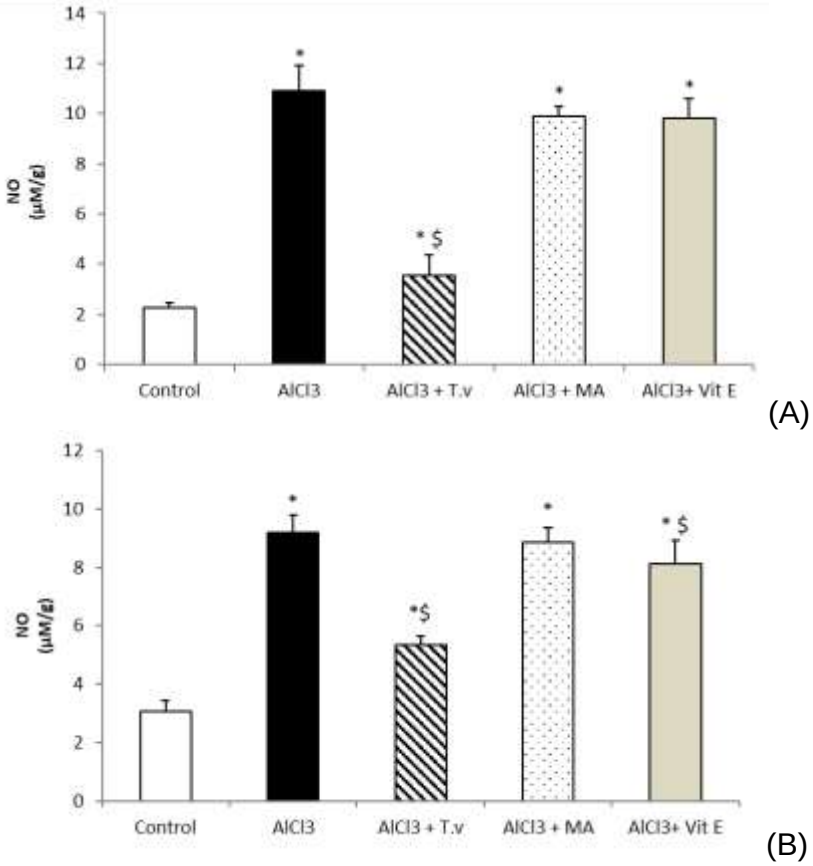

Figure 4. The Effect of Treatment on NO level (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

Table 3. The Effect of Treatment on Aluminum Content in the Liver and Kidney Tissues.

Groups	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
Liver Al (µg/g)	2.63 ± 0.41	8.84 ± 0.57*	3.60 ± 0.45$^{\$}$	8.16 ± 0.57*	8.88 ± 0.38*
Kidney Al(µg/g)	0.91 ± 0.11	4.72 ± 0.42*	3.19 ± 0.43$^{*\$}$	3.90 ± 0.38 $^{*\$}$	4.29 ± 0.31*

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($^{\$}$) Comparison with AlCl3 group.

tissues (Belaïd-Nouira et al., 2013; Tahari et al., 2016). It was also found out that the consumption of *T. vulgaris* L. induces a significant decrease ($p \leq 0.05$) in TBARS level.

Our study demonstrated that $AlCl_3$ administration caused a reduction in liver and kidney glutathione transferase (GSH-St) and glutathione peroxidase (GSH-PX) activities. The results are in accordance with Newairy et al. (2009). Furthermore, El-Sayed et al. (2011) reported a significant decrease in both enzymes in the liver of about (56 and 80%) respectively. A reduction of the kidney's GSH-Px activity was also noticed by Mahieu et al., 2009.

Nitrite resulted from the oxidation of NO, so Nitrite estimation is indirectly that of nitric oxide (NO) content in the biological samples. It has been suggested that Nitric oxide plays multiple roles in Al intoxication (Guo et al., 2001). The present investigation showed that $AlCl_3$ toxicity excessively increases NO products in the liver and kidney tissues which agree with many studies (Guo et al., 2005).

Recent studies demonstrated that aluminum may alter the activity of tissue antioxidative enzymes as TBARS and NO activity (Taïr et al., 2016; Benyettou et al., 2017). Our data support these results and the levels of these parameters were found to be elevated in $AlCl_3$-treated rats. However, GSH-Px and GSH- St activity were

decreased in the liver and kidney tissues. These observations are similar to previously published findings demonstrating that the aluminum intake promoted oxidative stress while the extract plants administration restored it (Kutlubay et al., 2007; Joshi et al., 2013).

Our results have indicated that a significant accumulation of $AlCl_3$ occurs in the liver and kidney tissues of $AlCl_3$-intoxicated rats. The same results were observed in other studies (Yeh et al., 2009) where $AlCl_3$ accumulation increased by 3 times in the liver and 6 times in the kidney (Missel et al., 2005; Cheng et al., 2014). However, the present study has shown that following *T. vulgaris* L. extract treatment; a significant decrease in $AlCl_3$ accumulation in the liver and kidney tissues was recorded.

It is well documented that the leaves and flowers of plants contain numerous aromatic chemical products which prevent the cellular damage caused by free radical molecules, ions or atoms (Dapkevicius et al., 2002; Al-Asmari et al., 2017). *T. vulgaris* L. is a famous herb cultivated all over the world, used essentially in food as a flavor and in medicine as an anti-microbial, anti-poisonous and deworming agent.

Intoxicated rats treated with thyme extract have shown low AlP and AcP levels in plasma which indicates the thyme's hepatoprotective impact. *T. vulgaris* L. aqueous extract also elevated the total serum protein, Alb and Uac levels. This explains the healing effect of thyme on aluminum toxicity and confirmed its nephroprotective effect as stated by El-Newary et al. (2017). This positive effect could be due to its secondary metabolites composition including alkaloids, polyphenols and flavonoids. The main compounds of *T. vulgaris* L. are the natural terpenoid thymol (12-61%) and its phenol isomer carvacrol (0.4-20.6%) which revealed a high antioxidative, antispasmodic and antibacterial effects (Amiri, 2012). In addition, *T. vulgaris* L. Contains high levels of vitamins B_3, C and E. It is also; very rich in potassium (K), Magnesium (Mg) and others trace elements (Komaki et al., 2016). Due to this composition, thyme is considered as a high regulator of homeostasis. This study also proclaimed that intoxicated rats given aqueous thyme extract showed remarkable lowered levels of MDA and NO and elevated levels of GSH-St and GSH-px in renal and hepatic tissues compared to control group. This implies the efficient and protective impact of thyme which is probably linked to its capacity to reduce oxidative stress as free radical scavenger or as a quencher of singlet oxygen formation preventing peroxidation of lipids.

El-Newary et al. (2017) found out that dietary supplementation of *T. vulgaris* L. reduced all the above measured parameters in the liver and kidney of rats. These results highlighted the benefit of *T. vulgaris* L. as a dietary antioxidant.

Other reports indicate that the essential oil of *T. vulgaris* L. has a potential antioxidant activity and a protective effect against toxicity of aflatoxins (El-Nekeety et al., 2011). The properties of the *T. vulgaris* L. constituents as polyphenols (Thymol and Carvacrol) and flavonoids (caffeic acid, rosmarinic acid, apigenine, thymonine and luteolin) protect the cellular membranes' integrity (Ündeger et al., 2009; Popovic-Milenkovic et al., 2014) from $AlCl_3$-induced oxidative damage and repair the antioxidant system. Moreover, other authors stated that thymol was found to decrease ROS production in dependent concentration and inhibit hepatotoxicity induced by CCl_4 in male Swiss albino mice as assessed by lipid peroxidation and by histopathological examination (Alam et al., 1999).

T. vulgaris L. has noticeably decreased the aluminum content in the liver and kidney. Some authors ascertained the fact that phenolic compounds were not able to chelate metal ions. Melidou et al. (2005) found that intracellular binding of iron is responsible for the protection offered by flavonoids against H_2O_2-induced DNA damage. On the other hand, complexation of plant extracts with metal ions might diminish the absorption of metal ions leading to chelating and blocking metal-mediators generating the ROS (Yang et al., 2013).

In the current study, it was also found that MA and Vit E treatments only partially alleviated the harmful effects of $AlCl_3$ toxicity as observed in previous research by El-Demerdash (2004) and Masoud et al. (2016). These results also concur with previously reported data indicating that administration of Aluminum with some plant preparations such as the *Crocus sativus* L. extract (Shati and Alamri, 2010), the *Zingiber Officinale* extract (Hasona and Ahmed, 2017) or Propolis (Turkez et al., 2010) or Selenium antioxidant products showed a recovery from hepatic damage (Viezeliene et al., 2011). Mahieu et al. (2009) and Yeh et al. (2009) have also revealed the beneficial effects of Melatonin and Taurine antioxidants against $AlCl_3$ nephrotoxicity.

Conclusion

Aluminum has adverse effects on human health and special attention must be paid to the sources of Al in food, water and personal care products. From the results presented in this study, it can be seen that exposure to $AlCl_3$ is capable of inducing significant harmful changes in some biochemical characteristics and redox status in liver and kidney tissues. However, this study has also demonstrated the therapeutic effects of the *T. vulgaris* L. plant in minimizing Aluminum liver and kidney toxicity. This treatment resulted in a significant improvement in body, liver and kidney weights and the plasma biochemical parameters (T. Protein, Alb, AlP, AcP and Uac). In addition, the *T. vulgaris* L. treatment led to a more efficient recovery from the oxidative tissue damage caused by Al intoxication (TBARS, GSH-Px, GSH-St and NO) when compared to the Malate and Vitamin E treatments. Therefore, using *T. vulgaris* L. extract could be a protective and preventative method in overcoming

the detrimental effects of Aluminum toxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

Advances in the research of *Adenanthera pavonina*: From traditional use to intellectual property

Maurycyo Silva Geronço[1], Roberta Cardoso Melo[2], Hugo Leonardo Mendes Barros[3], Samille Rodrigues Aquino[3], Fátima de Cássia Evangelista de Oliveira[5], Muhammad Torequl Islam[4], Claudia do Ó Pessoa[5], Marcia dos Santos Rizzo[3,6]* and Marcília Pinheiro da Costa[1,2]

[1]Graduate Program of Science and Engineering of Materials, Interdisciplinary Laboratory for Advanced Materials, Federal University of Piauí, Teresina - PI, Brazil.
[2]Graduate Program in Pharmaceutical Sciences, Federal University of Piauí, Teresina - PI, Brazil.
[3]Graduate Program in Dentistry, Federal University of Piauí, Teresina – PI, Brazil.
[4]Department of Pharmacy, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh.
[5]Graduate Program of Physiology and Pharmacology, Federal University of Ceará, Fortaleza - CE, Brazil.
[6]Department of Morphology, Health Sciences Center, Federal University of Piauí, Teresina – PI, Brazil.

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***Adenanthera pavonina*, an Asian native leguminous, is a well known highly used plant in traditional medicine. Such broad herbal medicine applications motivated several researchers to study its chemical composition, mainly of its leaves, seeds, and barks, besides the pharmacological effects aiming to demonstrate scientific evidence of its empirical uses. Most studies have been conducted with plant derivatives, and more recent studies have focused on isolated compounds. The phytochemical screening revealed various secondary metabolites with diverse biological effects, including flavonoids, alkaloids, saponins, tannins, steroids, triterpenoids, polyphenols, anthraquinones, coumarins, glycosides and polysaccharides. Experimental studies of this plant have shown numerous pharmacological activities such as antidiarrheal, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, anticancer, and others. Advances in the studies of phytochemistry and biological activity on this plant species over the last years led to the increasing interest of researchers in the protection of inventions focusing on its cosmetic, pharmaceutical and food applicability. This review summarizes the tremendous therapeutic and technological potential of *A. pavonina* that should be explored, opening new perspectives for future researches and the development of new products.**

Key words: Febaceae, herbal medicine, leguminosae, plant extracts, toxicity.

INTRODUCTION

Medicinal plants have traditionally been used in almost all cultures as a relevant therapeutic resource. These herbs compounds that can be used in the synthesis and development of new drugs (Hosseinzadeh et al., 2015;

*Corresponding author. E-mail: marciarizzo@ufpi.edu.br.

Singh, 2015; Chen et al., 2016).

Among the plant families used in traditional medicine, Fabaceae (Leguminosae) is the second-largest family of medicinal plants (Gao et al., 2010). This family is divided into three subfamilies: Mimosoideae, Caesalpinioideae, and Papilionoideae. *Adenanthera pavonina* is an essential representative of the Mimosoideae subfamily (Souza et al., 2016). This native Asian tree has been naturalized in many parts of the world (Adedapo et al., 2009; Soares et al., 2015). The plant has a long history of traditional medical use for the treatment of many diseases, and its derivatives are used empirically for fever, vomiting, diarrhea, gout, rheumatism, furuncle, hypertension, stomach bleeding, hematuria and cancer (Pandhare et al., 2012a; Godoi et al., 2014; Kuruppu et al., 2019).

There are reports that A. *pavonina* has a wide variety of chemical compounds and biological activities (Pandhare and Sangameswaran, 2012). Phytochemical studies of different parts of this plant revealed the presence of several secondary metabolites, mainly including flavonoids, alkaloids, steroids, saponins, tannins, triterpenoids, polyphenols, anthraquinones, coumarins, glycosides, carbohydrates (Ara et al., 2010a; Dash et al., 2010; Moniruzzaman et al., 2015), and lipid derivatives (Soomro and Sherazi, 2012). Some studies have shown the predominance of some components such as linoleic acid, oleic acid, and palmitic acid in its seed's oil (Kitumbe et al., 2013).

Based on the traditional knowledge of the use of parts of this plant, several pharmacological effects of derivatives have been demonstrated, such as antinociceptive activity of leaf ethanol extract (Moniruzzaman et al., 2015), cytoprotective and anti-inflammatory activity of seed extract (Koodalingam et al., 2015); antihyperglycemic and hypolipidemic effect of the aqueous extract of seeds (Pandhare et al., 2012a); antimicrobial and antioxidant activity of bark extracts (Ara et al., 2010b); antifungal activity relates to antimicrobial peptides present in seeds (Soares et al., 2012), among others. Several studies on this species have helped to understand the toxicity and supported its traditional use.

This review is intended to provide information about botanic traditional use, phytochemistry, toxicity, biological activities, and technological prospecting of A. pavonina and offer insights into potential use of this plant in the development of new medicines, besides opening perspectives for future research. This work also considered articles published in the last years and patents related to A. pavonina. This broader approach will be valuable in assessing the scope of scientific studies and the technological potential of this species.

METHODS

An extensive research of articles and patent applications referring to the plant species *A. pavonina*, between 2003 and June of 2019, was carried on. The keyword "*Adenanthera pavonina*" was used to search scientific articles on electronic databases including Scopus, ScienceDirect, Web of Science, PubMed and Virtual Health Library. The technological databases used were the World Intellectual Property Organization (WIPO), the European Patent Office (Espacenet), the United States Patent and Trademark Office - USPTO) and National Institute of Intellectual Property (INPI). The selection of the patents was based on the following inclusion criteria: published patents containing the keyword "*Adenanthera pavonina*" in their title or abstract, without the restriction of the year of publication. The keyword was searched in Portuguese in the INPI databases, and in English in the other databases. All relevant abstracts, full-text articles, and full patent documents written in English and Portuguese were studied and included.

Overview of scientific data

Concerning the results of the scientific productions related to *A. pavonina*, it was possible to identify, in all scientific papers database, a total of 142 articles (Figure 1A). Regarding the temporal evolution of articles, as can be seen in Figure 1B, the most significant number of publications was verified in 2012 with 13% of the total. Brazil and India were the countries with the most significant number of publications. Furthermore, based on all articles found during this time period, approximately 40% are related to the research of biological activity.

In this context, the work topics were subsequently elaborated, mainly based on the articles found in this time period, but older articles of relevance to the issue were also considered.

BOTANICAL INFORMATION AND TRADITIONAL USES

Botanical description

A.pavonina Linn. belongs to the family Fabaceae, subfamily Mimosoideae (Figure 2). This plant is a deciduous tree, usually erect, ranging in height between 18-24 m and in diameter up to 60 cm (Pandhare and Sangameswaran, 2012; Mujahid et al., 2013). It is a fast-growing tree of medium size with smooth bark of brown to greyish color, and many fissures (Rodrigues et al., 2009; Ara et al., 2010a; Dash et al., 2010; George et al., 2017). The spreading crown has few leaves. The leaves are bipinnate with 30 - 60 cm long with numerous oblong leaflets (2-5 x 0.7-2.5 cm) that are rounded at both ends and have a small point at the apex. The leaf axis is channeled on the upper surface. The flowers have corolla measured at approximately 4 mm. The fruit is a pod about 22 x 1.6 cm with quite hard seeds (George et al., 2017). The trees produce a large number of red seeds with a single seed weight on average of 0.27 g (Olajide et al., 2004; Soomro and Sherazi, 2012).

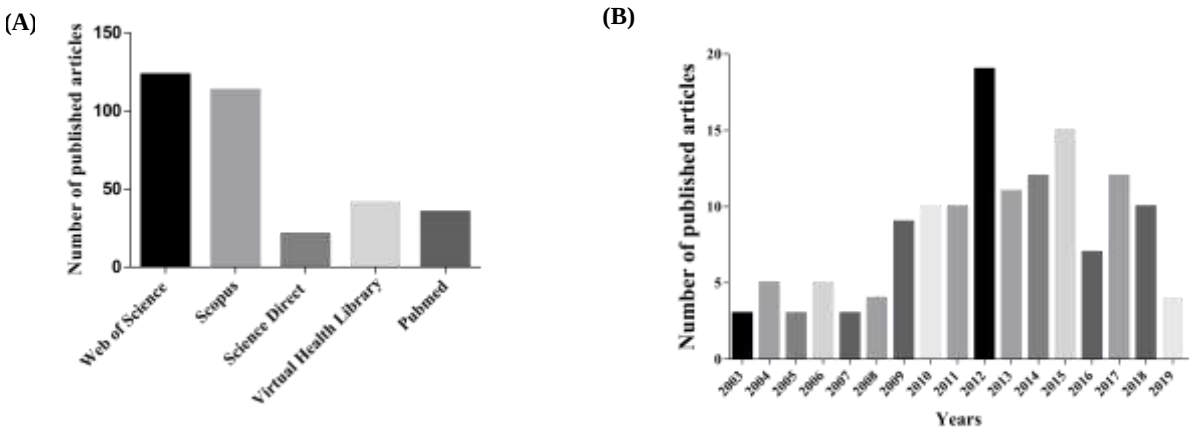

Figure 1. (A) Total number of articles by databases and (B) distribution of articles of all databases searched per year.

Figure 2. Imagens of parts of the plant *Adenanthera pavonina* L. (A) Cup of the tree (B) Seeds (C) Leaves (D) Green beans or fruits (E) Trunk (F) Inflorescence (G) Flowers.

Based on location, this plant is known as a red sandalwood tree, peacock flower fence, bead tree, coral tree, redwood, red-bread tree, Carolina tree, pigeon's eye, and dragon's eye (Olajide et al., 2004; Ara et al., 2010a; Silva et al., 2012; Mujahid et al., 2013; Godoi et al., 2014; Moniruzzaman et al., 2015). *A. pavonina* is also called a food tree because its seeds and leaves are often consumed by people (Zeid et al., 2012; Nwafor et al., 2017).

It is a species native of tropical Asia, with the first recorded appearences in India. It is common within the tropics of the old world and endemic in Southeast China and India (Olajide et al., 2004; Adedapo et al., 2009; Zeid et al., 2012; Nwafor et al., 2017). Moreover, it is an easily adaptable tree to grow on a variety of soils in humid and seasonally humid tropical climates (Olajide et al., 2004). It was introduced throughout the humid tropics and naturalized in Malaysia, Western and Eastern Africa and most island nations of both the Pacific and the Caribbean (Jaromin et al., 2006; Adedapo et al., 2009).

A. pavonina is a plant of diversified use. It is cultivated as forage, as an ornamental or urban tree and in reforestation. There are also reports of its use in perfumery and the manufacture of handicrafts. In addition to being used as food, especially the seeds for their high nutritional value, this species has received considerable attention for its use as a medicinal plant (Oni et al., 2009; Zeid et al., 2012; Godoi et al., 2014; Nwafor et al., 2017; Afolabi et al., 2018).

Ethnomedicinal use

A. pavonina is a species that has been traditionally used as an herbal medicine for the treatment, prevention, and control of several diseases (Oni et al., 2009; Dash et al., 2010). According to the literature, this plant was used in ancient Indian medicine, where crushed seeds were used to treat boils and inflammations (Olajide et al., 2004). In general, the literature reports its empirical use for diabetes, lipid disorders, diarrhea, ulcers, stomach bleeding, hematuria, rheumatism, asthma, hypertension, pulmonary infections and chronic ophthalmia (Pandhare et al., 2012a; Zeid et al., 2012; Mujahid et al., 2013; Godoi et al., 2014; Moniruzzaman et al., 2015; Dissanayake et al., 2018) and cancer (Lindamulage and Soysa, 2016). Zeid et al. (2012) reported its use as a tonic. These reports of traditional use were most common in Asian countries (Wickramaratne et al., 2016; Koodalingam et al., 2015; Dholvitayakhun et al., 2012; Arshad et al., 2010).

Regarding the widespread use of specific parts of the plant, bark and/or leaves derivates are used as anthelmintic, in colonorrhea, ulcers, pharyngoplasty, gout, rheumatism (Dash et al., 2010) and different painful conditions (Moniruzzaman et al., 2015); heartwood is beneficial in dysentery and haemorrhages (Dash et al., 2010). According to Olajide et al. (2004), redwood powder is also used as an antiseptic paste. Seed extract is used for the treatment of boils, inflammations, blood disorders, arthritis, rheumatism, cholera, paralysis, epilepsy, convulsion, spasm, indigestion (Oni et al., 2009; Mujahid et al., 2013), gout, burning sensation, hyperdipsia, vomiting, fever and giddiness (Dash et al., 2010). Seed powder is used as a poultice to induce abscess suppuration (Nwafor et al., 2017).

Phytochemical profile

There has been a growing interest in products derived from higher plants around the world. Besides being used in modern therapies, these plant derivatives are relevant for the synthesis of more complex molecules. *A. pavonina* is a plant that presents a complex and chemically varied group of compounds. Preliminary phytochemical studies on this plant revealed the presence of various secondary metabolites including mainly alkaloids, flavonoids, glycosides, carbohydrates, saponins, steroids, tannins, terpenoids and proteins (Olajide et al., 2004; Adedapo et al., 2009; Arshad et al., 2010; Dash et al., 2010; Mujahid et al., 2015).

Phytochemical analysis of leaves

Phytochemical screening of *A. pavonina* leaves identified the presence of alkaloids, carbohydrates, proteins, flavonoids, glycosides, saponins, steroids, tannins and resins (Moniruzzaman et al., 2015; Mujahid et al., 2015).

In a search for phytocompounds, a new five-membered lactone named pavonin with an exo-cyclic double bond was identified from the methanol soluble part of *A. pavonina* leaves (Ali et al., 2005).

The presence of flavonoids in leaves, seeds, and barks of *A. pavonina* has been implicated in some biological effects of this herb (Zeid et al., 2012; Adedapo et al., 2014; Mujahid et al., 2015). Three flavonoid compounds were elucidated from the methanolic extract of the seeds of this plant: 3,5,7,3',4'-pentahydroxy flavone-3'-O-a-Lrhamnopyranosyl-(1→4)-O-a-L-arabinopyranosyl-1→3)-O-b-D-xylopyranoside (A) along with two known compounds 2,4,7-trihydroxyisoflavone (B) and isovitexin (C) (Appendix A1) (Yadava and Vishwakarma, 2013).

In another study, ten methoxy flavonol glycosides were isolated from aqueous EtOH extract from *A. pavonina* leaves (Appendix A2). These compounds were named as quercetin 3-O-α-dirhamnopyranosyl-(1‴→2″,1‴→6″)-β-glucopyranoside-4′-methoxy (A), kaempferol-3-O-α-dirhamnopyranosyl-(1‴→2″,1‴→6″)-β-glucopyranoside (B), isovitexin (C), quercetin-3-O rhamnopyranosyl(1‴→4″)-β-glucopyranoside (D), quercetin-3-O-β-glucopranoside-4′-O-rhamnopyranoside (E), kaempferol-3-O-α-rhamnopyranosyl(1‴→2″)-β-glucopyranoside (F), quercetin-3-O-rhamnopyranosyl(1‴→2″)-β-glucopyranoside (G), quercetin-3-O-β-glucopyranoside (H), kaempferol (I) and

quercetin (J) (Mohammed et al., 2014).

A chemical assay was performed to identify the lipoidal content of powdered leaves of *A. pavonina*. Analysis by gas chromatography coupled mass spectrometry of the unsaponifiable fraction (USM) identified that fifty phytocompounds represented 80.1% of the total fraction, with squalene (16.38%) as the main compound followed by n- hentriacontane (14.61%), phytol (10.29%) and 2,2-diethoxy ethanamine (8.34%). Oxygenated compounds represented 27.22% of the total fraction while the fatty acids methylated fraction presented twenty-four compounds (88.49% of the total fraction), with methyl hexadecanoate (19.25%) as the main compound followed by methyl 9,12,15- octadecatrienoate (12.69%), methyl eicosanoate (10.14%), methyl-9- octadecenoate (10.06%) and methyl 9,12-octadecadienoate (9.23%). Unsaturated fatty acids represented 32.52% of the total fraction. The gas-liquid chromatography analysis of the sterol fraction (Appendix A3) revealed a mixture of stigmasterol (A) (62.28%), β-sitosterol (B) (29.43%), campesterol (6.75%) and cholesterol (1.54%) (Zeid et al., 2012).

Other stigmasterol glucosides, including octacosanol and dulcitol, have also been reported in the leaves of this plant (Mayuren and Ilavarasan, 2009; Moniruzzaman et al., 2015). Zeid et al. (2012) also isolated at the first time three triterpenoid compounds identified as 22-hydroxy hopan-3-one (A), 24-methylene cycloartenol (B) and betulinic acid (C) (Appendix A4).

Phytochemical analysis of seeds

Phytochemical screening of *A. pavonina* seeds detected the presence of cardiac glycosides, tannins, saponins, alkaloids, and flavonoids, but cyanogenetic glycosides and anthraquinones were absent (Olajide et al., 2004; Adedapo et al., 2009; Adeyemi et al., 2015).

Among the various compounds isolated and identified, O-acetylethanolamine, was extracted from the seeds of this plant (Appendix A5) (Hayman and Gray, 1987; Pandhare et al., 2017).

A study carried out to evaluate the constituents of *A. pavonina* seed oil showed their rich composition of long-chain fatty acids and fatty alcohols. Thus, a total of twelve wax ester was identified, including myristic acid arachidyl ester (C34), stearic acid stearyl ester (C36), nonadecanic acid stearyl ester (C37), arachidic acid oleoyl ester (C38), henicosanoic acid stearyl ester (C39), arachidic acid arachidyl ester (C40), henicosanoic acid arachidyl ester (C41), arachidic acid behenyl ester (C42), behenic acid behenyl ester (C44), heptacosanyl stearyl ester (C45), behenic acid lignoceryl ester (C46), lignoceryl acid lignoceryl ester (C48). Long-chain fatty esters have ranged between C34 and C48, with the most portion between C40 and C48. Among the long-chain wax esters, C46 was the dominant wax ester (22.2%). The essential wax esters of *A. pavonina* were produced mainly from the fatty acids and fatty alcohols C22 to C24 (Soomro and Sherazi, 2012).

Some work has reported the presence of proteins or peptides in *A. pavonina* seeds (Soares et al., 2012; Silva et al., 2012; Sasaki et al., 2015; Souza et al., 2016). Recently, Lavudi and Seshagirirao (2018) found an amount of protein in the crude seed extract of 82.11198 mg/ml, and a different protein profile was verified by an SDS-PAGE assay.

The seed endosperm of *A. pavonina* contains a reserve polysaccharide consisting predominantly of a galactomannan, with the general molecular structure shown in Appendix A6 (Prajapati et al., 2013). These polysaccharides present in legumes endosperm are the second largest group of reserve polysaccharides in plants (Macedo et al., 2010a). The galactomannan of *A. pavonina* L. has the ratio of mannose/galactose 1.35 and contains small amounts of other monosaccharides (Macêdo et al., 2013; Santos et al., 2015). Several studies have demonstrated the importance of this biopolymer due to its properties of water solubility and thickener, presenting thus, high versatility of application in several fields (Santos et al., 2015). Many studies using this galactomannan have been exploring the development of new formulations just like edible coatings (Lima et al., 2010), emulsion (Zarnowski et al., 2004; Jaromin et al., 2006), drug delivery (Nobre et al., 2018) and association with other materials, such as hydroxyapatite (Aquino et al., 2017).

Phytochemical analysis of barks

Phytochemical screening of *A. pavonina* barks revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponin, phytosterol, phenolics, tannins, terpenoids (Dash et al., 2010; Ara et al., 2010a), proteins, amino acids and acidic compounds (Arshad et al., 2010). The phytochemical analysis also showed the presence of stigmasterol glucoside in *A. pavonina* barks (Pandhare and Sangameswaran, 2012; Pandhare et al., 2017).

Biological activities

A. pavonina is a medicinal plant of traditional use that presents several scientific studies related to its biological activities. Based on their different derivatives, several activities were evaluated and confirmed by *in-vitro* and *in-vivo* studies. A brief overview of the evaluated activities of the different plant derivatives can be seen in Table 1.

Antidiarrheal activity

Using *A. pavonina* seeds, Pandhare et al. (2017) prepared three concentrations of aqueous extract of seeds (50, 100 and 200 mg/kg, p.o.) to the antidiarrheal test in experimental animals. The reference drug used was loperamide. This study showed that the extract

Table 1. Pharmacological activities evaluated using different derivatives of *A. pavonina.*

Evaluated activity	Derivative	Reference
Antidiarrheal	Aqueous seeds extract	Pandhare et al. (2017)
Antimalarial	MeOH seeds extract	Adedapo et al. (2014)
Anti-inflammatory	MeOH seeds extract	Olajide et al. (2004)
	EtOH leaves extract	Mayuren and Ilavarasan (2009)
	PE barks extract DCM barks extract EtOAc barks extract MeOH barks extract	Ara et al. (2010b)
	MeOH leaves extract	Jayakumari et al. (2012)
	Aqueous leaves extract EtOH leaves extract $CHCl_3$ leaves extract EtOAc leaves extract PE leaves extract	Zeid et al. (2012)
Antinociceptive	Aqueous seeds extract	Pandhare et al. (2012b)
	EtOH leaves extract	Moniruzzaman et al. (2015)
Anticancer	EtOH fruits extract	Sowemimo et al. (2009)
	EtOH seeds extract	Ferreira et al. (2011)
	EtOH leaves extract	Mohammeda et al. (2014)
	$CHCl_3$ leaves extract EtOAc leaves extract Acet leaves extract MeOH leaves extract EtOH leaves extract	Sophy et al. (2016)
	Barks decoction	Lindamulage and Soysa (2016)
	Seeds powder treated enzymatically	Araújo et al. (2019)
Hepatoprotective	MeOH leaves extract	Mujahid et al. (2013)
Antibacterial	Fixed oil from seeds	Chourasia and Rao (2005)
	PE barks extract DCM barks extract EtOAc barks extract MeOH barks extract	Ara et al. (2010a) and Hussain et al. (2011)
	MeOH leaves extract	Jayakumari et al. (2012)
	Aqueous leaves extract EtOH leaves extract Hexane leaves extract	Dholvitayakhun et al. (2012)
	Aqueous leaves extract Toluene leaves extract	Thippeswamy et al. (2015)
	$CHCl_3$ leaves extract EtOAc leaves extract EtOH leaves extract $CHCl_3$ leaves extract MeOH leaves extract PE leaves extract Acet leaves extract $CHCl_3$ barks extract	Sophy et al. (2015)

Table 1. Contd.

	MeOH barks extract PE barks extract Acet barks extract $CHCl_3$ seeds extract MeOH seeds extract PE seeds extract Acet seeds extract	Matin et al. (2015)
	MeOH seeds extract Hexane seeds extract Chromatographic fractions of seeds	Adeyemi et al. (2015)
Antifungal	MeOH roots extract MeOH barks extract MeOH seeds extract	Rodrigo et al. (2007)
	Seed peptides	Sousa et al. (2012)
Anthelmintic	EtOH barks extract	Dash et al. (2010)
Antiviral	Aqueous seeds extract Aqueous fruits extract	Chiang et al. (2003)
	Sulfated galactomannan of seeds	Marques et al. (2015) and Godoi et al. (2014)
	Native and sulfated galactomannan of seeds	Godoi et al. (2015)
Antioxidant	MeOH leaves extract MeOH roots extract MeOH barks extract MeOH seeds extract	Rodrigo et al. (2007)
	PE barks extract DCM barks extract EtOAc barks extract MeOH barks extract	Ara et al. (2010a)
	Aqueous EtOH leaves extract EtOH leaves extract PE leaves extract EtOAc leaves extract $CHCl_3$ leaves extract	Mohammeda et al. (2014)
	Aqueous leaves extract Toluene leaves extract	Thippeswamy et al. (2015)
	MeOH leaves extract MeOH barks extract	Partha and Rahaman (2015)
	Sulfated galactomannan of seeds	Marques et al. (2015)
	Aqueous leaves extract MeOH leaves extract EtOAc leaves extract PE leaves extract	Wickramaratne et al. (2016)
	Galactomannan of seeds	Melo et al. (2018)
	Seeds powder treated enzymatically	Araújo et al. (2019)
Antihypertensive	MeOH seeds extract	Adedapo et al. (2009)
Antihyperglycaemic	Aqueous seeds extract	Pandhare et al. (2012a)
	Galactomannan enriched food	Vieira et al. (2018)
Antihyperlipidaemic	EtOAc and n- butanol fractions of EtOH bark extract	Das et al. (2011)
	Aqueous seeds extract	Pandhare et al. (2012a)
	Galactomannan enriched food	Vieira et al. (2018)

Table 1. Contd.

Renal protective	Aqueous seeds extract	Pandhare and Sangameswaran (2012)
Anti-emetic	MeOH leaves extract	Hasan et al. (2012)
Anticonvulsant	MeOH seeds extract	Oni et al. (2009)
Central depressant	MeOH seeds extract	Oni et al. (2009)

Etanolic (EtOH); petroleum ether (PE); dichloromethane (DCM); ethyl acetate (EtOAc); metanol (MeOH); chloroform ($CHCl_3$); acetone (Acet).

exhibited dose-dependent significant antidiarrheal potential against castor oil and magnesium sulfate-induced diarrhea. The extract also reduced the number of diarrheal feces and the total weight of feces in a dose-dependent manner. These results confirm the antidiarrheal potential of the aqueous extract of *A. pavonina* seeds, justifying its traditional use for diarrhoea.

Antimalarial activity

Adedapo et al. (2014) studied the different concentration of the methanol seed extract of *A. pavonina* (100, 200, 400, 600 and 800 mg/kg, p.o.) in mice infected with *Plasmodium berghei*, with chloroquine as the reference drug. The percentage parasitemia decreased significantly in the treated group with the crude extract in a dose-dependent manner. The crude extract, at a dose of 800 mg/kg exerted an antimalarial effect (92.11%) higher than that of the chloroquine (88.73%).

Anti-inflammatory activity

Several experiments, including carrageenan-induced rat paw edema, acetic-acid-induced vascular permeability in mice, carrageenan-induced pleurisy in rats, acetic-acid-induced writhing in mice, and formalin-induced paw licking in mice were conducted to test the methanol extract of the seeds of *A. pavonina* at 50, 100 and 200 mg/kg (p.o.) using indomethacin as the standard. In all doses, the extract produced statistically significant inhibition of the carrageenan-induced paw edema in the rat. After treatment with the indomethacin and seed extract (50, 100 and 200 mg/kg), there was an inhibition of the carrageenan-induced paw edema in rats of 90.2, 34.4, 47.5 and 49.2% respectively. The extract also produced statistically significant inhibition in the acetic-acid-induced vascular permeability in mice. At a dose of 200 mg/kg, the extract caused inhibition of leakage of 61%, while indomethacin produced 75.2% inhibition. In carrageenan-induced pleurisy test, only at doses 100 and 200 mg/kg, there was a statistically significant reduction in both total and differential cell counts. In acetic-acid-induced writhing, extract inhibited the writhing syndrome in a dose-dependent manner, reflecting its analgesic effect. Indomethacin and seed extract (50, 100 and 200 mg/kg) showed an inhibition of writhing in mice by 78.6, 32.1, 44.7 and 64.7% respectively. The extract also produced a statistically significant reduction in licking time in both the early and late phases of the formalin-induced paw licking in mice, in a dose-dependent manner. Thus, results generated from this study demonstrated the anti-inflamatory and analgesic potential effects of the methanolic extract obtained from *A. pavonina* seeds (Olajide et al., 2004).

Using the ethanolic extract from *A. pavonina's* leaves, Mayuren and Ilavarasan (2009) evaluated their anti-inflammatory effects in Wistar rats at doses of 250 and 500 mg/kg in carrageenan-induced hind paw edema tests. Also, the chronic inflammation was measured using the cotton pellet-induced granuloma formation assay. The extract caused a significant reduction in paw edema from the third hour using 250 mg/kg dose and from the second hour at the 500 mg/kg dose. At both dosages, the extract caused a significant reduction in the wet and dry weights of the cotton pellets. In the sequential study, castor oil-induced diarrhea tests were performed to assess whether the mechanism of anti-inflammatory action could be related to the inhibition of prostaglandin synthesis. Indomethacin was the standard drug. The extract significantly retarded castor oil-induced diarrhoea, suggesting the involvement of prostaglandins in its mechanism. Then, both acute and chronic inflammatory models demonstrated anti-inflammatory effects of leaf extract. These data suggest a possible anti-inflammatory activity of the active constituents contained in the leaves, such as β-sitosterol and stigmasterol.

Another study using carrageenan-induced rat hind paw edema model evaluated the anti-inflammatory effect of the extracts of the barks of *A. pavonina* prepared with different organic solvents (petroleum ether, dichloromethane, ethyl acetate, and methanol). The extracts were administered orally at the doses of 200 and 400 mg/kg b.w., and diclofenac sodium was the reference drug. The results showed that the fractions exhibited significant anti-inflammatory effects in a dose-dependent manner. Inhibition of inflammation between different fractions showed that the methanolic extract (400 mg/kg) reduced 37.1% of paw edema at the first hour, while the dichloromethane fraction showed, in the same dose, 33.11% of inhibition after three hours of the study when compared to diclofenac sodium. This study confirms the traditional use of extracts of the bark of *A. pavonina* to

the treatment of some inflammatory processes (Ara et al., 2010b).

Zeid et al. (2012) also evaluated the acute anti-inflammatory activity of the extracts of *A. pavonina* leaves prepared with different solvents in carrageenan-induced rat hind paw edema model. The ethanolic extract was used at the doses of 50 and 100 mg/kg body weight (p.o.), while the other extracts were administered at the dose of 100 mg/kg using indomethacin as the standard. Ethanolic extract at a dose of 100 mg/kg showed a high efficacy (91.27%) followed by aqueous-ethanol extract (89.15%), chloroform extract (79.89%), ethyl acetate extract (70.10%) and petroleum ether extract (65.34%) in comparison to the indomethacin (100%). The results suggest a possible anti-inflammatory activity of active constituents contained in the leaves such as flavonoids, β-sitosterol, and stigmasterol. In other findings, an active anti-inflammatory principle, O-acetylethanolamine (Appendix A5), was isolated and identified of *A. pavonina* seeds (Hayman and Gray, 1987; Pandhare et al., 2017).In another study, the anti-inflammatory activity of methanolic leaves extract was assessed by formalin-induced rat paw edema model for acute inflammation (200 and 400 mg/kg body weight, p.o.) and cotton pellet granuloma model for chronic inflammation (400 mg/kg body weight, p.o.). The results showed that the extract was satisfactory in the experimental models of acute and chronic inflammation (Jayakumari et al., 2012).

Koodalingam et al. (2015) investigated the mechanism of anti-inflammatory activity of the seed extract of *A. pavonina* on lipopolysaccharide-stimulated rat peritoneal macrophages. The results showed that the pre-treatment with the seed extracts possess beneficial anti-inflammatory effects by suppressed nitric oxide production and superoxide anion generation, cell death, and nuclear fragmentation by inhibiting the H_2O_2 mediated generation of oxidative damage in rat peritoneal macrophages, suggesting that the extract has a cytoprotective property against intracellular peroxide production.

Antinociceptive activity

Pandhare et al. (2012b) evaluated the ameliorative effect of seeds of *A. pavonina* aqueous extract in attenuating neuropathic pain in streptozotocin-induced diabetic rats during twelve weeks of treatment. Diabetic rats orally received the test extract in 50, 100, or 200 mg/kg per day and pregabalin as a standard drug. Cold and hot water tail immersion test, photo Actometer, and Rotarod tests were performed. Methods for the determination of tissue superoxide anion and total calcium levels in sciatic nerve were conducted, besides histopathological evaluation of the sciatic nerve. Surprisingly, the results evidenced that extract increased tail-flick latency significantly in diabetic rats, however, did not produce any significant effect on motor coordination, and spontaneous motor activity of the rats. The extract also reduced superoxide anion and total calcium levels in a dose-dependent manner. Besides, the extract attenuated histopathological changes in the sciatic nerve. This study suggests that *A. pavonina* extract may attenuate the development of diabetic neuropathy in diabetic rats when compared with pregabalin and be effective in preventing the progression of diabetic nephropathy.

In another study, Moniruzzaman et al. (2015) assessed the antinociceptive activity of ethanol extract of leaves of *A. pavonina* at the doses of 50, 100, and 200 mg/kg b.w. (p.o.) using different nociceptive models in mice, including thermal tests (hot plate and tail immersion), acetic acid-induced writhing, and glutamate- and formalin-induced licking protocols. Besides, the possible mechanisms of action by the involvement of opioid receptor in analgesic activity were evaluated using naloxone and cyclic guanosine monophosphate (cGMP) signalling pathway by methylene blue. This study evidenced that the tested extract caused the reduction of nociceptive responses in a dose-dependent manner. A significant increased latency time was also observed in both thermal tests and reduction of the number of abdominal constrictions induced by acetic acid in all tested doses, evidencing the inhibition of acetic acid-induced visceral nociception, and also noting a significant inhibition of the glutamate and formalin-induced nociception. Concerning the possible mechanisms of action, this study suggests that opioid receptors and cGMP pathway may contribute to the antinociceptive actions observed in the extract. These findings demonstrate the antinociceptive activity of this extract which may be associated with its chemical compounds (e.g., alkaloids, carbohydrates, proteins, flavonoids, glycosides, saponins, steroids, and tannins) and support the traditional use of this plant in the treatment of different painful conditions.

Anticancer activity

In the literature, there are some reports on the traditional use of *A. pavonina* derivatives to the treatment of cancer (Lindamulage and Soysa, 2016). Some *in vitro* assays have shown the anticancer activity of plant derivatives.

In a preliminary study, Sowemimo et al. (2009) demonstrated the absence of cytotoxicity of ethanolic fruit extract of *A. pavonina* against the HeLa cell line.

A study conducted by Ferreira et al. (2011) evaluated the cytotoxic potential of the ethanolic extract obtained from *A. pavonina* seeds (50 µg/mL) in cancer cell lines. After 72 h of treatment, a low inhibition of cell proliferation against human cancer cells was observed, together with, colon HCT-8 (30.8 ± 5.2%), glioblastoma SF-295 (23.7 ± 3.2%), melanoma MDA/MB-435 (4.5 ± 2.4%), and leukemia HL-60 (1.2 ±13.2%) cells.

In another study, the ethanolic (EtOH) leaves extract of *A. pavonina* showed significant cytotoxic activity against human hepatoma HepG2 cells (IC_{50} = 2.50 µg) compared to cisplatin (IC_{50} > 10 µg) (Mohammed et al., 2014).

Sophy et al. (2016) evaluated the antiproliferative effect of the *A. pavonina* leaf extracts (chloroform, ethyl acetate, acetone, methanol, and ethanol) in four cancer cell lines (HCT116, NCIH460, U251, and MCF7) by sulphorhodamine B (SRB) assay with camptothecin used as a positive control. All the extracts presented better growth inhibition of breast cancer cell line (MCF 7). However, the chloroform extract showed the best growth inhibition. On the other hand, the ethanol extract showed low growth inhibition against all the cancer cell lines.

Lindamulage and Soysa (2016) observed that a decoction prepared with barks of *A. pavonina* and *Thespesia populnea* in equal proportion, exhibited antiproliferative activity and induced apoptosis in the Hep-2 cancer cells, 24 h post-treatment.

Araujo et al. (2019) evaluated the influence of antiproliferative activity against cancer cells of *A. panonina* seed powder treated enzymatically with amylase, cellulase, and protease. The enzymatic treatment of *A. pavonina* seed powder with protease and cellulase has been shown to improve antiproliferative activity in the prostate (PC-3) and kidney (786-0) tumour cell lines.

Hepatoprotective activity

Mujahid et al. (2013) evaluated the hepatoprotective effect of methanolic extract of leaves of *A. pavonina* against isoniazid and rifampicin-induced liver damage in rats. Animals were treated with isoniazid and rifampicin for 28 days orally to induce hepatotoxicity. Subsequently, isoniazid and rifampicin treated groups received the methanolic (50%) extract at a dose of 100 or 200 mg/kg as well as silymarin orally once daily for 28 days as the reference drug. After treatment with the extract, the serum enzymatic activities of glutamic oxaloacetic transaminase, glutamate pyruvate transaminase, alkaline phosphatase, bilirubin, and lactate dehydrogenase were restored to nearly normal levels in a dose-dependent manner. There was an increase in the levels of total protein and albumin towards normal in the methanolic extract-treated group. Restoration of hepatic antioxidant function was also verified by a significant increase in the levels of glutathione, catalase and superoxide dismutase. Interestingly, the extract avoided the elevation of hepatic malondialdehyde (MDA) formation in the liver of intoxicated rats by isoniazid and rifampicin. An histological examination observed a significant reduction in tissue damage along with minimal evidence of inflammation in liver tissue of rats treated with the extract. It is suggested that the hepatoprotective activity of the methanolic extract of *A. pavonina* may be related to the presence of flavonoids, alkaloids, glycosides, and saponins.

Antibacterial and antifungal activities

There have been many studies on antimicrobial activity of parts of *A. pavonina* species, and one of the oldest found was the work of Chourasia and Rao (2005) that evaluated the antimicrobial activity of fixed oil from seeds. The results showed that the seed oil showed weak activity against *B. anthracis*, *S. paratyphi,* and *B. mycoides* was ineffective against other Gram positive and Gram-negative bacteria tested.

Rodrigo et al. (2007) showed evidence of antifungal activity of methanolic extracts of roots, bark, and seeds of *A. pavonina*.

Antimicrobial activity of *A. pavonina* bark extracts prepared by petroleum ether, dichloromethane, ethyl acetate, and methanol were evaluated by disc diffusion method. All extracts were tested at different concentration (100, 200 and 400 µg/disc) against thirteen test bacteria: five Gram-positive (*Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus sereus*), eight Gram-negative (*Escherichia coli*, *Pseudomonas aureus*, *Salmonella paratyphi*, *Salmonella* Typhi, *Shigella dysenteriae*, *Shigella boydii*, *Vibrio mimicus*, *Vibrio parahemolyticus*), and three fungi (*Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae*). The antibiotic kanamycin (30 µg/disc) was the positive control. The methanolic extract had better results in the highest concentration of 400 µg/disc, with higher inhibitory activity against *S. aureus*, *S. lutea*, and *V. mimicus*. The other extracts showed the lowest inhibitory activity at the same dose level. At a dose of 100 µg/disc, with the exception of the methanolic extract, all were insensitive to all tested microorganisms. The authors suggest that saponins, alkaloids, tannins, and flavonoids compounds could be related to the antibacterial activity of the methanolic extract (Ara et al., 2010a).

Another study using extracts from *A. pavonina* bark at the concentration of 25, 50 and 75 mg/well, there was effective inhibition of the diameter of zones against both Gram-positive (*B. subtilis* and *S. epidermidis*) as well as Gram-negative bacteria (*Enterobacter aerogenes*, *P. aeruginosa*, *Salmonella typhimurium*) by disc diffusion method. However, the ethanolic and aqueous extracts evidenced the highest activity against all the tested bacteria (Hussain et al., 2011).

A study carried out by Dholvitayakhun et al. (2012) investigated the antibacterial activity of aqueous, ethanolic, and hexane extracts of leaves of *A. pavonina* against foodborne pathogens. Initially, the efficacy of extraction methods was assessed using the disc diffusion assay against *Campylobacter jejuni,* and erythromycin discs (10 µg/disc) were used as a reference. After minimum inhibitory concentrations (MICs) and minimum

bactericidal concentrations (MBCs), the best extract in the disc test were evaluated against Gram-positive (*B. cereus, L. monocytogenes, S. aureus*) and Gram-negative (*C. jejuni, E. coli, S. typhimurium*) bacteria. It was observed that the extraction method influenced bacterial activity. The aqueous extract was the most efficient to extract antibacterial constituents by the polar nature of these. However, the aqueous extract had a potent inhibition against *C. jejuni* but no activity against the other bacteria. Thippeswamy et al. (2015) also observed a significant antibacterial activity of the aqueous leaves extract against *S. aureus* and *E. coli* while the toluene extract showed activity against *E. coli, Klebsiella pneumoniae, Proteus vulgaris, P. aeruginosa, S.* Typhi*, S. aureus,* and *Streptococcus faecalis*.

Another study, in the same year, conducted by Soares et al. (2012) evaluated the antifungal activity of peptides extracted from *A. pavonina* seeds. The results of some fractions (H11 and H22) exhibited marked inhibition in the growth of *S. cerevisiae* and *C. albicans*.

Matin et al. (2015) conducted an antibacterial screening with extracts from leaves, seeds, and barks of *A. pavonina* prepared with petroleum ether, acetone, chloroform, and methanol. All extracts were tested at the concentration of 50 and 200 µg/disc, against fifteen test bacteria: six Gram-positive (*S. aureus*, *S. lutea*, *B. subtilis*, *B. megaterium*, *B. cereus* and *Streptococcus*-β–*haemolyticus*) and nine Gram-negative (*S. typhi*, *S. dysenteriae*, *E. coli*, *S. boydii*, *Shigella sonnei*, *P. vulgaris*, *K. pneumoniae* and *P. aeruginosa*). Ciprofloxacin was the positive control. Minimum inhibitory concentrations (MICs) of the chloroform extract of seeds and stem wood of *A. pavonina* were also evaluated against pathogenic bacteria. Chloroform fraction of the seed extract presented the highest average zone of inhibition found to be 25 mm away from *S.* β-*haemolyticus*. The chloroform fraction of the seed extracts also showed promising antibacterial activity against *Serratia dysenteriae* and *E. coli*. The susceptibility order of the extracts tested was seed > leaf > stem. The MIC values of the chloroform seed extract were 256 µg/mL against *B. cereus*, 128 µg/mL against *S.*-β–*haemolyticus*, *S. dysenteriae* and 64 µg/mL against *Klebsiella sp.* While MIC values of the chloroform extract of stem wood were 16, 32, 64 and 128 µg/mL against *S.* β–*haemolyticus*, *B. megaterium*, *S. sonnei*, and *S. typhi*, respectively. Satisfactory results of antimicrobial activity were also found in leaf extract extracted with chloroform, ethanol and ethyl acetate. All the three extracts displayed intense activity against *S. aureus*, *K. pneumoniae,* and *B. subtilis* (Sophy et al., 2015).

Another research about the antimicrobial activity of seed was also conducted, this time with crude extracts and chromatographic fractions of *A. pavonina* (Adeyemi et al., 2015). In this study, the crude extracts (hexane and methanolic) and 15 chromatographic fractions from methanolic extract were evaluated against different strains of *S. aureus* (PHM 001, PHM 002, 003, PHM 004, PHM 005) in different concentrations (6.25, 12.5, 25, 50 and 100 6.25 mg/mL). Gentamicin (500 µg/mL) was used as the positive control. The methanolic extract was effective only at the highest concentration against two strains (PHM 002 and PHM 004) while hexane extract was effective at concentrations of 50 and 100 mg/mL on PHM 001. Chromatographic fraction ST 10-12F produced inhibition on PHM 002 at 50 and 100 mg/mL while fraction ST-13-15F exhibited inhibition on PHM 002 at all concentrations.

In a study of the antibacterial mechanism of action, Vasavi et al. (2015) determined the quorum sensing (QS) of ethanol extract of *A. pavonina*. The extract showed anti-QS activity in *C. violaceum* CV026 biosensor bioassay and inhibition of QS-regulated violacein production in *C. violaceum* ATCC12472. The ethyl acetate fraction was tested and resulted in changes in the virulence factor production of *P. aeruginosa* PAO1. This data showed that this extract was active as an anti-QS agent.

Anthelmintic activity

The anthelmintic activity of the ethanolic extracts from the bark of *A. pavonina* was assessed against *Pheretima posthuma* and *Ascaridia galli*. Thus, a bioassay was developed to measure the time of paralysis and time of death of the worms in concentrations of 25, 50, and 100 mg/mL of the ethanolic extract and compared to piperazine citrate as a positive control. The results demonstrated that the ethanolic extract caused paralysis and death of worms in a comparable time to piperazine citrate, especially at higher concentration of 100 mg/mL. Dash et al. (2010) believed that the phenolic compounds in the *A. pavonina* bark extracts could be responsible for the compromise of the energy generation in the parasites by uncoupling oxidative phosphorylation, which might have paralyzed and causally led to the death of both worm species.

Antioxidant activity

In a more incipient work, Rodrigo et al. (2007) assessed the antioxidant activity of methanolic extracts of parts of the *A. pavonina* plant by the standard 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. α-Tocopherol was used as the reference. It was possible to observe a significant antioxidant activity in stem bark extract and moderate activity in root extract.

The antioxidant activity of the extracts from *A. pavonina* bark prepared with different organic solvents (petroleum ether, dichloromethane, ethyl acetate, and methanol) was measured by DPPH assay. Ascorbic acid and tert-butyl-1-hydroxytoluene (BHT) were used as positive controls. The ethyl acetate and methanol extracts presented

higher radical scavenging activity (IC_{50} value 8.72 ± 0.11 and 6.44 ± 0.04 µg/mL) when compared with the standard BHT (IC_{50} value 27.42 ± 0.99 µg/mL). The activities of these two extracts were also comparable to the ascorbic (IC_{50} value 5.71 ± 0.01 µg/mL), whose values were very close to free radical scavenging activity. It is suggested that the antioxidant effect may be due to the presence of flavonoids and tannins in ethyl acetate and methanol extracts of bark (Ara et al., 2010a).

A study carried out by Mohammed et al. (2014) evidenced the *in vivo* antioxidant activity of the *A. pavonina* leaf extracts. The result demonstrated that the ethyl acetate (EtOAc) extract exhibited the highest free radical scavenging activity (95.78%) followed by the ethanol extract (EtOH) at 100 mg/kg b.w. (75.35%), and chloroform extract (60.57%) as compared with vitamin E (100%). Partha and Rahaman (2015) also demonstrated the antioxidant activity by DPPH of methanolic extracts of leaf and bark (200 mg/mL), with a radical scavenging activity of 32.31 and 30.23%, respectively, when compared with ascorbic acid.

In a more complex study, Thippeswamy et al. (2015) demonstrated the antioxidant activity of extracts from leaves prepared with water and toluene using DPPH radical scavenging, *β*-carotene/linoleic acid bleaching inhibition and H_2O_2 assays. All tests showed the significant antioxidant activity of both extracts.

Marques et al. (2015) evaluated the antioxidant activity of sulfated galactomannan isolated from the endosperm of seeds of *A. pavonina*. The galactomannan exhibited high scavenging activity (IC_{50} value 7.51 ± 0.03 µg/mL). The data obtained suggest that antioxidant activity could be related to the sulfation degree and probably the mechanism of action associated with the intrinsic hydrogen-donating ability of sulfate groups.

Wickramaratne et al. (2016) demonstrated the antioxidant activity by DPPH scavenging property, and the total phenolic content of different solvent extracts of *A. pavonina* leaves. The EtOAc fraction of *A. pavonina* leaves showed the highest total phenolic content (34. 62 ± 1.14 mg/g extract) and the highest DPPH scavenging activity with an IC_{50} of 249.92 ± 3.35 µg/mL.

Galactomannan from *A. pavonina* has also been shown to have a significant antioxidant effect by the reduction of DPPH radicals (Melo et al., 2018).

More recently, Araujo et al. (2019) evaluated the influence of the antioxidant activity by DPPH assay of *A. panonina* seeds powder enzymatically treated with amylase, cellulase, and protease. After treatment with protease and cellulase, an increase in antioxidant activity was observed due to improvement in the extraction of phenolic compounds.

Antihypertensive activity

Antihypertensive effects of the methanolic seed extract of *A. pavonina* on the blood pressure of normotensive Wistar rats were assessed. Animals were treated with the seed extract at a dose of 200 mg/kg (p.o.) once daily for 28 days and propranolol (1 mg/kg) used as the positive control. On the 29th day, the mean arterial blood pressure of the animals was measured by using a Condon manometer. The mean arterial blood pressure of the groups treated with normal saline, propranolol, and seed extract were 60, 23, and 30 mmHg, respectively. Analysis of biochemical parameters showed that the total bilirubin, total protein and the globulin fraction were significantly higher in the extract treated groups compared to the control group, suggesting that the extract has a tonic effect on the kidney and liver and these organs play a central role on its metabolism. Histopathological examination showed an insignificant change in the kidney, liver, and testes. Therefore, this study evidenced the potential of the seed extract of *A. pavonina* to cause a decrease in blood pressure in normotensive rats (Adedapo et al., 2009).

Antihyperglycemic and antihyperlipidemic activities

Das et al. (2011) examined the antihyperlipidemic activity of *A. pavonina* L. ethanolic bark extract fractions on Triton WR-1339-induced hyperlipidemia in Wistar rats. Animals were treated, once daily for one week, with petroleum-ether fraction, diethyl ether fraction, ethyl acetate fraction and n-butanol fraction of ethanolic extract at a dose of 400 mg/kg (p.o.). Other groups received 0.3% w/v carboxy methylcellulose, CMC (vehicle control group) or atrovastatin (positive control group) 1 mg/kg. On the seventh day, 200 mg/kg Triton WR 1339 was injected (i.p.), into all the groups of animals shortly after drug treatment. Total serum cholesterol and triglycerides were measured for individual animals on the seventh day previous to drug treatment and after 24 h of Triton administration. The fractions of ethanolic extract were also administrated at the same dosage in high-fat diet-induced hyperlipidemic rats. In the results, it was observed that the ethyl acetate fraction and n-butanol fraction inhibited the rise in serum cholesterol and triglyceride levels on Triton WR 1339 administration rats. The extract fractions also significantly attenuated the elevated serum total cholesterol and triglycerides in high-fat diet-induced hyperlipidemic rats. These findings may be related to the presence of triterpenoids, flavonoids, tannins, and saponins in the fractions studied. The conclusions of the study exhibited that ethyl acetate fraction and n-butanol fraction of the ethanolic extract can adequately control the blood lipid levels in dyslipidemic situations by interfering with the biosynthesis of cholesterol and utilization of lipids.

A study conducted by Pandhare et al. (2012a) investigated the antihyperglycemic and antihyperlipidemic effects of *A. pavonina* seed aqueous extract in rats.

Initially, the effect of the extract on normoglycemic rats was evaluated. Three animal groups received seed extract orally at 50, 100, and 200 mg/kg/day (b.w.), respectively, and the control group received distilled water. Blood glucose levels were checked before and at 1, 2, 3, and 4 h after treatment. The oral glucose tolerance test was also evaluated in normal rats. Again, three animal groups received seed extract orally at 50, 100, and 200 mg/kg/day (b.w.), respectively, and the control group received distilled water. After 30 min of dosing, all the animals were given glucose (2 g/kg). Blood samples were collected before (0 h) and 1, 2, 3, and 4 h after the glucose loading and blood glucose levels were measured. The results showed that the extract at all doses did not significantly modify the blood glucose of the normoglycemic rats. On the other hand, at all doses, the extract reduced the blood glucose level in the animals that received glucose significantly after 3 h of oral administration. The antihyperglycemic activity was evaluated using streptozotocin-induced diabetes in rats (55 mg/kg, b.w., i.p.). After induction of diabetes, the rats received the extract orally at doses of 50, 100, and 200 mg/kg/day (b.w.) for 30 days, and glibenclamide, used as the reference standard. Blood glucose levels and body weight were measured on 1st, 10th, 20th, and the 30th day of the study. Treatment with seed extract in streptozotocin-induced diabetic rats showed a significant reduction in plasma glucose when compared with the control group. After treatment with the glibenclamide and seed extract (50, 100 and 200 mg/kg) there was a decrease in serum glucose levels of 74.27, 69.55, 71.45, and 72.66%, respectively, when compared with the control group. At the end of the experiment, the animals were sacrificed and blood collected to estimate biochemical parameters to the evaluation of the antihyperlipidemic activity, including plasma glucose, glycated haemoglobin (HbA1c), serum triglyceride, cholesterol, low-density lipoprotein (LDL)-cholesterol, very low-density lipoproteins (VLDL) and high-density lipoprotein (HDL)-cholesterol. It was possible to observe that treatment with the seed extract showed a significant decrease in HbA1c levels when compared to the control groups. Besides, the extracts at the doses of 50, 100, and 200 mg/kg reduced the lipid profile in diabetic rats. Lipid profile of animals showed significant reduction of 13.82, 18.08, and 22.34% cholesterol, 44.21, 51.57 and 60.00% LDL-cholesterol, 11.60, 18.13 and 18.86% VLDL and 27.43, 30.08 and 31.85% triglyceride, when compared with the control group. Significant increase in HDL-cholesterol level of 54.12, 66.62, and 70.75% was observed after treatment in the same doses. Therefore, this study speculates that seed aqueous extract *A. pavonina* has the potential to treat diabetes condition and associated lipid disorders.

In another work, galactomannan from *A. pavonina* seeds was evaluated for the antidiabetic effect in mice with streptozotocin-induced diabetes. Animals were divided into five groups: Negative control (non-diabetic animals), diabetic control (no treatment), diabetic mice treated with 1% galactomannan enriched food, diabetic mice treated with 2% galactomannan enriched food and diabetic mice treated with metformin®. Blood samples were checked at 0, 21, and 30 days of the treatment to ascertain fasting glycemia. Total cholesterol and triacylglycerol were evaluated at the end of treatment. It was observed that the feed enriched with 1 and 2% galactomannan decreased the glycemia, total cholesterol, and triacylglycerol of the animals. This work also highlights the potential of seed biopolymer as a therapeutic alternative for the control and treatment of diabetes (Vieira et al., 2018).

Renal protective effect

Another study investigated the renal protective effect ofthe same *A. pavonina* seed aqueous extract at doses of 50, 100 and 200 mg/kg/day in streptozotocin-induced diabetic rats. Results showed that after 13 weeks of treatment, the seed extract significantly reduced proteinuria, albuminuria, lipid levels, and HbA1c deposition in diabetic rats, suggesting the potential use of the extract in the reduction in the progression of diabetic nephropathy (Pandhare and Sangameswaran, 2012).

Anticonvulsant and depressant activities

Central nervous system activities of the *A. pavonina* seed methanolic extract were conducted by Oni et al. (2009). The methanolic extract was prepared with the seed powder (80%) using a soxhlet extractor. This study used three doses of extract (50, 100 and 200 mg/kg, i.p.) which consisted of two protocols, one evaluated the anticonvulsant activity, and the other evaluated the depressant activity in Swiss albino mice. In the anticonvulsant protocol, the picrotoxin, pentylenetetrazole and strychnine were used to induce convulsions in mice, and diazepam used as a reference anticonvulsant drug for comparison. The tonic hind limb extension of the animals was considered as a manifestation of seizure. The potential of the extract to prevent the seizures or delay/prolong the latency of or onset of the hind limb extensions was considered as a sign of the anticonvulsant effect. At all doses, the seed extract not only protected mice significantly and dose-dependently against picrotoxin and pentylenetetrazole-induced seizures but also delayed the onset of seizures induced by them. The most effective protection against picrotoxin and pentylenetetrazole-induced convulsions suggest that the anticonvulsant activity of the seed extracts could be related to GABAergic neurotransmission interference or in the stabilization of nerve cells membrane in the brain. In phenobarbitone-induced sleep protocol, three doses of

the extract (50, 100 and 200 mg/kg, i.p.) were tested, and chlorpromazine used as a reference sedative drug for comparison and 2% tween 80 as the negative control. After thirty minutes, all animals received phenobarbitone. The time of loss and gain of righting reflex was considered as a measure of sleep time. In all doses, the seed extracts prolonged the phenobarbitone-induced sleeping time in mice significantly and dose-dependently. It is suggested in this study that the extract interacts with the barbiturate allosteric site on the GABA receptors, boosting the action of phenobarbitone, the effect of which might induce prolonged sleeping time.

Antiviral activity

Antiviral effect of the *A. pavonina* seed and fruits aqueous extracts were conducted by Chiang et al. (2003). The extracts were tested against adenoviruses (ADV) and herpes simplex viruses (HSV). In the *in vitro* assays, it was observed that the aqueous extracts were effective only against ADV.

Sulfated galactomannan from *A. pavonina* has also been shown to have a relevant antiviral activity against dengue virus (Marques et al., 2015). Other researches also have confirmed the antiviral activity of sulfated galactomannan from *A. pavonina* against herpes simplex virus (Godoi et al., 2015), or of native or sulfated galactomannan from *A. pavonina* against poliovirus type 1 (PV-1) (Godoi et al., 2014).

Anti-emetic activity

Only one study about the anti-emetic activity was reported. The crude methanol extract of the leaves of *A. pavonina* was assessed for anti-emetic activity in male chicks. Emesis was induced by copper sulphate 50 mg/kg body weight (p.o.). The anti-emetic activity was ascertained by calculating the mean decrease in the number of retching in comparison with the control. The extract (150 mg/kg body weight orally) showed an anti-emetic activity of 50.17% when compared with standard chlorpromazine at the same dose (Hasan et al., 2012).

Other activities

Because it is a medicinal plant, the focus of this research towards pharmacological activities. However, other biological activities have been conducted as can be seen below.

Silva et al. (2012) demonstrated the bioinsecticidal effect of the *A. pavonina* seed proteinase inhibitor (ApTI) for the control of *Diatraea saccharalis*. In another study, Sasaki et al. (2015) showed that the *A. pavonina* seed proteinase inhibitor (ApTI) also caused a significant effect on *Aedes aegypti* larvae exposed to a non-lethal concentration of ApTI during short- and long-duration assays, decreasing survival, weight and proteinase activities of midget extracts of larvae. Other research conducted with the same seed proteinase inhibitor showed that purified ApTI resulted in an inhibition of growth of *Anagasta kuehniella* (Lepidoptera: Pyralidae) (Macedo et al., 2010b). Based on this seed proteinase inhibitor (ApTI), Rodrigues et al. (2018) developed a new promisor synthetic antimicrobial peptide called Adenovin.

In another study, Ito et al. (2018) were able to prove the inhibited effect of different extracts (bark and fruit) of *A. pavonina* against tyrosinase and collagenase.

TOXICITY STUDIES

Some studies have been conducted to evaluate the toxicity of derivatives from *A. pavonina*. Leaf extracts of this plant were screened for toxicity to brine shrimp *Artemia salina* and presented absence of cytotoxicity (Wickramaratne et al., 2016). However, the root and stem bark extracts showed cytotoxicity against *A. salina* (Rodrigo et al., 2007; Zeid et al., 2012).

Acute oral toxicity test of the ethanol extract of *A. pavonina* leaves was performed in mice. This test revealed nontoxicity up to 5000 mg/kg, demonstrating the safety of this extract (Mayuren and Ilavarasan, 2009). Another study conducted with the ethanolic extract from the leaves of *A. pavonina* revealed that the median lethal dose (LD_{50}) of the total ethanol extract was found to be 5.8 g/kg (b.w.). This result showed that this plant derivative is considered safe (Zeid et al., 2012). As expected, Moniruzzaman et al. (2015) did not observe the toxicity of the ethanolic extract from the leaves at 2000 mg/kg. Mujahid et al. (2013) performed the acute oral toxicity study of the methanolic extract of leaves of *A. pavonina* according to the OECD 425, 2001. The results showed that the methanolic extract was safe up to a dose of 2000 mg/kg. Adedapo et al. (2014) observed that the methanol extract of *A. pavonina* exhibited an LD_{50} bigger than 8000 mg/kg.

In another study, the brine shrimp lethality assay against extracts of the leaves of *A. pavonina* prepared with chloroform, ethyl acetate, acetone, methanol, and ethanol was conducted. The LD_{50} values of ethanol, methanol, acetone, ethyl acetate, and chloroform were 256, 602, 681, 910 and 1387 µg, respectively (Sophy et al., 2016).

Literature reports reveal that raw seeds are toxic but can be eaten after cooking (Zeid et al., 2012). Based on this information, some studies with the seed extracts were performed. A study performed with an emulsion containing seed oil of *A. pavonina* decreased in hemolytic activity and protective effect against sheep erythrocytes (Jaromin et al., 2006). Acute intraperitoneal toxicity of the methanolic seed extract of *A. pavonina* in mice demonstrated an LD_{50} value of 1.36 g/kg. However, the

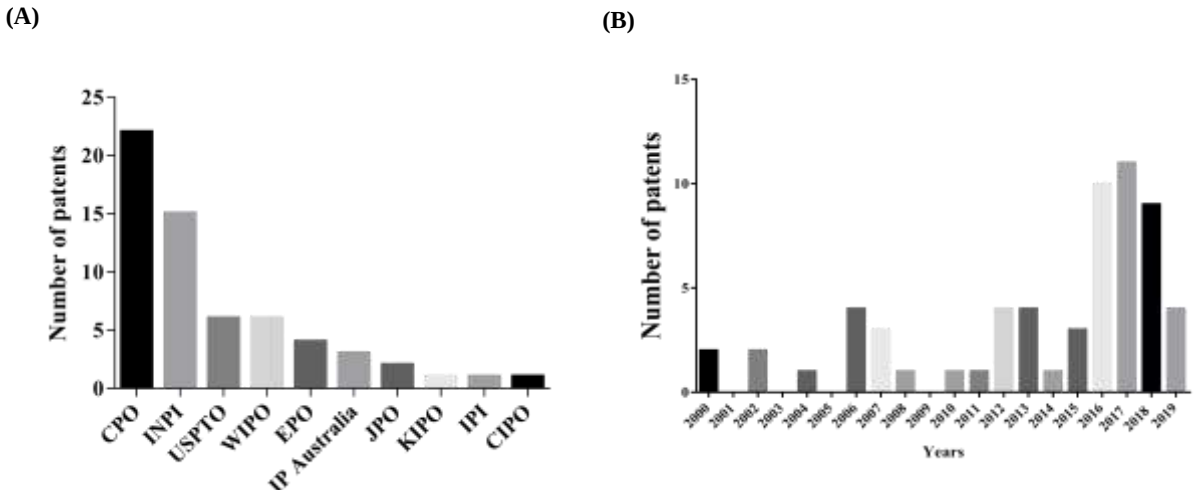

Figure 3. (A) Total number of patent documents by patent offices and (B) distribution of patent applications of all databases searched per year.

extract generated a dose-dependent reduction of motor activity, with 800, 1600, and 3200 mg/kg (b.w.) doses (Olajide et al., 2004). On the other hand, the LD_{50} of the seed aqueous extract of *A. pavonina* was higher than 2,000 mg/kg (b.w.) when administered orally (Pandhare et al., 2012a). Adedapo et al. (2009) observed an absence of toxicity in the kidney, liver, and testes of rats treated with a methanolic extract of the seeds of *A. pavonina* (200 mg/kg, p.o.). An interesting study evaluated the toxic potential of the purified trypsin from seeds of *A. pavonina* using *A. salina* lethality test. The results showed that a concentration of 0.16 mg/mL was enough to kill 100% of *A. salina* after 72 h (Souza et al., 2016).

In a recent study, Melo et al. (2018) proved the absence of toxicity of the seed biopolymer by the *A. salina* test. Therefore, it can be concluded that the *A. pavonina* derivatives were more often non-toxic. These results helped to conduct the pharmacological tests with more safety.

TECHNOLOGICAL PROSPECTING

The technological databases used were the INPI (National Institute of Industrial Property, Brazil), USPTO (United States Patent and Trademark Office), WIPO (World Intellectual Property Organization), EPO (European Patent Office - Espacenet), and The Patent Lens (the patent data, Cambia and Queensland University of Technology). The selection of the patents was based on the following inclusion criteria: published patents containing the keyword "*Adenanthera pavonina*" in their title, abstract or body without the restriction of the year of publication. The keyword was searched in English. All patent documents written in English were evaluated and included.

Despite the large number of publications with *A. pavonina*, this work showed a discrete number of patent applications related to this species. From the search of the patent databases, it was possible to identify a total of 61 patent documents, with 17 granted patents and 44 patent applications. Besides, the Chinese Patent Office (CPO) obtained the highest number of patent filings involving *A. pavonina* (Figure 3A). Several patent applications for the same invention were carried out in independent offices. It was possible to observe that the same inventions were applicated in several countries. Therefore, the decision of a company or institution to patent in a specific country signals a purpose to enter into a local market, and the intention to patent in several countries signals to expand this market (Dechezleprêtre et al., 2017).

It is believed that the advances in researches on this plant species and the increase in the general number of publications over the last years led to the growing interest of researchers in protecting inventions.

Several countries have filed patent applications, highlighting China, Brazil and the United States. Brazil leads the number of published articles. On the contrary, China presents more patent applications than scientific publications (Appendix B).

The analysis of patent evolution in all databases showed that the first registration involving A*. pavonina* occurred in 2000. The patent entitled 'Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/microorganisms or enzymes compositions thereof/modified by introduction of foreign genetic material' (WO 2000/005406 A1 and AU 1999/048985 A) was the first document found when comparing all databases.

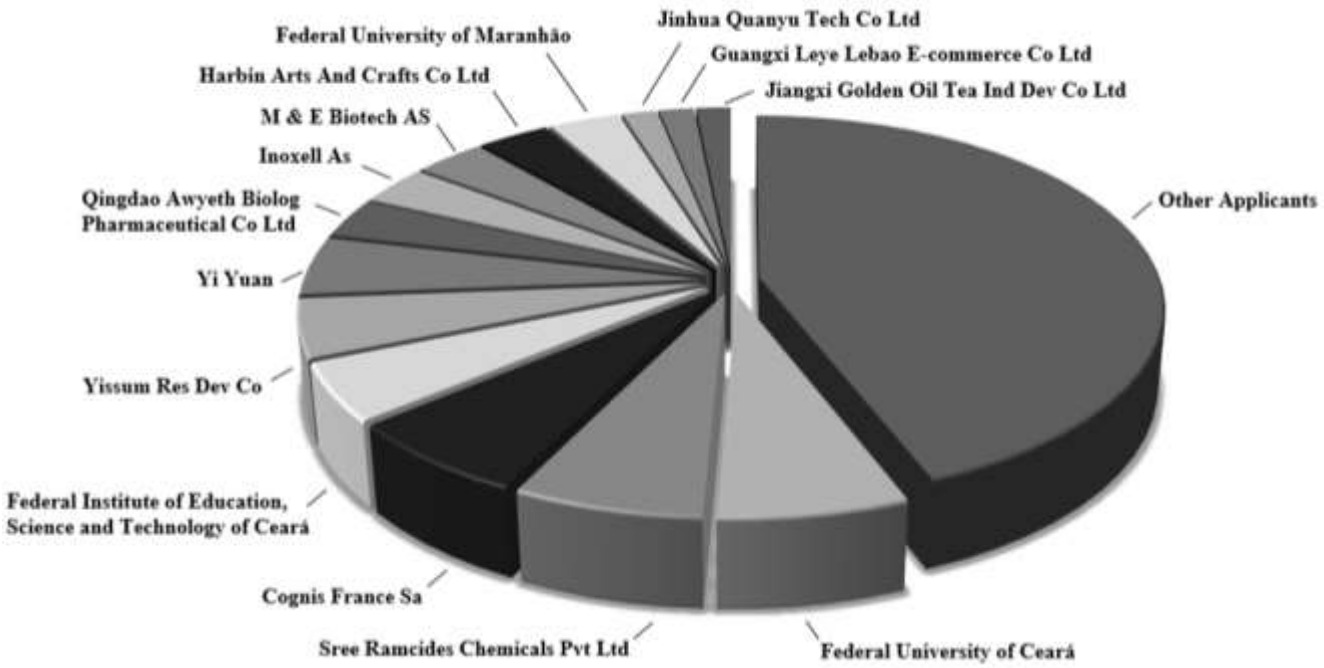

Figure 4. Representation of all applicants of patents.

As can be seen in Figure 3B, in 2017, the most significant number of patent applications was carried out. It is believed that with increased investment in research

on the plant, researchers have awakened the interest of protecting their inventions.

Figure 4 shows the main entities applying for patents with companies, academic institutions, and independent researchers among the main assignees.

The patent documents were analyzed according to the International Patent Classification (IPC) (Figure 5), and it was observed that Section A, regarding the human needs, had the largest number of applications, followed by Section C (chemical and metallurgical) and B (processing and transportation). It was possible to observe, assessing the contents of the patents, that several of them involved the use of the biopolymer of *A. pavonina* seeds. These biopolymers are hydrocolloids which form highly viscous and stable aqueous solutions, applied as excellent reinforcers and stabilizers for emulsions, thickeners, flocculants, emulsifiers, and gellants. Due to the excellent properties of this biopolymer, it becomes an attraction for use in the textile, pharmaceutical, biomedicine, cosmetics, and food industries (Cerqueria et al., 2009). Other records involve the plant extracts for soap manufacturing and the seed extracts for the preparation of dermatological formulas for the treatment of the skin and scalp as well as the preparation of foods such as sweets and beverages (Appendix B).

It is believed that a higher number of proposals of patent applications of the plant in the biomedical area is related to the significant part of researches on its chemical and medicinal properties. However, compared with other plant species, the search for protection of this species is still superficial. Therefore, this work showed the potential of the *A. pavonina* species in the biomedical area and opens perspectives for future discoveries.

CONCLUSION

In this review, it was possible to verify the range of knowledge related to the *A. pavonina* species. The spread of this native Asian species to other continents and its popularity as an herbal medicine aroused particular interest in studying this plant. This species has a long history of ethnomedical use with indications for various diseases such as diarrhoea, inflammations, diabetes, rheumatism, asthma, hypertension, among others. This broad application motivated many researchers to study the phytochemistry, mainly of leaves, seeds, and barks, besides the pharmacological effects aiming to demonstrate scientific evidence of these empirical uses. Experimental studies of this plant have shown numerous pharmacological activities, such as antidiarrheal, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, anticancer, etc. Interestingly, the extracts did not present high toxicity in the experimental models evaluated. It was also observed that most studies are conducted with plant derivatives, and more recent studies have been concerned with isolating, characterizing, and biologically evaluating these

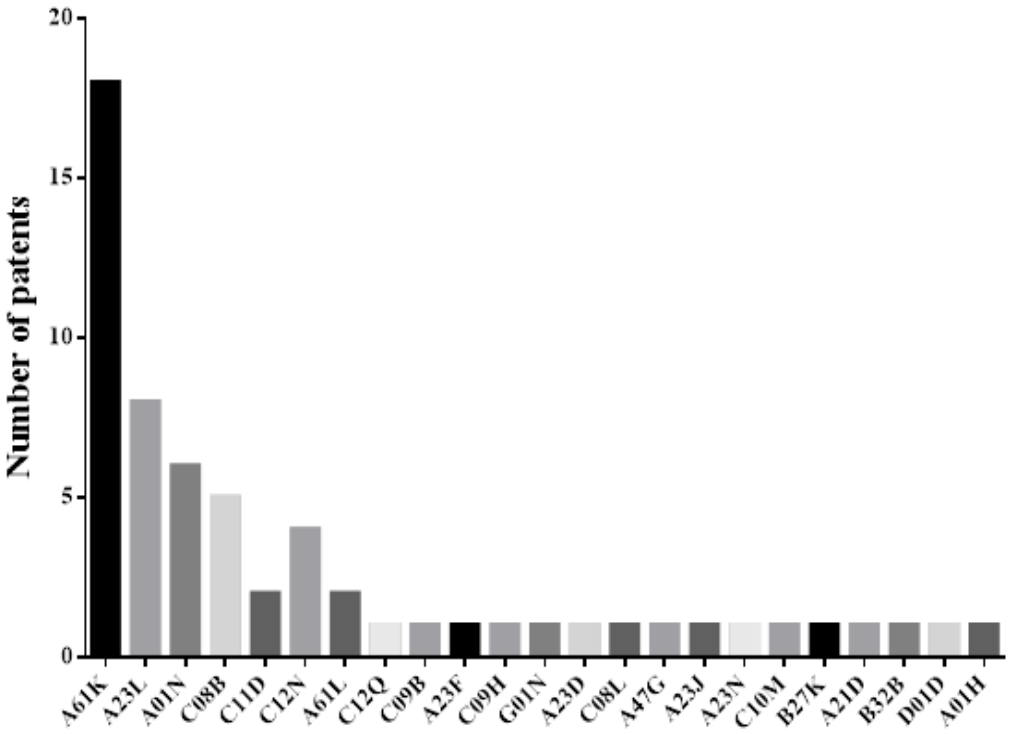

Figure 5. Distribution by International Patent Classification (IPC) of the patent documents found in the all databases.

compounds. Nevertheless, further research is necessary to identify and investigate the pharmacological mechanisms of these main active molecules. Despite the number of studies performed, few preclinical and clinical studies have been conducted to ensure efficacy and safety in traditional therapy. In this context, considering the risk-benefit of the use of medicinal plants, more research needs to be carried out to evaluate the potential toxicity after sub-chronic, and chronic administration of this species. Therefore, this work has shown the potential of the *A. pavonina* species in the human health area and reveals outlooks for future studies. Thus, it is believed that further research with this species will lead to the discovery of prototype molecules that can be used in the development of new herbal medicines, and those new species-related patents will be filed in the near future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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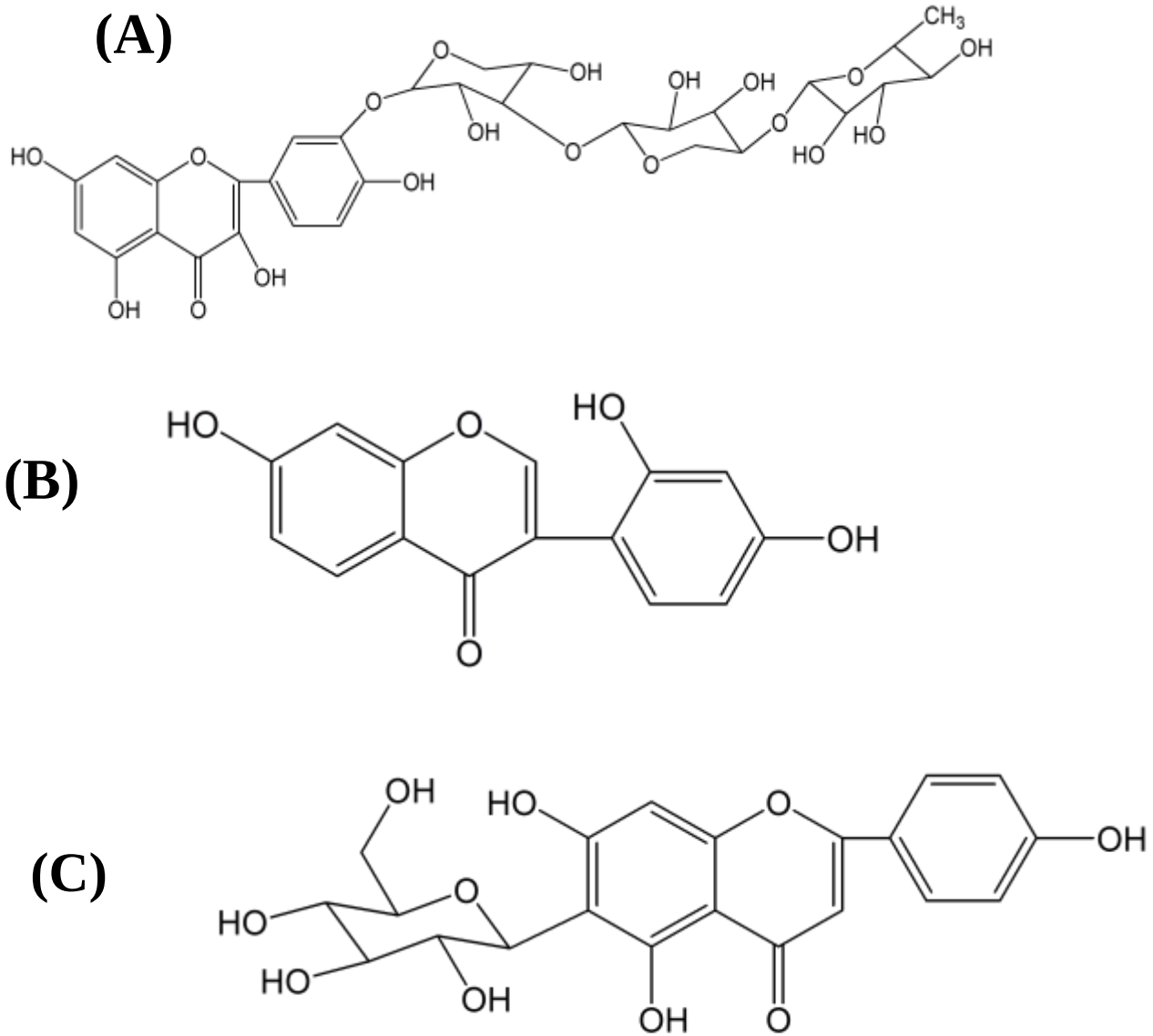
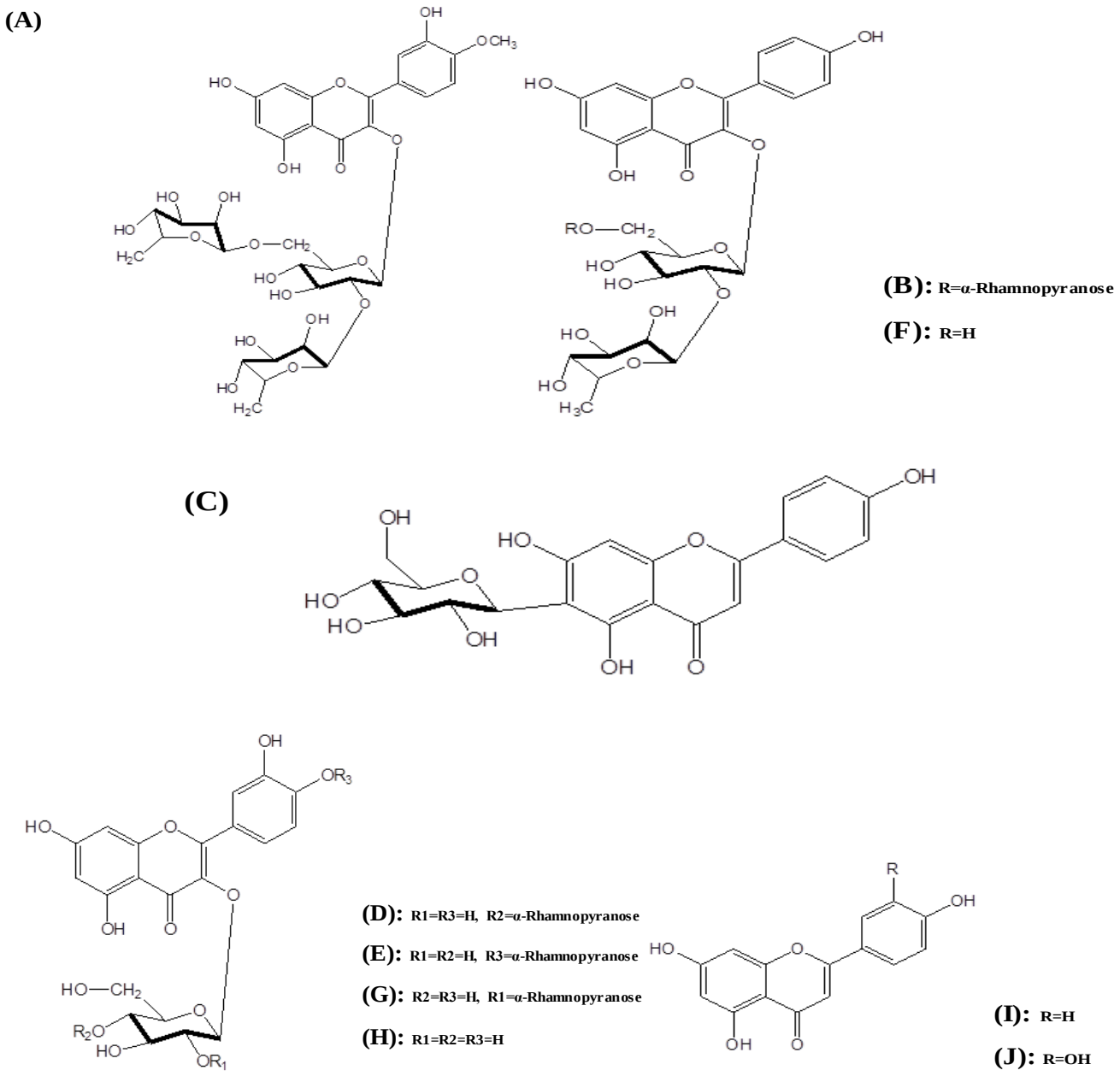
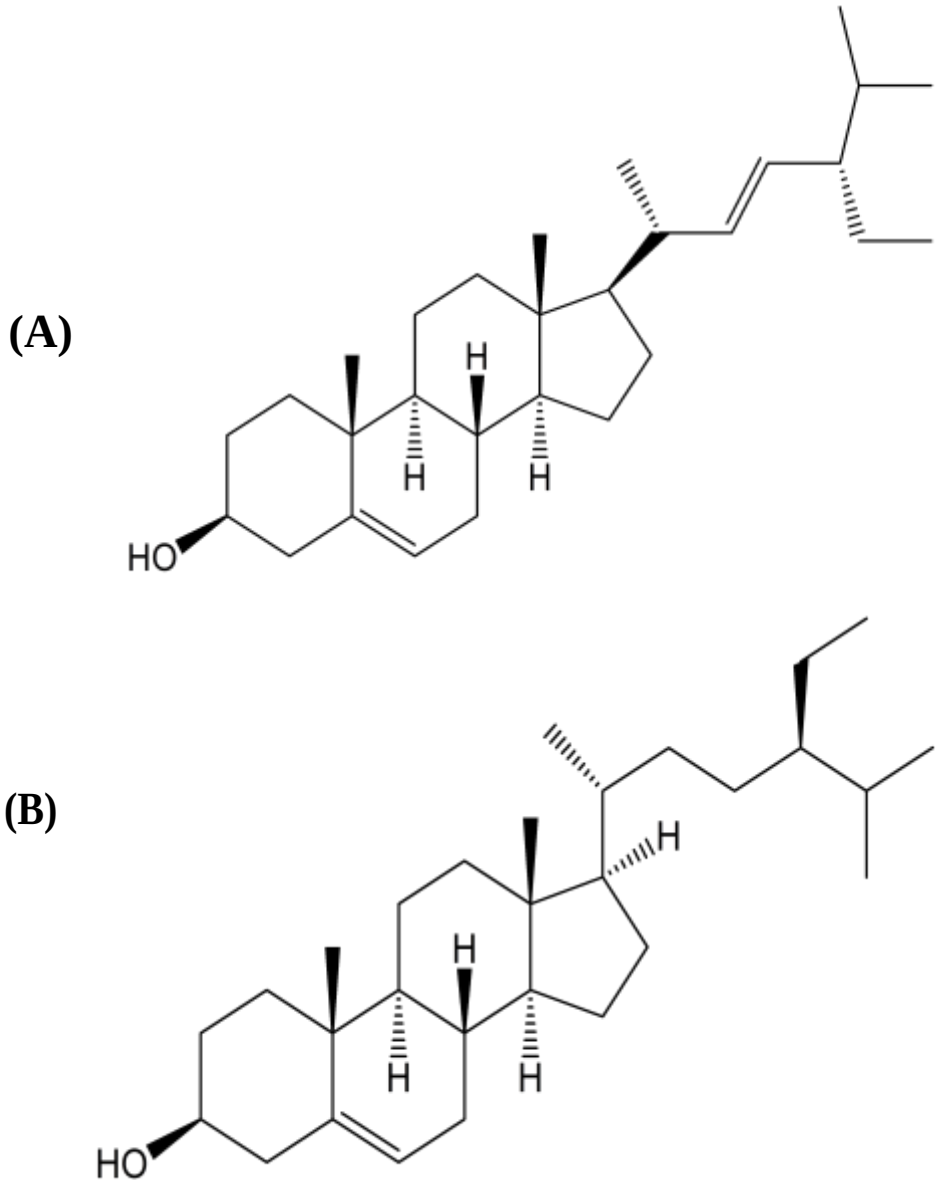
APPENDIX



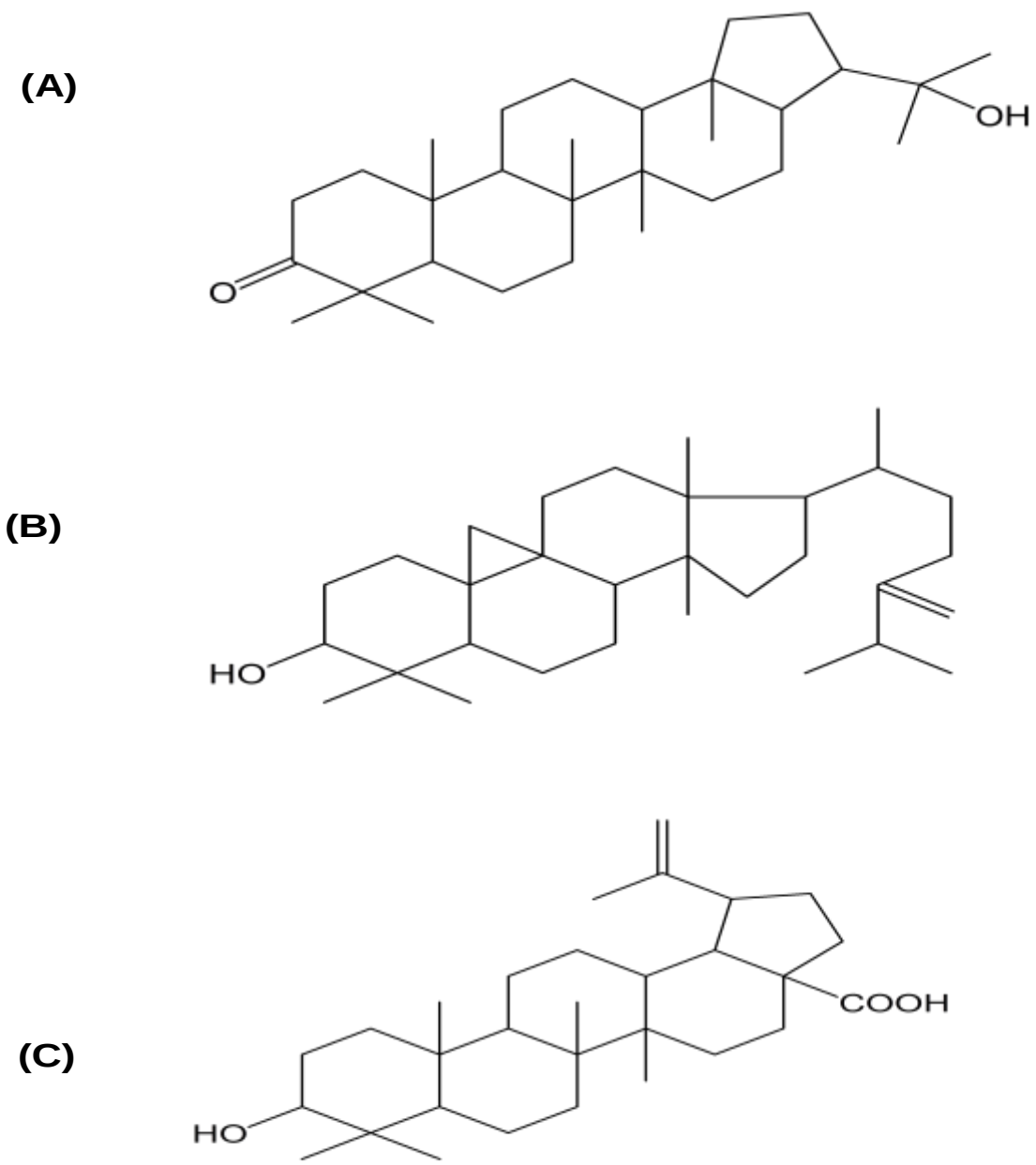
Scheme A1. Chemical structures of compounds A-C.



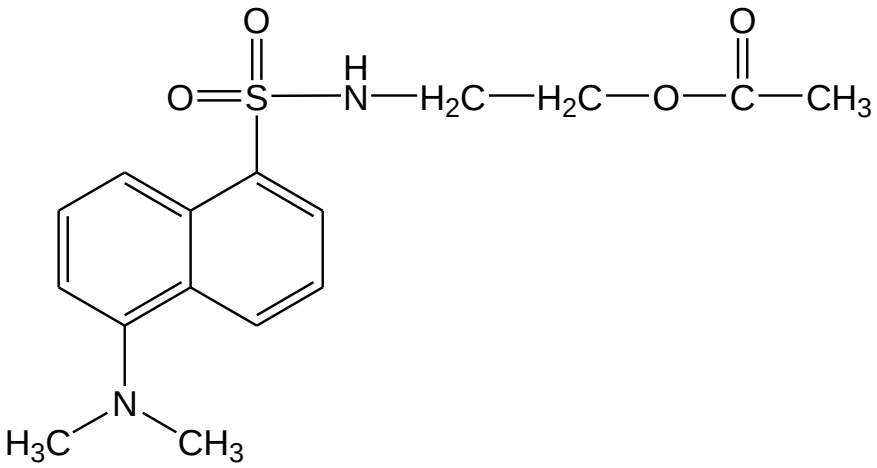
Scheme A2. Chemical structures of compounds A-J.



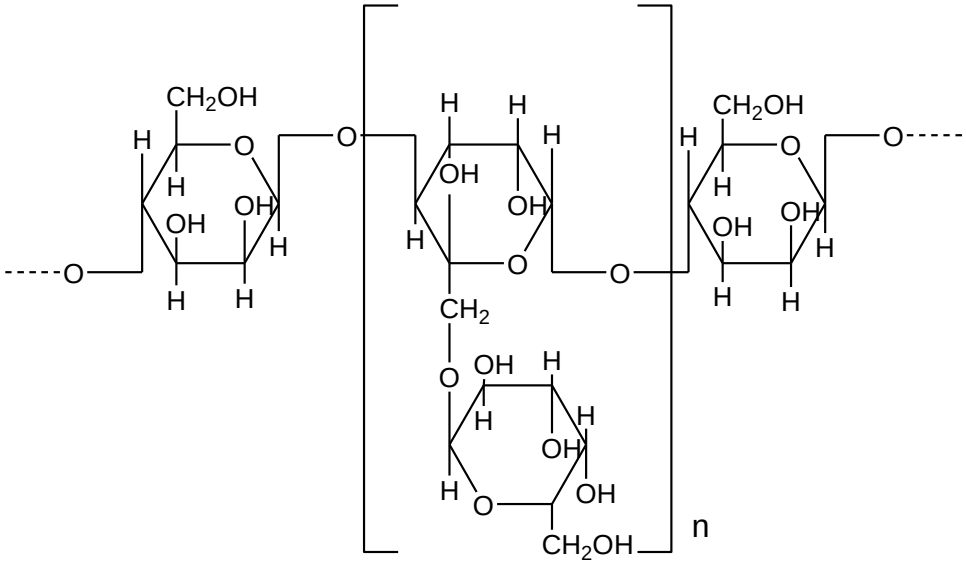
Scheme A3. Chemical structures of compounds A-B.
Source: Sriraman et al. (2015).



Scheme A4. Chemical structures of compounds A-C.



Scheme A5. Chemical structures of compound O-acetylethanolamine.
Source: Hayman and Gray (1987).



Scheme A6. Chemical structures of compound galactomannan.

Table B.1: Patent search document type.

Year of publication	Document types	Document type	IPC Classifications*	Title
2019	Granted patent	US 10308726 B2	C08B37/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/polysaccharides derivatives thereof/preparation of polysaccharides not provided for in groups Derivatives thereof cellulose	Crosslinked Polymer, Hydrogel Or Water-based Fracturing Fluid Comprising The Same, And Methods Of Making And Using Thereof
	Granted patent	CN109673955-A	A23L11/00	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/pulses, i.e. fruits of leguminous plants, for production of fodder or food Products from legumes Preparation or treatment thereof	Rapidly cooking Adenanthera pavonina plant of regular diet food comprises e.g. washing Adenanthera pavonina in cold water, and soaking the cleaned Adenanther apavonlna in glass or ceramic utensils in cold water for two times
	Granted patent	BR102017027235-4 A2	A23L33/21	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/modifying nutritive qualities of foods Dietetic products Preparation or treatment thereof/Addition of substantially indigestible substances, e.g. dietary fibres addition of gelling or thickening agents	Uso da galactomanana parcialmente hidrolisada de adenanthera pavonina como estabilizante, substituto de gordura e fonte de fibra alimentar em sobremesa láctea tipo mousse e seus respectivos processos de extração, hidrolisação e obtenção
	Patent application	WO 2019/023685 A1	D01D5/08	Textiles paper/natural or man-made threads or fibres spinning/mechanical methods or apparatus in the manufacture of man-made filaments, threads, fibres, bristles or ribbons	Polymer films with antimicrobial agents
2018	Granted patent	CN 105853341 B	A61K8/9789	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/cosmetics or similar toilet preparations	Anti-aging skincare product containing epidermal stem cell secretin and preparation method thereof
	Patent application	BRPI0925163	B32B23	Performing operations transporting/layered products/layered products, i.e. products built-up of strata of flat or non-flat, e.g. cellular or honeycomb, form/layered products essentially comprising cellulosic plastic substances	Procedimentos para obtenção de filmes a partir de galactomananas de adenanthera pavonina e de blendas dessa galactomananas com outros biopolímeros
	Patent application	BR102016027846-A2	A21D13/062	Human necessities/baking equipment for making or processing doughs doughs for baking/treatment, e.g. preservation, of flour or dough for baking, e.g. by addition of materials baking bakery products preservation thereof/finished or partly finished bakery products/Products with modified nutritive value/with modified sugar content Sugar-free products	Galactomannan-enriched bread useful for preventing acute and chronic complications of type 2 diabetes mellitus and improving intestinal transit, contains wheat flour, sugar, yeast, egg and galactomannan from Adenanthera pavonina seeds
	Granted patent	CN107913373-A	A61K36/899	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Poaceae or Gramineae (Grass family)	Traditional Chinese medicine composition useful for treating hematological system malignant disease including leukemia and aplastic anemia, comprises e.g. cockroach, Camptotheca acuminata, radix Polygonum multiflorum, dodder and nux vomica
	Patent Application	BR102016021174-A2	C10M109/02	Chemistry metallurgy/petroleum, gas or coke industries technical gases containing carbon monoxide fuels lubricants peat/lubricating compositions/Lubricating compositions characterised by the base-material being a compound of unknown or incompletely defined constitution takes precedence	Preparing lubricant for chains and gears, involves using base of plant source, where plant source is derived from Adenanthera pavonina trees, which protects corrosion, prevents entrance of impurities, and in simple, eco-friendly manner
	Patent application	BR102016026312	A61K	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes	Pasta para cimento odontólógico e processo de obtenção

Table B.1. Contd.

	Patent application	CN 108606338 A	A23N12/06	Human necessities/foods or foodstuffs their treatment, not covered by other classes/machines or apparatus for treating harvested fruit, vegetables, or flower bulbs in bulk/Machines for cleaning, blanching, drying or roasting fruits or vegetables	High-efficiency energy-saving *Adenanthera Pavonina* Linn washing equipment
	Patent application	BR102015021292-A2	A01N65/20	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof/biocides, pest repellants or attractants, or plant growth regulators containing material from algae, lichens, bryophyta, multi-cellular fungi or plants, or extracts thereof containing compounds of determined constitution/Fabaceae or Leguminosae [Pea or Legume family]	Composition for controlling or combating larval form of Aedes aegypti mosquito which transmits yellow fever and dengue, contains proteinase inhibitor in aqueous solution which is extracted from seeds of Adenanthera pavonina
	Patent application	US 2018/0273649 A1	C08B37/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/polysaccharides derivatives thereof/preparation of polysaccharides not provided for in groups Derivatives thereof cellulose	Crosslinked Polymer, Hydrogel Or Water-based Fracturing Fluid Comprising The Same, And Methods Of Making And Using Thereof
2017	Patent application	BR102016013958	A23J	Human necessities/foods or foodstuffs their treatment, not covered by other classes/protein compositions for foodstuffs working-up proteins for foodstuffs phosphatide compositions for foodstuffs	Produto alimentício à base de proteína vegetal e castanha de cajú
	Patent application	CN 106860825 A	A61K31/045	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations containing organic active ingredients	Traditional Chinese Medicine Ointment For Arthralgia Caused By Wind-damp-heat Pathogens, And Preparation Method Thereof
	Patent application	BR102015017021-1 A2	C09H05	Chemistry metallurgy/dyes paints polishes natural resins adhesives compositions not otherwise provided for applications of materials not otherwise provided for/preparation of glue or gelatine/stabilisation of solutions of glue or gelatine	Produção de cola biodegradável a partir da semente de adenanthera pavonina l
	Granted patent	CN107281422-A	A61K36/904	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Stemonaceae (Stemona family), e.g. croomia Feedba	Ointment used to control tick in sheep, contains ivermectin tablets, Fritillaria, European grape fruit, Viola, radix stemonae, herba gymnopteridis auriculatae, Chamaesium spatuliferum, mulberry leaf, Chinese asafetida, bezoar and Aloe
	Patent application	BR102015017019-A2	A61K8/97	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/Cosmetics or similar toilet preparations/from algae, fungi, lichens or plants from derivatives thereof	Cream for treating hyperkeratosis, comprises galactomannan extracted from endosperm of Adenanthera pavonina and gel extracted from leaf of Aloe barbadensis homogenized in acetic acid
	Patent application	BR102015017020-3 A2	C08B37/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/polysaccharides derivatives thereof/preparation of polysaccharides not provided for in groups derivatives thereof cellulose	Galactomannan is extracted from endosperm of Adenanthera pavonina, where galactomannan is cross-linked with glutaraldehyde to form network like structure
	Patent application	CN 106819773 A	A23L5/10	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/general methods of cooking foods, e.g. by roasting or frying	Method For Rapidly Cooking *Adenanthera pavonina* Plant
	Patent application	BR102015024624-A2	A01N31/08	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof/Biocides, pest repellants or attractants, or plant growth regulators	Micro-capsules obtained from the nordestine biomass used for potential larvicide activity against the dengue transmitter mosquito, comprises unprecedented

Table B.1. Contd.

				containing organic oxygen or sulfur compounds	combination of aqueous solutions of natural polysaccharides as galactomannans
	Patent application	CN 106615428 A	A23F3/34	Human necessities/foods or foodstuffs their treatment, not covered by other classes/coffee tea their substitutes manufacture, preparation, or infusion thereof	Dendrobium Officinale Kimura Tea And Manufacture Method Thereof
	Granted patent	CN107259906-A	A47G9/10	Human necessities/furniture domestic articles or appliances coffee mills spice mills suction cleaners in general/household or table equipment/pillows pillow holders	Healthcare pillow useful for e.g. treating cervical spondylosis, premature ejaculation, insomnia and night sweats, comprises pillow structure which is filled with plant seed mixture containing black millet seed, grape seed and okra seed
	Patent application	CN 106509606 A	A23L11/00	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/pulses, i.e. fruits of leguminous plants, for production of fodder or food Products from legumes Preparation or treatment thereof	Cereals Capable Of Recuperating Constitutions Of Pregnant Women And Production Technology Of Cereals
2016	Patent application	BR102014023575-A2	A61K36/185	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Magnoliopsida (dicotyledons)	Obtaining galactomannan used for preparing galactomannan film, involves extracting Adenanthera pavonina seed endosperm
	Granted patent	CN105944041-A	A61K36/9066	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Curcuma	Topical ointment useful for e.g. treating verruca plana, comprises honeysuckle, common andrographis herb, rhizoma coptidis, Conyza blinii, red paeony, Spatholobus stem, gallnut, cherry kernel, tuckahoe, oyster, Forsythia and licorice
	Patent application	BR102014027289-A2	A61K36/886	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Aloeaceae (Aloe family)	Obtaining galactomannan used for preparing film for treating wound, involves utilizing Adenanthera pavonina seed endosperm powder
	Patent application	WO 2016/188446 A1	C08B1/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/polysaccharides derivatives thereof	Crosslinked polymer, hydrogel or water-based fracturing fluid comprising the same, and methods of making and using thereof
	Patent application	CN 105853341 A	A61K8/97	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/cosmetics or similar toilet preparations	Anti-aging skincare product containing epidermal stem cell secretin and preparation method of anti-aging skincare product
	Patent application	CN 105767934 A	A23L15/00	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/egg products preparation or treatment thereof	Honeysuckle Low-alkaline Preserved Egg And Preparation Method Thereof
	Granted Patent	CN105441230-A	C11D9/38	Chemistry metallurgy/animal or vegetable oils, fats, fatty substances or waxes fatty acids therefrom detergents candles/detergent compositions use of single substances as detergents soap or soap-making resin soaps recovery of glycerol/compositions of detergents based essentially on soap compositions containing resin soap/products in which the composition is not	Tea oil cooling soap comprises e.g. composition A comprising e.g. tea oil extract, composition B comprising e.g. tea seed powder extract, composition C comprising e.g. Adenanthera pavonina extract, mountain spring water and brown sugar

Table B.1. Contd.

				well defined	
	Patent application	CN 105441230 A	C11D9/02	Chemistry metallurgy/animal or vegetable oils, fats, fatty substances or waxes fatty acids therefrom detergents candles/detergent compositions use of single substances as detergents soap or soap-making resin soaps recovery of glycerol	Camellia Oil Cold Processed Soap
	Patent application	CN 105249160 A	A23L33/10	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment/modifying nutritive qualities of foods dietetic products preparation or treatment thereof	Blood enriching and qi nourishing porridge
	Patent application	CN 105249197 A	A23L33/00	Human necessities/foods or foodstuffs their treatment, not covered by other classes/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment	Health noodles
2015	Granted patent	CN104255531-A	A01H4/00	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/new plants or processes for obtaining them plant reproduction by tissue culture techniques/lant reproduction by tissue culture techniques	Method for breeding Adenanthera Pavonina, involves sterilizing Adenanthera Pavonina leaves, inoculating sterilized leaves into culture medium, and culturing embryogenic suspension cells followed by inducing embryonic cells and roots
	Patent application	CN 104861697 A	C09B61/00	Chemistry metallurgy/dyes paints polishes natural resins adhesives compositions not otherwise provided for applications of materials not otherwise provided for/organic dyes or closely-related compounds for producing dyes mordants lakes fermentation or enzyme-using processes to synthesise a desired chemical compound	Formula used for preparing springgreen wheat straw dye and production method
	Patent application	CN 104861693 A	B27K9/00	Performing operations transporting/working or preserving wood or similar material nailing or stapling machines in general/processes, apparatus or selection of substances for impregnating, staining, dyeing or bleaching of wood, or for treating of wood with permeant liquids, not otherwise provided for chemical or physical treatment of cork, cane, reed, straw or similar materials	Formula used for preparing springgreen wheat straw dye and production method
2014	Patent Application	US20140045220	C12Q1/68	Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/measuring or testing processes involving enzymes, nucleic acids or microorganisms immunoassay compositions or test papers therefor processes of preparing such compositions condition-responsive control in microbiological or enzymological processes	Composition for biosample treatment and method for nucleic acid amplification using the same
2013	Granted Patent	IN201103883-I4	C08L00/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/compositions of macromolecular compounds	Bioplastic composition used to obtain compostable bioplastic granules comprises biological waste which act as a prime ingredient; dilute hydrochloric acid; dilute sodium hydroxide; sodium hypochloride; and water
	Granted Patent	CN102719309	A23D9/007	human necessities/foods or foodstuffs their treatment, not covered by other classes/edible oils or fats, e.g. Margarines, shortenings, cooking oils/other edible oils or fats/characterised by ingredients other than fatty acid triglycerides	Tea oil for reducing weight and processing technology
	Patent Application	BR201104726-A2	G01N33/52	Physics/measuring testing/investigating or analysing materials by determining their chemical or physical properties/investigating or analysing	Use of lectin-galactose binding molecule is claimed for diagnosing prostate diseases, where the lectin-

Table B.1. Contd.

				materials by specific methods not covered by groups	galactose binding molecule or frutalin utilizes Artocarpus altilis seeds for treating prostatic basal cells
	Patent application	US 2012/0294959 A1	A01N65/20	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof	A Botanical Pesticide For Agriculture/horticulture Crops
2012	Granted patent	CN102416057-A	A61K36/736	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines/Prunus	Traditional Chinese medicinal composition, useful as a whitening composition, comprises Adenanthera pavonina, radix Angelica, Euphorbia lathyris leaf, cherry leaf and jasmine flower, prepared by e.g. crushing, soaking and spray drying
	Patent application	BR200910090-A2	C08B37/00	Chemistry metallurgy/organic macromolecular compounds their preparation or chemical working-up compositions based thereon/polysaccharides derivatives thereof/preparation of polysaccharides not provided for in groups Derivatives thereof cellulose	Method for obtaining galactomannan from Adenanthera pavonina, involves boiling seed of Adenanthera pavonina in distilled water and then seed is kept in order to swell
	Patent application	EP2531037	A01N65/20	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof	A botanical pesticide for agriculture/horticulture crops
	Patent application	AU 2011/210381 A1	A01N65/20	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof	A botanical pesticide for agriculture/horticulture crops
2011	Patent application	WO 2011/092721 A1	A01N65/20	Human necessities/agriculture forestry animal husbandry hunting trapping fishing/preservation of bodies of humans or animals or plants or parts thereof	A botanical pesticide for agriculture/horticulture crops
2010	Granted patent	EP 1850889 B1	A61K8/73	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/cosmetics or similar toilet preparations	Depolymerized polysaccharide-based hydrogel adhesive and methods of use thereof
2008	Patent application	US 2008/0160118 A1	A61K36/00	Human necessities/ preparations for medical, dental, or toilet purposes	Treatment of skin with cosmetic and dermatological preparations containing extracts from seeds of plants of the Genus Adenanthera
2007	Granted patent	JP2007246429-A	A61K36/18	Human necessities/medical or veterinary science hygiene/preparations for medical, dental, or toilet purposes/medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof	New food/beverage products from an extract of one or more types of plants comprising preferably, Glirichidia sepium, useful in inhibiting lipase activity for decreasing blood triglyceride levels for treating obesity
	Patent application	JP 2007246429 A	A23L1/30	Human necessities/foods, foodstuffs, or non-alcoholic beverages, not covered by subclasses or their preparation or treatment	Lipase inhibitor, and food and drink
	Patent application	US 2007/0104676 A1	A61K36/00	Human necessities/ preparations for medical, dental, or toilet purposes	Cosmetic and/or dermatological preparations containing an extract from the seeds of plants of the genus Adenanthera.
2006	Patent application	WO/2006/085329	A61L15/60	Human necessities/medical or veterinary science hygiene/methods or apparatus for sterilising materials or objects in general disinfection, sterilisation, or deodorisation of air chemical aspects of bandages, dressings, absorbent pads, or surgical articles materials for bandages, dressings, absorbent pads, or surgical articles/liquid-swellable gel-forming materials	Depolymerized polysaccharide-based hydrogel adhesive and methods of use thereof
	Patent application	CA 2609790	A61L15/60	Human necessities/medical or veterinary science hygiene/methods or apparatus for sterilising materials or objects in general disinfection, sterilisation, or deodorisation of air chemical aspects of bandages, dressings, absorbent pads, or surgical articles materials for bandages, dressings, absorbent pads, or surgical articles/liquid-swellable gel-forming materials	Depolymerized Polysaccharide-based Hydrogel Adhesive And Methods Of Use Thereof

Table B.1. Contd.

	Patent application	EP1635910	A61K36/00	human necessities/ preparations for medical, dental, or toilet purposes	Cosmetic and/or dermatological preparations containing an extract from the seeds of plants of the genus Adenanthera.
	Patent application	KR 20060028389 A	A61K36/00	Human necessities/ preparations for medical, dental, or toilet purposes	Cosmetic and/or dermatological preparations containing an extract from the seeds of plants of the genus Adenanthera.
2004	Patent Application	WO 2004/100909 A1	A61K36/00	Human necessities/ preparations for medical, dental, or toilet purposes	Cosmetic and/or dermatological preparations containing an extract from the seeds of plants of the genus Adenanthera.
2002	Granted Patent	EP 1098991 B1	C12N1/15	Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/microorganisms or enzymes compositions thereof/modified by introduction of foreign genetic material	Novel methods for the identification of ligand and target biomolecules
	Granted Patent	AU 751055 B2	C12N1/15	Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/microorganisms or enzymes compositions thereof/modified by introduction of foreign genetic material	Novel methods for the identification of ligand and target biomolecules
2000	Patent Application	WO 2000/005406 A1	C12N1/15	Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/microorganisms or enzymes compositions thereof/modified by introduction of foreign genetic material	Novel methods for the identification of ligand and target biomolecules
	Patent Application	AU 1999/048985 A	C12N1/15	Chemistry metallurgy/biochemistry beer spirits wine vinegar microbiology enzymology mutation or genetic engineering/microorganisms or enzymes compositions thereof/modified by introduction of foreign genetic material	Novel methods for the identification of ligand and target biomolecules

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Phytochemical screening and in vitro evaluation of antibacterial activity of aqueous and ethanolic extracts of root and stem bark of *Bridelia ferruginea*. Benth. (Euphorbiaceae)

Mela Ilu Luka[1], Stanley Chukwudozie Onuoha[2*], Vincent Olasoji Oladele[3] and John Aguiyi[4]

[1]Department of Medical Microbiology, Faculty of Medical Sciences, University of Jos, P.M.B 2084 Jos, Plateau State, Nigeria.
[2]Department of Biotechnology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
[3]Department of Educational Services, Universal Basic Education, P.M.B 163 Garki, Abuja, Nigeria.
[4]Phytomedicine Research and Development, University of Jos, Plateau State, Nigeria.

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This study aimed at analysing the phytochemical content and antimicrobial activity of *Bridelia ferrugine* against selected bacteria. Total saponin, alkaloids, tannins, flavonoids and total anthraquinone contents were evaluated using spectrophotometric equivalents of the standards. The antibacterial activity of the plant extracts were determined using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays against selected bacteria. The root and stem revealed the presence of the phytochemicals tested except the stem that lacked anthraquinones. In vitro antimicrobial activity of the plant extracts against the gram-positive bacteria tested showed that *Bacillus cereus* was most susceptible to the plant extract having MIC and MBC of 25 and 50 mg/ml, respectively for the stem-bark and root-bark ethanolic extract, while gram-negative bacteria the plant extracts were most active against *Proteus mirabilis* with MIC and MBC of 50 and 100 mg/ml, respectively. The aqueous extract was most active against *Staphylococcus epidermidis* with MIC and MBC of 50 and 100 mg/ml for stem-bark and 25 and 50 mg/ml for root-bark extract. Concentration dependent study showed the plant extracts were either bacteriostatic or bactericidal. Only the stem-bark aqueous extract showed no primary effect on the control strains. The study confirmed the presence of some phytochemicals which revealed that the plant is of pharmacological importance going by the ability of these phytochemicals to elicit antibacterial activity.

Key words: Antibacterial, phytochemical screening, *Bridelia ferruginea*, plant extracts.

INTRODUCTION

Traditionally, the use of plants in treating diseases and ailments has its origin in the history of man as it dates

*Corresponding author. E-mail: sconuoha@yahoo.com.

back to antiquity (Ogunyemi, 1979; Grabley and Thiericke, 1999). The use of plant by different ethnic groups for medicinal purposes prevails among the Nigerian people; even in the developed countries, plant derived drugs may be of some importance. In the United States of America, 25% of drug prescribed in the community pharmacies from 1959 to 1980 contain plant extracts or active ingredients obtain from them (Fransworth, 1999). As pointed out by Baker et al. 1995, plants have contributed significantly in ensuring the health care of about 80% of the world's population. Most of the drugs produced from plant products arise from the traditional use of the plants. For instance, 74% of the drugs derived from plant products listed by WHO arise from studies that isolated the active ingredients that accounted for using the plant in traditional medicine (Fransworth, 1999). Long before now, reports of resistance by pathogenic microorganisms to many synthetic drugs has been alarming (Ozumba, 2003; Aibinu, 2004). The continued increase in strains of microorganisms having resistant to antibiotic result in the development of a more effective antibiotic such as the 3rd and 4th generations of cephalosporins by pharmaceutical industries (Odugbemi, 2006). Several reports showing the potency of plant extract against microorganisms have been published. From these reports, plants became the foundation for synthetic drugs (Evans et al., 2002).

B. ferruginea belongs to the family *Euphorbiaceae*. It mostly grows as a shrub but under favourable condition can grow well reaching the size of a tree. The tree is about 6 to 15 m high, and 1.5 m in girth. The bark is dark grey, rough and often scaly (Rashid et al., 2000). Its common names are Kirni (Hausa), Marehi (Fulfulde), Ira lodan (Yoruba), Ola (Igbo); and KensangeAbia (Boki). It's mostly found in the savannah, most especially the moisture regions that extend from Guinea to the Democratic Republic of the Congo and Angola.

B. ferruginea has diverse uses. Extract from the bark have been used in the coagulation of milk and lime juice to formulate a traditional gargle The bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle "egunefu" (Orafidiya et al., 1990). Reports have also indicated its usage in the treatment of water (Kolawole and Olayemi 2003) In Togo, the bark of the root is used I the remedy of intestinal disorders and for the treatment of skin diseases (De Bruyne et al., 1997). Some other reported activity of the bark extract are trypanocidal (Ekanem et al., 2008), antimicrobial (Adeoye et al., 1988), molluscidal (Iwu, 1984), anti-inflammatory (Olajide et al., 1999). The bark, leaves and roots are ingredients of Yoruba (in Nigeria) infusions chiefly administered to children (Burkil, 1994).

A large proportion of the population of African countries still rely on the use of local herbs in the management of many ailments ranging from surgical to medical either infectious and non-infectious, with different degree of success or claim of beneficial effects. The bark and the bright red infusion from it are commonly sold in Nigerian markets and shops for use as a mouth-wash and remedy for thrush in children. In Congo, a bark decoction is used for toothache and in the Ivory Coast for dysentery and diarrhoea or as a laxative (Gill, 1992). The bark is used as antidote against poison and arrow poison (Burkil, 1994). A leaf extract in saline solution is reported to produce a marked reduction of blood-sugar in laboratory rats and clinical trials have given a drop from 250 mg to the normal less than 120 mg after eight weeks of daily treatment (Iwu, 1980). A bark preparation is used for immunity against arrow poison and syphilis. Extract from the bark is mixed with the stem of *Costus* for the treatment of minor epilepsy (Akubue and Mittal 1982). Root and stem barks are used for skin disease and eruption. They are found to be rich in tannins and are used as chewing sticks/mouth washes (Burkil, 1994). The boiled root and stem bark (boiled water extract) have recorded positive action against gram positive bacteria; *Sarcinalutea* and *Staphylococcus aureus* (Malcolm and Sofowora 1969).

Antimicrobial properties of stem bark of *Bridelia ferruginea* against facultative gram negative rods have been reported by Ndukwe et al., 2007. The activities of the methanol, petroleum ether and chloroform bark extracts of *B. ferruginea* against some potential pathogenic organisms have been extensively investigated (Iwu, 1984; Adeoye et al., 1988; Olajide et al., 1999). *B. ferruginea* had been found to contain various organic compounds according to Sofowora et al., 1982. The plant was presumed to contain alkaloids, tannins, terpenoids, glycosides, flavonoids, saponins, anthraquinones and steroids.

The occurrence of multiple antibiotics resistance developed by microorganisms against the development of resistance to both the old and newer drugs calls for active search for more effective as well as affordable antimicrobial agents. This problem has prompted tremendous effort to explore for more potent antimicrobial agents, especially of natural origin to counter the resistance.

Previous studies have shown that, *B. ferruginea* has tremendous potentials as important sources of remedy for certain bacterial infections and as a source of new compounds for antimicrobial drugs syntheses (Sofowora, 1982).The plants are the sleeping giants of pharmaceutical industry (Hostettmann and Hamburger 1991), may provide natural source of antimicrobial drugs that will provide novel compounds that may be employed in the management some infections caused by microorganism.

Herbal preparations of *B. ferruginea,* have been found to be useful in treating some conditions but the extent of use is often met with a major setback from the fact that little scientific evidence is available for many of such preparations. In addition, the phytochemical properties of

this plant have been found to vary with the habitat or geographical locations (Hostettmann and Hamburger 1991). Therefore, there may be variations in the antibacterial effect of this plant from different part of Nigeria. This study was aimed at finding and establishing a scientific basis for the use of the plant in search of effective and affordable cure for certain bacterial infections. The specific objective is to determine the phytochemical contents and antibacterial activity of the ethanolic and aqueous extracts of the stem and root bark of *B. ferruginea.*

MATERIALS AND METHODS

Collection of plant materials

Fresh stem and root bark of *B. ferruginea* were collected from the field between July and October, 2015 and were identified by a botanist from the Federal College of Forestry, Jos, Plateau State.

Preparation of extracts

The plant materials (fresh stem and root bark of *B. ferruginea*) were dried at room temperature for 14 days followed by further drying in the oven at 50°C for 5 days to enable it dry completely. The dried stem and root bark were then pounded using a pestle and mortar and the powder stored in an air-tight container. The ground stem and root-bark of the plant was extracted using water and ethanol. About 20 g each of powdered root and stem-bark was poured in 120 ml of ethanol and water in a conical flask and the content sealed properly to avoid evaporation. In each case, the solution was filtered using Whatmann No. 1 filter paper after allowing the mixture to stand overnight. The filtrates were evaporated to dryness in an evaporating dish in a water bath at 70°C. The extracts were then scrapped and stored in a sterile container.

Phytochemical screening

The extracts of the stem and root bark of *B. feruginea* were analysed for the presence of the following phytochemicals; alkaloids, saponins, tannins, anthraquinones, and flavonoids according to standard methods (Sofowora 1982; Ngbede et al., 2008).

Source of the test organisms

The bacteria used for this study consist of six (6) clinical isolates; *Pseudomonas aeroginosa, P. mirabilis*, *Escherichia coli*, *S. aureus, Bacillus cereus and Stapylococcus epidermidis.* These isolates were obtained from the laboratory stock of central diagnostic, National Veterinary Research Institute, Vom, Jos. The gram positive and gram negative control strains; *E. coli* (NCTC 10418) and *S. aureus* (NCTC 6571) were also obtained from the Institute's Molecular Laboratory. The pure cultures of these isolates were prepared in nutrient broth in test tubes and kept in the refrigerator at 4°C, until needed for use.

Constitution of the stock concentration of the extracts

About 5 g each of the aqueous stem and root-bark extracts were dissolved in 50 ml of distilled water to constitute a stock solution of 100 mg/ml. In similar manner 5 g of the ethanolic stem and root-bark extracts were dissolved in 50 ml of Dimethyl Sulphuroxide (DMSO) to constitute a stock concentration of 100 mg/ml. DMSO was used for ethanolic extract because it does not dissolve completely in water as in the case of aqueous extracts.

Determination of minimum inhibitory concentration (MIC)

Tube dilution method described by Scott, 1989) was used. Exactly 8 tubes labelled 1 to 8 (2 sets) with each tube containing 4 ml of nutrient broth. 4 ml of the crude extracts in the stock concentration (100 mg/ml) was introduced to tube 1 and diluted using a double dilution to yield a concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml. Tube 8 contain a standard antibiotic (ciprofloxacin) serving as a positive control. To the first set of tubes, 0.02 ml (200µl) of 24 h broth cultures of the test organisms was added after dilution to yield 0.5 McFarland standard (equivalent to 1.5×10^8 Cfu/ml) using a Nephelometric turbidity metre. The tubes were then incubated at 37°C for 24 h after which they were examined for microbial growth. The second set of tube was not inoculated and serves as a control to observe for change in turbidity by comparing with the first set.The MICs of the ethanolic and aqueous extract of the stem and rootbark for each test organism is the smallest concentration of such extract that is capable of inhibiting the growth of specific inoculum of the test organism evidenced by absence of turbidity in the particular tube.

Determination of the minimum bactericidal concentration

The Minimum Bactericidal Concentrations (MBCs) was determined by identifying the tubes that showed no growth (evident by absence of turbidity) during MIC determination. One loop full from each of these tubes selected was inoculated over the surface of nutrient agar in Petri-dish and incubated for 18 to 24 h at 37°C. Lowest concentration of the extract at which no growth was observed is taken as the MBC.

RESULTS

The phytochemical evaluation of the aqueous extracts of the root and stem-bark, reveals the presence of some phytochemicals. The stem-bark extract contains alkaloids, saponins, tannins, and flavonoids. While root-bark extract demonstrated the presence of saponins, tannins, alkaloids, flavonoids and anthraquinones (Table 1).

The result of the phytochemical analysis of ethanolic extracts of the root and stem-bark of *B. ferruginea* is shown in Table 2. The analysis of the stem extract revealed the presence of saponins, tannins, flavonoids, alkaloids, while anthraquinones was not present. The analysis of the root-bark extract demonstrated an increased presence of saponins, tanins and alkaloids, while, flavonoids and anthraquinones were present in moderate amounts.

Table 3 shows the MIC and MBC of aqueous extract of the stem and root-bark of *B. ferruginea*. For stem-bark extract, amongst the gram positive bacteria tested, *S. aureus* (NCTC 6571) and *S. aureus* had no MIC and

Table 1. Phytochemical constituents from the aqueous extract of the stem- and root-bark of *B. ferruginea.*

Phytochemicals	Aqueous extract	
	Stem-bark	Root-bark
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Anthraquinones	−	+

Key: + = present ; − = absent.

Table 2. Phytochemical component of ethanolic extracts of the stem and root bark of *B. ferruginea.*

Phytochemicals	Ethanolic extracts	
	Stem-bark	Root-bark
Saponins	+ +	+ +
Tannins	+ +	+ +
Alkaloids	+	+ +
Flavonoids	+ +	+
Anthraquinones	−	+

Key:+ = present ;+ + = present in increased quantity; − = absent.

MBC, while *S. epidermidis* and *B. cereus* both recorded MIC and MBC of 50 and 100 mg/ml, respectively. For gram negative bacteria, only *P. mirabilis* had an MIC of 100 mg/ml. There was no MIC and MBC for all the other gram negative bacteria tested (*E. coli* (NCTC 10418), *E. coli*, and *P. aeruginosa*). In the case of root-bark extract, *S. aureus* (NCTC 6571) and *S. aureus* had MIC of 100 mg/ml but no MBC was recorded. *S. epidermidis* had MIC and MBC of 25 and 50 mg/ml, respectively, while *Bacilluscereus* had MIC and MBC of 50 and 100 mg/ml. Amongst the gram negative bacteria tested, *E. coli* (NCTC 10418) and *P. mirabilis* had MIC and MBC of 50 and 100 mg/ml, respectively, while *E. coli* and *P. aeruginosa* had MIC of 100 mg/ml but no MBC was observed.

Table 4 shows the MIC and MBC of ethanolic extracts of the root- and stem-bark of *B. ferruginea* against selected bacteria. For the stem-bark extract, amongst the gram positive bacteria tested, *S. aureus* (6571), *S. aureus* and *S. epidermidis* recorded MIC and MBC of 50 and 100 mg/ml, respectively, while *B. cereus* recorded MIC and MBC of 25 and 50 mg/ml, respectively. For the gram negative bacteria tested, *E. coli* (NCTC 6571), *E. coli*, and *P. aeruginosa* recorded MIC of 100 mg/ml with no MBC, while *P. mirabilis* had MIC and MBC of 50 and 100 mg/ml, respectively.

For the root-bark extract, amongst the gram positive bacteria tested, *S. aureus* (NCTC 6571) had MIC of 100 mg/ml with no recorded MBC. *S. aureus*, *S. epidermidis* and *B. cereus* had MIC and MBC of 25 and 50 mg/ml, respectively. For the gram negative bacteria, *E. coli* (10418), *E. coli* and *P. mirabilis* had MIC and MBC of 50 and 100 mg/ml, respectively, while *P. aeruginosa* had MIC of 100 mg/ml with no MBC.

Table 5 shows the summary of the primary activity of the root- and stem-bark extract of both the aqueous and ethanolic extract of *B. ferruginea* against the selected bacteria. The result indicates that, aqueous root-bark extract had more activity than aqueous stem-bark extract. On the other hand, the root-bark ethanolic extract had more activity than the stem-bark ethanolic extract. On a general note, the ethanolic root- and stem-bark extract had more activity than the aqueous root- and stem-bark extract with gram positive bacteria been more susceptible than the gram negative bacteria

DISCUSSIONS

Phytochemical analysis of the aqueous and ethanolic extracts of the root and stem bark of the plant revealed the presence of saponins, tannins, alkaloids, flavonoids and anthraquinones in the root-bark, while the stem-bark also possesses all the phytochemicals analysed, except for anthraquinones(Table 1 and 2).The presence of these phytochemicals reveals that, the extract of the plant is possibly active. The presence of these phytochemicals in the aqueous root-bark extract is in agreement with the findings of Adebayo and Ishola (2009) whereby all the phytochemical elements identified in this study were also found in their study. The absence of anthraquinones in the stem-bark extract, do not agree with the work done by Owoseni et al (2012) in which anthraquinones was present in the stem-bark extract.

The result from this study also revealed that there was no variation in terms of the active components in aqueous stem- and root-bark extract and that of ethanolic stem and root-bark however, there were variation in terms of the amount of the phytochemicals present in each of the extracts(Tables 1 and 2).There was an increase in the amount of saponins, tannins and flavonoids in ethanolic stem-bark extract, while ethanolic root-bark extract revealed an increase in the amount of saponins, tannins and alkaloids. In similar vein there was an increased extraction in the amount of the phytochemicals by ethanol when compared to aqueous agent. This finding is in line with Arya et al (2012) who observed higher yield using ethanol than other solvents such as petroleum ether, chloroform and water because ethanol is a more effective solvent in the extraction of bioactive molecules (Arya et al., 2012).

It has been discovered that the functional property of a plant relies upon the different secondary metabolites it

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous extracts of the stem and root-bark of *B. ferruginea* against selected bacteria.

Bacteria		Aqueous Extracts (mg/ml)			
		Stem-bark		Root-bark	
		MIC	MBC	MIC	MBC
Gram-positive	*Staphylococcus aureus* (NCTC 6571)	–	–	100	–
	Staphylococcus aureus	–	–	100	–
	Staphylococcus epidermidis	50	100	25	50
	Bacillus cereus	50	100	50	100
Gram-negative	*Escherichia coli* (NCTC 10418)	–	–	50	100
	Escherichia coli	–	–	100	–
	Proteus mirabilis	100	–	50	100
	Pseudomonas aeruginosa	–	–	100	–

Key: = no MIC/MBC at highest concentration of the extract tested.

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic extracts of the stem- and root-bark of *B. ferruginea* against selected bacteria.

Bacteria		Ethanolic Extracts (mg/ml)			
		Stem-bark		Root-bark	
		MIC	MBC	MIC	MBC
Gram-positive	*Staphylococcus aureus*(NCTC 6571)	50	100	100	–
	Staphylococcus aureus	50	100	25	50
	Staphylococcus epidermidis	50	100	25	50
	Bacillus cereus	25	50	25	50
Gram-negative	*Escherichia coli* (NCTC 10418)	100	–	50	100
	Escherichia coli	100	–	50	100
	Proteus mirabilis	50	100	50	100
	Pseudomonas aeruginosa	100	–	100	–

Key = no MIC/MBC at highest concentration of the extract tested.

possesses such as: phenolics, terpenoids, or alkaloids (Murugan and Parimelazhagan 2014). Among the phytochemicals isolated from the plant, flavonoids have been proven to be of more significance because of its ability to assist the body in fighting diseases. Flavonoids act as potent antioxidants but depending on the structure of the molecule and the hydroxyl group in the chemical structure (Iqbal et al., 2015). Phenols, flavonoids and flavonols are polyphenolic compounds of plants which bring about substantial antioxidant activity and several biological activities including: anti-helmintic, analgesic, anti-inflammatory, anti-microbial and anti-allergic properties (Oyedemi et al., 2012; Alabri et al., 2014). The activity of these phytochemicals mainly results from their ability to form a large complex with extracellular protein and the cell walls of bacteria. Flavanoids can also alter the membranes of a bacterial cell (Tsuchiya et al., 1996). The presence of saponins was indicated in *B. ferruginea* extracts which warrant the use of the plant in the management of inflammation. Saponins is known to prevent inflammation and is the main constituents of traditional medicinal plant and thus responsible for majority of the biological effects observed. This support reasons for using the plant in traditional medicine. Just et al., 1998 and Igbinosa et al., 2013 demonstrated the inhibitory effect of saponins on inflamed cells.

The presence of alkaloids is also observed in the extracts. A heterocyclic compound of nitrogen associated with a significant range of antimicrobial activity. Plants containing alkaloids are commonly used in traditional medicine due to their inhibitory effects against protozoa, bacteria and fungi (Kim et al., 2002). The common biological activity of alkaloids is toxicity against the cells of foreign organisms. It has the ability to accumulate in

Table 5. Primary effects of the aqueous and ethanolic extracts of the stem- and root-bark of *B. ferruginea* against selected bacteria.

Bacteria		Aqueous extracts		Ethanolic extracts	
		Stem-bark	Root-bark	Stem-bark	Root-bark
Gram positive	*S. aureus* (NCTC 6571)	–	Static	Cidal	Static
	S. aureus	–	Static	Cidal	Cidal
	S. epidermidis	Cidal	Cidal	Cidal	Cidal
	B. cereus	Cidal	Cidal	Cidal	Cidal
Gram negative	*E. coli* (NCTC 10418)	–	Cidal	Static	Cidal
	E. coli	–	Static	Static	Cidal
	P. mirabilis	Static	Cidal	Cidal	Cidal
	P. aeruginosa	–	Static	static	Static

Key: No effect.

cells due to the variation in membrane potentials and it is a very good DNA introducer (Iwasa et al., 2001) and effective against many microorganisms targeting the RNA polymerase, nucleic acid, RNA gyrase and topoisomerase (Yi et al., 2007).

Plants that have extracts containing tannins are known to be used commonly in traditional medicine for treating haemorrhage, diarrhoea, and detoxification (Okwu and Emenike, 2006). The tannins content as observed in the study especially in the ethanolic extracts justify the use of the plant in traditional medicine for the treatment of diarrhoea. Anthraquinones have been found to have wide range of antibacterial (also anti-mycobacterial) property resulting in loss of function and inactivation of bacterial proteins, cell wall, polypeptides, adhesins and membrane bound organelles with resultant death of the bacterial cell (Kurek et al., 2011).

The antibacterial activity of the plant extracts is tested on gram positive and gram negative bacteria. This is done by determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous and ethanolic extracts of the stem and root-bark of *B. ferruginea* (Tables 3 and 4). The presence of the phytochemicals is assumed to be responsible for the anti-inflammatory, antioxidant, antibacterial and cytotoxicity activity of the plants. From the results as shown in Table 3 and 4, the extracts have been found to be more active against gram-positive bacteria than gram-negative bacteria.

Among gram-positive bacteria tested, *B. cereus* was most susceptible to the plant extract having MIC and MBC of 25 and 50mg/ml, respectively for the stem-bark and root-bark ethanolic extract. For gram-negative bacteria, the plant extracts were most active against *P. mirabilis* with MIC and MBC of 50 and 100 mg/ml, respectively. The aqueous extract was most active against *S. epidermidis* with MIC and MBC of 50 and 100 mg/ml, respectively for stem-bark and 25 and 50 mg/ml, respectively for root-bark extract. The reason for the difference in sensitivity between gram negative and gram positive could be ascribed to the morphological differences between these microorganisms. As reported by Nostro et al., 2000; Nikaido and Vaara, (1985), gram-negative bacteria are characterized by an outer covering of phospholipidic membrane made up of lipopolysaccharide components, making the cell wall slightly impenetrable, whereas the gram-positive bacteria possessing only an outer peptidoglycan layer (not an effective permeability barriers) are more susceptible.

The level of resistance demonstrated by *P. aeruginosa, E. coli* and *S. aureus* is not strange since the resistances of these organisms to many antibacterial agents have been reported (Kunin, 1993). Also in another study by Dada-Adegbola et al, 2010, on “the activity of crude extract of *B. ferruginea* ” against *P. aeruginosa*, *E. coli* and *P. mirabilis* showed similar results as obtained from this study.

The ethanolic extracts of both the stem- and root-bark demonstrated an increase in activity than that of aqueous stem- and root-bark extracts (Tables 3 and 4). The result from this study is consistent with the study by Owoseni et al. (2010), where the ethanolic extracts of leaves and stem-bark of *B. ferruginea* had an increase antibacterial activity than other extracting solvents used. Also, this result is in tandem with the findings of Olukemi et al. 1997, where ethanolic extract of stem-bark of *Paekia filicoidea* demonstrated ten-fold increase in activity to that of aqueous extracts. This is due to the concentration of active substances responsible for inhibiting the growth of microorganisms which is high in ethanolic extract than aqueous extract of both the root- and stem-bark of the plant. It could also be due to the fact that ethanol as extracting solvent is more effective in extracting the active compounds than aqueous solvent (Arya et al., 2012). The extracts from the root-bark were found to be more effective against the tested pathogens than the stem-bark for both the aqueous and ethanolic extracts (Table 3 and 4). Similar results were obtained by

Adebayo and Ishola (2009) and Owoseni et al. (2012), where the root-bark ethanolic extract had higher activity than stem-bark extract against tested pathogens. The presence of anthraquinones in the roots of both the ethanolic and aqueous extract as against its absence in the stem extract could have been an attribute to its higher antibacterial activity as anthraquinones has been reported to have a wide range of antimicrobial activity against pathogenic organisms.

The primary effects of the aqueous and ethanolic extracts of the root- and stem-bark of the plant against selected bacteria as shown in Table 5 revealed that the antimicrobial activity of the plant is concentration dependent. Within the range of concentrations used for the study, the plant extracts were found to have the primary effect of being bacteriostatic or bactericidal, depending on the concentration against the test organisms. Only the stem-bark aqueous extract showed no primary effect on the control strains, *S. aureus*, *E. coli*, and *P. aeruginosa*. According to Oboh and Abulu (1997) antimicrobial activity is a function of the active ingredient having effect on the target organism.

Conclusion

Findings from this study confirmed the presence of phytochemicals such as saponins, tannins, alkaloids, flavonoids and anthraquinones which has revealed that the plant is of pharmacological importance going by the ability of these phytochemicals to elicit antibacterial activity.This study has discovered the great potential in *B. ferruginea* as one of the medicinal plant in serving as an alternative in treating human infection with less cost and toxicity.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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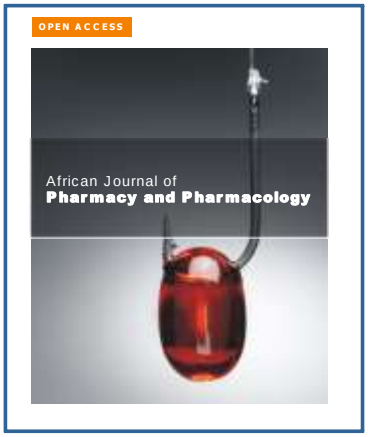

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