PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON ASSISTIGMA MANUBRIATUM

Ijazah: SARJANA MUDA (KIMIA INDUSTRI)

SESI PENGAJIAN: 2004/05

Saya AMUTHA MANIAN

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[/]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[ ]	TIDAK TERHAD	

Amutha

(TANDATANGAN PENULIS)

Alamat Tetap: 522, LORONG KENARI 42A, TAMAN KENARI, 06000 KULIM, KEDAH

Tarikh: 12/4/07

Disahkan oleh

(TANDATANGAN PUSTAKAWAN)

Nama Penyelia

Tarikh:

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON *FISSISTIGMA MANUBRIATUM*

AMUTHA MANIAN

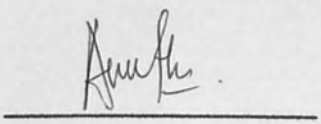
THIS DISSERTATION IS PRESENTED TO FULFILL THE PARTIAL REQUIREMENT TO OBTAIN A BACHELOR DEGREE OF SCIENCE WITH HONOURS

INDUSTRIAL CHEMISTRY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITY MALAYSIA SABAH

APRIL 2007

DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries each of which have been fully acknowledged.



APRIL, 2007

AMUTHA MANIAN
HS2004-1204

VERIFICATION

Name : Amutha Manian

Title : Phytochemical and Biological Studies on *Fissistigma manubriatum*

DR. HOW SIEW ENG

MR. MOH PAK YAN

MR. JAHIMIN ASIK

DEAN

APRIL, 2007

ACKNOWLEDGEMENT

Throughout this experiment, there are many people who have played a role in assisting me to the top. For this I would like to send my heartiest regards and thanks to them. Firstly, I would like to thank my supervisor, Dr. How Siew Eng for always helping me to find alternatives and answer to questions that surfaced from the experiment. Her concerns, guidance and encouragements offered to me throughout this project have eased my lab works. Special thanks to all of the other lecturers for their support, knowledge, direction and understanding.

My thanks reach out to the people in the lab, particularly my course mates who have always been patiently guiding me whenever I needed advise. To biotechnology students for always being there to guide me in the microbial works as well as for their gift of the bacterial strain.

My thanks to lab assistants, Mr. Sani and Mr. Samudi for always help me with the chemicals I need. Thanks to my parents and all my course mates, who I could not thank them individually.

My thesis would never have been finished without these people who continued provide essential knowledge, information, encouragement and support. My heartiest thanks to all of them.

ABSTRACT

The plant *Fissistigma manubriatum* (Annonaceae) or known more commonly as Gagon has long been a source of traditional remedy curing rheumatism and fever. In this study, *Fissistigma manubriatum* root bark was screened for phytochemical, antioxidant and antimicrobial activities. Four types of extracts, i.e. aqueous extract 2.705 g (0.54 %), hexane extract 0.637 g (0.13 %), dichloromethane extract 0.578 g (0.12 %) and butanol extract 2.810 g (0.56 %) were obtained by extraction of *Fissistigma manubriatum* root using solvent-solvent extraction method. Phytochemical screenings indicated that secondary metabolites such as alkaloids, saponins, tannins and flavonoids were found in dichloromethane, butanol and aqueous extracts. Antioxidation test using Ferric Thiocyanate method (FTC) indicated that butanol, aqueous and dichloromethane extracts of the sample demonstrated moderate antioxidative property compared to *butylated hydroxytoulene* (BHT, a synthetic antioxidant). The relative absorbance values of all these three extracts almost similar to each other indicating that they possessed a similar efficiency in inhibitory activity against linoleic acid peroxidation. An antimicrobial test using the disc diffusion method indicated that the butanol and aqueous extracts of the sample were partially active against *Bacillus cereus* (B 43/04B) and *Staphylococcus aureus* (S 277), but ineffective against *Escherichia coli* (E 91/026) and *Pseudomonas aeruginosa* (ATCC 10145). In conclusion, *Fissistigma manubriatum* extracts showed the presence of certain classes of phytoconstituents and acted as moderate antioxidants with weaker antioxidation activity than *butylated hydroxytoulene* (BHT).

KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI KE ATAS FISSISTIGMA MANUBRIATUM

ABSTRAK

Fissistigma manubriatum *atau lebih dikenali sebagai Gagon telah lama digunakan sebagai perubatan tradisional untuk mengubati sakit tulang dan demam. Dalam kajian ini, penyaringan fitokimia, ujian agen antioksida dan ujian anti-bakteria dari kulit akar Fissistigma manubriatum telah di lakukan dengan menggunakan kaedah pengekstrakan secara sistem pelarut-pelarut. Empat jenis ekstrak iaitu ekstrak akueus 2.705 g (0.54 %), ekstrak heksana 0.637 g (0.13 %), ekstrak diklorometana 0.578 g (0.12 %) dan ekstrak butanol 2.81 g (0.56 %) telah diperolehi daripada akar* Fissistigma manubriatum *melalui proses pengekstrakan. Penyaringan fitokimia menunjukkan kehadiran sebatian metabolit sekunder seperti alkaloid, saponin, flavonoid dan tanin di dalam ekstrak diklorometana, butanol dan akueus. Kajian ini menunjukkan bahawa ketiga-tiga ekstrak iaitu butanol, akueus dan diklorometana adalah anti-oksida yang sederhana di mana aktiviti antioksidaannya kurang cekap berbanding dengan* butylated hydroxytoulene *(BHT). Telah dibuktikan melalui ujian anti-bakteria bahawa ekstrak butanol dan akueus menunjukkan aktiviti yang merencat* Bacillus cereus *(B 43/04B) dan* Staphylococcus aureus *(S 277), tetapi tidak menunjukkan perencatan terhadap* Escherichia coli *(E 91/026) dan* Pseudomonas aeruginosa *(ATCC 10145). Kesimpulannya, ekstrak* Fissistigma manubriatum *menunjukkan kehadiran sebatian metabolit sekunder dan merupakan anti-oksida yang sederhana cekap.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

g	gram
L	Liter
M	Molar
µl	micro liter
mL	milli Liter
No	Number
° C	degree Celsius
%	percent
sp.	species
UV	Ultra Violet
w/v	weight per volume
F. manubriatum	*Fissistigma manubriatum*
F. species	*Fissistigma* species
E.coli	*Escherichia coli*
S.aureus	*Staphylococcus aureus*

CHAPTER 1

INTRODUCTION

1.1 Medicinal Plants

Uses of traditional medicinal plants in relieving symptoms of disease and curing various infections are as old as human civilization. In recent years, considerable interest has developed in Asian countries in the collection and extended use of indigenous plants for medicinal purposes (Kulip, 1997). Many surveys on medicinal plants have been carried out and a total of about 200 plant species used by Kadazan Dusun and Murut ethnic communities in treating various ailments and personal adornment was reported (Kulip, 2000).

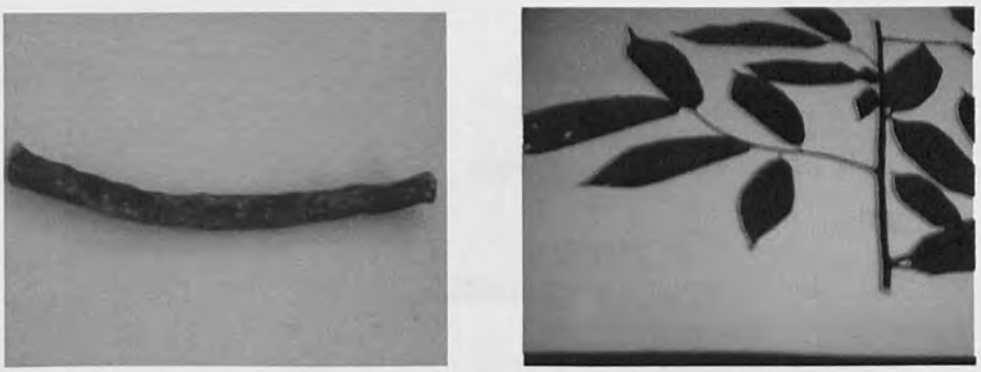
People who live far from town still rely on traditional cures handed down to them through the generations. Today, even though the practice of modern medicine is widely spread, the traditional methods of healing using plants are still popular in Sabah because of either people cannot afford to buy them or cannot avail themselves to hospital facilities. Regular indigenous medicinal plants easily found in 'Tamu' or local markets in Sabah.

Medicinal plants are used in one form or another to cure or alleviate a variety of ills such as headache, diabetes, toothache, stomachache and malaria. Remedies for various

ailments normally involve the use of roots, leaves and bark of the plants, while flowers, fruits and seeds are very seldom used because they are only available at certain times of the year (Ahmad & Raji, 1993). Some of the important medicinal plants that are commonly used include *Eurycoma longifolia* (Tongkat Ali) which is used as a tonic, *Morinda citrifolia* (mengkudu) for diabetes and Gagon root is used to cure fever and rheumatism (Salleh, 1985).

Plants have served as a primary source of useful natural products which currently constitutes about 25% of the world drug market. The biodiversity of Malaysia's plant resources offer 15,000 species of higher plants (Intan *et al.*, 1995). About 1,000 of these plants have undergone simple chemical screening, but much less have been subjected to phytochemical and biological studies (Intan *et al.*, 1995). This study focuses on *Fissistigma manubriatum* which is a traditional medicinal plant used by Kadazan dusun ethic in Sabah. Even though *Fissistigma manubriatum* also has high medicinal property like other medicinal plants but it is less popular among the local population (Kulip, 1997).

In this study, traditionally used *Fissistigma manubriatum* was studied scientifically via phytochemical and biological screenings. Alkaloids, tannins, saponin and flavonoids of this plant used medicinally were decided to undertake a phytochemical investigation. Screenings for biological activity using simple and fast bioassays have been added to give a better indication of the usefulness of the plants. A root of *F. manubriatum* used in this study is shown in Figure 1.1 (a), whereas the leaves are shown in Figure 1.1 (b).



(a) (b)

Figure 1.1 *F. manubriatum*. (a) Root, (b) Leaves.

1.2 Objectives of this Study

Objectives of this study were to screen the phytochemical contents in root part of *F. manubriatum* and to evaluate its antioxidative and antimicrobial properties.

1.3 Scope of the Study

This study involved a phytochemical screening to determine the presence of secondary metabolites such as alkaloids, tannins, saponins and flavonoids and biological screenings such as antioxidative and antimicrobial activity of the *Fissistigma manubriatum* extracts. Antioxidation activity of the plant extracts was studied semi-quantitatively relative to BHT using UV spectrophotometer and the autoxidation linoleic acid assay in an alcohol-aqueous system to measure antioxidation activity against lipid peroxidation (linoleic acid). Antimicrobial test was carried out by the disc diffusion method and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Features of *Fissistigma manubriatum*

Fissistigma manubriatum is a traditional medicinal plant, commonly used for the treatment of a variety of ailments under the local name Gagon or Subagon by Kadazan-Dusun ethnic in Sabah (Kulip, 1997).

F. manubriatum is a member of Annonaceae family. Annonaceae is a large floral family comprising of 120 genera and more than 2000 species (Leboeuf *et al.*, 1982). On the basis of morphology and habitat, Annonaceae is a very homogeneous plant family. All species are trees or shrubs, sometimes climbing, usually evergreen, with resin canals and septet pith in the stems. Annonaceae plants are recognized, in tropical and subtropical regions, by the alternate, exstipulate leaves, mostly trimerous flowers, numerous and often truncate free stamens, free carpels and seeds with ruminate endosperm (Watson & Dallwitz, 1999).

F. manubriatum is a shrub growing 3 to 5 meters high and usually has only one main stem. The leaves are alternate and evergreen whereas the bark and root are fibrous.

The root is in reddish-brown color. *F. manubriatum* is widely found in Malaysian Peninsula, Sabah and Sarawak (Perry, 1980).

2.2 Medicinal Uses of *Fissistigma* Species

In Malaysia eight species of *Fissistigma* are known such as *F. cylindricum, F. fulgens, F. kinabaluensis, F. kingii, F. lanuginosum, F. latifolium, F. manubriatum* and *F. mobiforme* (Jubri *et al.*, 1993). Species of *Fissistigma* have been used in traditional medicine by local community world wide and each one of this species has its own value. *Fissistigma* species such as *F. balancae, F. glaucescens* and *F. oldhamii* are also found in the Southern part of Yunnan in China and in Vietnam (Chia *et al.*, 1998; Lu *et al.*, 1985).

In contrast, *F. manubriatum* root is used by Kadazan Dusun community for the treatment of feverish diseases, rheumatism, as a tonic, stomachache and postparturation aids to mother (Salleh, 1985). The roots and stems of *F. glaucescens* have been used for muscular atrophy, hepatomegaly and hepatosplenomegaly in Taiwan and China (Lu *et al.*, 1985).

F. fulgens leaves are used to cure rheumatism, as a tonic, postparturation aids to mother, to stop bleeding in wounds or cuts and boils (Salleh, 1985). *F. kingii* and *F. lanuginosum* can be used for treatment of stomachache and digestion problem (Burkill, 1966). Further more, *F. lanuginosum* root is used as tonic for postparturation aids to mother. *F. latifolium* is used to cure rheumatism (Salleh, 1985). While, *F. cylindricum* is suitable for the treatment of diarrhea and snack bite (Salleh, 1985). Traditional uses of *Fissistigma* species are shown in Table 2.1.

Table 2.1 Traditional uses of *Fissistigma* species.

Traditional Uses	*Fissistigma* species
1. Postparturation aids to mother	• *F. fulgens* • *F. lanuginosum* • *F. manubriatum*
2. Diarrhea	• *F. kingii* • *F. lanuginosum* • *F. cylindricum*
3. Feverish diseases include malaria, cholera and typhoid	• *F. manubriatum*
4. Rheumatism	• *F. fulgens* • *F. latifolia* • *F. manubriatum*
5. Wound and boils	• *F. fulgens*
6. Tonic	• *F. manubriatum* • *F. fulgens* • *F. lanuginosum*
7. Stomachache	• *F. manubriatum* • *F. kingii* • *F. lanuginosum*
8. Muscular atrophy, hepatomegaly and hepatosplenomegaly	• *F. glaucescens*
9. Snake bite	• *F. cylindricum*

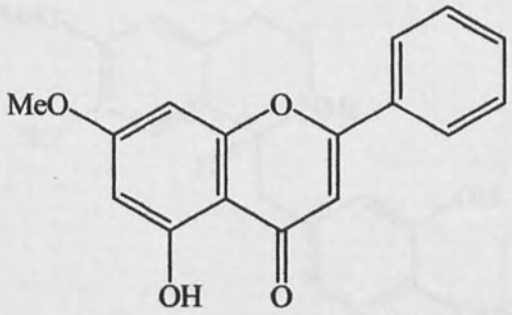
(Source: Salleh, 1985)

2.3 Phytochemicals

Phytochemical surveys are now seen as the first step towards the discovery of useful drugs because the tropical rain forest has been identified as a potential source due to its diverse richness in flora. Phytochemical separations are now routinely guided by bioassays which will ensure the isolation of the bioactive principles irrespective of whether they belong to a certain class of compounds or not. Isolated chemical compounds may be used for medicinal purposes in treating various ailment and minor complaints such as diabetes, high blood pressure, cancer and so on. Phytochemical and biological information about *Fissistigma* species is very important to phytochemical researchers because they believe that plants from same family, most probably have relatively same or same phytochemical components (Ahmad & Raji, 1993).

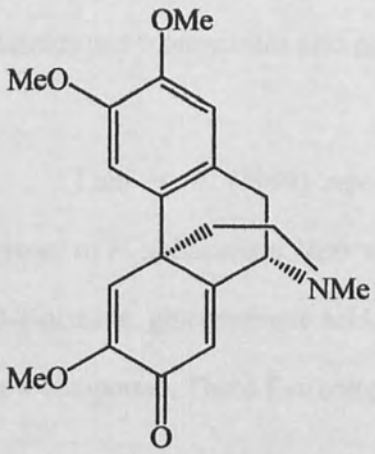
The genus *Fissistigma* is a genus of woody climber belonging to Annonaceae family found in tropics of the old world (Burkill, 1966). Annonaceae family is well known for its alkaloid bearing plants that have the isoquinoline structures. Among the alkaloidal components, isoquinolines which have been found to have benzylisoquinolines as *in vivo* precursors are the main alkaloidal constituents of Annonaceae. Aporphinoids are the largest class of compounds occurring in this family. Many species elaborate aporphines with their corresponding oxoaporphines, which support the view that oxoaporphines are formed in plants from corresponding aporphines (Leboeuf *et al.*, 1982).

A previous study by Jubri *et al.* (1995) led to an isolation of 5-hydroxy-7-methoxyflavone or tectochrysin **(1)** from petroleum extract of *F. latifolium*. Tectochrysin crystal was in yellow color and the melting point was between 165-166 °C.

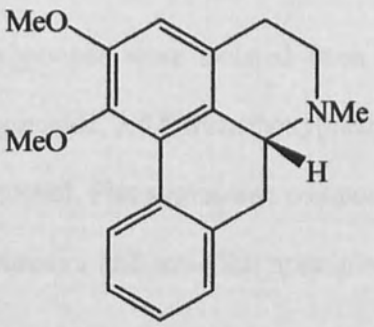


(1) tectochrysin

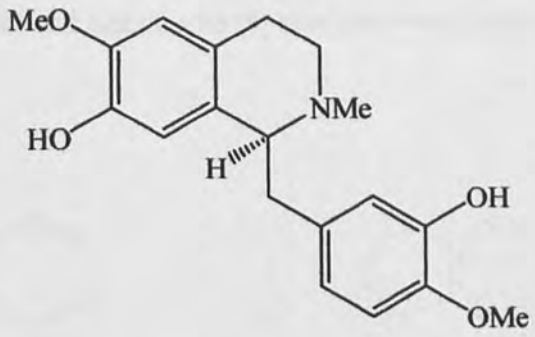
Among the alkaloids studied by Geh *et al.* (1995), (+)sebeferine **(2)**, (-)nuciferine **(3)** and (+)reticuline **(4)** from *F. latifolium* were appeared to be the most potent in lowering blood pressure and heart rate.



(2) (+) Sebeferine



(3) (-) Nuciferine



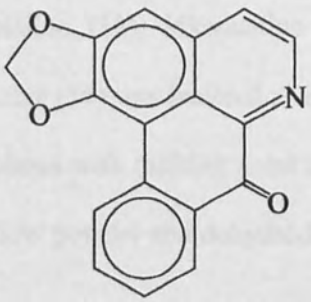
(4) (+) Reticuline

According to Husain *et al.* (1995), plants belonging to *Fissistigma* species such as *F. kinabaluensi*, *F. kingii*, *F. latifolium* and *F. manubriatum* contain secondary metabolites, for example alkaloid, saponin, steroid and triterpena. Alkaloids present mostly in the bark or roots and some in leaves, twigs, stem bark or woods. Besides, steroids and triterpenoids also present in all parts of the plants.

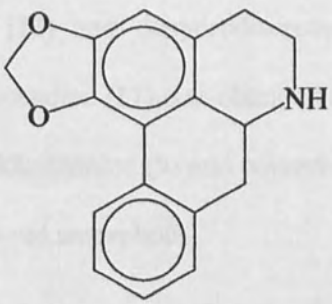
Liao *et al.* (1999) reported that five compounds were isolated from ethanolic extract of *F. polyanthum Merr* which were fissistigmoside, 3,4,5-trimethoxyphenyl-1-O-β-D-glucoside, glucosyringic acid, dacosterol and inositol. Fissistigmoside compound was a new compound. These five compounds showed antitumor and anti-viral principles.

Previous phytochemical work on *F. fulgens* (Hadi *et al.*, 1990) showed the presence of five alkaloid compounds which were isolated using chromatographic techniques from a crude alkaloid extract, for example anonaine, argentinine, discretamine, kikemanine and liriodenine. Liriodenine alkaloid **(5)** was isolated as a yellow needle with melting point around 277-279 °C. Anonaine alkaloid **(6)** was a reddish-brown amorphous. Argentinine alkaloid **(7)** was a colorless crystal with melting point around 235-237 °C.

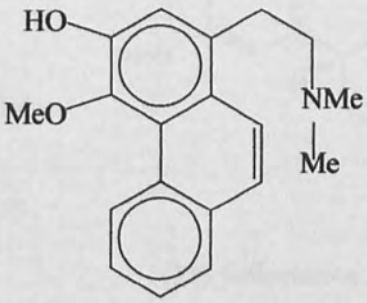
Besides, discretamine **(8)** and kikemanine **(9)** alkaloids were isolated as brown amorphous (Hadi *et al.*, 1990).



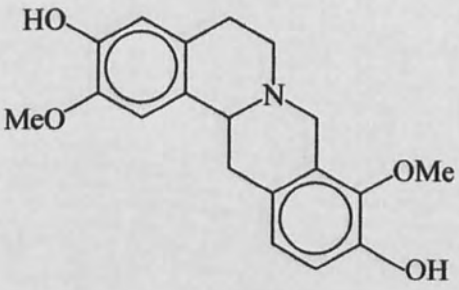
(5) liriodenine



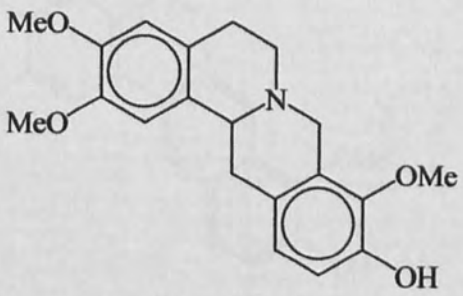
(6) anonaine



(7) argentinine

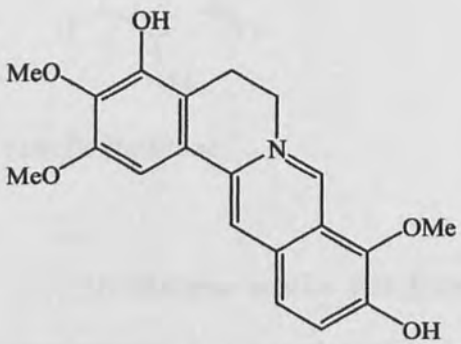


(8) discretamine

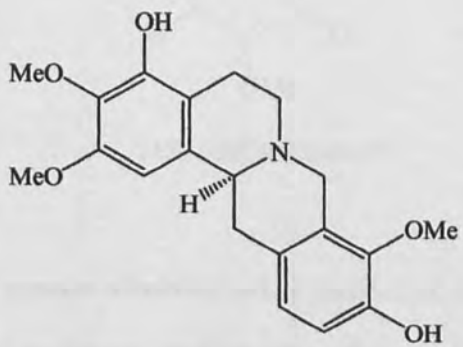


(9) kikemanine

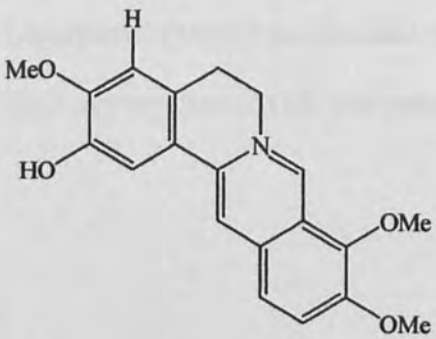
A series of studies on *Fissistigma* species was reported by Chia *et al.* (1998) that an ethanolic extracts from the twigs of *Fissistigma balansae* led to an isolation of a new proberberine alkaloid, fissisaine **(10)**, along with four known protoberberine alkaloids thaipetaline **(11)**, kikemanine **(9)**, columbamine **(12)** and dehydrodiscretamine **(13)**. Fissisaine **(10)** was isolated as a yellow needle. Thaipetaline **(11)** was obtained as a yellow amorphous with melting point around 229-232 °C, kikemanine **(9)** and columbamine **(12)** as yellow powder and dehydrodiscretamine **(13)** as a red amorphous.



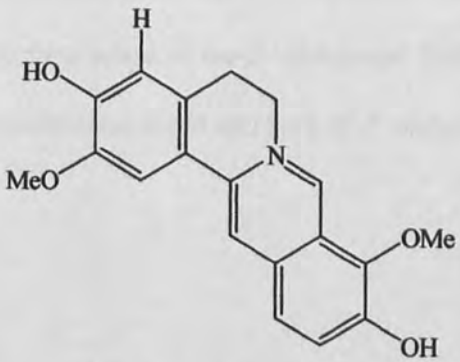
(10) fissisaine



(11) thaipetaline



(12) columbamine



(13) dehydrodiscretamine

Chia *et al.* (1998) reported the isolation and characterization of two new p-quinonoid aporphine alkaloids named fissilandione **(14)** and norfissilandione **(15)** from

REFERENCES

Ahmad, F. and Raji, H., 1993. *Kimia Hasilan Semulajadi dan Tumbuhan Ubatan.* Dewan Bahasa dan Pustaka, Kuala Lumpur.

Ahmed, A., Khan, K. A., Ahmad, V. U. and Qazi, S., 1986. Antibacterial activity of Juliflorine isolated from *Prosopis juliflora. Planta Med* **4**, 285-288.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C. and Fasure, K. A., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicilin resistant *Staphylococcus aureus* activity. *BMC Complementary and Alternative Medicine* **5**, 1-10.

Ali-shtayet, M. S., Yagmour, R. M., Faidi, Y. R., Salem, K. and Al-Nuri, M. A., (1998). *J. Ethnopharmac* **60**, 265.

Burkill, I. H., 1966. *A Dictionary of the Economic Products of the Malay Peninsula.* Ministry of Agriculture and Cooperative Malaysia, Kuala Lumpur. 2nd ed. 1 & 2: 132-2347.

Chia, Y. C., Chang, F. R., Li, C. M. and Wu, Y. C., 1998. Protoberberine Alkaloids from *Fissistigma balansae*. *Phytochemistry* **48** (2), 367-369.

Cowan, M. M., 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews* **12** (4), 564-582.

Geh, S. L., Rampal, R. M., Lee, K. H. and Goh, S. H., 1995. Hypotensive Alkaloids from Annonaceae. *Chemical Prospecting in the Malaysian Forest*, Sarawak, 143-150.

Hadi, H. A. and Khalijah, A., 1990. Alkaloids of *Fissistigma fulgens* Merr (Annonaceae), Proceedings of 7th National Seminar on Natural Products, University Sains Malaysia, Penang, 1-5.

Hadi, S. and Bremner, J. B., 2001. Initial Studies on Alkaloids from Lombok Medicinal Plants. *Molecules* **6,** 117- 129.

Huang, D. J., Lin, C. D., Chen, H. J. and Lin, Y. H., 2004. Antioxidant and antiproliferative activities of sweet potato constituents. *Bot. Bull. Acad. Sin.* **45**, 179-186.

Husain, K., Yusoff, N. I., Salleh, K. M., Said, I. M., Jubri, Z., Ariffin, J., Jalil, J. and Marzuki, M. A., 1995. Phytochemical and Biological Screening of Some Annonaceae Plants from Sabah. *Chemical Prospecting in the Malaysian Forest*, Sarawak, 17-26.

Intan, S. I., Ali, A. M., El-Sharkawy, S. H. and Kawazu, K., 1995. Bioassay Guided Isolation of Cytotoxic Constituents of *Juniper*-var-*scopulorum*. *Chemical Prospecting in the Malaysian Forest*, Sarawak, 151.

Islam, A., Sayeed, A., Sadik, G., Rahman, M. M. and Astaq Mohal Khan, G. R. M., 2001. Antimicrobial activity and Cytotoxicity of Clerodane Diterpines from *Polyalthia longifolia* seed. *The Sciences* **1** (5), 320-323.

Jubri, Z., Yusoff, N. I. and Said, I. M., 1995. Preliminary Studies on the Chemical Constituents of *Fissistigma latifolium* (Dunal) Merr and *F. kinabaluensis* (Stapf) Merr. *Chemical Prospecting in the Malaysian Forest*, Sarawak, 107-112.

Kie, R., 1985. The Phytochemical Survey. *Aspects of Natural Products Chemistry*, University Pertanian Malaysia, Selangor.

Kulip, J. 1997. A Preliminary Survey of Traditional Medicinal Plants in the West Coast and Interior of Sabah. *Journal of Tropical Forest Science* **10** (2), 271-274.

Kulip, J. and Majawat, G., 2000. Medicinal and other useful plants of the Lundayeh Community of Sipitang, Sabah, Malaysia. *Journal of Tropical Forest Science* **12** (4), 810-816.

Lavault, M., Guinaudeau, H., Bruneton, J., Sevenet, T. and Hadi, H. A., 1990. (-)-Thaipetaline, a Tetrahydroprotoberberine from a Malaysian Annonaceae. *Phytochemistry* **29** (12), 3845-3847.

Leboeuf, M., Cavě, A., Bhaumik, P. K., Mukherjee, B. and Mukherjee, R., 1982. *Phytochemistry* **21** (12), 2783-2813.

Liao, Y. H., Guo, J., Xu, L. Z. and Yang, S. L., 1999. The Chemical Constituents of *Fissistigma polyanthum* Merr. *Acta Pharmaceutica Sinica* **34** (3).

Lu, S. T., Wu, Y. C. and Leou, S. P., 1985. Alkaloids of Formosan *Fissistigma* and *Goniothalamus* Species. *Phytochemistry* **24** (8), 1829-1834.

Moody, C. S. and Hassan, H. M., 1982. Mutagenicity of oxygen free radicals. *Proc Natl Acad Sci* (USA) **79**, 2855-2859.

Osawa, T. and Namiki, M., 1981. A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agric. Biol. Chem.* **45** (3), 735-739.

Perry, L. M., 1980. *Medicinal plants of East and South East Asia.* Cambridge. Massachusett Institute of Technology Press. 620.

Rahmat, A., Kumar, V., Fong, L. M., Endrini, S. and Sani, H. A., 2003. Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines. *Asia Pacific J Clin Nutr* **12** (3), 292-295.

Salleh, K. M., 1985. *Comparative chemotaxonomy of Peninsular Malaysian Annonaceae*. MSc. Thesis, Department of Plant Science. Aberdeen: University of Aberdeen.

Silver, L. L. and Bostian, K. A., 1993. Discovery and development of new antibiotics, the problem of antibiotic resistance. *Antimicrob Agents Chemother* **37**, 377-383.

Solomons, T. W. G., 2004. *Organic Chemistry*. 8th edition. New York: John Wiley & Sons.

Subramaniam, V., Adenan, M. I., Ahmad, A. R. and Sahdan, R., 2003. Natural Antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). *Mal J Nutr* **9** (1), 41-51.

Watson, L. and Dallwitz, M. J., 1999. The Families of Flowering Plants: Descriptions, Illustrations, Identification and Information Retrieval.

Wu, Y. C., Kao, S. C., Huang, J. F., Duh, C. Y. and Lu, S. T., 1990. Two Phenanthrene Alkaloids from *Fissistigma glaucescens*. *Phytochemistry* **29** (7), 2387-2388.