STUDIES ON THE ISOLATION AND CHARACTERISATION OF FLAVONES AND TRITERPENOIDS FROM A FEW PLANTS

THESIS SUBMITTED TO
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY UNDER THE FACULTY OF SCIENCE

BY

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July 2002

CERTIFICATE

This is to certify that the thesis entitled '**Studies on the isolation and characterization of flavones and triterpenoids from a few plants**' submitted for the award of degree of Doctor of Philosophy in Chemistry under the Faculty of Science of Cochin University of Science and Technology is an authentic record of work carried out by Ms N. K. Leela under our supervision and the same has not been submitted elsewhere for the award of any other degree or diploma.

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DECLARATION

I hereby declare that the thesis entitled 'Studies on the isolation and characterization and flavones and triterpenoids from a few plants' submitted for the award of degree of Doctor of Philosophy in Chemistry under the Faculty of Science of Cochin University of Science and Technology is an authentic record of the work done by me under the joint supervision of Prof. P. Madhavan Pillai and Dr. G.Vijay Nair and no part of this thesis has been submitted earlier for the award of any other degree or diploma.

N.K Leela

ACKNOWLEDGEMENTS

It is with great pleasure that I express my deep sense of gratitude to my research supervisors ***Dr.P Madhavan Pillai and Dr G.Vijay Nair*** *for their guidance, encouragement and valuable suggestions during the course of this work.*

I am grateful to the Director, Indian Institute of Spices Research, Calicut for granting study leave and Director, Regional Research Laboratory , Thiruvanathapuram for providing all facilities to carry out the work .

I sincerely thank Dr. J. Madhusudana Rao, IICT , Hyderabad with whom I started this work , and Dr. Mangalam S. Nair for the timely help and encouragement.

Thanks are also due to Dr. N. Sasidharan, Kerala Forest Research Institute, Peechi for helping me in identifying the plants.

I am greatly indebted to many of my friends and colleagues especially, Dr. Beena Joy, Dr.B. Chempakam, Dr. S. Devasahayam, Mr. Sudhakaran, Mr. N. Sudhilal, Mr K.S.G. Kurup and Joly S. for the timely help in various ways which enabled me to complete this work.

I wish to thank Dr. P. Shanmugam and Ms. Soumini Mathew, RRL, Trivandrum, for providing NMR spectra and Mass Spectrometry group, IICT Hyderabad for mass spectra. Partial financial assistance from CSIR, New Delhi in the form of Senior Research Fellowship is gratefully acknowledged.

Thanks are also due to all the present and former colleagues and friends of Regional Research Laboratory for their help and cooperation during the course of my work.

I am very much indebted to my family for their immense patience, encouragement and support throughout the period of this work.

N.K.Leela

CONTENTS

Chapter 1

Introduction

Introduction

Plants serve as a vast reservoir of many complex organic compounds. The wide variety of compounds such as alkaloids, steroids and terpenoids produced in plants, which are not primary metabolites, are termed as secondary metabolites. The secondary metabolites endow some unique and species-specific characteristics to the plants. Many secondary metabolites have well defined biological functions. This realization has given a new impetus to the scientific study of secondary metabolites.

In the present scenario, there is an increasing demand for natural products in food industry, pharmaceuticals, cosmetics and agricultural sectors. In this context phytochemical study to identify newer chemicals has got great relevance. Phytochemical studies have become more reliable and encouraging with the development of modern analytical techniques.

In the present work the leaves of *Piper colubrinum* (Piperaceae), aerial parts of *Mussaenda frondosa* (Rubiaceae) and *Humboldtia vahliana* (Leguminosae) and the pericarp of fruits of *Artocarpus heterophyllus* (Moraceae) were investigated for their secondary metabolites. The major compounds isolated belong to the groups of flavonoids and triterpenoids.

Naturally occurring flavonoids have been used widely in chemotaxonomic studies of plants. Flavones and flavonols constitute a group of biosynthetically related natural products. No universal function has been established for flavones and flavonols in plants. However, many functions in individual plants have been demonstrated. These include protection of plants from ultraviolet light, insects and pests; pollinator attractants; antioxidants; plant hormone controllers; enzyme inhibitors and allelopathic agents. Flavonoids are attracting the attention of medical scientists in recent years because of their anticarcinogenic, antiallergic and anti-inflammatory properties. The recent discovery that flavonoids are involved in the process of nitrogen fixation in plants also opens the way for agricultural application of these constituents.

Triterpenoids are another class of compounds that are ubiquitous in plants. Some triterpenoids present in the latex and resins of plants are believed to be involved in chemical defence against pathogens and herbivores. Triterpenoids possess various biological properties including anti-inflammatory, antifeedant, pesticidal, fungitoxic and antimicrobial activities. Triterpenoids with cytotoxic activity and inhibitory effect on seed germination are also known.

The thesis comprises of six chapters. After this chapter on Introduction, Chapters II, III, IV and V deal with the isolation and characterization of crystalline substances from *P. colubrinum*, *M. frondosa*, *A. heterophyllus* and *H. vahliana*, respectively.

Chapters II, III and IV are divided into six major sections. The first section of each chapter gives a brief introduction. Section 2 deals with the phytochemicals isolated earlier from the respective genus and their biological activities. A brief account of the present work is given in Section 3 of all the chapters. The isolation procedures, identification of compounds and the relevant references are presented in the sections 4, 5 and 6, respectively. In chapter V the sections 1 and 2 are combined.Chapter VI summarises results of the present study.

Chapter 2

Chemical examination of Piper colubrinum Link

Chemical examination of *Piper colubrinum* Link

2.1 Introduction

The genus *Piper* belongs to the family Piperaceae, which is represented by more than 700 species distributed throughout the tropical and subtropical regions of the world [1]. Several species occur in Kerala, among which the most important economic being black pepper (*Piper nigrum)*. Most of the *Piper* species are reputed in the Indian Ayurvedic system of medicine for their medicinal properties [2]. Due to their economic and medicinal importance, several *Piper* species have been investigated worldwide for their chemical constituents and pharmacological properties and several reviews have appeared on these subjects [3-6]. A comprehensive review by Parmar *et. al.* lists the secondary metabolites isolated from *Piper* species up to June 1996[1].

2.2 Chemistry of genus *Piper* - an overview

An extensive survey on the phytochemical literature has been carried out as a part of the present work and the trends in the phytochemistry of the genus is overviewed here. The secondary metabolites isolated from the genus are broadly classified as

1. Amides
2. Lignans
3. Terpenes

4. Kawapyrones
5. Flavonoids
6. Miscellaneous compounds

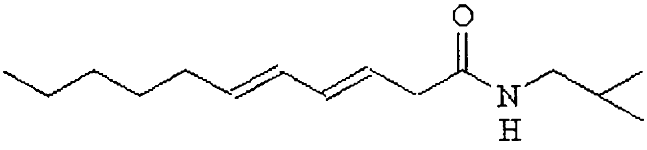
A brief and general discussion is intended for the above groups, while a complete compilation of flavonoids is given for a comparison of the compounds from the studied plant.

2.2.1 Amides

Amides are the largest class of compounds isolated from *Piper* species and about 160 amides have been reported from the genus. These are further grouped into isobutyl amides, piperidine amides, pyrrolidine amides and miscellaneous amides depending on the amine part of the molecule. A brief account of the amides and structures of some of the representative compounds are given in the following sections.

2.2.1.1 Isobutyl amides

Forty isobutyl amides have been reported from *Piper* [1, 7-34]. Majority of these amides are aromatic amides of $\Delta^{2,4}$ fatty acids. (2*E*, 4*E*)-*N*-isobutyldecadienamide [pellitorine **(1)**][16-22], guineensine **(2)**[23-27] and piperlonguminene [28-33] have been isolated from several species.



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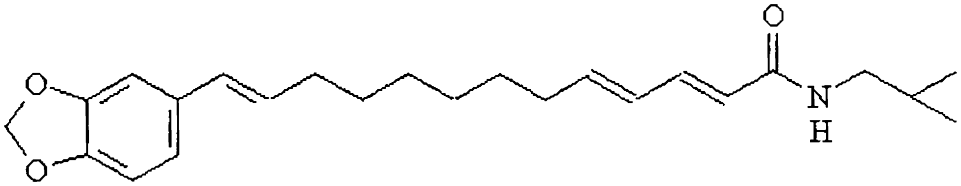

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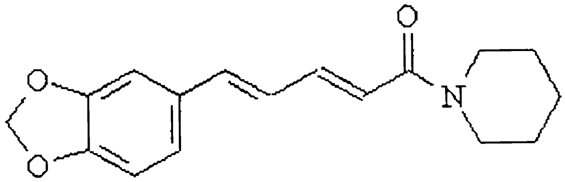
2.2.1.2 Piperidine amides



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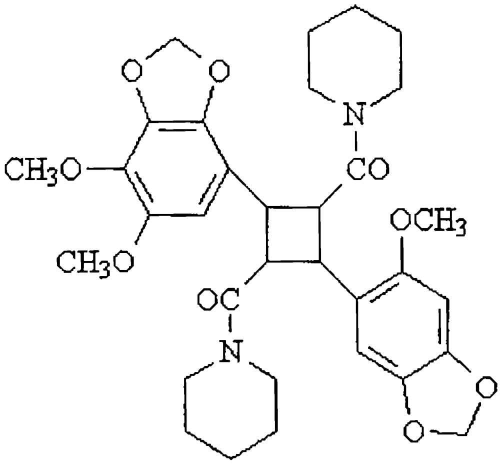
Thirty-four piperidine amides have been isolated from several *Piper* species. Piperine **(3)**, the pungent principle of black pepper occurs in 24 species [1, 35-37]. Two piperidine amide dimers **(4)** have been isolated from *P. peepuloides* [38].



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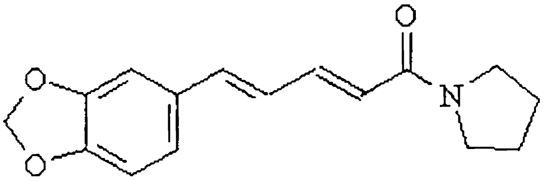
2.2.1.3 Pyrrolidine amides

Pyrrolidine amides (5) occur mainly in *P. trichostachyon* and *P. brachystachyum,* although these are reported from other species [1,15, 39-41]. Dimers of pyrrolidine amides (6) have been isolated from *P. peepuloides* [41].



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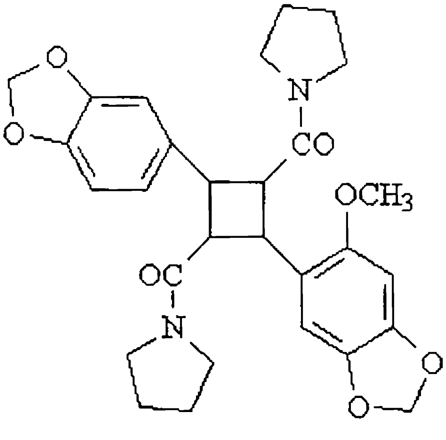

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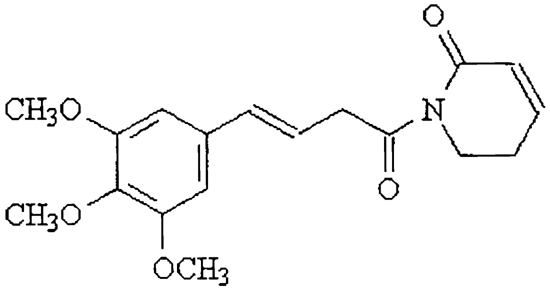
2.2.1.4 Miscellaneous amides

Several amides other than isobutyl, piperidine and pyrrolidine amides have been isolated from *Piper* species. These include pyridone alkaloids [36, 42, 53] (7), aristolactams [36, 45](8), dioxoaporphines [46-49](9), tetrahydropyridine alkaloids [43], benzyl isoquinoline alkaloids [44] and optically active aurantiamide [50, 51] and auranamide [52]. Recently, two alkaloids possessing cyclobutane ring named pipercyclobutanamides A and B have been isolated from *P. nigrum* [53].



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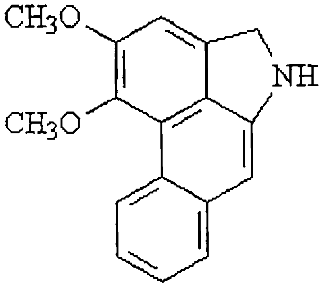

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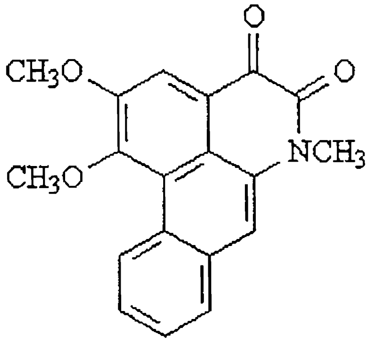

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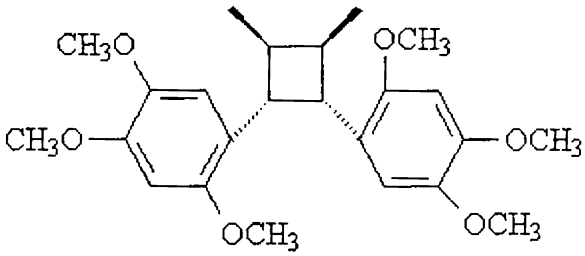
2.2.2 Lignans

Lignans are the second largest class of compounds occurring in *Piper* species and about 125 lignans have been reported from the genus. Lignans are composed of two C_6, C_3 units linked at the β-carbons of the side chain. The lignans from *Piper* may be divided into nine groups. Only brief accounts of the recently isolated lignans are given here and no attempt is made for compiling all the reported compounds.

2.2.2.1 1,4- Diaryl -2,3-dimethyl cyclobutane lignans

Three isomers of this lignan **(10)** are recorded from two species namely, *P. cubeba*[52] and *P. sumatranum*[55].



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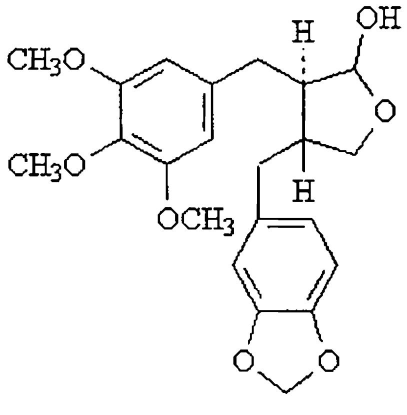
2.2.2.2 3,4-Dibenzyl- γ-butyrolactol lignans

Most of these lignans **(11)** are reported from *P. clusii*[56] and *P. cubeba*[54, 57]. However (-) cubebin has been reported from *P. cuneifolium*[1], *P. lacunosum*[1], *P. ribesoides*[17], *P. trichostachyon*[58] and *P. nigrum*[59].



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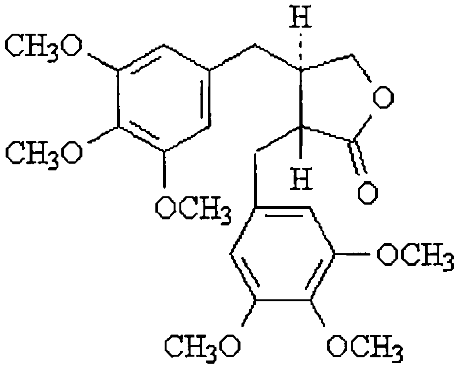
2.2.2.3 γ-Butyrolactones

Lignans of this group **(12)**, reported so far from the genus are from *P. cubeba*[57, 60-62]. (-) Hinokinin and (-) yatein occur in *P. clusii* also[1].



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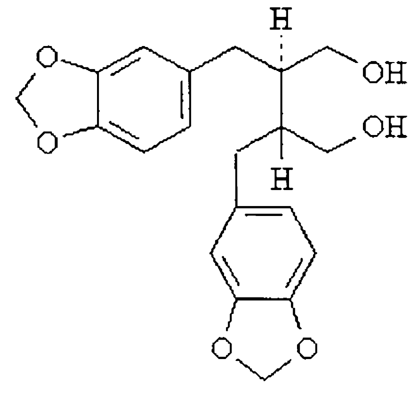
2.2.2.4 2,3-Dibenzylbutan-1, 4-diol lignans

These lignans **(13)** co-occur with the corresponding dibenzylbutyrolactols and lactones. These are found in *P. guineense* [1], *P. clusii* [56], *P. cubeba* [57] and *P. trichostachyon* [58].



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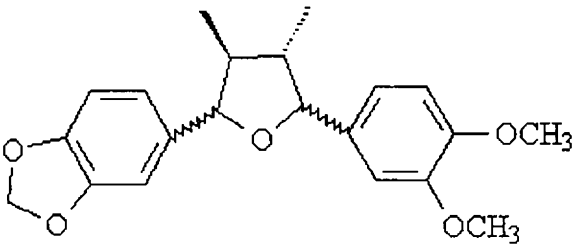
2.2.2.5 2,5-Bisaryl-3, 4-dimethyltetrahydrofurans

Tetrahydrofuran lignans **(14)** have been reported from *P. schmidtii* [63-64], *P. solmsianum* [65] and *P. wallichii* [66]. (-)Grandisin was isolated from *P. solmsianum* [65] while (+) grandisin from *P. polysiphorum* [67].



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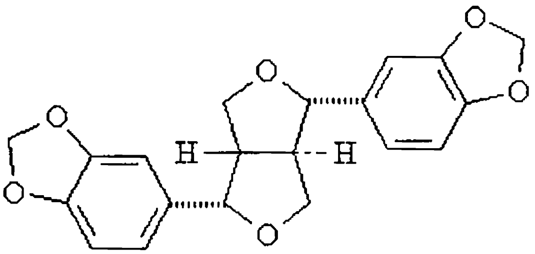
2.2.2.6 2,6-Bisaryl -3,7- dioxa- [3,3,0]-bicyclooctane lignans

Lignans of this basic skeleton occur in many *Piper* species [68-75]. Among these (+) sesamin **(15)** has been reported from 10 species[1].



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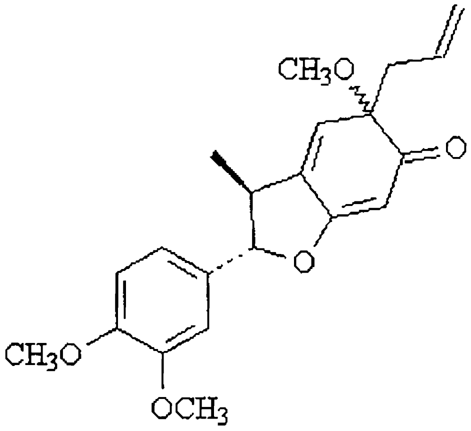
2.2.2.7 Benzofuran lignans



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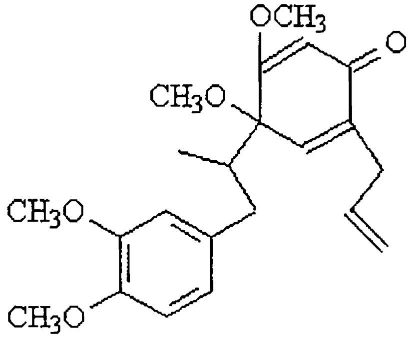
P. rignellii [76] and *P. aequale* [77] are rich sources of benzofuran lignans **(16)**, although these have been reported from *P. futokadsura*[1], *P. wallichi* [1], *P. hancei*[1], *P. schmidtii*[1], *P. capens* [1], *P. clarkii* [6], *P. interruptum* [1] and *P. magnibacum* [78].

2.2.2.8 1,2-Diarylpropanes

This group of lignans **(17)** has been recorded from several *Piper* species [79-83]and generally co-occurs with benzofurans.

2.2.2.9 Miscellaneous lignans

Besides the above mentioned groups of linans, a few lignans of some unique structures have also been reported from several *Piper* species [1,82]and these belong to miscellaneous lignans.



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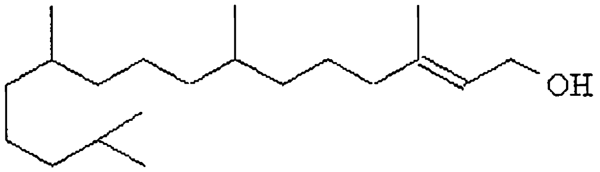
2.2.3 Terpenes

Terpenes form an important class of compounds in *Piper*. Most of the terpenes isolated from *Piper* species are either monoterpenes or sesquiterpenes. These constitute the volatile oils from different plant parts. About 90 constituents belonging to monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes have been characterized from *Piper* species by GC-MS analysis of volatile oils [1, 84-90, 148-150]. Camphene, 3-carene, caryophyllene, caryophyllene oxide, 1,8-cineol, cubebene, ρ-cymene, α-elemene, α-humulene, limonene, linalool, myrcene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, α-thujene and terpenolene are encountered in most of the *Piper* species[1, 84-90, 148-150]. A few uncommon sesquiterpenes have been reported from the genus namely, 1,5-epoxy salvial-4 (14)-ene from *P. obliuqum*, α-selenene and β-selenene from *P. fimbriulatum* [85], ishwarol from *P. amalago* [130] and capentin from *P. capens* [131]. The volatile oil from *P. pierrie* contained two rare esters namely, α-methyl benzylcinnamate and methyl benzylcinnamate [88]. Iswarane and trans-anesole, two unusual sesquiterpene hydrocarbons were identified as the major constituents of *P. fulvescens* leaves [150].

Transphytol **(18)** is the only diterpene isolated from the genus *Piper* [91-93]. Triterpenes reported from the genus are β-amyrin [1], friedelin [64, 94], epifriedelanol [94], ursolic acid [95] and ursolic acid-3β-acetate [95].



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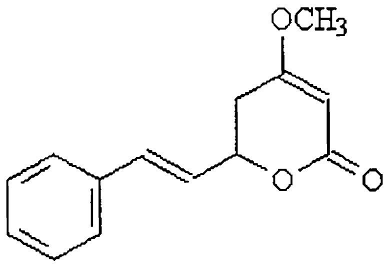
2.2.4 Kawapyrones and piperolides



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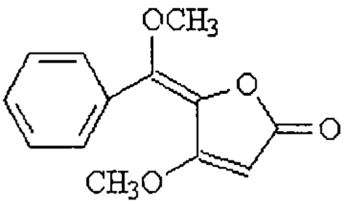
Kawapyrones **(19)** are δ-lactones having styryl and dihydrostyryl substituents. These were originally derived from kawa-kawa (*P. methysticum*) and known as kawa lactones/ pyrones. Besides *P. methysticum* [1, 47, 97-102], the only *Piper* species that has yielded kawalactones is *P. sanctum*[1]. Two 6-substituted 5,6-dihydropyran-2-ones have been isolated from *P. reticulatum* [96].



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Piperolides **(20)** are cinnamalidone butenolides. Only five piperolides have been recorded from *Piper* species namely *P. fadyenii* [103], *P. sanctum*[104-106], *P. aduncum* [107] and *P. hispidum* [107].

2.2.5 Flavonoids

Flavonoids are a group of natural pigments with benzopyrone skeleton and occur as glycosides or aglycones. The flavonoids isolated from *Piper* species may be classified into flavones, flavanones, chalcones and dihydrochalcones.

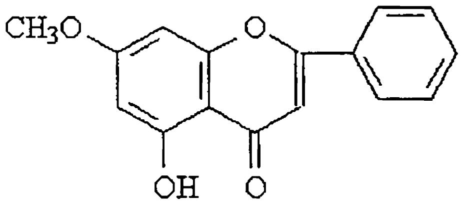
2.2.5.1 Flavones

Although flavones **(21)** are ubiquitous in nature, only a few flavones have been reported from *Piper* species. Most of the flavones isolated are tri or tetra-oxygenated as shown in **Table 2.1**. Flavonols are 3-hydroxyflavones. The flavonols isolated from *Piper* species are indicated in **Table 2.2**.

Mass spectra of flavones give intense molecular ion peaks. The molecular fragments produced by retro-Diels Alder reaction are important in determining the distribution of substituents between A and B-rings [108].

In ^{1}H NMR spectrum the aromatic protons of flavones are observed between δ 6.0-8.0. H-6 and H-8 generally resonate in the ranges δ 6.0-6.4 and δ 6.3-6.9 respectively [108]. H-3 protons also give singlets in this region. Chelated hydroxyl gives a one-proton sharp singlet between δ 12.7-12.9 and methoxyl protons give singlet (3H) in the range δ 3.8-3.9. The signals of B-ring protons are generally observed at lower field than those of A-ring protons.

^{13}C NMR resonances of flavone nuclear carbons occur in the range δ 90-185. Carbonyls and oxygenated aromatic carbons resonate at low field (δ 130-185) where as the resonances for the hydrocarbon and other aliphatic carbons are observed in the range δ 0-110 [108].



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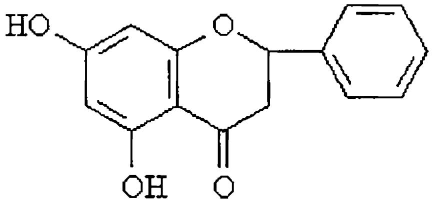
2.2.5.2 Flavanones

Flavanones **(22)** are 2,3-dihydroflavones. The flavanones except sakuranetin isolated from *Piper* species are oxygenated only in A-ring. Sakuranetin isolated from *P. aduncum* has a hydroxyl group in the B-ring. The flavanones isolated from *Piper* species are indicated in **Table 2.3**.



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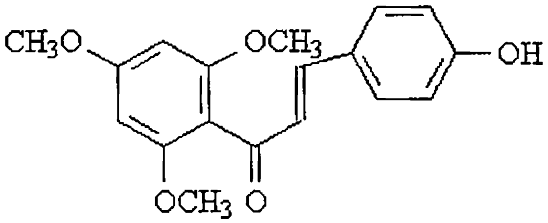
2.2.5.3 Chalcones



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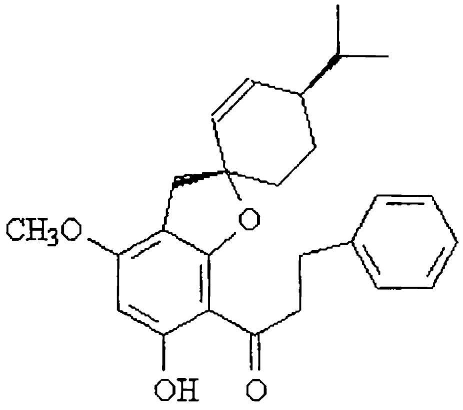
Chalcones **(23)** are characterised by the presence of a three carbon bridge consisting of an α, β-unsaturated carbonyl unit between the phenyl rings. Most of the chalcones from *Piper* are oxygenated at C-4' and C-6' positions. A few unusual monoterpene substituted dihydrochalcones, adunctins **(24)**, were isolated from *P. aduncum* by Orjala *et.al.* . The chalcones isolated from *Piper* species are indicated in **Table 2.4**.



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2.2.6 Miscellaneous compounds

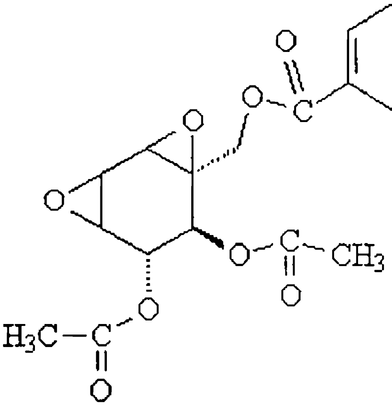
This group of compounds includes sterols, cyclohexanes, aromatic compounds and aliphatic compounds.

2.2.6.1 Sterols

β-Sitosterol occurs in 40 *Piper* species. β-Sitosterol palmitate is reported from *P. betle* leaves [1]. Other sterols reported from the genus are campesterol, cholesterol, cholestanol, daucosterol and stigmasterol [1].

2.2.6.2 Cyclohexanes

Seven oxygenated cyclohexanes namely, crotepoxide **(25)**, pipoxide, pipoxide chlorohydrin, piperonal A, piperonal B, acetyl piperonal A and (+) zeylenol are known to occur in *Piper* species[1]. Among these, crotepoxide is the most common constituent and has been reported from 11 *Piper* species [1].



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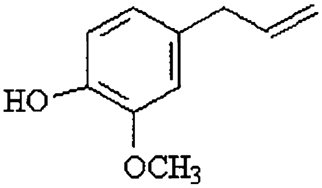
2.2.6.3 Aromatic compounds

Many aromatic organic acids such as benzoic and cinnamic acid derivatives have been isolated from *Piper* species [1]. Recently, several phenylpropanoids **(26)** [65,76], prenylated benzoic acids **(27)** [127-128, 132-133, 162], alkenylphenols [134], stilbenes [110] and hydroxycinnamicacid esters [135] have been recorded from the genus.



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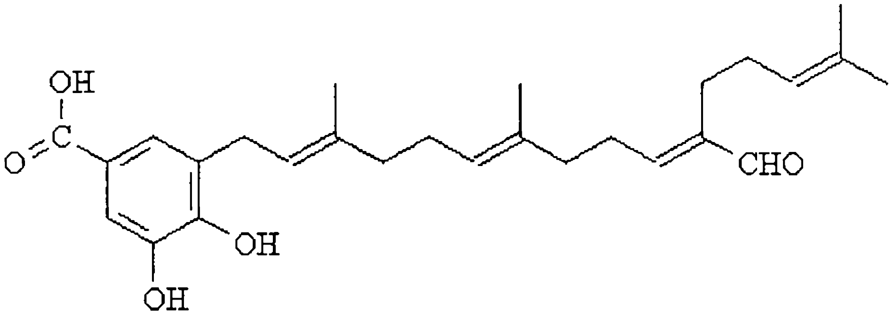

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2.2.6.4 Aliphatic compounds

Aliphatic compounds isolated from *Piper* include hydrocarbons, alcohols, acids, esters [14], aldehydes and ketones [1,136]. n-Triacontane, n-triacontanol, stearic acid and palmitic acid are very common in *Piper* species. Four 1-(3,4-methylenedioxyphenyl) alkanes having linear 10, 11, 12 and 14 carbon atoms were isolated from the roots of *P. darrience* [136].

2.2.7 Biological activity studies

Several compounds from *Piper* are associated with various biological activities [1]. *P. piscatorum* yielded an isobutyl amide piperovatine with piscicidal properties[9]. *N*-[7-(3',4'-methylenedioxyphenyl)-2(*Z*),4(*Z*)-

heptadienoyl]pyrrolidine from *P. hispidum* showed antifungal activity against *Cladosporium sphaerospermum* [38]. Methyl taboganate and 2,2-dimethyl-6-carboxy-chroman-4-one methylester from *P. dilatatum* possessed antifungal property against *Cladosporium cucumerinum*, a plant pathogenic fungus [127]. Piperine is reported to have antipyretic, anti-inflammatory and analgesic activities [137]. Pipericide, dihydropipericide and guineensine isolated from *P. nigrum* were shown to be toxic to male adult adzuki bean weevils [138]. Aduncamide from *P. aduncum* exhibited antibacterial activity against *Bacillus subtilis* and *Micrococcus luteus* [139]. Eugenol isolated from *P. betle* exhibited antifungal activity against *Aspergillus flavus* [141]. Dillapiole isolated from *P. aduncum* showed molluscicidal activity against *Biomphalaria glabrata* [142]. Several benzoic acid derivatives from *Piper* have shown antibacterial and molluscicidal activities [142]. Crotepoxide has been reported to be an antitumour principle [143].

The alkenylphenols from *P. gibbilimbum, namely,* gibbilimbol A [(*E*)-4-(4-decenyl)phenol], gibbilimbol B [(*E*)-4-(3-decenyl)phenol], gibbilimbol C [(*E*)-4-(4-octenyl)phenol] and gibbilimbol D [(*E*)-4-(3-octenyl)phenol] possessed antibacterial and cytotoxic activities [144]. 2*E*, 8*E*-*N*-9-(3,4-methylenedioxyphenyl)- nonadienoylpiperidine isolated from *P. nigrum* was reported to cause dilation of heart in rabbits [140]. Antifungal amides against *Cladosporium sphaerrospermum* have been isolated from *Piper hispidum* and *Piper tuberculatum* [145]. 1,3-benzodioxole-5-(2,4,8-triene-methylnonanoate), 1,3-

benzodioxole-5-(2,4,8-triene-isobutylnonanoate), myristicin, asarinin, sesamin and fargesin isolated from *P. mullesua* possessed antifeedant activity against the fourth instar larvae of *Spilarctia obliqua* [68],[146]. Very recently, amides and prenylated benzoic acids with antifungal activity have been isolated from *P. tuberculatum*, *P. arboreum* [147] and *P. lanceaefolium* [162]. Lanceaefolic acid methylester and pinocembrin chalcone isolated from *P. lanceaefolium*[162] displayed antifungal activity against *Candida albicans* with a minimal inhibitory concentration value of 100 μg/ml in both the cases. 3,4-dihydroxyphenyl-ethanol glucoside and 3,4-dihydroxy-6-(ethylamino)-benzamide isolated from green berries of *P. nigrum* inhibited growth of food-borne bacteria namely, *Salmonella typhimurinum*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* [151].

Table 2.1: Flavones from *Piper*

No.	Compound	Source	Ref.
1	5,7-Dimethoxyflavone	*P. canninum*	110
2	5-Hydroxy-7-methoxyflavone (Tectochrysin)	*P. falconeri* *P. manii* *P. sylvaticum*	111 1 74
3	5-Hydroxy-7,4'-dimethoxyflavone	*P. falconeri* *P. khasiana* *P. manausense* *P. manii* *P. peepuloides* *P. sylvaticum*	111 1 1 1 1,117 112
4	5-Hydroxy-7,3',4'-trimethoxyflavone	*P. peepuloides* *P. sylvaticum*	1,117 74
5	5,3'-Dihydroxy-7,4'-dimethoxyflavone	*P. auritum*	92
6	5,4'-Dihydroxy-7,3'-dimethoxyflavone (velutin)	*P. clarkii*	113
7	5,7,4'-Trihydroxyflavone-8-C-glucoside (Vitexin)	*P. clarkii*	114
8	Marginatoside	*P. marginatum*	114
9	6-C-β-D-Galactopyranosyl-acacetin-7-O-glucoside	*P. brachystachium*	1
10	6-C-β-D-Glucopyranosyl-acacetin-7-O-glucoside	*P. brachystachium*	1
11	5-Hydroxy-7-methoxy-8-C-β-D-glucosyl flavone (kaplanin)	*P. lhotzkyanum*	115

Table 2.2: Flavonols from *Piper*

No.	Compound	Source	Ref.
1	3,5-Dihydroxy-7,4'-dimethoxyflavone	*P. sylvaticum*	116
2	3,5,7,3', 4'-Pentahydroxyflavone-3-O-glucoside (Isoquercitrin)	*P. nigrum*	117
3	3,5,7,3'-Tetrahydroxy-4'-methoxyflavone-3-O-β-D-rutinoside(Isorhamnetin-3-O-β-D-rutinoside)	*P. nigrum*	117
4	3,5,7,4'-Tetrahydroxyflavone-3-O-arabinoside-7-rhamnoside (Kaempferol-3-O-arabinoside -7- rhamnoside)	*P. nigrum*	117
5	3,5,7,4'-Tetrahydroxy flavone-3-O-β-D-glucoside	*P. nigrum*	117
6	3,5,7,3', 4'-Pentahydroxyflavone-3-O-β – D-galactoside (Quercetin 3-O-β-D-galactoside)	*P. nigrum*	117
7	Quercetin-3-O-β-D-rutinoside	*P. nigrum*	117
8	Quercetin-3-O-β-D-rhamnoside	*P. nigrum*	117
9	Rhamnetin-tri-O-glucoside	*P. nigrum*	117

Table 2.3: Flavanones from *Piper*

No.	Compound	Source	Ref.
1	5,7-Dihydroxyflavanone	*P. hostmannianum* *P. steerni*	118 119
2	6-Hydroxy-5,7-dimethoxy flavanone	*P. hispidum*	120
3	8-Hydroxy-5,7-dimethoxy flavanone	*P. hispidum*	120
4	5-Hydroxy-7-methoxy-6,8-dimethylflavanone	*P. hostmannianum*	118
5	5-Hydroxy-7-methoxyflavanone (pinostrobin)	*P. methysticum* *P. aduncum* *P. fadyenii* *P. hispidum* *P. steerni*	122,123 107,121 107 121, 119
6	5,4'-Dihydroxy-7-methoxyflavanone (sakuranetin)	*P. aduncum* *P. lhotzkyanum*	124 115
7	5,7,8-Trimethoxyflavanone	*P. hispidum*	120
8	7-Hydroxy-5-methoxy-flavanone (alpinetin)	*P. methysticum*	122, 123

Table 2.4: Chalcones and dihydrochalcones from *Piper*

No.	Compound	Source	Ref.
1	2',3'-Dihydroxy-4',6'-dimethoxychalcone	*P. hispidum*	120
2	2'-Hydroxy-4,4',6'–trimethoxychalcone (Flavokawain A)	*P. methysticum*	124-126
3	2'-Hydroxy-4',6'–dimethoxychalcone (Flavokawain B)	*P. methysticum* *P. dilatatum*	124-126 127
4	4,2'-Dihydroxy-4',6'–dimethoxychalcone (Flavokawain C)	*P. methysticum* *P. murrayanum*	122, 124-126 128
5	2'-Hydroxy-2,4',5'-trimethoxychalcone	*P. hispidum* *P. dilatatum*	120 127
6	2',4'-Dihydroxy-6'-methoxychalcone (alpinetin chalcone)	*P. dilatatum* *P. methysticum*	127 1,5, 123
7	Adunctin A	*P. aduncum*	93
8	Adunctin B	*P. aduncum*	93
9	Adunctin C	*P. aduncum*	93
10	Adunctin D	*P. aduncum*	93
11	Adunctin E	*P. aduncum*	93

(Table 2.4 contd...)

No.	Compound	Source	Ref.
12	2',6'-Dihydroxy-4'-methoxydihydrochalcone	*P. longicaudatum*	118
13	4,2',6'-Trihydroxy-4'-methoxydihydrochalcone (asebogenin.)	*P. longicaudatum*	118
14	Piperadunctin B	*P. longicaudatum*	118
15	Longicaudatin	*P. longicaudatum*	118
16	2'-Hydroxy-3',4',6'-trimethoxychalcone	*P. hispidum*	120
17	Piperadunctin A	*P. aduncum*	129
18	Piperadunctin B	*P. aduncum*	129
19	Piperadunctin C	*P. aduncum*	129

2.3 Present work

Piper colubrinum Link (Piperaceae) is a multiple disease resistant species, native of Brazil[152]. *P. colubrinum* is valuable for its unique genetic make up, which renders it resistant to the *Phytophthora*-nematode complex causing *Phytophthora* foot-rot disease in black pepper [153-154]. Hence *P. colubrinum* has immense potential as the donor parent in breeding programme for the improvement of the cultivated species *P. nigrum*. A thorough literature survey revealed that no phytochemical investigation of this plant has been done. Hence a systematic chemical investigation was undertaken to study the chemistry of *P. colubrinum* leaves.

2.4 Experimental details

2.4.1 Materials and methods

Melting points are uncorrected. Silica gel (60-120 mesh) of E-Merck was used for column chromatography. Silica gel-G containing 13% calcium sulphate as binder was used for TLC. The plates were dried at room temperature for 12 h, activated in an air oven at 120 ^{0}C for 20 minutes. Samples for analysis were routinely dried under vacuum. UV spectra were recorded on Shimadzu UV-160A spectrometer. IR spectra were recorded as KBr pellets on Shimadzu FT-IR- 8101A spectrometer. ^{1}H NMR spectra were recorded using Brucker 300 MHz spectrometer in $CDCl_3$, CD_3COCD_3 or DMSO-d_6 and ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts are in ppm (δ values) using TMS as internal standard. EI mass spectra were recorded by means of direct insertion probe at ionisation energy of 70 eV.

2.4.2 Extraction

The leaves of *P.colubrinum* were procured from the Indian Institute of Spices Research farm at Calicut. A voucher specimen of the sample is available at the herbarium of I.I.S.R Calicut. Shade dried and powdered leaves (1 kg) were extracted successively with hexane (60-80 ^{0}C), chloroform and methanol in a soxhlet apparatus for 30h in each case. The extracts were separately concentrated to dryness under reduced pressure. The dark green residue obtained in each case was subjected to column chromatography over silica gel.

The dark residue from hexane extract of the leaves showed three prominent spots on TLC. Their separation and purification are discussed below.

2.4.3 Chromatographic separation of hexane extract

The dark green residue (20 g) from the hexane extract of the leaves was dissolved in 50 ml of hexane and transferred to a column of silica gel (500 g) set up with hexane. The column was eluted successively with hexane, hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (50:50) and finally with ethyl acetate (100%). Fractions of 100 ml were collected and concentrated. These fractions were monitored by TLC and similar fractions were combined and grouped as shown in **Table 2.5.**

Groups I-II

No crystalline compound could be obtained from these fractions by repeated crystallisation in different solvents. Hence it was not examined further.

Group III

The fractions, 25-41 were combined and concentrated. The white solid separated was filtered and recrystallised from ethyl acetate. It was designated as compound I (23 mg), R_f 0.84 (hexane: ethyl acetate 95:5).

Group IV

TLC examination of these fractions in different solvent systems revealed non-homogeneous behaviour and no crystalline compound could be isolated by repeated crystallisation. Hence further isolation was discontinued.

Group V

The fractions, 49-56 when concentrated gave a white solid, which was recrystallised from ethyl acetate. It was designated as compound II (21 mg), R_f 0.56 (hexane: ethyl acetate, 80:20).

Groups VI-VIII

The fractions from these groups showed heterogeneous behaviour on TLC and no crystalline material could be isolated by repeated crystallisation.

Table 2.5: Fractionation of hexane extract

Eluant	**Fraction No.**	**Group No.**	**Compd.**
Hexane	1-17	I	-
Hexane-ethyl acetate 95:5	18-24	II	-
	25-41	III	I
	42-48	IV	-
Hexane-ethyl acetate 90:10	49-56	V	II
	57-70	VI	-
Hexane-ethyl acetate 80:20	71-84	VII	-
Hexane-ethyl acetate 50:50	85-95	VIII	-
Ethyl acetate (100%)	96-102	IX	III

Group IX

The fractions, 96-102 upon concentration yielded a white solid, which was separated and recrystallised from ethyl acetate to give compound III (9 mg), R_f 0.54 (chloroform: methanol 90:10).

Compound I

Compound I was crystallized from ethyl acetate as white solid, (23 mg), R_f 0.84 (hexane – ethyl acetate 95:5), mp 92 ^{0}C

IR ν_{max} (KBr):

3410 (OH), 2928, 2858, 1710(CO), 1471, 939, 724 cm^{-1}

EIMS (70eV) : m/z (rel. int.)

Relative abundance below 10% not given.

452[M^+], 424, 407, 392, 382, 369(15), 354, 340, 326, 99(21), 87(11), 85(35), 73(91), 57(93), 55(51), 43(100), 41(45).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)

2.37 (2H, t, *J* = 7.3 Hz), 1.63 (4H,m), 1.25 (50H,br s), 0.86 (3H, t, *J* = 6.6 Hz)

^{13}C NMR (75MHz, $CDCl_3$): δ (ppm)

179.63, 33.99, 31.94, 29.71, 29.61, 29.45, 29.38, 29.25, 29.08, 24.70, 22.71, 14.12.

Compound II

Compound II was obtained as colourless needles from ethyl acetate, (21mg), R_f 0.56 (hexane-ethyl acetate, 80:20), mp 136 ^{0}C,undepressed on admixture with an authentic sample of β-sitosterol . It gave positive Liebermann-Burchard reaction (violet-blue-green) for steroids.

Acetylation:

Compound II (10 mg) was taken in pyridine (1ml) and treated with freshly distilled acetic anhydride (1ml) and left at room temperature for 48 h. The reaction mixture was worked up in the usual manner. The residue was separated and recrystallised from methanol as colourless needles (8mg),mp 125 ^{0}C.

Compound III

White amorphous powder (9 mg), R_f 0.54 (chloroform: methanol, 90:10), mp 278 ^{0}C .

IR ν_{max} (KBr):

3440(OH), 2980, 2815, 1470, 1465, 1385, 1381, 1070,1023cm^{-1}

FAB-MS:

599(M^+ + Na)

EIMS 70eV: m/z (rel. int.):

Relative abundance below 10% not given.

414(10), 396(11), 383(12), 255, 213, 192, 182, 136(35), 92 (100).

^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)

5.33 (1H, m, H-6), 4.87 (1H, br s, H-6'α), 4.43 (2H, br d, H- 1', H-6'β), 4.22(1H, d, *J* = 8 0 Hz, H-3'), 3.63(1H, m, H-3), 3.51(1H, m, H-4'), 3.04(1H, m, H-2'), 3.01(1H, m, H-5'), 2.84-1.00 (CH_2), 0.93 (3H,s, H-19), 0.87 (3H, d, *J* = 6.3Hz, H-26), 0.80 (3H, br s, H-21) , 0.78 (3H, br s, H-29), 0.76 (3H, d, *J* = 6.3 Hz, H-27), 0.65 (3H, s, H-18).

^{13}C NMR (75MHz, DMSO-d_6): δ(ppm)

140.97, 121.74, 101.29, 77.45, 77.26, 73.99, 70.62, 61.62, 56.70, 55.94, 50.13, 45.39, 42.37, 38.82, 37.35, 36.74, 36.00, 33.87, 31.94, 29.78, 29.23, 28.31, 25.96, 24.38, 23.13, 21.11, 20.24, 19.62, 19.46, 19.14, 12.30, 12.19.

2.4.4 Chromatographic separation of chloroform extract

The residue (12 g) from the chloroform extract of *Piper colubrinum* leaves indicated two yellow spots on TLC. The residue was dissolved in diethyl ether, adsorbed on 20 g silica gel and the powder was chromatographed on a silica gel (500 g) column. The column was eluted with hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (70:30), hexane-ethyl acetate (50:50) and ethyl acetate (100%). Fractions of 100 ml were collected, concentrated and similar fractions as monitored by TLC were combined and grouped as indicated in **Table 2.6**

Groups I-III

These fractions, when concentrated yielded a waxy material, which showed heterogeneous nature on TLC. No crystalline material could be isolated from these fractions by repeated crystallisation. Hence it was not examined further.

Group IV

The fractions, 29-50 were combined and concentrated to give a yellow solid. It was separated and recrystallised from ethyl acetate as pale yellow needles (96 mg). It was designated as compound IV., R_f 0.80 (chloroform : methanol 95:5), mp 285 ^{0}C.

Group V

The fractions, 51-65 were combined and concentrated to give a yellow solid, which was separated and recrystallised from ethyl acetate .It was designated as compound V (47 mg), R_f. 0.6 (chloroform : methanol, 95:5), mp 258 ^{0}C .

Groups VI-VII

These fractions did not give any crystalline material upon repeated crystallisation from different solvents and suggested waxy nature. It was not examined further.

Table 2.6: Fractionation of Chloroform extract

Eluant	Fraction No.	Group No.	Compd.
Hexane	1-10	I	-
Hexane-ethyl acetate 95:5	11-20	II	-
Hexane-ethyl acetate 90:10	21-28	III	-
Hexane-ethyl acetate 80:20	29-50	IV	IV
Hexane-ethyl acetate 70:30	51-65	V	V
Hexane-ethyl acetate 50:50	66-77	VI	-
Ethyl acetate (100%)	78-89	VII	-

Compound IV

Compound IV was obtained as yellow needles (96 mg) from ethyl acetate, R_f 0.80 (chloroform:methanol, 95:5), mp 285 ^{0}C.

UV λ_{max} nm

MeOH: 268,335nm

$AlCl_3$: 276, 300, 345, 380

$AlCl_3$ + HCl: 277, 300, 340, 380

NaOAc: 267, 336

NaOAc+H_3BO_3: 267, 336

IR ν_{max} (KBr):

3439(OH), 2928, 1667(CO), 1606,1498 cm^{-1}

EIMS (70eV): m/z (rel. int.):

Relative abundance below 10% not given.

284$[M]^+$ (75), 255 (28), 241(32), 212, 167, 166, 157, 138 (42), 118(50), 95(100), 69(90).

^{1}H NMR (300 MHz, acetone-d_6): δ(ppm)

12.99 (chelated OH), 9.62 (OH), 7.96 (2H, d, *J* =8.6 Hz, H-2', 6'), 7.02 (2H, d, *J* =8.6 Hz, H-3', H-5'), 6.71(1H, s, H-8), 6.69 (1H, s, H-3), 6.34 (1H, s, H-6), 3.94 (3H, s, OCH_3)

^{13} C NMR (75MHz, DMSO-d_6): δ(ppm)

181.83 (C-4), 165.04 (C-7), 163.98 (C-2), 161.21 (C-4'), 161.10 (C-9), 157.14 (C-5), 128.47 (C-2', 6'), 120.95 (C-1'), 115.88 (C-3', 5'), 104.57 (C-10), 102.92 (C-3), 97.86 (C-6), 92.60 (C-8), 55.95 (OCH_3)

Compound V

Compound V was obtained as yellow amorphous powder (47 mg) from ethyl acetate, R_f 0.60 (chloroform : methanol, 95:5), mp 258 ^{0}C .

UV λ_{max} nm

MeOH: 255, 266sh, 348nm

$AlCl_3$: 272, 295sh, 345,380sh

$AlCl_3$ +HCl: 277, 300sh, 340, 380sh

IR ν_{max} (KBr):

3415, 2921, 1664, 1606,1452, 1042 cm^{-1}

EIMS 70eV: m/z (rel. int.):

Relative abundance below 10% not given.

300 (100) $[M]^+$, 272 (33), 258 (21), 168 (18), 138 (14), 133 (11), 95 (30), 69 (30), 43 (29).

^1H NMR (300 MHz, acetone-d_6): δ(ppm)

12.99 (chelated OH), 7.98(OH), 7.96 (OH), 7.0 (1H, d, J = 8.1Hz), 7.52 (1H, m) and 7.48(1H,d) merged and appeared as a doublet, 6.69 (1H, br s), 6.63 (1H, br s), 6.33 (1H, br s), 3.94 (3H, s)

^{13}C NMR (75MHz, DMSO-d_6): δ(ppm)

181.72, 165.02, 164.16, 161.10, 157.11,149.77, 145.68, 121.27, 119.00, 115.87, 113.38, 104.55, 102. 93, 97.85, 92.48, 55.93.

2.4.5 Methanol extract of *Piper colubrinum* leaves

Methanol extract of *Piper colubrinum* leaves did not show any interesting spots on TLC. Hence it was not examined further.

2.5 Results and discussion

2.5.1 Hexane extract

The dark brown residue from the hexane extract of *P. colubrinum* leaves yielded three compounds. The bar diagram for their separation is given in **Chart 2.1.**

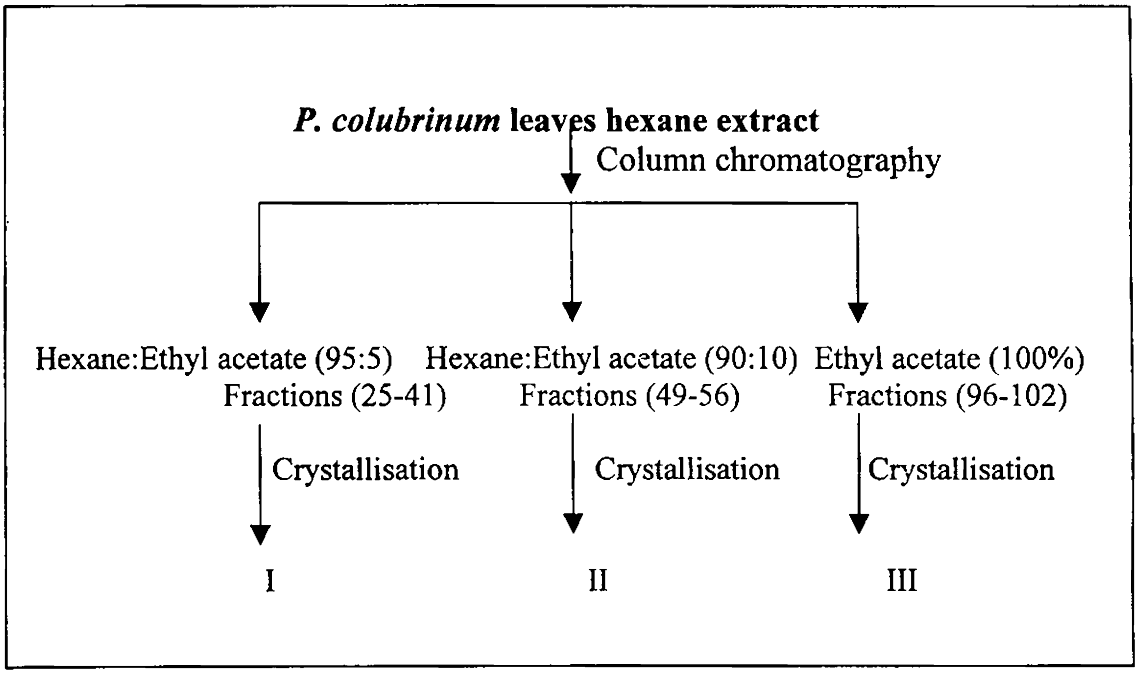


Chart 2.1

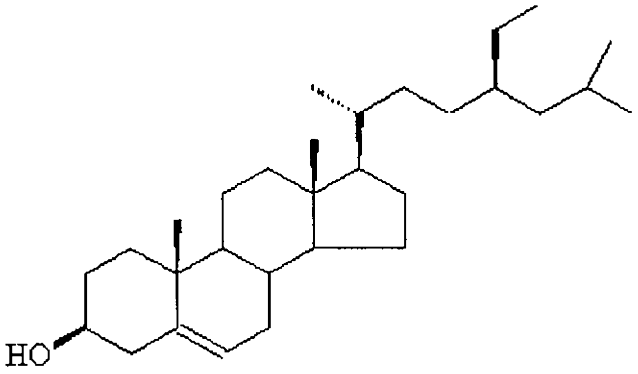
Structure of compound I

Compound I was obtained as white solid from ethyl acetate, mp 92 ^{0}C . The mass spectrum displayed molecular ion peak at m/z 452 corresponding to the molecular formula $C_{30}H_{60}O_2$. IR spectrum indicated carbonyl and hydroxyl absorptions at 1710 and 3410 cm^{-1} respectively. A two-proton triplet centered on δ 2.37 (J = 7.3 Hz) in the ^{1}H NMR spectrum was indicative of the methylene protons adjacent to the carbonyl group. It showed a methyl triplet at δ 0.86 (3H, t, *J* = 6.6 Hz) for the terminal methyl. A broad singlet at δ 1.25 (50 H) and a multiplet centered on δ 1.63 (4H) were assigned to the methylene protons. The absence of (M^+-15) ion together with the consecutive loss of 14 and 28 mass units suggested it to be a straight chain aliphatic compound [155, 156]. Its mass spectrum was identical with that of n-triacontanoic acid reported in literature (lit. mp 94 ^{0}C) [157]. Hence it was identified as **n- triacontanoic acid** , whose structure is given below.

$$CH_3(CH_2)_{28}COOH$$

Structure of compound II

Compound **II** was recrystallised as colourless needles from ethyl acetate , mp 136 ^{0}C. It was identified as **β-sitosterol (28)** by direct comparison of its mp (m mp 136 ^{0}C) [163], super imposable IR and co-TLC with an authentic sample of β-sitosterol. Prepared its acetate, mp 125 ^{0}C and was found to be identical with the melting point of β-sitosterol acetate (lit. mp 127 ^{0}C) [159].



(28) R=H **(29)** R=Glc

Structure of compound III

Compound III was obtained as white amorphous powder from chloroform-methanol, mp 278°C. It gave positive reaction to Liebermann-Burchard test for steroids and Molish test for sugars. Its IR spectrum showed the presence of strong absorptions at 3401 and 1070 cm^{-1} characteristic of a glycoside. The FAB mass spectrum gave a quasimolecular ion at m/z 599 (M^{+} + Na), which suggested the molecular weight to be 576. EI mass spectrum of compound III showed a molecular fragment at m/z =396 due to the loss of glucose moiety from the molecular ion . The ^{13}C NMR spectrum showed 34 carbon atoms in the molecule. An anomeric signal at δ 100.29 indicated the presence of a single monosaccharide moiety. ^{13}C-DEPT NMR spectrum revealed the presence of six methyl, 12 methylene and 14 methine carbon atoms. Four methine carbon resonances at δ 70.62, 77.26, 73.99, 77.26 and one methylene carbon at δ 61.62 were assigned to C-2', C-3', C-4', C-5'and C-6' respectively.[158] The olefinic signals at δ 140.97 and δ 121.74 corresponded to C-5 and C-6 of sterol moiety. ^{1}H NMR spectrum displayed 2 tertiary methyls [δ 0.65 (3H,s, H-18) and δ 0.93 (3H,s, H-19)] and four secondary methyls [δ 0.87 (3H,d, *J* = 6.3 Hz, H-26), δ 0.80 (3H, br s, H-21), δ 0.78 (3H,br s, H-29) and δ 0.76 (3H,d, *J* = 6.3 Hz, H-27)]. The protons of the sugar moiety were observed between δ 3.05

and δ 4.88. All other peaks in the ^{1}H NMR spectrum were identical with that of β-sitosterol reported earlier[159].

Table 2.7: ^{13}C NMR assignments of compound III

C.No.	δ_C	C.No.	δ_C	C.No.	δ_C	C.No.	δ_C
1	37.35	11	21.11	21	19.14	2’	70.62
2	33.87	12	38.82	22	28.31	3’	77.26
3	77.45	13	42.37	23	25.96	4’	73.99
4	36.74	14	56.70	24	45.39	5’	77.26
5	140.97	15	24.38	25	29.23	6’	61.62
6	121.74	16	29.78	26	20.23		
7	31.94	17	55.94	27	19.46		
8	31.94	18	12.30	28	23.13		
9	50.13	19	19.62	29	12.19		
10	-	20	36.00	1’	101.29		

Hydrolysis of compound III with 10% sulphuric acid gave an aglycone, which was identified as β-sitosterol by direct comparison with an authentic sample of β-sitosterol. Hence compound III was identified as **sitosterol-3-O-β-D-glucopyranoside (29)**, (lit.mp 283-286 °C)[163].

2.5.2 Chloroform extract

Two crystalline compounds obtained from the chloroform extract of *Piper colubrinum* leaves were designated as compounds IV and V. The bar diagram for their separation is given in **Chart 2.2**.

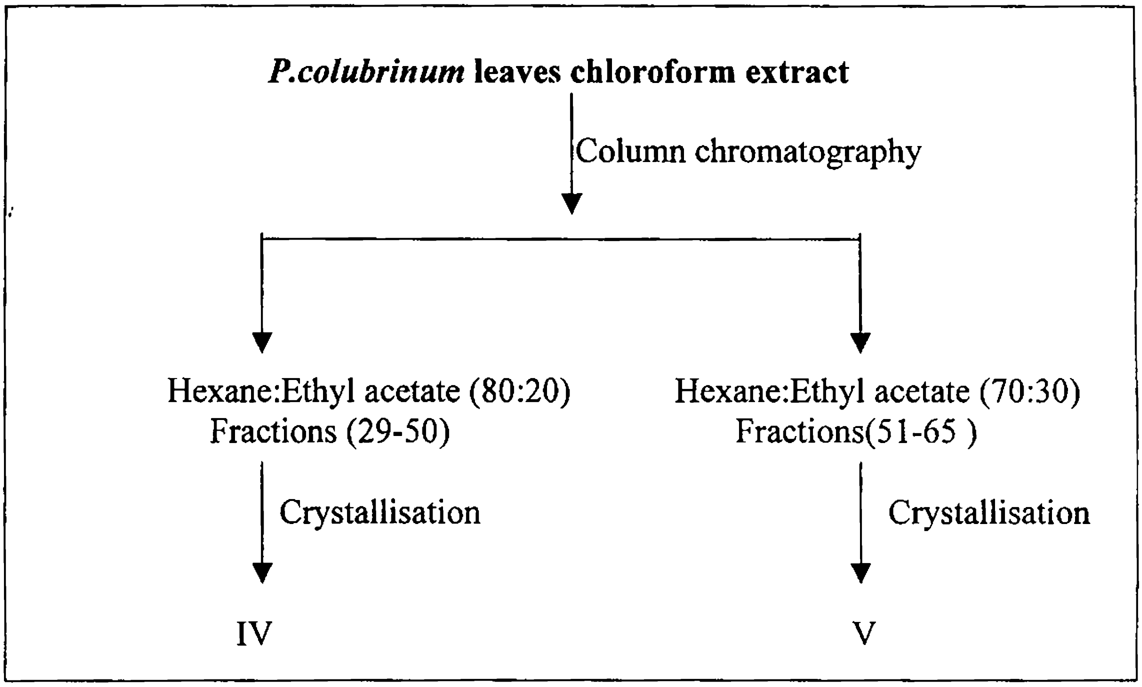
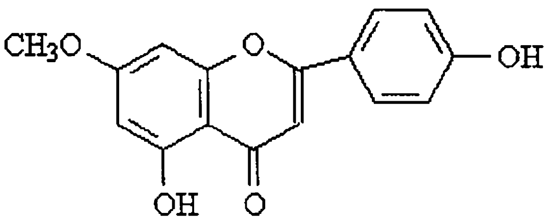


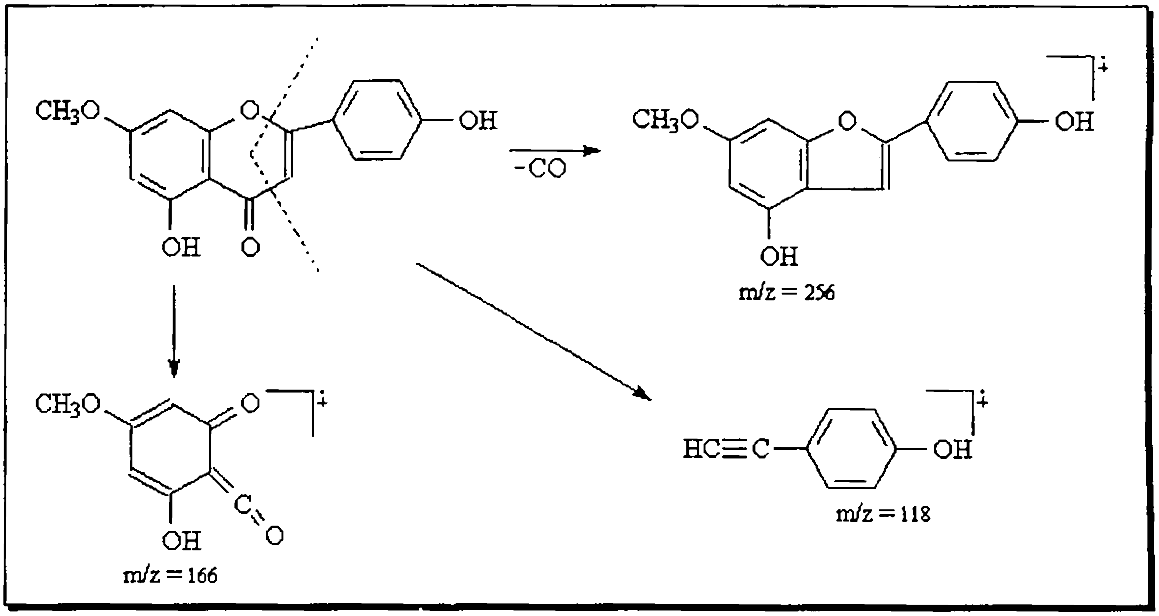
Chart 2.2

Structure of compound IV

Compound IV was recrystallised as yellow needles from ethyl acetate,mp 285 ^{0}C. The mass spectrum indicated molecular ion peak at the m/z 284 corresponding to the molecular formula $C_{16}H_{12}O_5$. It gave positive reaction to Mg-HCl test for flavonoids. IR spectrum showed carbonyl and hydroxyl absorptions at 1667 cm^{-1} and 3439 cm^{-1} respectively. ^{13}C NMR spectrum indicated 16 carbons in the molecule. A sharp one-proton singlet at δ 12.99 in the ^{1}H NMR spectrum was characteristic of a chelated hydroxyl (OH-5) and a three-proton singlet at δ 3.94 revealed the presence of a methoxyl group. A pair of doublets at δ 7.96 (2H,d, *J* =8.6 Hz) and δ 7.02 (2H, d, *J* = 8.6 Hz) were characteristic of H-2', H-6', H-3' and H-5' respectively of the 1,4-disubstituted aromatic ring system [108]. The one-proton singlet at δ 6.69 was assigned to H-3 of the flavone. Two one-proton broad singlets at δ 6.34 and δ 6.71were assigned to the meta-coupled protons at H-6 and H-8 respectively [109]. The molecular fragments at m/z 118,166 and 167 in the mass spectrum, formed by the retro Diels Alder fragmentation of flavones, suggested that the methoxyl group was in ring A and the hydroxyl in ring B [108]. The UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectra of compound IV were identical with that of 5,4'-dihydroxy-7-methoxy-flavone reported earlier (lit. mp 286°C)[160,108,109]. Hence compound IV was identified as **5,4'-dihydroxy-7-methoxy-flavone**. This is the first report of 5,4'-dihydroxy-7-methoxy-flavone from *Piper* genus.



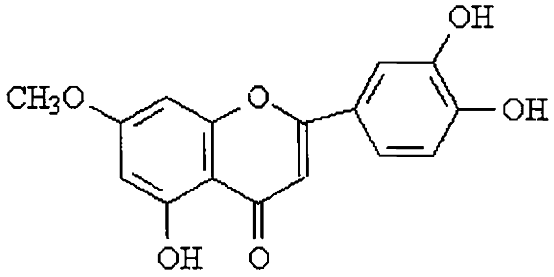
(30)



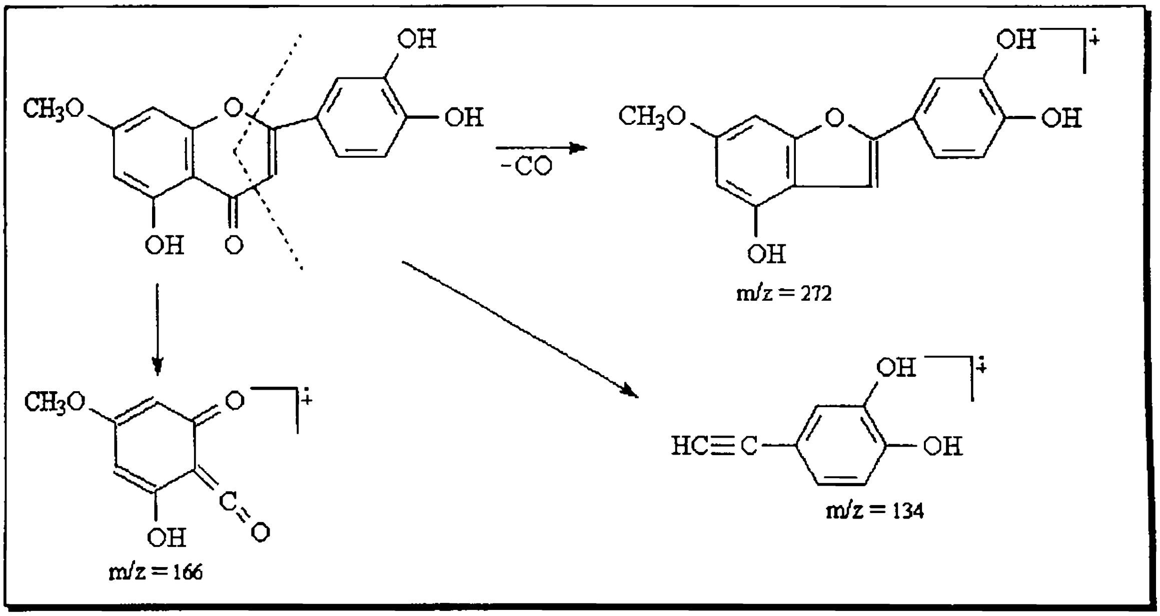
Scheme 2.1: Mass spectral fragmentation of compound IV

Structure of compound V

The compound V was obtained as yellow amorphous powder from ethyl acetate mp 258 ^{0}C . The mass spectrum of compound V displayed molecular ion peak at m/z 300 which accounted for the molecular formula $C_{16}H_{12}O_6$. IR spectrum indicated hydroxyl and carbonyl absorptions at 3415 cm^{-1} and 1664 cm^{-1} respectively. It gave positive reaction to Mg-HCl test for flavonoids. ^{13}C NMR spectrum indicated sixteen carbon atoms in the molecule. ^{1}H NMR spectrum showed a sharp one-proton singlet at δ 12.99 for a chelated hydroxyl (H-5) and a three-proton singlet at δ 3.94 for a methoxyl group. It also showed two hydroxyls at δ 7.98 and δ 7.96. The one-proton singlet at δ 6.63 was assigned to H-3. The one-proton broad singlets at δ 6.33 and δ 6.69 were assigned to the meta-coupled protons H-6 and H-8 respectively. The one-proton mutiplet at δ 7.52 (1H, H-6') [illegible] δ 7.48 (1H, H-2') and δ 7.0(1H,d, *J* = 8.1 Hz, H-5') were assigned to the protons of 1, 3, 4-trisubstituted benzene ring[161]. The typical retro Diels Alder fragments at m/z 133, 134,166 and 168, in the EI mass spectrum revealed that the methoxyl group was in ring-A and two hydroxyls in ring B of the flavone. The, IR, UV, ^{1}H NMR , ^{13}C NMR and mass spectra of compound V were identical with that of 5, 3', 4'-trihydroxy-7-methoxy flavone reported earlier (lit. mp 261°C) [161,108,109]. Hence compound V was identified as **5, 3', 4'-trihydroxy-7-methoxy flavone (31)**. This is the first report of this flavone from the genus *Piper*.



(31)



Scheme 2.2 : Mass spectral fragmentation of V

2.6. References

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Chapter 3

Crystalline constituents from Mussaenda frondosa L.

Crystalline constituents from *Mussaenda frondosa* L.

3.1 Introduction

The genus *Mussaenda* (Rubiaceae) consisting of shrubs and rarely herbs is distributed chiefly in tropical and subtropical regions of the world. About 15 species occur in India; a few of which are ornamental and cultivated in gardens. Some species are reputed for their medicinal value. The leaves and roots of *M. glabra* are used to relieve cough; its flowers are reported to possess diuretic properties and used in asthma, recurrent fevers and dropsy [1]. Various parts of *M. macrophylla* are used to treat persons with sore mouths and sore throats in Nepal [2]. In Chinese folk medicine *M. pubescens* is used as diuretic, antiphlogistic and antipyretic [3]. It is also used to detoxify mushroom poisoning and to terminate early pregnancy [4].

3.2 Chemistry of genus *Mussaenda* - An overview

An extensive survey of literature on phytochemicals of *Mussaenda* indicated that most of the studies were centered on *M. pubescens.* The compounds so far isolated from *Mussaenda* belong to the following groups.

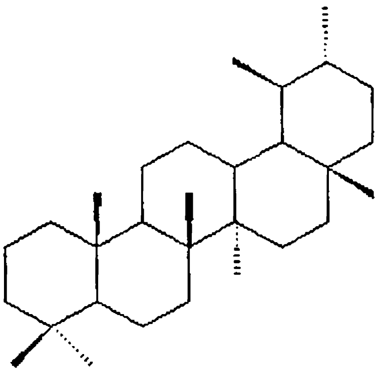
1. Triterpenoids
2. Flavonoids
3. Miscellaneous compounds

3.2.1. Triterpenoids

Triterpenoids form the largest class of compounds isolated from *Mussaenda.* These occur in *Mussaenda* either in the free state or as saponins. The saponins of *Mussaenda* are generally called mussaendosides. Mussaendosides yield the respective aglycones on hydrolysis. These are identified by spectral studies. NMR spectroscopy, particularly ^{13}C NMR, offers the most precise technique in the elucidation of structure of triterpenoids. Triterpenoids isolated from *Mussaenda* belong to ursanes **(1)**, oleananes **(2)** and cycloartane **(3)** groups. The triterpenoids are formed through the cyclisation of their precursor, squalene-2, 3-epoxide [5].

3.2.1.1 Ursanes

Most of the ursanes found in *Mussaenda* are reported from *M. pubescens.* These are derivatives of urs-12-en-28-oic acid as well as their glucosides. The glucosides are identified by hydrolysis with HCl into aglycones and glucose. The number of glucose moieties is revealed by the number of anomeric signals in the ^{1}H NMR spectrum and ^{13}C NMR spectrum. The glucose units are linked through hydroxyl bonds or ester bonds to the aglycones by β-linkage. The β-glucosidic linkages are cleaved by hydrolysis using the enzyme emulsin. The ursanes isolated from *Mussaenda* are indicated in **Table 3.1**.



(1)

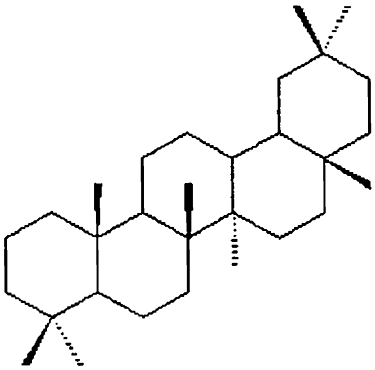
Table 3.1: Ursanes from *Mussaenda*

No.	Compound	Source	Ref.
1	3β-Hydroxy-urs-12-en-28-oic acid (ursolic acid)	*M. pubescens*	6
2	3β,23-Dihydroxy-urs-12-en-28-oic acid	*M. pubescens*	7
3	3β,19α,23-Trihydroxy-urs-12-en-28-oic acid (Rotundic acid)	*M. pubescens,* *M. macrophylla*	7,8
4	3β,19α, 24-Trihydroxy-urs-12-en-28-oic acid	*M. pubescens*	7
5	3β,19α, 23, 24-Tetrahydroxy-urs-12-en-28-oicacid (Clethric acid)	*M. pubescens*	7
6	3β, 19α-Dihydroxy- urs-12-en-24-oic acid - 28-O-β -D-glucopyranoside (Ilex saponin A)	*M. pubescens*	7
7	3β-O-β-D-Glucopyranosyl-19α-hydroxy-urs-12-en-24-oicacid-28-O-β-D-glucopyranoside (Mussaendoside R)	*M. pubescens*	9
8	3β, 19α-Dihydroxy-urs-12-en-24,28-dioicacid-24,28-di-O-β-D-glucopyranoside (Mussaendoside V)	*M. pubescens*	7
9	3β-O-β-D-Glucopyranosyl-urs-12-en- 27,28-dioicacid-28-O-β -D-glucopyranoside	*M. pubescens*	9
10	3-O-β-D-Glucopyranosyl (1→2)O-β-D-gluco pyranosyl-3β,19α-dihydroxy-urs-12-en-28-oic acid-28-O-β-D-glucopyranoside	*M. pubescens*	10

3.2.1.2 Oleananes

Majority of oleananes are isolated from *M. macrophylla.* Glucosides as well as rhamnosides of olean-12-enes have been reported from *M. macrophylla.* In ^{1}H NMR spectrum, glucosyl and rhamnosyl protons give signals between δ 4.0-5.6 and δ 1.84-6.16 respectively. The corresponding carbon signals are observed between δ 62.0-105.1 and δ 18.9-101.9 respectively.[8]

In ^{1}H NMR spectrum, all methyls of oleananes are observed as singlets while two methyls in ursanes (H-29 and H-30) give doublets. Olean-12-enes and urs-12-enes can be unambiguously distinguished by the chemical shift values of C-12 and C-13 by ^{13}C NMR spectrum. The signals for C-12 and C-13 appear at δ 120 and δ 144 in olean-12-enes while the corresponding signals are at δ 125 and δ 140 in urs-12-enes [8]. The oleananes occurring in *Mussaenda* are given in **Table 3.2.**



(2)

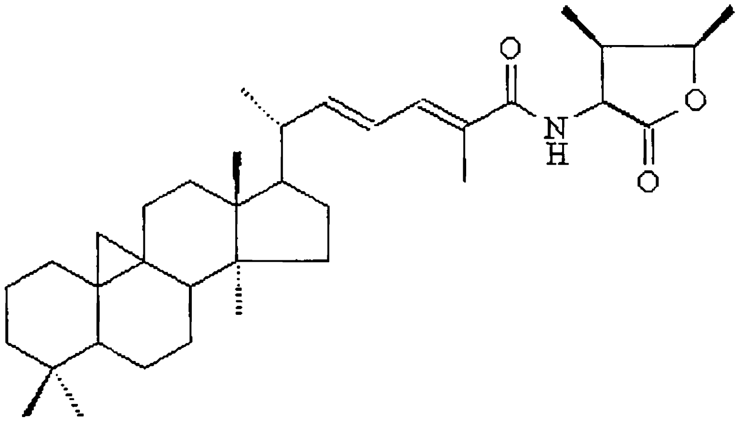
Table 3.2: Oleananes from *Mussaenda*

No.	Compound	Source	Ref.
1	3-O-Acetyl-6β-hydroxy-olean-12-ene	*M. macrophylla*	8
2	3-O-Acetyl-olean-12-en-28-oic acid	*M. macrophylla*	8
3	2α,3β,19α-Trihydroxy-olean-12-ene-28-oic acid (arjunolic acid)	*M. pubescens*	11
4	2β,3β,6β,16α,23-Pentahydroxy-olean-12-en-28-oic acid (16α-hydroxyprotobassic acid)	*M. macrophylla*	8
5	28-O-β-D-Glucopyranosyl-16α-hydroxy-23-deoxyprotobassic acid	*M. macrophylla*	8
6	3-O-β-D-Glucopyranosyl-28-O-α-L-rhamno pyranosyl-16α-hydroxy-23-deoxyprotobassic acid	*M. macrophylla*	8
7	3-O-β-D-Glucopyranosyl-28-O-α-L-rhamno pyransoyl-1 6α-hydroxyprotobassic acid	*M. macrophylla*	8
8	3-O-α-L-Rhamnopyranosyl (1→2)-O-β-D-gluco pyranosyl oleanolic acid-28-O-β-D-glucopyranosyl ester (Mussaendoside J)	*M. pubescens*	10
9	3β,19α-Dihydroxy-olean-12-en-24, 28-dioic acid-24,28-di-O-β-D-glucopyranoside (Mussaendoside K)	*M. pubescens*	12
10	3β-O-β-D-Glucopyranosyl-olean-12-en-27, 28-doicacid-28-O-β-D-glucopyranoside (Mussaendoside S)	*M. pubescens*	9,13

3.2.1.3 Cycloartanes

Majority of cycloartane type triterpenes from *Mussaenda* contains an α-amino-γ-lactone in an amide form in the side chain. These, on acid hydrolysis with 2N-H_2SO_4/C_6H_6 (1:1) yield a common aglycon namely, heinsiagenin-A which is *N*-[(2*S*,3*R*,4*R*)-3-methyl-4-pentanolid-2-yl]-3-β-hydroxy-9,19-

cyclolanosta-(22*E*,24*E*)-dien-27-amide [17]. ^{1}H NMR spectrum of heinsiagenin-A displays the characteristic proton signals at δ 5.6 (1H, dd, H-22), δ 6.4 (1H, dd, H-23) and δ 7.3 (1H, d, H-24) for the conjugated diene moiety; δ 9.15 (1H,d) for amide; δ 0.22 (1H,d) and δ 0.46 (1H,d) for cyclopropyl methylenes [11]. In ^{13}C NMR spectrum, the olefinic carbons appear at δ 148 (C-22), δ 123.5 (C-23), δ 134.8 (C-24) and δ 129 (C-25) [11]. The cycloartanes isolated from *Mussaenda* are listed in **Table 3.3.**



(3)

Table 3.3: Cycloartanes from *Mussaenda*

No.	Compound	Source	Ref.
1	HeinsiageninA-3-O-β-D-glucopyranosyl(1→2)O-β-D-glucopyranoside (Mussaendoside D)	*M. pubescens*	13
2	HeinsiageninA-3-O-β-D-glucopyranosyl(1→6)-3-O-β-D-glucopyranosyl(1→2)-O-β-D-glucopyranoside (Mussaendoside E)	*M. pubescens*	13
3	18-Hydroxyheinsiagenin A-3-O-[α-L-rhamnopyranosyl (1→2)-O-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl(1→4)O-β-D-glucopyranoside (Mussaendoside H)	*M. pubescens*	13

(Table 3.3 contd….)

No.	Compound	Source	Ref.
4	HeinsiageninA-3-O-{α-L-rhamnopyranosyl(1→2)-O-[β-D-glucopyranosyl(1→6)]-O-β-D-glucopyranosyl(1→2)}-α-L-rhamnopyranosyl (1→4) -O-β-D-glucopyranoside (Mussaendoside G)	*M. pubescens*	12
5	Heinsiagenin A-3-O-β-D-glucopyranoside (Mussaendoside A)	*M. pubescens*	14
6	Heinsiagenin A-3-O-β-D-xylopyranoside (Mussaendoside B)	*M. pubescens*	14
7	HeinsiageninA-3-O-β-D-glucopyranosyl-(1→2)-O-β-D-xylopyranoside (Mussaendoside C)	*M. pubescens*	14
8	HeinsiageninA-3-O-α-L-rhamnopyranosyl(1→2)-O-β-D-glucopyranosyl-(1→2)-[O-α-L-rhamnopyranosyl(1→4)]O-β-D-xylopyranoside (Mussaendoside M)	*M. pubescens*	15 16
9	HeinsiageninA-3-O-α-L-rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→6)]-O-β-D-glucopyranosyl-(1→2)[O-α-L-rhamnopyranosyl(1→4)]-O-β-D-xylo pyranoside (Mussaendoside N)	*M. pubescens*	16
10	Heinsiagenin A-3-O-{[β-D-glucopyranosyl(1→2)-(O-β-D-glucopyranosyl)(1→6)]-O-α-L-rhamnopyranosyl -(1→2) O-β-D-glucopyranosyl] (1→2)}-O-α-L-rhamno pyranosyl(1→4)-O-β-D-glucopyranoside (Mussaendoside U)	*M. pubescens*	7
11	3-O-{[β-D-glucopyranosyl(1→6)]O-α-L-rhamnopyranosyl(1→2)-O-β-D-glucopyranosyl (1→2)}O-β-D-glucopyranosyl(1→3)-O-β-D-glucopyranosyl-cycloart-22,24-dien-27-oicacid (Mussaendoside W)	*M.macrophylla*	8
12	Mussaendoside O	*M. pubescens*	17
13	Mussaendoside P	*M. pubescens*	17
14	Mussaendoside Q	*M. pubescens*	17
15	Mussaendoside F	*M. pubescens*	

3.2.2 Flavonoids from *Mussaenda*

Ten flavonoids have been isolated from *Mussaenda*. Most of them are isolated from *M. hirsutissima*. The flavonoids isolated from *Mussaenda* are listed in **Table 3.4.**

Table 3.4: Flavonoids from *Mussaenda*

No.	Compound	Source	Ref.
1	Quercetin	*M. frondosa* *M. hirsutissima*	19
2	Rutin	*M. hirsutissima* *M. arcuata*	19,20
3	Hypenin	*M. frondosa* *M. hirsutissima*	19
4	Astragalin	*M. arcuata*	20
5	Isoquercitrin	*M. arcuata*	20
6	Kaempferol-3-O-β-D-rutinoside	*M. arcuata*	20
7	Aureusidin-4-glucoside	*M. hirusutissima*	21
8	Aureusidin-6-glucoside	*M. hirusutissima*	21
9	Aureusidine-4,6-diglucoside	*M. hirusutissima*	21
10	Cernuoside	*M. hirusutissima*	21

3.2.3 Miscellaneous compounds

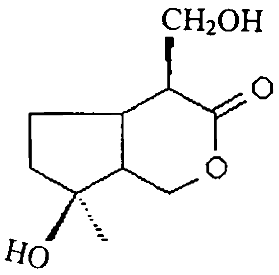
These include aromatic compounds, iridoids and sterols. The aromatic compounds isolated from *Mussaenda* are listed in **Table 3.5.**

3.2.3.1 Iridoids

The monoterpene argyol and three monoterpene lactones namely, mussaenin A **(4)**, mussaenin B **(5)** and mussaenin C **(6)** were isolated from *M. pubescens*.[22] Iridoid glucosides namely mussaenoside **(7)** and shanzhiside methyl ester **(8)** have been isolated from *M. parviflora, M. shikokiana* and *M. pubescens*.[17, 23, 24] Shanzhiside methyl ester and 8-acetyl shanzhiside have been isolated from *M. arcuata* roots.[25]

Table 3.5:Aromatic compounds from *Mussaenda*

No.	Compound	Source	Ref.
1	Ferulic acid	*M. pubescens* *M. frondosa* *M. hirsutissima*	6 19 19
2	Sinapic acid	*M. frondosa* *M. hirsutissima*	19 19
3	Caffeic acid	*M. pubescens*	6
4	p-Coumaric acid	*M. pubescens*	6
5	Melilotoside	*M. arcuata*	20
6	Dihydro melilotoside	*M. arcuata*	20



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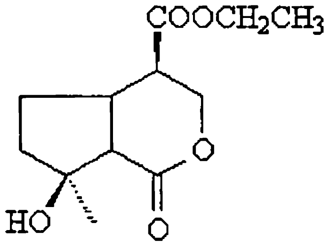

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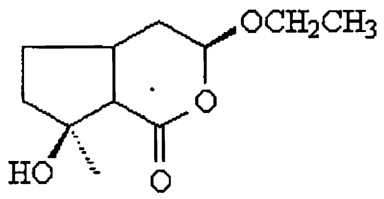

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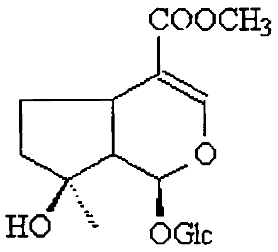

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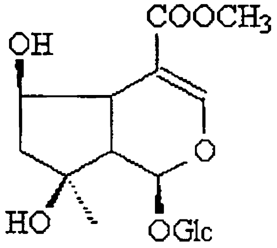


(8)

3.2.3.2 Sterols

The ubiquitous phytosterol, β-sitosterol has been isolated from *M. pubescens, M. frondosa* and *M. hirsutissima* [6,19]. β-Sitosterol glucoside was isolated from *M. frondosa* and *M. hirutissima* [6,19]. Other sterols reported from the genus are stigmasterol and doursterol from *M. pubescens*[11,19].

3.2.4 Bioactivity studies

Mussaenda species in general are rich sources of triterpenoids. Oleanolic acid derivatives isolated from the root bark of *M. macrophylla* exhibited differential inhibitory activity on the growth of *Porphyromonas gingivalis*, the gram-negative anaerobic oral bacterium, and most commonly associated with human gum disease [8]. 3-O-Acetyloleanolic acid isolated from *M. macrophylla* inhibited the growth of *Porphyromonas gingivalis* with the MIC value of 39 μg/ml [8]. Mussaendoside F isolated from aerial part of *M. pubescens* was found to be a muscarinic antagonist [19]. Ursolic acid and oleanolic acid lowered blood sugar levels in normal and alloxan-diabetic mice [26]. Oleanolic and ursolic acids are known to possess significant anti-inflammatory activities. Both these acids are effective in preventing chemically induced liver injury in laboratory animals. Oleanolic acid has been marketed in China as an oral drug for human liver disorders. Recently these compounds have been noted for their antitumour promotion effects [27].

Ferulic acid and caffeic acid (250mg/kg) terminated early pregnancy (1-2 days) in mice following subcutaneous administration [7].

3.3 Present work

M. frondosa L., known as 'Vellila' in Malayalam, is often found along the river sides of Kerala. The leaves and flowers of *M. frondosa* are used in the external application of ulcers. A weak decoction of its dried roots is given to children to relieve cough. The bitter roots are used in the treatment of white leprosy. Its white leaves are useful in the treatment of jaundice [28]. Several triterpenoids and their saponins have been isolated from the genus; a few of them are reported to have biological activities. However, no detailed phytochemical investigation on *M. frondosa* has been reported except the isolation of quercetin, hypenin, ferulic acid, synapic acid, β-sitosterol and β-sitosterol glucoside from its sepals[19]. Hence the present study was undertaken to identify the secondary metabolites from the aerial parts of *M. frondosa.*

3.4 Experimental details

Aerial parts of *M. frondosa* L., were collected from Trichur district of Kerala and were identified by Dr. N. Sasidharan of Kerala Forest Research Institute, Peechi, Trichur where a voucher specimen has been deposited.

3.4.1 Extraction

Air-dried and finely powdered aerial parts of *M. frondosa* (600 g) were successively extracted with hexane (60-80 ^{0}C), chloroform and methanol in a soxhlet apparatus for 30 h in each case. The extracts were separately concentrated to dryness, under reduced pressure and the residue was subjected to column chromatographic separation of the compounds.

3 .4.2 Chromatographic separation of hexane extract

Preliminary examination of the residue from the hexane extract of *M. frondosa* aerial parts showed three prominent spots on TLC when developed in different solvent systems.

The residue (15 g) was dissolved in 30 ml hexane, and transferred on to a column of silica gel (500 g) set up with hexane. The column was eluted with hexane followed by hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (50:50) and finally with ethyl acetate (100%). Fractions of 100 ml were collected, concentrated and similar fractions as monitored by TLC were combined and grouped as indicated in **Table 3.6.**

Group I

The yellow mass obtained from these fractions resisted crystallisation and suggested a waxy nature. It was not examined further.

Group II

Repeated crystallisation of these fractions from hexane-ethyl acetate mixture yielded a colourless solid. It was designated as compound VI (257 mg), mp 81-82 ^{0}C, R$_f$ 0.70 (hexane -ethyl acetate 95:5)

Group III

No crystalline compound could be obtained from this group by repeated crystallization in different solvents.

Table 3.6: Fractions from hexane extract

Eluant	Fraction No.	Group No.	Compd.
Hexane	1-17	I	-
Hexane:ethyl acetate 95:5	18-27	II	VI
	28-42	III	-
Hexane:ethyl acetate 90:10	43-54	IV	II
	55-68	V	-
Hexane:ethyl acetate 80:20	69-85	VI	VII
	86-96	VII	-
Hexane:ethyl acetate 50:50	97-107	VIII	VIII
Ethyl acetate	108-119	IX	-

Group IV

A colourless needle-like compound could be isolated from these fractions by repeated crystallisation from ethyl acetate, (13 mg), mp 136 ^{0}C, R_f 0.56 (hexane-ethyl acetate 8:2). It was identified as **β-sitosterol** by comparison with an authentic sample of β-sitosterol.

Group V

This group did not give any crystalline material upon repeated crystallisation from different solvents. It was not examined further.

Group VI

The residue obtained from these fractions upon repeated crystallisation from ethyl acetate yielded a white powder, which was designated as compound VII (170 mg), mp 244 ^{0}C, R_f 0.50 (chloroform-methanol 95:5).

Group VII

No crystalline compound could be isolated from this group by repeated crystallisation in different solvents.

Group VIII

The residue from this group (97-107) yielded colourless flakes upon repeated crystallisation from methanol. It was designated as compound VIII (1.7 mg), mp 201 ^{0}C, R_f 0.30 (chloroform -methanol 95:5).

Group IX

Repeated crystallization of these fractions did not yield any crystalline material. It was not examined further.

Compound VI

Compound VI was recrystallised from hexane-ethyl acetate mixture as white amorphous powder (257 mg), mp 81-82 ^{0}C.

IR ν_{max} (KBr):

3365, 2926, 2857, 1470, 1060, 723 cm^{-1}

EI-MS (70MeV): m/z (% rel. intensity)

Relative intensity below 10% not given.

420 (M^+-18) (15), 392 (13), 125 (20), 97 (90), 83 (91), 69 (72), 57 (100), 43 (90).

^{1}H NMR (300 MHz CDCl$_3$): δ (ppm)

3.64 (2H, t, J = 6.8 Hz), 1.55 (4H, m), 1.25 (54H, s), 0.86 (3H, t, J = 6.6 Hz)

^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm)

63.12, 32.81, 31.93, 29.70, 29.44, 29.37, 25.74, 22.70, 14.13.

Compound VII

Compound VII was obtained as white amorphous powder from chloroform (170 mg), mp 244 ^{0}C.

IR ν_{max} (KBr):

3365, 2933, 2866, 1699, 1462, 1385, 1280, 1037 cm^{-1}.

EI-MS (70MeV): m/z (% rel. intensity)

Relative intensity below 10% not given.

456 (M^+) (15), 248 (64), 208, 207 (16), 203 (43), 189, 133 (31), 69 (41), 55 (54), 45 (100), 43 (91).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)

5.25 (1H, m), 3.21 (1H, m), 2.82 (1H, m), 2.17 (1H, m), 2.0 (1H, m), 1.90 (1H, m), 1.60-1.42 (remaining protons), 1.25 (2x3H, s), 1.13 (3H, s), 1.08 (3H, d), 0.99 (3H, s), 0.95(3H, s), 0.93 (3H, s), 0.77 (3H, s)

^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm)

(Additional peaks are underlined)

179.03, $\underline{144.30}$, 138.67, 125.10, $\underline{122.05}$, $\underline{79.45}$, 77.54, 55.30, $\underline{52.90}$, $\underline{47.53}$, 47.39, $\underline{46.19}$, 46.0, 42.11, 41.79, $\underline{41.49}$, 36.98, $\underline{36.82}$, 33.78, $\underline{33.31}$, 33.20, 32.81, 31.15, $\underline{30.80}$, 30.64, 28.70, $\underline{28.00}$, 27.29, 26,06, 24.28, $\underline{23.81}$, 23.73, 23.34, $\underline{23.08}$, 21.54, 18.49, 17.48, $\underline{17.42}$, 16.51, 15.67, $\underline{15.54}$.

Compound VIII

The compound was obtained as colourless flakes (1.7 mg) from methanol, mp 201 ^{0}C, R_f =0.30 (chloroform-methanol 95:5). It could not be identified due to paucity of the sample.

3.4.3 Chromatographic separation of the chloroform extract

Chloroform extract of the *M. frondosa* aerial parts indicated three prominent spots on TLC. The residue (10 g) was dissolved in diethyl ether (25 ml) and adsorbed on silica gel (25 g). The ether was removed under vacuum and transferred on to a column of silica gel (250 g) set up with hexane-ethyl acetate

(95:5). The column was eluted successively with hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (50:50) and finally with ethyl acetate (100%). 100 ml fractions were collected and concentrated. All fractions were monitored by TLC and grouped as shown in **Table 3.7.**

Groups I - II

The yellow residue obtained from these fractions showed heterogeneous nature on TLC and no crystalline material could be isolated. It was not examined further.

Group III

Repeated crystallization of these fractions (28-42) from chloroform yielded a white powder (75 mg), melting point (mp 244 ^{0}C) and R_f 0.50 (chloroform- methanol 95:5). It was identified as compound VII by direct comparison with the compound isolated from the hexane extract.

Group IV

These fractions (43-54) upon concentration yielded a white solid, which was separated and recrystallised from ethyl acetate as an amorphous powder (21 mg), R_f 0.54 (chloroform: methanol 90:10), mp 278 ^{0}C. It was identified as **sitosterol-3-O-β-D-glucopyranoside** by direct comparison with the compound isolated from *P.colubrinum* leaves.

Group V

Repeated crystallization of these fractions in different solvent systems did not yield any crystalline material. It was not examined further.

Table 3.7: Fractions from chloroform extract

Eluant	**Fraction No.**	**Group No.**	**Compd.**
Hexane: ethyl acetate 95:5	1-17	I	-
Hexane: ethyl acetate 90:10	18-27	II	-
Hexane: ethyl acetate 80:20	28-42	III	VII
Hexane: ethyl acetate 50:50	43-54	IV	III
Ethyl acetate	55-68	V	-

3.4.3 Methanol extract of *M. frondosa* aerial parts

TLC examination of methanol extract of *M. frondosa* aerial parts showed streaks and no interesting spots on TLC. Hence its isolation was not attempted.

3.5 Results and discussion

3.5.1.Hexane extract

The dark brown residue from the hexane extract of the aerial parts of *M. frondosa* yielded four crystalline compounds VI, II, VII and VIII of which, the first three were characterised. The bar diagram for their isolation is given in **Chart 3.1.**

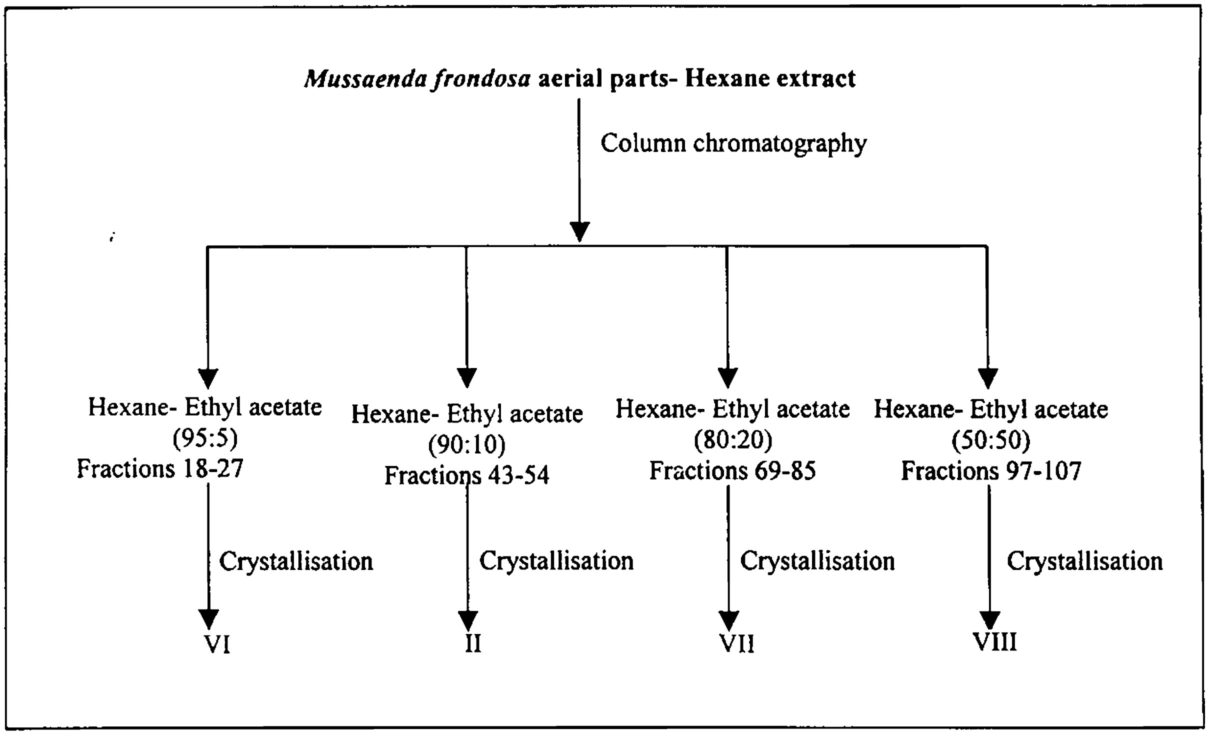


Chart 3.1

Structure of compound VI

Compound VI was recrystallized from ethyl acetate as a colourless solid, mp 81-82 ^{0}C. IR spectrum indicated a hydroxyl group at 3365 cm^{-1}. Absorptions at 1470 and 723 cm^{-1} are diagnostic of the bending vibrations of $(CH_2)_n$ when n is more than four [30].

The mass spectrum of VI showed molecular ion peak at m/z 438 corresponding to the molecular formula $C_{30}H_{62}O$. An intense peak at m/z 420 (M^+-H_2O) indicated the presence of a terminal hydroxyl group[31]. Other peaks corresponding to the consecutive loss of 14 and 28 mass units were characteristic of straight chain aliphatic compound [32].

A two-proton triplet at δ 3.64 (J = 6.8 Hz) in the ^{1}H NMR spectrum indicated the protons attached to a hydroxylated carbon. ^{1}H NMR spectrum also displayed the signals for a terminal methyl at δ 0.86 (3H, t, J=6.6 Hz) and methylenes at δ 1.25 (54H, s) and δ 1.55 (4H, m).

^{13}C NMR spectrum showed the hydroxylated carbon signal at δ 63.11. The signals at δ 14.13, 22.7, 25.74, 29.37, 29.44, 29.7, 31.93 and 32.81 indicated aliphatic nature of the compound. The physical and spectral properties of the compound were identical with those of n-triacontanol already reported in literature (lit. mp 83 ^{0}C) [29]. Hence it was identified as **n-triacontanol** whose structure is given below.

$$CH_3\,(CH_2)_{28}\,CH_2OH$$

Structure of compound VII

Compound VII, was obtained as white amorphous powder from hexane - ethyl acetate (8:2), mp 244 ^{0}C. It gave single spot on TLC (R_f = 0.5). Its IR spectrum exhibited absorptions at 3445 (broad) and 1699 cm^{-1} for hydroxyl and carboxyl functions respectively. ^{13}C NMR spectrum indicated the presence of thirty intense carbon signals.

The EI- mass spectrum showed molecular ion peak at m/z 456, which accounted for the molecular formula $C_{30}H_{48}O_3$. The mass spectrum revealed the fragmentation peaks at m/z 248, 207, 203, 189 and 133, which are characteristic of Δ^{12} pentacyclic triterpenoids[33, 34]. The retro-Diels Alder fragments at m/z 248 and 203 indicated that C, D, and E rings of compound VII were identical to that of ursolic acid [33, 34].

^{1}H NMR spectrum indicated seven methyls between δ 0.77 and δ 1.13. ^{1}H NMR spectrum showed a one-proton multiplet at δ 5.25 (H-12) and a one-proton multiplet at δ 2.17 (H-18) and a one-proton broad singlet at δ 3.2 (H-3).

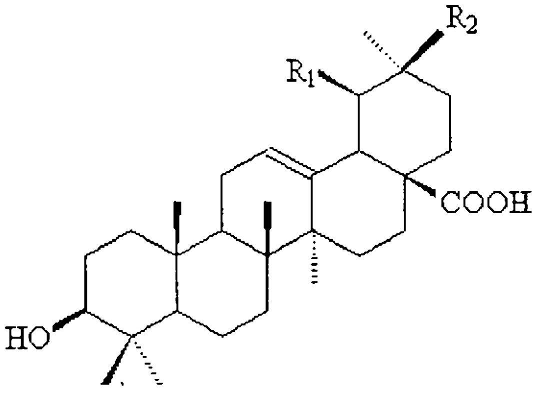
^{13}C NMR signals at δ 125.10 (C-12) and δ 138.67 (C-13) indicated that M_3 belonged to urs-12-ene series [35-37] and at δ 77.54 revealed the presence of a hydroxyl group. The ^{13}C NMR signal at δ 179.03 (COOH) and rest of the peaks in ^{13}C NMR spectrum of compound VII were identical with that of 3-β-hydroxy-urs-12-en-28-oic acid reported earlier [36] . The mass spectral fragmentation of 3β-hydroxy-urs-12-en-28-oic acid is indicated in **Scheme 3.2.**

Although ^{13}C NMR spectrum of compound VII showed close resemblance with that of 3-β-hydroxy-urs-12-en-28-oic acid, a careful examination of it revealed the presence of twenty extra carbon signals indicating that it was an isomeric mixture. These signals were less intense and very close to the signals assigned for 3-β-hydroxy-urs-12-en-28-oic acid. In the ^{13}C NMR spectrum, a small peak at δ 79.40 indicated the presence of another hydroxylated carbon and a small signal coinciding with δ179.03, for a second carboxyl function. The peaks at δ 122.05, 144.30, 41.49, 30.80, 33.31 and 23.81 are diagnostic of C-12, C-13, C-18, C-20, C-29 and C-30 of oleanolic acid. The chemical shift values of other signals were very close to oleanolic acid. Hence compound VII was identified as a mixture of **3-β-hydroxy-urs-12-en-28-oic**

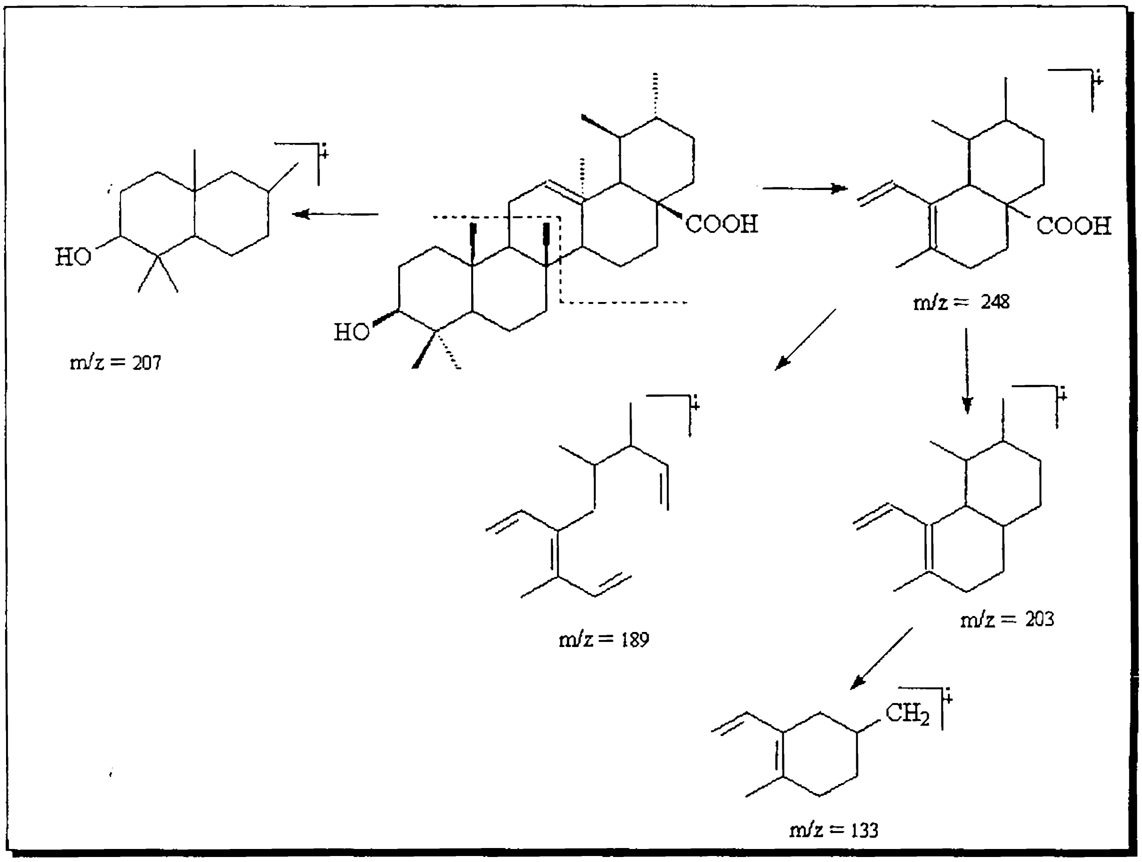
acid (9) and **3-β-hydroxy-olean-12-en-28-oic acid (10)** in which the former predominated. The existence of similar isomeric mixtures in *Salvia sclerea* and their characterisation by ^{13}C NMR spectrum was reported by Shawl *et.al* [38]. The ^{13}C NMR assignment of compound VII is given in **Table 3.8.**

Table 3.8 : ^{13}C NMR (75MHz) assignments of VII (DMSO-d_6)

C.No.	Ursolic acid	Oleano-lic acid	C.No.	Ursolic acid	Oleano-lic acid	C.No.	Ursolic acid	Oleano-lic acid
1	-	-	11	23.34	23.09	21	30.64	33.78
2	27.29	27.65	12	125.1	122.05	22	36.98	32.81
3	77.54	79.45	13	138.66	144.30	23	28.69	-
4	-	-	14	42.12	41.79	24	15.66	15.54
5	55.30	-	15	28.01	27.65	25	16.51	16.51
6	18.47	-	16	24.28	-	26	17.48	17.41
7	33.20	32.81	17	46.01	46.19	27	23.73	26.06
8	-	-	18	52.90	41.50	28	179.03	179.03
9	47.39	47.53	19	-	-	29	17.48	33.31
10	36.82	-	20	-	30.84	30	21.54	23.81



(9) $R_1 = CH_3$, $R_2 = H$ (10) $R_1 = H$, $R_2 = CH_3$



Scheme 3.2: Mass spectral fragmentation of 3β-hydroxy-urs-12-en-28-oic acid

Structure of compound VIII

Compound VIII crystallized as colourless flakes (1.7 mg) from methanol, mp 201 ^{0}C. It could not be identified due to paucity of the sample.

3.5.2 Chloroform extract

The dark brown residue from the chloroform extract of *M. frondosa* aerial parts yielded two crystalline substances which were identified as a mixture of **3β-hydroxy-urs-12-en-28-oicacid** and **3β-hydroxy-olean-12-en-28-oicacid** (compound VII) and **sitosterol-3-O-β-D-glucopyranoside** (compound III). The bar diagram for their isolation is given in **Chart 3.2.**

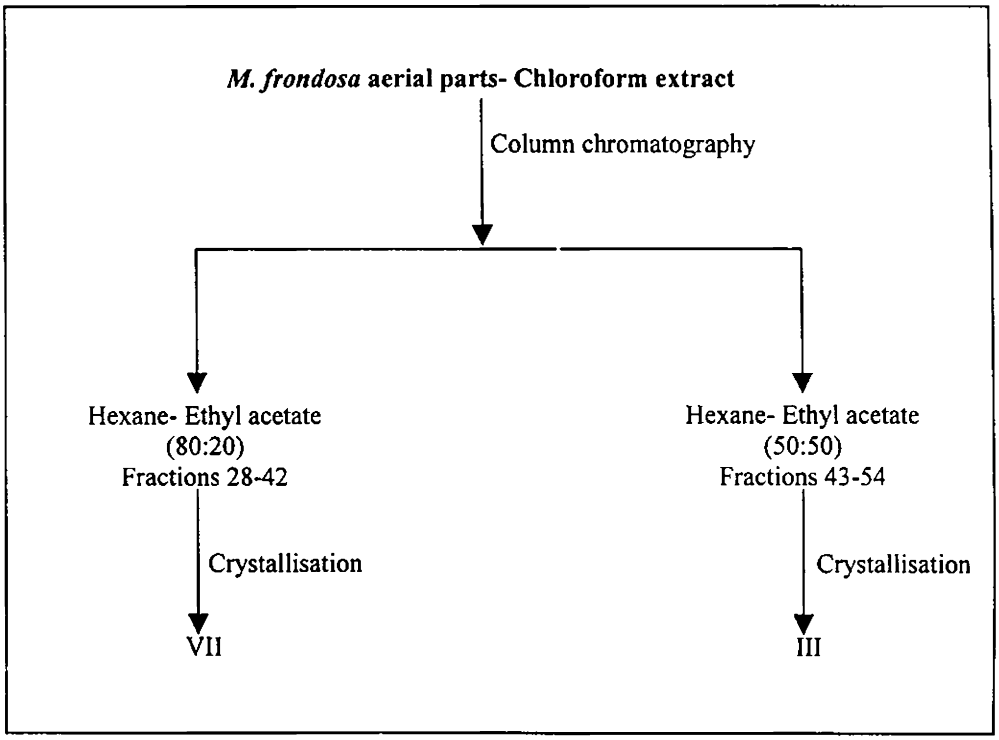


Chart 3.2

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Chapter 4

Phytochemical studies of Artocarpus heterpollus Lamk

Phytochemical studies of *Artocarpus heterophyllus* Lamk

4.1 Introduction

Artocarpus (Moraceae) is a genus of small to large evergreen trees distributed in the area from Sri Lanka and India to South China and through Malaysia to the Solomon Islands. Nine species are recorded from India[1]. The fruits of *A. communis*, *A. integer*, *A. heterophyllus* and *A. polyphema* are edible. *A. chaplasha, A. lakoocha* and *A. hirsutus* are important timber trees. Some members of the genus are used in folk medicines. *A. chaplasha* and *A. lakoocha* are used for the treatment of tapeworm infection [2]. Prenylated flavonoids with potent inhibitory activity against the action of arachidonate-5-lipoxygenase from porcine leucocytes have been isolated from *Artocarpus* [3]. Stilbenes with antimalarial activity have been reported from *A. integer* [4]. Stilbenes and flavones from *A. gomezianus* roots possessed tyrosinase inhibitory activity [5-7].

4.2 Chemistry of *Artocarpus* - an overview

An extended survey of the literature revealed that *Artocarpus* is a rich source of secondary metabolites and these can be broadly listed into

1. Isoprenylated flavonoids
2. Triterpenoids
3. Stilbenes
4. Miscellaneous compounds

4.2.1 Isoprenylated flavonoids

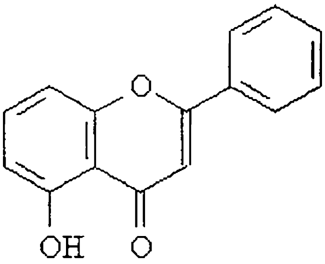
Isoprenylated flavonoids represent the most abundant class of compounds in *Artocarpus.* A characteristic feature of all *Artocarpus* flavonoids is the β-resorcylic orientation of hydroxyl groups in the B-ring. The flavonoids from *Artocarpus* are further grouped into flavones, flavanones, Diels Alder adducts, miscellaneous flavonoids and flavonol glycosides.

4.2.1.1 Flavones

Majority of *Artocarpus* flavonoids are flavone derivatives. Flavones with mono, di and tri-prenyl as well as dimethyl chromeno (DMC) and furano derivatives are encountered in the genus.

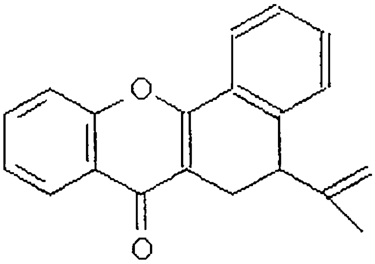
In ^1H NMR spectrum, γ,γ-dimethyl allyl proton signals are observed at δ 1.64-1.89 (3H, S), δ 1.76-1.85 (3H, S), δ 3.35-3.71 (2H, d, J =7 Hz), δ 5.27-5.50 (1H, m).[10-12] 2,2-Dimethyl chromeno protons give ^1H NMR signals at δ 1.45 (3H, S), δ 1.47 (3H, S), δ 5.59-5.75 (1H, d, J = 10 Hz), and δ 6.40-6.76 (1H, d, J=10 Hz)[10-12].

In ^{13}C NMR spectrum, γ,γ-dimethyl allyl carbons are observed at δ 22.2-24.8 (CH_2-), δ 122.8-124.5 (CH=), δ 131.7-133.0 (C=), δ 25.7-26.6 CH_3) and δ 18.0-18.8 (CH_3). 2,2-dimethyl chromeno carbons give signals at δ 28.1-28.4 (CH_3), δ 78.0-79.1 (C-O), CH_3), δ 114.0-116.3 and δ 127.5-129.1 (olefinic carbons)[9,18]. **Tables 4.1, 4.2 and 4.3** reveal the flavones of structure **(1)**, **(2)** and **(3)** isolated from *Artocarpus.*

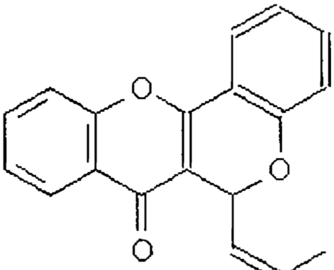


(1)

Flavones having dihydrobenzoxanthone skeleton **(4)** are biogenetically derived from 3-isoprenylated 2',4',5'-trioxygenated flavones through the phenol oxidative cyclisation . In these flavones C-C linkage takes place between C-6' position of B-ring and C-10 position of isoprenoid moiety located at C-3 position. The flavones of this type isolated from *Artocarpus* are indicated in **Table 4.4.**



(2)



(3)

Table 4.1: Flavones of structure (1)

No.	Compound	Structure	Source	Ref.
1	Morin	3,5,7,2’, 4’-(OH)$_5$	*A.integrifolia*	8,9
2	Artonin U	5,4’-(OH)$_2$ 7-OMe 8-(3,3-DMA)	*A.heterophyllus*	10
3	Artonin Y	5,7,2’, 4’-(OH)$_4$ 8-(3,3-DMA)	*A.heterophyllus*	11
4	Artonin E	5,2’, 4’, 5’-(OH)$_4$ 3-(3,3-DMA) 7,8-(2,2-DMC)	*A.heterophyllus* *A.communis*	12 13,29
5	Heteroartonin A	5,7,2’,5’-(OH)$_4$ 4’-OMe 3,3’-(3,3-DMA)$_2$	*A.heterophyllus*	14
6	Artocarpesin	5,7,2’, 4’-(OH)$_4$ 6-(3,3-DMA)	*A.heterophyllus* *A.elasticus*	15 16
7	Artelasticin	5,7,2’,4’-(OH)$_4$ 3,6,8-(3,3-DMA)$_3$	*A. elasticus*	16
8	Artocarpetin B	5,4’-(OH)$_2$ 7,2’- (OMe)$_2$ 8-(3,3-DMA)	*A.heterophyllus*	14
9	Artocarpetin A	5,2’,4’-(OH)$_3$ 7-(OMe) 8-(3,3-DMA)	*A.heterophyllus*	17
10	Artocarpetin	5,2’, 4’-(OH)$_3$ 7- (OMe)	*A.heterophyllus*	17
11	Norartocarpetin	5, 7,2’, 4’-(OH)$_4$	*A.integrifolia* *A.heterophyllus*	17 18

(Table 4.1 contd....)

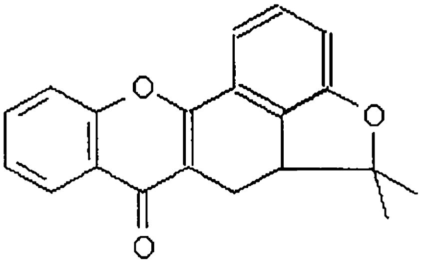
No.	Compound	Structure	Source	Ref.
12	Cycloartocarpesin	5,2', 4'-$(OH)_3$ 6,7- (2,2-DMC)	*A.heterophyllus*	19
13	Oxydihydroartocarpesin	5, 7,2', 4'-$(OH)_4$ 6-(3-Hydroxy-3-methyl butyl)	*A.heterophyllus*	19
14	Isoartocarpin	5,4'-$(OH)_2$ 7- (OMe) 6-(3-methyl-but-1-enyl) 2'-(O-3, 3 -DMA)	*A.heterophyllus*	20
15	Artocarpin	5, 2', 4'-$(OH)_3$ 7- (OMe) 3-(3,3 -DMA) 6-(3-methyl-but-1-enyl)	*A.heterophyllus* *A.integrifolia*	17 21,22
16	Integrin	5, 2', 4'-$(OH)_3$ 7- (OMe) 3-(3,3 -DMA)	*A.integer*	23
17	KuwanonT	5,7, 2', 4'-$(OH)_4$ 3,3'-$(3,3\text{-DMA})_2$	*A.heterophyllus*	14
18	Artobilochromene	5, 2', 4', 5'-$(OH)_4$ 3'-(3,3 -DMA) 6,7-(2,2-DMC)	*A.nobilis*	24 25 26
19	Heterophyllin	5, 2', 4', 5'-$(OH)_4$ 3,8-$(3,3\text{ -DMA})_2$ 6,7-(2,2-DMC)	*A.heterophyllus*	14

DMA - dimethyl allyl

DMC - dimethyl chromeno

Table 4.2: Flavones of structure (2)

No.	Compound	Structure	Source	Ref.
1	Artonin N	5, 7,2', 5'-$(OH)_4$ 6-(3,3 -DMA) 3',4'-(2,2-DMC)	*A.rigida*	27
2	Artonin B	5, 2', 4', 5'-$(OH)_4$ 6,7-(2,2-DMC) 8-(3,3-DMA)	*A.heterophyllus*	28
3	Artobiloxanthone	5, 2', 4',5'-$(OH)_4$ 7,8-(2,2-DMC)	*A.nobilis* *A.communis*	24 29
4	Artonol C	5, 2', 5'-$(OH)_3$ 3', 4'-(2,2-DMC) 7,8-(2,2-DMC)	*A.communis*	29
5	Artonol E	5, 2', 5'-$(OH)_3$ 7-(OMe) 3', 4'-(2,2-DMC)	*A.communis*	29
6	Artomunoxanthone	5, 2', 5'-$(OH)_3$ 4'-(OMe) 7,8-(2,2-DMC)	*A.communis*	30



(4)

Table 4.3: Flavones of structure (3)

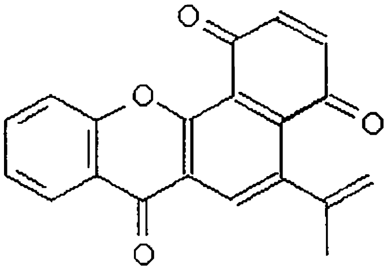
No.	Compound	Structure	Source	Ref.
1	Cycloartomunin	5,5’-(OH)$_2$ 4’-(OMe) 7,8-(2,2-DMC)	*A.communis*	31
2	Cycloartocarpesin	5,2’, 4’-(OH)$_3$ 6,7-(2,2-DMC)	*A.heterophyllus*	19
3	Cyclocommunol	5,7,4’-(OH)$_3$	*A.communis*	32
4	Cyclocommunin (Isocyclomulberrin)	5,7,4’-(OH)$_3$ 6-(3,3-DMA)	*A.communis* *A.altilis*	32 26
5	Cyclomulberrin	5,7,4’-(OH)$_3$ 8-(3,3-DMA)	*A.communis*	32
6	Artelastin	5,7,4’-(OH)$_3$ 6,8-(3,3-DMA)$_2$	*A.elasticus*	16
7	Artelastochromene	5, 4’-(OH)$_2$ 6,7-(2,2-DMC) 8-(3,3-DMA)	*A.elasticus*	16
8	Dihydrocycloartomunin	5,4’, 5’-(OH)$_3$ 7-OMe 8-(3,3-DMA)	*A.communis*	31
9	Dihydro isocycloartomunin	5,7,4’-(OH)$_3$ 5’-OMe 8-(3,3-DMA)	*A.communis*	32
10	Cycloheterophyllin	5, 3’, 4’-(OH)$_3$ 6,7-(2,2-DMC) 8-(3,3-DMA)	*A.heterophyllus*	17 33
11	Cycloartocarpin	5, 4’-(OH)$_2$ 6-(3-methyl-but-1-enyl) 7-OMe	*A.heterophyllus*	34
12	Cycloartocarpin A	5, 7-(OH)$_2$ 4’-OMe 6-(3-methyl-but-1-enyl)	*A.heterophyllus*	35
13	Cyclochampedol	5,7,3’, 4’-(OH)$_4$	*A.champeden*	36

Table 4.4: Dihydrobenzoxanthones of structure (4)

No.	Compound	Structure	Source	Ref.
1	Artonin A	5, 2’,4’-$(OH)_3$ 8-(3,3 -DMA) 6,7-(2,2-DMC)	*A.heterophyllus* *A. champeden*	28 37
2	Artonin J	5,7, 2’,4’-$(OH)_4$ 3’-(3,3 -DMA)	*A.heterophyllus*	38 39
3	Artonin K	5, 2’, 4’-$(OH)_3$ 7-OMe	*A.heterophyllus*	38 29
4	Artonin F	5, 2’, 4’-$(OH)_3$ 6-(3,3 -DMA) 7,8-(2,2-DMC)	*A.communis*	13
5	Artonin L	5, 4’-$(OH)_2$ 7,2’-$(OMe)_2$	*A.heterophyllus*	38
6	Artonin G	5, 7, 2’, 4’-$(OH)_4$ 6,3’-(3,3 -DMA$)_2$	*A.heterophyllus*	40
7	Artonin T	5, 2’, 4’-$(OH)_3$ 7-OMe 3’-(3,3 -DMA)	*A.heterophyllus*	10
8	Cycloartomunoxanthone	5, 2’-$(OH)_2$ 4’-OMe 7,8-(2,2-DMC)	*A.communis*	31
9	Cycloartobiloxanthone	5, 2’, 4’-$(OH)_3$ 7,8-(2,2-DMC)	*A.nobilis* *A.teysmanii* *A.communis*	24 39 29

4.2.1.2 Quinonoid pyrano benzoxanthones

Only three quinonoid pyrano benzoxanthones (5) have been reported from *Artocarpus*; artonin O from *A. rigida* [27] and artonol D and artomunoxanthenetrione from *A.* communis [29,30].



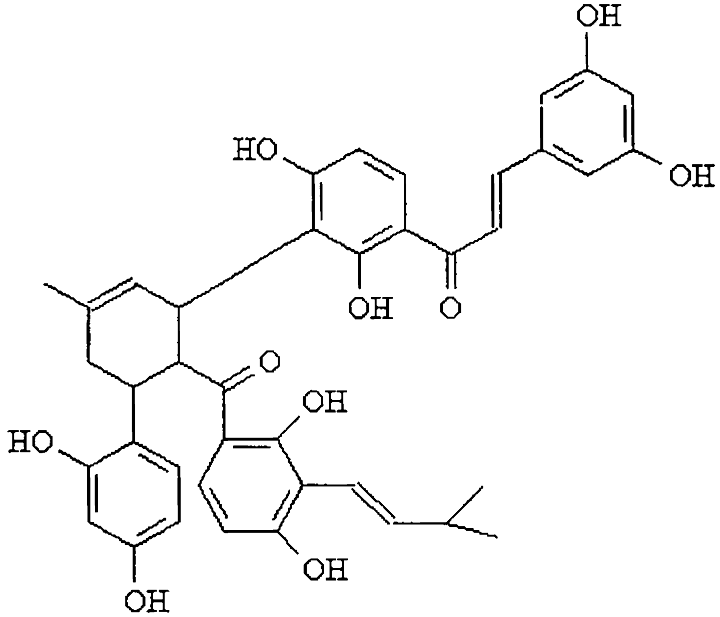
(5)

4.2.1.3 Flavanones

Five flavanones namely artocarpanone[17],artocarpanone A,[17] heteroflavanone B,[41] heteroflavanone C[35] and Dihydromorin[17] have been isolated from *Artocarpus.* All of them are from a single species, *A. heterophyllus.*

4.2.1.4 Diels Alder adducts

Some of the isoprenoid-substituted phenols from *Artocarpus* are regarded as the natural Diels Alder adducts of dehydroprenyl phenols and chalcone derivatives. Five such Diels Alder adducts have been isolated from *A. heterophyllus.* These are artonin C (6), artonin D, artonin I, artonin X and kuwanon R from *A . heterophyllus.*[42-44]



(6)

4.2.1.5 Miscellaneous Flavonoids

In addition to the above-mentioned groups of flavonoids, flavonoids of some unique structures have also been isolated from *Artocarpus*. These include artonin F,[13] artonol A[29] and artonol B[29] from *A. communis*, cyclointegrin,[23] oxycyclointegrin,[23] oxyisocyclointegrin[23] from *A. integer*, isocyclomorusin[26] and Isocyclomulberrin[26] from *A. altilis*, artonin M and artonin P from *A. rigida*,[27] artoindonesianin A[37] and artoindonesianin B[37] from *A. champeden*, artoindonesianin C[39] from *A. teysmanii*, artonin S[10], cyanomaclurin,[17] heterophyllol[45], isocycloheterophyllin[47] and artelastofuran[48] from *A. heterophyllus*, chaplashin[46] from *A. chaplasha* and artocarpols A, B, C, D, E and F from *A. rigida*[50-52]. Morin calcium chelate has been recorded from *A. heterophyllus*[49].

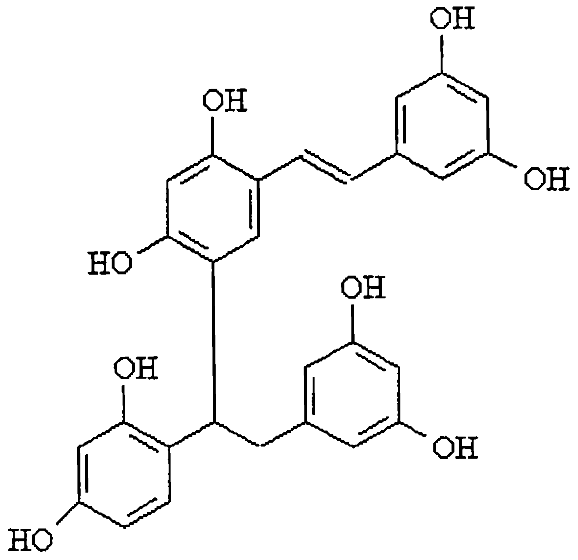
4.2.1.6 Flavonol glycosides

Three flavonol glycosides have been recorded from *A. lakoocha*. These are galangin-3-O-β-galactopyranosyl(1→4)α-L-rhamnopyranoside,[53,54] quercetin-3-O-α-L-rhamnopyranoside[55] and Kaempferol-3-O-β-D-xylanopyranoside.[54]

4.2.2 Triterpenoids

Most of the triterpenoids isolated from *Artocarpus* belong to cycloartane group. The triterpenoids isolated from *Artocarpus* are indicated in Table 4.5.

4.2.3 Stilbenes



(7)

Only five stilbenes have been recorded from *Artocarpus*. Two stilbene dimers, Artogomezianol (7), Andalasin A were isolated from *A. gomezianus* [5]. The stilbenes from *Artocarpus* are shown in **Table 4.6.**

Table 4.5: Triterpenoids from *Artocarpus*

No.	Compound	Source	Ref.
1	Cycloartenone	*A.heterophyllus* *A.champeden*	56,57,58 36,60
2	Cycloartenol	*A.altilis* *A.heterophyllus*	59 60
3	Cycloart-23-en-3β,25-diol	*A.altilis*	59
4	Cycloart-25-en-3β,24-diol	*A.altilis*	59
5	Lupeol	*A.lakoocha*	61
6	Lupeol acetate	*A.communis* *A.lakoocha*	30 62
7	α-Amyrin	*A.lakoocha* *A.altilis*	61 59
8	α-Amyrin acetate	*A.lakoocha*	62
9	Betulin	*A.heterophyllus*	35
10	Betulinic acid	*A.heterophyllus*	35
11	Ursolic acid	*A.heterophyllus*	35
12	Glutinol	*A.champeden*	46
13	Cycloeucalynol	*A.champeden*	46
14	24-Methylene cycloartanone	*A.champeden*	46
15	24R, -9,19-Cyclolanost-25-en-3β, 24-diol	*A.heterophyllus*	60
16	24S, -9,19-Cyclolanost-25-en-3β, 24-diol	*A.heterophyllus*	60

Table 4.6: Stilbenes from *Artocarpus*

No.	Compound	Structure	Source	Ref.
1	Stilbene	3,5,2', 4'-$(OH)_4$ 4-(3,3 DMA)	*A.integer*	4
2	Stilbene	3,5,2', 4'-$(OH)_4$ 4-(3-methyl -*E*-but-1-enyl)	*A.integer*	4
3	Artocarbene	3,2', 4'-$(OH)_3$ 4,5-(2,2-DMC)	*A.integer* *A.incisus*	4 63
4	Stilbene	2,4,3', 5' -$(OH)_4$	*A.lakoocha* *A.chaplasha*	65 46
5	Resveratrol	3,5, 4'-$(OH)_3$	*A.chaplasha*	46

4.2. 4 Miscellaneous compounds

This group includes sterols and aliphatic compounds reported from *Artocarpus.*

4.2.4.1 Sterols

The only sterol reported from *Artocarpus* is β-sitosterol. It has been isolated from *A. champeden, A. heterophyllus*[35,57], *A. communis*[30] , *A. elasticus*[16,23] , *A. altilis*[64] *and A. chaplasha*[61].

4.2.4.2 Aliphatic compounds

About forty-five volatile constituents have been identified from *A. heterophyllus* and *A. polyphema.* These include alcohols, aldehydes, ketones, carboxylic acids and esters. Methyl-3-methyl butanoate, ethyl-3-methyl butanoate, butan-1-ol, 3-methyl-butan-1-ol and 3-methyl butanoic acid have been recorded from *A. heterophyllus* and *A. polyphema.* 2-Methyl-propan-1-ol, 3-hydroxy-butan-2-one and 2-phenyl-ethanol were recorded from *A. polyphema* only [66-68] . Two long chain aliphatic esters namely, 4-hydroxy undecyl docosanoate [56] and 9-hydroxy tridecyl docosanoate [35] have been isolated from *A. heterophyllus*. Capric acid, myristic acid, palmitic acid, lauric acid, oleic acid, stearic acid and linoleic acid occur in different parts of *A. heterophyllus*. All parts of *A. heterophyllus* except bark contain glucose, fructose and sucrose.

4.2.5 Biological activity studies

Stilbene dimers from *A. gomezianus viz.,* andalasin A, and artogomezianol showed moderate tyrosinase activity with IC_{50} values of 39 and 68 μM.[5] Diels Alder adducts from *Artocarpus* showed hypotensive effect, inhibitory activity against arachidonate-5-lipoxygenase, anti tumour promoting activity *etc.* Trans-4-(3-methyl-*E*-but-1-enyl)-3,5,2',4'-tetrahydroxystilbene exhibited antimalarial activity against *Plasmodium falciparam* in culture with EC_{50} value of 1.7μ g/ml.[4] *Artocarpol A* isolated from the root bark of *A. rigida* strongly inhibited superoxide formation in phorbol-12-myristate-13-acetate (PMA) stimulated rat neutrophils with an IC_{50} value of 13.7±0.7 μM.[50] . Artoindonesianin A and B isolated from *A. champeden* roots exhibited cytotoxic activity against murine leukemia (P-388) cells with IC_{50} values of 21.0 and 3.9

μg/ml respectively.[49] Cycloartocarpin and artocarpin isolated from the roots of *A. gomezianus* exhibited tyrosinase inhibitory activity.[6] 2-Geranyl-3, 4, 2', 4'-tetrahydroxychalcone was isolated as an antiallergy agent from *A. communis*.[54]

4.3 Present work

A. heterophyllus Lamk is an evergreen tree, which is valuable for its timber as well as fruits. Prenylated flavones with varied structural features, triterpenes, and aliphatic esters have been isolated from its stem bark and roots. However chemical constituents of its fruit pericarp has not been investigated so far. Therefore a systematic study was undertaken to isolate and characterise the compounds from the pericarp of the fruits of *A. heterophyllus.*

4.4 Experimental

4.4.1 Extraction

Fruits of *A. heterophyllus* were collected from a single tree, a voucher specimen of which is available at the Botany department, University of Kerala. The shade-dried pericarp (1.1 kg) of fruits was extracted successively with hexane (60-80 ^{0}C), chloroform and methanol for 30 h in each case. The extracts were concentrated to dryness and subjected to column chromatography over silica gel.

4.4.2 Chromatographic separation of hexane extract

Preliminary examination of hexane extract of pericarp of *A. heterophyllus* fruits on TLC indicated four prominent spots. The residue (10 g) from the hexane extract was dissolved in 25 ml hexane and transferred on to a column of silica gel. The column was eluted successively with hexane-ethyl acetate mixtures in the order of increasing polarity [hexane-ethyl acetate (98:2), hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20)] and finally with ethyl acetate. Fractions of 100 ml were collected and concentrated. Similar fractions as judged by TLC were combined and grouped as shown in **Table 4.7**.

Table 4.7: Hexane extract

Eluant	**Fraction No.**	**Group No.**	**Compd.**
Hexane	1-20	I	-
Hexane-ethyl acetate (98:2)	21-29	II	-
	30-40	III	IX
	41-47	IV	-
Hexane-ethyl acetate (95:5)	48-58	V	X
	59-70	VI	-
Hexane-ethyl acetate (90:10)	71-78	VII	-
	79-91	VIII	II
Hexane-ethyl acetate (80:20)	92-110	IX	-
Ethyl acetate	111-121	X	-

Groups I- II

The yellow semisolid obtained from these fractions did not give any crystalline material after repeated crystallization from different solvent systems. Hence no further separation was attempted.

Group III

The fractions 30-40 were combined and concentrated. The white solid separated was filtered off and recrystallised from chloroform as sugar-like crystal. It was designated as compound IX (1.3 g), mp 104^{0}C, R_f 0.60 (hexane-ethyl acetate 95:5).

Group IV

These fractions were combined and concentrated. It showed heterogeneous nature on TLC and did not yield any crystalline material and was not examined further.

Group V

These fractions were concentrated. The solid separated was recrystallised from chloroform. It was designated as compound X (73 mg), mp 74^0 C, R_f 0.40 (hexane-ethyl acetate 95:5).

Groups VI- VII

No crystalline material could be isolated from these fractions. Hence no further separation was attempted.

Group VIII

These fractions, when concentrated, a white solid separated. It was recrystallised from chloroform as colourless needles (12 mg), mp 136^0 C, R_f 0.56 (hexane-ethyl acetate 80:20). This was identified as **β-sitosterol** by direct comparison with authentic sample.

Group IX - X

These fractions were combined and concentrated. No crystalline material could be isolated from these fractions. Therefore further separation was discontinued.

Compound IX

Compound IX was obtained as colourless plates from chloroform (1.3 g), mp 104 ^{0}C, R_f 0.60 (hexane-ethyl acetate, 95:5)

IR ν_{max} (KBr):

3040, 2946, 2875, 1708(CO), 1462, 1380, 1113 cm^{-1}

EIMS (70eV) m/z (rel. int.):

Relative abundance below 10% not given.

424 $[M]^+$ (20), 409, 340, 313, 304, 286, 111, 109 (21), 107 (21), 105 (18), 95 (25), 93 (21), 98 (23), 69 (78), 55 (70), 41(100).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)

5.10 (1H, m, H-24), 2.73 (1H, d t, J = 6.4 Hz, 13.8 Hz, H-2α), 2.32 (1H, m, H-2β), 1.68 (3H, s, H-26), 1.60 (3H, s, H-27), 1.10 (3H, s, H-29), 1.05 (3H, s, H-

30), 1.00(3H, s, H-18), 0.90 (3H, s, H-28), 0.88 (3H, d, $J = 6.1$ Hz, H-21), 0.78 (3H, d, $J = 4.0$ Hz, H-19α), 0.57 (3H, d, $J = 4.0$Hz, H-19β)

^{13}C NMR (75MHz, $CDCl_3$): δ(ppm)

δ 216.67, 130.95, 125.21, 52.29, 50.24, 48.73, 48.44, 47.89, 45.32, 37.49, 36.33, 35.87, 35.57, 33.43, 32.79, 28.14, 29.57, 26.73, 25.96, 25.87, 25.74, 24.94, 22.18, 21.51, 21.10, 20.78, 19.29, 18.23, 18.09, 17.54

Compound X

Compound X was obtained as white amorphous powder from chloroform (73mg), mp 74^{0}C, R_f 0.40 (hexane-ethyl acetate, 95:5)

IR ν_{max} (KBr):

3380(OH), 2927, 2850, 1740 (CO), 1473, 1180, 726, cm^{-1}

EIMS (70eV) m/z (rel. int.):

Relative abundance below 10% not given.

539 (M^++1), 538 (M^+), 482 (12), 481, 452 (10), 451, 362, 341(15), 334, 323, 313 (18), 295 (12), 87(35), 69 (80), 57 (90), 43 (100).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm)

4.05 (2H, t, $J = 6.6$ Hz), 3.57 (1H, br s), 2.29 (2H, t, $J = 7.4$ Hz), 1.59 (8H, br s), 1.26 (50H, br s), 0.86 (6H, t, $J = 6.8$ Hz).

^{13}C NMR (75MHz, $CDCl_3$): δ(ppm)

174.04, 72.05, 64.41, 37.52, 31.94, 29.71 (br), 29.51, 29.37, 29.28, 29.18, 28.67, 25.94, 25.68, 25.05, 22.70, 14.12.

4.4.3 Chromatographic separation of chloroform extract

The residue (8 g) from the chloroform extract of pericarp of *A. heterophyllus* fruits was dissolved in 15 ml diethyl ether, adsorbed on 15 g silica gel and the solvent was removed under reduced pressure. The powder so obtained was transferred on to a column of silica gel (160 g) set up with hexane-ethyl acetate (90:10). The column was eluted successively with hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (50:50) and finally with ethyl acetate. Fractions of 100 ml were collected, concentrated and similar fractions as monitored by TLC were combined and grouped as shown in **Table 4.8.**

Table 4.8: Chloroform extract

Eluant	Fraction No.	Group No.	Compd.
Hexane-ethyl acetate (90:10)	1-15	I	-
Hexane-ethyl acetate (80:20)	16-32	II	-
Hexane-ethyl acetate (50:50)	33-41	III	XI
Ethyl acetate	42-49	IV	III
	50-58	V	
Methanol	59-65	VI	-

Group I

The yellow semisolid separated from these fractions showed a waxy nature and no crystalline material could be separated. It was not examined further.

Group II

These fractions did not give any crystalline material upon repeated crystallization from different solvents. Hence no further isolation was attempted.

Group III

These fractions were concentrated and a pale yellow powder separated out. It was filtered and recrystallised from ethyl acetate. It was designated as XI (9 mg), mp 246 ^{0}C, R_f 0.40 (chloroform : methanol 95:5).

Group IV

These fractions, when concentrated a white solid separated. It was filtered off and recrystallized from ethanol (12 mg), mp 278 ^{0}C, R_f 0.54 (chloroform: methanol 90:10). It was identified as **sitosterol-3-O-β-D-glucopyranoside** by comparison with the sample isolated earlier.

Group V-VI

These fractions were combined and concentrated. No crystalline material could be isolated. Hence further isolation was discontinued.

Compound XI

Compound XI was obtained as yellow amorphous powder from ethyl acetate (9 mg), mp 246 ^{0}C, R_f 0.40 (chloroform: methanol, 95:5)

UV $\lambda_{max.}$ (nm.)

MeOH : 355, 286sh, 270, 250.

IR ν_{max} (KBr):

3420, 1665, 1610, 1540, 1500 cm^{-1}.

EIMS (70eV) m/z (rel. int.):

Relative abundance below 10% not given.

354 (32), 311 (90), 299 (93) 178 (11) 177 (15), 166 (22), 165 (20), 136 (10), 135 (22), 134, 55 (75), 43 (98), 41 (100).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm)

13.28 (1H,s), 10.79 (2H, br s), 10.22 (1H, s), 7.72 (1H, d, *J*=8.6 Hz), 6.97 (1H s), 6.48 (1H, d *J*=2.0 Hz), 6.45 (1H, s), 6.42 (1H, d d, *J*=8.3, 2.2 Hz).

4.4.4 Methanol extract

The residue from the methanol extract did not show any interesting spots on TLC. Hence no further separation was attempted.

4.5 Results and discussion

4.5.1 Hexane extract

The dark brown residue from the hexane extract of pericarp of fruits from *A. heterophyllus* yielded three compounds. The flow chart for their separation is shown in **Chart 4.1**

Structure of compound IX

Compound IX was obtained as colourless needles from chloroform, mp104 ^{0}C.[58] It gave positive reaction to Liebermann Burchard test indicating triterpene nature. The EI mass spectrum showed molecular ion peak at m/z 424 accounting for the molecular formula $C_{30}H_{48}O$. The IR spectrum indicated absorptions for carbonyl and cyclopropane methylene at 1708 and 3040 cm^{-1} respectively. ^{13}C NMR and DEPT-^{13}C NMR spectra revealed thirty carbons including seven methyls (six singlets and a doublet), eleven methylenes, five methines and seven quaternary carbons. In the ^{1}H NMR spectrum a one-proton multiplet at δ 5.10 indicated the proton on the trisubstituted olefinic carbon. ^{1}H NMR spectrum exhibited six methyl singlets (δ 0.90 -1.68) and a methyl doublet (δ 0.88, *J*= 6.1Hz). The relatively down field shift of two methyls (δ 1.60, 1.68) could be assigned to a terminal isopropylidene group.[69] A pair of one-proton doublets at δ 0.57 and δ 0.78 (*J*= 4.0 Hz each) was characteristic of the cyclopropyl methylenes. The deshielding of one of the cyclopropane protons at δ 0.78 suggested the presence of a carbonyl at C_3 [70]. This was further supported by a pair of one-proton multiplets at δ 2.73 (dt, J= 6.4, 13.8 Hz, H-2α) and δ 2.32 (m, H-2β) for the methylene protons of a ketomethylene group. These results suggested that the compound was a cycloartane derivative.[56,69] The EI-mass spectrum indicated a molecular fragment at m/z 313 (**8**), generated by the loss of the whole side chain, which was characteristic of cycloartanes. It also indicated that the olefinic bond was located in the side chain.

^{13}C NMR spectrum showed the resonance for a carbonyl carbon at δ 216.67 and olefinic carbons at δ 130.95 and δ 125.21 and seven methyls between δ 17.54-25.64.

Further, the ^{1}H NMR, IR and mass spectra of compound IX were identical with that of 9,19-cyclolanost-24-en-3-one (cycloartenone) reported earlier (lit.mp.109 ^{0}C)[58] . Hence the compound was identified as **9,19-cyclolanost-24-en-3-one (cycloartenone)**. Its structure is represented as **(9)**. The ^{13}C NMR spectral assignment of compound IX is indicated in **Table 4.9**.

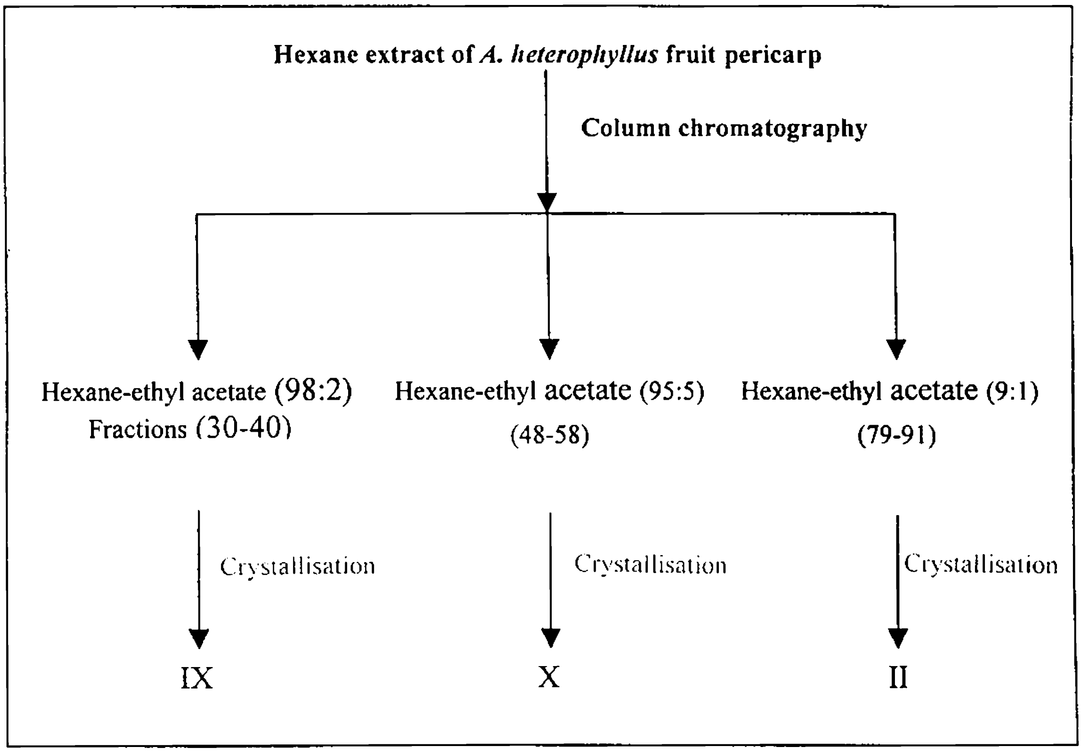
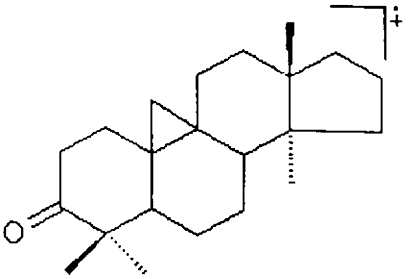


Chart 4.1



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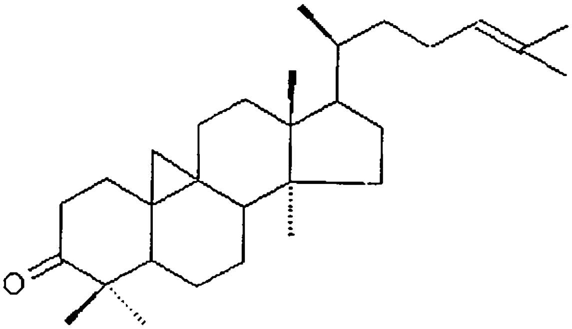


(9)

Table 4.9: ^{13}C NMR spectral assignment of IX

C.No.	δ_C	C.No.	δ_C	C.No.	δ_C
1	33.43	11	25.74	21	18.09
2	37.49	12	35.57	22	24.94
3	216.67	13	45.32	23	36.33
4	50.24	14	48.73	24	125.21
5	48.44	15	32.79	25	130.95
6	21.51	16	26.73	26	25.87
7	28.14	17	52.29	27	19.29
8	47.89	18	17.65	28	18.23
9	21.10	19	29.57	29	22.18
10	25.96	20	35.87	30	20.78

Structure of Compound X

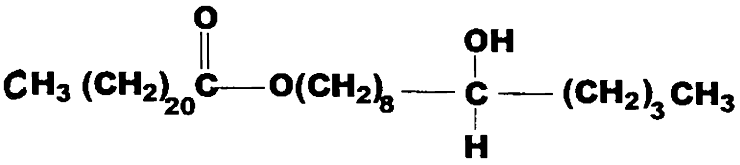
Compound X was obtained as amorphous powder from chloroform, mp 74 ^{0}C, [35]. The EI mass spectrum displayed molecular ion peak at m/z 538, which accounted for the molecular formula $C_{35}H_{70}O_3$. The ion peak at m/z 539 (M^++1) denoted the presence of a free hydroxyl group and m/z 520 indicated the loss of a molecule of water [71] .

The IR spectrum exhibited an ester carbonyl and hydroxyl groups at 1740 and 3380 cm^{-1} respectively. The absorptions at 1473, 1180 and 726 cm^{-1} in the IR spectrum were characteristic of the bending vibrations of $(CH_2)_n$ where n is more than four [72-74]. The IR absorptions at 2927 and 2850 cm^{-1} suggested the stretching vibrations of methylene group. In the ^1H NMR spectrum, the signal at δ 2.29 (2H, t, J=7.4 Hz) was assigned to the methylenes adjacent to the ester carbonyl. The one-proton broad singlet at δ 3.57 accounted for the proton linked to a hydroxylated carbon. It also showed a two-proton triplet at δ 4.05 (J= 6.6 Hz) due to the protons of the methylene ester. A six-proton triplet at δ 0.86 (J= 6.8Hz) indicated the presence of two terminal methyls. The broad singlets at δ 1.26 (50H) and δ 1.59(8H) were assigned to the methylene protons.

^{13}C NMR spectrum of the compound revealed a carbonyl carbon at δ 174.05, two oxygenated carbons at δ 72.05 (CH-OH) and δ 64.41($-OCH_2$) and two methyl carbon signals at δ 14.12. Rest of the carbons appeared between δ 37.52 and δ 22.70 with a broad signal at δ 29.71. From these results it was evident that compound X was a hydroxy ester.

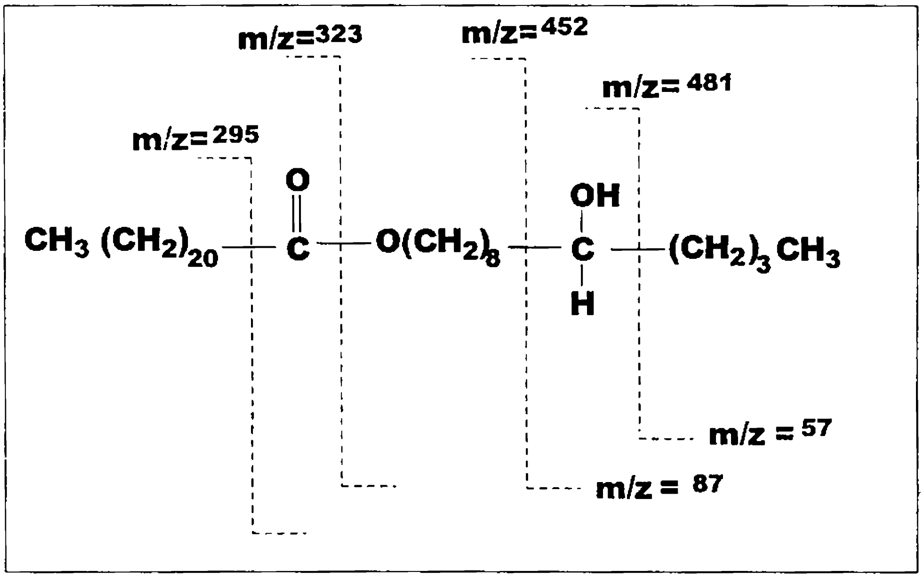
The molecular fragments at m/z 341, 324, 323 and 295 in the mass spectrum accounted for the docosanoate moiety [75]. The fragments at m/z 482, 481, 452, 87 and 57 indicated that the attachment of hydroxyl was at C-9 of the tridecyl moiety. The IR, NMR and ^{13}C NMR spectra of compound X were identical with that of 9-hydroxy tridecyl docosnoate reported earlier (lit.mp. 75-

76 ^{0}C) [35]. Hence compound X was identified as **9-hydroxy tridecyl docosanoate (10).** Its mass spectral fragmentation is shown in **Scheme 4.1.**



$$CH_3(CH_2)_{20}\overset{O}{\overset{\|}{C}}-O(CH_2)_8-\underset{H}{\underset{|}{\overset{OH}{\overset{|}{C}}}}-(CH_2)_3CH_3$$

(10)



Scheme 4.1: Mass spectral fragmentation of compound X

4.2.2 Chloroform extract

The residue from the chloroform extract yielded two compounds. The bar diagram for their separation is shown in **Chart 4.2.**

Structure of compound XI

The compound was obtained as yellow amorphous powder from ethyl acetate, mp 246 ^{0}C . It gave positive reaction for Mg-HCl test indicating flavonoid nature. The UV spectrum of this compound (ν_{max} 250, 270, 286sh, 355nm) indicated the same oxygenation pattern as that of artocarpanone and norartocarpetin and the absence of 3-OH group. The IR spectrum showed a chelated carbonyl at 1660 cm^{-1} and a broad hydroxyl band at 3410 cm^{-1}.

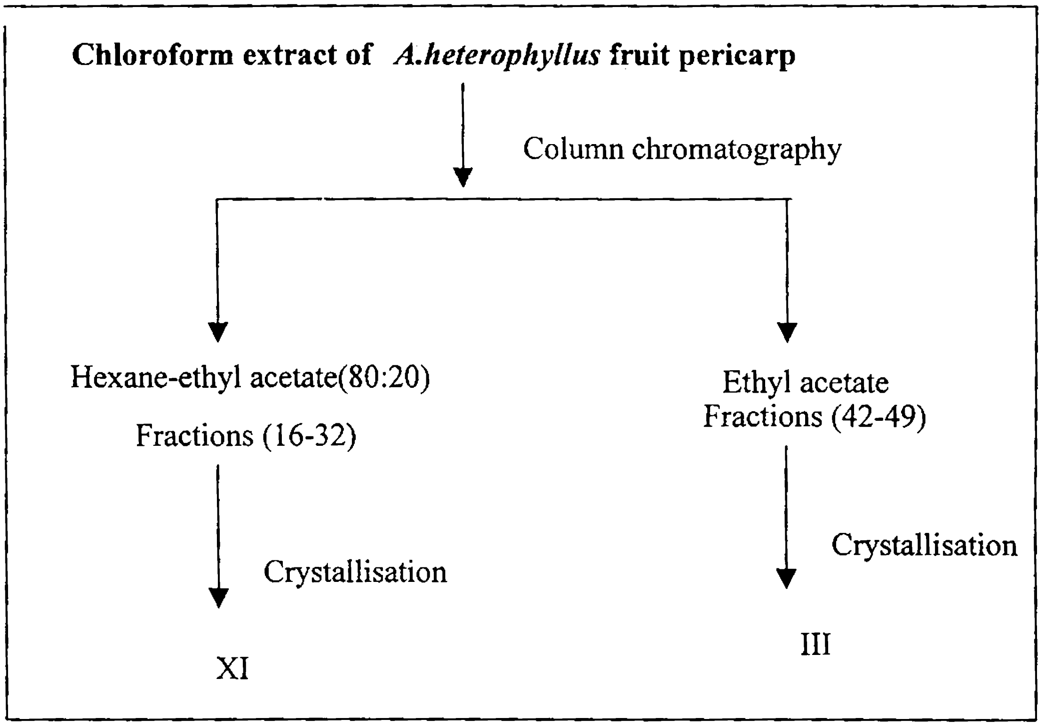
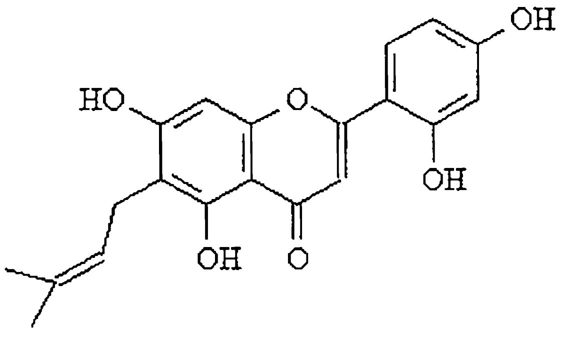


Chart 4.2

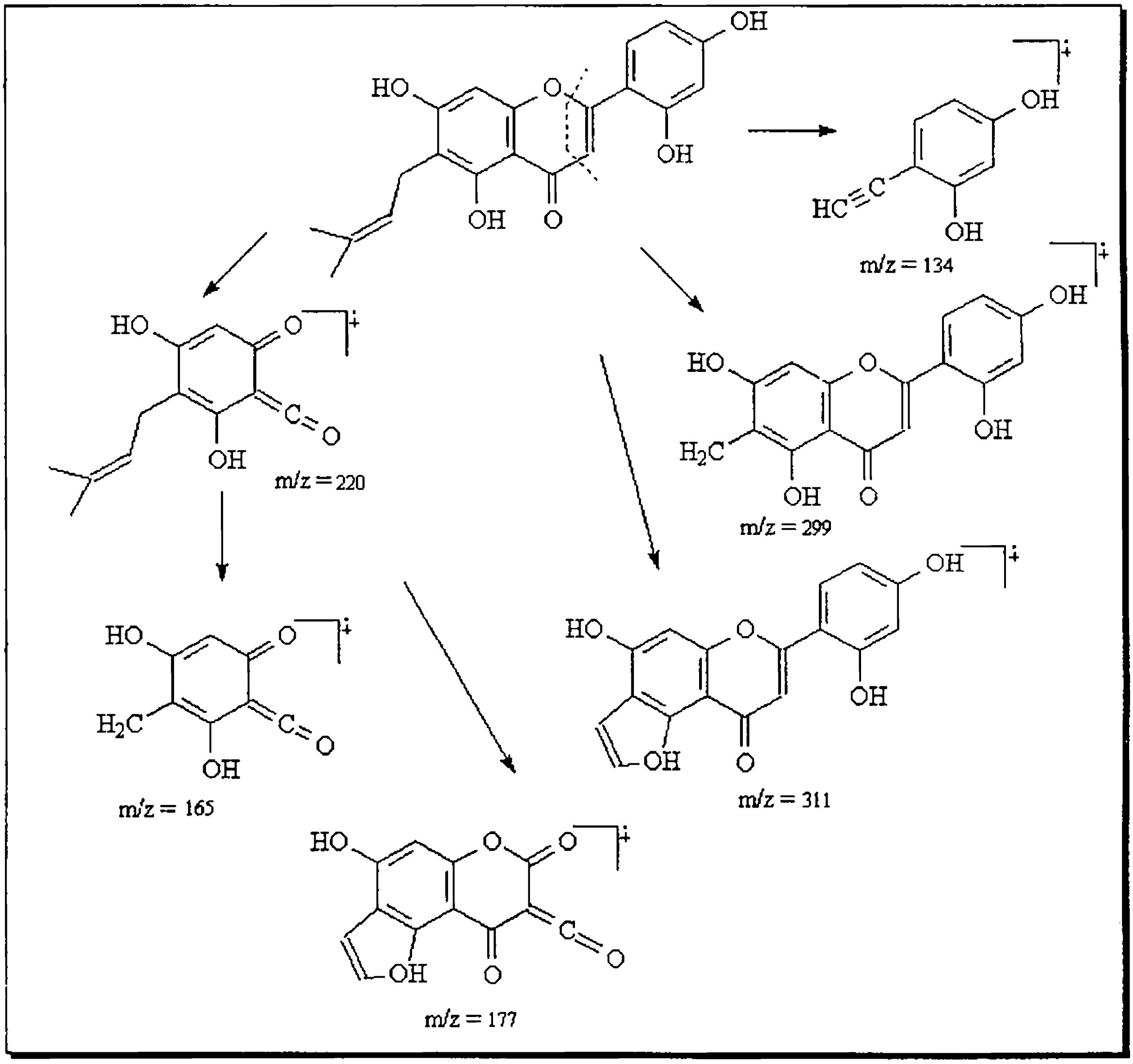
The EI-mass spectrum displayed molecular ion at m/z 354 corresponding to the molecular formula $C_{20}H_{18}O_6$. ^1H NMR spectrum revealed two methyl singlets at δ 1.61 and δ 1.72, a two-proton doublet at δ 3.19 (J=7.0Hz) and a one-proton multiplet at δ 5.16, which were characteristic of γ, γ-dimethyl allyl group. A sharp one-proton singlet at δ 13.29 was diagnostic of a chelated hydroxyl. The broad singlets at δ 10.22 (1H) and δ10.79 (2H) were assigned to three hydroxyl groups. The one-proton singlet at δ 6.97 was assigned to H-3.

The peaks at δ 7.72 (1H, d, *J*= 8.3Hz,), δ 6.42 (1H, dd, *J*= 8.3, 2.2Hz) and δ 6.48 (1H, d, *J*= 2.0 Hz) were assigned to H-6', H-5'and H-3' respectively. The one-proton singlet at δ 6.45 could be due to H-6 or H-8.

The molecular fragments at m/z 219, 177, 165, 135 and 134 in the EI mass spectrum indicated that two hydroxyls and γ, γ-dimethyl allyl group were located in ring A and two hydroxyls in ring B of the flavone. The location of γ,γ-dimethyl allyl group was confirmed by the presence of molecular fragments (M^+-55) and (M^+-43) in the mass spectrum,, which are characteristic of 6-prenylated flavones.[10,76] Further the UV, IR, ^{1}H NMR and mass spectra of compound XI are in agreement with that of 5,7,2',4'-tetrahydroxy-6-γ, γ-dimethyl allyl flavone (artocarpesin) reported in literature(lit.mp 250 ^{0}C) [15,46,58]. Hence the compound was identified as 5,7,2', 4'-tetrahydroxy 6-γ, γ-dimethyl allyl flavone **(10)**. The mass spectral fragmentation of compound XI is shown in **Scheme 4.2.**



(10)



Scheme 4.2:Mass spectral fragmentation of XI

4.6 References

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Chapter 5

Chemical examination of Humboldtia vahliana Wight

Chemical examination of *Humboldtia vahliana* Wight

5.1 Introduction

Humboldtia (Leguminosae) is a small genus of trees and shrubs distributed in India, Sri Lanka and tropical Africa. Six species occur in India. From Western Ghats, *H. bourdillonii, H. brunonis, H. decurrens, H. unijuga* and *H. vahliana* have been reported.[1] *H. vahliana* is a medium sized tree with rough, dark brown bark mottled with grey. Its bark is used for the treatment of ulcers, biliousness, leprosy and epilepsy. Phytochemical studies on the genus are very scanty except the one on *H. laurifolia* reported by Samaraweera *et. al.* [2]. They have isolated O-acetyl oleanolic aldehyde, sitosterol, sitosteryl ester, lupeol, apigenin, (2*R*,3*R*)-3,5,7,3',5'-pentahydroxyflavan and 3α-methoxyfriedelan from *H. laurifolia*. Hence a systematic investigation was taken up on *H. vahliana* aerial parts in order to isolate as many compounds as possible.

5.2 Experimental

5.2.1 Extraction

Aerial parts of *H. vahliana* were collected from the premises of Neyyar dam and identified by Dr. N. Sasidharan of Kerala Forest Research Institute, Peechi, where a voucher specimen has been deposited. The air-dried plant material (1 kg) was extracted successively with hexane (60-80 ^{0}C), chloroform and methanol for 30 h in each case. The extracts were separately concentrated to dryness and subjected to column chromatography over silica gel.

5.2.2 Chromatographic separation of hexane extract

Preliminary examination of the residue from the hexane extract of *H. vahliana* aerial parts indicated several spots on TLC. The residue (20 g) was dissolved in 40 ml hexane and transferred on to a column of silica gel (400 g) set up with hexane. The column was first eluted with hexane, followed by hexane-ethyl acetate (98:2), hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20) and finally with ethyl acetate (100%). Fractions of 100 ml were collected, concentrated and similar fractions as monitored by TLC were combined and grouped as indicated in **Table 5.1.**

Group I

The fractions 1-15 when concentrated yielded a yellow mass which showed heterogeneous nature on TLC. It did not yield any crystalline material upon repeated crystallisation from different solvents. Hence it was not examined further.

Group II

The fractions (16-20) from this group yielded a waxy residue, which resisted crystallisation. It was not examined further.

Group III

These fractions (21-32), when combined and concentrated a white solid separated. It was recrystallised from chloroform as sugar like crystals (115 mg), R_f 0.88 (hexane- ethyl acetate 95:5), mp 298-300 ^{0}C. It was designated as compound XII.

Group IV

The residue from this group showed heterogeneous nature on TLC and no crystalline compound could be isolated. It was not examined further.

Group V

These fractions were combined and concentrated. The residue was recrystallised as colourless needles (30 mg) from ethyl acetate, which was identified as **β-sitosterol**, R_f 0.56 (hexane-ethyl acetate 80:20), mp 136 ^{0}C.

Table 5.1: Hexane extract

Eluant	**Fraction No.**	**Group No.**	**Compd.**
Hexane	1-15	I	-
Hexane- ethyl acetate 98:2	16-20	II	-
	21-32	III	XII
Hexane- ethyl acetate 95:5	33-37	IV	-
	38-43	V	II
Hexane- ethyl acetate 90:10	44-54	VI	-
	55-58	VII	-
Hexane- ethyl acetate 80:20	59-65	VIII	-
Ethyl acetate (100%)	66-72	IX	-

Groups VI - IX

No crystalline compound could be isolated from these fractions by repeated crystallisation from different solvents. Hence no further isolation was attempted.

Compound XII

IR ν_{max} (KBr):

2942,2929,2866, 1451,1386,1050, 999 cm^{-1}

EIMS 70eV: m/z (rel.int.)

Relative abundance below 10% not given.

442 (10), 428 (12), 410, 395, 289, 269, 257, 247, 231, 221, 207, 205, 202, 189, 177 (22), 149, 148, 125 (51), 68 (100), 54 (90).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm)

3.31 (3H, s, -OMe), 2.81 (1H, m, H-3), 2.20 (1H, m, H- 4), 1.74 (1H, m, H-2α), 1.62 (1H, m, H-2β), 1.28-1.19 (CH_2), 1.16 (3H, s, Me), 1.00 (3H, s, Me), 0.99 (3H, s, Me), 0.98 (3H, s, Me), 0.94 (3H, s, Me), 0.81 (3H, s, Me), 0.77 (3H, s, Me), 0.83 (3H, d, J = 6.6 Hz).

^{13}C NMR (75MHz, $CDCl_3$): δ(ppm)

80.95, 60.03, 56.28, 52.90, 51.39, 42.77, 41.36, 39.64, 39.21, 38.25, 38.03, 36.96, 36.00, 35.48, 35.29, 35.02, 32.75, 32.30, 32.07, 31.76, 30.56, 29.96, 29.64, 28.14, 20.14, 19.25, 18.64, 18.12, 17.82, 14.56, 9.82.

5.2.3 Chromatographic separation of chloroform extract

The residue (12 g) from the chloroform extract showed a number of spots on TLC. The residue (10 g) was dissolved in diethyl ether (15 ml), adsorbed on silica gel (15 g) and the powder so obtained was transferred on to a column of silica gel (250 g) set up with hexane. This was first eluted with hexane, followed by hexane-ethyl acetate (95:5), hexane-ethyl acetate (90:10), hexane-ethyl acetate (80:20), hexane-ethyl acetate (70:30) and finally with ethyl acetate. Fractions of 100 ml were collected, concentrated, and similar fractions as judged by TLC were combined and grouped as indicated in **Table 5.2**.

Table 5.2: Chloroform extract

Eluant	Fraction No.	Group No.	Compd.
Hexane	1-10	I	-
Hexane- ethyl acetate 95:5	11-18	II	II
Hexane- ethyl acetate 90:10	19-29	III	-
Hexane- ethyl acetate 80:20	30-42	IV	-
Hexane- ethyl acetate 70:30	43-57	V	III
Ethyl acetate (100%)	58-67	VI	-

Group I

These fractions did not yield any crystalline material up on repeated crystallisation. Hence it was not examined further.

Group II

These fractions were combined and concentrated. The solid separated was recrystallised from ethyl acetate as colourless needles (18 mg), R_f 0.56 (hexane-ethyl acetate 80:20), mp 136 ^{0}C, which was identified as **β-sitosterol.**

Groups III - IV

No crystalline compounds could be isolated from these fractions by repeated crystallisation. Hence no further isolation was attempted.

Group V

These fractions when concentrated, a white solid separated out. This was recrystallized from ethyl acetate as white amorphous powder (53 mg) from chloroform-methanol, mp 278 ^{0}C, R_f 0.54 (chloroform: methanol 9:1). It was identified as **sitosterol-3-O-β-D-glucopyranoside.**

Group VI

The residue from this group did not yield any crystalline material upon repeated crystallisation from different solvents. Further isolation was discontinued.

5.2.4 Chromatographic separation of methanol extract

Methanol extract was concentrated to 250 ml and 250 ml distilled water was added and shaken well. This was extracted with ethyl acetate (300 ml x 3). The ethyl acetate soluble fraction was separated and concentrated to dryness and subjected to column chromatography over silica gel. The residue (3 g) was dissolved in 5 ml acetone, adsorbed on silica gel (6 g) and transferred on to a column of silica gel (60 g) set up with hexane. The column was eluted with hexane, followed by hexane-ethyl acetate 75:25, hexane-ethyl acetate 50:50, hexane-ethyl acetate 25:75 and ethyl acetate 100%. Finally the column was washed with methanol. Several 100 ml fractions were collected, and similar fractions as monitored by TLC were combined and grouped as shown in **Table 5.3**.

Groups I - II

These fractions showed heterogeneous nature on TLC and did not yield any crystalline material upon repeated crystallisation. Hence no further isolation was attempted.

Group III

These fractions were combined and concentrated. The residue obtained was recrystallised from methanol as yellow powder (18 mg), which was designated as XIII R_f 0.52(chloroform-methanol 90:10), mp 345 ^{0}C.

Group IV

The dark residue from this group resisted crystallisation from different solvent systems. It was not examined further.

Group V

These fractions were combined and concentrated, a white solid (60mg), separated from this group R_f 0.54 (chloroform-methanol 90:10), mp 278 ^{0}C. It was identified as **sitosterol-3-O-β-D-gluco pyranoside**.

Group VI

The residues from these groups did not yield any crystalline material up on repeated crystallisation. It was not examined further.

Table 5.3: Methanol extract

Eluant	Fraction No.	Group No.	Compd.
Hexane	1-7	I	-
Hexane-ethyl acetate 75:25	8-12	II	-
Hexane-ethyl acetate 50:50	13-16	III	XIII
Hexane-ethyl acetate 25:75	17-21	IV	-
Ethyl acetate (100%)	22-25	V	III
Methanol	26-31	VI	-

Compound XIII

Compound XIII was obtained as yellow powder from methanol (18 mg), mp 345 ^{0}C.

UV λ_{max} nm:

MeOH: 267, 296 sh, 335.

IR ν_{max} (KBr):

3338, 2927, 1655, 1607,1446, 1356, 829 cm^{-1}.

EIMS 70eV: m/z (rel. int.)

Relative abundance below 10 % not given.

270 (12), 242, 241, 153, 152, 150, 149, 119, 118, 90 (100), 69 (91), 60 (53).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm)

12.90 (1H, s), 10.82 (1H, br s), 10.37 (1H, br s), 7.86 (2H, d, J=8.6 Hz), 6.86 (2H, d, J=8.6 Hz), 6.73 (1H, s), 6.43 (1H, d, J=2.3 Hz), 6.14 (1H, d, J=2.3 Hz).

^{13}C NMR (75MHz, $CDCl_3$): δ(ppm)

182.32, 164.71, 164.34, 162.00, 161.72, 157.89, 129.06, 121.73, 116.55, 104.25, 103.40, 99.41, 94.56.

5.3 Results and discussion

5.3.1 Hexane extract of *H. vahliana* aerial parts

Two crystalline compounds were isolated from the hexane extract of aerial parts of *H. vahliana.* The bar diagram for their isolation is given in **Chart 5.1**.

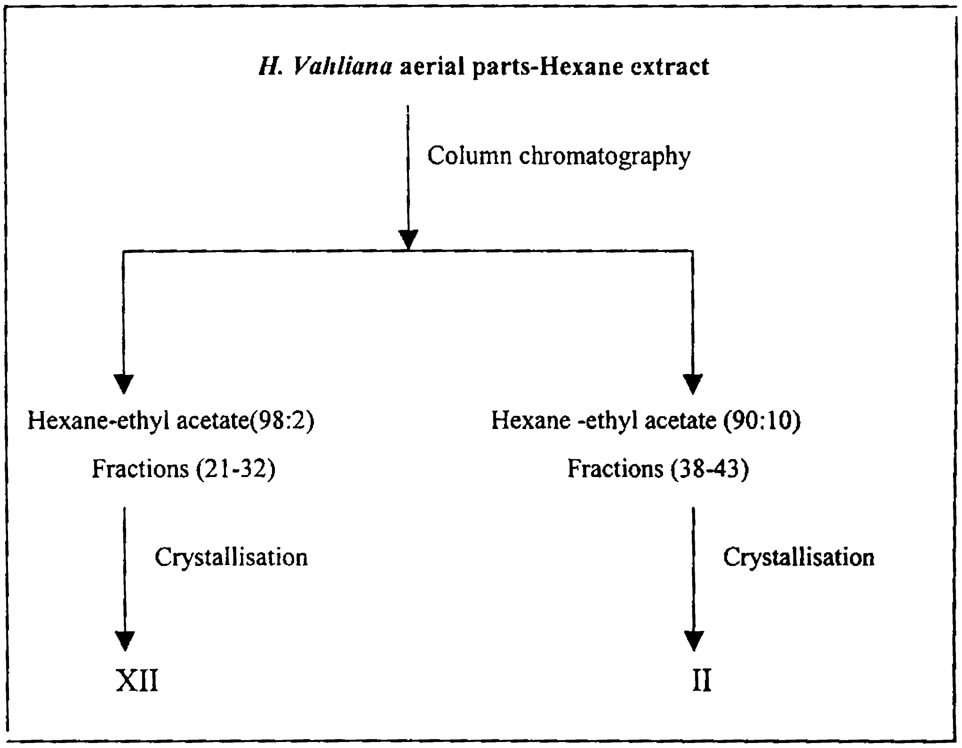
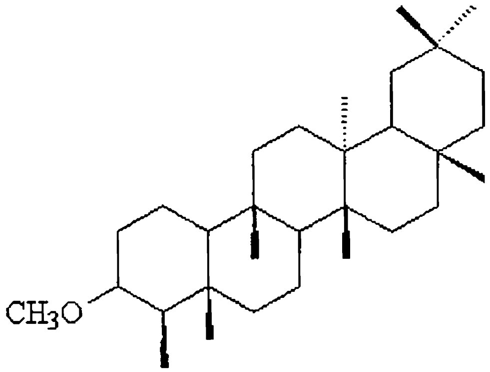


Chart 5.1

Structure of compound XII

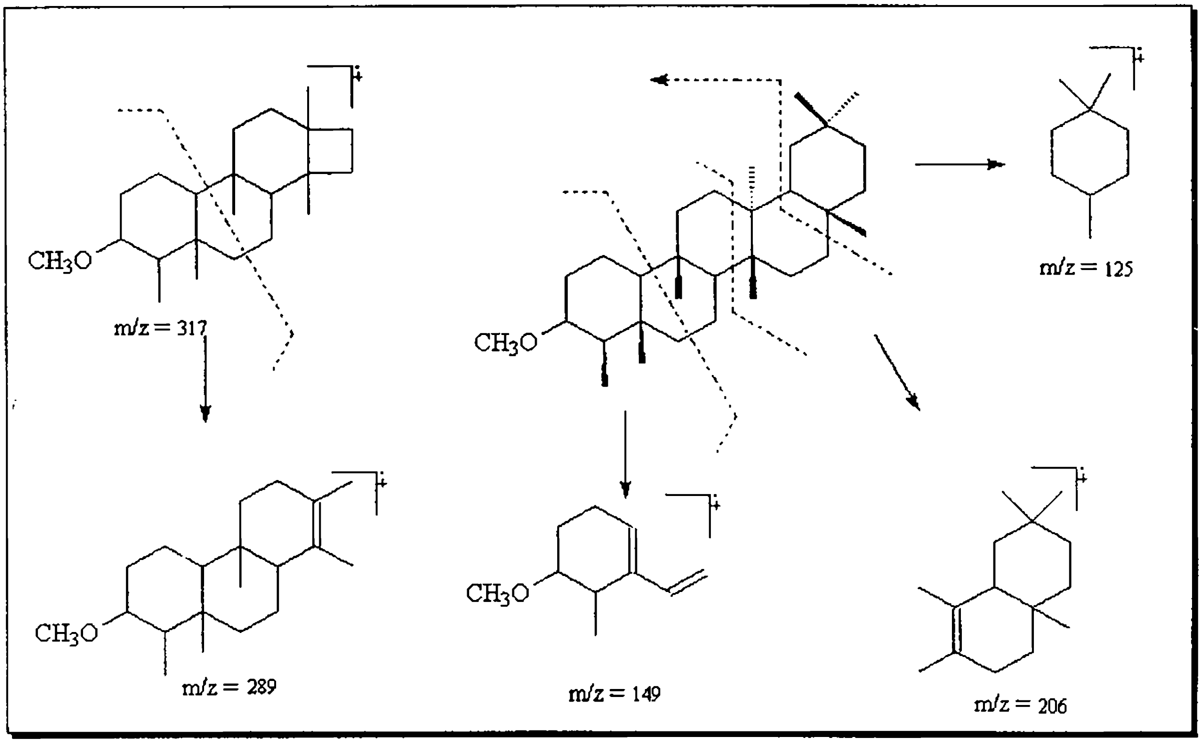
Compound XII was obtained as colourless needles from hexane-ethyl acetate, mp 298-300^0 C. It gave positive reaction to Liebermann-Burchard test, indicating triterpene nature. IR spectrum indicated aliphatic nature of the compound with strong C-H stretching absorptions at 2929 and 2866 cm^{-1} and methoxyl absorptions at 1451, 1386 and 1050 cm^{-1}. ^{13}C NMR spectrum indicated 31 carbon signals. The EI mass spectrum of the compound exhibited molecular ion peak at m/z 442 corresponding to the molecular formula $C_{31}H_{54}O$. ^{13}C NMR and DEPT- ^{13}C NMR spectra indicated the presence of 9 methyls, 11 methylenes, 5 methines, and 6 quaternary carbons. A three-proton singlet at δ 3.31 in ^{1}H NMR spectrum and the signal at δ 56.28 in ^{13}C NMR spectrum were diagnostic of a methoxyl group. ^{1}H NMR spectrum revealed 7 tertiary methyls as singlets (δ 1.16, 1.00, 0.99, 0.98, 0.94, 0.81 and 0.77), one secondary methyl as doublet (δ 0.83, *J* = 6.6 Hz), three protons α- to the methoxylated carbon [δ 2.20 (1H, m, H-4), δ 1.74 (1H, m, H-2α), δ 1.62 (1H, m, H-2β)] and the absence of any olefinic protons, which were characteristic of friedelanes [3]. A one-proton multiplet at δ 2.28 was assigned to the proton on methoxylated carbon. The molecular fragments with m/z 289, 206, 149 and 125 indicated that methoxyl group was located in ring A of the triterpene. ^{1}H NMR spectrum and EI mass spectrum of the compound were identical with that of 3α-methoxyfriedelan (lit. mp 301^0 C) reported in literature [2]. Hence compound XII was characterised as **3α-methoxyfriedelan (1)**. However, its ^{13}C NMR spectral data has not been reported. The ^{13}C NMR spectral assignment of XII is given in **Table 5.5** by comparison with that of 3α-hydroxy friedelan reported earlier [4]. The mass spectral fragmentation of the compound is shown in **Scheme 5.1**.



(1)

Table 5.4: ^1H NMR (300 MHz) spectral assignments of compound XII ($CDCl_3$)

H. No.	δ_H	H. No.	δ_H
23	0.83(3H,d, *J*=6.6 Hz)	30	0.94(3H,s)
24	0.77 (3H,s)	3	2.81(1H,m)
25	0.81 (3H,s)	4	2.20 (1H,m)
26	0.98(3H,s)	2α	1.74 (1H,m)
27	0.99 (3H,s)	2β	1.62 (1H, m)
28	1.16 (3H,s)	-OCH_3	3.31 (3H,s)
29	1.00 (3H,s)		



Scheme 5.1: Mass spectral fragmentation of 3α-methoxy friedelan(XII)

Table 5. 5: ^{13}C NMR (75 MHz) spectral assignments of compound H_1 ($CDCl_3$)

C. No.	δ$_C$	C. No.	δ$_C$	C. No	δ$_C$
1	19.25	11	35.48	21	32.75
2	29.65	12	30.56	22	39.21
3	80.95	13	39.64	23	9.82
4	51.39	14	38.25	24	14.56
5	38.03	15	32.30	25	18.12
6	41.36	16	36.00	26	18.36
7	17.82	17	29.96	27	20.14
8	52.90	18	42.77	28	32.07
9	36.96	19	35.02	29	31.76
10	60.03	20	28.14	30	35.29
				OCH_3	56.28

5.3. 2 Chloroform extract of *H. vahliana* aerial parts

Two crystalline compounds were isolated from the chloroform extract of aerial parts of *H. vahliana* which were identified as **β-sitosterol** and **sitosterol-3-O-β-D-glucopyranoside** . The bar diagram for their isolation is given in **Chart 5.2.**

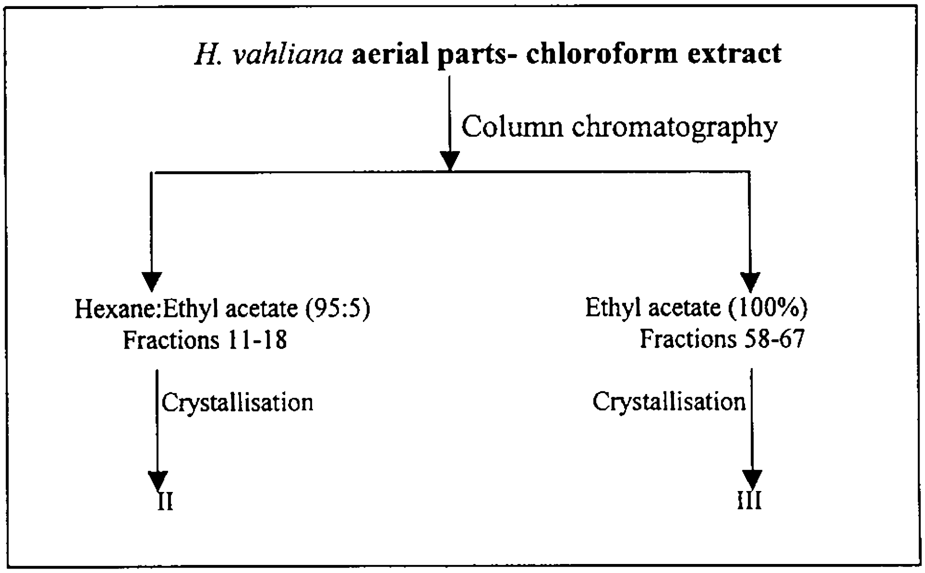


Chart 5.2

5.3.3 Methanol extract of *H. vahliana* aerial parts

The brown residue from the methanol extract of *H. vahliana* aerial parts yielded two crystalline compounds XIII and **sitosterol-3-O-β-D-glucopyranoside**.. The bar diagram for their separation is given in **Chart 5.3.**

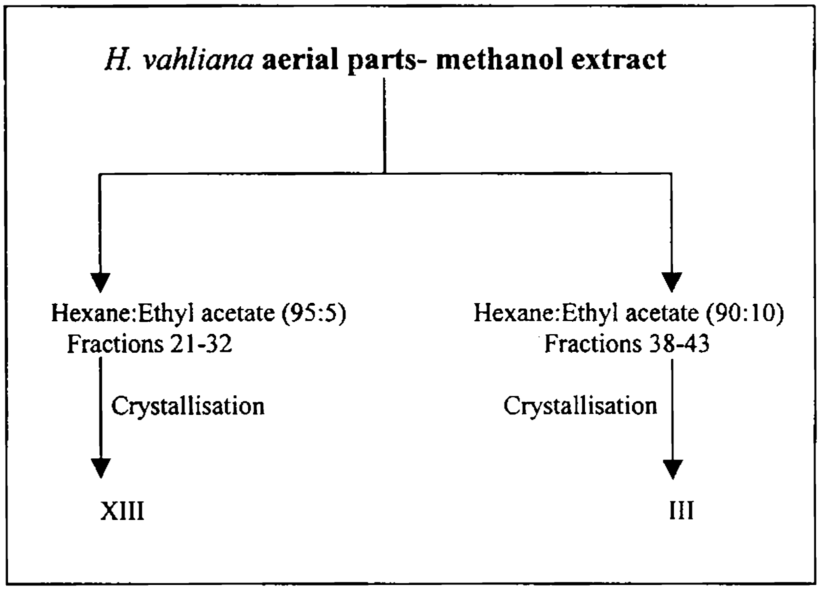
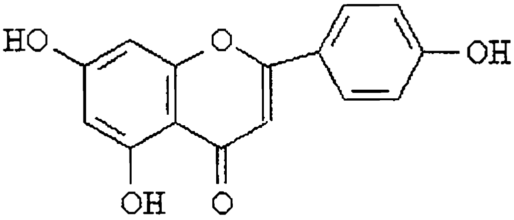
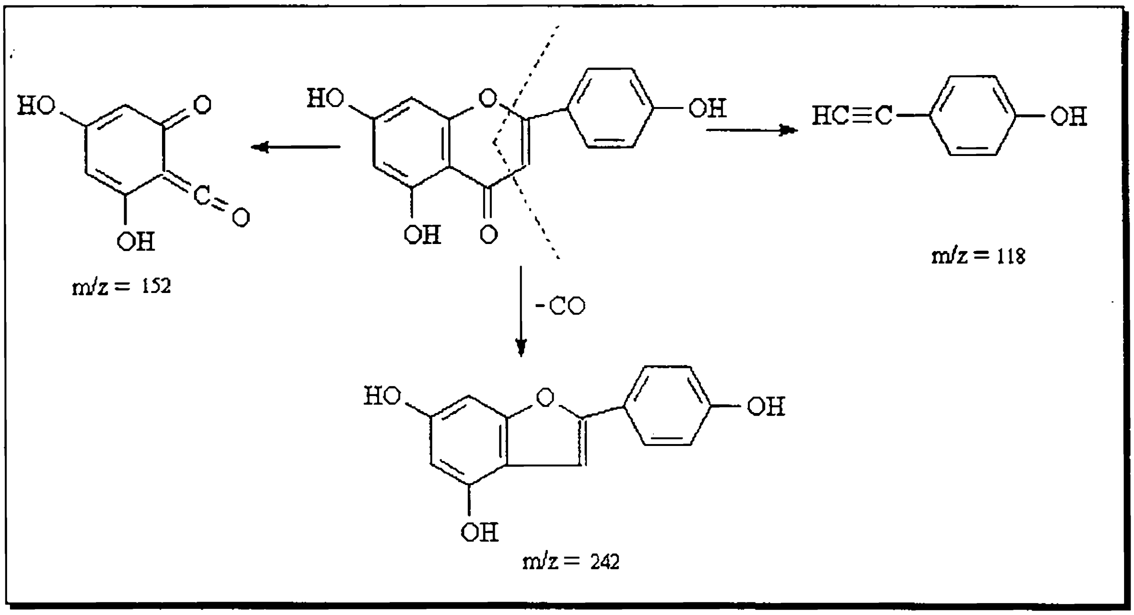

H. vahliana aerial parts- methanol extract
Hexane:Ethyl acetate (95:5)
Fractions 21-32
Crystallisation
XIII
Hexane:Ethyl acetate (90:10)
Fractions 38-43
Crystallisation
III

Chart 5.3


HO
O
OH
OH
O

(2)



Scheme 5.2: Mass spectral fragmentation of compound XIII

Structure of compound XIII

Compound XIII was obtained as yellow powder, from methanol, mp 345 ^{0}C. It gave positive reaction (red) to Mg-HCl test for flavonoids. IR spectrum showed hydroxyl group at 3338 cm^{-1} and a chelated carbonyl at 1655 cm^{-1}. EI mass spectrum of the compound indicated molecular ion peak at m/z 270, which accounted for the molecular formula $C_{15}H_{10}O_5$. ^{13}C NMR spectrum of the compound displayed 15 signals. A sharp singlet at δ 12.90 in ^{1}H NMR spectrum was characteristic of a chelated hydroxyl. It also showed two other hydroxyls as broad singlet at δ 10.37 and δ 10.82. A couple of two proton doublets at δ 7.86 and δ 6.86 (*J*=8.6 Hz each) were assigned to the protons of a 1,4-disubstituted aromatic ring. The one-proton singlet at δ 6.73 was assigned to H-3 of the flavone. The one-proton doublets at δ 6.14 and δ 6.43 (*J*=2.3 Hz each) were assigned to H-6 and H-8 of ring A of the flavone. The molecular fragments at m/z 153, 152, 119 and 118 indicated that two hydroxyls were located in ring A and one in ring B. The ^1H NMR and ^{13}C NMR and EI mass spectra of compound XIII were identical with those of 5,7,4'-tri hydroxy flavone reported earlier (lit. mp 347 ^0C) [2,5,6]. Hence the compound XIII was identified as **5,7,4'-trihydroxy flavone (2)**. The mass spectral fragmentation of the compound is shown in **Scheme 5.2.**

Table 5.6: ^{13} C NMR (75MHz) spectral assignments of compound XIII (DMSO-d_6)

C.No.	δ_C	C.No.	δ_C
2	164.34	10	104.40
3	103.40	1'	121.73
4	182.32	2'	129.06
5	162.00	3'	116.55
6	99.41	4'	161.72
7	164.71	5'	116.55
8	94.56	6'	129.06
9	157.89		

5.4 References

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3. Anjaneyulu, A. S. R., Rao, M. N., *Phytochemistry*, 1980, **19**, 1163.
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Chapter 6

Summary

Summary

The thesis deals with the isolation and identification of crystalline constituents from four plants namely *Piper colubrinum*, *Mussaenda frondosa*, *Artocarpus heterophyllus*, and *Humboldtia vahliana* by IR, UV, ^{1}H NMR ,^{13}C NMR and MS. Each chapter from II to V begins with an overview of phytochemical studies of the relevant genus followed by a detailed experimental section and results and discussion.The references are given at the end of the chapter .

Chapter II deals with chemistry of the genus *Piper* and characterization of five compounds from *P. colubrinum* leaves. The compounds isolated are n-triacontanoic acid, β-sitosterol, sitosterol-3-O-β-D-glucopyranoside, 5,4'-dihydroxy-7-methoxyflavone and 5,3',4'-trihydroxy-7-methoxyflavone. The last two compounds were reported for the first time from this genus. The results of these studies were presented in the 'UGC-DRS National symposium on Synthetic protocols and Structure elucidation in Chemistry' held at Madurai, during April 20-22, 1998.

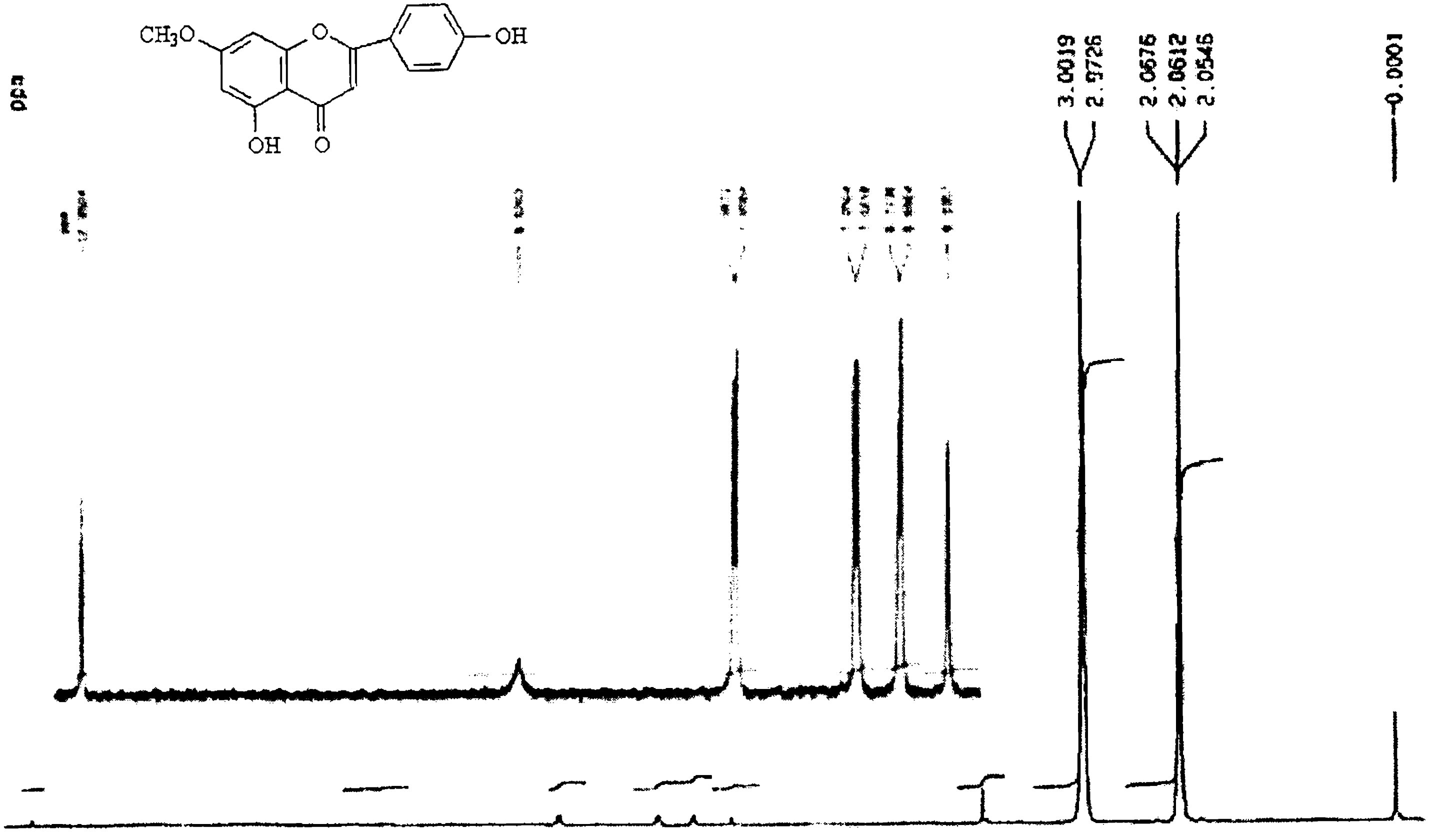
Chemical investigation of *M. frondosa* aerial parts resulting in the identification of four crystalline substances is described in Chapter III. The compounds identified are n-triacontanol, β-sitosterol, sitosterol-3-O-β-D-glucopyranoside and a mixture of 3β-hydroxy-urs-12-en-28-oic acid and 3β-hydroxy-olean-12-en-28-oic acid. 3β-hydroxy-urs-12-en-28-oic acid and 3β-hydroxy-olean-12-en-28-oic acid are well known as anti-inflammatory agents. Hence *M. frondosa* can serve as a new source for these two compounds. The results have been communicated to *Fitoterapia*.

Chapter IV describes the isolation and structure elucidation of five compounds from the pericarp of fruits of *A. heterophyllus*. These are cycloartenone, 9-hydroxytridecyldocosanoate, β-sitosterol, 5,7,2'4'-tetrahydroxy-6-γ,γ-dimethylallylflavone and sitosterol-3-O-β-D-glucopyranoside. This is the first report on the chemistry of pericarp of fruits of *A. heterophyllus*. The results have been communicated to *Asian Journal of Chemistry*.

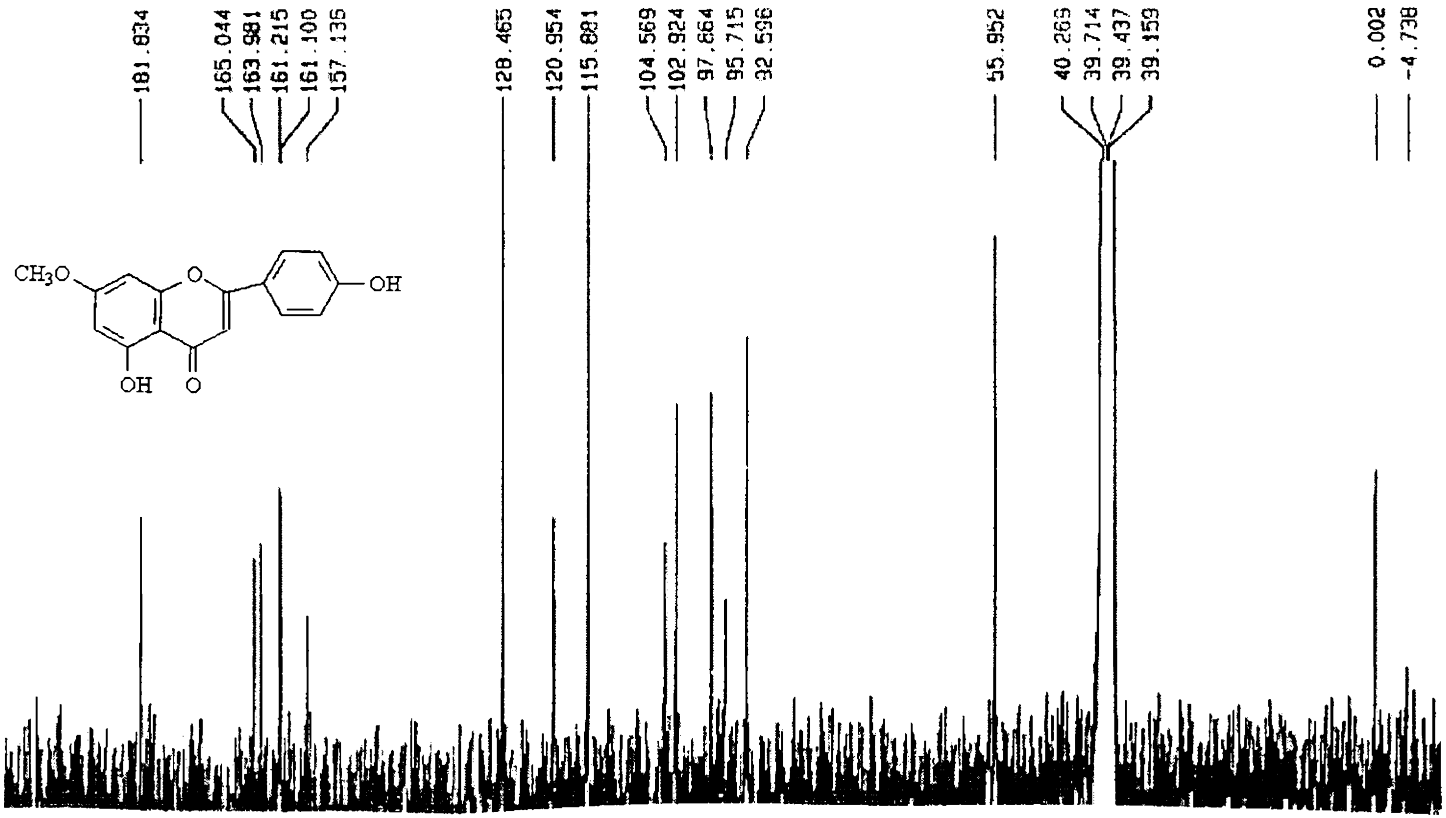
The isolation and characterization of 3α-methoxy friedelan, β-sitosterol, sitosterol-3-O-β-D-glucopyranoside and 5,7,4'-trihydroxyflavone from the aerial parts of *Humboldtia vahliana* are presented in Chapter V. This is the first report on the chemistry of *Humboldtia vahliana*.

Appendix

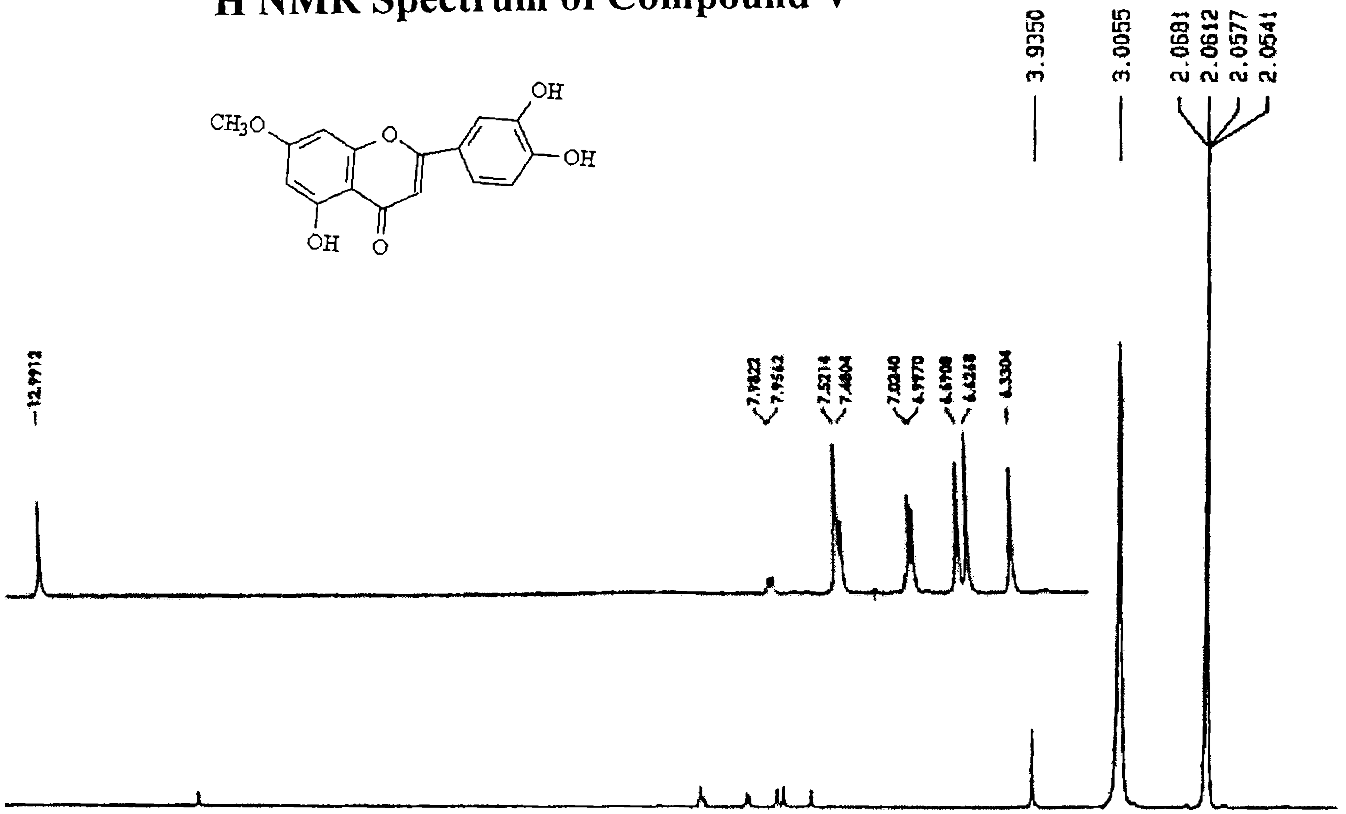
^{1}H NMR Spectrum of Compound IV



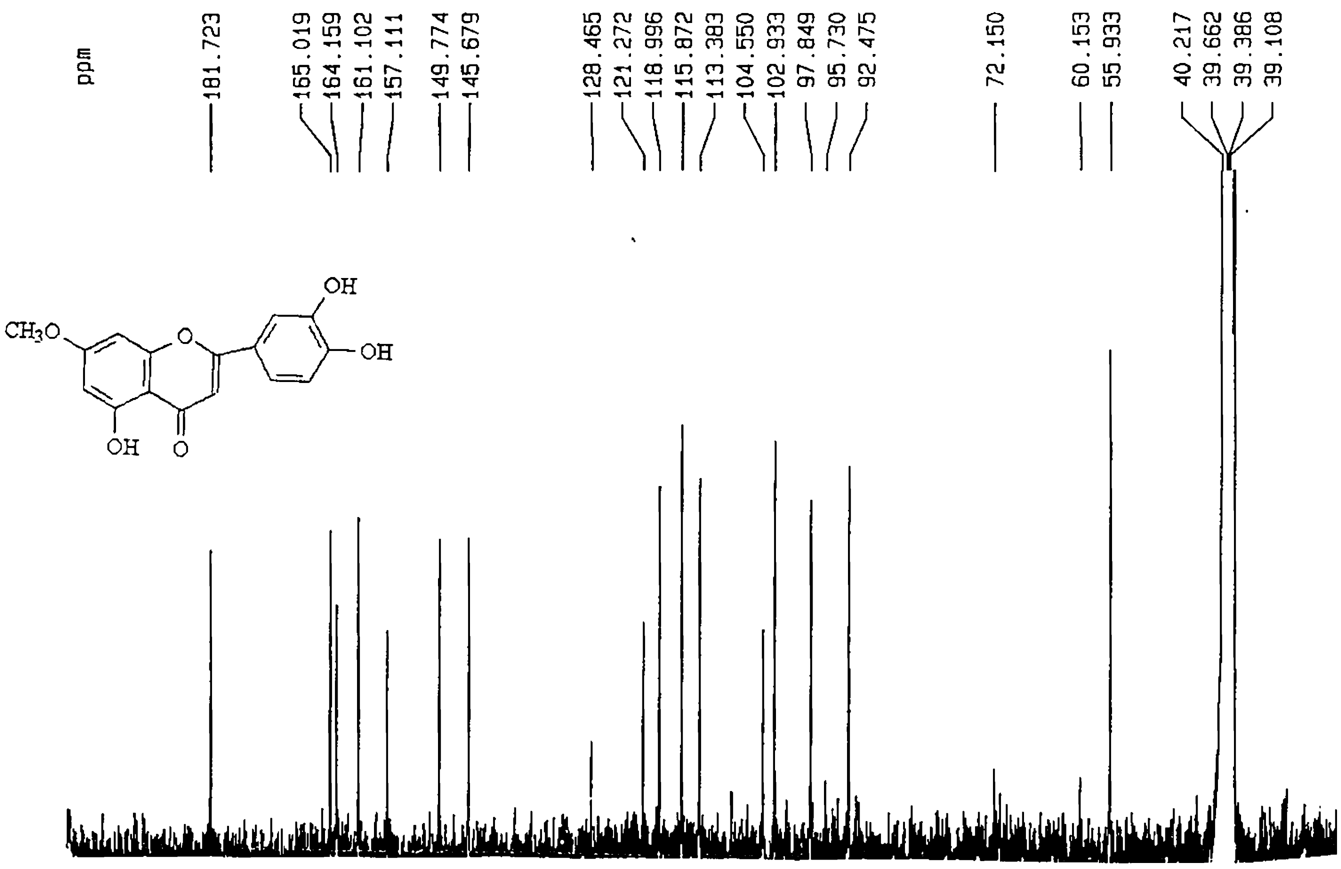
^{13}C NMR Spectrum of compound IV



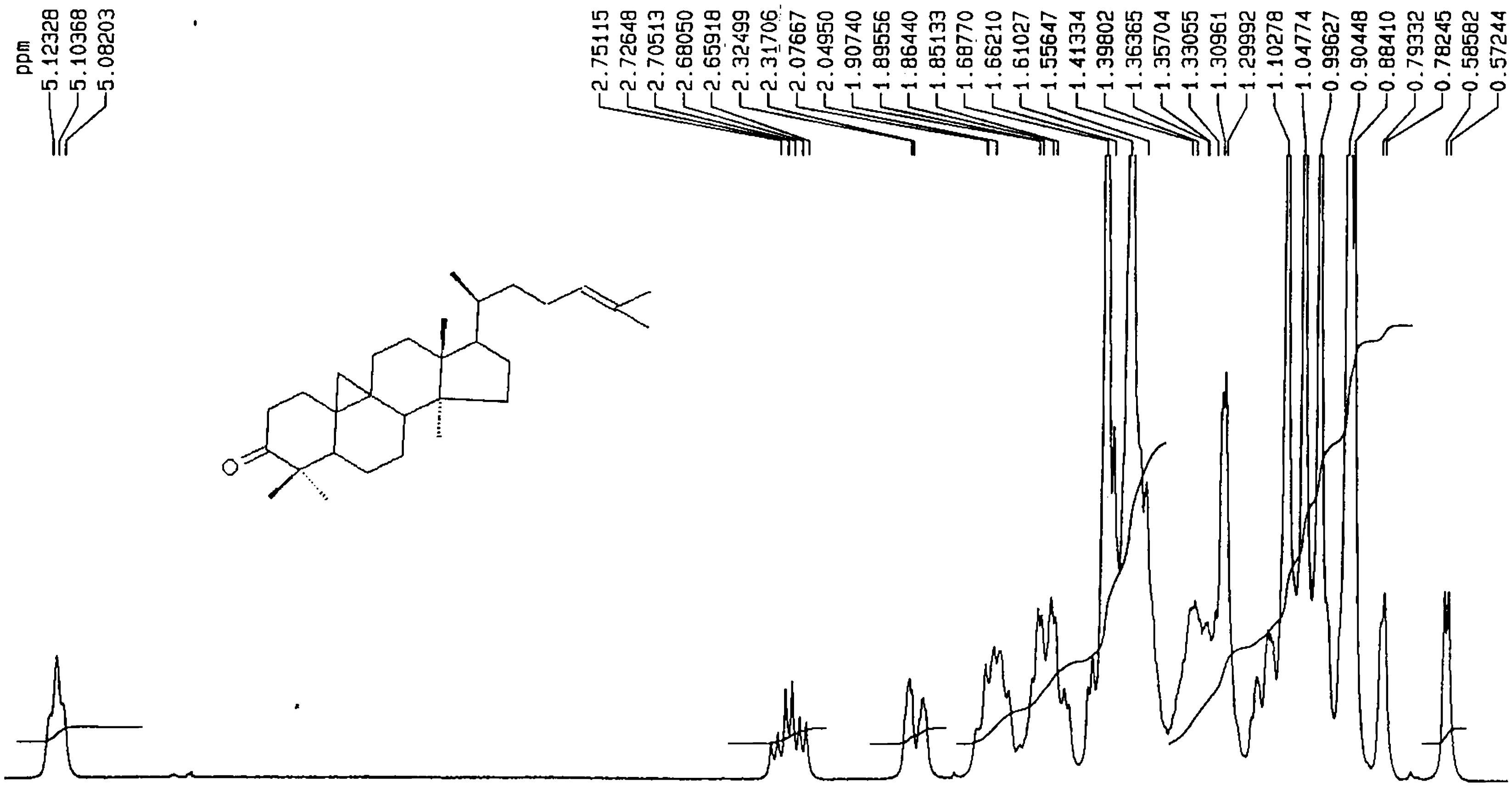
^{1}H NMR Spectrum of Compound V



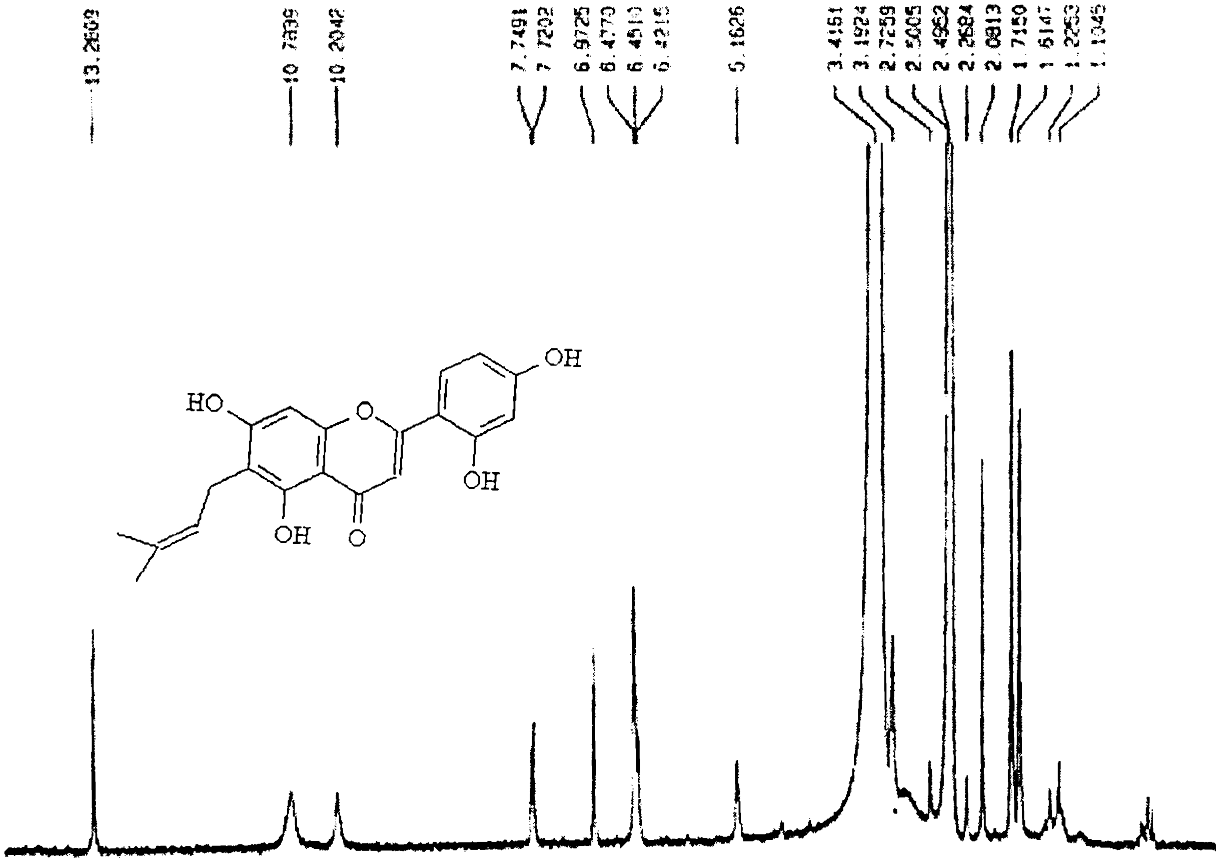
^{13}C NMR Spectrum of compound V



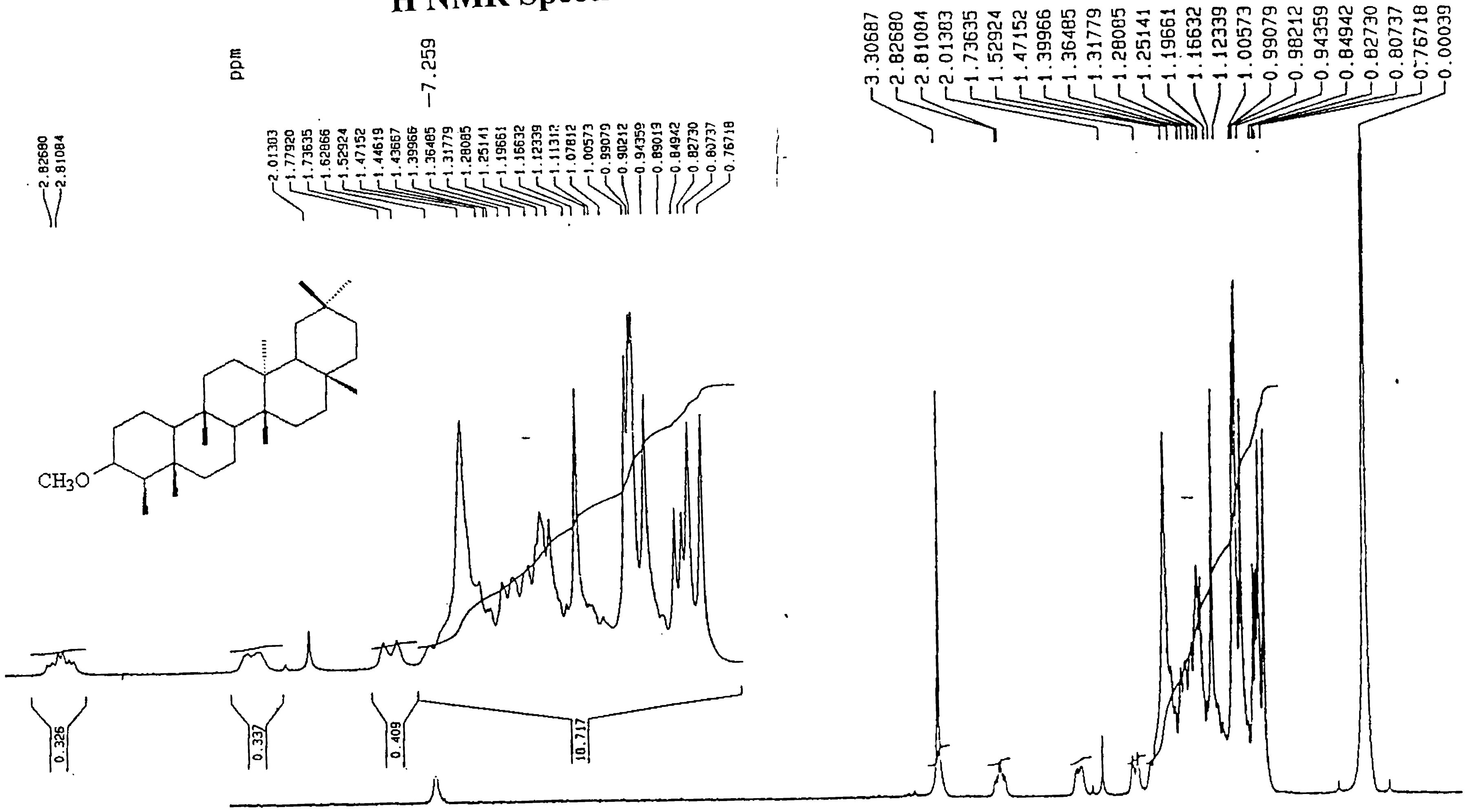
^{1}H NMR Spectrum of Compound IX



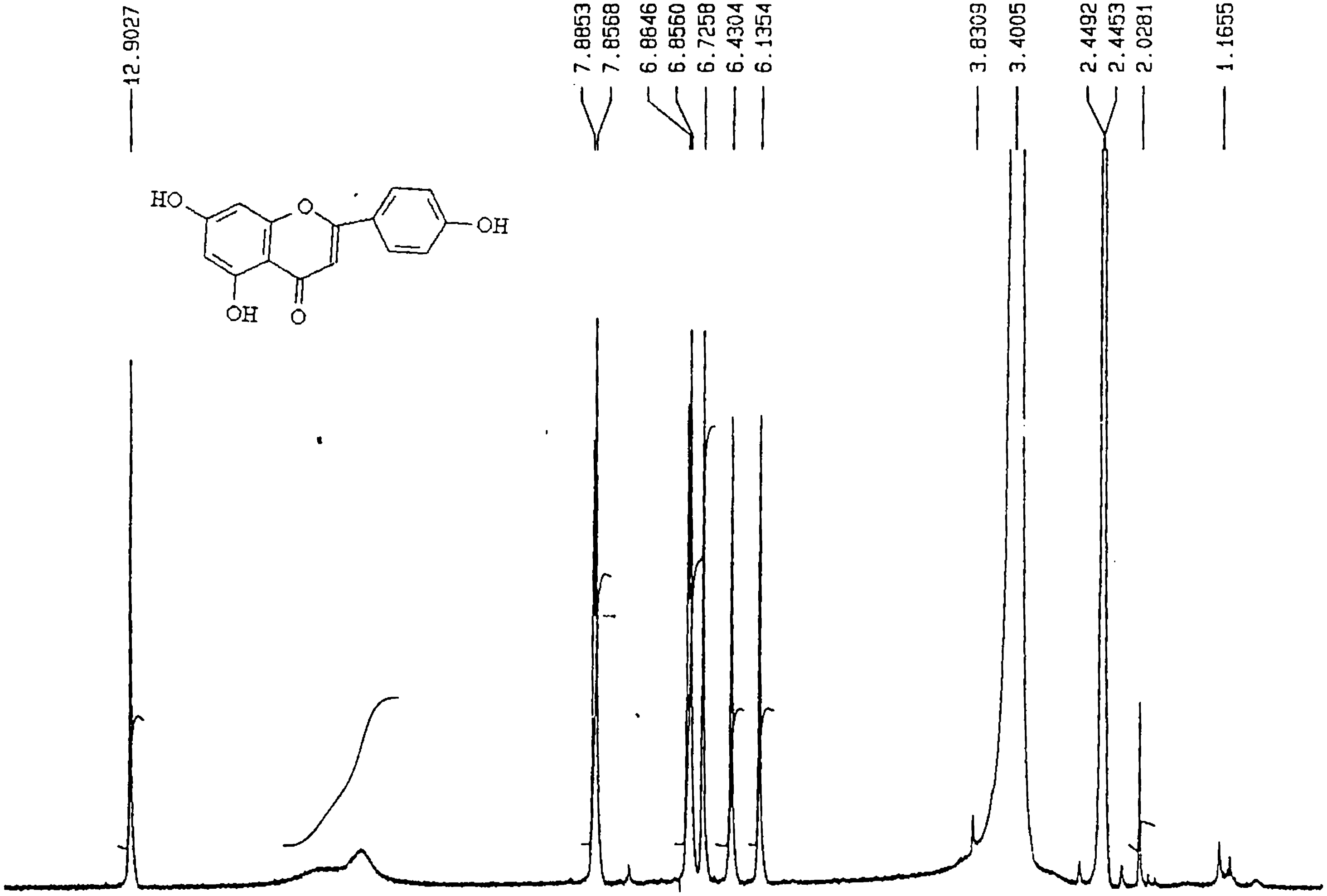
^{1}H NMR Spectrum of compound XI



^{1}H NMR Spectrum of Compound XII



¹H NMR Spectrum of Compound XIII



^{13}C NMR Spectrum of Compound XIII