



UNIVERSITI PUTRA MALAYSIA

**ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES
OF CHEMICAL CONSTITUENTS OF *OPHIORRHIZA*
AND *HEDYOTIS* SPECIES**

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AND *HEDYOTIS* SPECIES

by

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LIST OF ABBREVIATIONS

acetyl CoA	acetyl coenzyme-A
br	broad
BuOH	butanol
dd	doublet of doublet
EtOH	ethanol
IR	infra red
MeOH	methanol
MS	mass spectrum/ mass spectrometry
NMR	nuclear magnetic resonance
pet. ether	petroleum ether
plc	preparative layer chromatography
sh	shoulder
tlc	thin layer chromatography
UV	ultra violet



Abstract of the thesis presented to the Senate of Universiti
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degree of Master of Science

**ISOLATION, CHARACTERIZATION AND BIOLOGICAL
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OPHIORRHIZA AND *HEDYOTIS* SPECIES**

by

AHMAD SAZALI BIN HAMZAH
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Chairman: Prof. Dr. Md. Nordin Hj. Lajis
Faculty: Science and Environmental Studies

Chemical investigation on the leaves of *Ophiorrhiza* species, *O. communis* and *O. tomentosa*, has resulted in the isolation of two indole type alkaloids, harman and strictosidinic acid. Strictosidinic acid was isolated from the butanol extract of both *Ophiorrhiza communis* and *O. tomentosa* while harman was isolated from *O. communis* only. The structures of these alkaloids were elucidated using modern spectroscopic techniques such as UV, IR, NMR, MS and also by comparison with the literature.

A study on the constituents of *Hedyotis verticillata* has resulted in the isolation of kaempferitrin and Hv 2 whereas from *Hedyotis herbacea*, kaempferol-3-O-rutinoside, ursolic acid, kaempferol-3-O-arabinopyranoside and quercetin galactoside were isolated. These structures were determined using modern spectroscopic techniques and also by comparison with the literature.



A bioassay was also carried out using brine shrimp and anti bacterial tests on the pure and crude compounds. The brine shrimp tests showed that some of the compounds were very toxic whereas the anti-bacterial tests showed that some compounds have anti-bacterial properties and certainly these compounds have potential for further investigations for their medicinal and practical uses.



Abstrak tesis dikemukakan kepada Senat Universiti Pertanian
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**PENGASINGAN, PENCIRIAN DAN KEAKTIFAN BIOLOGI SEBATIAN SEBATIAN
KIMIA DARI SPESIS *OPHIORRHIZA* DAN *HEDYOTIS*.**

oleh

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Pengerusi: Prof. Dr. Md. Nordin Hj. Lajis
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Kajian kimia ke atas dua spesis *Ophiorrhiza* iaitu *Ophiorrhiza communis* dan *O. tomentosa* telah memberikan dua alkaloid indola iaitu harman dan asid striktosidinik . Asid striktosidinik telah diasingkan daripada ekstrak butanol dari kedua spesis *Ophiorrhiza* tersebut manakala harman diperolehi hanya daripada ekstrak kloroform *Ophiorrhiza communis*. Struktur alkaloid ini telah dikenalpasti dengan menggunakan kaedah spektroskopi seperti UV, IR, NMR, MS dan juga perbandingan dengan literatur.

Kajian yang berasingan ke atas dua spesis *Hedyotis*, iaitu *Hedyotis verticillata* telah menghasilkan kaempferitrin dan Hv 2 manakala daripada *Hedyotis herbacea*, kaempferol-3-O-rutinosida, kaempferol-3-O-arabinopyranosida , asid ursolik dan kuersetin galaktosida telah diasingkan. Struktur struktur tersebut telah dikenalpasti melalui kaedah spektroskopi dan perbandingan dengan literatur.



Satu biocerakinan juga telah dijalankan melalui kaedah 'brine shrimp' dan anti bakteria ke atas sebatian sebatian tulen dan ekstrak mentah spesis *Ophiorrhiza* dan *Hedyotis*. Keputusan nya menunjukkan sesetengah sebatian mempunyai nilai ketoksikan yang tinggi dan juga mempunyai sifat anti-bakteria yang berguna untuk kajian seterusnya.

PART 1

**CHEMICAL CONSTITUENTS OF *OPHIORRHIZA COMMUNIS* AND
*OPHIORRHIZA TOMENTOSA***



INTRODUCTION

Plants play a very important role in human life. Besides being the major source of food, plants have numerous other practical applications such as for shelters, food, flavourings and preservatives. Fine chemicals derived from plants have also been widely used as dyes and pharmaceuticals, in perfumery and in pest management.

The study on efficacy and chemistry of plant constituents has resulted in the discovery of a number of pharmaceutical drugs such as codeine, vinblastine, vincristine and reserpine. Despite being one of the oldest fields in science, natural product chemistry still remains an active field of research. With the emergence of biotechnology the study on chemistry of natural products has become even more important since the findings in this study can be used as the basis for biotechnology. This is clearly shown by the recent interest in the search for new drugs from tropical plants conducted by several multinational pharmaceutical firms and various other foreign organizations. These studies have shown that some of the medicinal plant used traditionally indeed have the curative phenomena associated with it.

Malaysia, being in the tropics stores a large collection of plants species. It is estimated that there are about 10,000 species of higher plants and about 2000 species of lower plants available in



Malaysia with approximately 16 percent of these claimed to be used for medicinal purposes (Lattif *et al.*, 1984).

The study on the chemistry of Malaysian plants began in late fifties when Douglas and Kiang published their first phytochemical survey report (Douglas and Kiang, 1957). For the past decade, there has been a resurgence of interests amongst the local scientists in the research on medicinal plants. Individuals and groups of scientists are engaged in the activities related to medicinal values of many plant species that are available throughout the country (Zurinah, 1989). The flora of Malaysia has provided a wealth of material for investigation and it is therefore not surprising that many chemists are involved in this area of research.

The earliest records on the use of plants as medicine in Malaysia were given by Gimlett and Burkill (1939), Burkill and Haniff (1930) and Burkill (1935). Recently, a compound named Calanolide A, isolated from *Callophyllum lenigerum* which was found in Sarawak was shown to destroy the HIV virus. Further studies are now being carried out to test the potency of this compound in combating against the dreadful disease (Kashman *et al.*, 1992).

A number of phytochemical surveys conducted by chemists and botanists have helped in selecting plants for their research undertakings based on its chemical class.

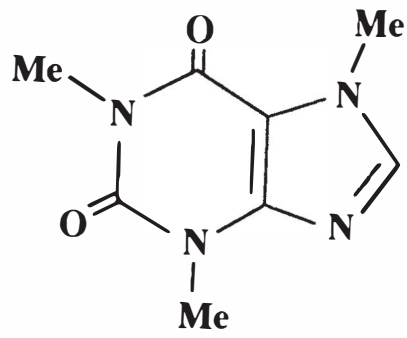


In this project, some species from two genera, *Ophiorrhiza* and *Hedyotis* were selected due to their various medicinal claims and their chemotaxonomic correlation. These two genera belong to the sub-family Rubioideae. According to the literature, there are about 80 genera comprising of 550 species of Rubiaceae family found in Malaysia. Most of the species are known to contain alkaloids, flavonoids, steroids, terpenoids and fatty acids. Compounds such as caffeine (1), quinine (2) and emetine (3) isolated from the family Rubiaceae are of major pharmaceutical importance.

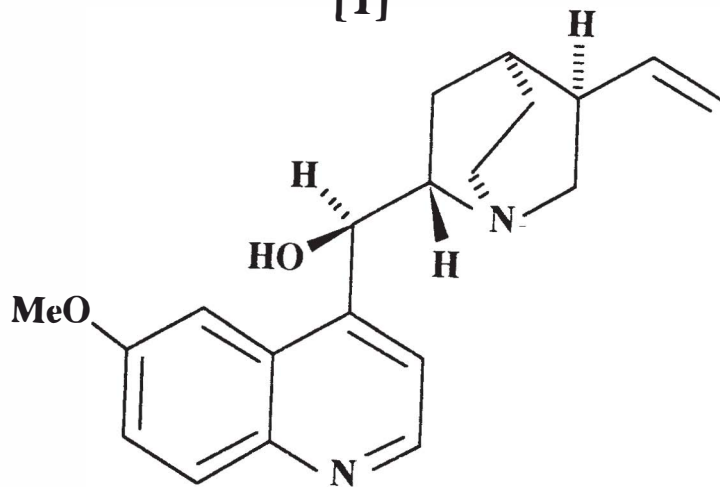
Plants from the family Rubiaceae are among the very commonly found throughout Malaysian forests. They mostly appear as herbs, shrubs, trees or climbers. Plants of this family are easily recognised by their morphological characteristics of having opposite, simple leaves, with stipules between the base of each pair of leafstalks, one on either side of the twig.

Genus *Ophiorrhiza*

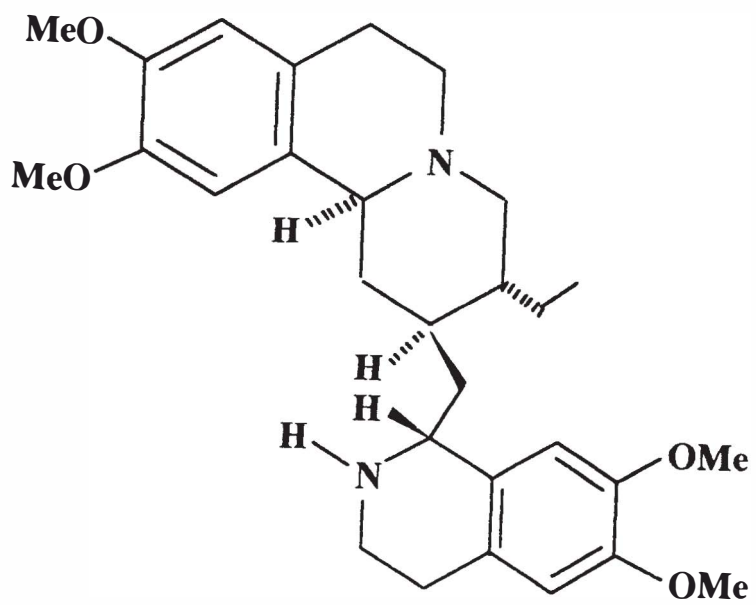
Ophiorrhiza is a small herbs mainly found in the forests of Southern India and eastward as far as the Pacific Islands. The generic name means 'snake root' as *Ophiorrhiza mungos* was known for treatment of snake bite. According to Ridley (1923), there are about 50 species of *Ophiorrhiza* in tropical Asia of which 21 were identified in Peninsular Malaysia. *Ophiorrhiza communis* is known locally as pokok peparu (lung plant) or sometimes called pengerak nasi. Being a soft and easily pulped plant, it is used as a poultice



[1]



[2]



[3]

and also for treating coughs. *Ophiorrhiza tomentosa* was also reported to be used as poultice (Burkill, 1935).

Objectives of the Study:

In view of their medicinal values and of the high alkaloid contents of *Ophiorrhiza*, a research work was carried out on this genus with the following objectives:

- 1) To extract and isolate the alkaloids from *Ophiorrhiza communis* and *O. tomentosa*.
- 2) To elucidate the structures of the compounds isolated using modern spectroscopic methods.
- 3) to carry out the bioassay on the crude extracts and/or the pure compounds.

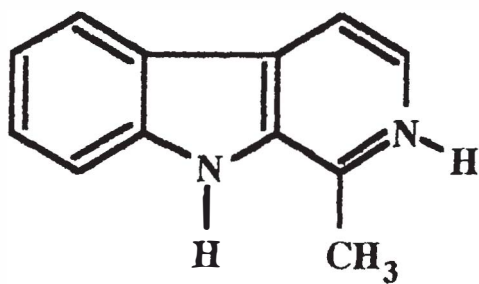
Previous Work On Genus *Ophiorrhiza*:

Aimi *et al.*, (1985, 1986, 1989, 1990) reported the isolation of alkaloids from three *Ophiorrhiza* species, *O. kuroiwai* (Aimi *et al.*, 1985), *O. japonica* (Aimi *et al.*, 1986) and *O. pumila* (Aimi *et al.*, 1989, Aimi *et al.*, 1990). The compounds isolated from these are summarised in Table 1.

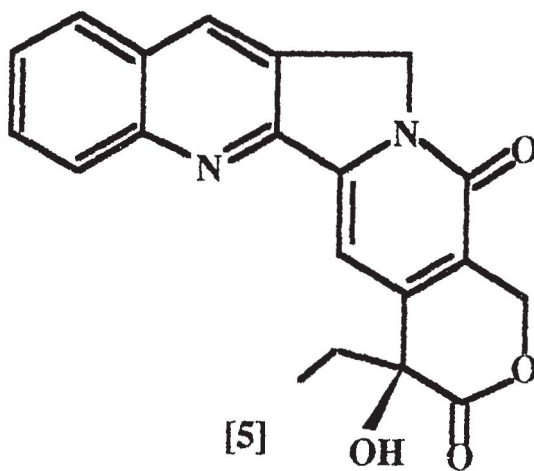
Table 1 Alkaloids Isolated from *O. kuroiwai*,
O. japonica and *O. pumila*.

Species	Alkaloids Isolated
<i>O. kuroiwai</i>	harman (4), camptothecine (5), 9-methoxycamptothecine, lyalosidic acid, ophiorine A and ophiorine B.
<i>O. japonica</i>	harman, lyalosite, 6-hydroxy harman lyalosidic acid, ophiorine A and B, 10-hydroxylyalosidic acid, ophiorine A methyl ester, ophiorine B methyl ester.
<i>O. pumila</i>	pumiloside, deoxypumiloside, camptothecine, chaboside.

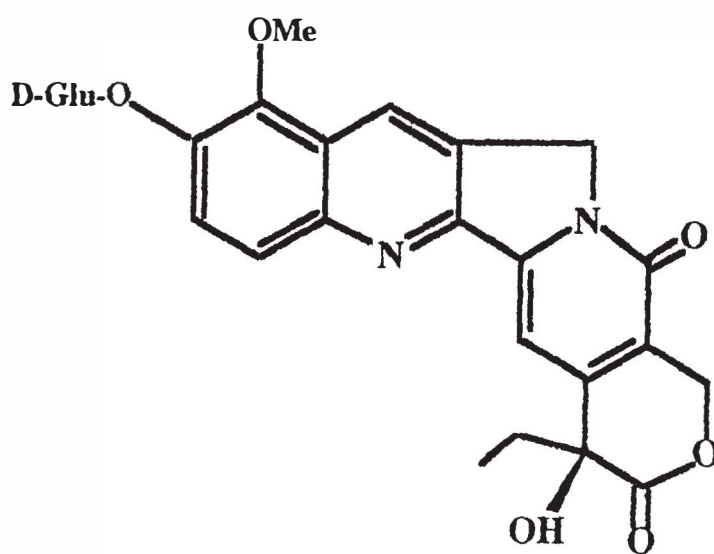
Table 1 shows that camptothecines were isolated from both *Ophiorrhiza kuroiwai* and *O. pumila*. Camptothecine (5) is one of the most important anticancer compounds derived from natural sources following its first isolation from a Chinese tree, *Camptotheca acuminata* in 1966 (Wall *et al.*, 1966). Apart from being anti-tumour it has also been shown to have specific inhibitory activity on mammalian topoisomerase I (Aimi *et al.*, 1989). Numerous investigations had been carried out on this compound due to its



[4]



[5]



[6]

remarkable biological activities and also for its novel structure (Aimi *et al.*, 1990). Another novel compound isolated from *O. pumila* is chaboside (6) and it represents a natural glucoside of true camptothecinoid. Following this interesting finding camptothecinoid glycosides has been synthesised successfully (Yaegashi *et al.*, 1989).

Arbain *et al.*, (1992, 1993) reported the alkaloidal constituents of four *Ophiorrhiza* species; *O. discolor*, *O. filistipula*, *O. major* and one unidentified *Ophiorrhiza* species. *O. filistipula* is used in traditional West Sumatran medicine as a remedy for scalp infections in children. One of the compounds isolated from this species, 7-methoxycamptothecin, was isolated for the first time from natural sources although the racemic compound had been synthesised previously (Baxmann and Winterfeldt, 1978). A summary of the alkaloids isolated by Arbain *et al.*, (1992) is given in Table 2.