

**PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION ON
PAEONIA EMODI WALL. EX ROYL AND *BERGENIA LIGULATA*
SENSU BLATTER**



Ph.D. Thesis

By

ANWAR SADAT

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR,
PESHAWAR PAKISTAN,
JANUARY 2014**

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SENSU BLATTER**

By

Anwar Sadat

**Dissertation submitted to the University of Peshawar in
partial fulfillment of the requirements for the degree of
doctor of philosophy in organic chemistry**

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR,
PESHAWAR PAKISTAN,
JANUARY 2014**



DEDICATED TO

MY PARENTS,

MY FAMILY

&

TEACHERS

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Acknowledgments

In the name of Allah who is the Creator of the Universe who gave me puissance to consummate this research work and to explore the physique of the molecules originating in nature.

I feel great elatedness to express my indefinable appreciativeness thanks to my encouraging, animate and learnt supervisor Prof. Dr. Ghias Uddin (Foreign Professor, Institute of Chemical Sciences, University of Peshawar) whose personal interest, guidance and consequential advices, suggestions and discussions enfranchised me to complete my research work.

The research work illustrated in the current dissertation would have never been accomplished without the momentous and endless encouragement of my co-supervisor Prof. Dr. Bina Shaheen Siddiqui (HEJ Research institute of Chemistry, University of Karachi) who provided me with advanced research facilities available to any student of the institute.

I convey my deepest gratitude and appreciation to Prof. Dr. Yousaf Iqbal (Director Institute of Chemical Sciences, University of Peshawar) who guided me during the course of my Ph.D. in the right direction and facilitated my research work and thanks to all the faculty members particularly to all professors of organic chemistry for their moral support.

I am highly thankful to Higher Education Commission of Pakistan for financial support under the indigenous fellowship scheme and access to modern spectroscopic and biological assay techniques.

The contribution of Prof. Dr. Abdur Rashid (Ex-chairman Botany Department, University of Peshawar) and Abdul Majid (Lecturer Department of Botany, Hazara University, Mansehra) in plant identification and collection is worth mentioning.

Special thanks to all my lab fellows, Dr. Hamid Hussain, Dr. Abdul Latif, Dr. Taj-ur-Rehman, Mr. Ashfaq Ahmad and Mr. Muhammad Alam and Mr. M. Akram for their encouragement and providing friendly environment for my research work. I am also very much thankful to our lab assistants Mr. Waqas and Mr. Saqib for their assistance during the course of my research.

In the last but not least, I have no word to thank my respectable and loving parents for their unflagging efforts, lots of prayers, encouragements, guidance, moral and financial support. Thanks to my siblings and wife for their well wishing and prayers.

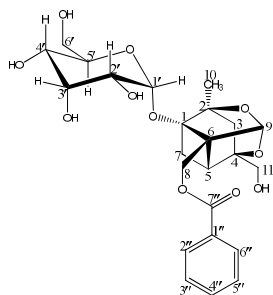
Anwar Sadat

Abstract

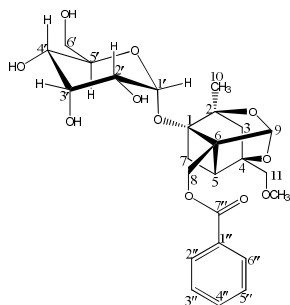
The research work illustrated in the dissertation is inhere of two parts mainly converged on the isolation of bioactive secondary metabolites from two well-known medicinal plants *P. emodi* and *B. ligulata*, their structural elucidation utilizing modern spectroscopic techniques such as EI, ESI, ESI-HR-MS, ^1H , ^{13}C NMR and 2D NMR. Different bioassay techniques were used to figure out the bioactive potential of various isolated secondary metabolites.

Part A

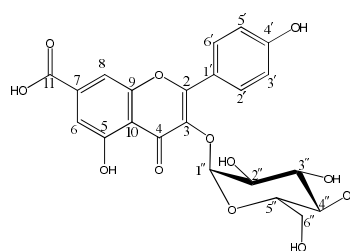
Part A described the phytochemical investigation of *P. emodi*, natural grown medicinal plant of Pakistan, which led to the isolation, structural elucidation and characterization of eighteen secondary metabolites, including two new peoniflorines (**6**, **7**), three new flavonoids (**12**, **15**, **16**) and fifteen reported compounds, six of them are hitherto unreported from the ethyl acetate fraction of aerial parts of *Paeonia emodi*. The flavonoids displayed remarkable radical scavenging activity and urease inhibitory potential.



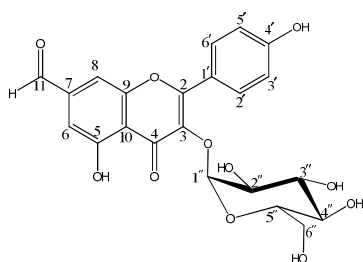
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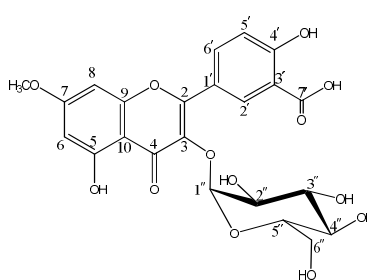
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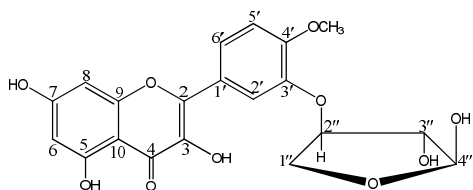
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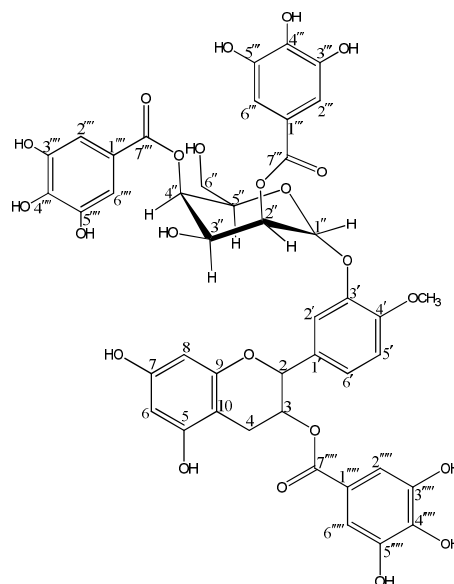
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Part B

This part of the research work is amid to the isolation and characterization of two new compounds (**22**, **29**) together with nine known constituents, five are hitherto unreported from the ethyl acetate fraction of *B. ligulata*. Their bioactive potential were evaluated by the use of different bioassay techniques and was established that the bergenin and its isolated derivatives displayed moderate to significant antiplasmodial activity while the flavonoids exhibited significant antioxidant and urease inhibitory potential.



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List of abbreviations

Ar	Aryl
AcO	Acetate
BB	Broad Band
COSY	Correlation spectroscopy
D	Deuterium
DCM	Dichloromethane
DMSO	Dimethyl sulphoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EIMS	Electron impact mass spectrometry
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HSQC	Heteronuclear single quantum coherence
HRESIMS	High resolution electrospray ionization mass spectrometry
Hz	Hertz
¹ H-NMR	Hydrogen-1-nuclear magnetic resonance
¹³ C-NMR	Carbon-13-nuclear magnetic resonance
IC ₅₀	Inhibitory concentration 50 percent
IR	Infrared
<i>J</i>	Coupling constant
mM	Millimole
<i>m/z</i>	Mass to charge ratio
Me	Methyl
MeO	Methoxy
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy

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GENERAL INTRODUCTION

1.0. Introduction

The phytotherapeutics were used through the ages by humankind as a core of the treatment for perilous medical conditions like microbial infections, viral disease, cancer, heart diseases and many other physiological and pathological conditions. Although most of the medicaments are currently synthesized or have semi synthetic origin but most of these are modeled onto the structure of the plant derived biologically active secondary metabolites.¹

About 80% of the population of the developing countries uses traditional medicines for their primary health care according to the World Health Organization (WHO) and this trend is pronounced in Asia where extensive use of the medicinal plants is well documented. Up till now 119 chemical substances of known properties derived from the plant origin are currently used as a major medicine throughout the world e.g. morphine, vincristine and vinblastine etc.²

Plants and natural remedies are used by human dated million of years back. The ancient civilizations dead remains have shown that plants were used for various physiologic dysfunction, pathological conditions, to promote the human health and treated diseases with the first written account of herbal remedies from China (2800 BC).³

The Chinese system of medicines has been extensively affirmed over centuries the use of medicinal plant species. Based on the literature 5000 years ago, Shen Nung was the first Chinese known physician interested in the medical craft and tested different plants. Consequently, he documented a number of drugs and toxins by experimenting on his followers. Pen Taso “The Great Herbal” is his amassment contained the illustration of

more than thousand drugs, several of which like croton, rhubarb, opium and aconite are still in practice in the traditional Chinese Medicine.^{4, 5}

From the start of human race, they depend on plant sources for their food, shelter and maintenance of health. With the passage of time, they knew about the use of plants species for health and food, which led to development of various disciplines of science. Benzoic acid is the first natural product isolated from the plant origin. Subsequently, F.W. Serturner isolated morphine from the opium and subsequently quinine, strychnine, brucine, caffeine, cinchonine were isolated from various plants by Pelletier and his co-workers, however their structures were elucidated by Schiff in 1870 and synthesized by Ladenburg.⁶

In the beginning of the 20th century, research in synthetic drugs have taken over importance, however phytochemistry has also been gained a considerable interest of scientists in herbal drugs with the discovery of penicillin and other antibiotics which played an important role in the treatment of malaria, cancer, cardiovascular diseases and mental disorder. In this contest reserpine and ajmaline hydrochloride were isolated from *Rauwolfia serpentina* (Indian snakeroot) to treat hypertension and cardiac arrhythmias, respectively.⁷

The alkaloids; vinblastine and vincristine, isolated from *Catharanthus roseus* have been found to be effective in the treatment of choriocarcinoma, testicular cancer, breast cancer, epidermoid carcinoma, Hodgkin's disease and acute lymphocytic leukemia in children. Similarly, Taxol, a diterpene isolated from a number of *Taxus* species, is a drug of choice for the treatment of ovarian and breast cancers while artemisinin a

sesquiterpene isolated from *Artemisia annua* as a potent antimalarial drug against resistant strains *Plasmodium falciparum*.⁷

During the middle ages, Muslim scientists dominated in the field of medicine. Ibn Sina (908-1037 C.E) was the great Muslim physician and philosopher, wrote a book on phytomedicines (Qanun fi al-Tibb) which is an immense encyclopedia of medicine extending over a millions words. Another great Muslim scientist Al-Razi (864-930 C.E.) contributed significantly and has written many books such as Kitab-al-Mansoori, Al-Hawi, Kitab al-Mulooki and Kitab al-Judari wa al-Hasabahearned in the field of medicines. Ibn-al-Baitar (1197-1248 C.E) a great Spanish Muslim scientist also contributed in the field of botany and pharmacy and wrote a book Kitab al-Jami fi al-Adwiya al-Mufrada on medicinal plants. Al-Idrisi (1099-1166 C.E.) a well-known Muslim scientist, studied medicinal plants and documented in the book Kitab al-Jamili-sifat Ashtat-al-Nabat.⁸⁻¹⁰

Furthermore, the discovery of therapeutic agents from natural sources have been increasingly rationalized that the bioactive natural products serve for the dual purpose of nurturing the pharmacopoeial spectrum and capitalizing valuable guidelines for the synthesis of new therapeutic agents modeled onto the skeletal structure of these natural products.^{11, 12}

With the invention of the sophisticated instruments and softwares led to new approaches such as computer based molecular modeling and combinatorial chemistry which is being in practice to aid on in drug discovery, however natural product chemistry is playing the key role in drug discovery and development. Isolation of bioactive

secondary metabolites from the plants is the key interest for natural product chemist. In this context exploiting of the plants to isolate secondary metabolites and evaluate these chemical substances for various biological activities, is the modern method of drug discovery. A very small number of plant species have been subjected to phytochemical screening and isolation of secondary metabolites out of the whole Kingdom of Plantae so there is intense need to explore the flora photochemically in order to develop new therapeutic agents that can be safer, more selective and easily available.^{13, 14}

The current research project is based on the literature to explore two medicinal plants i.e. *Paeonia emodi* and *Bergenia ligulata* phytochemically which have been used extensively in the traditional medicines from centuries but very few secondary metabolites of therapeutic importance have been isolated.

BIOSYNTHESIS

Biosynthesis or biogenesis is enzyme-catalyzed complex chemical processes occur within the living cells; resulting in formation of complex molecules from relatively simple fragments. Sequencing of biosynthetic pathways and divulging of the biochemical reactions takes place which required multidisciplinary approaches of the biological amalgamation, isotopic labeling of the precursors and genetic information etc.¹⁵

For all these consequent biosynthetic reactions, energy and substrates are provided by photosynthesis occurring in chlorophyll containing plants as well as bacteria. Enzyme catalyzed reactions modeled these simple substrates into complex molecules called metabolites which are classified in to two major metabolite categories i.e. primary and secondary.¹⁶

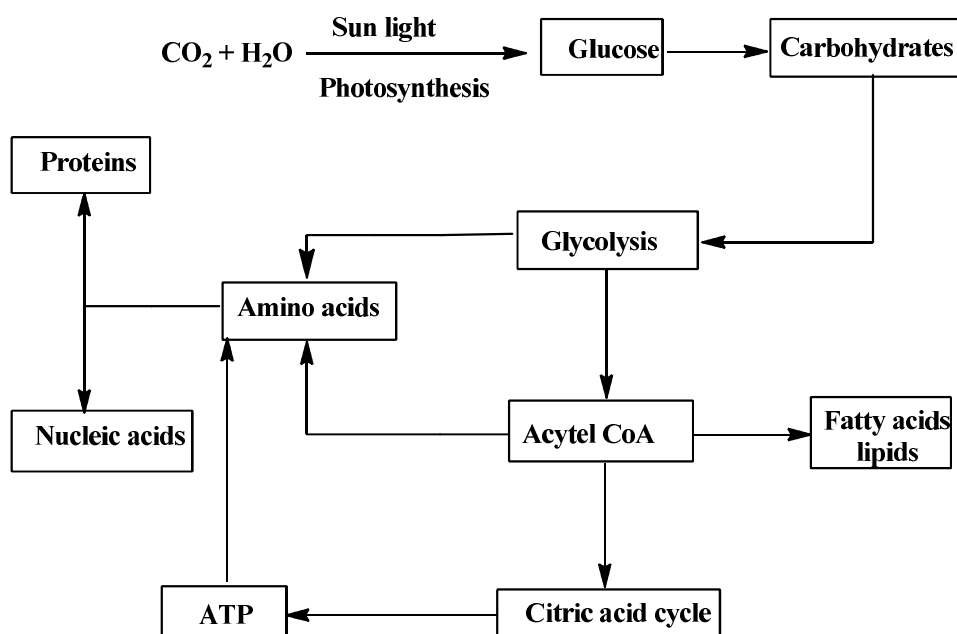
Nucleic acid, polysaccharides and proteins are the building rudiment of all living cells and therefore label as primary metabolites synthesize by primary metabolic pathways as these are pivotal for their growth and existence. Still there are other kinds of metabolites which are synthesized by the plants species according to their type and these are not mandatory for their existence and growth i.e. terpenes, alkaloids, flavonoids, spaponins and irodides etc. are termed as secondary metabolites.¹⁷

Basic steps involved in the primary and secondary metabolites biosynthesis are as follow.

2.1 Primary metabolites

These metabolites are indispensable and forthrightly involved in the growth, reproduction and photosynthesis by which carbon dioxide is converted into carbohydrate which are then transmute to adenosin triphosphate (ATP) *via* krebs cycle (TCA) which is

involved in amino acid biosynthesis controlled by the genetic information stored within nucleic acids as outlined below (**Scheme-2.1**).¹⁸

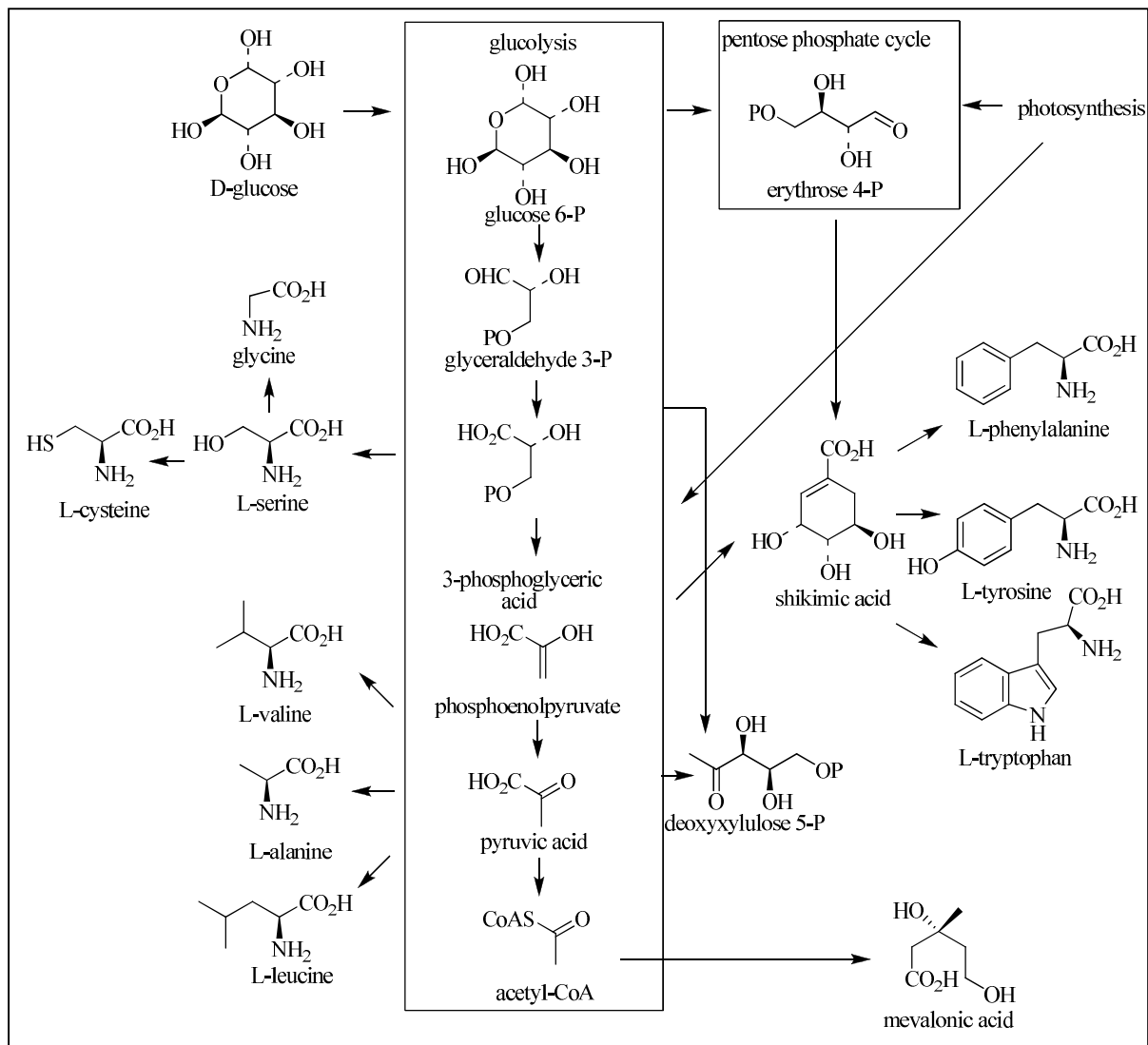


Scheme-2.1: Primary metabolites biosynthesis

2.2. Secondary metabolites

These metabolites are not directly involved in the growth, development or reproduction and biosynthesize by primary metabolites, which are found mostly in the plants and microorganisms e.g. alkaloids, flavonoids, phenols, steroids, terpenes and oligosaccharides etc.¹⁸

Many of the secondary metabolites displayed excellent biological activities such as anti-inflammatory, analgesic and antioxidant etc.¹⁸ and their structures are derived from primary metabolites (**Scheme-2.2**).



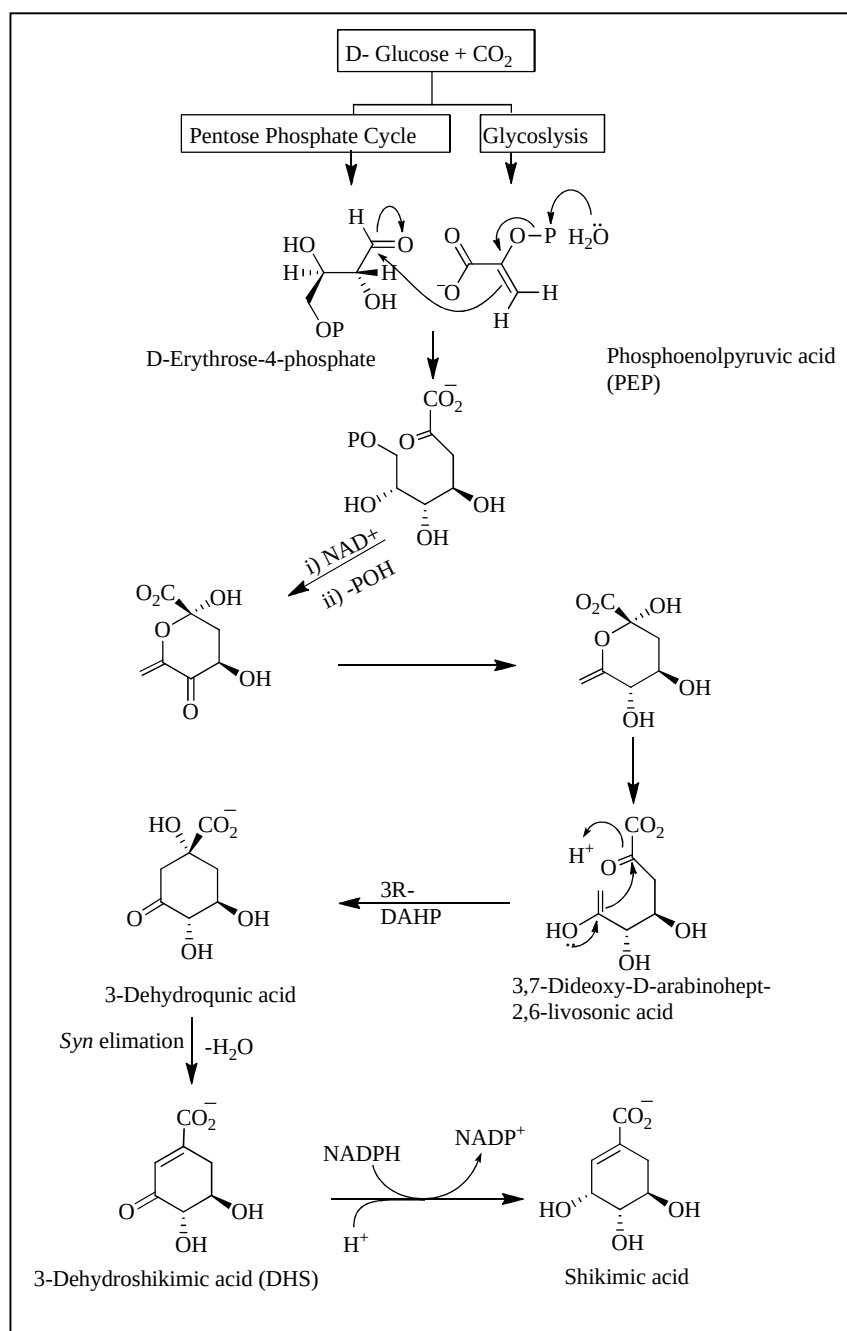
Scheme-2.2: Biosynthesis of secondary metabolites¹⁸

2.3. Biosynthesis of flavonoids

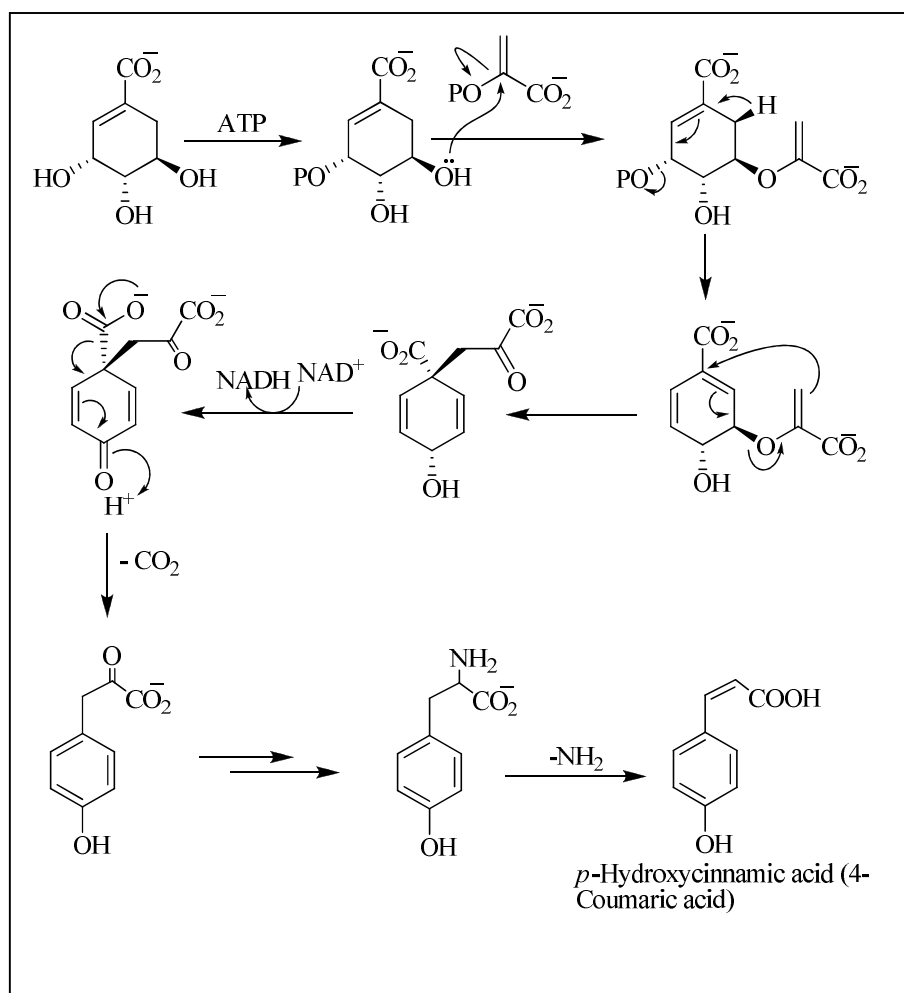
The term flavonoid is derived from “flavus” a Greek word signify yellow. These are also termed as anthoxanthins due to the coloring co-pigments of the plants which give range of colors and shades to the flowers and leaves. Owing to their structural variation, these can be divided into several classes e.g. flavonoid, isoflavonoid, neoflavonoid and homoflavonoid. The parent flavonoid nucleus comprises of fifteen carbon atoms in which three carbons’ chain from diphenylpropane derivatives linked two phenyl rings known as chalconoids which then react with oxygen to form a five member (aurochalconoids) or six member heterocyclic ring derived from 1,3-diphenylpropane nucleus (flavonoid). Those derived from 1,2-diphenylpropane nucleus i.e. 3-phenylcoumarin skeleton is known as isoflavonoid while those obtained from 1,1-diphenylpropane nucleus are termed as neoflavonoid. Homoflavonoid has an additional carbon atom at position C-11 in flavonoid nucleus. In certain cases, the flavonoids have hydroxyl group attached to a sugar unit *via* an acid labile hemiacetal bond result in the formation of *O*-glucoside flavonoid while there are some other flavonoids in which sugar is attached to the carbon atom to constitute C-glycoside flavonoid, which are acid resistant.^{19, 20}

Research studies showed that malonyl-CoA and 4-coumaroyl-CoA (*p*-hydroxycinnamic acid CoA ester) are the main progenitor of chalcones biosynthesis. Glycolysis intermediate acetyl-CoA and carbon dioxide react to form malonyl-CoA and the reaction is catalyzed by the acetyl-CoA carboxylase while 4-coumaroyl-CoA is synthesized *via* shikimate pathway, which is the main route to synthesize phenylalanine, tyrosine and aromatic amino acids in the higher plants.²¹

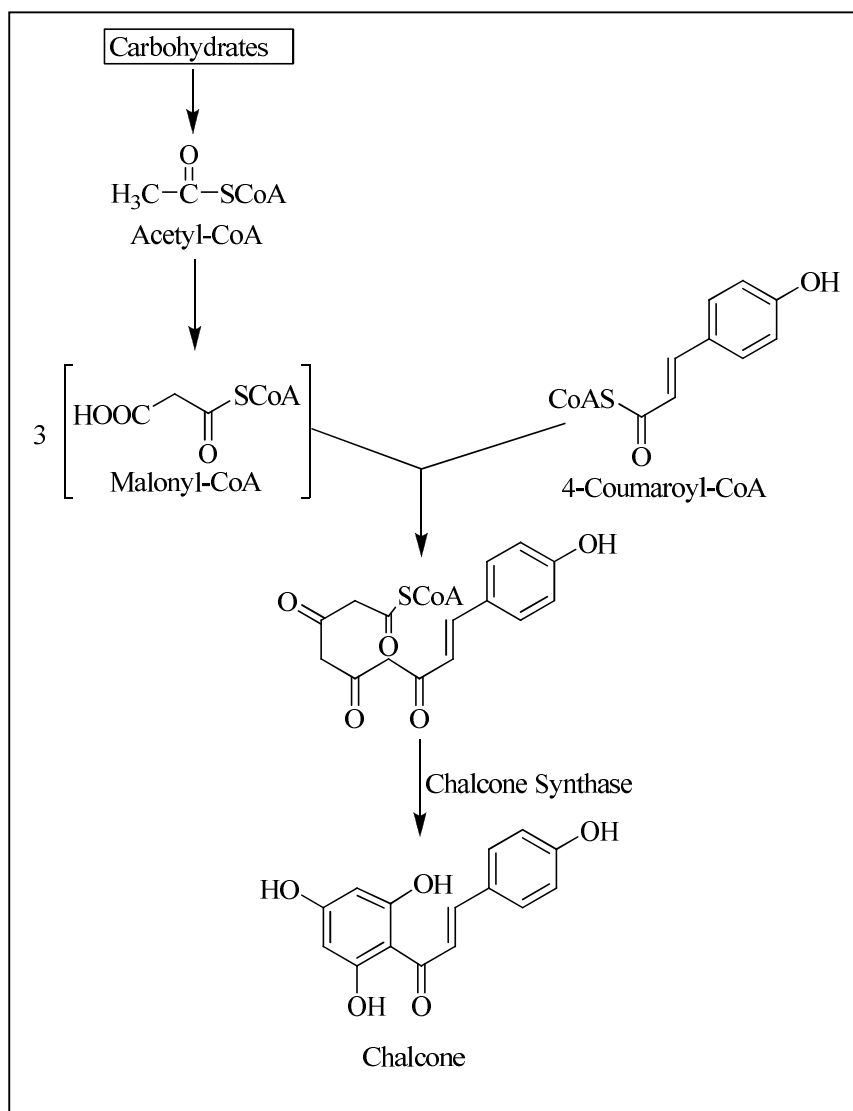
The Shikimic acid biosynthesis (**Scheme-2.3**) is initiated by the condensation of D-erythrose-4-phosphate from pentose phosphate pathway and phosphoenolpyruvic acid from glycolysis. After a sequence of biosynthetic reactions, shikimic acid is converted to *p*-hydroxycinnamic acid (**Scheme-2.4**). The 4-coumaroyl-CoA and malonyl-CoA then couple together to form chalcone (**Scheme-2.5**) followed by cyclization catalyzed by chalcone synthase.²²



Scheme-2.3: Shikimic acid biosynthesis.²²



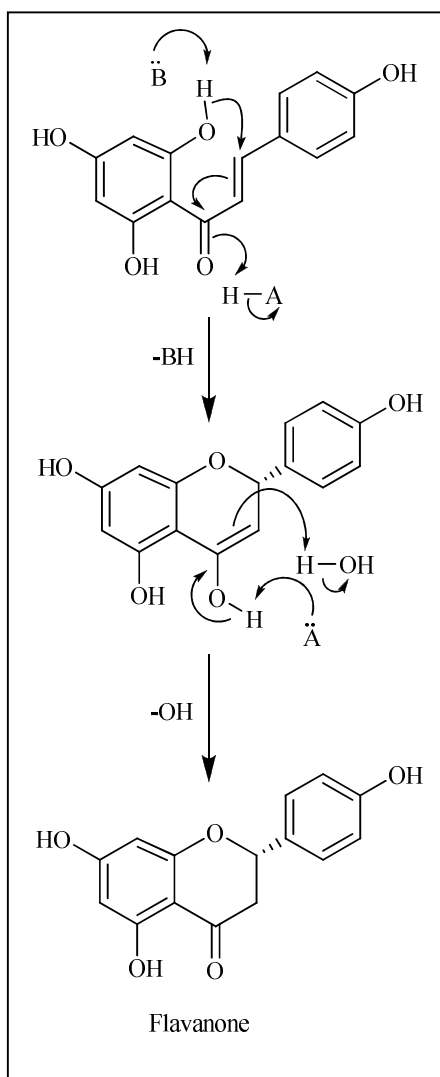
Scheme-2.4: Conversion of shikimic acid into *p*-hydroxycinnamic acid.²²



Scheme-2.5: Coupling of malonyl-CoA and 4-cumaroyl CoA to form chalcone.²²

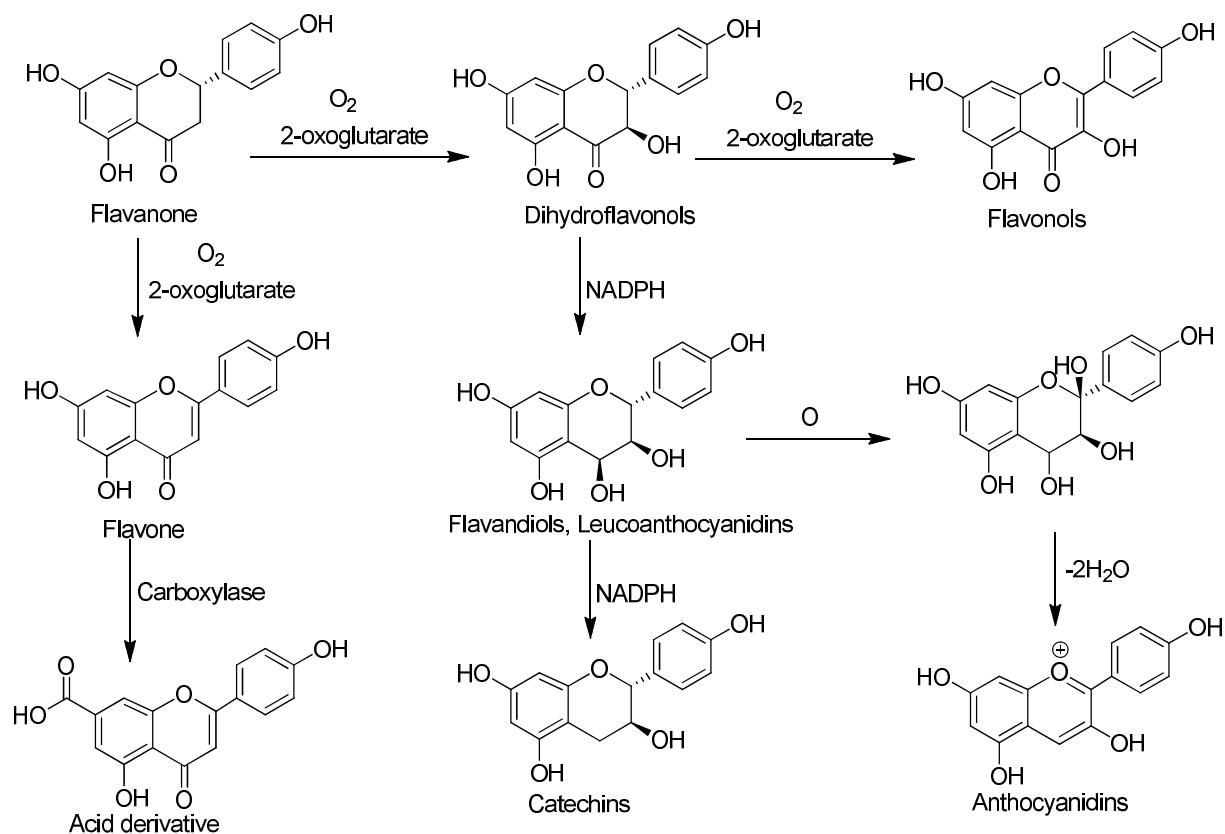
The enzyme chalcone flavanone isomerase subsequently isomerizes chalcone into flavanone, which is a stereospecific enzymatic reaction. Therefore, naturally occurring flavanones have (*S*) configuration at C-2, while the equilibrium completely shifts toward flavanone in aqueous medium (**Scheme-2.6**). Furthermore, a carbonyl group with *ortho*-

phenolic hydroxyl group having strong hydrogen bonding also stabilized the equilibrium towards flavanone.²²



Scheme-2.6: Flavanone biosynthesis.²²

Flavanone is converted by different enzymatic reactions into its different natural derivatives by dehydrogenation, dehydration or reduction (**Scheme-2.7**).



Scheme-2.7: Different enzymatic reactions convert flavanone to different derivatives.²²

PLANT INTRODUCTION (part A)

3.1 Plant introduction

Plant description

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Saxifragales
Family:	Paeoniaceae
Genus:	<i>Paeonia</i>
Species:	<i>Paeonia emodi</i>
Binomial name:	<i>Paeonia emodi</i> Wall. ex Royle



Photographs of the *P. emodi* in the field

3.1.1. Family Paeoniaceae

The family Paeoniaceae is also known by the name of “Ranunculaceae” consisting of 33 genera distributed mainly in warm temperate region of Europe, Asia, and North-Western America. They are perennial herbs or sometime shrubby plants up to 2 m tall and grow from stout rootstocks.²⁴

3.1.2. Genus *Paeonia*

The genus *paeonia* comprises of 38 species, native to Northern Temperate region of Mediterranean and Asia. These species are mostly herbaceous while eight are of woody kind and *Paeonia emodi* is the only species found in Northern areas of Pakistan (Hazara Division). These species grow well in the cold climate of hilly area and flourish well in moist and loamy soil. The herbaceous paeonias are mostly used for ornamental purposes. In dainty of tincture and aroma they are similar to roses.²⁴

3.1.3. *Paeonia emodi* Wall. ex Royle

P. emodi is a perennial herb, which is about 50 cm tall with an erect leafy and glabrous appearance, having biternate or ternate leaves with pale lamina while flowers are of white color showing up solitary and axillaries arrangement. The bracts are leafy, petals are 8 and seeds vary from 3-5.²⁴

3.2. Pharmacological importance of the genus *Paeonia*

P. emodi is an important medicinal plant of the subcontinent. The first person who discovered this plant was Pliny, Roman author, naturalist, and natural philosopher and given the name *Paeonia* derived from Paeon means the physician of god because he discovered the efficacy of the plant in many illnesses.²⁵

In the indigenous system of medicine, it is placed at very high profile based on its potential physiologic activities. The tubers of the *P. emodi* are used for the treatment of convulsions, colic, hysteria, uterine diseases and bile duct obstructions and also given to the children as a blood purifier while seeds are cathartic and emetic in nature. The dried flowers are used for the treatment of diarrhea while tea made up of petals of various peony species are used for the treatment of cough, varicose veins and hemorrhoids. *P. emodi* is medicinally famous plant f to treat hypertension, palpitations and asthma.^{26, 27}

M. A Tantry *et al.*, in 2012 isolated nortriterpenoid (6 α ,7 α -epoxy-1 α ,3 β ,4 β ,13 β -tetrahydroxy-24,30-dinor-olean-20-ene-28,13 β -olide) from the ethyl acetate-chloroform fraction of air dried roots of *P. emodi* and tested for cytotoxicity against human cancer cell lines. The nortriterpen was the most active one with IC₅₀ value of 12 $\pm$ 10.05 mg/mL against HCT116.²⁸

F. Haq *et al.*, in 2012 analyzed *P. emodi* extract for the presence of different elements such as sodium, potassium, calcium (66.06 mg/c), magnesium, copper, zinc, iron, cobalt (0.039 mg/c), manganese.²⁹

Ghayur *et al.*, in 2008 tested 70% ethanolic extract of peony roots for airway relaxant activity and aganist arachiodonic acid inducted platelet aggregation, which showed significant airway relaxant activities and antiplatelet aggregation activities.³⁰

K. Taous *et al.*, in 2007 screened the ethanolic extract derived from the aerial parts of *P. emodi* for enzyme inhibition activities against urease (Jack Bean and *Bacillus pasteurii*) and α -chymotrypsin and the extract showed excellent urease inhibition

potential and moderate inhibition potential against α -chymotrypsin. In addition, the extract also displayed significant radical scavenging activity using DPPH assay.²⁵

K. Taous *et al.*, in 2007 also tested the crude extract for spasmolytic effect using the isolated rabbit jejunum and showed significant spasmolytic activity in a dose dependent manner and inhibited the spontaneous motility of the rabbit jejunum by 76% at 5 mg/mL concentration. The crude extract was subsequently fractionated into *n*-hexane, chloroform and ethyl acetate. The chloroform and ethyl acetate fractions exhibited excellent spasmolytic activities and were more potent than the native crude extract.²⁶

R. Naheed *et al.*, in 2003 isolated monoterpene glycosides paeonins A and B from the chloroform soluble fraction of *P. emodi* roots, which showed lipoxygenase inhibitory activity.³¹

In 2004 N. Riaz *et al.*, isolated paeoninol, paeonin C, oligostilbene and monoterpene galactoside from the methanolic extract of *P. emodi* fruits. The paeoninol and paeonin C displayed potent inhibitory potential with IC₅₀ (0.77 and 99.5 μ M) against enzyme lipoxygenase in a concentration-dependent fashion.³²

H. R. Nawaz *et al.*, in 2000 isolated a β -glucuronidase inhibiting triterpene from the chloroform fraction of *P. emodi* and established the structure as 1 β , 3 β , 5 α , 23,24-pentahydroxy-30-norolean-12,20-(29)-dien-28-oic acid.³³

P. Muhammad *et al.*, in 1999 isolated monoterpene glycosides i.e. wurdin and benzoylwurdin from the ethyl acetate fraction of *P. emodi*.³⁴

M. Asif *et al.*, in 1983 analyzed *P. emodi* roots' oil. The unsaponifiable lipid was found to contain a mixture of C14-33 *n*-alkanes, β -amyrin, butyrospermol, cycloartenol, lupeol, 24-methylenecycloartanol, two unidentified triterpene diols, cholesterol, campesterol, and sitosterol. The saponifiable lipid contained octanoic, decanoic, lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, and linoleic acids.³⁵

In 1972, Rashid *et al.*, carried out chemical analysis on the roots of *P. emodi* which contained starch (9.5%), sucrose (5.4%), reducing sugars (3.4%), malic acid (0.47%), oxalic acid (0.36%), tartaric acid (0.34%) and benzoic acid (0.02%).³⁶

3.3. Literature survey on the genus *Paeonia*

The *Paeonia* is the flowering plants containing a verity of phytochemicals ranging from simple acetylpyrrole and gallic acid derivatives to complex analogues of albiflorins and paeoniflorins along with flavonoids. The detail literature survey of phytochemistry of the genus *Paeonia* is summarized in the table 3.1.

Table-3.1. List of isolated compounds from genus *Paeonia*

S. No.	Name of Compounds	Molecular Formula	Sources
1.	2-Acetylpyrrole	C ₆ H ₇ NO	<i>P. moutan</i> ³⁷
2.	Acylsucroses-1'-benzoyl	C ₁₉ H ₂₆ O ₁₂	<i>P. obovata</i> ³⁸
3.	Albiflorin	C ₂₃ H ₂₈ O ₁₁	<i>P. albiflora</i> ³⁹
4.	Albiflorin-6'-O-benzoyl	C ₃₀ H ₃₂ O ₁₂	<i>P. delavayi</i> ³⁹
5.	Albiflorin-debenzoyl, 6'-O-(3,4,5-trihydroxybenzoyl)	C ₂₃ H ₂₈ O ₁₄	<i>P. obovata</i> ³⁹
6.	Albiflorin-4''-hydroxy, 6'-O-benzoyl	C ₃₀ H ₃₂ O ₁₃	<i>P. delavayi</i> ³⁹
7.	Albiflorin-4-Me ether	C ₂₄ H ₃₀ O ₁₁	<i>P. albiflora</i> ⁴⁰
8.	Albiflorin-4-O-(3,4,5-trihydroxy benzoyl)	C ₃₀ H ₃₂ O ₁₅	<i>P. lactiflora</i> ⁴⁰
9.	Albiflorin-6'-O-(3,4,5-trihydroxy benzoyl)	C ₃₀ H ₃₂ O ₁₅	<i>P. lactiflora</i> ⁴⁰
10.	Albiflorin R ₁	C ₂₃ H ₂₈ O ₁₁	<i>P. lactiflora</i> ⁴¹
11.	Debenzoylpaeoniflorin-8-benzoyl-1-O-(6-O-acetyl-β-D-glucopyranoside)	C ₂₅ H ₃₀ O ₁₂	<i>P. veitchii</i> ⁴²
12.	Debenzoylpaeoniflorin-8-benzoyl-1-O-(6-O-benzoyl-β-D-galactopyranoside)	C ₃₀ H ₃₂ O ₁₂	<i>P. emodi</i> ⁴³
13.	Debenzoylpaeoniflorin-4-benzoyl-1-O-(6-O-benzoyl-β-D-glucopyranoside)	C ₃₀ H ₃₂ O ₁₂	<i>P. emodi</i> ⁴³
14.	Debenzoylpaeoniflorin-8-benzoyl-1-O-(4-O-benzoyl-β-D-glucopyranoside)	C ₃₀ H ₃₂ O ₁₂	<i>P. suffruticosa</i> ⁴⁴

15.	Debenzoylpaeoniflorin-8-benzoyl-1-O-(6-O-benzoyl- β -D-glucopyranoside)	C ₃₀ H ₃₂ O ₁₂	<i>P. suffruticosa</i> ⁴⁴
16.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[3,6-bis-O-(3,4,5-trihydroxybenzoyl)- β -D-glucopyranoside]	C ₃₇ H ₃₆ O ₁₉	<i>P. obovata</i> ⁴⁵
17.	Debenzoylpaeoniflorin-8-benzoyl-1-O- β -D-galactopyranoside	C ₂₃ H ₂₈ O ₁₁	<i>P. emodi</i> ⁴⁶
18.	Debenzoylpaeoniflorin-1-O-(6-O-benzoyl- β -D-glucopyranoside)	C ₂₃ H ₂₈ O ₁₁	<i>P. suffruticosa</i> ⁴⁷
19.	Debenzoylpaeoniflorin-4-benzoyl-1-O- β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₁	<i>P. emodi</i> ⁴⁸
20.	Debenzoylpaeoniflorin-8-benzoyl-1-O- β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₁	<i>P. japonica</i> ⁴⁸
21.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[4-hydroxybenzoyl-6- β -D-glucopyranoside]	C ₂₉ H ₃₈ O ₁₆	<i>P. lactifolia</i> ⁴⁸
22.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[4-hydroxy-3-methoxybenzoyl-6- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₃	<i>P. suffruticosa</i> ⁴⁹
23.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[4-hydroxy-3-methoxybenzoyl-6- β -D-glucopyranoside]	C ₃₁ H ₃₄ O ₁₄	<i>P. obovata and P. suffruticosa</i> ⁵⁰
24.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[4-methoxybenzoyl-6- β -D-glucopyranoside]	C ₃₁ H ₃₄ O ₁₃	<i>P. suffruticosa</i> ⁵¹
25.	Debenzoylpaeoniflorin-8-benzoyl-4-sulfite, 1-O- β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₃ S	<i>P. lactiflora</i> ⁵²
26.	Debenzoylpaeoniflorin aglycone-8-benzoyl-1-O-[3,4,5-trihydroxybenzoyl-3- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₅	<i>P. lactiflora</i> ⁵²
27.	Debenzoylpaeoniflorin-8-benzoyl-1-O-[3,4,5-trihydroxybenzoyl-3- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₅	<i>P. lactiflora</i> ⁵²

28.	Debenzoylpaeoniflorin-8-benzoyl-benzoyl, 1- <i>O</i> -[3,4,5-trihydroxybenzoyl-6- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₅	<i>P. suffruticosa</i> ⁵³
29.	Debenzoylpaeoniflorin-4-Et ether-8-benzoyl-1- <i>O</i> - β -D-glucopyranoside	C ₂₅ H ₃₂ O ₁₁	<i>P. delavayi</i> ⁵⁴
30.	Debenzoylpaeoniflorin-1- <i>O</i> - β -D-glucopyranoside	C ₁₆ H ₂₄ O ₁₀	<i>P. lactiflora</i> ⁵⁵
31.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> -[6- <i>O</i> -benzoyl- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₃	<i>P. suffruticosa</i> ⁵⁶
32.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl), 1- <i>O</i> - β -D-galactopyranoside	C ₂₃ H ₂₈ O ₁₂	<i>P. emodi</i> ⁵⁷
33.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> - β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₂	<i>P. albiflora</i> ⁵⁸
34.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> - β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₂	<i>P. albiflora</i> ⁵⁸
35.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> -[4-hydroxybenzoyl-6- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₄	<i>P. suffruticosa</i> ⁵⁹
36.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> -[4-hydroxybenzoyl-6- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₄	<i>P. suffruticosa</i> ⁶⁰
37.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl)-1- <i>O</i> -[4-hydroxy-3-methoxybenzoyl-6- β -D-glucopyranoside]	C ₃₁ H ₃₄ O ₁₅	<i>P. suffruticosa</i> ⁶⁰
38.	Debenzoylpaeoniflorin-8- <i>O</i> -(4-hydroxybenzoyl), 1- <i>O</i> -[4-methoxybenzoyl-6- β -D-glucopyranoside]	C ₃₁ H ₃₄ O ₁₄	<i>P. suffruticosa</i> ⁶⁰

39.	Debenzoylpaeoniflorin-8-O-(4-hydroxybenzoyl)-1-O-[3,4,5-trihydroxybenzoyl-6- β -D-glucopyranoside]	C ₃₀ H ₃₂ O ₁₆	<i>P. suffruticosa</i> ⁶⁰
40.	Debenzoylpaeoniflorin-8-O-(4-hydroxy-3-methoxybenzoyl)-1-O-(6-O-benzoyl- β -D-glucopyranoside)	C ₃₁ H ₃₄ O ₁₄	<i>P. delavayi</i> ⁶¹
41.	Debenzoylpaeoniflorin-8-O-(4-hydroxy-3-methoxybenzoyl)-1-O- β -D-glucopyranoside	C ₂₄ H ₃₀ O ₁₃	<i>P. suffruticosa</i> ⁶²
42.	Debenzoylpaeoniflorin-4-methyl ether-8-benzoyl-1-O-(6-O-benzoyl- β -D-glucopyranoside)	C ₃₁ H ₃₄ O ₁₂	<i>P. albiflora</i> ⁶³
43.	Debenzoylpaeoniflorin-4-methyl ether-8-benzoyl-1-O- β -D-glucopyranoside	C ₂₄ H ₃₀ O ₁₁	<i>P. lactifoli</i> ⁶⁴
44.	Debenzoylpaeoniflorin aglycone-4-methyl ether-8-benzoyl-1-O-[3,4,5-trihydroxybenzoyl-6- β -D-glucopyranoside]	C ₃₁ H ₃₄ O ₁₅	<i>P. albiflora</i> ⁶⁵
45.	Debenzoylpaeoniflorin-4-methyl ether-1-O- β -D-glucopyranoside	C ₁₇ H ₂₆ O ₁₀	<i>P. albiflora</i> ⁶⁵
46.	Debenzoylpaeoniflorin-4-methyl ether-8-O-(4-hydroxy-3-methoxybenzoyl)-1-O- β -D-glucopyranoside	C ₂₅ H ₃₂ O ₁₃	<i>P. delavayi</i> ⁶⁶
47.	Debenzoylpaeoniflorin-8-O-(4-methoxybenzoyl), 1-O- β -D-glucopyranoside	C ₂₄ H ₃₀ O ₁₂	<i>P. suffruticos</i> ⁶⁷
48.	Debenzoylpaeoniflorin-8-O-(3-methylbutanoyl)-1-O- β -D-glucopyranoside	C ₂₁ H ₃₂ O ₁₁	<i>P. obovata</i> ⁶⁸
49.	Debenzoylpaeoniflorin-1-O-[3,4,5-trihydroxybenzoyl-6- β -D-glucopyranoside]	C ₂₃ H ₂₈ O ₁₄	<i>P. obovata</i> ⁶⁸
50.	Debenzoylpaeoniflorin-8-O-(3,4,5-trihydroxybenzoyl)-1-O- β -D-glucopyranoside	C ₂₃ H ₂₈ O ₁₄	<i>P. obovata</i> ⁶⁸

51.	<i>p</i> -Digallic acid	C ₁₄ H ₁₀ O ₉	<i>P. lactiflora</i> ⁶⁹
52.	2,3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol; (S)-form	C ₁₀ H ₁₂ O ₃	<i>P. suffruticosa</i> ⁷⁰
53.	2,3-Dihydro-3-(hydroxymethyl)-6-methyl-2,5-benzofurandiol; (2 <i>R</i> ,3 <i>R</i>)-form-2-methyl ether-1'-benzoyl	C ₁₈ H ₁₈ O ₅	<i>P. albiflora</i> ⁷¹
54.	2,3-Dihydro-3-(hydroxymethyl)-6-methyl-2,5-benzofurandiol-2-methyl ether-1'-benzoyl	C ₁₈ H ₁₈ O ₅	<i>P. albiflora</i> ⁷¹
55.	2',4'-Dihydroxyacetophenone-4'-methyl ether	C ₉ H ₁₀ O ₃	<i>P. moutan</i> ⁷²
56.	2',4'-Dihydroxyacetophenone-4'-methyl ether- <i>O</i> -[β -D-apiofuranosyl(1-6)- β -D-glucopyranoside]	C ₂₀ H ₂₈ O ₁₂	<i>P. suffruticosa</i> ⁷³
57.	2',4'-Dihydroxyacetophenone-4'-methyl ether, <i>O</i> -[β -D-apiofuranosyl-(1-6)-[3,4,5-trihydroxybenzoyl-(4)]- β -D-glucopyranoside]	C ₂₇ H ₃₂ O ₁₆	<i>P. suffruticosa</i> ⁷³
58.	2',4'-Dihydroxyacetophenone-4'-methyl ether- <i>O</i> -[α -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]	C ₂₀ H ₂₈ O ₁₂	<i>P. suffruticosa</i> ⁷³
59.	2',4'-Dihydroxyacetophenone-4'-methyl ether- <i>O</i> -[α -L-arabinopyranosyl-(1-6)-[β -D-glucopyranosyl-(1-3)]- β -D-glucopyranoside]	C ₂₆ H ₃₈ O ₁₇	<i>P. suffruticosa</i> ⁷³
60.	2',4'-Dihydroxyacetophenone-4'-methyl ether <i>O</i> -[α -L-arabinopyranosyl-(1-6)-[3,4,5-trihydroxybenzoyl-(4)]- β -D-glucopyranoside]	C ₂₇ H ₃₂ O ₁₆	<i>P. suffruticosa</i> ⁷³
61.	2',4'-Dihydroxyacetophenone-4'-methyl ether- <i>O</i> - β -D-glucopyranoside	C ₁₅ H ₂₀ O ₈	<i>P. suffruticosa</i> ⁷³

62.	2',4'-Dihydroxyacetophenone-4'-methyl ether-O-[3,4,5-trihydroxybenzoyl-(-5)- β -D-apiofuranosyl-(1-6)- β -D-glucopyranoside]	$C_{27}H_{32}O_{16}$	<i>P. suffruticosa</i> ⁷⁴
63.	2',4'-Dihydroxyacetophenone-4'-methyl ether-O-[3,4,5-trihydroxybenzoyl-(-4)- α -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]	$C_{27}H_{32}O_{16}$	<i>P. suffruticosa</i> ⁷⁴
64.	3,4-Dihydroxy-24,30-dinor-12,20(29)-oleanadien-28-oic acid; (3 β ,4 β)-form	$C_{28}H_{42}O_4$	<i>P. veitchii</i> ⁷⁵
65.	1,6-Dihydroxy- <i>p</i> -menthan-9,3-olide; (1 <i>S</i> ,3 <i>R</i> ,6 <i>S</i> ,8 <i>R</i>)-form	$C_{10}H_{16}O_4$	<i>P. lactifolia</i> ⁷⁶
66.	1,6-Dihydroxy- <i>p</i> -menthan-9,3-olide-6- <i>O</i> - β -D-Glucopyranoside	$C_{16}H_{26}O_9$	<i>P. lactifoli</i> ⁷⁶
67.	2',5'-Dihydroxy-4'-methylacetophenone	$C_9H_{10}O_3$	<i>P. suffruticosa</i> ⁷⁷
68.	3,23-Dihydroxy-30-nor-12,20(29)-oleanadien-28-oic acid; 3 β -form	$C_{29}H_{44}O_4$	<i>P. japonica</i> ⁷⁸
69.	4-(3,4-Dihydroxyphenyl)-3,4-dihydro-5,6,7-trihydroxy-1 <i>H</i> -2-benzopyran-1-one; (3 <i>R</i> ,4 <i>S</i>)-form, 3'-Me ether	$C_{17}H_{16}O_7$	<i>P. albiflora</i> ⁷⁹
70.	1,8-Dihydroxy-2-pinen-4-one-1- <i>O</i> - β -D-glucopyranoside	$C_{16}H_{24}O_8$	<i>P. suffruticosa</i> ⁸⁰
71.	1,8-Dihydroxy-2-pinen-4-one-8- <i>O</i> - β -D-Glucopyranoside	$C_{16}H_{24}O_8$	<i>P. suffruticosa</i> ⁸⁰
72.	1,8-Dihydroxy-2-pinen-4-one-2 β ,3-dihydro, 8- <i>O</i> -(3,4,5-trihydroxybenzoyl)	$C_{17}H_{20}O_7$	<i>P. albiflora</i> ⁸⁰
73.	Ellagic acid-2-methyl ether-7- <i>O</i> - β -D-glucopyranoside	$C_{21}H_{18}O_{13}$	<i>P. delavayi</i> ⁸¹
74.	1,8-Epoxy- <i>p</i> -menthane-3-Oxo	$C_{10}H_{16}O_2$	<i>P. albiflora</i> ⁸²

75.	11,12-Epoxy-3,4,23-trihydroxy-24,30-dinor-20,29-oleanen-28,13-olide; (3 β ,4 β ,11 α ,12 α ,13 β)-form	C ₂₈ H ₄₀ O ₆	<i>P. veitchii</i> ⁸³
76.	11,12-Epoxy-3,13,23-trihydroxy-30-nor-20,29-oleanen-28-oic acid-28-13-lactone	C ₂₉ H ₄₂ O ₅	<i>P. lactiflora</i> ⁸³
77.	11,12-Epoxy-3,13,23-trihydroxy-28-oleananoic acid-28,13-lactone	C ₃₀ H ₄₆ O ₅	<i>P. japonica</i> ⁸³
78.	Gnetin H	C ₄₂ H ₃₂ O ₉	<i>P. lactiflora</i> , <i>P. suffruticosa</i> ⁸⁴
79.	Gnetin H; 2,5-Diepimer	C ₄₂ H ₃₂ O ₉	<i>P. emodi</i> ⁸⁵
80.	2-Hydroxybenzaldehyde- <i>O</i> -[α -L-Arabinopyranosyl-(1-6)- β -D-glucopyranoside]	C ₁₈ H ₂₄ O ₁₁	<i>P. clusii</i> , <i>P. veitchii</i> ⁸⁶
81.	3-Hydroxy-24,30-dinor-4,23,12,20,29-oleanatrien-28-oic acid; 3 β -form	C ₂₈ H ₄₀ O ₃	<i>P. veitchii</i> ⁸⁶
82.	5-Hydroxy-6-methyl-1 <i>H</i> -indole-3-carboxaldehyde	C ₁₀ H ₉ NO ₂	<i>P. albiflora</i> ⁸⁷
83.	2-Hydroxy-1-(5-methyl-1 <i>H</i> -pyrrol-2-yl)-1-propanone- <i>O</i> - α -D-Glucopyranoside	C ₁₄ H ₂₁ NO ₇	<i>P. suffruticosa</i> ⁸⁸
84.	3-Hydroxy-11,13,18-oleanadien-28-oic acid; 3 β -form	C ₃₀ H ₄₆ O ₃	<i>P. japonica</i> ⁸⁹
85.	3-Hydroxy-11-oxo-12-oleanen-28-oic acid; 3 β -form	C ₃₀ H ₄₆ O ₄	<i>P. japonica</i> ⁸⁹
86.	10-Hydroxy-4-pinane	C ₁₀ H ₁₆ O ₂	<i>P. lactifolia</i> ⁹⁰
87.	Kaempferol 3,7-diglycosides-3- <i>O</i> - β -D-Arabinopyranoside, 7- <i>O</i> - β -D-glucopyranoside	C ₂₆ H ₂₈ O ₁₅	<i>P. suffruticosa</i> ⁹¹
88.	Kaempferol 3,7-diglycosides-3,7-Di- <i>O</i> - β -D-glucopyranoside	C ₂₇ H ₃₀ O ₁₆	<i>P. albiflora</i> ⁹¹

89.	Kaempferol-3- <i>O</i> -[β -D-Galactopyranosyl-1 β -D-galactopyranoside]	C ₂₇ H ₃₀ O ₁₆	<i>P. decora</i> ⁹²
90.	Lactiflorin	C ₂₃ H ₂₆ O ₁₀	<i>P. lactiflora</i> ⁹³
91.	20(29)-Lupene-3,23,28-triol-28-carboxylic acid	C ₃₀ H ₄₈ O ₄	<i>P. japonica</i> ⁹⁴
92.	2-Methoxy-5-(1-propenyl)phenol- <i>O</i> -[α -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]	C ₂₁ H ₃₀ O ₁₁	<i>P. lactiflora</i> ⁹⁵
93.	6-Methyl-3-benzofuranmethanol- <i>O</i> -[α -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]	C ₂₁ H ₂₈ O ₁₁	<i>P. hybrida</i> ⁹⁶
94.	Methyl 2-hydroxybenzoate- <i>O</i> -[α -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]	C ₁₉ H ₂₆ O ₁₂	<i>P. anomala</i> ⁹⁷
95.	Mudanoside A	C ₁₄ H ₁₈ O ₉	<i>P. suffruticosa</i> ⁹⁸
96.	Mudanpinoic acid	C ₃₀ H ₄₆ O ₃	<i>P. suffruticosa</i> ⁹⁸
97.	11,13(18)-Oleanadiene-3,23,28-triol-28-Carboxylic acid	C ₃₀ H ₄₆ O ₄	<i>P. suffruticosa</i> ⁹⁹
98.	Paeobrin	C ₂₃ H ₂₈ O ₁₀	<i>P. hybrida</i> ¹⁰⁰
99.	Paeonianin A	C ₇₅ H ₅₄ O ₄₇	<i>P. lactiflora</i> ¹⁰¹
100.	Paeonianin A-3'-7''' Analogue	C ₇₅ H ₅₄ O ₄₇	<i>P. lactiflora</i> ¹⁰¹
101.	Paeonianin A-4'-7''' Analogue	C ₇₅ H ₅₄ O ₄₇	<i>P. lactiflora</i> ¹⁰¹
102.	Paeonianin A-6'-7''' Analogue	C ₇₅ H ₅₄ O ₄₇	<i>P. lactiflora</i> ¹⁰¹
103.	Paeonianin E	C ₄₂ H ₃₀ O ₂₆	<i>P. lactiflora</i> ¹⁰¹

104.	Paeonidanin	$C_{24}H_{30}O_{11}$	<i>P. hybrida</i> ¹⁰²
105.	Paeonidanin-Aglycone	$C_{18}H_{20}O_6$	<i>P. albiflora</i> ¹⁰³
106.	Paeonidanin-6'-Benzoyl	$C_{31}H_{34}O_{12}$	<i>P. albiflora</i> and <i>P. suffruticosa</i> ¹⁰³
107.	Paeonidanin-debenzoyl	$C_{17}H_{26}O_{10}$	<i>P. lactiflora</i> ¹⁰³
108.	Paeonidanin-8-debenzoyl-8-O-(4-hydroxybenzoyl), 6'-benzoyl	$C_{31}H_{34}O_{13}$	<i>P. albiflora</i> ¹⁰³
109.	Paeonidanin; 9-Epimer	$C_{24}H_{30}O_{11}$	<i>P. hybrida</i> ¹⁰⁴
110.	Paeonidanin-6'-O-(3,4,5-trihydroxybenzoyl)	$C_{31}H_{34}O_{15}$	<i>P. albiflora</i> ¹⁰⁴
111.	Paeonidanin D	$C_{47}H_{56}O_{21}$	<i>P. albiflora</i> ¹⁰⁴
112.	Paeonidanin E	$C_{46}H_{54}O_{21}$	<i>P. albiflora</i> ¹⁰⁴
113.	Paeonidaninol A	$C_{30}H_{32}O_{12}$	<i>P. peregrina</i> ¹⁰⁵
114.	Paeonidaninol A; 9-Epimer	$C_{30}H_{32}O_{12}$	<i>P. peregrina</i> ¹⁰⁵
115.	Paeoniflorigenone	$C_{17}H_{18}O_6$	<i>P. albiflora</i> ¹⁰⁶
116.	Paeoniflorigenone debenzoyl	$C_{10}H_{14}O_5$	<i>P. suffruticosa</i> ¹⁰⁷
117.	Paeoniflorigenone debenzoyl, 7-epimer	$C_{10}H_{14}O_5$	<i>P. suffruticosa</i> ¹⁰⁷
118.	Paeonihybridin	$C_{41}H_{50}O_{21}$	<i>P. hybrida</i> ¹⁰⁸
119.	Paeoniisothujone	$C_{10}H_{14}O_3$	<i>P. suffruticosa</i> ¹⁰⁹

120.	Paeonilactone A	C ₁₀ H ₁₄ O ₄	<i>P. spp</i> ¹¹⁰
121.	Paeonilactone A-9-hydroxy	C ₁₀ H ₁₄ O ₅	<i>P. delavayi</i> ¹¹¹
122.	Paeonilide	C ₁₇ H ₁₈ O ₆	<i>P. delavayi</i> ¹¹¹
123.	Paeonin B	C ₁₆ H ₂₂ O ₉	<i>P. lactiflora</i> ¹¹²
124.	Paeonin B-9-Me ether	C ₁₇ H ₂₄ O ₉	<i>P. lactiflora</i> ¹¹²
125.	Paeonin C	C ₁₇ H ₂₄ O ₉	<i>P. lactiflora</i> ¹¹²
126.	Paeoninol	C ₄₂ H ₃₂ O ₉	<i>P. emodi</i> ¹¹³
127.	Paeonisuffrone	C ₁₀ H ₁₄ O ₄	<i>P. suffruticosa</i> ¹¹⁴
128.	Paeonisuffrone-8-benzoyl-1-O-β-D-glucopyranoside	C ₂₃ H ₂₈ O ₁₀	<i>P. lactiflora</i> <i>P. hybrida</i> ¹¹⁵
129.	Paeonisuffrone-8-deoxy	C ₁₀ H ₁₄ O ₃	<i>P. suffruticosa</i> ¹¹⁶
130.	Paeonisuffrone-1-O-β-D-glucopyranoside	C ₁₆ H ₂₄ O ₉	<i>P. lactifolia</i> ¹¹⁷
131.	Palbinone	C ₂₂ H ₃₀ O ₄	<i>P. albiflora</i> ¹¹⁸
132.	1,2,3,4,6-Pentagalloylglucose-β-D-pyranose-form, O-3,4,5-trihydroxybenzoyl	C ₄₈ H ₃₆ O ₃₀	<i>P. lactifolia</i> ¹¹⁹
133.	1,2,3,4,6-Pentagalloylglucose-β-D-bis(3,4,5-trihydroxybenzoyl)	C ₅₅ H ₄₀ O ₃₄	<i>P. lactifolia</i> ¹¹⁹
134.	1,2,3,4,6-Pentagalloylglucose-β-D-bis(3,4,5-trihydroxybenzoyl)	C ₅₅ H ₄₀ O ₃₄	<i>P. lactiflora</i> ¹¹⁹
135.	1,2,3,4,6-Pentagalloylglucose-β-D-bis(3,4,5-trihydroxybenzoyl)	C ₅₅ H ₄₀ O ₃₄	<i>P. lactiflora</i> ¹²⁰

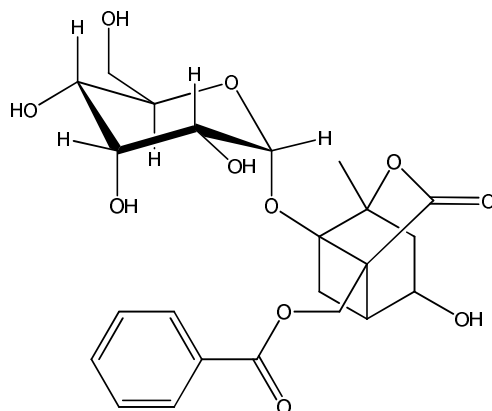
136.	1,2,3,4,6-Pentagalloylglucose- β -D-tris(3,4,5-trihydroxybenzoyl)	C ₆₂ H ₄₄ O ₃₈	<i>P. lactiflora</i> ¹²⁰
137.	1,2,3,4,6-Pentagalloylglucose- β -D-O-galloylgalloyl	C ₅₅ H ₄₀ O ₃₄	<i>P. lactiflora</i> ¹²⁰
138.	1,3,5,23,24-Pentahydroxy-30-nor-12,20(29)-oleanadien-28-oic acid	C ₂₉ H ₄₄ O ₇	<i>P. emodi</i> ¹²⁰
139.	Suffruticosol A	C ₄₂ H ₃₂ O ₉	<i>P. suffruticosa</i> ¹²¹
140.	Suffruticosol A; 7 <i>b</i> -Epimer	C ₄₂ H ₃₂ O ₉	<i>P. suffruticosa</i> ¹²¹
141.	Suffruticosol C	C ₄₂ H ₃₂ O ₉	<i>P. suffruticosa</i> ¹²¹
142.	2',4,4',6'-Tetrahydroxychalcone-2'-O- β -D-glucopyranoside	C ₂₁ H ₂₂ O ₁₀	<i>P. trollioides</i> ¹²²
143.	3,4',5,7-Tetrahydroxy-3',8-dimethoxyflavone-3-O-[β -D-glucopyranosyl-(1-2)- β -D-glucopyranoside]	C ₂₉ H ₃₄ O ₁₈	<i>P. tenuifolia</i> ¹²²
144.	3,4',5,7-Tetrahydroxy-8-methoxyflavone-3-O-[β -D-glucopyranosyl-(1-2)- β -D-glucopyranoside]	C ₂₈ H ₃₂ O ₁₇	<i>P. tenuifolia</i> ¹²³
145.	3,4',5,7-Tetrahydroxy-3'-methoxyflavylium(1+)-3,5-di-O- β -D-glucopyranoside	C ₂₈ H ₃₃ O ₁₆	<i>P. spp</i> ¹²³
146.	2',3',4'-Trihydroxyacetophenone; 4'-Me ether	C ₉ H ₁₀ O ₄	<i>P. broteroi</i> <i>P. suffruticosa</i> ¹²³
147.	2',4',5'-Trihydroxyacetophenone-4'-methyl ether	C ₉ H ₁₀ O ₄	<i>P. suffruticosa</i> ¹²³
148.	3,4,5-Trihydroxybenzoic acid-3-O-[β -D-apiofuranosyl-(1-6)- β -D-glucopyranoside]	C ₁₈ H ₂₄ O ₁₄	<i>P. suffruticosa</i> ¹²³
149.	3,4,23-Trihydroxy-24,30-dinor-12,20(29)-oleanadien-28-oic acid; (3 β ,4 β)-form	C ₂₈ H ₄₂ O ₅	<i>P. delavayi</i> ¹²⁴

150.	3,4,23-Trihydroxy-24,30-dinor-12,20(29)-oleanadien-28-oic acid-4,23-Isopropylidene	$C_{31}H_{48}O_5$	<i>P. anomala</i> ¹²⁴
151.	1,3,23-Trihydroxy-12-oleanen-28-oic acid; (1 β ,3 β)-form	$C_{30}H_{48}O_5$	<i>P. emodi</i> ¹²⁴
152.	ϵ -Viniferin; (7Z,7'R,8'R)-form	$C_{28}H_{22}O_6$	<i>P. lactiflora</i> ¹²⁵
153.	ϵ -Viniferin; (7E,7'S,8'S)-form	$C_{28}H_{22}O_6$	<i>P. lactiflora</i> ¹²⁵

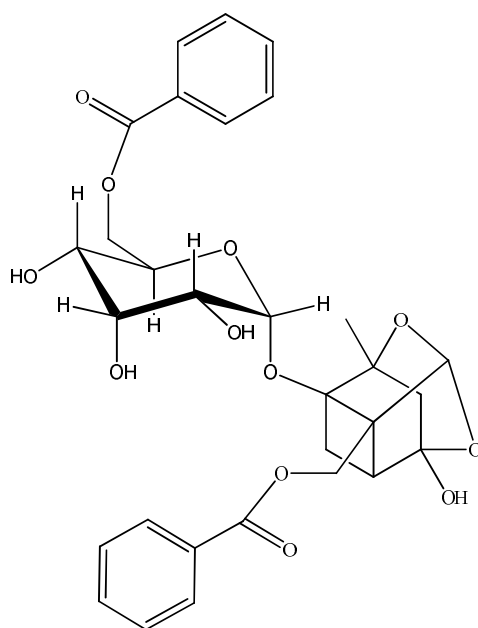
Albiflorin = 4-Hydroxy-6-methyl-(1R, 3R, 4R, 6S)-;7-oxatricyclo [4.3.0.03,9] nonan-8-one, 9-;[(benzoyloxy)methyl]-1-(β -D-glucopyranosyloxy)-;9-((Benzoyloxy)methyl)-1-(β -D-glucopyranosyloxy)-4-hydroxy-6-methyl-7-oxatricyclononan-8-one;9-((Benzoyloxy)methyl)-1-(β -D-glucopyranosyloxy)-4-hydroxy-6-methyl-7-oxatricyclononan-8-one.

Paeoniflorin=[(1R,2S,3R,5R,6R,8S)-3-(β -D-Glucopyranosyloxy)-6-hydroxy-8-methyl-9,10-dioxatetracyclo[4.3.1.0^{2,5}.0^{3,8}]dec-2-yl]methyl benzoate

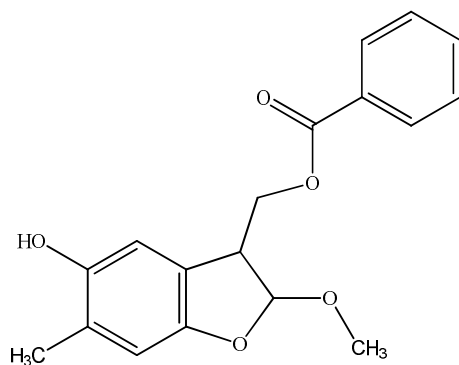
3.4. Structures of selected compounds reported from genus *Paeonia*



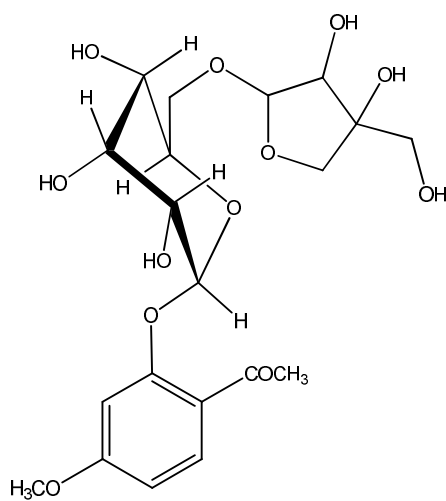
Debenzoylpaeoniflorin-8-benzoyl, 1-O-(6-O-acetyl- β -D-glucopyranoside)⁴²



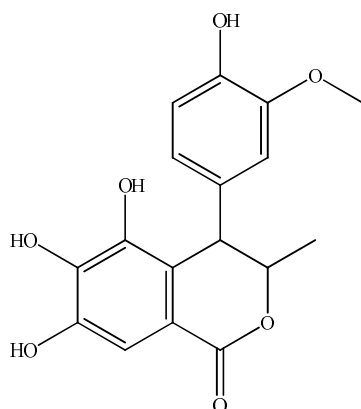
Debenzoylpaeoniflorin-8-Benzoyl, 1-O-(6-O-benzoyl- β -D-galactopyranoside)⁴³



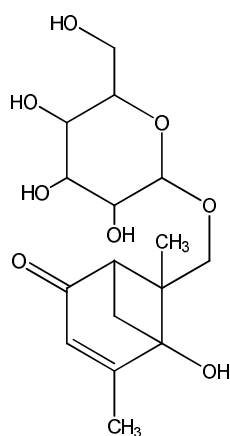
2,3-Dihydro-3-(hydroxymethyl)-6-methyl-2,5-benzofurandiol-2-methylether-1'-benzoyl⁷¹



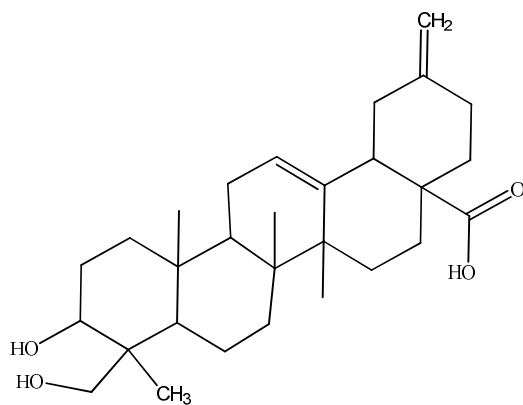
2',4'-Dihydroxyacetophenone-4'-methylether-O-[\beta-D-apiofuranosyl(1-6)-\beta-D-glucopyranoside]⁷³



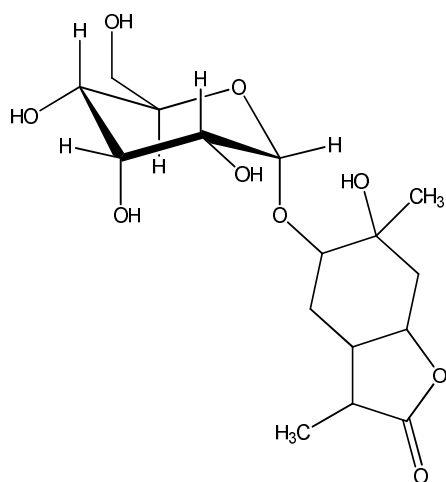
4-(3,4-Dihydroxyphenyl)-3,4-dihydro-5,6,7-trihydroxy-1*H*-2-benzopyran-1-one-3'-methylether⁷⁹



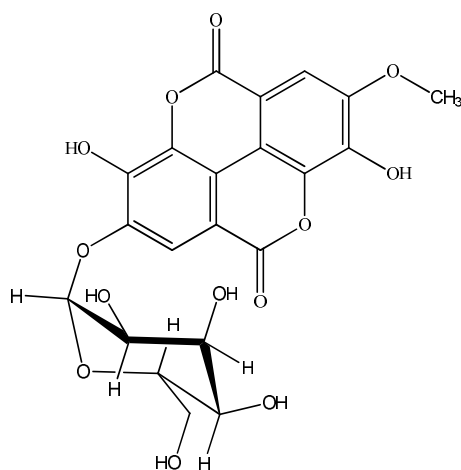
1,8-Dihydroxy-2-pinen-4-one-8-O- β -D-glucopyranoside⁸⁰



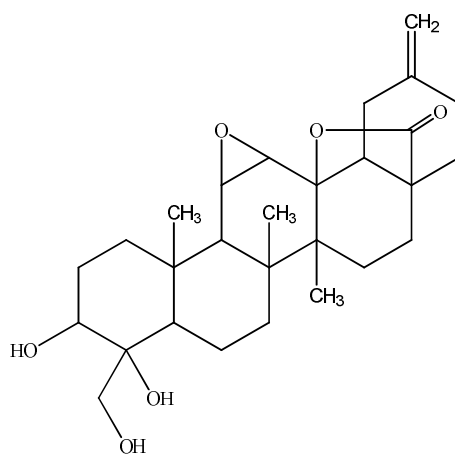
3,23-Dihydroxy-30-nor-12,20(29)-oleanadien-28-oic acid⁷⁸



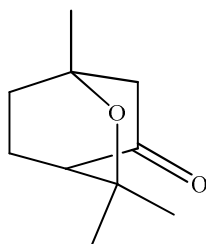
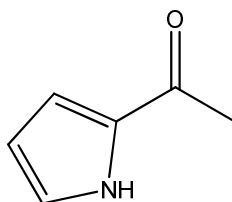
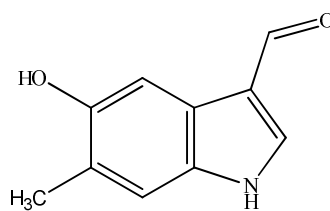
1,6-Dihydroxy-p-menthan-9,3-olide-6-O- β -D-glucopyranoside⁷⁶

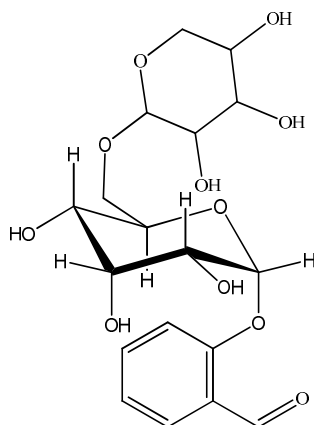


Ellagic acid-2-methylether-7-*O*- β -D-glucopyranoside⁸¹

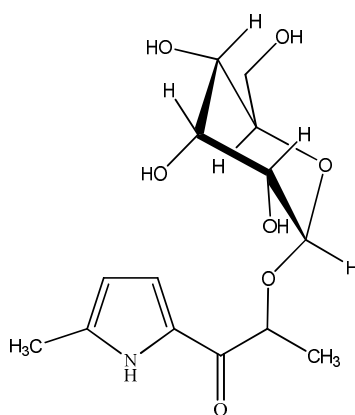


11,12-Epoxy-3,4,23-trihydroxy-24,30-dinor-20(29)-oleanen-28,13-olide⁸³

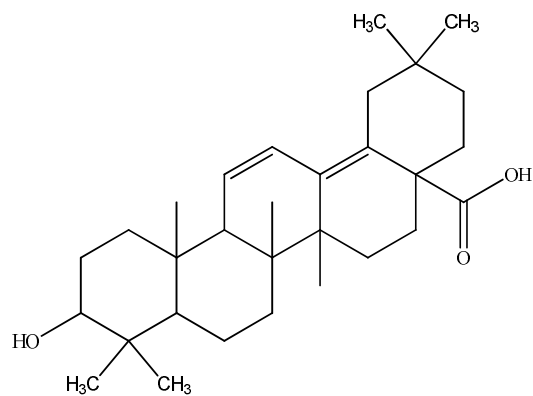
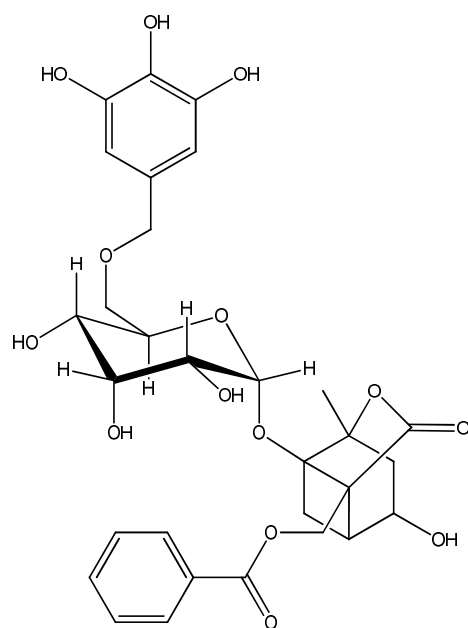
1,8-Epoxy-*p*-menthane-3-oxo⁸²2-Acetylpyrrole³⁷5-Hydroxy-6-methyl-1H-indole-3-carboxaldehyde⁸⁷



2-Hydroxybenzaldehyde-O- $[\beta$ -L-arabinopyranosyl-(1-6)- β -D-glucopyranoside]⁸⁶



2-Hydroxy-1-(5-methyl-1H-pyrrol-2-yl)-1-propanone-O- β -D-glucopyranoside⁸⁸

3-Hydroxy-11,13(18)-oleanadien-28-oic acid⁸⁹6'-O-(3,4,5-trihydroxybenzoyl)-paeonidanin¹⁰⁴

RESULTS AND DISCUSSION (part A)

This chapter illustrates the isolation, structural elucidation and characterization of eighteen secondary metabolites, which includes two new peoniflorines, three new flavonoids, six hitherto unreported and nine known constituents from the ethyl acetate fraction of the aerial parts of *Paeonia emodi*. Their structures were elucidated with the aid of sophisticated NMR and mass spectrometric techniques. The isolated compounds were tested for urease inhibition and antioxidant activities and displayed remarkable activity.

4.1. New constituents isolated from *P. emodi*

- Paeoniflorine C (6)
- Paeoniflorin D (7)
- Kaempferol-3-*O*- β -D-glucopyranosyl-7-oic acid (12)
- Kaempferol-7-*al*-3-*O*- β -D-glucopyranoside (15)
- Kaempferol-7-methoxy-3-*O*- β -D-glucopyranosyl-3'-oic acid (16)

4.2. Hitherto unreported constituents from *P. emodi*

- 2,3-Dihydroxy-4-methoxyacetophenone (3)
- Quercetin-3'-methoxy-3- β -D- glucopyranoside (5)
- Debenzoyl-8-galloypaeoniflorin (8)
- 2-*O*-Ethyl- β -D-glucopyranoside (9)
- 2, 3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol (17)
- *p*-Digallic acid (18)

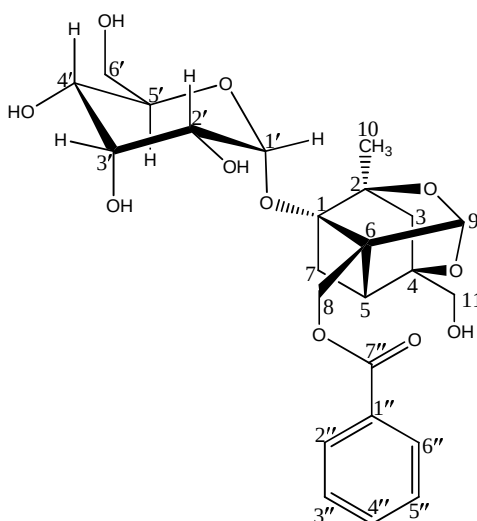
4.3. Known constituents from *P. emodi*

- Ethyl gallate **(1)**
- Methyl gallate **(2)**
- Debenzoylpaeoniflorin;4-methylether-8-benzoyl-1-*O*- β -D-glucopyranoside **(4)**
- Quercetin-3- β -D-glucopyranoside **(10)**
- Kaempferol-3- β -D-glucopyranoside (Astragalin) **(11)**
- 8-Debenzoylpaeoniflorin-1-*O*- β -D-glucopyranoside **(13)**
- Debenzoyl-8-galloylpaeoniflorin-1-*O*- β -D-glucopyranoside **(14)**

4.4. Structure elucidation of new compounds from *P. emodi*

4.4.1 Paenoflorin C (6)

Compound **6** was obtained as dark yellow powder soluble in methanol. The structural formula was determined as $C_{24}H_{30}O_{11}$ by HR-ESI-MS $[M+H]^+$ at m/z 495.1860 a.m.u. (calcd. 495.1854 a.m.u.) indicating nine degrees of un-saturation. The UV spectrum exhibited λ_{max} 285 and 182 nm corresponding to benzene ring and carbonyl functional groups. The IR spectrum showed characteristic bands at 3400 (hydroxyl group), 1690 (ester functional group) and 1620 (aromatic C=C stretching) and 1020 cm^{-1} (glycoside linkage).



6

The ^1H NMR spectrum (**Table-4.1**) indicated the presence of five aromatic protons, one glucose moiety, and a paeoniflorin nucleus. Two AB doublets appeared at δ_{H} 1.81 (1H, $J=12.5$ Hz, H-3a) and δ_{H} 2.19 (1H, $J=12.5$ Hz, H-3b) while AB double doublets for the geminal protons resonated at δ_{H} 1.96 (1H, dd, $J=10.7, 1.5$ Hz, H-7a) and δ_{H} 2.50 (1H, dd, $J=10.7, 1.5$ Hz, H-7b) due to presence of methine (C-5) in the vicinity of C-7 along with

one proton triplet at δ_H 2.57 ($J=6.5$ Hz, H-5) in the ^1H -NMR spectrum and supported by the ^{13}C -NMR (BB and DEPT) spectra; displayed signals at δ_C 44.5 (C-3), 23.4 (C-7) and 44 (C-5). Furthermore, the proton NMR spectrum showed a methyl singlet at δ_H 1.35 (H-10) along with methylene and methine groups resonated at δ_H 4.81 (H-8, s), δ_H 4.74 (H-11, s) and δ_H 5.41 (H-9, s) respectively which is also supported by ^{13}C -NMR spectrum (**Table-4.1**) where C-9 resonated at δ_C 100.2 applicably downfield as two oxygen atoms are σ -bonded to C-9.

The benzoate group exhibited two sets of aromatic protons; AA' (H-2'' and H-6'') and BB' (H-3'' and H-5'') appeared as a double doublet at δ_H 8.03 (2H, $J=7.5$, 2.5 Hz) and triplet at δ_H 7.45 (2H, t, $J=7.5$ Hz), respectively while H-4'' proton resonated at δ_H 7.60, (1H, t, $J=7.5$ Hz). ^{13}C -NMR and the mass fragment appeared at m/z 123.0440 a.m.u. confirmed that the molecule contains a benzoate group.

The characteristic peaks of a glucose moiety in the molecule was indicated at δ_H 5.41, (1H, $J=7.6$ Hz, H-1'), 4.08 (1H, t $J=7.6$ Hz, H-2'), 4.33, (1H, t, $J=7.6$ Hz, H-3'), 5.79 (1H, t, $J=7.6$ Hz, H-4'), 3.99 (1H, m, H-5'), 4.10 (1H, dd, $J=7.6$, 1.5 Hz, H-6a') and δ_H 4.21 (1H, dd, $J=7.6$, 1.5 Hz, H-6b') in the ^1H -NMR spectrum. These assignments were further supported by ^{13}C -NMR (BB & DEPT) (**Table-4.1**) and mass fragment at m/z 317.1384 a.m.u. which is due to loss of glucose moiety and acid hydrolysis.

The attachment of the monosaccharide unit, benzoate group and relative position of C-H were confirmed by HMBC (**Fig-4.1**) experiment. The anomeric carbon is correlated with the C-2 while H-8 has strong correlation with C-9. Other important HMBC correlations are H-11/C5, H-6, H-2''/C-7'' and H-8/C-7'' indicating and ester linkage.

Based on the above spectral data, the structure of **6** is elucidated as 11-hydroxy-4-*O*-galloyl-paeoniflorin (Paeoniflorin C). The absolute stereochemistry of paeoniflorin C is identical to 4-*O*-galloyl-paeoniflorin and supported by ^1H - and ^{13}C -NMR, ^1H - ^1H coupling constants.

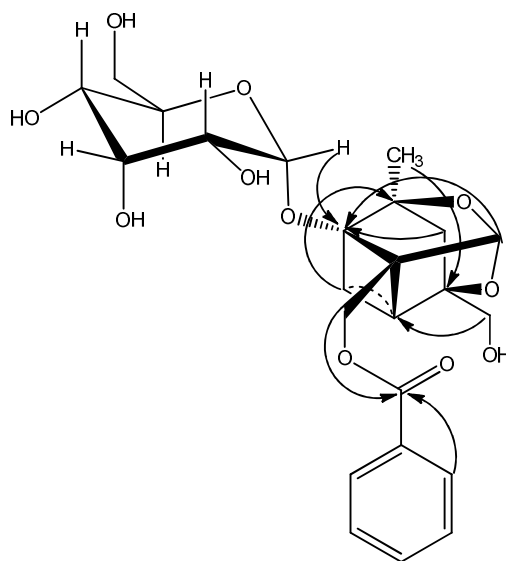


Fig-4.1. Selected key COSY (H---H) and HMBC (H—C) interactions of **6**

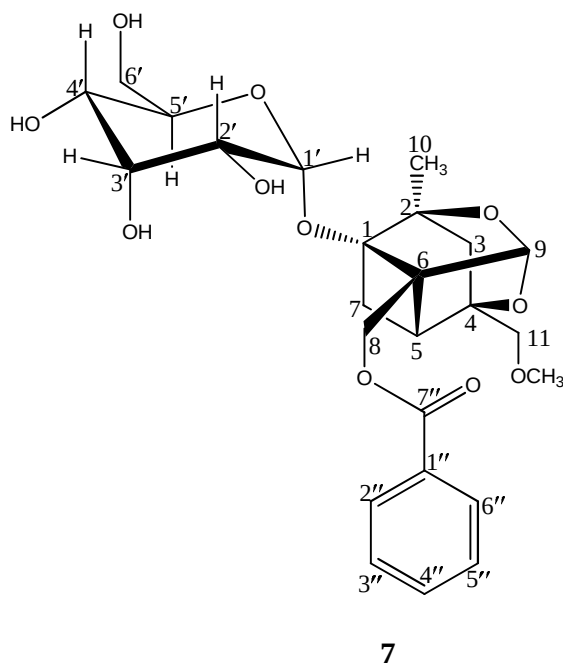
Table-4.1. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **6** in CD_3OD

C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	89.1	C	-
2	86	C	-
3	44.5	CH_2	1.81, d ($J=12.5$) 2.19, d ($J=12.5$)
4	106	C	-
5	44	CH	2.57, t ($J= 6.5$)
6	71.7	C	-
7	23.4	CH_2	1.96, dd ($J= 10.7, 1.5$) 2.50, dd ($J= 10.7, 1.5$)
8	60.8	CH	4.81, s
9	100.2	CH	5.41, s
10	19.6	CH_3	1.35, s
11	62.2	CH_2	4.74, s
1'	102.3	CH	5.41, d ($J=7.6$)
2'	75	CH	4.08, t ($J=7.6$)
3'	77.9	CH	4.33, t ($J=7.6$)
4'	72.2	CH	5.79, t ($J=7.6$)
5'	78.1	CH	3.99, m
6'	62.9	CH_2	4.10, dd ($J=7.6, 1.5$) 4.21, dd ($J=7.6, 1.5$)
1''	130.7	C	-
2'', 6''	130	CH	8.03 dd ($J=7.5, 2$)
3'', 5''	129.6	CH	7.48, t ($J=7.5$)
4''	134.4	CH	7.60, t ($J=7.5$)
7''	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.4.2. Paeoniflorin D (7)

Compound **7** was obtained as yellow gummy solid and its molecular formula ($C_{25}H_{32}O_{11}$) was calculated from ESI-HR-MS m/z 509.2014 a.m.u. $[M+H]^+$ (calcd. 509.2010 a.m.u.). The UV spectrum exhibited absorption peaks at $\lambda_{\max}$ 285 and 182 nm indicating benzene ring and a carbonyl group in the molecule. The IR bands showed the presence of ester, benzene ring, hydroxyl groups and a glycoside linkage (*vide experimental*).



The NMR of compound **7** is similar to **6** with a difference of one methoxy group. The ^1H NMR (**Table-4.2**) showed the presence of five aromatic protons i.e. a set of AA' protons (H-2'' and H6'') resonated at δ_H 8.24 (2H, dd, $J=7.5$, 2 Hz), a set of BB' protons (H-3'' and H5'') appeared as a triplet at δ_H 7.59 ($J=7.5$ Hz) along with one proton triplet at δ_H 7.86 ($J=7.5$ Hz, H-4''). The mass fragment at m/z 123.0440 a.m.u. suggesting the presence of benzoate functionality in the molecule.

Two AB doublets exhibited at δ_H 2.14 (1H, d, $J=12$ Hz) and δ_H 2.35 (1H, d, $J=12$ Hz) corresponding to geminal protons H-3a and H-3b, respectively while two AB doublets indicated at δ_H 2.10 (1H, dd, $J=11.5, 2$ Hz) and δ_H 2.81 (1H, dd, $J=11.5, 2$ Hz) are due to geminal protons H-7a and H-7b due to presence of methine (C-5) in the vicinity of C-7 and H-5 which showed a triplet at δ_H 2.64 ($J=7.5$ Hz) in the ^1H -NMR spectrum. The downfield singlets of oxygenated methylenes and methine were observed at δ_H 4.83 (2H, H-8), 4.92 (2H, H-11) and δ_H 5.62 (1H, H-9) in the ^1H -NMR spectrum respectively.

Methyl groups singlet appeared at δ_H 1.49 (H-10) and δ_H 3.50 (OCH_3) which was confirmed by ^{13}C -NMR spectrum (**Table-4.2**). Moreover, the presence of $\text{M}^+ - \text{OCH}_3$ peak in the ESI spectrum at m/z 479.1908 a.m.u. confirmed the presence of methoxy group in the molecule.

The glucose unit in the structure of **7** was indicated by the ^1H -NMR spectrum, which displayed signals at δ_H 5.64 (1H, $J=7.5$ Hz, H-1'), 3.98 (1H, t $J=7.5$ Hz, H-2'), 4.52 (1H, t, $J=7.5$ Hz, H-3'), 5.81 (1H, t, $J=7.5$ Hz, H-4'), 4.03 (1H, m, H-5'), 4.18 (1H, dd, $J=7, 1.5$ Hz, H-6a') and δ_H 4.39 (1H, dd, $J=7, 1.5$ Hz, H-6b'). These assignments were further confirmed by ^{13}C -NMR (BB and DEPT) (**Table-4.2**), mass fragment at m/z 331.1540 a.m.u. and acid hydrolysis.

The presence of a methyl carbon, a methoxy, five methylene, 12 methine and six quaternary carbons are evident from ^{13}C -NMR (BB and DEPT) spectra (**Table-4.2**). ^1H - ^1H COSEY correlation showed coupling of H-3a/H-3b and H-5/H-7a, H-7b.

The attachment of the glucose moiety and benzoate with the parent paeoniflorin nucleus was confirmed with HMBC (**Fig-4.2**) cross peak matching i.e. an anomeric proton is showed correlation with C-1 and H-8/C3, C-1/C-9 and methoxy protons with C-11. Other key HMBC correlations are H-11/C5 and H-3/C4.

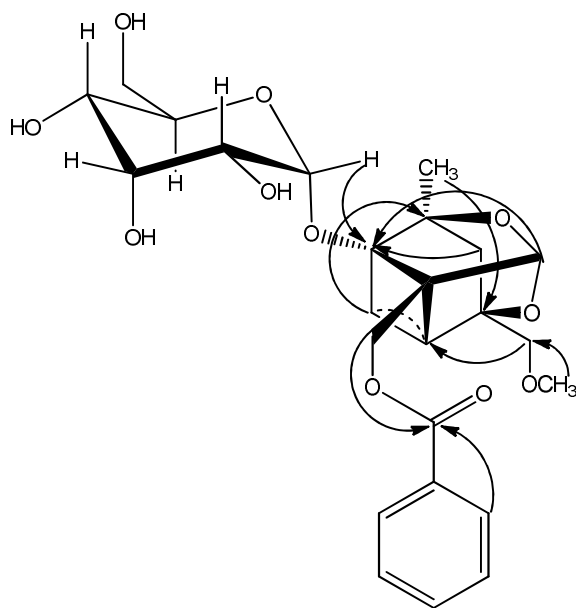


Fig-4.2. Selected key COSY (H---H) and HMBC (H—C) interactions of 7

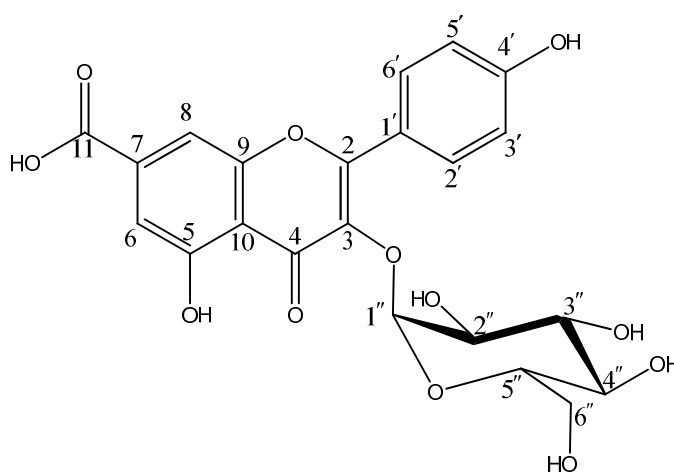
Table-4.2. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **7** in CD_3OD

C/H. No.	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	90.8	C	-
2	86.4	C	-
3	43.9	CH_2	2.14, d ($J=12$) 2.35, d ($J=12$)
4	105.6	C	-
5	45.1	CH	2.64, t ($J=7.5$)
6	72.4		-
7	22.9	CH_2	2.10, dd ($J=11.5, 2$) 2.81, dd ($J=11.5, 2$)
8	61.8	CH	4.83, s
9	99.8	CH	5.62, s
10	19.4	CH_3	1.49, s
11	62.4	CH_2	4.92, s
OCH_3	51.7	CH_3	3.50, s
1'	103.5	CH	5.64, d ($J=7.5$)
2'	75.4	CH	3.98, t ($J=7.5$)
3'	78.3	CH	4.52, t ($J=7.5$)
4'	72	CH	5.81, t ($J=7.5$)
5'	78.7	CH	4.03, m
6'	63.1	CH_2	4.18, dd ($J=7.5, 1.5$) 4.39, dd ($J=7.5, 1.5$)
1''	129.8	C	-
2'', 6''	130.4	CH	8.24 dd ($J=7.5, 2$)
3'', 5''	129.2	CH	7.59, t ($J=7.5$)
4''	135.2	CH	7.86, t ($J=7.5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.4.3. Kaempferol-3-O- β -D-glucopyranosyl-7-oic acid (12)

Compound **12** was isolated as white granules exhibited its $[M+H]^+$ at m/z 477.1014 a.m.u. (calcd. 477.1010 a.m.u.) in the ESI-HR-MS spectrum corresponding to molecular formula $C_{22}H_{20}O_{12}$. The IR showed absorption bands at 3480 (OH carboxyl), 3350 (OH phenolic), 1710 (C=O carboxyl), 1640 (α - β unsaturated C=O) and 1520 cm^{-1} (C=C) stretching and UV spectrum displayed λ_{max} 360 and 345 nm.



12

The NMR spectra are consistent with the presence of kaempferol nucleus. ^1H -NMR (**Table-4.3**) spectrum of **12** showed signals at δ_{H} 6.14 (1H, d, $J = 1.8\text{ Hz}$, H-6), 6.32 (1H, d, $J = 1.8\text{ Hz}$, H-8) indicating *meta* coupling of H-6 with H-8 together with four quaternary carbons appeared at δ_{C} 158.8, 135.4, 179.2, 105.1 and δ_{C} 159.6 in the ^{13}C -NMR spectrum which is characteristic of chromane-4-one ring. A set of AA'/BB' protons appeared in the ^1H -NMR spectrum at δ_{H} 8.04 (2H, d, $J = 8.5\text{ Hz}$, H-2', H-6') and δ_{H} 6.87 (2H, d, $J = 8.5\text{ Hz}$, H-3', H-5') pertains to *ortho-para* substituted benzene ring. The methines of kaempferol appeared at δ_{C} 100.8 (C-6), δ_{C} 95.4 (C-8), δ_{C} 132.2 (C-2, C6) and

δ_C 116.8, (C-3, C-5) together with two carbonyl carbons at δ_C 168 and δ_C 179.2 in the ^{13}C -NMR spectrum (**Table-4.3**). The ESIMS spectrum displayed peaks at m/z 433.1130 a.m.u. for $\text{M}^+ - \text{COOH}$, characteristics of carboxylic acid function, which suggested the presence of carboxyl group in the molecule.

The presence of β -D-glucose is evident from ^1H -NMR signals at δ_H 5.41 (1H, d, $J=7.6$ Hz, H-1''), 4.08 (1H, t, $J=7.6$ Hz, H-2''), 4.33 (1H, t, $J=7.6$ Hz, H-3''), 5.79 (1H, t, $J=7.6$ Hz, H-4''), 3.99 (1H, m, H-5''), 4.10 (1H, dd, $J=7.6, 1.5$ Hz, H-6a'') and δ_H 4.21 (1H, dd, $J=7.6, 1.5$ Hz, H-6b''). The ^{13}C -NMR spectrum (**Table-4.3**) also confirmed β -D-glucose. The presence of sugar moiety was also confirmed by the presence of peak at m/z 255.0652 a.m.u. in the ESI spectrum due to loss of sugar moiety from $\text{M}^+ - 45$ peak. The detailed analysis of chemical shifts, coupling constants, ^1H - ^1H COSY and HMBC of the moiety suggested that the sugar is β -D-glucose. Furthermore, acid hydrolysis of compound **12** was done and by comparing the aqueous phase TLC with authentic samples of monosaccharide depicted that the sugar unit is β -D-glucose.

The relative positions of carbon to hydrogen confirmed by HMBC and ^1H - ^1H COSY (**Fig-4.3**) experiments. The anomeric carbon showed correlation with C-3 indicating that the sugar is attached at position C-3 of the kaempferol. The H-6 and H-8 both showed strong correlation with C-11 (C=O) suggesting that COOH group is attached at C-7. Aside from this, H-2', H-6'/C2, H-3', H-5'/C-1' and H-6, H8/C10 are the other key HMBC correlations observed in the spectrum.

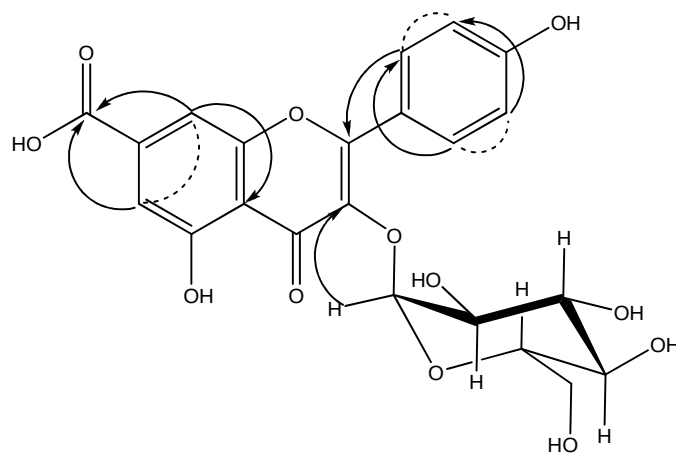


Fig-4.3. Selected key COSY (H---H) and HMBC (H—C) interactions of **12**

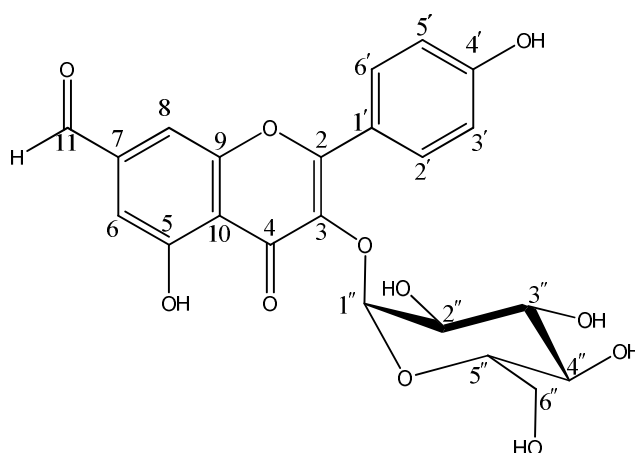
Table-4.3. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **12** in CD_3OD

C/H. No.	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	158.8	C	-
3	135.4	C	-
4	179.2	C	-
5	162.9	C	-
6	100.8	CH	6.14, d ($J=1.8$)
7.	129	C	-
8	95.4	CH	6.32, d ($J=1.8$)
9	158.6	C	-
10	105.1	C	-
11	168	C	-
1'	122.7	C	-
2', 6'	132.2	CH	8.04, d ($J=8.5$)
3', 5'	116.8	CH	6.87, d ($J=8.5$)
4'	161.6	C	-
1''	104.3	CH	5.18, d ($J=7.0$)
2''	75.7	CH	3.42, t ($J=6.5$)
3''	78	CH	3.40, t ($J=6.5$)
4''	71.3	CH	3.38, t ($J=6.5$)
5''	78.3	CH	3.1, m
6''	62.6	CH_2	3.53, dd ($J=11.5, 5.0$) 3.67, dd ($J=11.5, 5.0$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.4.4. Kaempferol-7-al-3-O- β -D-glucopyranoside (**15**)

Compound **15** was isolated as greenish white powder and established its molecular formula as $C_{22}H_{20}O_{12}$ [m/z 461.1076 a.m.u; $M+H$] from ESI-HR-MS, (calcd. 461.1072 a.m.u.). The IR showed absorption bands at 3390 (OH), 1690 (C=O), 1665 cm^{-1} (α - β unsaturated C=O) and 1540 cm^{-1} (aromatic C=C stretching) while UV λ_{max} 362 nm corresponding to benzene ring. ^{13}C -NMR (**Table-4.4**) spectra indicated the presence of one methyl, one methane, nine methine and eleven quaternary carbons, consistent with the molecular formula $C_{22}H_{20}O_{12}$.



15

^1H - and ^{13}C -NMR spectra of **15** is similar to that of **12** except with the presence of an aldehyde instead of acid group in **15**.

The ^1H -NMR (**Table-4.4**) spectrum showed aglycone protons signal including two *meta* protons at δ_H 6.24 (1H, d, J = 1.5 Hz, H-6) and δ_H 6.48 (1H, d, J = 1.5 Hz, H-8) and aldehydic proton resonated as a singlet at δ_H 8.19 (H-11) which are supported by the

corresponding carbons chemical shifts at δ_c 100.5, 95.2 and δ_c 170.8. The presence of $M^+ - 29$ peak in the ESI spectrum at m/z 433.1124 a.m.u. confirmed the aldehydic function. The ^1H -NMR spectrum further indicated two AA'BB' doublets at δ_H 7.96 (2H, d, $J = 8.5$ Hz) and δ_H 6.92 (2H, d, $J = 8.5$ Hz) are due to H-2', 6' and H-3', 5' respectively.

The presence of the glucose moiety was indicated by ^1H -NMR spectrum (**Table-4.4**) resonating at δ_H 5.21, (1H, d, $J=7$ Hz, H-1'), 3.61 (1H, t, $J=7$ Hz, H-2'), 3.72 (1H, t, $J=7$ Hz, H-3'), 3.18 (1H, t, $J=7$ Hz, H-4'), 3.02 (1H, m, H-5'), 3.50 (1H, dd, $J=12.5, 2.5$ Hz, H-6a') and δ_H 3.68 (1H, dd, $J=12.5, 2.5$ Hz, H-6b'). These assignments were confirmed by ^{13}C -NMR (**Table-4.4**) and 2D-NMR (^1H - ^1H COSY and HMBC; **Fig-4.4**) spectra, which was also supported by the presence of mass fragment at m/z 255.0646; suggested that the monosaccharide unit is β -D-glucose. Moreover, acid hydrolysis of compound **15** was done and by comparing the aqueous phase TLC with authentic samples of monosaccharide confirmed β -D-glucose moiety.

The connectivity of the neighboring protons confirmed by ^1H - ^1H COSY experiment i.e. H-6 is *meta* coupled with H-8, H-2' is coupled with H-3, and H-5' with H-6' while the HMBC experiment is consistent with correlation of H-6 and H-8 with carbonyl carbon, H-2' and H-6'/C-4' and anomeric protons with C-3 of the kaempferol nucleus suggesting the attachment of glucose at C-3.

In the light of the above information, the structure of **15** is established as kaempferol-7-al-3-O- β -D-glucopyranoside.

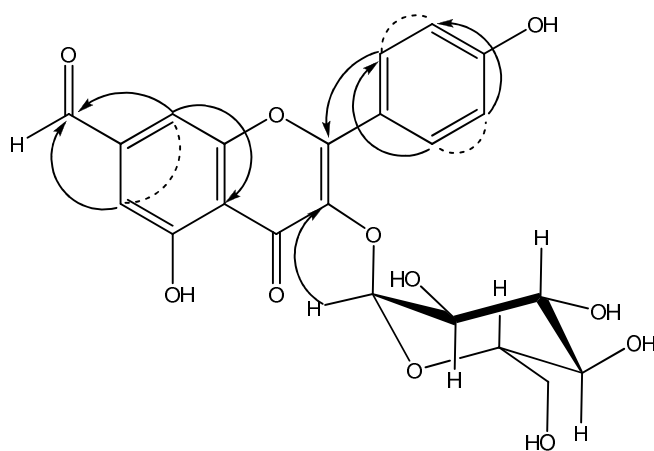


Fig-4.4. Selected key COSY (H---H) and HMBC (H—C) interactions of **15**

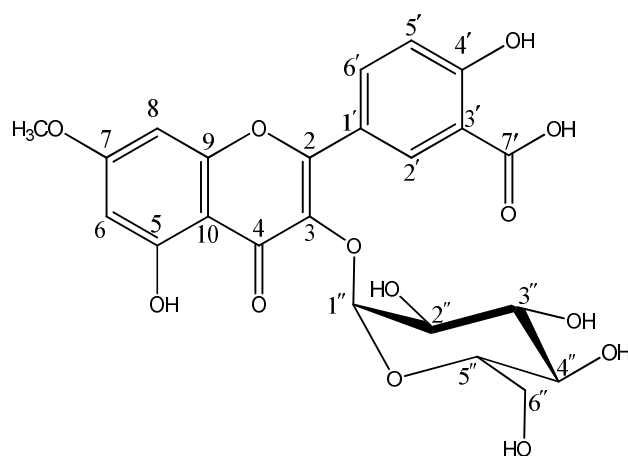
Table-4.4. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **15** in CD_3OD

C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	158.4	C	-
3	134.2	C	-
4	178.9	C	-
5	162.4	C	-
6	100.5	CH	6.24, d ($J=1.5$)
7.	130.1	C	-
8	95.2	CH	6.48, d ($J=1.5$)
9	158.4	C	-
10	105	C	-
11	170.8	C=O	8.19, s
1'	122.6	C	-
2', 6'	132.8	CH	7.96, d ($J=8.5$)
3', 5'	116	CH	6.92, d ($J=8.5$)
4'	162.1	C	-
1''	104.8	CH	5.21, d ($J=7$)
2''	75.7	CH	3.61, t ($J=7$)
3''	78.4	CH	3.72, t ($J=7$)
4''	71	CH	3.18, t ($J=7$)
5''	77.9	CH	3.02, m
6''	62.2	CH ₂	3.50, dd ($J=12.5, 2.5$) 3.68, dd ($J=12.5, 2.5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.4.5. Kaempferol-7-methoxy-3-O- β -D-glucopyranosyl-3'-oic acid (**16**)

Compound **16** was isolated as white amorphous powder. Molecular formula ($C_{23}H_{22}O_{13}$) was established by ESI-HR-MS at m/z 507.1130 a.m.u. (calcd. 507.1126 a.m.u.). The IR spectrum indicated the presence of OH carboxylic (3450 cm^{-1}), phenolic OH 3375 cm^{-1} , carbonyl group 1640 cm^{-1} and 1710 cm^{-1} for α,β -unsaturated ketone. The UV spectrum is also consistent with the presence of benzene ring and a carbonyl group showing λ_{max} at 355 nm.



16

The ^1H - and ^{13}C -NMR spectra (**Table-4.5**) of **16** are identical to **15** indicated ^1H -NMR peaks for two *meta* coupled protons at δ_{H} 6.28 (1H, d, $J = 1.8\text{ Hz}$, H-6) and δ_{H} 6.39 (1H, d, $J = 1.8\text{ Hz}$, H-8) along with additional *meta* coupled doublet at δ_{H} 7.70 (1H, d, $J = 2\text{ Hz}$, H-2') and a methoxy signal at δ_{H} 3.81. The ^1H -NMR spectrum further displayed a double doublet at δ_{H} 7.57 (1H, $J = 8.5, 2\text{ Hz}$) which was assigned to H-6' due to the presence of a *meta* proton (H-5') in the vicinity of C-6' where H-5' resonated as a doublet at δ_{H} 6.85 ($J=8.5$).

The glucose protons signals are observed in the ^1H -NMR spectrum resonated at δ_{H} 5.18 (1H, d, $J = 6.5$ Hz, H-1''), 3.42 (1H, t, $J = 6.5$ Hz, H-2''), 3.40 (1H, t, $J = 6.5$ Hz, H-3''), 3.38 (1H, t, $J = 6.5$ Hz, H-4''), 3.21 (1H, m, H-5''), 3.53 (1H, dd, $J = 6.5, 2.0$ Hz, H-6a'') and δ_{H} 3.67 (1H, dd, $J = 6.5, 2.0$ Hz, H-6b'') and was confirmed by ^{13}C -NMR (**Table-4.5**) and 2D-NMR (**Fig-4.5**) spectra. Furthermore, the presence of mass fragment at m/z 285.0754 a.m.u also suggested that the monosaccharide unit is β -D-glucose, which was confirmed by the acid hydrolysis of compound **16** and by comparing the aqueous phase TLC with authentic samples of monosaccharide.

The ^{13}C -NMR spectrum displayed signal at δ_{C} 168.3 for carboxyl carbon (C-7') and a signal at δ_{C} 145.9 was assigned to C-3', slightly downfield due to attachment of carboxyl carbon at C-3 which was confirmed by HMBC experiment (**Fig-4.5**).

The coupling of protons was confirmed by ^1H - ^1H COSY experiment which showed coupling of H-6/H-8, H-4'/H-5' and H-5'/H-1'.

The relative position of various groups with respect to each other was confirmed by the HMBC experiment (**Fig-4.5**) i.e. H-6 and H-8 both showed correlation with OCH_3 carbon indicating that OCH_3 carbon is in neighboring of both of these protons suggesting the attachment of a methoxy group at C-7. In addition, H-6 and H-8 are showing correlation with C-10. H-2' showed correlation with C=O carboxylic and also with C-2 while H-5' is showed correlation with C-1' and also with C-3'. The anomeric carbon is showed correlation with C-3 indicating the attachment of glucose with the C-3.

The ESIMS spectrum of compound **16** was analyzed and the presence of M^+ -45 peak at m/z 463.1235 a.m.u. indicated presence of carboxyl function, in addition the peak at m/z 477.1022 a.m.u is due to loss of OCH_3 .

Acid hydrolysis of compound **16** was done and by comparing the aqueous phase TLC with authentic samples of monosaccharide revealed that the sugar is β -D-glucose.

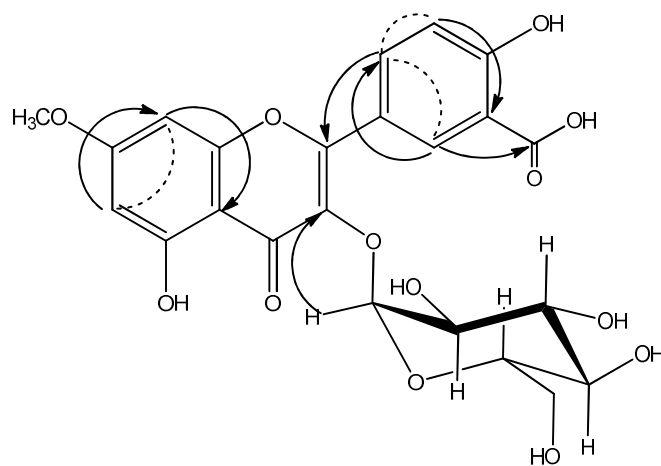


Fig-4.5. Selected key COSY (H---H) and HMBC (H—C) interactions of **16**

Table-4.5. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **16** in CD_3OD

C. No	^{13}C -NMR (δ_{C}) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ_{H}) coupling constants J_{HH} (Hz) ^{cd}
2	158.8	C	-
3	135.6	C	-
4	179.2	C	-
5	163	C	-
6	99.8	CH	6.28, d ($J=1.8$)
7.	132.2	C	-
8	94.7	CH	6.39, d ($J=1.8$)
9	159	C	-
10	104.3	C	-
OCH_3	56.6	CH_3	3.81, s
1'	123	C	-
2'	117.5	CH	7.70 d ($J=2.0$)
3'	145.9	C	-
4'	149.8	C	-
5'	116	CH	6.85, d ($J=8.5$)
6'	123.2	CH	7.57, dd ($J=8.5, 2.0$)
7'	168.3	$\text{C}=\text{O}$	-
1''	104.2	CH	5.25, d ($J=6.5$)
2''	75.7	CH	3.47, t ($J=6.5$)
3''	78.1	CH	3.42, t ($J=6.5$)
4''	71.2	CH	3.34, t ($J=6.5$)
5''	78.4	CH	3.21, m
6''	62.5	CH_2	3.57, dd ($J=6.5, 2.0$) 3.70, dd ($J=6.5, 2.0$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

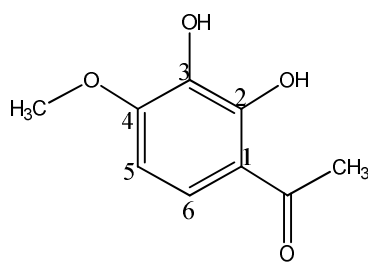
4.5. Hitherto Unreported Compounds

4.5.1. 2,3-Dihydroxy-4-methoxyacetophenone (3)

Compound **3** was isolated as pale yellow crystals and molecular formula was established by ESI-HR-MS showing $[M+H]^+$ peak at m/z 183.0652 a.m.u. calcd. 183.0648 a.m.u. corresponding to molecular formula $C_9H_{10}O_4$. IR spectrum indicated the presence of benzene ring (1675 cm^{-1}) and a carbonyl group (1710 cm^{-1}) and UV spectrum pertained to the presence of benzene ring (λ_{max} 290).

^1H -NMR spectrum (**Table-4.6**) displayed signals at δ_{H} 2.50 and at δ_{H} 3.83 for $\text{CH}_3\text{-CO}$ and OCH_3 respectively. The doublet at δ_{H} 6.60 ($J = 7.5$) is assigned to H-5 and a doublet at δ_{H} 7.22 ($J = 7.5$) is assigned to H-6.

^{13}C -NMR (**Table-4.6**) shows signals for two methyls, two aromatic methines and five quaternary carbons i.e. one carbonyl carbon and four aromatic quaternary carbons.



3

The spectral data of compound **3** were identical with reported compound 2,3-dihydroxy-4-methoxyacetophenone and the structure of **3** was established as 2,3-dihydroxy-4-methoxyacetophenone.¹²⁶

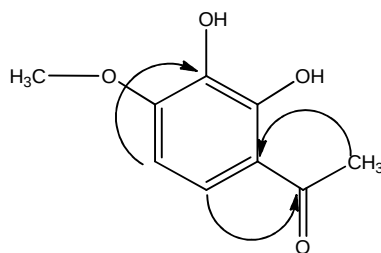


Fig-4.6. Selected key COSY (H---H) and HMBC (H—C) interactions of **3**

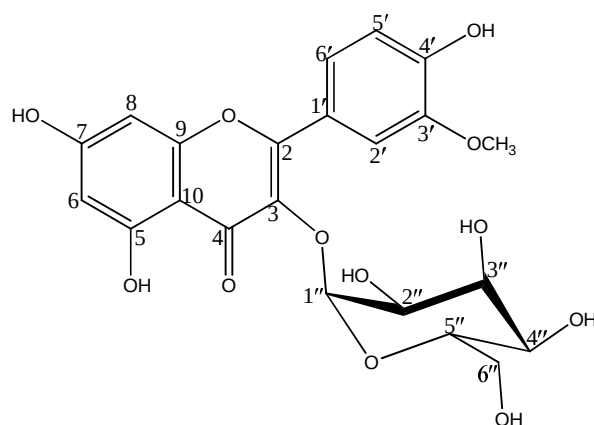
Table-4.6. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **3** in CD_3OD

C. NO	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1.	115.5 -	C	-
2.	152.3	C	-
3.	134.3	C	-
4	153.7	C	-
5	104.7	CH	6.60, d, $J = 7.5$ Hz
6	129.3	CH	7.22, d, $J = 7.5$ Hz
OCH_3	51.3	CH_3	3.83, s
COCH_3	26.6	CH_3	2.50, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.6.5. Kaempferol-3'-methoxy-3- β -D-glucopyranoside (5)

Compound **5** was isolated as white amorphous powder and the ESI-HR-MS m/z 479.1182 a.m.u. calcd. 479.1178 a.m.u. corresponding to molecular formula $C_{22}H_{22}O_{12}$. IR spectrum indicated the presence of phenolic group (3400 cm^{-1}), carbonyl carbon (1665 cm^{-1}) and a glycoside linkage (1030 cm^{-1}). UV spectrum indicated the presence of highly unsaturated benzene type system (λ_{max} 360 nm).



5

^1H - and ^{13}C -NMR spectra analysis of compound **5** revealed the presence of a kaempferol nucleus and a glucose moiety. In ^1H -NMR (**Table-4.7**), the signals appeared at δ_{H} 6.16 (1H, d, $J=1.8\text{ Hz}$, H-6), 6.34 (1H, d, $J=1.8\text{ Hz}$, H-8), 7.58 (1H, d, $J=2.0\text{ Hz}$, H-2'), 6.68 (1H, d, $J=8.0\text{ Hz}$, H-5') and δ_{H} 7.43 (1H, dd, $J=8.5, 2.0\text{ Hz}$, H-6'). The glucose protons appeared at their respective δ value i.e. δ_{H} -5.16, (1H, d, $J = 7.0\text{ Hz}$, H-1''), 3.42 (1H, t, $J = 6.5\text{ Hz}$, H-2''), 3.40 (1H, t, $J = 6.5\text{ Hz}$, H-3''), 3.36 (1H, t, $J = 6.5\text{ Hz}$, H-4''), 3.20 (1H, m, H-5''), 3.53 (1H, dd, $J = 11.5, 5.0\text{ Hz}$, H-6a'') and δ_{H} -3.68 (1H, dd, $J = 11.5, 5.0\text{ Hz}$, H-6b'').

^{13}C -NMR (BB & DEPT) spectra (**Table-4.7**) displayed signals for one methyl, one methylene, nine methin and nine quaternary carbons corresponding to molecular formula $\text{C}_{22}\text{H}_{22}\text{O}_{12}$. The correlation of neighboring protons and relative positions are confirmed by COSY and HMBC experiments.

The spectral data of **5** was compared with the spectral data of known compound; quercetin-3'-methoxy-3-*O*- β -D-glucopyranoside which was found identical. On this basis of spectral evidence compound **5** was elucidated as kaempferol-3'-methoxy-3-*O*- β -D-glucopyranoside.¹²⁷

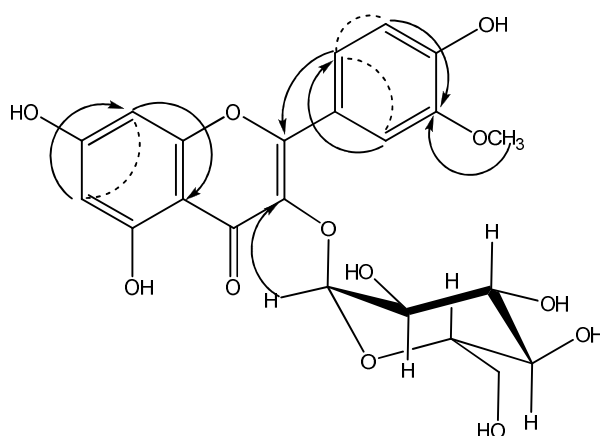


Fig-4.7. Selected key COSY (H---H) and HMBC (H—C) interactions of **5**

Table-4.7. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **5** in CD_3OD

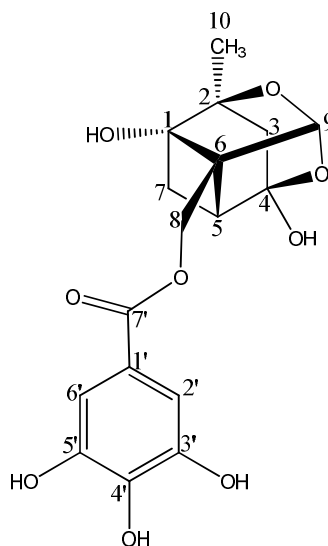
C/H. No	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	158.5	C	-
3	135.3	C	-
4	178.9	C	-
5	162.7	C	-
6	100.4	CH	6.16, d ($J=1.8$)
7	129.5	C	-
8	94.9	CH	6.34, d ($J=1.8$)
9	158.7	C	-
10	105.5	C	-
OCH_3	56.3	C	3.91, s
1'	123.2	C	-
2'	117.7	CH	7.58 d ($J=2$)
3'	145.6	C	-
4'	149.4	C	-
5'	116.2	CH	6.68, d ($J=8.5$)
6'	123.1	CH	7.43, dd ($J=9.5, 2$)
1''	104.4	CH	5.16, d ($J=7$)
2''	75.9	CH	3.42, t ($J=6.5$)
3''	78.2	CH	3.40, t ($J=6.5$)
4''	71.1	CH	3.36, t ($J=6.5$)
5''	78.6	CH	3.20, m
6''	62.4	CH_2	3.54, dd ($J=11.5, 5$) 3.68, dd ($J=11.5, 5$)

Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.5.3. Debenzoyl-8-galloylpaeoniflorin (**8**)

Compound **8** was isolated as white amorphous powder. Molecular formula was established by ESI-HR-MS m/z 367.1020 a.m.u. calcd. 367.1016 a.m.u. for $C_{17}H_{18}O_9$. IR spectrum indicated the presence of benzene ring (1580 cm^{-1}), carbonyl carbon (1710 cm^{-1}) and number of OH groups (3440 cm^{-1}). UV spectrum also corresponds to presence of benzene ring (λ_{max} , 275) and a carbonyl carbon ($n\text{-}\pi^*$ transition at. 190 nm).

^1H - and ^{13}C -NMR spectra of compound **8** revealed the presence of paeonoflorin nucleus.



8

^1H -NMR spectrum (**Table-4.8**) showed proton signals of compound **8** at δ_{H} 1.92 (1H, d, $J=12.5\text{ Hz}$, H-3a), 2.34 (1H, d, $J=12.5\text{ Hz}$, H-3b), 2.53 (1H, t, $J= 6.5\text{ Hz}$, H-5), 2.07 (1H, dd, $J= 10.7, 1.5\text{ Hz}$, H-7a), 2.67 (1H, dd, $J= 10.7, 1.5\text{ Hz}$, H-7b), 5.68 (2H, s, H-8), 5.41 (1H, s, H-9) and δ_{H} 1.41 (3H, s, H-10). The two aromatic protons H-2' and H6' appeared at δ_{H} 7.64.

^{13}C -NMR (BB and DEPT) (**Table-4.8**) experiments are consistent with the presence of one methyl, three methylene, five methine and eight quaternary carbons consistent with molecular formula $\text{C}_{17}\text{H}_{18}\text{O}_9$.

^1H - ^1H COSY and HMBC experiments were used to assign relative positions of the groups. The spectral data of **8** was matched with debenzoyl-8-galloylpaeoniflorin from the literature and showed similarities.^{70, 71}

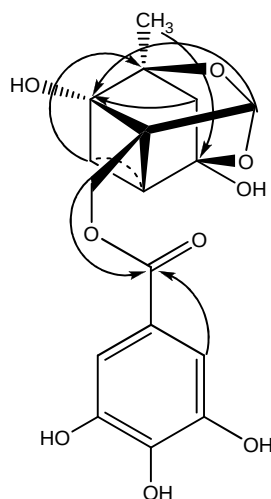


Fig-4.8. Selected key COSY (H---H) and HMBC (H—C) interactions of **8**

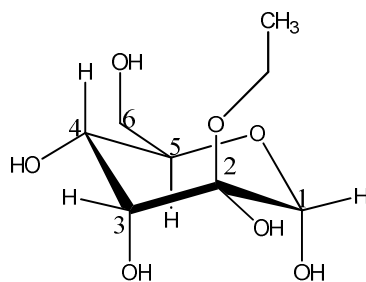
Table-4.8. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **8** in CD_3OD

C/H. No	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	88	C	-
2	87.1	C	-
3	43.9	CH_2	1.92, d ($J=12.5$) 2.34, d ($J=12.5$)
4	104.1	C	-
5	44.8	CH	2.53, t ($J= 6.5$)
6	72.1	C	-
7	23.8	CH_2	2.07, dd ($J= 10, 1.5$) 2.67, dd ($J= 10, 1.5$)
8	61.8	CH	4.94, s
9	100	CH	5.68, s
10	19.4	CH_3	1.41, s
1'	130.2	C	-
2', 6'	109.8	CH	7.64, s
3', 5'	147.7	C	-
4'	141.5	C	-
7'	168.9	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.5.4. 2-O-Ethyl- β -D-glucopyranoside (**9**)

Compound **9** was isolated as white needle like crystals. Molecular formula was determined by ESIHRMS m/z 225.0964 a.m.u. calcd. 225.0964 a.m.u. for $C_8H_{16}O_6$. IR spectrum indicated hydroxyl group (34200 cm^{-1}), saturated straight chain C-C stretching (1420 cm^{-1}) and C-O stretching was observed at 1040 cm^{-1} . UV spectrum indicated the absence of unsaturated system.



9

The ^1H -NMR spectral analysis showed seven different peaks for the molecule i.e. δ_{H} 4.02 (1H, d, $J = 7.8\text{ Hz}$, H-1), 3.15 (1H, t, $J = 8.4\text{ Hz}$, H-2), 3.25 (1H, m, H-3), 3.27 (1H, m, H-4) and δ_{H} 4.23 (1H, d, $J = 7.8\text{ Hz}$, H-1).

The spectral data of **9** was found identical with reported compound; 2-O-ethyl- β -D-glucopyranoside from the literature.¹²⁸

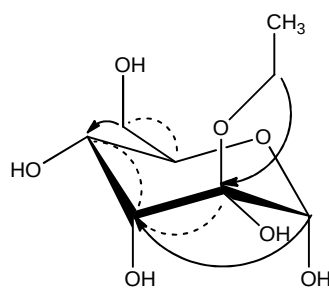


Fig-4.9. Selected key COSY (H---H) and HMBC (H---C) interactions of **9**

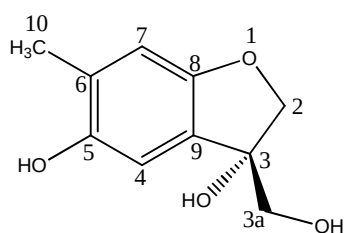
Table-4.9. ¹H- (600 MHz) and ¹³C-NMR (150 MHz) chemical shifts of **9** in CD₃OD

C. No	¹³ C-NMR (δ _c) ^a	Multiplicity (DEPT) ^{bd}	¹ H-NMR (δ _H) coupling constants <i>J</i> _{HH} (Hz) ^{cd}
1	104	CH	4.02, d (<i>J</i> = 7.8)
2	75	CH	3.15, t (<i>J</i> = 8.4)
3	77.9	CH	3.25, m
4	71.6	CH	3.27, m
5	78	CH	3.33, m
6	62.7	CH ₂	4.10, dd (<i>J</i> = 7.5, 1.5) 4.21, dd (<i>J</i> = 7.5, 1.5)
CH ₃	15.4	CH ₃	1.22, t (<i>J</i> = 7.2)
CH ₂	66.1	CH ₂	3.30, s

a. Broad Band; b. DEPT; c. ¹H-NMR; d. HSQC interactions

4.5.5. 2,3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol (**17**)

Compound **17** was isolated as white powder. Molecular formula was established by ESI m/z 181.0860 a.m.u. $[M+H]^+$, calcd. 181.0856 a.m.u for $C_{10}H_{12}O_3$ IR absorption bend is indicative of benzene ring (1675 cm^{-1}) and hydroxyl group (3440 cm^{-1}). UV spectrum also pertained to the presence of benzene ring ($\lambda_{\text{max}} 315\text{ nm}$).



17

The ^1H -NMR (**Table-4.10**) experiment showed signals for two aromatic protons at δ_{H} 6.42 (1H, s, H-4) and δ_{H} 6.87 (1H, s, H-7). In addition ^1H -NMR also displayed signals at δ_{H} 3.14 (1H, dd, $J = 11, 1.5\text{ Hz}$, H-2a), 3.36 (1H, dd, $J = 11, 1.5\text{ Hz}$, H-2b), 2.89 (1H, m, H-3) and δ_{H} 2.28 (3H, s, H-10) while 3a protons appeared at δ_{H} 3.92 (1H, dd, $J = 10.5, 1.5\text{ Hz}$, H-3a) and δ_{H} 3.52 (1H, d, $J = 10.5, 1.5\text{ Hz}$, H-3a'). ^{13}C -NMR (**Table-4.10**) indicated the presence of one methyl, two methylene, three methine and four quaternary carbons corresponding to molecular formula $C_{10}H_{12}O_3$.

HMBC correlation and ^1H - ^1H COSY also confirmed the structure of **17**. The spectral data of **17** was matched with the spectra of 2,3-dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol from the literature and found identical.⁷³

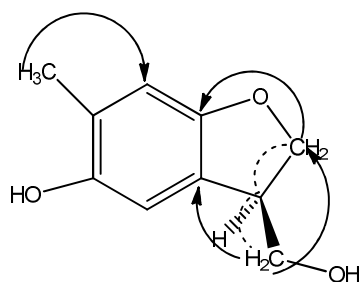


Fig-4.10. Selected key COSY (H---H) and HMBC (H—C) interactions of **17**

Table-4.10. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **17** in CD_3OD

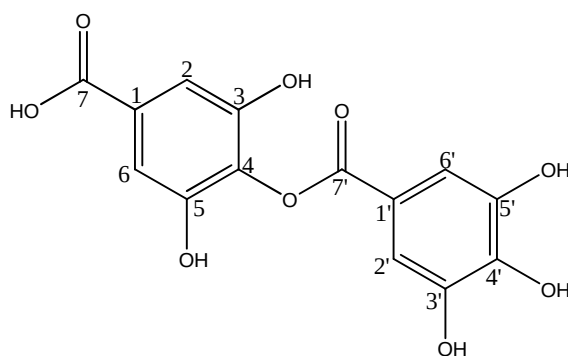
C/H. No.	^{13}C -NMR (δ_{C}) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ_{H}) coupling constants J_{HH} (Hz) ^{cd}
1	-	-	-
2	72.4	CH	3.36, dd ($J= 11.0, 1.5$)
		CH	3.14, dd ($J= 11.0, 1.5$)
3	49.7	CH	2.89, m
4	112.3	CH	6.42, s
5	146.1	C	-
6	122.2	C	-
7	117.1	CH	6.87, s
8	153.8	C	-
9	129.8	C	-
10	15.4	CH_3	2.28, s
3a	61.8	CH_2	3.92, dd ($J= 10.5, 1.5$)
			3.52, dd ($J= 10.5, 1.5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.5.6. *p*-Digallic acid (**18**)

Compound **18** was isolated as colorless fine crystals and molecular formula was established by ESI-HR-MS m/z 323.0394 a.m.u. calcd 323.0398 a.m.u. for $C_{14}H_{10}O_9$. IR spectrum indicated the presence of benzene ring (1540 cm^{-1}) and a carbonyl carbon of ester function (1680 cm^{-1}). UV spectrum also indicated the presence of benzene ring (λ_{max} 280 nm).

^1H -NMR experiment (**Table-4.11**) showed one singlet for two aromatic protons i.e. H-2 and H-6 at δ_{H} 6.65 and for protons H-2' and H-6' singlet at δ_{H} 7.15 as well. ^{13}C -NMR indicated the presence of one methyl, four methine and ten quaternary carbons i.e. two carbonyls four aromatic quaternary carbons consistent with the formula $C_{14}H_{10}O_9$.



18

HMBC experiment showed the correlation of H-2 and H-6 with that of carbonyl carbon and H-2' and H-6' showed correlation with the carbonyl carbon of ester function. The spectral data of **18** was identical with the spectral data of *p*-digallic acid.^{38, 129}

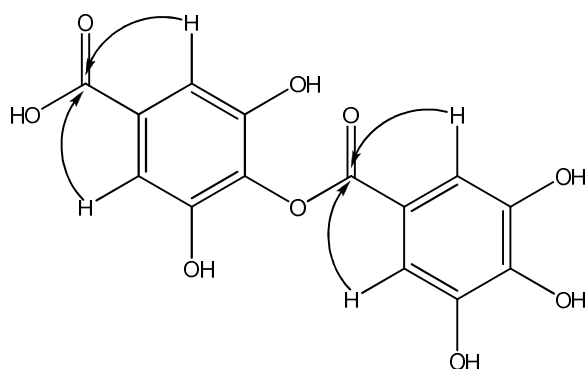


Fig-4.11. Selected key COSY (H---H) and HMBC (H---C) interactions of **18**

Table-4.11. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **18** in CD_3OD

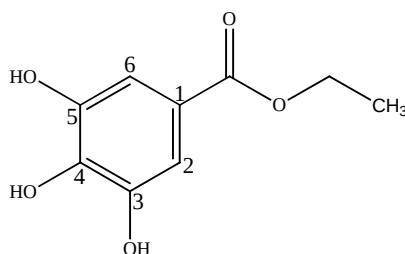
C. No	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	119.6	C	-
2, 6	108.5	CH	6.65 (s)
3, 5	145.5	C	-
4	138.3	C	-
7	168.7	C=O	-
1'	118.4	C	-
2',6'	108.2	C	7.15 (s)
3',5'	144.8	C	-
4'	136.9	C	-
7'	167.9	C=O	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.6. Known Compounds from *P. emodi*

4.6.1. Ethyl gallate (1)

Compound **1** was obtained as white needles. Molecular formula was established by ESI-HR-MS m/z 199.0598 a.m.u calcd. 199.0594 a.m.u IR bands analysis indicated the presence of an ester linkage (1690 cm^{-1}), benzene ring (1540 cm^{-1}) and a hydroxyl groups (3400 cm^{-1}). UV spectrum also supported the presence of benzene ring (λ_{max} 275 nm) and a carbonyl carbon in conjugation with the aromatic ring (220 nm).



1

$^1\text{H-NMR}$ (**Table-4.12**) showed peaks at δ_{H} 1.31 (3H, t, $J = 7.0$ Hz, CH_3), 4.20 (2H, q, $J = 7.0$, O- CH_2) and δ_{H} 6.67 (2H, s, H-2, H-6). $^{13}\text{C-NMR}$ (**Table-3.12**) displayed signals at δ_{C} 165.8 (C=O), 119.6 (C-1), 108.5 (C-2, C-6), 145.5 (C-3, C-5), 138.3 (C-4), 60 (-O- CH_2 -) and δ_{C} 14.2 (- CH_3).

The spectral data of **1** was compared to with the ethyl gallate from literature and found identical.¹³⁰

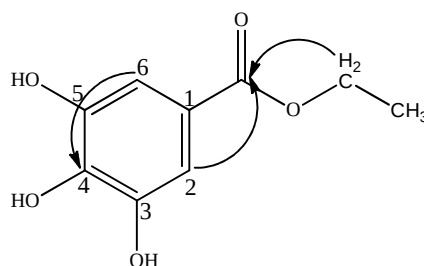


Fig-4.12. Selected key COSY (H---H) and HMBC (H—C) interactions of **1**

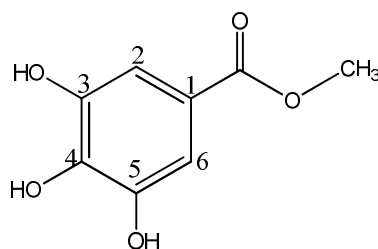
Table-4.12. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **1** in CD_3OD

C. No	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	119.6	C	-
2, 6	108.5	CH	6.67 (s)
3, 5	145.5	C	-
4	138.3	C	-
O-CH ₂	60.2	CH ₂	4.20 (q, $J = 7$)
CH ₃	14.2	CH ₃	1.32 (t, $J = 7$)
C=O	165.8	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interections

4.6.2. Methyl gallate (2)

Compound **2** was off-white needles and molecular formula was established by ESI-HR-MS m/z 185.0442 a.m.u calcd. 185.0438 a.m.u. for $C_8H_8O_5$ IR spectrum indicated presence of benzene ring (1540 cm^{-1}) and a carbonyl carbon of ester function (1690 cm^{-1}) together with hydroxyl groups (3400 cm^{-1}). UV spectrum indicated presence of benzene ring (λ_{max} 265 nm).



2

The $^1\text{H-NMR}$ (**Table-4.13**) of **15** demonstrated signals for two aromatic protons at δ_{H} 7.28 (2H, s, H-2, H-6) and singlet at δ_{H} 3.81 for three methyl protons. $^{13}\text{C-NMR}$ showed the presence of one methyl, two methine and five quaternary carbons i.e. one carbonyl four aromatic quaternary carbons consistent with the formula $C_8H_8O_5$.

HMBC experiment showed the correlation of three methyl protons with that of carbonyl carbon where two aromatic protons also showed correlation with the carbonyl carbon. The spectral data of **15** was found identical to the methyl gallate from the literature.¹³⁰

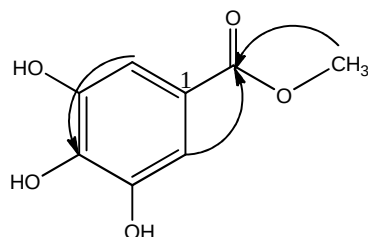


Fig-4.13. Selected key COSY (H---H) and HMBC (H---C) interactions of **1**

Table-4.13. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **2** in CD_3OD

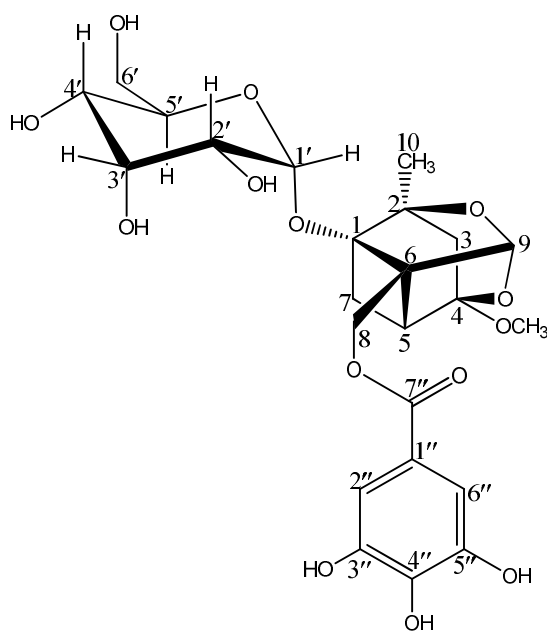
C. No	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	119.6	C	-
2, 6	108.5	CH	7.28 (s)
3, 5	145.5	C	-
4	138.3	C	-
O-CH ₃	60.2	CH ₃	3.81 (s)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interections

4.6.3. Debenzoylpaeoniflorin; 4-methylether-8-benzoyl-1- β -D-glucopyranoside (**4**)

Compound **4** was isolated as amorphous powder. The IR spectrum is consistent with the presence of benzene ring (1540 cm^{-1}), ester linkage (1710 cm^{-1}), glycoside linkage (1030 cm^{-1}) and hydroxyl groups (3410 cm^{-1}). UV spectrum also indicated presence of highly conjugated system ($\lambda_{\text{max}}\ 265\text{ nm}$). Molecular formula was established by ESI-HR-MS $m/z\ 543.1708\text{ a.m.u.}$ calcd. 543.1706 a.m.u. for $\text{C}_{24}\text{H}_{30}\text{O}_{11}$.

^1H - and ^{13}C -NMR spectra of **4** are identical to that of compound **14** with difference of additional methoxy group.



4

The ^1H -NMR (**Table-4.14**) showed signals at $\delta_{\text{H}}\ 1.78$ (1H, d, $J=11.5\text{ Hz}$, H-3a), 2.24 (1H, d, $J=11.5\text{ Hz}$, H-3b), 2.68 (1H, t, $J= 6.5\text{ Hz}$, H-5), 2.04 (1H, dd, $J= 10.5, 2\text{ Hz}$, H-7a), 2.61 (1H, dd, $J= 10.5, 2\text{ Hz}$, H-7b), 4.81 (2H, s, H-8), 5.51 (1H, s, H-9), 1.47 (1H, s, H-

10) and OCH_3 appeared at δ_{H} 3.97 as a singlet. The glucose moiety showed signals in ^1H -NMR spectrum at δ_{H} 5.62 (1H, d, $J=7.5$ Hz, H-1'), 4.21 (1H, t, $J=7.5$ Hz, H-2'), 4.52 (1H, t, $J=7.5$ Hz, H-3'), 5.68 (1H, t, $J=7.5$ Hz, H-4'), 3.84 (1H, m, H-5'), 4.08 (1H, dd, $J=7.5$ Hz, 1.5 Hz, H-6a') and δ_{H} 4.37 (1H, dd, $J=7.5$, 1.5 Hz, H-6b') while the two aromatic protons appeared at δ_{H} 7.68 as a singlet.

The spectral data of **4** was similar with the debenzoylpaeoniflorin; 4-methylether-8-benzoyl-1-*O*- β -D-glucopyranoside.^{44, 131}

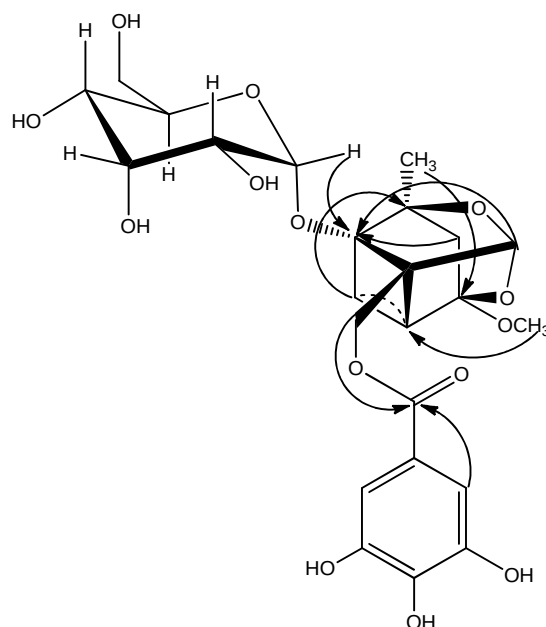


Fig-4.14. Selected key COSY (H---H) and HMBC (H—C) interactions of **14**

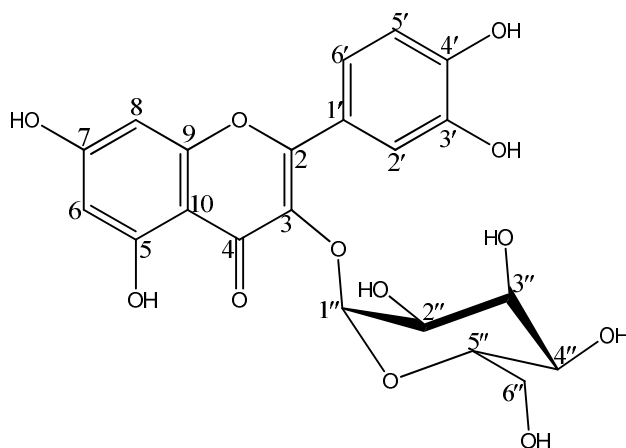
Table-4.14. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **4** in CD_3OD

C. No	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	88.7	C	-
2	86	C	-
3	44.5	CH_2	1.78, d ($J=11.5$) 2.24, d ($J=11.5$)
4	105.2	C	-
5	44	CH	2.68, t ($J=6.5$)
6	72	C	-
7	23.6	CH_2	2.04, dd ($J=10.5, 2$) 2.61, dd ($J=10.5, 2$)
8	61.4	CH	4.81, s
9	100.4	CH	5.51, s
10	19.2	CH_3	1.47, s
OCH_3	51.2	CH_3	3.97, s
1'	102.8	CH	5.62, d ($J=7.5$)
2'	75.6	CH	4.21, t ($J=7.5$)
3'	77	CH	4.52, t ($J=7.5$)
4'	72.4	CH	5.68, t ($J=7.5$)
5'	78.4	CH	3.84, m
6'	62.2	CH_2	4.08, dd ($J=7.5, 1.5$) 4.37, dd ($J=7.5, 1.5$)
1''	129.9	C	-
2'', 6''	110.2	CH	7.68, s
3'', 5''	147.8	C	-
4''	141.4	C	-
7''	168.7	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.6.4. Quercetin-3- β -D-glucopyranoside (10)

Compound **10** was isolated as yellowish amorphous powder. Molecular formula was confirmed by ESI-HR-MS showing molecular ion peak at m/z 465.1024 a.m.u. calcd 465.1020 a.m.u. corresponding to molecular formula $C_{21}H_{20}O_{12}$. IR spectrum indicated presence of benzene ring (1525 cm^{-1}), α,β -unsaturated carbonyl group (1665 cm^{-1}) and hydroxyl groups (3400 cm^{-1}). The UV spectrum is also consistent with presence of highly unsaturated conjugated benzene type system (λ_{max} 360 nm).



10

The ^1H - and ^{13}C -NMR spectra (**Table-4.15**) indicated the presence of quercetin nucleus and a glucose moiety attached at C-3. In ^1H -NMR peaks appeared at δ_{H} 6.12 (1H, d, $J = 1.5\text{ Hz}$, H-6), 6.28 (1H, d, $J = 1.5\text{ Hz}$, H-8), 7.58 (1H, d, $J = 2.0\text{ Hz}$, H-2'), 6.68 (1H, d, $J = 8.5\text{ Hz}$, H-5') and δ_{H} 7.43 (1H, dd, $J = 8.5, 2\text{ Hz}$, H-6'). The glucose protons resonated at δ_{H} 5.17 (1H, d, $J = 6.5\text{ Hz}$, H-1''), 3.42 (1H, t, $J = 6.5\text{ Hz}$, H-2''), 3.40 (1H, t, $J = 6.5\text{ Hz}$, H-3''), 3.38 (1H, t, $J = 6.5\text{ Hz}$, H-4''), 3.1 (1H, m, H-5''), 3.53 (1H, dd, $J = 11.5, 5\text{ Hz}$, H-6a'') and δ_{H} 3.67 (1H, dd, $J = 11.5, 5.0\text{ Hz}$, H-6b'').

^{13}C -NMR (BB and DEPT) spectra (**Table-4.15**) of **10** showed the presence of one methylene, twelve methine and ten quaternary carbons. The structure was further confirmed by 2D NMR techniques i.e. HMBC, NOSEY and COSY. H-6 and H-8 are correlated with C-7 and C-10 while H-2' and H-6' showed correlation with C-1 and with C4'. The anomeric proton showed correlation with C-3 indicating the attachment of glucose at C-3.

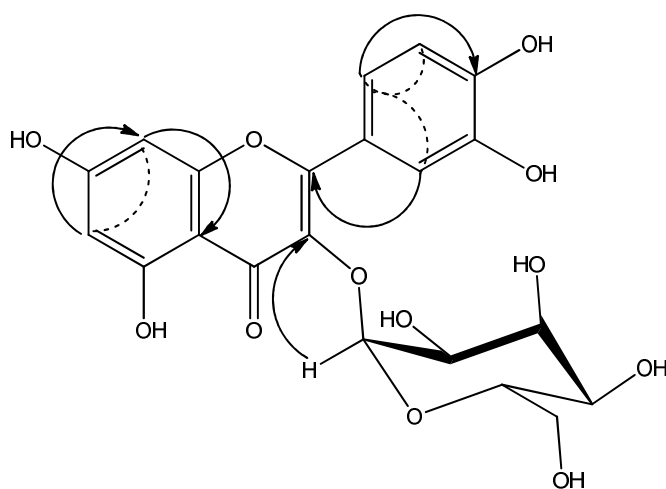


Fig-4.15. Selected key COSY (H $\cdots$ H) and HMBC (H $\cdots$ C) interactions of **10**

The spectral data of **10** was identical to the reported compound; quercetin-3- β -D-glucopyranoside in the literature.¹³²

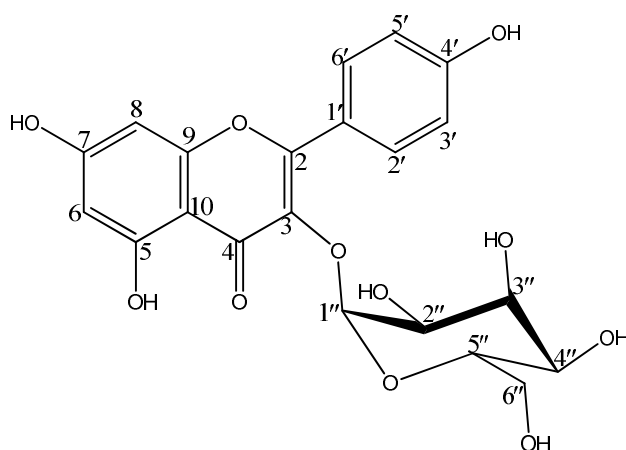
Table-4.15. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **10** in CD_3OD

C. No	^{13}C -NMR (δ_{C}) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ_{H}) coupling constants J_{HH} (HZ) ^{cd}
2	158.5	C	-
3	135.3	C	-
4	179.2	C	-
5	163.2	C	-
6	99.6	CH	6.12, d ($J=1.5$)
7.	132.4	C	-
8	94.6	CH	6.28, d ($J=1.5$)
9	159.1	C	-
10	104.2	C	-
1'	123.2	C	-
2'	117.7	CH	7.58 d ($J=2$)
3'	145.6	C	-
4'	149.4	C	-
5'	116.2	CH	6.68, d ($J=8.5$)
6'	123.1	CH	7.43, dd ($J=9.5, 2$)
1''	104.4	CH	5.17, d ($J=7.5$)
2''	75.6	CH	3.37, t ($J=6.5$)
3''	78.1	CH	3.29, t ($J=6.5$)
4''	71.3	CH	3.21, t ($J=6.5$)
5''	78.8	CH	3.18, m
6''	62.3	CH_2	3.45, dd ($J=11.5, 5$) 3.59, dd ($J=11.5, 5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.6.5. Kaempferol-3- β -D-glucopyranoside (astragalin) (**11**)

Compound **11** was isolated as yellow needles. The molecular formula ($C_{21}H_{20}O_{11}$) was determined by ESI-HR-MS m/z 449.1074 a.m.u. calcd. 449.1070 a.m.u. The IR spectrum indicated presence of phenolic groups (3380 cm^{-1}), and a carbonyl carbon (1680 cm^{-1}) while UV spectrum is consistent with the presence of highly conjugated system (λ_{max} 356 nm).



11

The ^1H - and ^{13}C -NMR of compound **11** are identical with the spectral data of reported compound astragalin from literature. The ^1H -NMR spectrum (**Table-4.16**) of compound **7** showed proton signals at δ_{H} 6.14 (1H, d, $J = 1.8\text{ Hz}$, H-6), 6.32 (1H, d, $J = 1.8\text{ Hz}$, H-8), 8.04 (2H, d, $J = 8.5\text{ Hz}$, H-2', H-6') and δ_{H} 6.87 (2H, d, $J = 8.5\text{ Hz}$, H-3', H-5'). The glucose protons appeared at their respective δ value i.e. δ_{H} 5.18 (1H, d, $J = 7.0\text{ Hz}$, H-1''), 3.42 (1H, t, $J = 6.5\text{ Hz}$, H-2''), 3.40 (1H, t, $J = 6.5\text{ Hz}$, H-3''), 3.38 (1H, t, $J = 6.5\text{ Hz}$, H-4''), 3.1 (1H, m, H-5''), 3.53 (1H, dd, $J = 11.5, 5\text{ Hz}$, H-6a'') and δ_{H} 3.67 (1H, dd, $J = 11.5, 5\text{ Hz}$, H-6b'').

^{13}C -NMR (**Table-4.16**) depicted the presence of one methylene, eleven methine and nine quaternary carbons corresponding to molecular formula $\text{C}_{21}\text{H}_{20}\text{O}_{11}$.

The HMBC experiment confirmed the correlation of H-6 and H-8 with H-10 while H-2' and H-6' showed correlation with C-4. The anomeric carbon showed correlation with C-3 showing the attachment of glucose to position C-3 of the kaempferol.

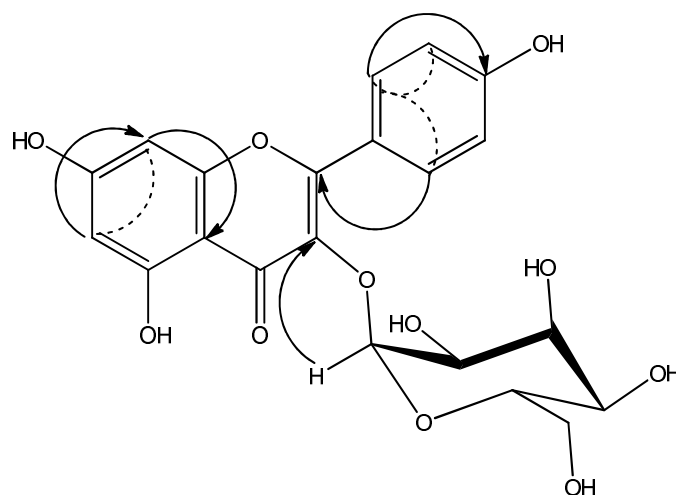


Fig-4.16. Selected key COSY (H----H) and HMBC (H—C) interactions of **11**

The spectra of **11** was compared with astragalin from the literature and it showed similarity.¹³³

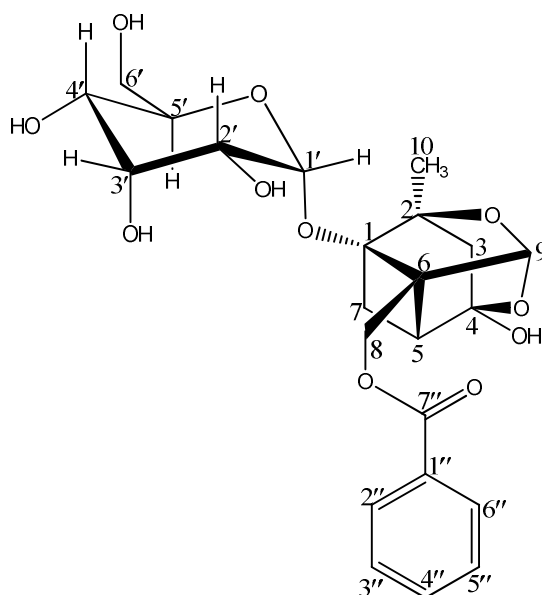
Table-4.16. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **11** in CD_3OD

C/H. No	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	158.8	C	-
3	135.4	C	-
4	179.2	C	-
5	162.9	C	-
6	100.8	CH	6.14, d ($J=1.8$)
7	129	C	-
8	95.4	CH	6.32, d ($J=1.8$)
9	158.6	C	-
10	105.1	C	-
1'	122.7	C	-
2', 6'	132.2	CH	8.04, d ($J=8.5$)
3', 5'	116.08	CH	6.87, d ($J=8.5$)
4'	161.6	C	-
1''	104.3	CH	5.18, d ($J=7.0$)
2''	75.7	CH	3.42, t ($J=6.5$)
3''	78	CH	3.40, t ($J=6.5$)
4''	71.3	CH	3.38, t ($J=6.5$)
5''	78.3	CH	3.1, m
6''	62.6	CH_2	3.53, dd ($J=11.5, 5$) 3.67, dd ($J=11.5, 5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interections

4.6.6. 8-Debenzoylpaeoniflorin-1-O- β -D-glucopyranoside (13)

Compound **13** was isolated as brown amorphous powder and its molecular formula was established as $C_{23}H_{28}O_{11}$ from ESI-HR-MS m/z 481.1702 a.m.u. calcd. 481.1700 a.m.u. IR spectrum indicated presence of benzene ring (1540 cm^{-1}), ester linkage (1710 cm^{-1}), hydroxyl groups (3370 cm^{-1}) and glycoside linkage (1030 cm^{-1}). The UV spectrum indicated presence of highly conjugated systems (λ_{max} 275 nm).



13

^1H - and ^{13}C -NMR spectra (**Table-4.17**) indicated the presence of a benzene ring, a glucose moiety and a paeoniflorin nucleus. ^1H -NMR (**Table-4.17**) showed signals at δ_{H} 1.79 (1H, d, $J=12.5\text{ Hz}$, H-3a), 2.38 (1H, d, $J=12.5\text{ Hz}$, H-3b), 2.42 (1H, t, $J=6.5\text{ Hz}$, H-5), 2.07 (1H, dd, $J=10.7, 1.5\text{ Hz}$, H-7a), 2.68 (1H, dd, $J=10.7, 1.5\text{ Hz}$, H-7b), 4.81 (2H, s, H-8), 5.62 (1H, s, H-9) and δ_{H} 1.42 (3H, s, H-10). The glucose protons resonated in the ^1H -NMR at δ_{H} 5.74 (1H, d, $J=7.6\text{ Hz}$, H-1'), 4.13 (1H, t, $J=7.6\text{ Hz}$, H-2'), 4.51 (1H, t,

$J=7.6$ Hz, H-3'), 5.85 (1H, t, $J=7.6$ Hz, H-4'), 4.02 (1H, m, H-5'), 4.16 (1H, dd, $J=7.6$, 1.5 Hz, H-6a') and δ_{H} 4.36 (1H, dd, $J=7.6$, 1.5 Hz, H-6b'). The five aromatic protons appeared at δ_{H} 8.16 (2H, dd, $J=7.5$, 2 Hz, H-2'', H-6''), 7.69 (2H, t, $J=7.5$ Hz, H-3'', H-5'') and δ_{H} 7.85 (1H, t, $J=7.5$ Hz, H-4'') in the ^1H -NMR spectrum.

^{13}C -NMR spectra (**Table-4.17**) indicated presence of one methyl, four methylenes, twelve methines and six quaternary carbons corresponding to molecular formula $\text{C}_{23}\text{H}_{28}\text{O}_{11}$.

The spectral data of **13** are identical to the reported compound; 8-Debenzoylpaeoniflorin-1-*O*- β -D-glucopyranoside.⁶³

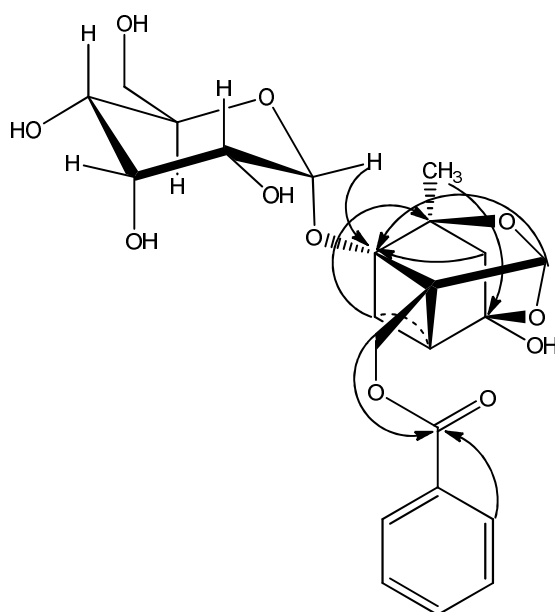


Fig-4.17. Selected key COSY (H---H) and HMBC (H—C) interactions of **13**

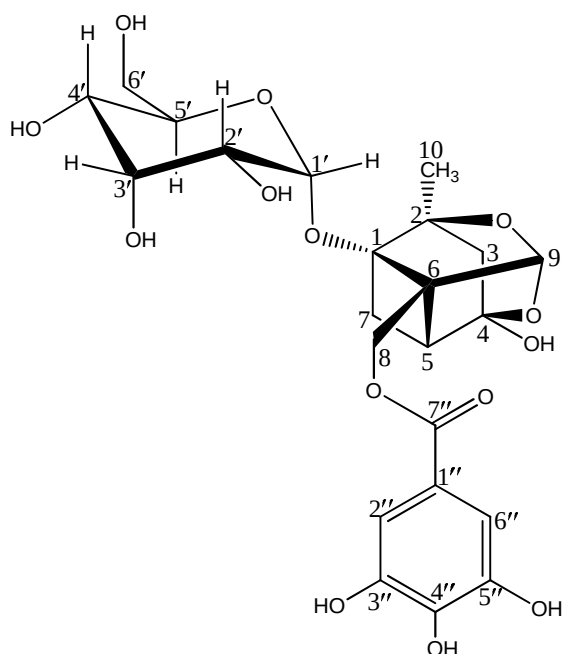
Table-4.17. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **13** in CD_3OD

C/H. No	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) coupling constants J_{HH} (Hz) ^{cd}
1	90	C	-
2	86.4	C	-
3	44.1	CH_2	1.79, d ($J=12.5$) 2.38, d ($J=12.5$)
4	105	C	-
5	44.8	CH	2.42, t ($J= 6.5$)
6	71.2	C	-
7	23.8	CH_2	2.07, dd ($J= 10.7, 1.5$) 2.68, dd ($J= 10.7, 1.5$)
8	61.2	CH	4.81, s
9	100.6	CH	5.62, s
10	19.6	CH_3	1.42, s
1'	102.7	CH	5.74, d ($J=7.6$)
2'	75.5	CH	4.13, t ($J=7.6$)
3'	78.2	CH	4.51, t ($J=7.6$)
4'	72.5	CH	5.85, t ($J=7.6$)
5'	77.9	CH	4.02, m
6'	62.4	CH_2	4.16, dd ($J=7.6, 1.5$) 4.36, dd ($J=7.6, 1.5$)
1''	130.2	C	-
2'', 6''	131.4	CH	8.16 dd ($J=7.5, 2$)
3'', 5''	129.2	CH	7.69, t ($J=7.5$)
4''	134.3	CH	7.85, t ($J=7.5$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.6.7. Debenzoyl-8-galloylpaeoniflorin-1-O- β -D-glucopyranoside (**14**)

Compound **14** was isolated as amorphous, tan. powder. The molecular formula was established by ESI-HR-MS m/z 543.1706 a.m.u. calcd. 543.1702 a.m.u. for $C_{23}H_{28}O_{14}$. The IR spectrum indicated presence of hydroxyl groups (3410 cm^{-1}), carbonyl carbon (1710 cm^{-1}), benzene ring (1540 cm^{-1}) and a glycoside linkage (1030 cm^{-1}). The UV spectrum also indicated presence of benzene ring (λ_{max} 280) and a carbonyl group ($n\text{-}\pi^*$ transition at 170 nm).



14

The ^1H - and ^{13}C -NMR spectra (**Table-4.18**) indicated the presence of a benzene ring, a glucose moiety and a paeoniflorin nucleus. ^1H -NMR (**Table-4.18**) showed protons signals at δ_{H} 1.92, d ($J=12\text{ Hz}$, H-3a), 2.37, d ($J=12\text{ Hz}$, H-3b), 2.72, t ($J=7\text{ Hz}$, H-5), 2.14, dd ($J=10.7, 2\text{ Hz}$, H-7a), 2.82, dd ($J=10.7, 2\text{ Hz}$, H-7b), 4.49, s (H-8), 5.64, s (H-9)

and δ_{H} 1.37, s (H-10). The glucose protons showed signals in the ^1H -NMR at δ_{H} 5.34 (1H, d, $J=7$ Hz, H-1'), 4.17 (1H, t, $J=7$ Hz, H-2'), 4.51 (1H, t, $J=7$ Hz, H-3'), 5.68 (1H, t, $J=7$ Hz, H-4'), 4.02 (1H, m, H-5'), 4.16 (1H, dd, $J=7, 1.5$ Hz, H-6a') and δ_{H} 4.27 (1H, dd, $J=7, 1.5$ Hz, H-6b') while the two aromatic protons i.e. H-2'' and H-6'' appeared at δ_{H} 7.76 as a singlet.

The key HMBC correlation also confirmed the relative position of carbons to protons.

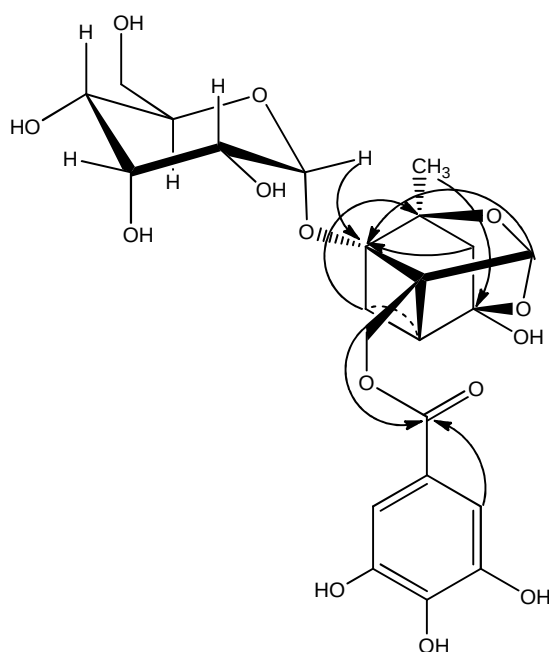


Fig-4.18. Selected key COSY (H----H) and HMBC (H—C) interactions of **14**

Spectral data of compound **14** was also found identical to the reported compound; debenzoyl-8-galloylpaeoniflorin-1-O-β-D-glucopyranoside from the literature.¹³⁴

Table-4.18. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **14** in CD_3OD

C/H. No	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	89.6	C	-
2	86.5	C	-
3	43.9	CH_2	1.92, d ($J=12$) 2.37, d ($J=12$)
4	104.7	C	-
5	44.6	CH	2.72, t ($J=7$)
6	71.2	C	-
7	22.8	CH_2	2.14, dd ($J=10.7, 2$) 2.82, dd ($J=10.7, 2$)
8	61.2	CH	4.49, s
9	100.2	CH	5.64, s
10	19.6	CH_3	1.37, s
1'	102.3	CH	5.34, d ($J=7$)
2'	75	CH	4.17, t ($J=7$)
3'	77.9	CH	4.51, t ($J=7$)
4'	72.2	CH	5.68, t ($J=7$)
5'	78.1	CH	4.02, m
6'	62.9	CH_2	4.16, dd ($J=7, 1.5$) 4.27, dd ($J=7, 1.5$)
1''	130.7	C	-
2'', 6''	110.4	CH	7.76, s
3'', 5''	147.3	C	-
4''	141.3	C	-
7''	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.7. DPPH antioxidant assay

Antioxidant activity of various compounds isolated from the aerial parts of *P. emodi* was evaluated using DPPH as a stable free radical (**Table-4.19**). As reported previously, the ethanolic extract showed about 83% DPPH radical scavenging activity.²⁵ Hence, the crude extract was subjected to solvent-solvent separation (**Scheme-5.1**) and observed that maximum radial scavenging activity was concentrated in ethyl acetate and aqueous fractions. The ethyl acetate fraction resulted in isolation of eighteen pure compounds i.e. three new flavonoids and two new paeoniflorins while the rest are known compounds including flavonoids, paeoniflorins, gallic acid derivatives and benzofuran. Compounds **1-18** were tested for DPPH radical scavenging assay to gauge their antioxidant potential and the flavonoids have remarkable antioxidant potentials as expected while the paeoniflorin compounds displayed very mild antioxidants activity. Moreover, amongst the flavonoids, the quercetin derivatives have higher antioxidant potential as compared to corresponding kaempferol derivatives, which can be accredited to the presence of an additional hydroxyl group in quercetins. It is also evident from the **Table-4.19** that if an acidic group is present in either of the aromatic ring of the flavonoid, it increases the antioxidant potential. The highest antioxidant activity was observed by **12** and **16**, which are new acid derivatives of kaempferol with IC₅₀ value of 29.4± 1.51 and 35.4±1.24 μ M respectively. It can also be concluded that when the COOH group is in ring A of the flavonoid, it exhibits higher antioxidant potential than the acid group is present in ring B suggesting that ring A has more influence in antioxidant activity.

Table-4.19. Antioxidant activity of isolated compounds against DPPH assay

S.No	Sample	% inhibition	IC ₅₀ ± SEM (μM)
1.	Paeoniflorin A (6)	61	Inactive
2.	Paeoniflorin B (7)	57	Inactive
3.	Kaempferol-3- <i>O</i> -β-D-glucopyranosyl-7-oic acid (12)	86	29.4± 1.51
4.	Kaempferol-7- <i>al</i> -3- <i>O</i> -β-D-glucopyranosid (15)	74	58.2±0.98
5.	Kaempferol-7-methoxy-3- <i>O</i> -β-D-glucopyranosyl-3'-oic acid (16)	84	35.4±1.24
6.	2,3-Dihydroxy-4-methoxyacetophenone (3)	68	68±1.31
7.	Quercetin-3'-methoxy-3-β-D-glucopyranoside (5)	57	Inactive
8.	Debenzoyl-8-galloylpaeoniflorin (8)	40	Inactive
9.	2- <i>O</i> -Ethyl-β-D-glucopyranoside (9)	32	Inactive
10.	2,3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol (17)	74	Inactive
11.	<i>p</i> -Digallic acid (18)	80	29.5±0.85
12.	Ethyl gallate (1)	94	4.96±0.31
13.	Methyl gallate (2)	91	4.18±0.31
14.	Debenzoylpaeoniflorin; 4-methylether-8-benzoyl-1- <i>O</i> -β-D-glucopyranoside (4)	37	Inactive
15.	Quercetin-3-β-D-glucopyranoside (10)	54	Inactive
16.	Kaempferol-3-β-D-glucopyranoside (Astragalin) (11)	57	Inactive
17.	8-Debenzoylpaeoniflorin-1- <i>O</i> -β-D-glucopyranoside (13)	22	Inactive
18.	Debenzoyl-8-galloylpaeoniflorin-1- <i>O</i> -β-D-glucopyranoside (14)	58	97±1.28
19.	Ascorbic acid (Std.)	84	6.31 ± 1.03
20.	Gallic acid (Std.)	87	4.53 ± 0.92
21.	Quercetin (Std.)	86	4.19 ± 4.41
22.	α-tocopherol (Std.)	79	32.50 ± 1.58

% age inhibition was evaluated using inhibitor at a concentration of 100 μM

4.8. Antibacterial activity

The result indicated that none of the crude fractions possesses significant antibacterial potential.

Table-4.20. Antibacterial activity of the crude fractions of *P. emodi*

Bacterial Species	Zone of inhibition (mm)					
	Imipenem 10 µg/disc(+)	DMSO (-)	<i>n</i> -Hexane	Chloroform	Ethyl Acetate	Aqueous
<i>Escherichia coli</i>	35	-	-	3	6	2
<i>Bacillus subtilis</i>	36	-	-	5	5	3
<i>Shigella flexneri</i>	36	-	-	-	10	6
<i>Staphylococcus aureus</i>	43	-	-	4	9	6
<i>Pseudomonas aeruginosa</i>	32	-	-	-	-	-
<i>Salmonella typhi</i>	40	-	-	6	10	10

4.8. Antifungal activity

No significant antifungal activity was observed indicating that the crude fractions do not possess any antifungal metabolites.

Table-4.21. Antifungal activities of various fractions of *P. emodi*

Fungal species	Std. Drug Mic µg/ml	% inhibition			
		<i>n</i> -Hexane	Chloroform	Ethyl acetate	Aqueous
<i>Trichophyton longifusus</i>	Miconazole 70	-	-	16	-
<i>Candida albicans</i>	Miconazole 110.8	-	6	4	-
<i>Aspergillus flavus</i>	Amphotericin 20	-	-	-	4
<i>Microsporum canis</i>	Miconazole 98.4	-	-	-	-
<i>Fusarium solani</i>	Miconazole 73	-	-	8	10
<i>Candida glaberata</i>	Miconazole 110.8	-	-	-	-

4.9. Urease inhibition assay

Urease inhibitory potential of compounds **1-18** isolated from the aerial part of *P. emodi* was evaluated. The results (**Table-4.22**) indicated that the isolated compounds having paeoniflorin nucleus are found to be inactive. Furthermore it is evident from the **Table-4.22** that those compounds having flavone nucleus showed promising urease inhibitory potential. Moreover the quercetin derivative have higher inhibitory effect than corresponding kaempferol derivatives. Higher activity of quercetin derivatives can be attributed to the presence of an additional hydroxyl group. The new flavonoids **12** and **16** which are the acid derivatives showed more potent inhibitory competence with IC_{50} of 31 ± 1.24 and $33 \pm 1.05 \mu M$ than the corresponding flavonoid derivatives with no carboxylic function suggesting that COOH group is effectively involved the process of enzyme inhibition.

Table-4.22. Inhibitory activity of isolated compound against Jack bean urease.

S.No	Sample	% inhibition	IC ₅₀ ± SEM (μM)
1.	Paeoniflorin A (6)	15	Inactive
2.	Paeoniflorin B (7)	14	Inactive
3.	Kaempferol-3- <i>O</i> -β-D-glucopyranosyl-7-oic acid (12)	84	31± 1.24
4.	Kaempferol-7- <i>al</i> -3- <i>O</i> -β-D-glucopyranosid (15)	64	98±1.48
5.	Kaempferol-7-methoxy-3- <i>O</i> -β-D-glucopyranosyl-3'-oic acid (16)	82.6	33±1.05
6.	2,3-Dihydroxy-4-methoxyacetophenone (3)	4	Inactive
7.	Quercetin-3'-methoxy-3-β-D-glucopyranoside (5)	74	78± 1.07
8.	Debenzoyl-8-galloylpaeoniflorin (8)	10	Inactive
9.	2- <i>O</i> -Ethyl-β-D-glucopyranoside (9)	57	Inactive
10.	2,3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol (17)	14	Inactive
11.	<i>p</i> -Digallic acid (18)	52	87±1.36
12.	Ethyl gallate (1)	61	87±1.92
13.	Methyl gallate (2)	58	92±1.08
14.	Debenzoylpaeoniflorin; 4-methylether-8-benzoyl-1- <i>O</i> -β-D-glucopyranoside (4)	20	Inactive
15.	Quercetin-3-β-D-glucopyranoside (10)	39	Inactive
16.	Kaempferol-3-β-D-glucopyranoside (Astragalin) (11)	42	Inactive
17.	8-Debenzoylpaeoniflorin-1- <i>O</i> -β-D-glucopyranoside (13)	19	Inactive
18.	Debenzoyl-8-galloylpaeoniflorin-1- <i>O</i> -β-D-glucopyranoside (14)	21	Inactive
19.	Thiourea (standard)	96.9	21.8 ± 1.6

% age inhibition was evaluated using inhibitor at a concentration of 100 μM

4.10. Brine shrimp (*Artemia salina*) lethality bioassay

As detailed in the **Table-4.23**, none of the fraction showed cytotoxicity indicating that the plant do not possess any cytotoxic ingredients.

Table-4.23. Brine shrimp (*Artemia salina*) lethality assay of various fractions from aerial parts of *P. emodi*

Fractions	Dose ($\mu\text{g/mL}$)	No. of shrimps/vial	No of survivors/vial	LD ₅₀ ($\mu\text{g/mL}$)	Std. Drug	LD ₅₀ ($\mu\text{g/mL}$)
F1	1000	10	8	>1000	Etoposide	7.4625
	100	10	9			
	10	10	9			
F2	1000	10	8	>1000	Etoposide	7.4625
	100	10	10			
	10	10	10			
F3	1000	10	7	>1000	Etoposide	7.4625
	100	10	9			
	10	10	10			
F4	1000	10	9	<1000	Etoposide	7.4625
	100	10	10			
	10	10	10			

F1 = *n*-hexane, F2 = chloroform, F3 = MeOH, F4 = aqueous

4.11. Antiplasmodial activity

No antiplasmodial activity was observed for any fraction suggesting that the plant do not possess any antiplasmodial phytochemicals.

Table-4.24. Antiplasmodial activity of *P. emodi* crude extracts against *P. falciparum* (D10)

S.No	Sample	D10 IC ₅₀
1	<i>n</i> -Hexane	Inactive
2	Chloroform	Inactive
3	Ethyl acetate	Inactive
4	Aqueous	Inactive
4	Chloroquine (nM)	28.07 ± 12.48
5	Artemisinin (nM)	26.81 ± 8.54

The crude extracts were tested at concentration of 100 µg/ml and standard at 50, 25 and 12.5 nM

EXPERIMENTAL (part A)

5.1. General experimental conditions

5.1.1. Physical contents

Melting points were measured using Stuart SMP 10 apparatus in glass capillary tubes which is uncorrected. Optical rotation was measured by using JASCO-DIP-360 digital polarimeter. Ultraviolet spectra were carried in methanol by using Hitachi-U-3200 spectrophotometer. Infrared spectra were measured on JASCO-A-302 spectrophotometer by direct placing of sample on the diamond cell. Proton NMR spectra were recorded on Bruker Aspect Spectrometer operating at 400 MHz, 500 MHz and 600 MHz respectively with TMS as an internal standard. Carbon-13 NMR spectra (BB and DEPT) were recorded on Bruker AV 500 and 600 spectrometer operating at 125 MHz and 150 MHz respectively. The 2D NMR experiments i.e. HSQC, HMBC, NOESY, ^1H - ^1H COSY, and *J*-resolved were performed on Bruker AV 500 Spectrometer.

Exact assignments of the proton NMR chemical shifts were made through double resonance experiment and 2D studies such as HSQC, HMBC, NOESY, ^1H - ^1H COSY, and *J*-resolved. ^{13}C -NMR spectral assignments of the purified compounds have been made partially through the DEPT and 2D spectra and partially through comparison of the chemical shift with reported compounds.

Mass spectra were recorded on Finnigan-MAT-311 and Varian MAT 312 spectrometer while exact masses have been measured through High Resolution (HR) and Fast Atom Bombardment (FAB +ve) mass spectra using JMS HX-110 double focusing mass

spectrophotometer where methanol was using as a solvent and glycerol as a matrix on the target. Xe gas was used to ionize the sample.

5.1.2. Chromatographic Techniques

Column chromatography (CC) was performed over Merck silica gel (200 μ m), Sephadex LH-20 (Sigma-Aldrich) and RP-18 (Merck). Preparative TLC silica gel 60 F₂₅₄ coated on aluminum sheets (Merck) with thickness of 0.2 mm while reverse phase preparative TLC glass plates coated with RP-18 F₂₅₄ (Merck) of 0.25 mm thickness were used. The purity of compounds was checked on silica gel coated plates under compact UV lamp (245/365 nm), spraying the plates with ceric sulphate reagent, vanilin, I₂ vapors and Dragendorff's reagent.

5.2. Materials and methods

5.2.1. Plant materials

The plant was collected from Hazara Division of Khyber Pakhtunkhwa Province of Pakistan in July of 2009 and identified by taxonomist Mr. Abdul Majid, Lecturer Department of Botany, Hazara University, Monshera where a voucher specimen was deposited at the herbarium. The plant species was further authenticated by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan.

5.2.2. Extraction and isolation

The shade dried roots and aerial parts were separately extracted with commercial grade ethanol (x 3) at room temperature. The thickish chocolate color residue obtained on removal of the solvent from the combined extract under Vacuo at 45 °C which was partitioned between *n*-hexane and H₂O, dichloromethane and H₂O and ethyl acetate H₂O in order to fractionate the complex mixture into non-polar, slightly polar and medium polar sub-fractions while highly polar compounds remain in the aqueous phase (**Scheme-5.1**).

The ethyl acetate fraction (**86 g**) was subjected to column chromatography (CC) on silica gel (GF) eluted with *n*-hexane, *n*-hexane; EtOAc and DCM; MeOH (**Scheme-5.2**). The elution was started from pure *n*-hexane and with increasing polarity with EtOAc. After every 5 liters of the solvent elution, the polarity was increased by 5% until the polarity has reached to 70% EtOAc and 30% *n*-hexane then the solvent system was changed to dichloromethane and methanol i.e started elution with pure dichloromethane and then increasing polarity with methanol by 1% after elution of 1 liter of solvent until reached to pure methanol which led to the isolation of semi purified fractions A to I based on TLC profile.

Fraction C was subjected to CC by using silica gel while MeOH and dichloromethane as a solvent system in order of increasing polarity. The DCM; MeOH elution furnished two pure compounds (**1 & 2**).

Semi pure fraction D was subjected to column chromatography over silica gel with elution by dichloromethane and methanol in order of increasing polarity to afford two major fractions (D1 & D2) which was further purified by size exclusion chromatography on Sephadex LH-20 with elution by methanol:dichloromethane (7:3) to purify **3**, **4** and **5** from D1 and **6**, **7** and **8** from fraction D2.

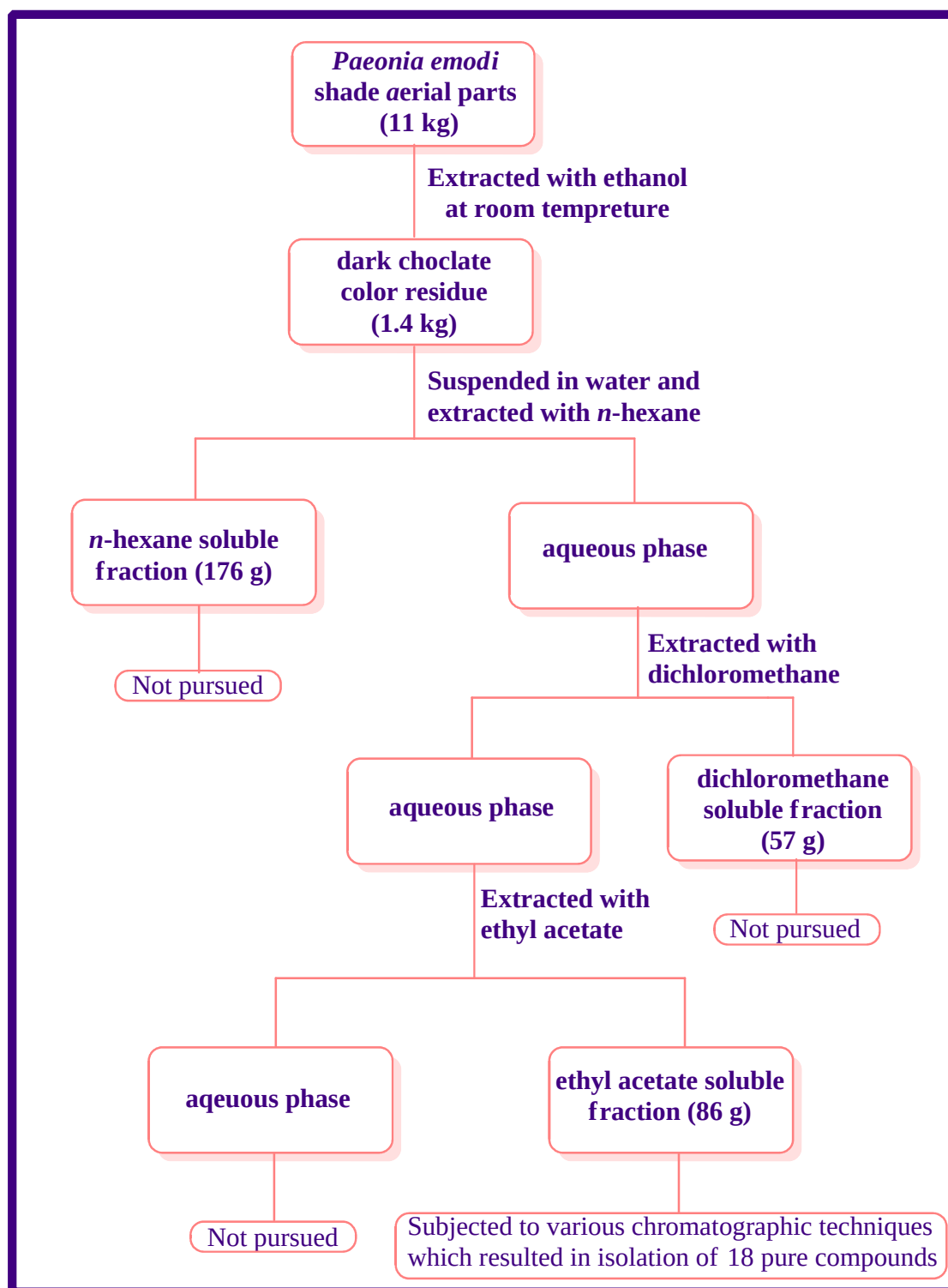
Fraction F was further purified and chromatograph over sephadex LH-20 using by elution of 70% (MeOH) and 30% (dichloromethane) to obtain one pure compound **9** along with sub fraction F1 which was subjected to CC using RP-18 as a stationary phase and water and MeOH (50:50) v/v as a mobile phase and yielded compound **10**.

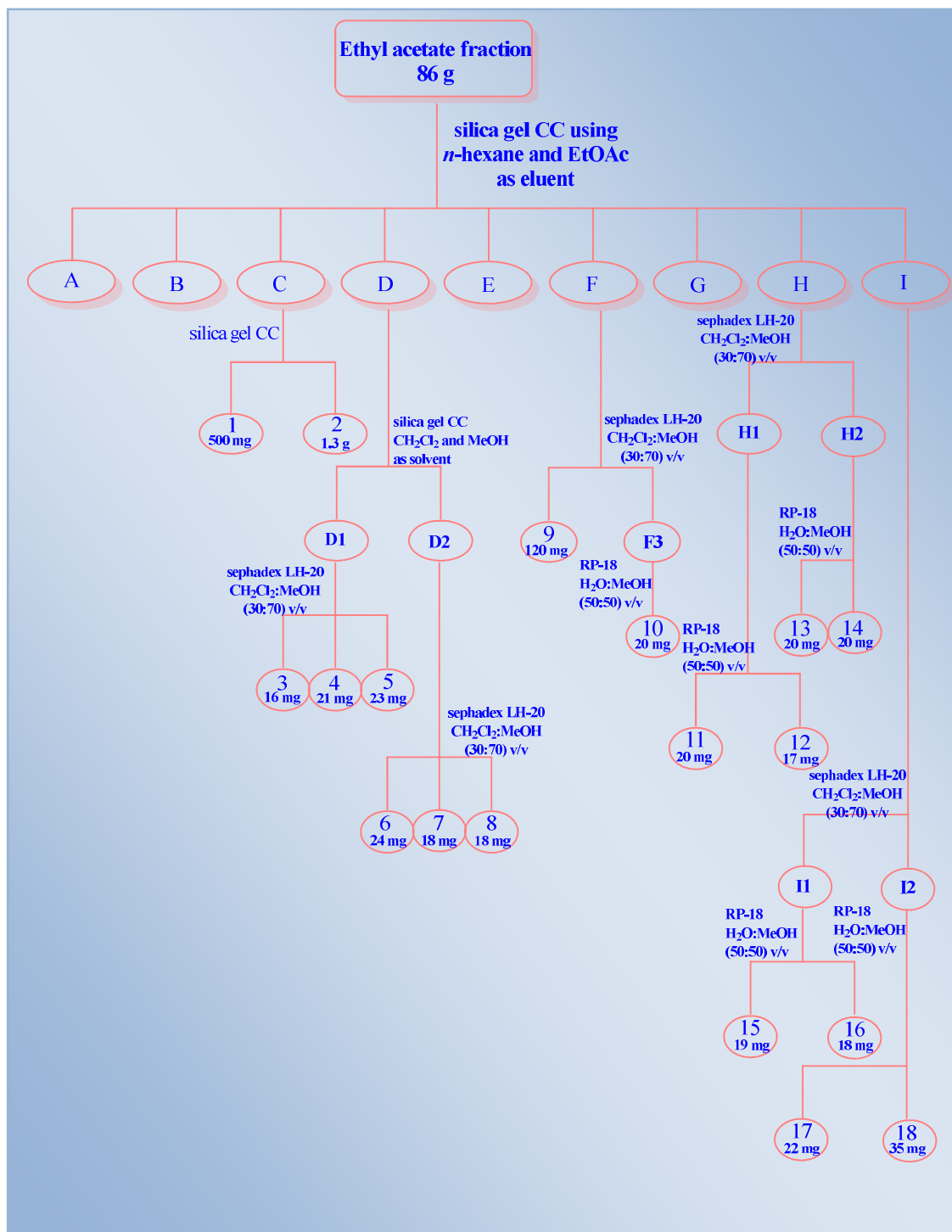
Further purification of fraction H was achieved by size exclusion chromatography over sephadex LH-20 with 70% MeOH and 30% dichloromethane as a solvent system which afforded two semi-purified fractions H1 and H2 which were than subjected to RP-18 CC with water and MeOH (50:50) v/v as a solvent system. Sub-fraction H1 afforded two compounds i.e. **11** & **12** and H2 yield two compounds i.e. **13** & **14**.

Fraction I afforded two subfractions I1 and I2 when subjected to size exclusion chromatography over sephadex LH-20 and eluted with 70% (MeOH) and 30% (dichloromethane). I1 yield two compounds i.e. **15** & **16** when subjected to reverse phase CC over RP-18 as a stationary phase and water and MeOH (50:50) v/v as a mobile phase. In similar fashion fraction I2 afforded two compounds i.e. **17** & **18**.

5.2.3. Acid hydrolysis

Acid hydrolysis of compounds containing sugar moiety was accomplished by dissolving 3-5 mg of the compound in 5 mL mixture of HCl-H₂O-EtOH (2:1:2) and maintained the reaction mixture at 80 °C for about 4 hours. The hydrolysate was extracted by EtOAc and the aqueous layer was compared with authentic sample of monosaccharide on silica gel TLC using DCM-MeOH (10:05) with a drop of 50% acetic acid as a solvent system.

**Scheme-5.1.** Extraction and fractionation of the crude extract



Scheme-5.2. Isolation of pure compounds from ethyl acetate fraction

5.3. Characterization of new compounds from *P. emodi*

5.3.1. Paeoniflorin C (6)

Physical State: Dark yellow sticky

Yield: 24 mg

IR: 3400, 1690, 1620, 1010 cm^{-1}

UV λ_{max} (MeOH): 285, 220 nm

HR-ESI-MS: m/z 495.1860 $[\text{M}+\text{H}]^+$

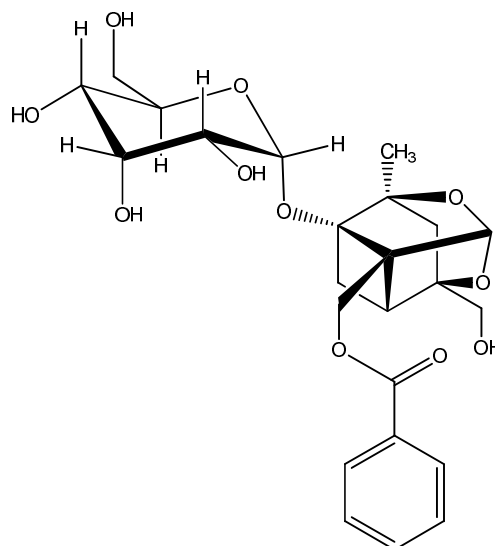
calcd. for $\text{C}_{24}\text{H}_{30}\text{O}_{11}$ 495.1854

ESI (+)-MS m/z : 495.1860, 317.1384

375.1650, 123.0440

^1H - and ^{13}C -NMR and HSQC: **Table-4.1**

HMBC: **Fig-4.1**



5.3.2. Paeoniflorin D (7)

Physical State: Yellow gum

Yield: 18 mg

IR: 3380, 1690, 1620, 1000 cm^{-1}

UV λ_{max} (MeOH): 288, 225 nm

HR-ESI-MS: m/z 509.2014 $[\text{M}+\text{H}]^+$

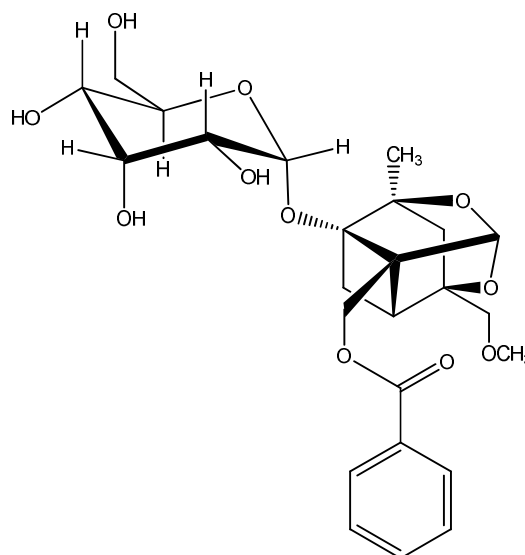
calcd. for $\text{C}_{25}\text{H}_{32}\text{O}_{11}$ 509.2010

ESI (+)-MS m/z : 509.2014, 479.1908,

331.1540, 123.0440

^1H - and ^{13}C -NMR and HSQC: **Table-4.2**

HMBC: **Fig-4.2**



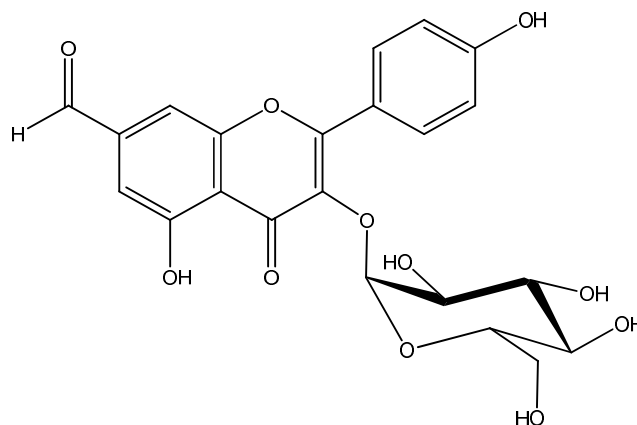
5.3.3. Kaempferol-3-O- β -D-glucopyranosyl-7-oic acid (12)

Physical State: White granules

Yield: 17 mg

IR: 3380, 1640, 1520, 1000 cm^{-1} UV λ_{max} (MeOH): 360, 345 nmHR-ESI-MS: m/z 477.1014 $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_{12}$ 477.1010ESI (+)-MS: m/z 477.1014, 433.1130,

255.0652

 ^1H - and ^{13}C -NMR and HSQC: **Table-4.3**HMBC: **Fig-4.3****5.3.4. Kaempferol-7-al-3-O- β -D-glucopyranosid (15)**

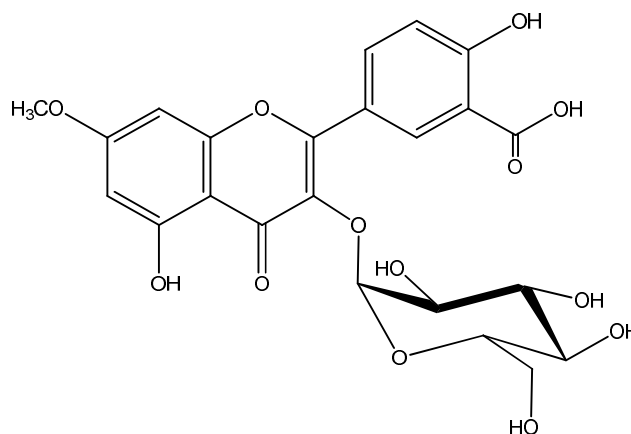
Physical State: Greenish white powder

Yield: 19 mg

IR: 2820, 1635, 1540 cm^{-1} UV λ_{max} (MeOH) 362, 340 nmHR-ESI-MS: m/z 461.1076 $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{22}\text{H}_{20}\text{O}_{11}$ 461.1072ESI (+)-MS m/z : 461.1076, 433.1124

255.0646

431.0973, 269.0445

 ^1H - and ^{13}C -NMR and HSQC: **Table-4.4**HMBC: **Fig-4.4**

5.3.5. Kaempferol-7-methoxy-3-O- β -D-glucopyranosyl-3'-oic acid (16)

Physical State: White amorphous powder

Yield: 14 mg

IR: 3380, 1640, 1710, 1550 cm^{-1}

UV λ_{max} (MeOH): 355, 320 nm

HRESIMS: m/z 507.1130 $[\text{M}+\text{H}]^+$

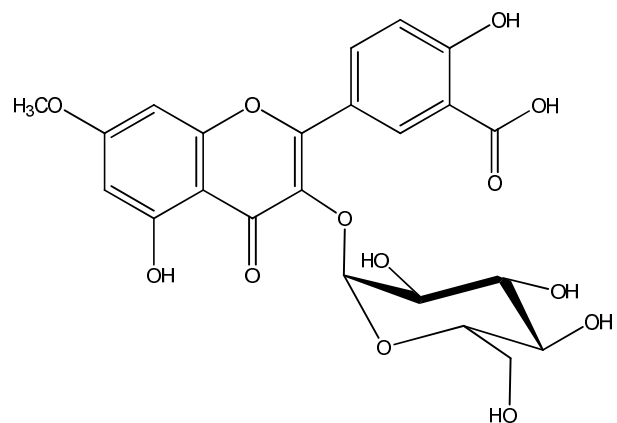
calcd. for $\text{C}_{23}\text{H}_{22}\text{O}_{13}$ 507.1126

ESI (+)-MS m/z : 507.1130, 477.1022

463.1235, 285.0754

^1H - and ^{13}C -NMR and HSQC: **Table-4.5**

HMBC: **Fig-4.5**



5.4. Hitherto unreported constituents from *P. emodi*

5.4.1. 2,3-Dihydroxy-4-methoxyacetophenone (3)

Physical State: oil

Yield: 16 mg

IR: 3420, 1710, 1675, 1540 cm^{-1}

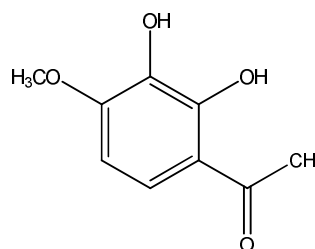
UV λ_{max} (MeOH): 290, 210 nm

HRESIMS: m/z 183.0652 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_9\text{H}_{10}\text{O}_4$ 183.0648

^1H - and ^{13}C -NMR and HSQC: **Table-4.6**

HMBC: **Fig-4.6**



5.4.2. Kaempferol-3'-methoxy-3- β -D-glucopyranoside (5)

Physical State: Amorphous powder

Yield: 23 mg

IR: 3400, 1665, 1655, 1525, 1030 cm^{-1}

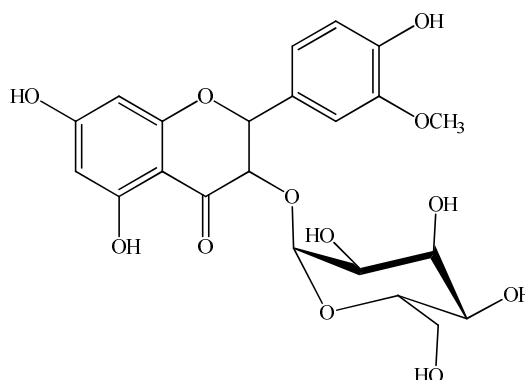
UV λ_{max} (MeOH): 360, 255 nm

HRESIMS: m/z 479.1182 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{22}\text{H}_{22}\text{O}_{12}$ 479.1178

^1H - and ^{13}C -NMR and HSQC: **Table-4.7**

HMBC: **Fig-4.7**



5.4.3. Debenzoyl-8-galloylpaeoniflorin (8)

Physical State: White amorphous powder

Yield: 18 mg

IR: 3440, 1710, 1580 cm^{-1}

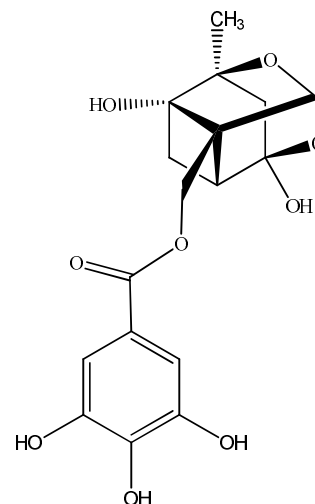
UV λ_{max} (MeOH): 275, 190 nm

HR-ESI-MS: m/z 367.1020 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{17}\text{H}_{18}\text{O}_9$ 367.1016

^1H - and ^{13}C -NMR and HSQC: **Table-4.8**

HMBC: **Fig-4.8**

**5.4.4. 2-O-Ethyl- β -D-glucopyranoside (9)**

Physical State: Milky white crystals

Yield: 120 mg

IR: 34200, 1420, 1040 cm^{-1}

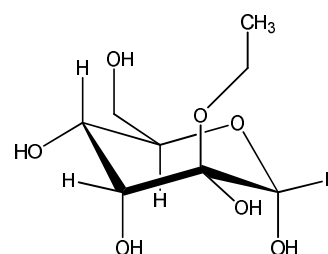
UV λ_{max} (MeOH): In active

HR-ESI-MS: m/z 225.0964 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_8\text{H}_{16}\text{O}_5$ 225.0960

^1H - and ^{13}C -NMR and HSQC: **Table-4.9**

HMBC: **Fig-4.9**



5.4.5. 2,3-Dihydro-5-hydroxy-6-methyl-3-benzofuranmethanol (17)

Physical State: White powder

Yield: 22 mg

IR: 3440, 1675, 1540 cm^{-1}

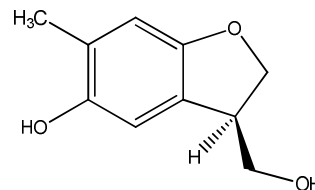
UV λ_{max} (MeOH): 315, 219 nm

HR-ESI-MS: m/z 181.0860 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{10}\text{H}_{12}\text{O}_3$ 181.0856

^1H - and ^{13}C -NMR and HSQC: **Table-4.10**

HMBC: **Fig-4.10**

**5.4.6. *p*-Digallic acid (18)**

Physical State: Colorless fine crystals

Yield: 35 mg

IR: 3420-3270, 1710, 1680, 1540

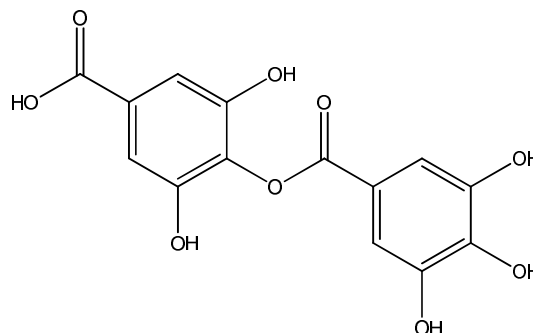
UV λ_{max} (MeOH): 280, 220 nm

HRESIMS: m/z 323.0394 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{14}\text{H}_{10}\text{O}_9$ 323.0398

^1H - and ^{13}C -NMR and HSQC: **Table-4.11**

HMBC: **Fig-4.11**



5.5. Known constituents from *P. emodi*

5.5.1. Ethyl gallate (1)

Physical State: White needles

Yield: 500 mg

IR: 3400, 2860, 1690, 1540 cm^{-1}

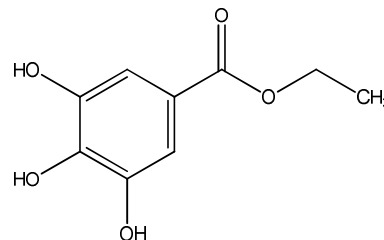
UV λ_{max} (MeOH): 275, 220 nm

ESIMS: m/z 199.0598 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_9\text{H}_{10}\text{O}_5$ 199.0594

^1H - and ^{13}C -NMR and HSQC: **Table-4.12**

HMBC: **Fig-4.12**



5.5.2. Methyl gallate (2)

Physical State: Half-white needles

Yield: 1.3 g

IR: 3400, 1690, 1540 cm^{-1}

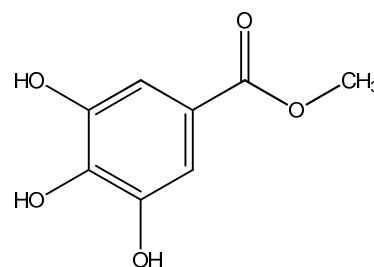
UV λ_{max} (MeOH): 265, 220 nm

HRESIMS: m/z 185.0442 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_8\text{H}_8\text{O}_5$ 185.0438

^1H - and ^{13}C -NMR and HSQC: **Table-4.13**

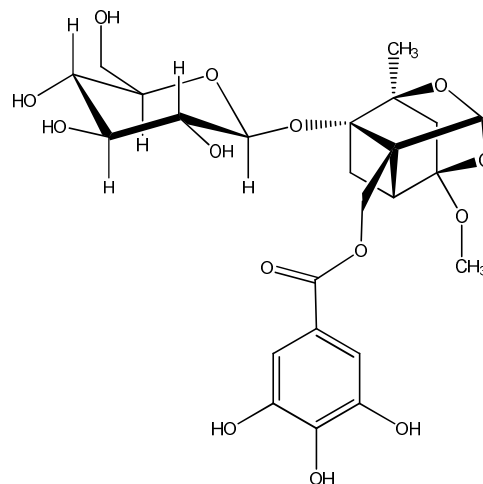
HMBC: **Fig-4.13**



5.5.3. Debenzoylpaeoniflorin; 4-methylether-8-benzoyl-1- β -D-glucopyranoside (4)

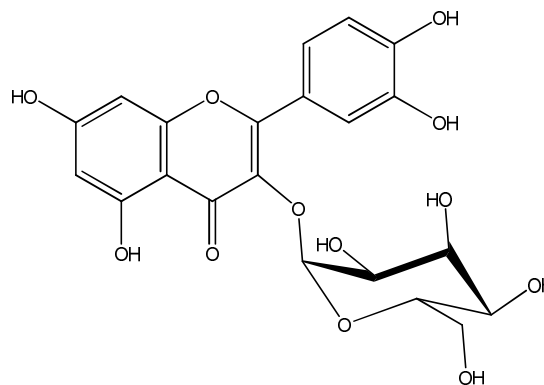
Physical State: Amorphous powder

Yield: 21 mg

IR: 3410, 1710, 1540, 1030 cm^{-1} UV λ_{max} (MeOH): 265, 225 nmHRESIMS: m/z 543.1708 $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{30}\text{O}_{11}$ 543.1706 ^1H - and ^{13}C -NMR and HSQC: **Table-4.14**HMBC: **Fig-4.14****5.5.4. Quercetin-3- β -D-glucopyranoside (10)**

Physical State: Amorphous powder

Yield: 18 mg

IR: 3400, 1665, 1655, 1525, 1030 cm^{-1} UV λ_{max} (MeOH): 360, 255 nmHR-ESI-MS: m/z 465.1024 $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{22}\text{H}_{22}\text{O}_{12}$ 465.1020 ^1H - and ^{13}C -NMR and HSQC: **Table-4.15**HMBC: **Fig-4.15**

5.5.5. Kaempferol-3- β -D-glucopyranoside (astragalin) (11)

Physical State: Yellow crystalline powder

Yield: 20 mg

IR: 3380, 1680, 1640, 1525, 1040 cm^{-1}

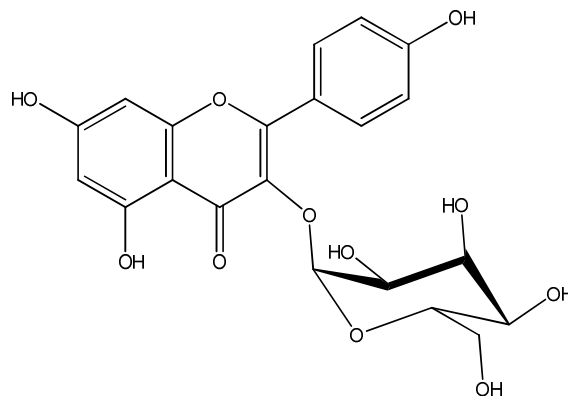
UV λ_{max} (MeOH): 356, 260 nm

HRESIMS: m/z 449.1074 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ 449.1070

^1H - and ^{13}C -NMR and HSQC: **Table-4.16**

HMBC: **Fig-4.16**



5.5.6. 8-Debenzoylpaeoniflorin-1-O- β -D-glucopyranoside (13)

Physical State: Yellow gum

Yield: 20 mg

IR: 3370, 1710, 1540, 1030 cm^{-1}

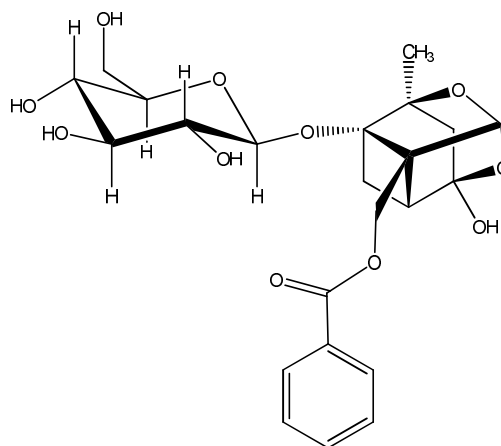
UV λ_{max} (MeOH): 275, 170 nm

HR-ESI-MS: m/z 481.1702 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{23}\text{H}_{28}\text{O}_{11}$ 481.1700

^1H - and ^{13}C -NMR and HSQC: **Table-4.17**

HMBC: **Fig-4.17**



5.5.7. Debenzoyl-8-galloylpaeoniflorin-1-O- β -D-glucopyranoside (14)

Physical State: Amorphous. tan powder

Yield: 20 mg

IR: 3410, 1710, 1540, 1030 cm^{-1}

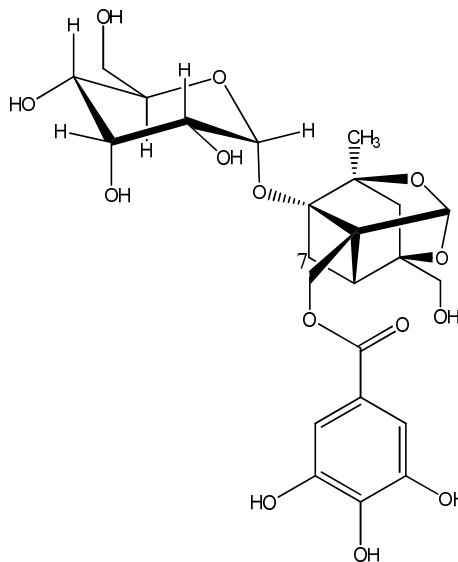
UV λ_{max} (MeOH): 280, 170 nm

HR-ESI-MS: m/z 543.1706 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{23}\text{H}_{28}\text{O}_{14}$ 543.1702

^1H - and ^{13}C -NMR and HSQC: **Table-4.18**

HMBC: **Fig-4.18**



5.6. Biological screening

Various fractions obtained and isolated pure compounds from *P. emodi* were subjected to various biological assays as illustrated below;

5.6.1. DPPH antioxidant assay

1 mL of 0.135 mM DPPH prepared in ethanol was mixed with 1 mL of aqueous extract ranging from 0.2-0.8 mg/mL and pure compound ranging from 20, 40, 60, 80, 100 μ M/mL. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability of the plant extract and pure compounds were calculated using the following equation;

$$\text{DPPH Scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{\text{Abs}_{\text{control}}} \times 100$$

Where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH + methanol, $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample i.e. plant extract.^{135, 136}

The IC_{50} values (50% inhibitory concentration) were obtained from nonlinear regression analysis via Microsoft Excel and GraphPad Prism v. 5.0.

5.6.2. Antibacterial activity

The antibacterial activity was evaluated by the agar well diffusion method in which one loop full of 24 hours old culture containing approximately 10⁴-10⁶ CFU was spread on the surface of Mueller-Hinton Agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solutions of the test samples in the

concentration of 3 mg/mL were prepared in dimethyl sulfoxide (DMSO) and 100 μ L dilutions were added in their respective wells. The antibacterial activity of each extract was compared with standard drug imipenem (positive control) and DMSO (negative control). The antibacterial activity was determined by measuring zones of inhibition usually of each sample wells.¹³⁷

5.6.3. Antifungal activities

The antifungal activity was determined by the Agar tube dilution Method. The crude extract was dissolved in DMSO (24 mg/mL). Sterile Sabouraud's dextrose agar medium (5 mL) was placed in a test tube and inoculated with the sample solution (400 μ g/mL) kept in slanting position at room temperature for overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29 °C. DMSO was used as negative control and Miconazole 70, Miconazole 110.8, Amphotericin 20, Miconazole 98.4 and Miconazole 73 were used as standard inhibitors. The growth inhibition was observed. Percentage growth inhibition was calculated with reference to the negative control by applying the equation.¹³⁸

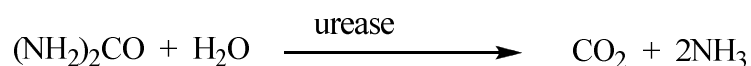
$$\% \text{Inhibition of fungal growth} = 100 - \frac{\text{linear growth in test (mm)}}{\text{linear growth in control (mm)}} \times 100$$

5.4.4. Brine shrimp (*Artemia salina*) lethality bioassay

To measure the cytotoxic potential of the selected plant species, Brine shrimp lethality bioassay was used. Briefly, a tray was half filled with brine solution and about 50 mg of Brine shrimp's eggs were dispersed in the solution and incubated at room temperature. Stock solution was prepared from each test sample by dissolving 20 mg of test sample in acetone and diluted with acetone to make the volume 20 mL which make the concentration of stock solution as 1 mg/mL. From the stock solution, final concentration of 10, 100 and 1000 $\mu\text{g/mL}$ were prepared. It took about two days for the Brine shrimp's eggs to mature as nauplii (larvae). Pasteur pipette was used to place 10 larvae per vial and then raised the volume up to 5 mL with seawater in each vile. Solvent was used as negative control and Etoposide was used as positive control while 1 mL of each test sample was added to rest of the vials. After 24 hours of incubation at 25-27 °C in illumination chamber, the number of survivors were counted and the data was analyzed to calculated LD₅₀ with from GraphPad Prism v. 5.0.¹³⁹

5.6.5. Urease inhibition assay

Urease inhibition potential was estimated by using indophenol method, which is based on the fact that hydrolysis of urea to ammonia and CO₂ is catalyzed by enzyme called urease (urea amidohydrolase EC 3.5.15). Urease catalyzes the conversion of urea to ammonia, which reacts, with a mixture of salicylate, hypochlorite and nitroprusside to yield a blue-green dye (indophenol) with λ_{max} 625 nm. The intensity of the color is proportional to the concentration of ammonia produced by the urea. The whole reaction can be summarized as follow.¹⁴⁰



A solution comprising 25 μL of Jack bean Urease, 55 μL of buffer and 100 mM urea were incubated with 5 μL (0.5 mM conc.) of the test compounds in ethanol at 30 °C for 15 min in well plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 μL , 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 μL , 0.5% w/v sodium hydroxide and 0.1% NaOCl) were added to each well and the increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). The change in absorbance per min was noted and the results processed using SoftMax Pro software (Molecular Device, USA). All the tests were performed in triplicate. The assays were performed at pH 8.2 (0.01 M $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 1.0 mM EDTA and 0.01 M LiCl_2).¹⁴⁰

Thiourea is used as standard inhibitor. The percentage inhibition was calculated from the formula;

$$100 - (\text{OD}_{\text{testwell}} / \text{OD}_{\text{control}}) \times 100$$

5.6.6. Antiplasmodial activity

For antiplasmodial activity the samples were tested in triplicate manner against chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen. Quantitative assessment of antiplasmodial activity *in vitro*

was determined *via* the parasite lactate dehydrogenase assay using a modified method described by makler.^{141, 142}

The samples were prepared to a 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. Chloroquine (CQ) and Artemisinin were used as the reference drugs in all experiments. Compounds were stored at -20°C until use. Test samples were tested at three concentrations which were 10 μ M, 5 μ M and 2.5 μ M. CQ and artemisinin were tested at three concentrations i.e. 50 nM, 25 nM and 12.5 nM. The IC₅₀ values (50% inhibitory concentration) were obtained from nonlinear dose-response curve fitting analysis *via* Microsoft Excel and GraphPad Prism v. 5.0.

PLANT INTRODUCTION (part B)

6.1. Plant introduction**Plant description**

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Saxifragales
Family:	Saxifragaceae
Genus:	<i>Bergenia</i>
Species:	<i>Bergenia ligulata</i>
Binomial name	<i>Bergenia ligulata</i> (wall.) Engl.



Photographs of *B. ligulata* in the field

6.1.1. Saxifragaceae

The family Saxifragaceae is represented by thirty genera and 580 species generally distributed in cold and temperate regions. Three genera and twenty-two species are reported in Pakistan mainly in Swat and Kashmir areas from 2000-3500 m height. The family is recognized by perennial herbs, alternate leaves, without stipules, flowers are hermaphroditic and actinomorphic, five merous, perigynous and fruit capsule.¹⁴³

6.1.2. Genus *Bergenia*

Bergenia is a genus, which has ten flowering plant species, native to central Asia and the Himalayan region. Two of them (*Bergenia ligulata* and *Bergenia ciliata*) are present in Pakistan. They are clamp-forming, rhizomatous evergreen perennial plants with a spirally arranged rosette of leaves (6–35 cm long and 4–15 cm broad), and produce pink flowers in a cyme. The leaves are glossy green in color and large in size, which turn into red or bronze in the fall season. The flowers grow on a stem similar in color to a rhubarb stalks and most varieties have cone-shaped flowers in varying shades of pink.¹⁴⁴

6.1.3. *Bergenia ligulata*

B. ligulata belongs to the genus *Bergenia*. It grows well on shady rocks in Himalayas at high altitude (2000-3500 m) mostly in Swat and Kashmir valleys. In ayurvedic system of medicine, it is known as Paashaanbhed and has been for various ailments from centuries.¹⁴⁴

6.2. Pharmacological importance of the genus *Bergenia*

Literature survey revealed that *B. ligulata* is medicinally important possess excellent therapeutic potentials such as antilithiatic, anticalcification, α -glucosidase inhibiting, antiviral (influenza virus A), antiinflammatory and antibacterial activities. The plant was also found to have antiurolithic effect and the study proved that the crude extract of *B. ligulata* has the potentiality of calcium oxalate monohydrate crystal growth inhibition.^{144, 145}

In 2000, J. Saijyo *et al.*, carried out bioassay guided isolation of α -glucosidase inhibitors from the ethyl acetate fraction of rhizomes of *B. ligulata* and isolated (+)-afzelechin as an active constituent for the aforementioned activity. Furthermore, the structure activity relation study of different derivatives of afzelechin revealed that (+)-afzelechin is the most potent α -glycosidase inhibitor.¹⁴⁶

T. S. Garimella *et al.*, in 2001 proclaimed that *B. ligulata* extract showed significant antilithiatic/anticalcification activity. Subsequently, M. Rajbhandari in 2003 studied inhibitory effect of *B. ligulata* on influenza virus A and reported that methanolic extract inhibited viral RNA synthesis and reduced viral peptide synthesis at 10 μ g/mL.¹⁴⁷

V. S. Joshi *et al.*, in 2005 reported that aqueous extract of *B. ligulata* inhibit oxalate crystal growth.^{148, 149}

6.3. Literature survey of the genus *Bergenia*

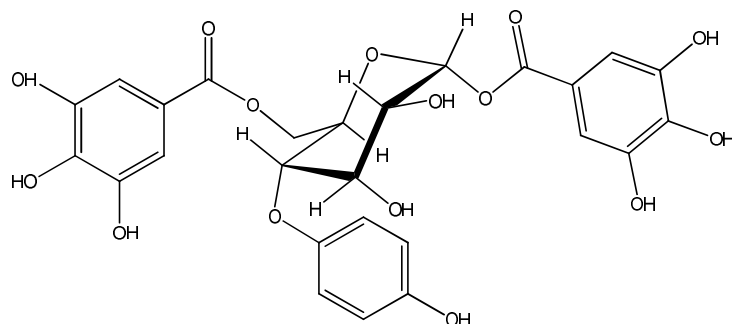
The literature record indicated that genus *Bergenia* has not been thoroughly investigated phytochemically, mainly found to contain bergenin derivatives, arbutin and flavanoids which are summarized below in the table form.

S. No.	Name of Compounds	Molecular Formula	Sources
1.	Arbutin-4,6- <i>bis</i> -O-(3,4,5-trihydroxybenzoyl)	C ₂₆ H ₂₄ O ₁₅	<i>B. purpurascens</i> ¹⁵⁰
2.	Arbutin-6-O-(3,4-dihydroxy benzoyl)	C ₁₉ H ₂₀ O ₁₀	<i>B. ciliata</i> ¹⁵¹
3.	Arbutin-6-O-(4-hydroxybenzoyl)	C ₁₉ H ₂₀ O ₉	<i>B. ciliata</i> ¹⁵¹
4.	Arbutin-6-O-(3,4,5-trihydroxy benzoyl)	C ₁₉ H ₂₀ O ₁₁	<i>B. purpurascens</i> ¹⁵²
5.	Bergenin	C ₁₄ H ₁₆ O ₉	<i>B. corylopsis</i> ¹⁵³
6.	Bergenin-O-demethyl	C ₁₃ H ₁₄ O ₉	<i>B. scopulosa</i> ¹⁵³
7.	Bergenin-11-O-(3,4-dihydroxy benzoyl)	C ₂₁ H ₂₀ O ₁₂	<i>B. ciliata</i> ¹⁵⁴
8.	Bergenin-11-O-(4-hydroxybenzoyl)	C ₂₁ H ₂₀ O ₁₁	<i>B. ciliata</i> ¹⁵⁴
9.	Bergenin-11-O-(3,4,5-trihydroxy benzoyl)	C ₂₁ H ₂₀ O ₁₃	<i>B. purpurascens</i> ¹⁵⁵
10.	Bergenin-4-O-(3,4,5-trihydroxy benzoyl)	C ₂₁ H ₂₀ O ₁₃	<i>B. purpurascens</i> ¹⁵⁵
11.	3,6-Digalloylglucose	C ₂₀ H ₂₀ O ₁₄	<i>B. scopulosa</i> ¹⁵⁶

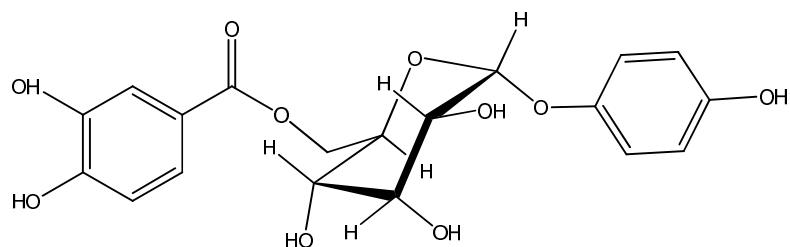
12.	3,3',4',5,7-Pentahydroxyflavan-3-O-(3,4,5-trihydroxybenzoyl)	C ₂₂ H ₁₈ O ₁₀	<i>B. crassifolia</i> ¹⁵⁷
13.	3,3',4',5,7-Pentahydroxyflavan-7-O-(3,4,5-Trihydroxybenzoyl)	C ₂₂ H ₁₈ O ₁₀	<i>B. purpurascen</i> ¹⁵⁷
14.	Quercetin-3-O-[α -L-rhamnopyranosyl-(1-2)- β -D-galactopyranosyl-(1-6)- β -D-glucopyranoside]	C ₃₃ H ₄₀ O ₂₁	<i>B. himalaica</i> ¹⁵⁸
15.	1,2,4,6-Tetragalloylglucose	C ₃₄ H ₂₈ O ₂₂	<i>B. crassifolia</i> ¹⁵⁹
16.	3,4,5,6-Tetrahydro-4-hydroxy-6-methyl-2H-pyran-2-one-O-[4-hydroxybenzoyl-(6)- β -D-glucopyranoside]	C ₁₉ H ₂₄ O ₁₀	<i>B. ciliata</i> ¹⁶⁰
17.	3,4,5,6-Tetrahydro-4-hydroxy-6-methyl-2H-pyran-2-one-O-(4- β -D-glucopyranosyloxybenzoyl)	C ₁₉ H ₂₄ O ₁₀	<i>B. ligulata</i> ¹⁶⁰
18.	2,4,6-Trigalloylglucose	C ₂₇ H ₂₄ O ₁₈	<i>B. purpurascens</i> ¹⁶¹

Bergenin = (2R,3S,4S,4aR,10bS)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3, 4,4a,10b-tetrahydro-2H-pyrano[3,2-c]isochromen-6-one.

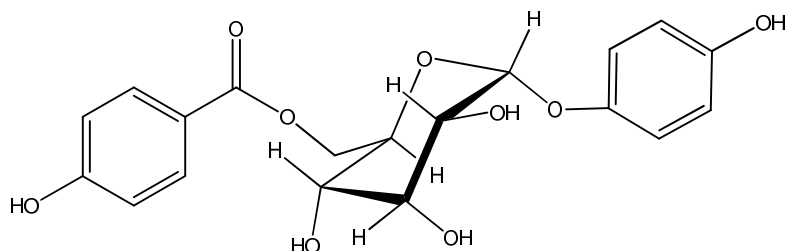
Arbutin = (2R,3S,4S,5R,6S)-2-Hydroxymethyl-6- (4-hydroxyphenoxy)oxane-3,4,5-triol

6.4. Structure of selected compounds reported from the genus *Bergenia*

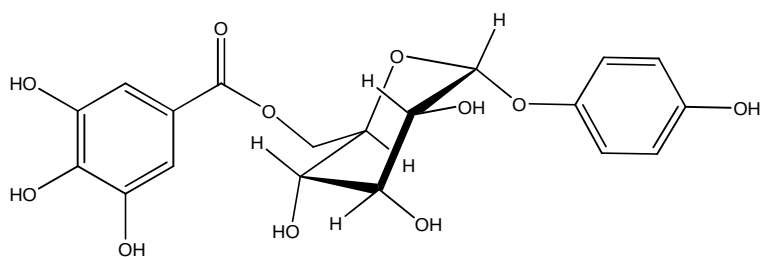
Arbutin-4,6-bis-O-(3,4,5-trihydroxybenzoyl)¹⁵⁰



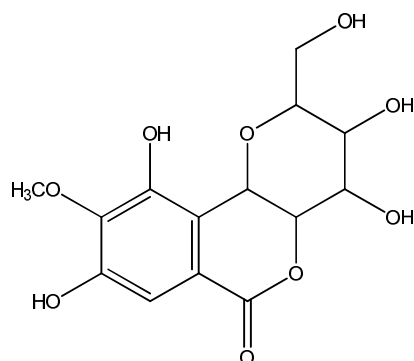
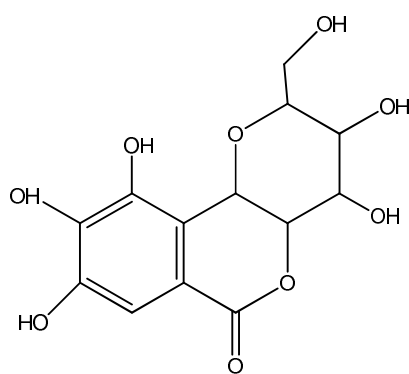
Arbutin-6-O-(3,4-dihydroxybenzoyl)¹⁵¹

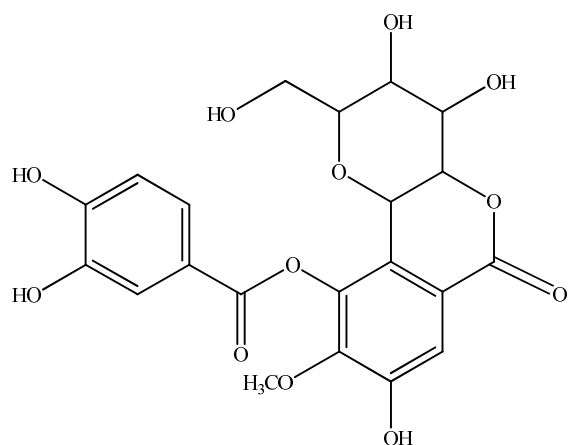
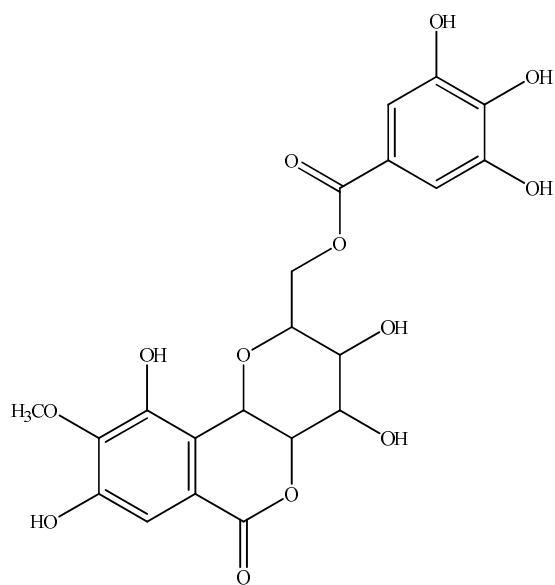


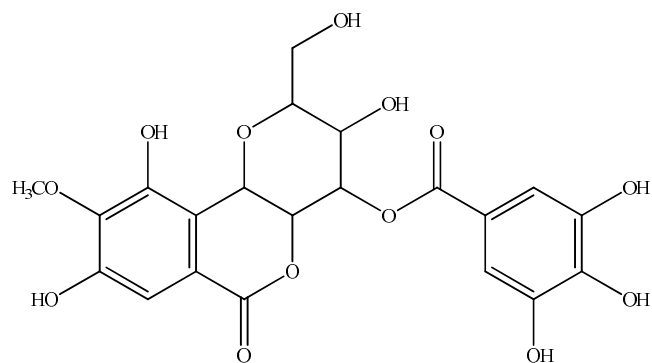
Arbutin-6-O-(4-hydroxybenzoyl)¹⁵¹



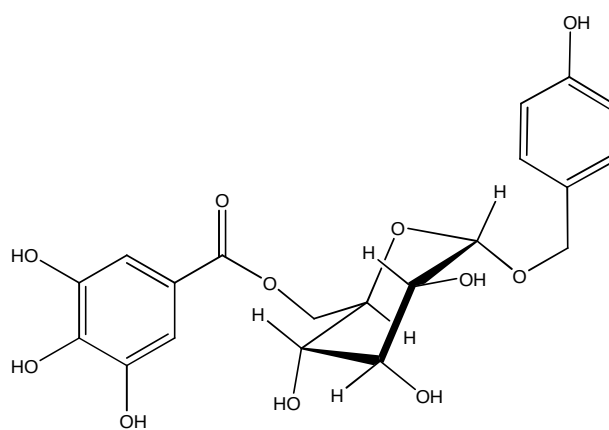
Arbutin-6-O-(3,4,5-trihydroxybenzoyl)¹⁵²

Bergenin¹⁵³Demethylbergenin¹⁵³

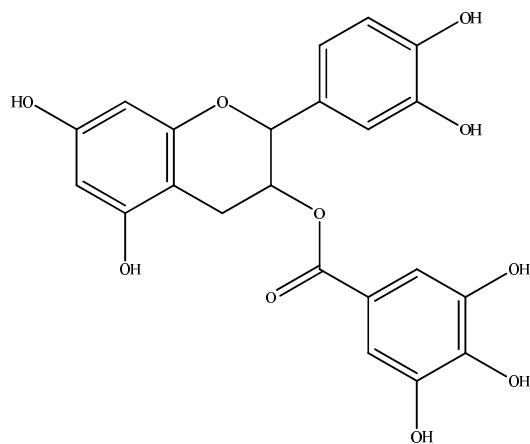
Bergenin-11-O-(3,4-dihydroxybenzoyl)¹⁵⁴Bergenin-11-O-(3,4,5-trihydroxybenzoyl)¹⁵⁵



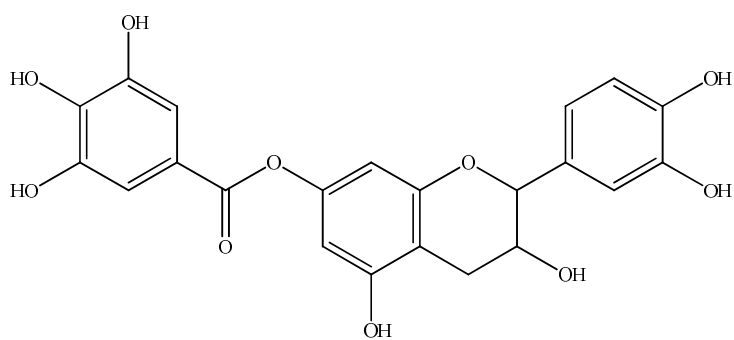
Bergenin-4-O-(3,4,5-trihydroxybenzoyl)¹⁵⁵



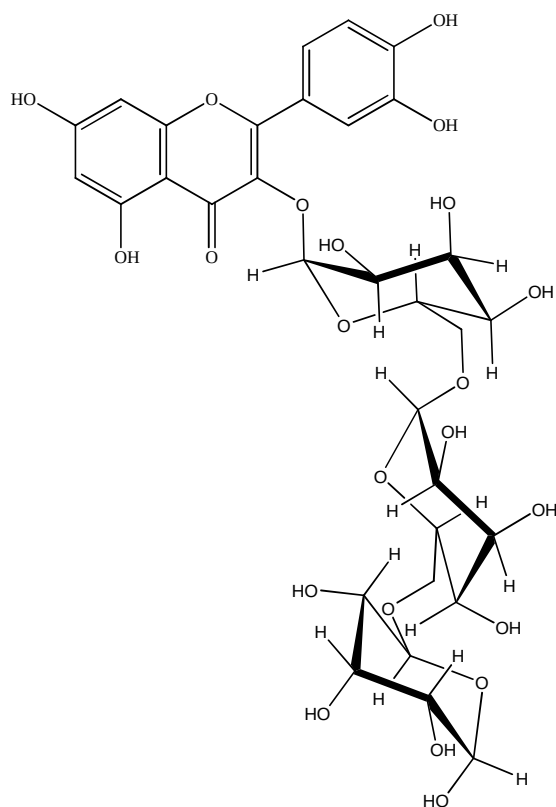
3,6-Digalloylglucose¹⁵⁶



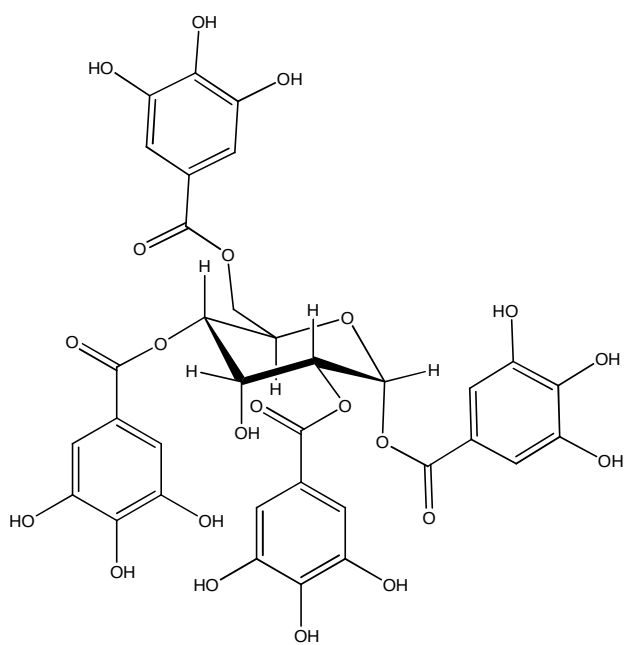
3-O-galloylcatechine



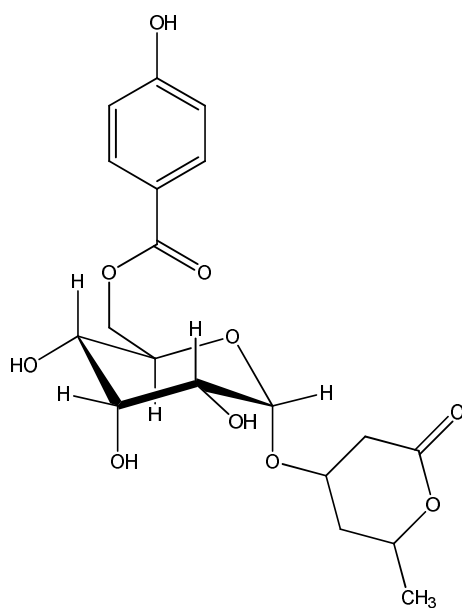
7-O-Galloylcatechine



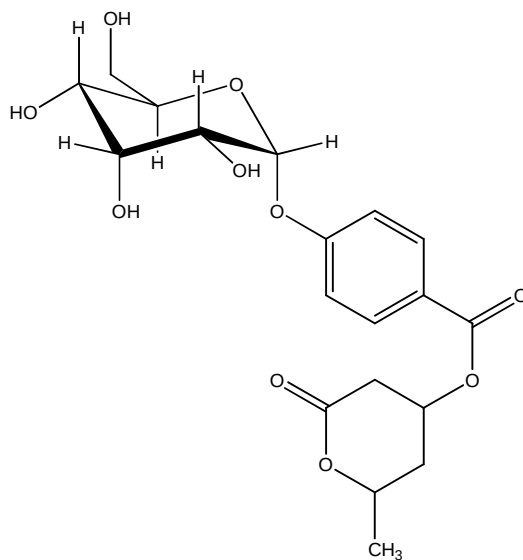
Quercetin-3-O- $[\alpha$ -L-rhamnopyranosyl-(1-2)- β -D-galactopyranosyl-(1-6)- β -D-glucopyranoside]¹⁵⁸



1,2,4,6-1,2,4-Tetragalloylglucose¹⁶¹



3,4,5,6-Tetrahydro-4-hydroxy-6-methyl-2H-pyran-2-one-4-O-[4-hydroxybenzoyl-6-O- β -D-glucopyranoside]¹⁶⁰



3,4,5,6-Tetrahydro-4-hydroxy-6-methyl-2H-pyran-2-one-4-O-(4- β -D-glucopyranosyloxybenzoyl)¹⁶⁰

RESULTS AND DISCUSSION (part B)

Shade dried plant materials were extracted with ethanol (x3, 50 L) and subjected to solvent-solvent separation resulted in the resolution of complex mixture into nonpolar (*n*-hexane) and polar fractions (chloroform and ethyl acetate). Ethyl acetate fraction was subjected to various chromatographic techniques such as silica gel CC, size exclusion CC and reverse phase CC, which resulted in isolation of two new compounds and nine known constituents out of which five are hitherto unreported from this specie. Modern spectroscopic techniques such as ¹H-NMR, ¹³C-NMR (BB and DEPT), 2D NMR, ESI and ESI-HR-MS were utilized to elucidate the structures of isolated secondary metabolites.

7.1. New Constituents from *B. ligulata*

- 4'-Methoxygalangin-3'-*O*- β -D-erythrofuranoside (**22**)
- 4'-Methoxycatechin-3'-galloyl-2'',4''-bis-*O*-(3,4,5-trihydroxybenzoyl)-3-*O*- β -D-glucopyranoside (**29**)

7.2. Hitherto unreported constituents from *B. ligulata*

- 11-*O*-*para*-Hydroxybenzoylbergenin (**20**)
- Meciadanol (**21**)
- 11-*O*-Galloylbergenin (**25**)
- 3-Galloylcatechine (**26**)
- 3,7-Digalloylcatechin (**28**)

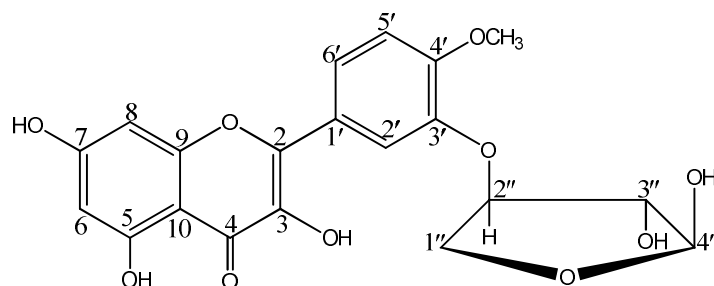
7.3. Known constituents from *B. ligulata*

- Bergenin (**19**)
- 6-*O*-Galloylarbutin (**23**)
- Catechine (**24**)
- Arbutin (**27**)

7.4. Structure elucidation of new constituents

7.4.1. 4'-Methoxygalangin-3'-O- β -D-erythrofuranoside (22)

Compound **22** was isolated as yellowish granules and its molecular formula was established as $C_{20}H_{18}O_{10}$ by HR-ESI-MS $[M+H]^+$ at m/z 419.0973 a.m.u. (calcd. 419.0968). The double bond equivalent (DBE) calculation pertains to 12 degree of unsaturation. The UV absorption bands indicated the presence of benzene ring while IR spectrum showed characteristic peaks at 3400 for OH groups, 1655 and 1525 for benzene ring and 1030 cm^{-1} for glycoside linkage.



22

The ^1H - and ^{13}C -NMR spectra of **22** was compared with galangin suggested that compound (**22**) contains galangin nucleus.¹⁶²

The NMR spectra indicated two *meta* coupled AB doublet at δ_{H} 6.19 ($J = 2.4\text{ Hz}$) and δ_{H} 6.39 ($J=2.4\text{ Hz}$), assigned to H-6 and H-8 protons respectively while the presence of nine carbons at δ_{C} 158, 135, 178, 160, 100, 163, 94.8, 157, 103 corresponding to carbons 2-10 respectively, which are the characteristic features of chromane ring. The ^1H -NMR also displayed one proton doublet resonated at δ_{H} 7.92 ($J = 1.8$, H-2') due to the presence of a *meta* proton (H-6') in vicinity of H-2' and H-6' proton appeared as double doublet at δ_{H}

7.58 ($J = 8.4, 1.8$ Hz) due to the presence of a *ortho* proton (H-5') and *meta* proton (H-2'). H-5' appeared as a doublet at δ_H 6.89 ($J = 8.4$ Hz) which confirm the assignments of chromane nucleus. A methoxy protons singlet resonated at δ_H 3.93 and confirmed by ESI spectrum displayed mass fragment at m/z 389.0868 corresponding to $M^+ - OCH_3$.

Analysis of ^{13}C -NMR (BB & DEPT) suggested that the sugar moiety has four carbons i.e. one methylene and three methines resonating at δ_c 62.5, 79, 78, 104 indicating the presence of furanose sugar which was supported by the mass fragment at m/z 301.0702. The ^1H -NMR spectrum also indicated furanose displayed two AA' doublet doublets at δ_H 3.72 (1H, dd, $J = 7.8, 5.4$ Hz, H-1''a) and δ_H 3.55 (1H, dd, $J = 7.8, 5.4$ Hz, H-1''b) corresponds to methylene group attached to a chiral centre (C-2''). An anomeric proton resonated at δ_H 5.41 (1H, d, $J = 7.2$, H-4') while two methine protons appeared at δ_H 3.23 (1H, m, H-2') and δ_H 3.44 (1H, t, $J = 7.2$, H-3'). The detailed analysis of the positions and coupling constant of the protons of the sugar moiety indicated that it is β -D-erythrofuranoise.

The ^1H - ^1H COSY showed coupling of H-6 with H-8, H-5' with H-6' and H-2' with H-6'. In the furanose, CH₂ protons showed coupling with each other and also with H-2'' protons, H-2'' proton also showed coupling with H-3'' proton. The anomeric proton showed coupling with H-3''. The HMBC (**Fig-7.1**) experiment showed correlation of H-2'' protons with C-3' which suggested the attachment of furanose moiety with C-3'.

In the light of spectral data, the structure of **22** elucidated as 4'-methoxygalangin-3'-O- β -D-erythrofuranoside.

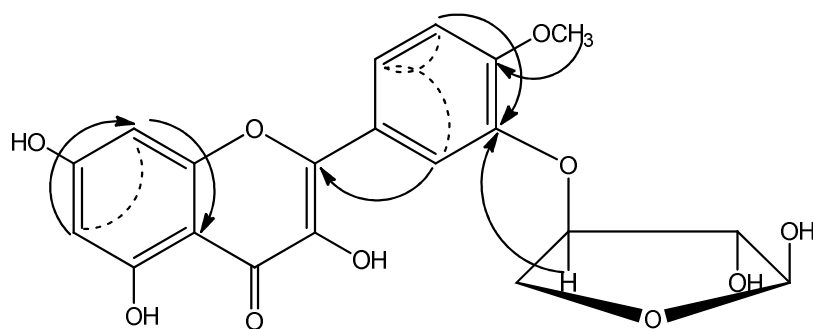


Fig-7.1. Selected key COSY (H---H) and HMBC (H—C) interactions of **22**

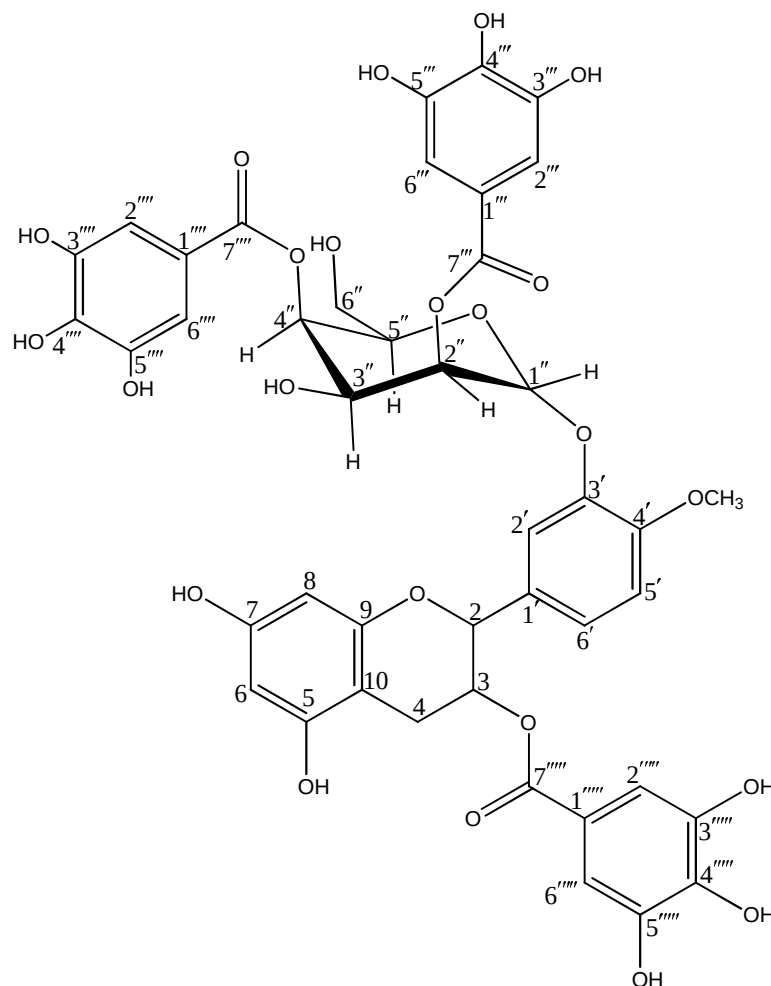
Table-7.1. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **22** in CD_3OD

C/H. No.	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	158	C	-
3	135	C	-
4	178	C	-
5	160	C	-
6	100	CH	6.19, d ($J = 2.4$)
7	163	C	-
8	94.8	CH	6.39, d ($J = 2.4$)
9	157	C	-
10	103	C	-
OCH_3	51.2	CH_3	3.93, s
1'	123	C	-
2'	114.3	CH	7.92, d ($J = 1.8$)
3'	146	C	-
4'	148	C	-
5'	116	CH	6.89, d ($J = 8.4$)
6'	123.7	CH	7.58, dd ($J = 8.4, 1.8$)
1''	62.5	CH_2	3.55 dd ($J = 7.8, 5.4$) 3.72, dd ($J = 7.8, 5.4$)
2''	79	CH	3.23, m
3''	78	CH	3.44, t ($J=6$)
4''	104	CH	5.41, d ($J=7.2$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.4.2. 4'-Methoxycatechin-3'-galloyl-2'',4''-bis-O-(3,4,5-trihydroxybenzoyl)-3-O- β -D-glucopyranoside (29)

Compound **29** was isolated as dark brown amorphous powder and its molecular formula was obtained as $C_{42}H_{36}O_{23}$ from HR-ESI-MS spectrum displayed molecular ion peak at m/z 909.1721 a.m.u. $[M+H]^+$ (calcd. 909.1716 a.m.u.). The IR spectrum exhibited characteristic peaks at 3420 (OH), 1700, 1690 (ester function groups), 1650 for aromatic ring (double bond), 1525 (aromatic C-H) and at 1030 cm^{-1} (glycoside linkage). The UV spectrum indicated the presence of an ester group along with the aromatic rings in the molecule.

**29**

The ^1H - and ^{13}C -NMR spectra (**Table-7.2**) are consistent with the presence of catechine nucleus compared with the NMR spectra of catechine.¹⁶³

The ^1H -NMR displayed signals for two AA'/B double doublets at δ_{H} 2.49 (1H, dd, $J = 16$, 5.4 Hz, H-4a) and δ_{H} 2.82 (1H, dd, $J = 16$, 5.4 Hz, H-4b) corresponding to methylene appeared at δ_{C} 28.5 in the ^{13}C -NMR spectrum. One proton multiplet resonated at δ_{H} 3.97 assigned to H-3 while H-2 is displayed as doublet at δ_{H} 4.56 ($J = 7.8$ Hz). Two *meta* coupled AB protons exhibited at δ_{H} 5.86 (1H, d, $J = 2$ Hz, H-6) and δ_{H} 5.92 (1H, d, $J = 2$ Hz, H-8). The ^1H -NMR further indicated signals at δ_{H} 6.71 (1H, dd, $J = 8$, 2.5 Hz, H-6'), 6.75 (1H, d, $J = 8$, H-5') and 6.83 (1H, d, $J = 2.5$ Hz, H-2') for *meta-para* tri-substituted benzene ring. In addition, three protons singlet was observed at δ_{H} 3.90, corresponding to chemical shift appeared at δ_{C} 51 confirmed the presence of OCH_3 group in the molecule. The partial structure was established as 2-phenyl chroman, which was also confirmed by the presence of mass fragment, appeared at m/z 291 a.m.u. in the ESI spectrum.

The ^{13}C -NMR is also with an agreement for the presence of a chroman ring, observed methylene signal at δ_{C} 28.5 (C-4), two methines signals at 82.8 (C-2) and 80.6 (C-3) along with aromatic methines and four quaternary carbons at δ_{C} 96.2 (C-6), 95.4 (C-8), 154.3 (C-5), 155.2 (C-7), 156.1 (C-9) and δ_{C} 98.9 (C10) respectively.

The presence of sugar moiety is evident from the ^1H - and ^{13}C -NMR spectra (**Table-7.2**) displayed signals for glucose protons at δ_{H} 5.41 (1H, d, $J = 7.6$ Hz, H-1''), 4.08 (1H, t, $J=7.6$ Hz, H-2''), 4.33 (1H, t, $J = 7.6$ Hz, H-3''), 5.79 (1H, t, $J = 7.6$ Hz, H-4''), 3.99 (1H, m, H-5''), 4.10 (1H, dd, $J=7.6$, 1.5 Hz, H-6a'') and δ_{H} 4.21 (1H, dd, $J = 7.6$, 1.5 Hz, H-6b'') which was confirmed by ^{13}C -NMR chemical shifts (**Table-7.2**).

The ^1H - and ^{13}C -NMR also indicated the presence of three 3,4,5-trihydroxybenzoyl moieties, observed three singlets at δ_{H} 7.88 (H-2''', H-6'''), 7.61 (H-2''', H-6''') and δ_{H} 7.42 is due to two aromatic protons of the trihydroxybenzoyl unit attached to catechin nucleus which was supported by ^{13}C -NMR displayed chemical shifts at (C-2''' and C-6''') at δ_{C} 110.4 while C-2'''' and C-6'''' displayed signal at δ_{C} 109.4. The C-2''''' and C-6''''' resonated at 108.3 along with the presence of three carbonyl groups exhibited signals at δ_{C} 168.7 (C-7'''), δ_{C} 168.4 (C-7''') and δ_{C} 168.1 (C-7''') in ^{13}C -NMR spectrum. The mass fragment at m/z 169.0 in the ESI spectrum also suggested the presence of galloly moieties in the molecule.

The relative position of carbons with respect to each other was confirmed by the HMBC and ^1H - ^1H COSY correlation (**Fig-7.2**). The anomeric proton showed HMBC correlation with C-3' confirming the attachment of sugar at this position. Furthermore, H-2'' showed HMBC correlation with a carbonyl carbon (δ_{C} 168.7, C-7''') to which H-6''' and H-2''' are also correlated validated the attachment of one galloly group at C-2''. In the similar fashion, H-4'' showed correlation with a carbonyl carbon (C-7''') appeared at δ_{C} 168.7 to which H-2'''' and H-6'''' showed correlation in HMBC experiment accredited the attachment of the second galloly group at C-4'' of the sugar moiety, while the attachment of the third galloyl group is affirmed by HMBC experiment at C-3 as H-3 showed correlation with C-7''''' resonated at δ_{C} 168.1 to which H-6''''' and H-2''''' are also correlated. The methoxy protons showed HMBC correlation with C-4' suggesting the attachment of OCH_3 at C-4'.

The ^1H - ^1H COSY spectrum analysis indicated the coupling of H-6/H-8, H-5'/H-6' and also H-2'/H-6' which can be observed in the structure of **29**.

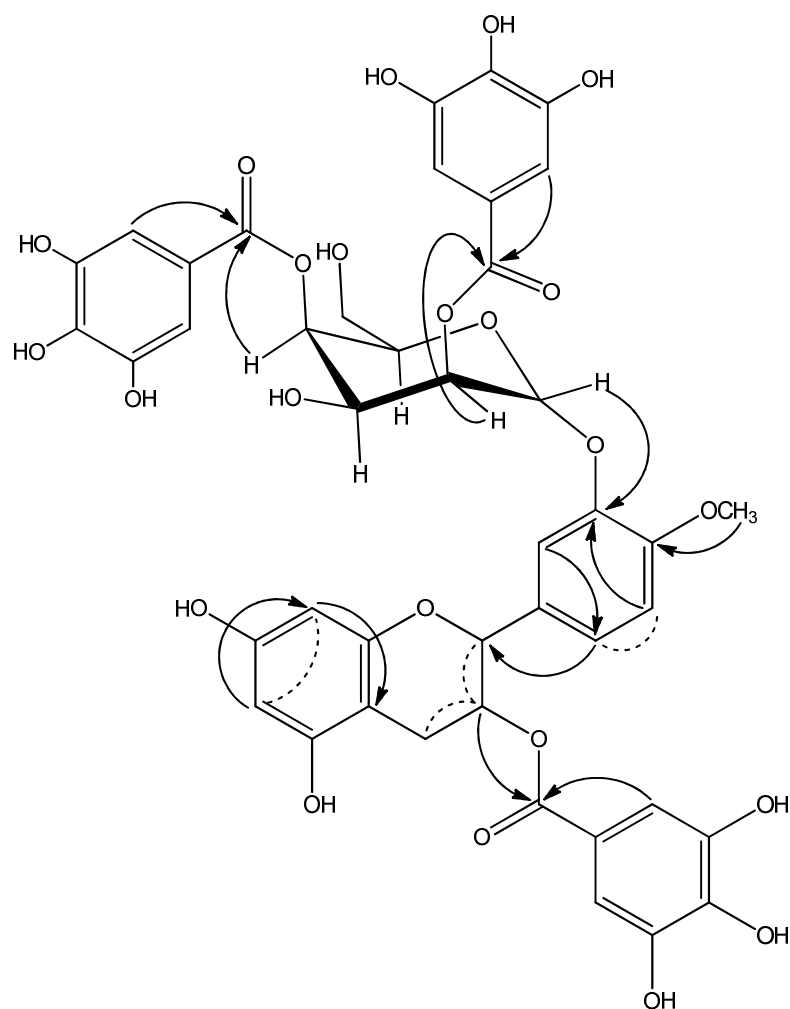


Fig-7.2. Selected key COSY (H---H) and HMBC (H—C) interactions of **29**

Table-7.2. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **29** in CD_3OD

C. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) Coupling Constants J_{HH} (Hz) ^{cd}
2	82.8	CH	4.56, d ($J=7.8$)
3	80.6	CH	3.97, m
4	28.5	CH_2	2.49, dd ($J=16.2, 8.4$) 2.82, dd ($J=16.2, 5.4$)
5	154.3	C	-
6	96.2	CH	5.86, d ($J = 2$)
7	155.2	C	-
8	95.4	CH	5.92, d ($J = 2$)
9	156.1	C	-
10	98.9	C	-
OCH_3	51	CH_3	3.90, s
1'	127.2	C	-
2'	115.3	CH	6.83, d ($J = 2.5$)
3'	144.8	C	-
4'	148.7	C	-
5'	120.3	CH	6.75, d ($J=8$)
6'	116	CH	6.71, dd ($J=8, 2.5$)
1''	102.3	CH	5.41, d ($J=7.6$)
2''	75	CH	4.08, t ($J=7.6$)
3''	77.9	CH	4.33, t ($J=7.6$)
4''	72.2	CH	5.79, t ($J=7.6$)
5''	78.1	CH	3.99, m
6''	62.9	CH_2	4.10, dd ($J=7.6, 1.5$) 4.21, dd ($J=7.6, 1.5$)
1'''	130.7	C	-
2''', 6'''	110.4	CH	7.88, s

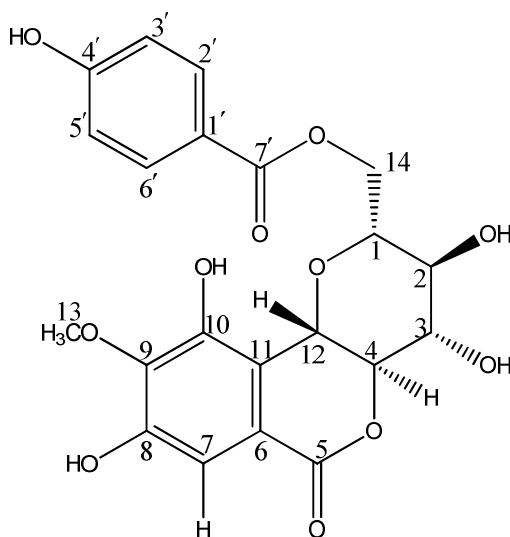
Chapter 7 (Part B)			Results & Discussion
3''', 5'''	147.3	C	-
4'''	141.3	C	-
7'''	168.7	C	-
2''', 6'''	109.4	CH	7.61, s
3''', 5'''	146.7	C	-
4'''	141	C	-
7'''	168.4	C	-
1'''	129.7	C	-
2''', 6'''	108.3	CH	7.42, s
3''', 5'''	146.1	C	-
4'''	140	C	-
7'''	168.1	C	-
a. Broad Band; b. DEPT; c. ¹ H-NMR; d. HSQC interactions			

7.5. Hitherto Unreported Constituents from *B. ligulata*

7.5.1. 11-*O*-*p*-Hydroxybenzoylbergenin (20)

Compound **20** was isolated as white granules from the EtOAc fraction of *B. ligulata*. The ESI spectrum showed molecular ion peak at m/z 449.1079 a.m.u. $[M+H]^+$ corresponding to molecular formula $C_{21}H_{20}O_{11}$. The IR spectrum displayed for at 3400, 1700 1685 and 1530 cm^{-1} corresponding to hydroxyl group, carbonyl carbon and benzene ring. The UV spectrum is also consistent with presence of benzene ring in the molecule (λ_{max} 380 nm)

Comparison of ^1H - and ^{13}C -NMR (**Table-7.3**) of **20** with reported compound 11-*O*-parahydroxybenzoylbergenin from the literature revealed that compound **20** is 11-*O*-parahydroxybenzoylbergenin, which was unreported from this specie.¹⁶⁴



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Table-7.3. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **20** in CD_3OD

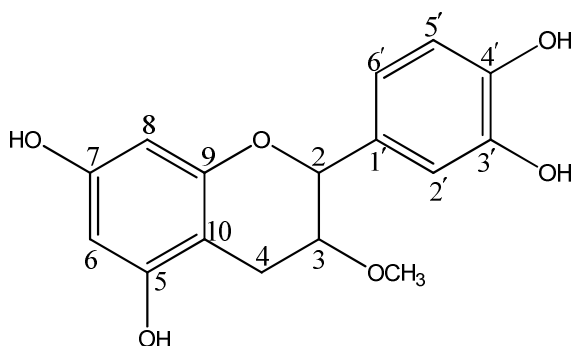
C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	80.6	CH	3.96, m
2	71.9	CH	3.52, t ($J = 9$)
3	75.4	CH	3.84, t ($J = 9$)
4	81.2	CH	4.10, t ($J = 9$)
5	167.8	C	-
6	152.4	C	-
7	111.1	CH	-
8	163.8	C	-
9	142.2	C	-
10	165.6	C	-
11	149.3	C	-
12	74.3	CH	5.03, d ($J = 10$)
13	60.8	CH_3	3.88, s
14	64.7	CH_2	4.36, dd ($J = 7.5, 5$)
			4.89 dd ($J = 10, 2$)
1'	130.7	C	-
2', 6'	132.2	CH	8.04, d ($J=8.5$)
3', 5'	116	CH	6.87, d ($J=8.5$)
4'	150.3	C	-
7'	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.5.2. Meciadanol (21)

Compound **21** was isolated as white needles and its molecular formula was established as $C_{16}H_{16}O_6$ from ESI spectrum showing molecular ion peak at m/z 305.1020 a.m.u.

$[M+H]^+$, calcd. 305.1016. The IR spectrum indicated the presence of benzene ring (1530 cm^{-1}) and hydroxyl groups (3420 cm^{-1}). The UV spectrum displayed λ_{max} at 360 nm corresponding to benzene ring.



21

The ^1H - and ^{13}C -NMR spectra (**Table-7.4**) of **21** were compared with reported compound mecidanol (catechine derivatives) from the literature which was found identical.¹⁶⁵

Table-7.4. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **21** in CD_3OD

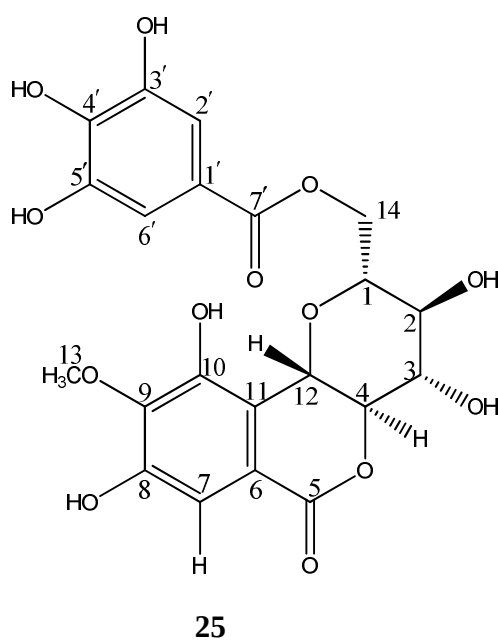
C. No.	^{13}C -NMR (δ_{C}) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ_{H}) coupling constants J_{HH} (Hz) ^{cd}
2	82.8	CH	4.14, d ($J=7.2$)
3	80.6	CH	4.13, m
4	28.5	CH_2	2.49, dd ($J=16.2, 5$) 2.75, dd ($J=16.2, 5$)
5	154.2	C	-
6	96.2	CH	5.91, d ($J = 2.4$)
7	154.8	C	-
8	95.4	CH	5.83, d ($J = 2.4$)
9	156.4	C	-
10	99	C	-
1'	126.7	C	-
2'	115.3	CH	6.79, d ($J = 1.5$)
3'	145.6	C	-
4'	149.4	C	-
5'	120.3	CH	6.75, d ($J = 8.4$)
6'	116	CH	6.71, dd ($J = 8.4, 1.5$)
OCH_3	58.1	CH_3	3.81, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.5.3. 11-O-Galloylbergenin (25)

Compound **25** was isolated as transparent needles. Molecular formula was established as $C_{21}H_{20}O_{13}$ from ESI-HR-MS showing molecular ion peak at m/z 481.0977 a.m.u. $[M+H]^+$, calcd. 481.0972 a.m.u. The IR spectrum indicated the presence of hydroxyl group (3410 cm^{-1}), ester function (1690 cm^{-1}) and benzene ring (1520 cm^{-1}). The UV spectrum is also in agreement with the presence of benzene ring (λ_{max} 380).

The ^1H - and ^{13}C -NMR spectra (**Table-7.5**) of **25** are identical with the reported compound 11-O-galloylbergenin^{166, 167}, which was further confirmed by single crystal x-ray (**Fig-7.3**).



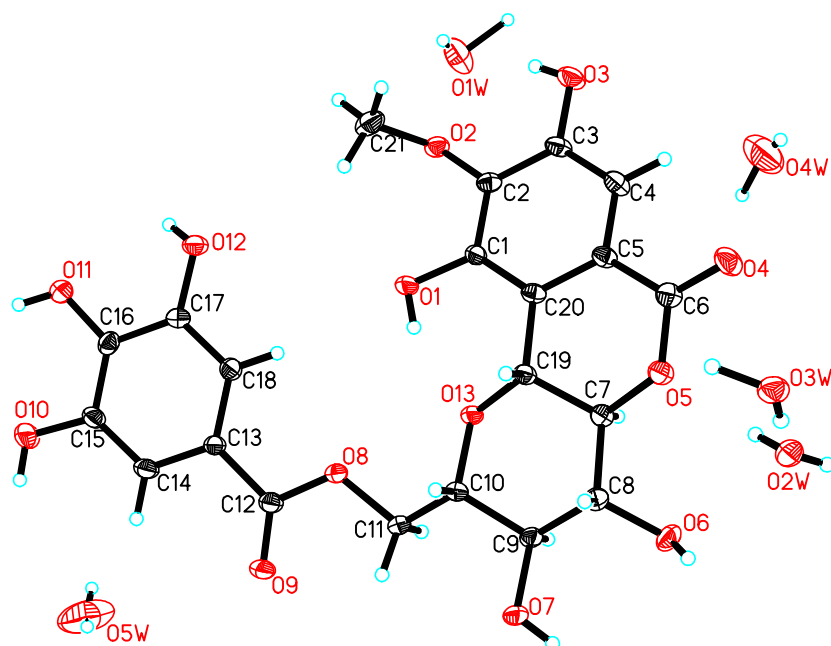


Fig-7.3. Single crystal x-ray photograph of 11-O-galloylbergenin pentahydrate

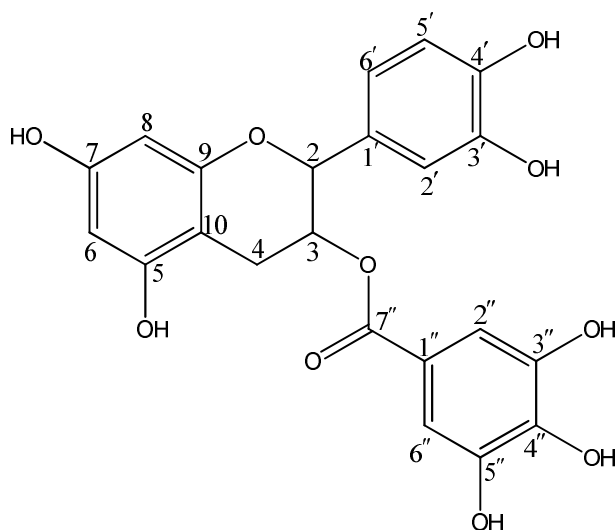
Table-7.5. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **25** in CD_3OD

C. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	81.2	CH	3.68, m
2	80.1	CH	3.71, t ($J = 8.5$)
3	75.8	CH	4.17, t ($J = 8.5$)
4	79.9	CH	4.97, t ($J = 8.5$)
5	167.4	C	-
6	152.1	C	-
7	111	CH	-
8	164	C	-
9	142.2	C	-
10	165.6	C	-
11	149.6	C	-
12	74	CH	4.96, d ($J = 10$)
13	59.9	CH_3	3.91, s
14	64.3	CH_2	4.48, dd ($J = 10, 5$) 4.68, dd ($J = 10, 2$)
1'	130.7	C	-
2', 6'	110.4	CH	7.63, s
3', 5'	147.3	C	-
4'	141.3	C	-
7'	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.5.4. 3-Galloylcatechine (26)

Compound **26** was isolated as amorphous powder. Its molecular formula was established by ESI-MS showing molecular ion peak at m/z 443.0973 a.m.u. $[M+H]^+$, calcd. 443.0968 a.m.u. corresponding to $C_{22}H_{18}O_{10}$. The IR spectrum displayed bends at 3410, 1675 and 1520 cm^{-1} corresponding to hydroxyl groups, ester group and benzene ring. The UV spectrum also indicated the presence of benzene ring (λ_{max} 365 nm).



26

Comparative study of ^1H - and ^{13}C -NMR spectra (**Table-7.6**) of compound **26** and 3-galloylcatechine suggested that compound **26** is actually 3-galloylcatechine.¹⁶⁸

Table-7.6. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **26** in CD_3OD

C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	83.1	CH	4.61, d ($J=7.2$)
3	80.4	CH	4.17, m
4	28.2	CH_2	2.61, dd ($J=16.2, 8.4$) 2.94, dd ($J=16.2, 5.4$)
5	153.8	C	-
6	96.2	CH	6.13, d ($J = 2.4$)
7	155.4	C	-
8	95.4	CH	5.79, d ($J = 2.4$)
9	155.7	C	-
10	98.2	C	-
1'	127	C	-
2'	115.3	CH	6.68, d ($J = 1.5$)
3'	145.6	C	-
4'	149.4	C	-
5'	119.2	CH	6.82, d ($J = 8.5$)
6'	115.8	CH	6.75, dd ($J = 8.5, 1.5$)
1''	130.7	C	-
2'', 6''	108.2	CH	7.20, s
3'', 5''	146	C	-
4''	140.3	C	-
7''	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.5.5. 3,7-Digalloylcatechin (28)

Compound **28** was isolated as amorphous powder and its molecular formula $C_{15}H_{14}O_6$ was determined from ESI mass spectrum showing molecular ion peak at m/z 595.1083 a.m.u. $[M+H]^+$ (calcd. 595.1078 a.m.u.). The IR spectrum indicated the presence of hydroxyl groups (3400 cm^{-1}), couple of carbonyl carbons ($1680, 1710\text{ cm}^{-1}$) and benzene ring (1520 cm^{-1}). The UV spectrum also indicated the presence of benzene ring (λ_{max} 370).

The spectral data of **28** (Table-7.7) was compared with the reported compound 3,7-bis(3,4,5-trihydroxybenzoyl) catechine confirmed the structure of **28** as 3,7-bis(3,4,5-trihydroxybenzoyl) catechine.^{169, 170}

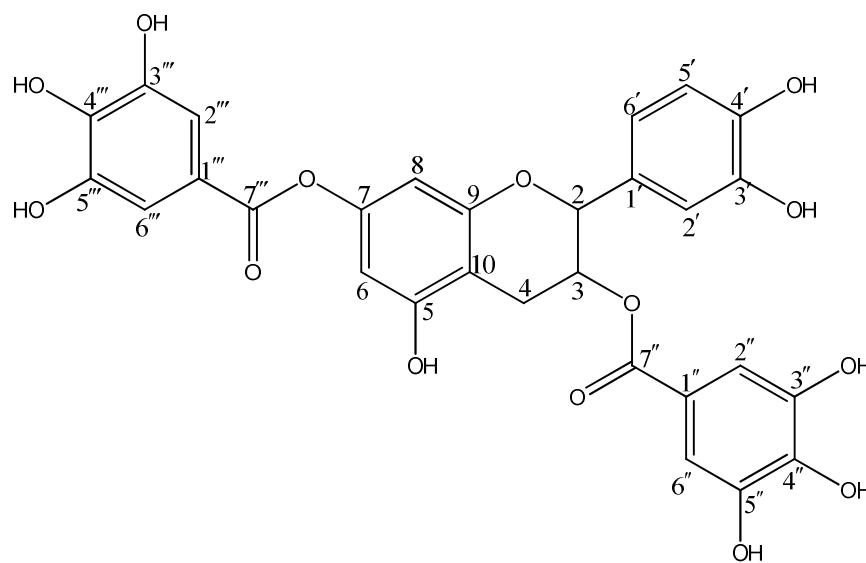


Table-7.7. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **28** in CD_3OD

C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	82.5	CH	4.71, d ($J=7$)
3	80.3	CH	3.84, m
4	28.1	CH_2	2.38, dd ($J=14.5, 2.5$) 3.15, dd ($J=14.5, 2.5$)
5	152.9	C	-
6	95.8	CH	5.86, d ($J = 2.4$)
7	155.1	C	-
8	95.4	CH	5.81,d ($J = 2.4$)
9	165.2	C	-
10	100.2	C	-
1'	127.1	C	-
2'	115.4	CH	6.71, d ($J = 2$)
3'	144.9	C	-
4'	149.2	C	-
5'	119.9	CH	6.79, d ($J = 8.2$)
6'	116.2	CH	6.52, dd ($J = 8.2, 2$)
1''	130.7	C	-
2'', 6''	108.2	CH	7.20, s
3'', 5''	146	C	-
4''	140.3	C	-
7''	168.4	C	-
1'''	130.7	C	-
2''', 6'''	110.4	CH	7.95, s
3''', 5'''	147.3	C	-
4'''	141.3	C	-
7'''	168.4		-

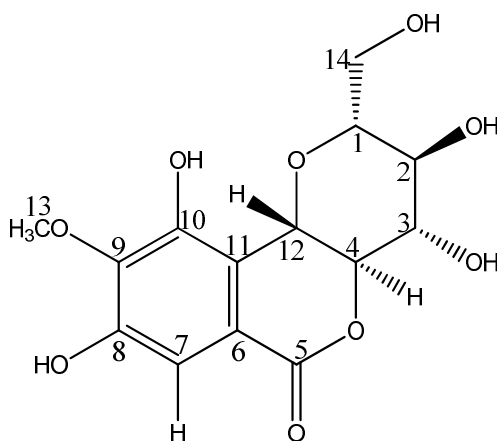
a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.6. Known Constituents from *B. Ligulata*

7.6.1. Bergenin (19)

Compound **19** was isolated as white prisms. Molecular formula was determined from ESI showing pseudo molecular ion peak at m/z 329.0868 a.m.u. $[M+H]^+$, calcd. 329.0864 a.m.u. corresponding to $C_{14}H_{16}O_9$. The IR spectrum indicated the presence of hydroxyl group (3400 cm^{-1}), carbonyl carbon (1680 cm^{-1}) and benzene ring (1525 cm^{-1}).

The spectral data (^1H - and ^{13}C -NMR) spectra (**Table-7.8**) of **19** showed similarity with the spectral data of bergenin from the literature. Moreover single crystal x-ray diffraction study (**Fig-6.6**) also confirmed the isolated compound as bergenin monohydrate.¹⁷¹



19

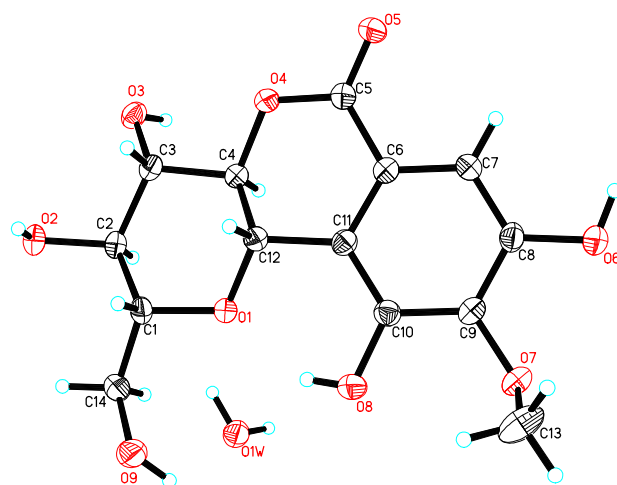


Fig-7.4. Single crystal x-ray photograph of Bergenin

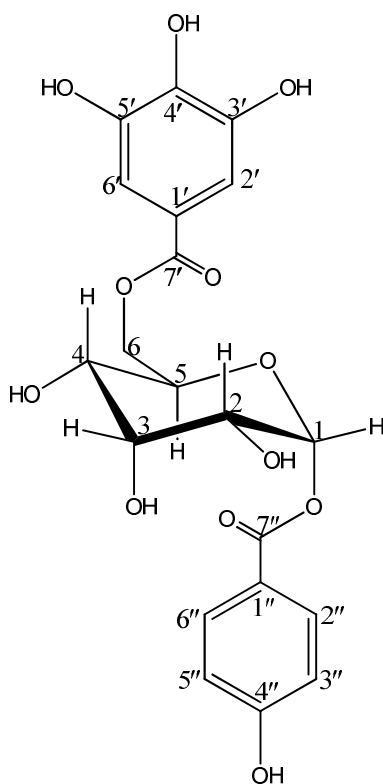
Table-7.8. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **19** in CD_3OD

C. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	80.4	CH	4.12, m
2	72.1	CH	3.49, t ($J = 8.4$)
3	75.8	CH	4.16, t ($J = 8.4$)
4	80.9	CH	4.24, t ($J = 8.4$)
5	167.5	C	-
6	152.7	C	-
7	110.8	CH	-
8	163.5	C	-
9	141.7	C	-
10	165.2	C	-
11	148.9	C	-
12	74.1	CH	4.97, d ($J = 10$)
13	60.4	CH_3	3.91, s
14	64.7	CH_2	4.42, dd ($J = 10, 2$) 5.16 dd ($J = 10, 2$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.6.2. 6-O-Galloylarbutin (23)

Compound **23** was isolated as white granules. The molecular ion peak $[M+H]^+$ was displayed at m/z 425.1079 a.m.u. (calcd. 425.1072 a.m.u.) in the ESI spectrum establishing the molecular formula as $C_{19}H_{20}O_{11}$. The IR spectrum indicated the presence of hydroxyl group (3400 cm^{-1}), ester function (1680 cm^{-1}) and benzene ring (1525 cm^{-1}).



23

The structure of **23** was elucidated as 6-O-galloylarbutin by comparison of its ^1H - and ^{13}C -NMR spectra (**Table-7.9**) with a known compound 6-O-galloylarbutin from literature.¹⁷²

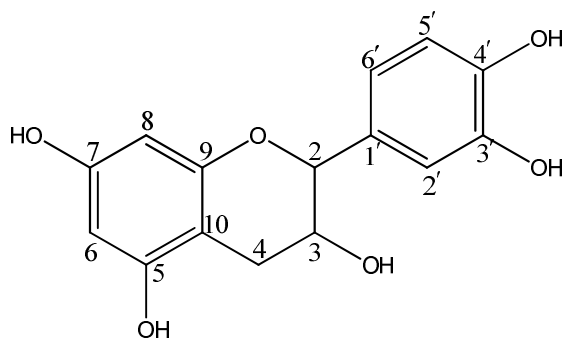
Table-7.9. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **23** in CD_3OD

C/H. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	104.4	CH	5.16, d ($J=7.0$)
2	75.9	CH	3.42, t ($J=6.5$)
3	78.2	CH	3.40, t ($J=6.5$)
4	71.1	CH	3.36, t ($J=6.5$)
5	78.6	CH	3.2, m
6	62.4	CH_2	3.54, dd ($J=11.5, 5.0$) 3.68, dd ($J=11.5, 5.0$)
1'	130.7	C	-
2', 6'	110.4	CH	7.79, s
3', 5'	147.3	C	-
4'	141.3	C	-
7'	168.4	C	-
1''	123.7	C	
2'', 6''	131.9	CH	8.04, d ($J=8.5$)
3'', 5''	116.2	CH	6.87, d ($J=8.5$)
4''	161.6	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.6.3. Catechine (24)

Compound **24** was isolated as white needles and its molecular formula was established as $C_{15}H_{14}O_6$ from ESI showing molecular ion peak at m/z 291.0864 $[M+H]^+$ (calcd. 291.0860 a.m.u). The IR spectrum displayed bends at 3420, 1660 and 1530 cm^{-1} corresponding to hydroxyl group and benzene ring.



25

The spectral data of **25** (**Table-7.10**) was compared with the catechine from literature and confirmed the structure of **25** as catechine.^{173, 174}

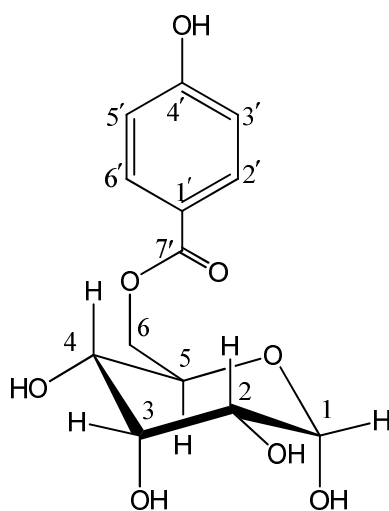
Table-7.10. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of **24** in CD_3OD

C. No.	^{13}C -NMR (δc) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
2	83	CH	4.81, d ($J=7.5$)
3	89.8	CH	4.16, m
4	27.6	CH_2	2.24, dd ($J=15.5, 2.5$) 3.16, dd ($J=15.5, 2.5$)
5	153.1	C	-
6	96.7	CH	6.17, d ($J = 2.4$)
7	152.5	C	-
8	94.8	CH	5.74, d ($J = 2.4$)
9	156.4	C	-
10	99.1	C	-
1'	126.7	C	-
2'	114.2	CH	6.51, d ($J = 8.2$)
3'	145.4	C	-
4'	148.2	C	-
5'	120.8	CH	6.42, d ($J = 8.2$)
6'	115.9	CH	6.81, dd ($J = 8.2, 2.4$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.6.4. Arbutin (27)

Compound **27** was isolated as snow-white needles. Molecular formula was established as $C_{12}H_{16}O_9$ from ESI showing molecular ion peak at m/z 333.0817 a.m.u. $[M+H]^+$ (calcd. 333.0812 a.m.u.). The IR spectrum is consistent with the presence of hydroxyl group (3410 cm^{-1}), ester group (1685 cm^{-1}) and benzene ring (1520 cm^{-1}).



27

By comparison of the spectral data (^1H - and ^{13}C NMR) of compound **27** (Table-7.11) with a known compound 6-O-benzoyl-β-D-glucopyranoside from literature, confirmed that **27** is 6-O-benzoyl-β-D-glucopyranoside.¹⁷⁵

Table-7.11. ^1H - (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of 27 in CD_3OD

C/H. No.	^{13}C -NMR (δC) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δH) coupling constants J_{HH} (Hz) ^{cd}
1	104.4	CH	4.94, d ($J=7$)
2	75.9	CH	3.74, t ($J=6.5$)
3	78.2	CH	3.68, t ($J=6.5$)
4	71.1	CH	3.79, t ($J=6.5$)
5	78.6	CH	2.94, m
6	62.4	CH_2	3.62, dd ($J=11.5, 5$) 3.94, dd ($J=11.5, 5$)
1'	130.7	C	-
2', 6'	110.4	CH	7.96, s
3', 5'	147.3	C	-
4'	141.3	C	-
7'	168.4	C	-

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.7. Biological activities

7.7.1. DPPH Antioxidant Assay

All the isolated compounds from *B. ligulata* were evaluated for their radical scavenging potential (**Table-7.12**) and it was found out that the new compound **29** is the most potent antioxidant with IC₅₀ value of 14.6±1.25 μ M as compared to catechine whose IC₅₀ value is 20.2±1.31 μ M. Moreover, the natural derivatives of bergenin were evaluated for their antioxidant effect and it was established that 11-O-Galloylbergenin with IC₅₀ value of 35.4±1.24 μ M is the most potent antioxidant amongst the naturally occurring three bergenin derivatives isolated from *B. ligulata* indicating that the OH groups attached to benzene ring have key function in scavenging of the free radicals.

Table-7.12. Antioxidant activity of isolated compounds against DPPH assay

S.No	Sample	% inhibition	IC ₅₀ ± SEM (μ M)
1.	4-Methoxygalangin-3'-O- β -D-erythrofuranoside (22)	61	Inactive
2.	4'-Methoxycatechin-3'-galloyl-2'',4''-bis-O-(3,4,5-trihydroxybenzoyl)-3-O- β -D-glucopyranoside (29)	93.4	14.6±1.25
3.	11-O-Parahydroxybenzoyl bergenin (20)	73.4	29.4± 1.51
4.	Mecidanol (21)	74	58.2±0.98
5.	11-O-Galloylbergenin (25)	84	35.4±1.24
6.	3-Galloylcatechine (26)	68	68±1.31
7.	3,7-Digalloylcatechin (28)	81	34±1.23
8.	Bergenin (19)	63	Inactive
9.	6-O-Galloylarbutin (23)	58	Inactive
10.	Catechine (24)	91.2	20.2±1.31
11.	Arbutin (27)	80	29.5±0.85

% age inhibition was evaluated using inhibitor at a concentration of 100 μ M

7.7.2Antibacterial activities

Antibacterial potential of the various fraction of *B. ligulata* was summarized in the **Table-7.13** and it is evident that the plant does not possess any significant antibacterial substances i.e. only the ethyl acetate and the aqueous fraction displayed weak antibacterial activity.

Table-7.13. Antibacterial activity of crude fractions of *P. emodi*

Bacterial Species	Zone of inhibition (mm)					
	Imipenem 10 µg/disc(+)	DMSO(-)	Hexane	Chloroform	Ethyl Acetate	Aqueous
<i>Escherichia coli</i>	35	-	-	3	6	2
<i>Bacillus subtilis</i>	36	-	-	5	5	3
<i>Shigella flexaneri</i>	36	-	-	-	10	6
<i>Staphylococcus aureus</i>	43	-	-	4	9	6
<i>Pseudomonas aeruginosa</i>	32	-	-	-	-	-
<i>Salmonella typhi</i>	40	-	-	6	10	10

7.7.3 Antifungal activity

No significant antifungal activity was observed (**Table-7.14**) indicating that the crude fractions do not possess any antifungal metabolites.

Table-7.14. Antifungal activities of various fractions of *B. ligulata*

Fungal species	Std. Drug Mic $\mu\text{g/ml}$	% inhibition			
		Hexane	Chloroform	Ethyl Acetate	Aqueous
<i>Trichophyton longifusus</i>	Miconazole 70	-	-	16	-
<i>Candida albicans</i>	Miconazole 110.8	-	6	4	-
<i>Aspergillus flavus</i>	Amphotericin 20	-	-	-	4
<i>Microsporum canis</i>	Miconazole 98.4	-	-	-	-
<i>Fusarium solani</i>	Miconazole 73	-	-	8	10
<i>Candida glabrata</i>	Miconazole 110.8	-	-	-	-

7.7.4. Urease inhibition assay

As reported previously (Khan, T *et al*,), *B. ligulata* crude extract showed moderate urease inhibitory effect so the urease inhibitory potential of compounds **19-29** isolated from the aerial part of *B. ligulata* was evaluated. The results were summarized (**Table-7.15**). The compounds containing flavonoid structure showed moderate urease inhibitory potential however the bergenin and its natural depravities displayed significant urease inhibitory potential and 11-*O*-parahydroxybenzoyl bergenin is the most potent inhibitor with IC_{50} value of $29.4 \pm 1.51 \mu\text{M}$ as compare to 11-*O*-gallolybergenin (IC_{50} $35.4 \pm 1.24 \mu\text{M}$) and bergenin. These results also indicated the structure activity relation which may establish that the two *meta* hydroxyl group in the benzoyl moiety are not involved in interaction with the enzyme and only the *p*-hydroxyl group is effectively involved. The

two new compounds (**22** & **29**) are inactive and did not display significant urease inhibitory potential.

Table 7-15. Urease inhibitory activity of isolated compound

S.No	Sample	% inhibition	IC ₅₀ ± SEM (μM)
1.	4-Methoxygalangin-3'-O-β-D-erythrofuranoside (22)	61	Inactive
2.	4'-Methoxycatechin-3'-galloyl-2''4''bis-O-(3,4,5-trihydroxybenzoyl)-3-O-β-D-glucopyranoside (29)	57	Inactive
3.	11-O-Parahydroxybenzoyl bergenin (20)	86	29.4± 1.51
4.	Meciadanol (21)	74	58.2±0.98
5.	11-O-Galloylbergenin (25)	84	35.4±1.24
6.	3-Galloylcatechine (26)	68	68±1.31
7.	3,7-Digalloylcatechin (28)	81	34±1.23
8.	Bergenin (19)	40	Inactive
9.	6-O-Galloylarbutin (23)	32	Inactive
10.	Catechine (24)	0	Inactive
11.	Arbutin (27)	80	29.5±0.85
12.	Thiourea (standard)	96.9	21.8 ± 1.6

% age inhibition was evaluated using inhibitor at a concentration of 100 μM

7.7.5. Brine shrimp (*Artemia salina*) lethality bioassay

The various crude fractions were assayed to estimate the cytotoxic potential but none of the fractions displayed significant cytotoxicity (**Table 7.16**) against Brine shrimp larvae.

Table 7-16. Brine shrimp (*Artemia salina*) lethality assay of various fractions from aerial parts of *B. ligulata*

Fractions	Dose ($\mu\text{g/mL}$)	No. of shrimps/vial	No of survivors/vial	LD ₅₀ ($\mu\text{g/mL}$)	Std. Drug	LD ₅₀ ($\mu\text{g/mL}$)
F1	1000	10	9	>1000	Etoposide	7.4625
	100	10	10			
	10	10	10			
F2	1000	10	10	>1000	Etoposide	7.4625
	100	10	10			
	10	10	10			
F3	1000	10	10	>1000	Etoposide	7.4625
	100	10	10			
	10	10	10			
F4	1000	10	10	>1000	Etoposide	7.4625
	100	10	10			
	10	10	10			

F1 = *n*-hexane, F2 = chloroform, F3 = MeOH, F4 = aqueous

7.7.6. Antiplasmodial activity

The ethyl acetate fraction of the *B. ligulata* showed weak antimalarial activity while all the other fractions were inactive (**Table 7.17**). It has been obvious from the literature that compounds containing flavonoid skeleton do not show antimalarial potential so only bergenin derivatives were subjected to antimalarial assay and the results were summarized in the table 0.00 and it was found that 11-O-galloylbergenin is the most potent antimalarial with IC₅₀ value of $2.34 \pm 0.2 \mu\text{M}$. Moreover, the structure activity relation can be established that the hydroxyl groups on the benzene ring have important role in the antimalarial activity. So this study predicts for the first time that the bergenin

nucleus have antimalarial potential and it is needed to conduct further study to modeled bergenin into a more potent antimalarial agent which could be more safer and easily available than the existing antimalarial agents.

Table 7.17. Antimalarial activity of *B. ligulata* against *P. falciparum* (D10)

S.No.	Samples	D10 IC ₅₀
1	<i>n</i> -hexane	Inactive
2	Chloroform	Inactive
3	Ethyl acetate	Inactive
4	Aqueous	Inactive
5	Bergenin (μ M)	2.41 $\pm$ 0.4
6	11- <i>O</i> - <i>p</i> -hydroxybergenin	2.40 $\pm$ 1.2
6	11- <i>O</i> -galloylbergenin	2.34 $\pm$ 0.2
4	Chloroquine	28.07 $\pm$ 12.48
5	Artemisinin	26.81 $\pm$ 8.54

Crude extracts were tested at concentration of 100 μ g/ml, pure compounds were tested at concentration of 10, 5, 25 μ M and the standards were tested at 50, 25 and 12.5 nM.

EXPERIMENTAL (Part B)

8.1. General experimental conditions

8.1.1. Physical contents

Melting points were measured using Stuart SMP 10 apparatus in a glass capillary, which are un-correct. Optical rotation was measured using JASCO-DIP-360 digital polarimeter and Ultraviolet spectroscopy (UV) was carried in methanol using Hitachi-U-3200 spectrophotometer while Infrared spectra (IR) were measured using JASCO-A-302 spectrophotometer by direct placing of sample on the diamond cell. Proton NMR spectra were taken by Bruker AV 400, 500 and 600 cryoprob machine at 400 MHz, 500 MHz and 600 MHz respectively with TMS as an internal standard. Carbon 13 spectra were taken by Bruker AV 500 and 600 cryoprob machine at 125 MHz and 150 MHz respectively and TMS was used as an internal standard. 2D NMR experiments i.e. HSQC, HMBC, NOESY, ^1H - ^1H COSY, and *J*-resolved, Bruker AV 500 spectrometer was used. Finnigan-MAT-311 and Varian MAT 312 spectrometers were used to determine the mass spectrum at 250 °C with ionization potential of 70 eV. Jeol JMS-600H mass spectrometer was used for High Resolution mass spectra (HRMS) utilizing PFK as an internal standard. Fast Atom Bombardment mass spectra (FAB +ve) was obtained using JMS HX-110 double focusing mass spectrophotometer where methanol was using as a solvent and glycerol as a matrix on the target and Xe gas was used to ionize the sample.

8.1.2. Chromatographic Techniques

Column chromatography (CC) was performed using Merck silica gel (200 μm), Sephadex LH-20 (Sigma-Aldrich) and RP-18 (Merck). The preparative TLC silica gel 60 F₂₅₄ coated on aluminum sheets (Merck) with thickness of 0.2 mm and for reverse phase preparative TLC glass plates coated with RP-18 F₂₅₄ (Merck) of 0.25 mm thickness were used. Visualization of the spots on the TLC plate was done under compact UV lamp (245/365 nm), spraying the plates with ceric sulphate reagent, vanilin, I₂ vapors and Dragendorff's reagent.

8.2. Materials and Methods

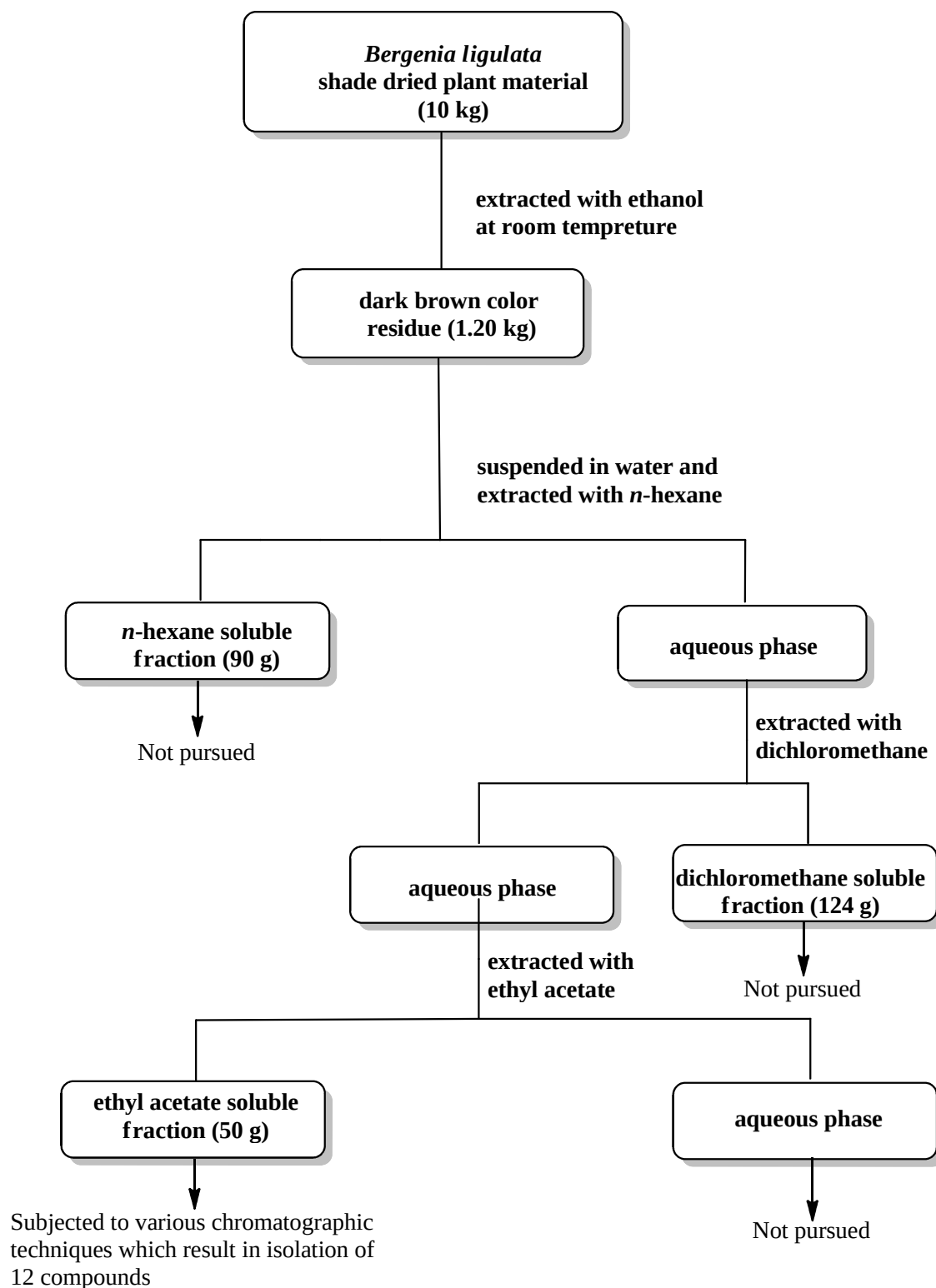
8.2.1. Plant materials

Whole plant was collected from Hazara Division of Khyber Pakhtunkhwa province of Pakistan in July of 2009 and identified by Mr. Abdul Majid, Lecturer Department of Botany, Hazara University, Monshera where a voucher specimen was deposited at the herbarium. The plant species was further authenticated by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar.

8.2.2. Extraction and isolation

The shade dried roots and aerial parts were separately extracted with commercial grade ethanol (x 3) at room temperature and concentrated in rotary evaporator at 45 °C to thick syrup which was further concentrated in water bath at 45 °C for three days to dark brown color solid mass. The combined extract was suspended in water and extracted with *n*-hexane, dichloromethane and ethyl acetate in order to fractionate the complex mixture

into non-polar, slightly polar and medium polar sub-fractions while highly polar compounds remained in the aqueous phase (**Scheme-8.1**).



Scheme-8.1. Fractionation of the crude extract of *B. ligulata*

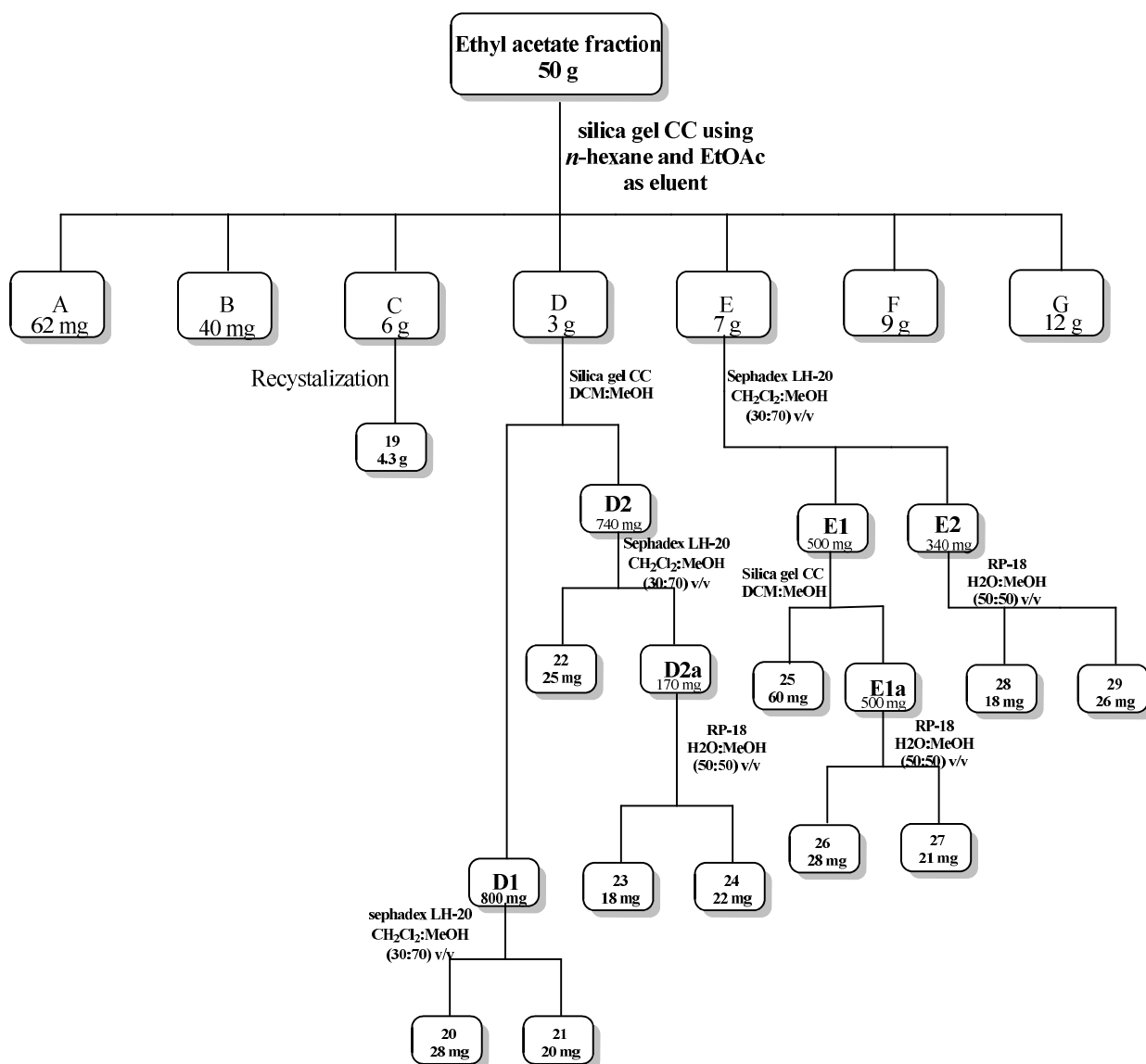
The ethyl acetate fraction (**50 g**) was subjected to column chromatography on silica gel and eluted with *n*-hexane and ethyl acetate in order to increasing polarity until 70% EtOAc and 30% *n*-hexane. Subsequently, the solvent system was changed to dichloromethane and methanol i.e started elution with pure dichloromethane and then increasing polarity with methanol by 1% after elution of 1 liter of solvent until to pure methanol. The ethyl acetate fraction was further partitioned into seven semi-purified fractions (**a-g**) based on the TLC profile.

Fraction A was green color oil while fraction B was yellow color oil so both of these fractions were not processed for the isolation of metabolites.

Fraction C on dryness yielded impure crystals, washed with ethyl acetate and cold methanol and re-crystallized in a mixture of methanol and acetone (50%) resulted 4.3 g pure white color crystals of compound **9**.

Fraction D was subjected to column chromatography (CC) over silica gel (GF) and started elution with dichloromethane and a polarity gradient was created by adding MeOH, which afforded two sub-fractions D1 & D2. Subsequently, D1 was subjected to size exclusion chromatography over Sephadex LH-20 and eluted with mixture of MeOH and dichloromethane (3:7) which resulted in purification of **20** (28 g) and **21** (20 g). Similarly, D2 fraction afforded **22** (25 mg) and a semipurified fraction D2a and subjected to size exclusion chromatography over Sephadex LH-20 with the same solvent systems used for D1. D2a was then subjected to reverse phase chromatography using RP-18 as a stationary phase and 50% mixture of water and methanol, which yielded two pure compounds i.e. **23** (18 mg) and **24** (22 mg).

Fraction E afforded two sub-fractions (E1 and E2) and subjected to size exclusion chromatography over Sephadex LH-20 using dichloromethane and MeOH (3:7) as a solvent system. Compound **25** (18 mg) along with a semipurified fraction E1a was obtained while fraction E1 was subjected to CC over Silica gel utilizing a mixture of MeOH and dichloromethane as a solvent system. E1a was then subjected to reverse phase chromatography using RP-18 as a stationary phase and 50% mixture of water and methanol as elute, which afforded two pure compounds **26** (28 mg) and **27** (21 mg). Fraction E2 was also subjected to reverse phase chromatography which yielded two pure compounds; **28** (18 mg) and **29** (26 mg).

**Scheme-8.2.** Isolation of pure compounds from ethyl acetate fraction

8.3. Characterization of new compounds from *B. ligulata*

8.3.1. 4-Methoxygalangin-3'-O- β -D-erythrofuranoside (22)

Physical State: Yellow granules

Yield: 25 mg

IR: 3400, 1690, 1655, 1525, 1030 cm^{-1}

UV λ_{max} (MeOH): 375, 260 nm

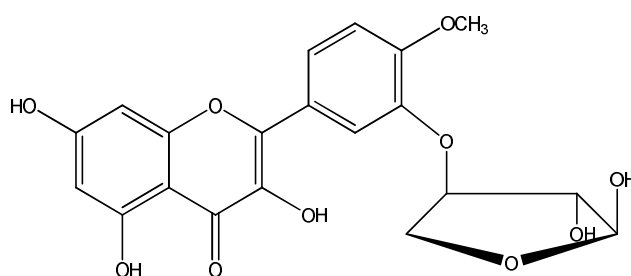
HR-ESI-MS: m/z 419.0973 $[\text{M}+\text{H}]^+$

calcd. for 419.0968

ESI (+)-MS m/z : 419.0973, 389.0868, 301.0702

^1H - and ^{13}C -NMR and HSQC: **Table-7.1**

HMBC: **Fig-7.1**



8.3.2. 4'-Methoxycatechin-3'-galloyl-2'',4''-bis-O-(3,4,5-trihydroxybenzoyl)-3-O- β -D-glucopyranoside (29)

Physical State: Dark brown powder

Yield: 26 mg

IR: 3420, 1665, 1700, 1690, 1650, 1525, 1030 cm^{-1}

UV λ_{max} (MeOH): 360, 270, 245 nm

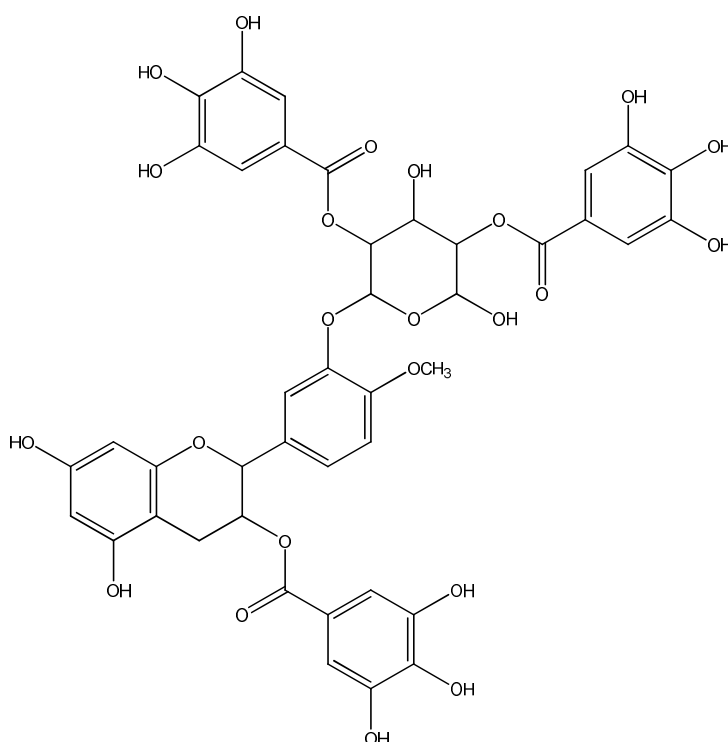
HR-ESI-MS: m/z 909.1720 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{42}\text{H}_{36}\text{O}_{23}$ 909.1716

ESI (+)-MS m/z : 457.1130, 471.0770, 305.1020, 171.0288

^1H - and ^{13}C -NMR and HSQC: **Table-**

HMBC: **Fig-7.1**



8.4. Hitherto Unreported Constituents

8.4.1. 11-*O-p*-Hydroxybenzoylbergenin (20)

Physical State: White granules

Yield: 28 mg

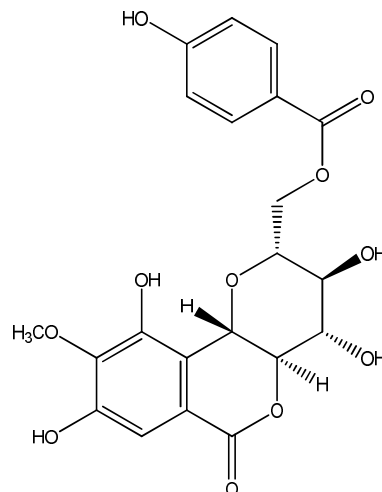
IR: 3400, 1700, 1685, 1620, 1530 cm^{-1}

UV λ_{max} (MeOH): 380, 295 nm

HR-ESI-MS: m/z 449.1079 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ 449.1066

^1H - and ^{13}C -NMR and HSQC: **Table-7.3**



8.4.2. Meciadanol (21)

Physical State: White needles

Yield: 20 mg

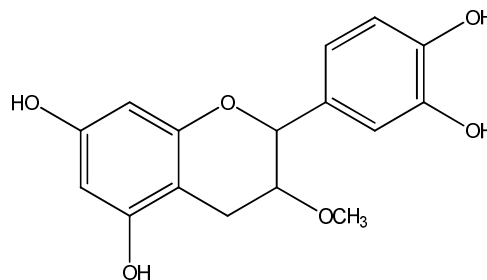
IR: 3420, 1660, 1530 cm^{-1}

UV λ_{max} (MeOH): 360 nm

HR-ESI-MS: m/z 305.1020 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{16}\text{H}_{16}\text{O}_6$ 305.1016

^1H - and ^{13}C -NMR and HSQC: **Table-7.4**



8.4.3. 11-O-Galloylbergenin (25)

Physical State: Transparent needles

Yield: 60 mg

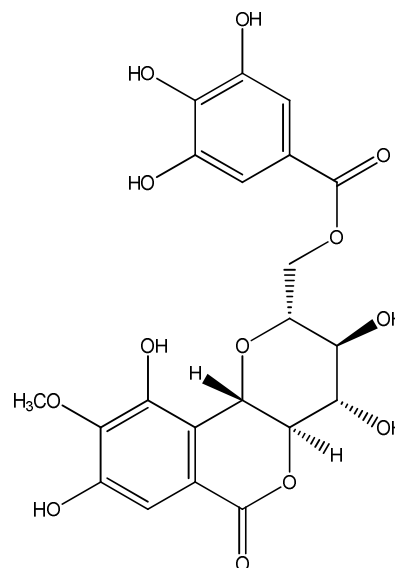
IR: 3410, 1665, 1690, 1625, 1520 cm^{-1}

UV λ_{max} (MeOH): 380, 295 nm

HR-ESI-MS: m/z 481.0977 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{21}\text{H}_{20}\text{O}_{13}$ 481.0972

^1H - and ^{13}C -NMR and HSQC: **Table-7.5**

**8.4.3. 3-Galloylcatechine (26)**

Physical State: Amorphous powder

Yield: 28 mg

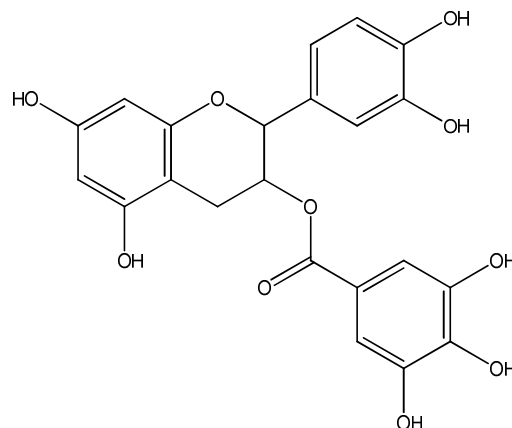
IR: 3410, 1675, 1650, 1520 cm^{-1}

UV λ_{max} (MeOH): 365, 285 nm

HR-ESI-MS: m/z 443.0973 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{22}\text{H}_{18}\text{O}_{10}$ 443.0968

^1H - and ^{13}C -NMR and HSQC: **Table-7.6**



8.4.4. 3,7-Digalloylcatechin (28)

Physical State: Amorphous powder

Yield: 18 mg

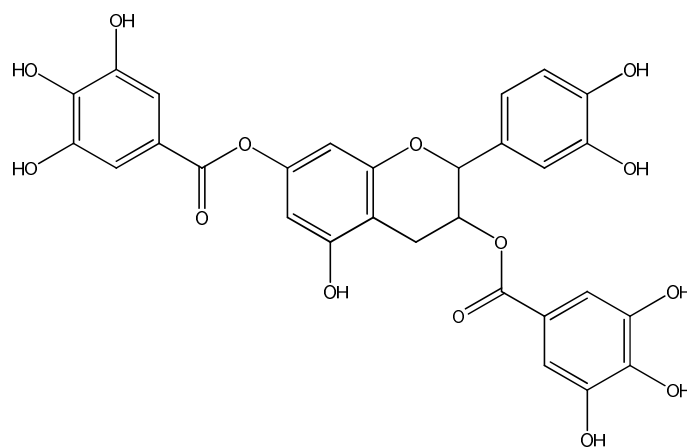
IR: 3400, 1680, 1710, 1650, 1520 cm^{-1}

UV λ_{max} (MeOH): 370, 285 nm

HR-ESI-MS: m/z 595.1083 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{29}\text{H}_{22}\text{O}_{14}$ 595.101078

^1H - and ^{13}C -NMR and HSQC: **Table-7.7**



8.5. Reported constituents from *B. ligulata*

8.5.1. Bergenin (19)

Physical State: White prisms

Yield: 4.3 g

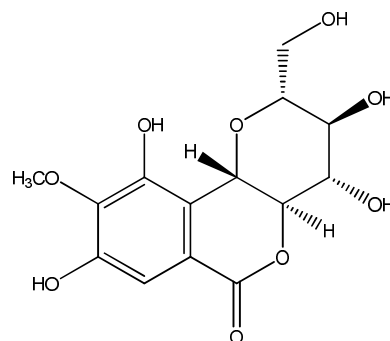
IR: 3400, 1680, 1620, 1525 cm^{-1}

UV λ_{max} (MeOH): 375, 280 nm

HR-ESI-MS: m/z 329.0868 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{14}\text{H}_{16}\text{O}_9$ 329.0864

^1H - and ^{13}C -NMR and HSQC: **Table-7.8**



8.5.2. 6-O-Galloylarbutin (23)

Physical State: Needles

Yield: 18 mg

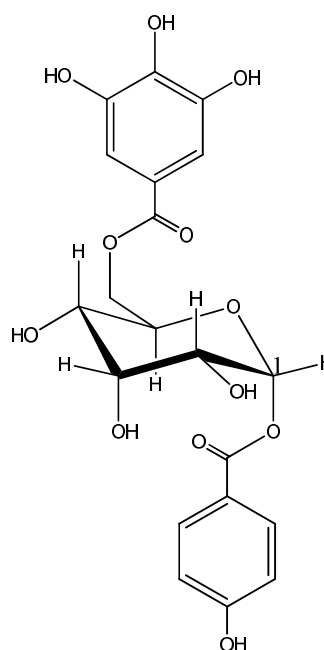
IR: 3390, 1690, 1650, 1520 cm^{-1}

UV λ_{max} (MeOH): 354, 265 nm

HR-ESI-MS: m/z 425.1079 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{19}\text{H}_{20}\text{O}_{10}$ 425.1072

^1H - and ^{13}C -NMR and HSQC: **Table-7.9**



8.5.3. Catechine (24)

Physical State: White needles

Yield: 22 mg

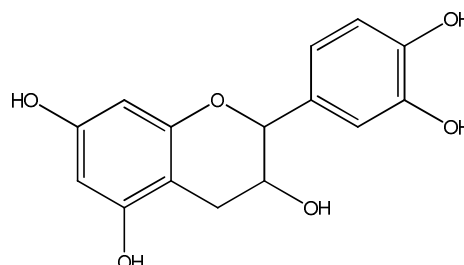
IR: 3420, 1660, 1530 cm^{-1}

UV λ_{max} (MeOH): 354 nm

HR-ESI-MS: m/z 291.0864 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{15}\text{H}_{14}\text{O}_6$ 291.0860

^1H - and ^{13}C -NMR and HSQC: **Table-6.9**

**8.5.4. Arbutin (27)**

Physical State: Snow white needles

Yield: 21 mg

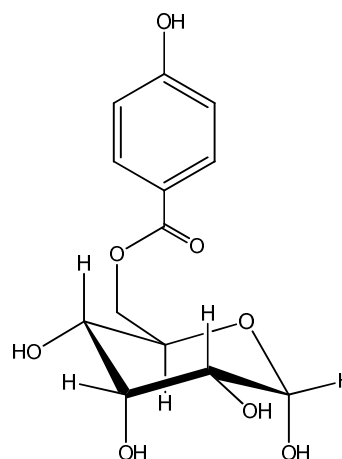
IR: 3410, 1685, 1710, 1650, 1520 cm^{-1}

UV λ_{max} (MeOH): 370, 284 nm

HR-ESI-MS: m/z 333.0817 $[\text{M}+\text{H}]^+$

calcd. for $\text{C}_{13}\text{H}_{16}\text{O}_{10}$ 333.0812

^1H - and ^{13}C -NMR and HSQC: **Table-7.10**



8.6. Biological Screening

8.6.1. DPPH antioxidant assay

Radical scavenging activity was estimated by the method as described in **Chapter 5 Part A**.

8.6.2. Antimicrobial activities

Antibacterial and antifungal activities were performed as per the protocols as stated in the **Chapter 5 Part A**.

8.6.3. Brine shrimp (*Artemia salina*) lethality bioassay

Cytotoxicity of the crude extracts was evaluated according to the protocol described in **Chapter 5 Part A**.

8.6.4. Urease inhibition assay

Urease enzyme inhibitory potential of the isolated compounds was analyzed by the method describe in **Chapter 5 Part A**.

8.6.5. Antiplasmodial activity

Antiplasmodial potential of isolated compounds was measured according to the protocol as discussed in **Chapter 5 Part A**.

References

1. Watt, G. A Dictionary of the Economic Products of India; Cosmo Publications, Delhi, India, 1893.
2. Morse, W. R., Chinese medicine (Clio Medica). New York, P.B. Hoeber, Vol. 7, 1934, p. 88.
3. Naheed, A. Studies in the chemical constituents of *plumeria obtusa* and sturcture and activity relationship studies in the *Hermine* series of alkaloids. Ph.D. Thesis, University of Karachi, Karachi, Pakistan, 1991.
4. Chen, K. K., Chinese drug stores. *Annals of Medical History* 1925, VII, 103-109.
5. Siddiqui, S. *Rauwolfia* alkaloidal complexes and process for isolating the same form *Rauwolfia* plant material. 3047562, 1962.
6. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, 277, 665-667.
7. Haque, I. U.; Saba, H., Vinblastine: A Review. *J. Chem. Soc. Pak.* **2010**, 32, 245-258.
8. Withington, E. T., *Medical History from the Earliest Times*. The Scientific Press, Ltd. Ed, 1894, p 141.
9. Kaviratna, A. C., *Works of Sushruta*. Trans **1907**.
10. Finlayson, J., Hippocrates. *The Glasgow Medical Journal* **1892**, 37, 748.
11. Sigerist, H. E. *Bulletin of the History of Medicine* **1934**, 2, 190-214.
12. Theophrastus, A. H., *De Historia Plantarum*. Loeb Library: 1916.
13. Walsh, J., Galen's Writings and Influences Inspiring Them. *Annals of Medical History* **1936**, 6, 1.
14. Chatard, J. A., Avicenna and Arabian Medicine. *The Johns Hopkins Hospital Bulletin* **1908**, 19, 157.

References

15. Kardar, M. N. Studies on The chemical constituents of the aerials parts of *Lawsonia alba*. Ph.D. Thesis, University of Karachi, Karachi, Pakistan, 2005.
16. J. A. Bassham and M. Calvin. The *Photosynthesis of Carbon Compounds*; 2nd ed; W. A.. Benjamin Co., New York, N.Y., 1962.
17. Barton, D.; Ollis, W.; Stoddart, J.; Sutherland, I.; Jones, D.; Sammes, P.; Haslam, E.; Drayton, S., *Comprehensive Organic Chemistry: the Synthesis and Reactions of Organic Compounds*. Pergamon New York: 1979; pp 869-1205.
18. Manitto, P.; Sammes, P., *Biosynthesis of Natural Products*. Horwood Chichester: Halsted Press, 1981, p 548.
19. Dewick, P., *Medicinal Natural Products: A Biosynthetic Approach*. 2nd ed.; John Wiley & Sons Inc: Chichester, UK, 2002.
20. Harborne, J.; Mabry, T.; Mabry, H., *The Flavonoids*. Chapman and Hall: London, 1975.
21. Zechmeister L. (ed.). "*Progress in The Chemistry of Organic Natural Products*; Springer-Verlag, Vienna: 1957; Vol. 14.
22. Sharad, S.; Ajay, K.; Singh, R. *J. Sci. Ind. Res.* **2008**, 67, 65-72.
23. Chauhan, S. K.; Singh, B.; Agrawal, S.; *J. AOAC. Int.* **2000**, 83, 1480-1483.
24. Wall. Ex, Royle, N., *Flora of West Pakistan* **1978**, 121, 1-3.
25. Khan, T.; Ahmad, M.; Nisar, M.; Lodhi, M. A.; Choudhary, M. I. *J. Enzyme. Inhib. Med. Chem.* **2005**, 20, 245-249.
26. Khan, T.; Ahmad, M. *Pharmazie* **2007**, 62, 476-467.
27. Prochazkova, M.; Dolezal, T.; Silva, J.; Krsiak, M. *J. Eur. neuropsychopharmacol.* **2005**, 15, 638-641.

References

28. Tantry, M.; Dar, J. A.; Khuroo, M. A.; Shawl, A. S. *Phytochem. Lett.* **2012**, *5*, 253-257.
29. Haq, F.; Rehman, S.; Ahmad, H.; Iqbal, Z.; Ullah, R. *Am. J. Biochem.* **2012**, *4*, 47-50.
30. Ghayur, M.; Anwarul, H.; Rasheed, H.; Khan, A.; Iqbal, Z.; Ismail, M.; Saeed, S. A.; Janssen, L. J. *Can. J. Physiol. Pharmacol.* **2008**, *86*, 793-803.
31. Raiz, N.; Anis, I.; Malik, A.; Ahmed, Z.; Choudhary, M. I. *Chem. Pharm. Bull.* **2003**, *51*, 252-254.
32. Raiz, N.; Malik, A.; Rehman, A.; Ahmed, Z.; Muhammad, P.; Nawaz, S. A.; Siddiqui, J.; Choudhary, M. I. *Phytochemistry* **2004**, *65*, 1129-1135.
33. Nawaz, H. R.; Malik, A.; Khan, P. M.; Shujaat, S.; Atta-Ur-Rehman. *Chem. Pharm. Bull.* **2000**, *48*, 1771-1773.
34. Pir, M.; Saeed, A.; Hafiz, R.; Ullah, N.; Abdul, M. Z. *Naturforsch.* **1999**, *4*, 544-548.
35. Asif, M.; Ahmad, M. S.; Mannan, A.; Itah, T.; Matsumoto, T. *J. Am. Oil. Chem. Soc.* **1983**, *80*, 581-583.
36. Rashid, M.; Begum, S.; Malik, M. N. *Pak. J. Forest* **1972**, *22*, 109-111.
37. Ito, M. *Agric. Biol. Chem.* **1991**, *55*, 2117.
38. Tanaka, T.; Kataoka, M.; Tsuboi, N. *Chem. Pharm. Bull.* **2000**, *48*, 201-207.
39. Kaneda, M.; Iitaka, Y. *Tetrahedron* **1972**, *28*, 4309-4317.
40. Wu, S. H.; Luo, X. D.; Ma, Y. B.; Hao, X. J.; Wu, D. G. *J. Asian Nat. Prod. Res.* **2002**, *4*, 135-140.
41. Haroon ur Rashid, M.; Begum, S.; Malik, M. N. *Pak. J. Forest* **1972**, *22*, 109-111

References

42. Wang, X. L.; Jiao, W.; Liao, X.; Peng, S. L.; Ding, L. S. *Chin. Chem. Lett.* **2006**, *17*, 916-918.
43. Ma, Y. B.; Da-Gang, W. U.; Ji-Kai, L. I. U. *Chin. Chem. Lett.* **1999**, *10*, 771-774.
44. Duan, W. J.; Yang, J. Y.; Chen, L. X.; Zhang, L. J.; Jiang, Z. H. Cai, X. D.; Zhang, X.; Qiu, F. *J. Nat. Prod.* **2009**, *72*, 1579-1584.
45. Yamasaki, K.; Kaneda, M.; Tanaka, O. *Tetrahedron. Lett.* **1976**, *17*, 3965-3968.
46. Zhang, X. Y.; Gao, C. K.; Wang, J. H.; Li, X. *Yaoxue Xuebao* **2002**, *37*, 705-708.
47. Aimi, N.; Inaba, M.; Watanabe, M.; Shibata, S. *Tetrahedron* **1969**, *25*, 1825-38.
48. Kaneda, M.; Iitaka, Y. *Acta Cryst. B* **1972**, *28*, 1411-1420.
49. Yu, J.; Elix, J. A. *Phytochemistry* **1990**, *29*, 3859-3863.
50. Yoshikawa, M.; Uchida, E.; Kawaguchi, A.; Kitagawa, I.; Yamahara, J. *Chem Pharm. Bull.* **1992**, *40*, 2248-2250.
51. Corey, E. J.; Wu, Y. J. *JACS.* **1993**, *115*, 8871-8872.
52. Hatakeyama, S.; Kawamura, M.; Takano, S. *J. Am. Chem. Soc.* **1994**, *116*, 4081-4082.
53. Murakami, N.; Saka, M.; Shimada, H.; Matsuda, H.; Yamahara, J.; Yoshikawa, M. *Chem. Pharm. Bull.* **1996**, *44*, 1279-1281.
54. Lin, H. C.; Ding, H. Y.; Wu, T. S. *Phytochemistry* **1996**, *41*, 237-242.
55. Lemmich, J. *Phytochemistry* **1996**, *41*, 1337-1340.
56. Ding, H. Y.; Wu, Y. C.; Lin, H. C.; Chan, Y. Y.; Wu, P. L.; Wu, T. S. *Chem. Pharm. Bull.* **1999**, *47*, 652-655.
57. Sarker, S. D.; Whiting, P.; Dinan, L.; ik, V.; Rees, H. H. *Tetrahedron* **1999**, *55*, 513-524.

References

58. Tanaka, T.; Kataoka, M.; Tsuboi, N. *Chem. Pharm. Bull.* **2000**, *48*, 201-207.
59. Ding, H. Y.; Lin, H. Y.; Wu, Y. C. *Chin. Chem. Soc.* **2000**, *47*, 381-388.
60. Park, E. K.; Ross, G. W.; Petrovith, H. *Arch. Pharmacol. Res.* **2001**, *24*, 105-108.
61. Matsuda, H.; Ohta, T.; Kawaguchi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 69-72.
62. Wu, S. H.; Zhou, J. Y.; Chen, Z. G. *Chin. Chem. Lett.* **2002**, *13*, 430-431.
63. Stavri, M.; Mathew, K. T.; Bucar, F. *Planta Med.* **2003**, *69*, 956-959.
64. Riaz, N.; Nawaz, S. A.; Malik, A.; Afza, N.; Ali, S.; Ullah, S.; Muhammad, P. *Phytochemistry* **2004**, *65*, 1129-1135.
65. An, R. B.; Kim, H. C.; Lee, S. H.; Jeong, G. S.; Sohn, D. H.; Park, H.; Kwon, D. Y.; Lee, J. *Arch. Pharmacol. Res.* **2006**, *29*, 815-820.
66. Wu, S. H.; Chen, T. W.; Yang, L. Y.; Li, S. L.; Li, Z. Y. *Fitoterapia* **2007**, *78*, 76-78.
67. Wang, Q.; Liu, X.; Yaowu, F. Z. *Zhongcaoyao* **2007**, *38*, 972-976.
68. Braca, A. Kiem, P. V.; Yen, P. H.; Nhiem, N. X.; Quang, T. H.; Cuong, N. X. *Fitoterapia* **2008**, *79*, 117-120.
69. Okasaka, M.; Kashiwada, Y.; Kodzhimatov, O. K.; Ashurmetov, O.; Takaishi, Y. *Phytochemistry* **2008**, *69*, 1767-1772.
70. Washida, K.; Itoh, Y.; Iwashita, T.; Nomoto, K. *Chem. Pharm. Bull.* **2009**, *57*, 971-974.
71. Washida, K.; Itoh, Y.; Iwashita, T.; Nomoto, K. *Chem. Pharm. Bull.* **2009**, *57*, 1150-1152.

References

72. Ren, M. L.; Zhang, X.; Ding, R.; Dai, Y.; Tu, F. J.; Cheng, Y. Y. *J. Asian Nat. Prod. Res.* **2009**, *11*, 670-674.
73. Ha, D. T.; Ngoc, T. M.; Lee, I.; Lee, Y. M.; Kim, J. S.; Jung, H.; Lee, S.; Na, M.; Bae, K. *J. Nat. Prod.* **2009**, *72*, 1465-1470.
74. Duan, W. J.; Jin, X.; Chen, L. X. *J. Asian Nat. Prod. Res.* **2009**, *11*, 299-305.
75. Li, J.; Kadota, S.; Kawata, Y.; Hattori, M.; Xu, G. J.; Namba, T. *Chem. Pharm. Bull.* **1992**, *40*, 3133-3137.
76. De Guttiérrez, A. N. *Phytochemistry* **1995**, *39*, 795-800.
77. Kuwajima, H.; Shibano, N.; Baba, T.; Takaishi, K.; Inoue, K.; Shingu, T. *Phytochemistry* **1996**, *41*, 289-292.
78. Lin, Y. L. Lin, T. C.; Kuo, Y. H. *J. Nat. Prod.* **1997**, *60*, 368-370.
79. Yasuda, T. Kon, R.; Nakazawa, T.; Ohsawa K. *J. Nat. Prod.* **1999**, *62*, 1142-1144.
80. Naeimi, H.; Moradi, L. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 284-287.
81. Kazantzoglou, G.; Magiatis, P.; Kalpoutzakis, E.; Skaltsounis, A. L. *J. Nat. Prod.* **2009**, *72*, 187-189.
82. Wu, S. H.; Yang, S. M.; Wu, D. G. *Helv. Chim. Acta.* **2005**, *88*, 259-265.
83. Lin, H. C.; Ding, H. Y.; Ko, F. N.; Teng, C.; Wu, Y. *Planta Med.* **1999**, *65*, 595-599.
84. Ikuta, A.; Kamiya, K.; Satake, T.; Saiki, Y. *Phytochemistry* **1988**, *27*, 2813-2815.
85. Kumar, N. Hah, S.; Kalla, A. K.; Dhar, K. L. *Phytochemistry* **1986**, *25*, 250-254.
86. Kamiya, K.; Satake, T.; Saiki, Y. *Phytochemistry* **1997**, *44*, 141-144.
87. Ikuta, A.; Kamiya, K.; Satake, T.; Saiki, Y. *Phytochemistry* **1995**, *38*, 1203-1207.

References

88. Lins, A.P.; Yoshida, M.; Gottlieb, O. R.; Gottlieb, H. E.; Kubitzki, K. *Bull. Soc. Chim. Belg.* **1986**, *95*, 737-748.
89. Kim, H. J.; Chang, E. J.; Cho, S. H. *Arch. Pharmacol. Res.* **2002**, *25*, 293-299.
90. Wang, R. Mihashi, K.; Cosentino, L. M.; Morris-Natschke, S. L. *J. Asian Nat. Prod. Res.* **2006**, *8*, 277-280.
91. Xiao, K. Z.; Zhang, Z. Y.; Su, Y. M.; Liu, F.Q.; Yan, Z. Z. *Nat. Prod. Res.* **2008**, *22*, 1614-1619.
92. Markham, K. R.; Andersen, O. M. *Phytochemistry* **1990**, *29*, 3919-3920.
93. Budzianowski, J. *Phytochemistry* **1991**, *30*, 1679-1682.
94. Wu, H.; Dushenkov, S.; Ho, C. T.; Sang, S. *Food Chem.* **2009**, *115*, 592-595.
95. Yu, J. Elix, J.; Iskander, M. *Phytochemistry* **1990**, *29*, 3859-3863.
96. Guo, D. Ye, G.; Guo, H. *Fitoterapia* **2006**, *77*, 613-614.
97. Robertson, A. j. *cell. sci.* **1932**, 2770-2771.
98. Lin, H. C.; Ding, H. Y.; Wu, T. S. *J. Nat. Prod.* **1998**, *61*, 343-346.
99. Tanaka, T.; Fukumori, M.; Ochi, T.; Kouno, I. *J. Nat. Prod.* **2003**, *66*, 759-763.
100. Kostova, I. N.; Simeonov, M. F.; Todorova, D. I. *Phytochemistry* **1998**, *47*, 1303-1307.
101. Wang, H. B.; Gu, W. F.; Chu, W. J.; Zhang, S.; Tang, X. C.; Qin, G. W. *J. Nat. Prod.* **2009**, *72*, 1321-1324.
102. Kostova, I. N.; Simeonov, M. F.; Todorova, D. I.; Petkova, P. L. *Phytochemistry* **1998**, *48*, 511-514.
103. Shimizu, M.; Hayashi, T.; Morita, N. *Chem. Pharm. Bull.* **1983**, *31*, 577-583.

References

104. Yoshikawa, M.; Hayashi, E.; Kawaguchi, A. *Chem. Pharm. Bull.* **1993**, *41*, 630-632.
105. Yoshikawa, M.; Murakami, M.; Saka, H.; Shimada, H.; Matsuda, J. *Chem. Pharm. Bull.* **1994**, *42*, 736-738.
106. Hayashi, T.; Shinbo, T.; Shimizu, M. *Tetrahedron. Lett.* **1985**, *26*, 3699-3702.
107. Liu, J.; Ma, Y.; Wu, D.; Lu, Y.; Shen, Z.; Zheng, Q.; Chen, Z. *Biosci., Biotechnol. Biochem.* **2000**, *64*, 1511-1514.
108. Wang, H. B.; Gu, W. F.; Chu, W. J.; Zhang, S.; Tang, X. C.; Qin, G. W. *J. Nat. Prod.* **2009**, *72*, 1321-1324.
109. Kim, J. S.; Yean, M. H.; Lee, J. Y.; Kim, Y. J.; Lee, E. J.; Lee, S. Y.; Kang, S. S. *Helv. Chim. Acta.* **2008**, *91*, 85-89.
110. Kadota, S.; Terashima, S.; Basnet, P. *Chem. Pharm. Bull.* **1993**, *41*, 487-490.
111. Nonaka, G.; Ishimatsu, M.; Tanaka, T.; Nishioka, I.; Nishizawa, M.; Yamagishi, T. *Chem. Pharm. Bull.* **1987**, *35*, 3127-3131.
112. Kandil, F. E.; El-Sayed, N. H.; Micheal, H. N.; Ishak, M. S. *Phytochemistry* **1996**, *42*, 1243-1245.
113. Baumgartner, R. R.; Steinmann, D.; Heiss, E. H.; Atanasov, A. G. *J. Nat. Prod.* **2010**, *73*, 1578-1581.
114. Nawaz, H. R.; Malik, A.; Khan, P. M.; Shujaat, S. *Chem. Pharm. Bull.* **2000**, *48*, 1771-1773.
115. Tanaka, T.; Nakaya, K.; Iinuma, M.; Takahashi, Y.; Naganawa, H.; Ohyama, M. *Phytochemistry* **2000**, *54*, 63-69.
116. Bohlmann, F.; Grenz-M, J.; Ulubelen, A. *Phytochemistry* **1984**, *23*, 1338-1339.

References

117. Stosic, D.; Gorunovic, M.; Skaltsounis, A. I.; Tillequin, F.; Koch, M. *Helv. Chim. Acta.* **1988**, *71*, 348-353.
118. Ferreres, F.; Tomas-Barberan, F. A.; Tomas-Lorente, F.; Nieto, J. L.; Rumbero, A.; Olias, J. M. *Phytochemistry* **1989**, *28*, 1901-1903.
119. Pomilio, A. B. *Phytochemistry* **1973**, *12*, 218-220.
120. Ali, S. Yasmeen, S.; Afza, N.; Malik, A. I.; Iqbal, L.; Lateef, M.; Raiz, N.; Ashraf, M. *J. Asian Nat. Prod. Res.* **2009**, *11*, 457-464.
121. Wu, S.H. *Chin. Chem. Lett.* **2001**, *12*, 345-346.
122. Li, X.; Li, S.; Pu, J.; Huang, S.; Sun, H. *Yunnan Zhiwu Yanjiu* **2007**, *29*, 259-262.
123. Riaz, N.; Nawaz, S. A.; Mukhtar, N.; Malik, A. I.; Afza, N.; Ali, S.; Ullah, S.; Muhammad, P. *Nat. Prod. Res.* **2003**, *17*, 247-251.
124. Iinuma, M.; Ohyama, M.; Tanana, T.; Lang, F. A.; *Phytochemistry* **1994**, *37*, 1157-1159.
125. Oshima, Y.; Kamijou, A.; Ohizumi, Y.; Niwa, M.; Ito, J.; Hisamichi, K.; Takeshita, M. *Tetrahedron* **1995**, *51*, 11979-11986.
126. *In Aldrich Library of ¹³C and ¹H FT NMR Spectra 1992*; Vol. 2, p 851.
127. Rigano, D.; Formisano, C.; Basile, A.; Lavitola, A.; Senatore, F.; Rosselli, S.; Bruno, M. *Phytother. Res.* **2007**, *21*, 395-397.
128. Uddin, N. studies on the bioactive constituents of *Lawsonia alba* (Henna) (Part-A) and analysis of salicylic acid and its derivatives using MALDI-MS and HPLC-ESI-MS (Part-B). Ph.D. Thesis, University of Karachi, Karachi, 2011.
129. Nishizawa, M.; Yamagishi, T.; Nonaka, G.; Nishioka, I. *J. Chem. Soc. Perkin Trans.* **1982**, *1*, 2963-2968.

References

130. Nazrul-Hisham, M.; Mohd-Lip, J.; Mohd-Noh, J.; Normah, A. *J. Trop. Agric. Fd. Sc.* **2011**, 39, 279-284.
131. Braca, A.; Keim, P.; Yen, P.;Nhien, N.; Quang, T.; Cuong, N.; Minh, C. *Fitoterapia* **2008**, 79, 117-120.
132. Eldahshan, O. A. *Curr. Res. J. Biol.Sci.* **2011**, 3, 52-55.
133. Mok, S. Y.; Kim, H. M.; Lee, S. *Hort. Environ. Biotechnol.* **2013**, 54, 450-455.
134. Matsuda, H.; Ohta, T.; Kawaguchi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, 49, 69-72.
135. Blois, M. S. *Nature* **1958**, 26, 1199-1200.
136. Yildirim, A.; Mavi, A.; Kara, A. A. *J. Sci. Food. Agric.* **2001**, 49, 4083-4089.
137. Carron, R. A.; Maran, J. M.; Montero, L.; Fernandezlago; Dominguez, A. *Plantas. Medicinales et Phytotherapie* **1987**, 21, 195-202.
138. Choudhary, M. I.; Dur-e-Shahwar; Perveen, Z.; Jabbar, A.; Ali, I.; Atta-ur-Rahman. *Phytochemistry* **1995**, 40, 1243-1246.
139. Meyer, B.N.; Ferrigni, N. R.' Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, 45, 31-34.
140. Weatherburn, M. W. *Anal. Chem.* **1967**, 39, 971-974.
141. Trager, W.; Jensen, J. B. *Science.* **1976**, 193, 673-675.
142. Makler, M. T.; Rase, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. *Am J Trop Med Hyg.* **1993**, 48, 739-741.
143. Sternb, H.; Yeo N.; *Flora of West Pakistan* **1977**, 108, 4-6.
144. Rajbhandari, M.; Wegner, U.; Schopke, T.; Lindequist, U.; Mentel, R. *Pharmazie* **2003**, 58, 268-271.

References

145. Rajbhandari, M.; Wegner, U.; Julich, M.; Schopke, T.; Mentel, R. *J. Ethnopharmacol.* **2001**, *74*, 251-255.
146. Saijyo, J.; Suzuki, Y.; Okuno, Y.; Yamaki, H.; Suzuki, T.; Miyazawa, M. *J. Oleo. Sci.* **2008**, *57*, 431-5.
147. Garimella, T. S.; Jolly, C. I.; Narayanan, S. *Phytother. Res.* **2001**, *15*, 351-355.
148. Joshi, V. S.; Parekh, B. B.; Joshi, M. J.; Vaidya, A. D. *Urol. Res.* **2005**, *33*, 80-86.
149. Ballabh, B.; Chaurasia, O. P.; Ahmed, Z.; Singh, S. B. *J. Ethnopharmacol.* **2008**, *118*, 331-339.
150. Chen, X. M.; Yoshida, T.; Hatanc, T.; Fukushima, M.; Okuda, T. *Phytochemistry* **1987**, *26*, 515-517.
151. Kursar, C.; Coley, P. D.; Solis, P. N.; Gupta, M. P. *J. Nat. Prod.* **2003**, *52*, 858-860.
152. Sakar, M. K.; Berkman, M. Z.; Calis, I.; Ruedi, P. *Fitoterapia* **1991**, *62*, 176-177.
153. Lutterbach, R.; Stöckigt, J. *Helv. Chim. Acta.* **1992**, *75*, 2009-2011.
154. Machida, K.; Kikuchi, M. *Chem. Pharm. Bull.* **1993**, *41*, 248-251.
155. Posternak, T.; Dürr, K. *Helv. Chim. Acta.* **1958**, *41*, 1159-1162.
156. Yoshida, T.; Seno, K.; Takama, Y. *Phytochemistry* **1982**, *21*, 1180-1182.
157. Taneyama, M.; Yoshida, S.; Michio, K. *Phytochemistry* **1983**, *22*, 1053-1054.
158. Frick, W.; Schmidt, R. R. *Carbohydr. Res.* **1991**, *209*, 101-107.
159. Piacente, S. Pizza, C.; Tommasi, N. D.; Mahmood, N. *J. Nat. Prod.* **1996**, *59*, 565-569.
160. Sumino, M.; Sekine, T.; Ruangrunsi, N.; Igarashi, K.; Ikegami, F. *Chem. Pharm. Bull.* **2002**, *50*, 1484-1487.

References

161. Nawwar, M. A.; Sahar, A.; Hussein, M. *Phytochemistry* **1994**, *36*, 1035-1037.
162. Rubens, F. V.; Wangner, F. *Spectrochimica Acta Part A* **2005**, *61*, 1985-1990
163. Malterud, K. E.; Bremnes, T. E.; Faegri, A.; Moe, T. *J. Nat. Prod.* **1985**, *48*, 559-563.
164. Fuji, M.; Miyaichi, Y.; Tomimori, T. *Nat. Med.* **1996**, *50*, 404-407.
165. Saito, K.; Matsumoto, M.; Sekine, T.; Murakoshi, I.; Morisaki, N.; Iwasaki, S. *J. Nat. Prod.* **1989**, *52*, 1221-1226.
166. Tanaka, T.; Nonaka, G.; Nishioka, I. *Phytochemistry* **1983**, *22*, 2575-2578.
167. Finger, A.; Engelhardt, U. H.; Wray, V. *Phytochemistry* **1991**, *30*, 2057-2060.
168. Haddock, E.A. Gupta, R. K.; Al-Shafi, S. M. K.; Haslam, E. *J. Chem. Soc., Perkin Trans.* **1982**, 2515.
169. Chandraredy, U. D.; Reddy, C.; Amrik, S.; Chawla. *Phytochemistry* **1998**, *47*, 907-909.
170. Chen, X. M.; Yoshida, T.; Hatanc, T.; Fukushima, M.; Okuda, T. *Phytochemistry* **1987**, *26*, 515-517.
171. Weon, J. B.; Lee, B. H.; Yun, B. R.; Ma, C. J. *Planta Med.* **2012**, *78*, 71.
172. Parker, W. H.; Bohm, B. A. *Phytochemistry* **1975**, *14*, 553-555.
173. Mehla, K.; Balwani, S.; Sulshreshtha, A.; Nandi, D. *J. Ethnopharmacol.* **2011**, *137*, 1245-1352.
174. Yu, J. Elix, J.; Iskander, M. *Phytochemistry* **1990**, *29*, 3859-3863.
175. Wang, K. J.; Yang, C. R.; Zhang, Y. *Food Chem.* **2007**, *101*, 365-371.

List of Publications

Uddin, G, **Sadat, A**, Siddiqui, B.S. Comparative antioxidant and antiplasmodial activities of 11-O-galloylbergenin and bergenin isolated from *Bergenia ligulata*. *Tropical Biomedicine* 2014, 31(1); 143-148

<http://www.msptm.org/files/143 - 148 Uddin G.pdf>

Ghias Uddin, Muhammad Alam, Naveen Muhammad, Bina S. Siddiqui and **Anwar Sadat**. Bioassay-guided isolation of an antinociceptive, anti-inflammatory and antipyretic Benzofuran Derivative from *Viburnum grandiflorum*. *Pharmaceut. Anal. Acta* 2013, 4-10

<http://dx.doi.org/10.4172/2153-2435.1000274>

Ghias Uddin, **Anwar Sadat**, Bina Shaheen Siddiqui. Phytochemical Screening, in vitro Antioxidant and Antimicrobial Activities of the crude fractions of *Paeonia emodi* Wall. Ex Royle. *Middle-East Journal of Scientific Research* 2013, 17(3): 367-373.

<http://www.idosi.org/mejsr/mejsr17%283%2913/17.pdf>

Ghias Uddin, Ashfaq Ahmad Khan*, Muhammad Alamzeb, Saqib Ali, Mamoon-Ur-Rashid, **Anwar Sadat**, Muhammad Alam, Abdul Rauf and Wali Ullah. Biological screening of ethyl acetate extract of *Hedera nepalensis* stem. *African Journal of Pharmacy and Pharmacology* 2012, 6(42); 2934-2937.

<http://www.academicjournals.org/AJPP>.

Ghias Uddin, Waliullah, Bina Shaheen Siddiqui, Muhammad Alam, **Anwar Sadat**, Ashfaq Ahmed and AlaUddin "Chemical Constituents and Biological Screening of *Grewia optiva* Drummond ex Burret Whole Plant. *Middle-East Journal of Scientific Research*, 2011, 8(1); 85-91.

<http://www.idosi.org/mejsr/mejsr8%281%2911/15.pdf>

Muhammad Alam, Ghiasuddin, **Anwar Sadat**, Naveed muhammd, Ashfaq Ahmad Khan and Bina S. Siddiqui "Evaluation of *Viburnum grandiflorum* for its in-vitro pharmacological screening." *African Journal of Pharmacy and Pharmacology* 2012, 6(22); 1606-1610.

http://www.academicjournals.org/article/article1380795680_Alam%20et%20al.pd