CHAPTER 1

INTRODUCTION

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1.1 General

Malaysia, widely recognized as one of the centres of biological diversity, is richly endowed with plant, animal and microbial genetic resources. If exploited and managed wisely, these genetic resources could provide renewable useful products for not only the present but also the future generation[1].

Traditional medicine which uses medicinal plants as an important ingredient in preparations has been linked to human culture since early time. In 1993, the World Health Organization (WHO) estimated that 80% of the world's populations are dependent on traditional medicine for health care. In other words, despite the advances of modern medicine, the practice of traditional medicine persists. In Malaysia, the practice of traditional medicine is still common among various ethnic groups, i.e. Malay, Chinese and Indian. This traditional medicine knowledge has been passed on through many generations[2].

Recently, there is a trend that people are turning towards the use of natural products as remedies. One of the main reasons stems from the fact that modern pharmaceuticals frequently employ chemicals and synthetic drugs that may have adverse impact on one's health. Furthermore, these drugs have so far failed to cure certain chronic disease, e.g. cancer, hypertension, AIDS, etc. Medicinal plants which are believed to have lesser side effects are therefore become an alternative for disease prevention and treatment. The medicinal value of plants is found in roots, leaves or other plant parts. Their effectiveness

and popularity depend on new research findings and experience of traditional usage, ethnic beliefs and the availability of plant materials, etc.[2]

In recent years a rich harvest of novel natural products had been made, some of which possess cytotoxic or insecticidal activities. Some of the diverse classes of natural products, e.g acetogenins, alkaloids, biflavonoids, coumarins, terpenoids and xanthonoids, isolated from Malaysian plant families such as Annonaceae, Apocynaceae, Guttiferae, Meliaceae, Rubiaceae, Rutaceae, Verbenaceae, etc. have been encountered.[3]

1.2 Lauraceae: General Appearance and Morphology

In Malaysia, Lauraceae family is known as *Medang* or *Tejur*. The Lauraceae comprises a group of flowering plants included in the order Laurales. The family contains about 62 genera and over 2000 (perhaps as many as 4000) species world-wide, mostly from warm or tropical regions, especially Southeast Asia and tropical America[4]. The tree of Lauraceae are usually evergreen, shrubs, and without buttresses.

Ecology of Lauraceae is dependent on type of the lands, whether lowlands or highlands. In the lowlands, Lauraceae are typically small trees except for a view species which may reach 30 m tall. In the highlands, Lauraceae like fagaceae, becoming more abundant reaches the top layer of the forest, which lies at 1200-1600 m. Such Oak-Laurel Forest is a feature of the mountains of tropical Asia from Himalayas to New Guinea[5].

The bark is usually smooth, rarely fissured, scaly or dippled, often covered with large lenticels, grey-brown to reddish-brown. The inner bark is usually very thick, granular, mottled or laminated, often with strong aromatic smell, yellow, orange-brown, pinkish or

reddish. Sapwood is pale yellow to pale brown with satiny luster when freshly cut. Terminal bud naked or covered with bud scales which sometimes appear like small leaves.

The leaves are simple, without stipules, and usually alternate, spiral, opposite, subopposite, or whorled (*Actinodaphne*), entire and leathery. The secondary veins pinnate have only one pair as in *Cinnamommum*. The colour of the new leaves is vary from nearly white to pink, purple or brown.

The flowers of Lauraceae are actinomorphic and usually small, regular, greenish, white or yellow, fragrant or with rancid smell, bisexual or unisexual, perianth free or united with six petals in two rows. The flowers are pollinated chiefly by flies and beetles which are attracted by the smell emitted from the flowers.

The fruit of this family are small to large (one seed berry), sometimes enveloped by the accrescent perianth tube or persiting and clasping the base of fruit. In some genera perianth lobes are dropping but the tube developing into a shallow or deep cup at base of fruit. The fruits stalk enlarging and becoming highly coloured in some species of *Dehaasia* and *Alseodaphne*. The seed of Lauraceae are without albumen, with thin testa. Cotyledons large, flat, convex, pressed against each other.

Wood of Lauraceae is soft to moderately hard, light to moderately heavy to varying from 350-880 kg/m^3 air dry. Grain straight or slightly interlocked, texture moderately fine and even. Sapwood usually is a distinctly lighter shade than the heartwood. The heartwood is yellow-white (Beilschmiedia), yellow brown or red-brown in most species of *Cinnamomum, Cryptocarya* and *Endiandra*, olive green in species of *Litsea, Actinodaphne, Alseodaphne, Notaphoebe, Phoebe,* and dark olive green-brown in *Dehaasia*.

The family of Lauraceae provided many useful economic products. Most of the economically important species other than sources of excellent wood are spices or flavoring agent. For example avocado (*Persea*) is one of important tropical fruit.

1.3 Classification of Tribe

There are many primitive and archaic features, which characterize the Lauraceae family. The determination is dependent on a combination of characters. Scheme 1 and list below illustrated the classification proposed by Hsuen Keng. There are over 30 genera, mainly tropical and sub-tropical Asia and America, about 16 are found in Malaysia. This classification is according to Malayan seed plants [6].

Kingdom : Plantae

Divison : Magnoliophyta

Class : Magnoliopsida

Order : Laurales

Family : Lauraceae

Genera :

1. *Actinodaphne*
2. *Adenodaphne*
3. *Aiouea*
4. *Alseodaphne*
5. *Anaueria*
32. *Lindera*
33. *Litsea*
34. *Machilus*
35. *Mezilaurus*
36. *Mochinnodaphne*

6. *Aniba*

7. *Apollonias*

8. *Aspidostemon*

9. *Beilschmiedia*

10. *Brassiodendhron*

11. *Caryodaphnopsis*

12. *Cassytha*

13. *Chlorocardium*

14. *Cinnadenia*

15. *Cinnamomum*

16. *Clinostemon*

17. *Cryptocarya*

18. *Dahlgrenodendrone*

19. *Dehaasia*

20. *Dicypellium*

21 .*Dodecadenia*

22. *Endiandra*

23. *Endlicheria*

24. *Eusideroxylon*

25. *Gamanthera*

26. *Hexapora*

27. *Hypodaphnis*

28. *Iteadaphne*

29. *Kubitzkia*

37. *Mutisiopersea*

38. *Nectandra*

39. *Neocinnamomum*

40. *Neolitsea*

41. *Notaphoebe*

42. *Ocotea*

43. *Paraia*

44. *Parasassafras*

45. *Persea*

46. *Phoebe*

47. *Phyllostemonodaphne*

48. *Pleurothyrium*

49. *Potameia*

50. *Potoxylon*

51. *Povedadaphne*

52. *Ravensara*

53. *Rhodostemonodaphne*

54. *Sassafras*

55. *Sextonia*

56. *Sinosassafras*

57. *Syndiclis*

58. *Triadodaphne*

59. *Umbellularia*

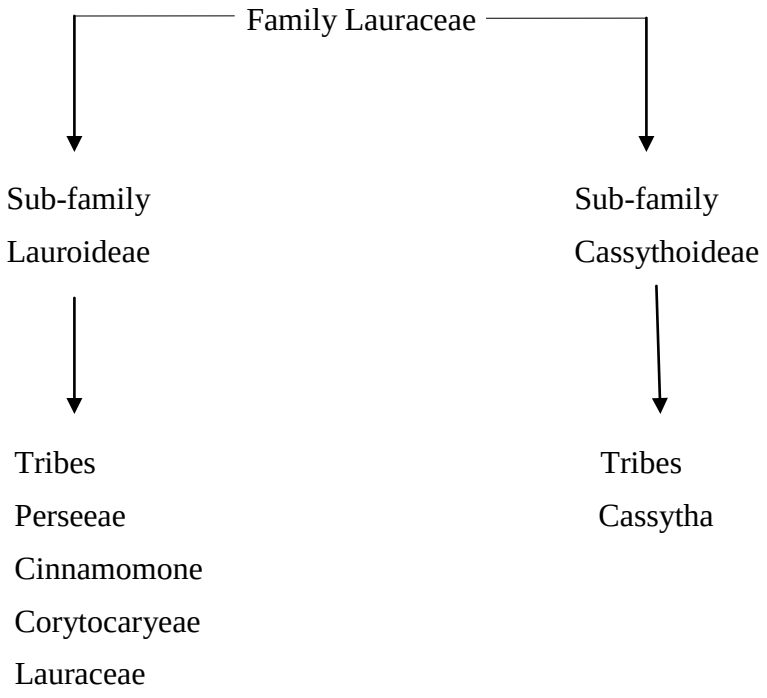
60. *Urbanodendron*

30. *Laurus*

31. *Licaria*

61. *Williamodendron*

62. *Yushunia*



Scheme 1.1: Classification of Family Lauraceae

1.4 The Genus Cryptocarya

Cryptocarya is a genus of evergreen trees belonged to the Lauraceae family. The genus includes more than 350 species[7] and 19 species are found in Malaysia[8]. This genus is distributed through the Neotropic, Afrotropic, Indomalaya and Australasia ecozones.

In Malaysia, the species of *Cryptocarya are C. bracteolata, C. caesia, C. costata, C. crassinervia, C. densiflora, C. enervis, C. ferrea, C. griffithiana, C. impressa, C. infectoria, C. kurzii, C. laevigata, C. nitens, C. rugulosa, C. scortehinii, C. teysmanniana, C. tomantosa, C. wrayi and C. zollingeriana*.

The leaves are alternate, pinnatinerved, rarely trinerved. Inflorescenses in axillary or sub terminal panicles. The flowers are bisexual (perianth tube turbinate or ovoid), lobes 6, stamens usually 9. The fruits entirely included in the enlarge perianth tube leaving only a minute opening at apex.

1.5 *Cryptocarya densiflora*

Cryptocarya densiflora is a medium sized tree up to 20 m tall, 135 cm girth. Bark (Figure 1.3) is grey-brown, smooth with scattered large lenticels. The inner bark are reddish brown and granular while their sapwood is pale yellow. Leaves stalk (Figure 1.1) 1-1.2 cm long, blade leathery, elliptic to oblong, 9.5-21 x 4.5-14 cm; apex acuminate; base cuneate to rounded; secondary nerves up to 3 pairs, trinerved at base, tertiary nerves scalariform; reticulations circular, distinctly visible below, faint or inconspicuous above. The flowers (Figure 1.2) are in axillary panicles, pale yellow, sessile, perianth funnel shaped, densely hairy; perianth lobes oblong, as long as perianth tube. The fruits are green ripening blue, globose, 2 cm across when dry; surface shallow ridged and warty.

The species can be found in hill and mountain forests up to 1500 m in Malaysia (Kelantan, Perak, Pahang, Selangor) and Indonesia[8].

Figure 1.1: Leaves of *Cryptocarya densiflora*.

Figure 1.2: Flowers of *Cryptocarya densiflora*

Figure 1.3: Bark of *Cryptocarya densiflora*

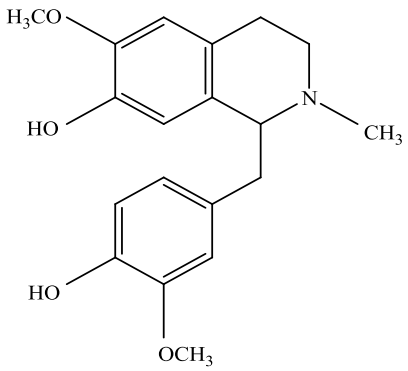
1.6 Alkaloids Isolated from *Cryptocarya* species

Chemical investigation on several species *Cryptocarya* have been done before, which is known to produce interesting alkaloids and other chemical constituents. Several types of alkaloids were isolated and reported including some new compounds. Some of the alkaloids isolated from the *Cryptocarya* species are shown in Table 1.1

Table 1.1: Alkaloids Isolated from the genus of *Cryptocarya*

Plant	Alkaloids Isolated
Cryptocarya amygdalina[9]	Orientaline **1**
	Laudanidine **2**
Cryptocarya chinensis[10]	(+)-eschscholtzidine-*N*-oxide **3**
(wood)	(-)-12-hydroxycrychine **4**
	(-)-12-hydroxy-*O*-methylcaryachine **5**
	(-)-*N*-demethylcrychine **6**
	Isocryprochine **7**
	Prooxocryptochine **8**
	Isoamuronine **9**
	(+)-8,9-dihydrostepharine **10**
Crptocarya crassinervia[11]	2-hydroxyatherosperminine **11**
	N-demethyl-2-methoxyatherosperminine **12**
	Lirioferine **13**
	Reticuline **14**
	Lirinine **15**
	2-methoxyatherosperminine **16**

	2-methoxyatherosperminine *N*-oxide **17**
Crptocarya phyllostemon[12]	Antofine **18**
	Dehydroantofine **19**
	Cryptowoline **20**
	Cryptowolidine **21**
	Cryptowolinol **22**
Cryptocarya konishii[13]	Oblongine **23**
	Methyloblongine **24**
	Xanthoplanine **25**



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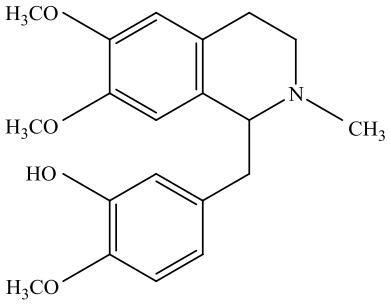

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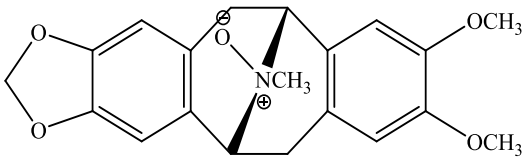

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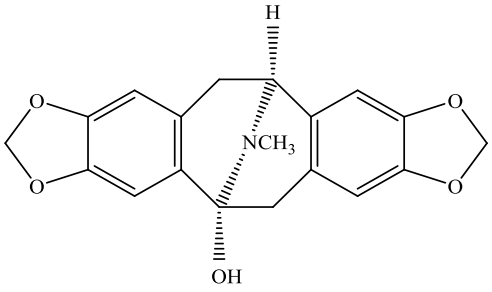

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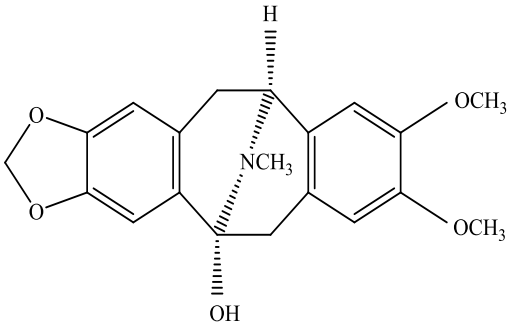
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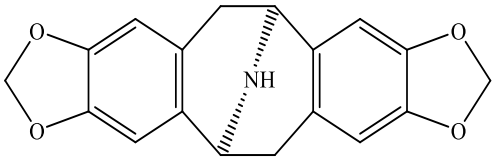
O
O
NCH3
OCH3
OCH3
OH

5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NH
O
O

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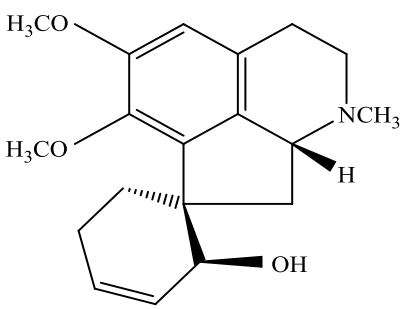
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NCH3
H
OH

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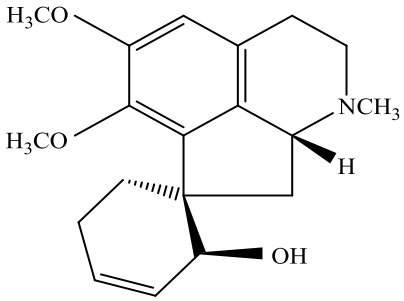
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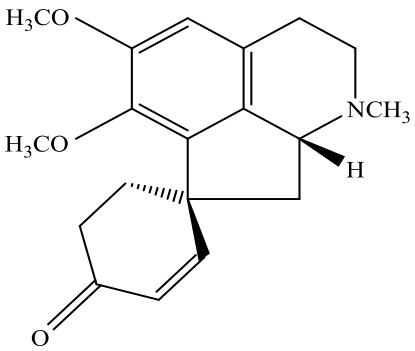
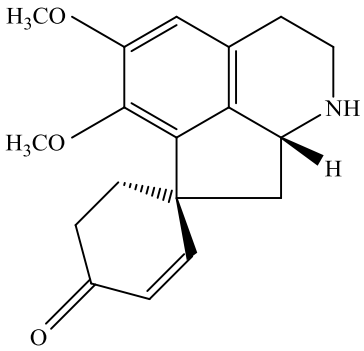
H3CO
NCH3
H
OH

8


H3CO
H3CO
NCH3
H
O

9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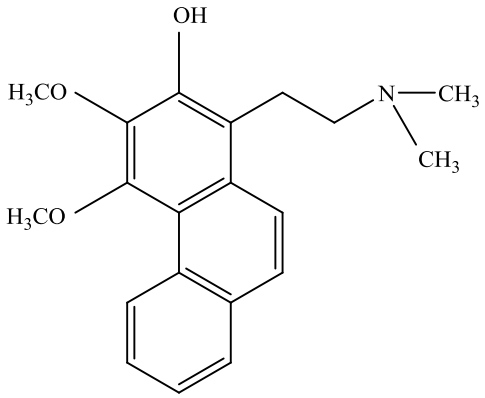
H3CO
NH
H
O

10



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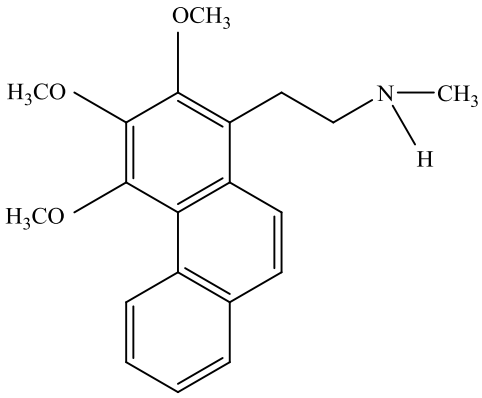

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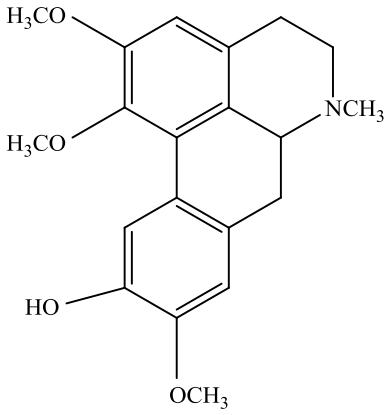

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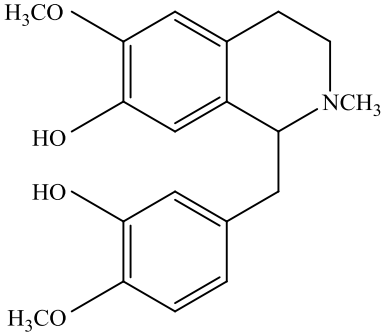

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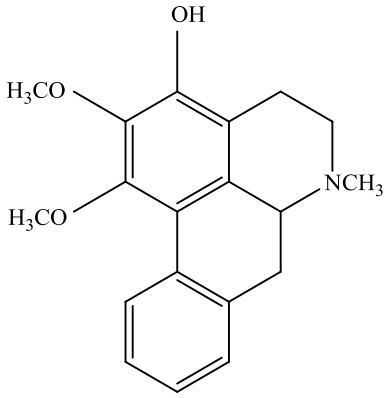

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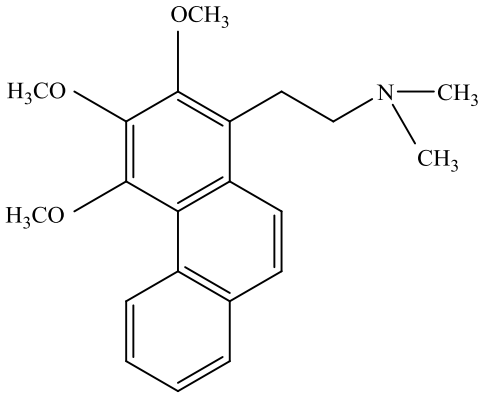

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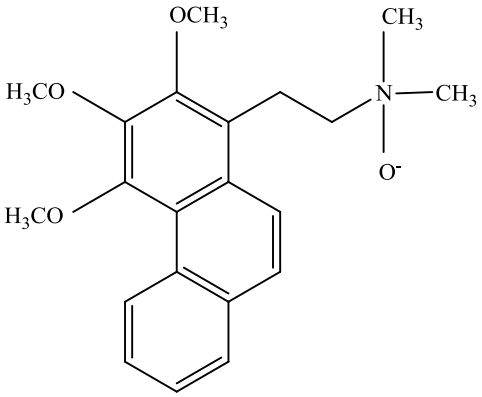

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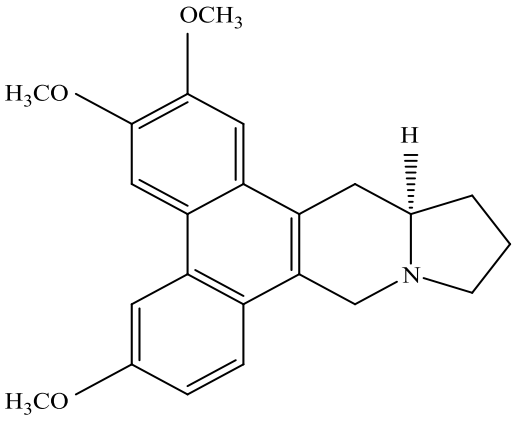

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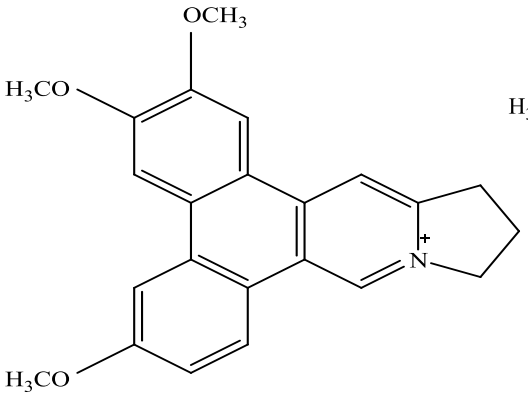

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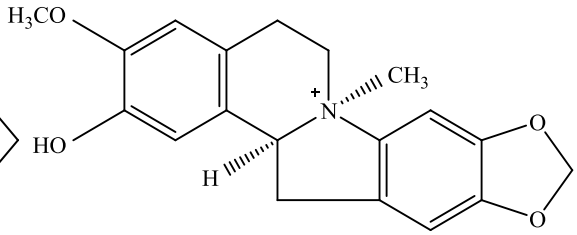

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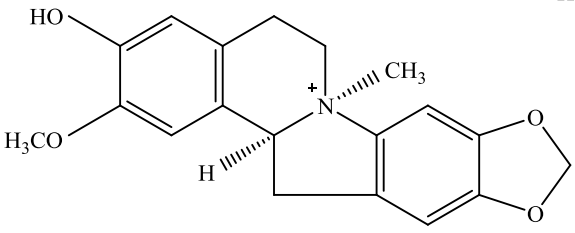

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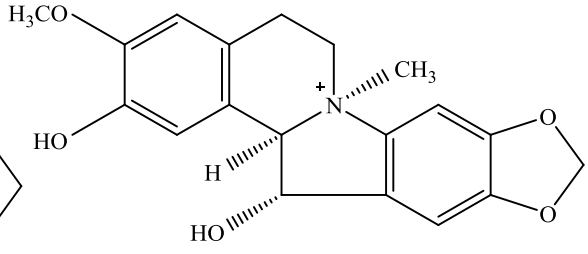

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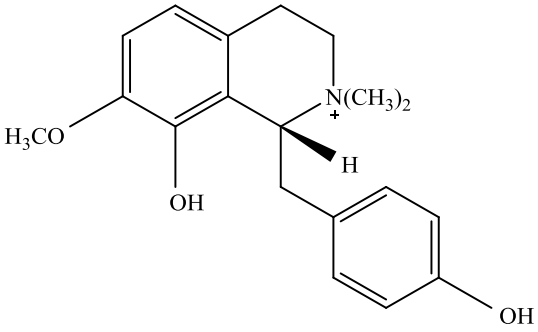
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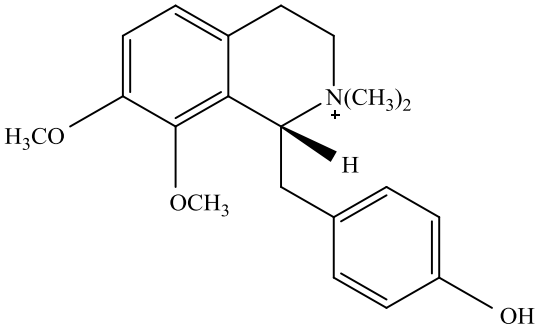
N(CH3)2
H
OH
OH

23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OCH3
OH

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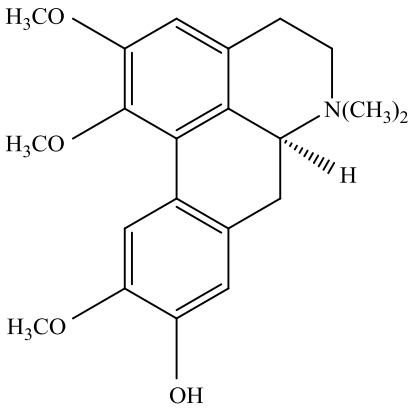
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N(CH3)2
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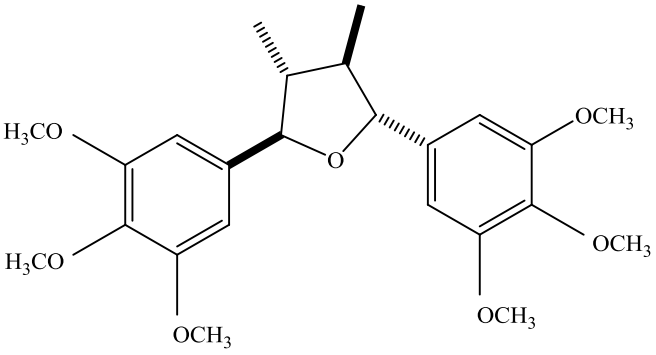
H3CO
H3CO
O
OCH3
OCH3
OCH3
OCH3

26

Table 1.2: Different species of the genus *Cryptocarya* and their distribution[8].

Species	Distribution
C. bracteolata Gamb	Widely distributed from lowland including swamps, to medium forest in Kedah, Kelantan, Terengganu, Pahang, Selangor and Java.
C. caesia BI	Rare; in lowland forest; Malacca, Singapore, Andaman Island, Java.
C. costata BI	Lowland forests by streams; locally abundant; Kedah, Pahang, Johore, Java.
C. crassinervia Miq	The species occurrence from lowlands to hill forest up to 900 m and distributed in Kedah, Perak, Terengganu, Pahang, Johore and Java.
C. enervis Hk. f.	Very rare; Selangor (peat swamp); Malacca, Borneo.
C. ferrea BI	Widely distributed from lowlands including swamps, to mountain forests Kedah, Kelantan, Terengganu, Pahang, Selangor, Java.
C. griffithiana Wight	Uncommon; Perak, Pahang, Selangor, Negeri Sembilan, Malacca, Johore, Singapore in lowland forests, Burma, Thailand, Indonesia, Borneo, the Philippines.
C. impressa Miq	Rare; known only from lowland forests, including swamps, from Negeri Sembilan, Malacca and Johore, Indo-Chinna, Sumatra, Java.

C. infectoria Miq	Scattered occurance in lowlands, hills and upper hills up to 600 m; Kedah, Perak, Kelantan, Pahang, Selangor, Negeri Sembilan, Johore, Java.
C. kurzii Hk. f.	Scattered in lowland forests; Kedah, Perak, Terengganu, Pahang, Malacca, Johore, Java, Borneo.
C. laevigata BI	Rare, known only from a single collection, SFN 28958 from swamp forest in Johore, Borneo, the Philippines, Madagascar.
C. nitens (BI.) K. et V. (Lat., shiny)	Uncommon tree of riversides in lowland forests including limestone in Kedah, Perak, Terengganu, Pahang and Selangor, Java.
C. rugulosa Hk. f.	Lowlands and hill forest in Kedah, Penang, Perak, Terengganu, Malacca, Johore, Singapore, Indonesia and Sarawak.
C. teysmanniana Miq	Rare; Selangor, Johore; in hill forests, Sumatra.
C. tomentosa BI	Rare, lowland to upper hill forests up to 1000 m; by streams, Kedah, Perak, Indonesia, Borneo.
C. wrayi Gamb.	Common; mountain forests between 900-1200 m; Perak, Kelantan, Pahang, Johore, Endemic.

1.7 Medicinal Value

The medicinal value of plants is inestimable. The tropical rain forest plants are biologically and chemically diverse resources as they synthesize various chemicals to defend themselves again pests, diseases and predators. Thus, they are also an excellent reservoir of medicines and chemical leads. In fact, about 25% of the drugs used in modern medicine own their origins to plants from tropical rain forest[14].

The exploration of the plant kingdom for chemical compounds of medicinal value has been going on for thousands of years, and herbalism and folk medicine, ancient and modern, have been the source of much useful therapy. During the nineteenth century, organic chemists took up the study of many plant principles, the physiological effect of which had been recognized. A new impetus was given to the search for medicinal plant principles by the discovery of the clinical usefulness of alkaloids of *Rauwolfia* species and during the past dozen years this has provided fresh stimulus for an enlarged and concentrated attack upon the still unexplored botanical resources of the world[14].

Cryptocarya massoy is a native of New Guinea. In Peninsular Malaysia, the bark of this species is used by the women after childbirth, and also is added to tonics and cigarettes. In Indonesia, it is used against diarrhea and spasmodic bowel trouble, usually in combination with a *Cinnamon*. Furthermore, it is an ingredient of various native medicines and has a characteristic odor. In New Guinea, the aromatic bark is employed to treat fever, and often it is used by the natives to treat bad cases of tuberculosis of the lungs. A small plug of the bark is placed so as to close a fresh wound. It is chewed and the saliva rubbed over the limbs to ease muscular pains. In small quantities, mostly in combination with other vegetable drugs, it is ingested for violent headache, pain in the joints, puerperal infection,

distention of the stomach, vomiting and chronic constipation; it is also mixed with cloves and sandalwood and used by the natives as rubefacient. The volatile oil of the bark contains pinene, limonene, dipentene, eugenol and safrol. In Peninsular Malaysia, the bark of the species *C.griffithiana* Wight is mentioned as poisonous as is that of *C.tomantosa* B.I. The natives heat the leaves of *C.multipaniculata* Teschn and then apply them to sore eyes[15].

Past studies of the species of *Cryptocarya chinensis* have found that it contains many pavine and proaphorpine alkaloids[16-20]. The pavine alkaloids have been noted to possess antiviral and immunological activity[21], behavioral and electrophysiological effects, and antiarrhythmic potential[18]. Several other compounds were isolated by Horn et al. from the leaves and bark of the South African plant. *Cryptocarya latifolia*, which has long been noted for its medicinal properties[22]. These range from the treatment of headaches and morning sickness to that of cancer, pulmonary diseases and various bacterial and fungal infections[23]. Phytochemical studies on steam bark and leave of *Cryptocarya moschata* from Atlantic Rain Forest show the occurrence of styrylpyrones and flavonoid glycosides on this species. Some of the pyrones and styrylpyrones isolated from this genus have shown larvicidal and antifertility activities, as well as inhibition of breast cancer cell lines[24].

(-)-Grandisin **26**, isolated from *Cryptocarya crassinervia* collected from Selangor (Malaysia), exhibited a weak activity against human lung carcinoma (A-549), human breast carcinoma (MCF-7) and human colon adenocarcinoma (HT-29) with an ED_{50} value of >10 μg/ml. Its analogues have been reported as an antagonist of platelet activating factor (PAF), useful for the treatments of inflammation, cardiovascular disorders, asthma, lung edema, adult respiratory distress syndrome, pain and platelet aggregation[25]. In Brazilian Atlantic rain forest, the fruits of species *Cryptocarya mandioccana* Meisner are distinct by their aroma and pungent flavor, being carminative and with stomachical properties. Its bark,

bitter and scented is also considered to be stomachical and helpful in fighting colic and diarrhea. The tea from its seeds is used against stomachache, and it's crushed leaves against aches and colics[26].

1.8 The Objectives of Study

The objectives of study are:

1. To extract, isolate and purify the compounds, mainly alkaloids from *Cryptocarya densiflora*.

2. To elucidate the structure of isolated compounds using spectroscopic methods such as 1D-NMR (^{1}H, ^{13}C, DEPT, NOE), 2D NMR (COSY, NOESY, HMQC, HMBC), UV, IR and MS (GCMS, LCMS and HRMS).

3. To classify the types of alkaloid.

1.9 The importance of study

The importances of study are:

1. To discover new compounds and other alkaloids from *Cryptocarya densiflora*, (this is the first report on the chemical constituents of *Cryptocarya densiflora* and provide opportunities for the discovery of bioactive compounds and

2. to provide the chemotaxonomy of the plant species besides providing literature reviews for future needs.