CHEMICAL AND BIOLOGICAL STUDIES OF EXTRACTS OF MEDICINAL PLANTS

SUMMARY

THESIS

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Summary

The present thesis entitled "Chemical and biological Studies of Extracts of Medicinal Plants", is divided into six chapters. The chapter first includes a critical review of the plant chemistry of natural products with special reference to flavonoids, coumarins and terpenoids and high lighted the recent advances in the analytical techniques applied for the isolation of natural products and their structure elucidation.

The chapters second to fifth deal with the isolation and identification of naturally occurring compounds from the following four indigenous medicinally important plants. Overall thirty constituents have been isolated, out of which eight are being reported for the first time. Their structures have been established by chemical and physical data (IR, $^1$H NMR, $^{13}$C NMR, DEPT-135, UV and MS spectra). The chapter sixth deals with the biological studies of the following plants and some of their new products.

A. *Ficus benjamina* (Var. comosa)
B. *Xylosma longifolium*
C. *Crataegus crenulata*
D. *Rhus alata*

**Chapter Second: Chemical Constituents from the leaves of Ficus benjamina (Var. comosa).**

Following six compounds have been isolated and characterized from *Ficus comosa.*

**Fc-1: pentacontanyl decanoate**

\[
\text{M. F.} \quad C_{60}H_{120}O_2 \\
\text{M. P.} \quad 265^\circ C \\
\text{M. W.} \quad 872
\]

(not observed)

Crystallized from chloroform-acetone as white granular crystals.
Summary

Structural elucidation is based on spectral studies, IR, $^1$H NMR, and MS.

**Fc-2 : $\beta$-sitosterol**

M. F. C$_{29}$H$_{50}$O

M. P. 136-37°C

M. W. 414

Crystallized from chloroform-ethanol afforded white needle shaped crystals.

Ac$_2$O/Pyridine (mild)-Monoacetate

M. P. 114-15°C

Crystallized from chloroform-methanol as colorless flakes.

Benzoyl chloride/pyridine – Benzoate

M. P. 145-46°C

Crystallized from methanol

Structure has been established on the basis of spectral studies, IR, $^1$H NMR, and MS.

**Fc-3 : Friedelin**

M. F. C$_{30}$H$_{50}$O

M. P. 262-64°C

M. W. 426
Crystallized with chloroform-methanol as white crystalline solid.

Structure has been elucidated with the help of spectral studies, IR, $^1$H NMR and MS.

**Fc-4**: (9,11),(18, 19)-disecoolean-12-en- 28-oic acid (new compound)

M. F. C$_{30}$H$_{52}$O$_2$

M. P. m.p. 195°C

M. W. 444

Crystallization in chloroform -methanol yielded white crystals.

Structural elucidation is based on modern spectral techniques i.e. IR, $^1$H NMR, and MS.

**Fc-5**: Serrata-3-one (New compound)

M. F. C$_{30}$H$_{50}$O

M. P. m.p. 230°C

M. W. 426

Crystallized from chloroform-methanol as colourless crystals (40 mg). Its structure has been elucidated on the basis of spectral studies, IR, $^1$H NMR, $^{13}$C NMR, DEPT-135 and MS.
Fc-6 : β-amyrrin

M. F. C_{30}H_{50}O.

M. P. m.p. 198°

M. W. 426

Crystallized with chloroform- methanol as white crystalline solid.

Structural elucidation is based on spectral studies, IR, \(^1\)H NMR and MS.

Chapter Third: Chemical Constituents from the leaves of Xylosma longifolium

Following ten compounds have been isolated and characterized from Xylosma longifolium.

XI-1: n-Hentriacontane

M.F. C_{31}H_{64} \quad \text{CH}_3 (\text{CH}_2)_{29} \text{CH}_3

M. P. 68°C

M.W. 436

Crystallized with chloroform-ethanol as colorless crystals (110 mg). Structure has been established on the basis of spectral studies, IR, \(^1\)H NMR, and MS.

XI-2 : β-sitosterol

M. F. C_{29}H_{50}O

M. P. 133-35°C

M. W. 414
Summary

Crystallized from chloroform-ethanol afforded white crystalline solid (80 mg).

Structure has been established on the basis of spectral studies, IR, $^1$H NMR, and MS.

**XI-3 : β-amyrrin**

M. F. $C_{30}H_{50}O$.

M. P. m.p. 192-93°C

M. W. 426

Crystallized with chloroform- methanol as shining needles (120 mg).

Structural elucidation is based on spectral studies, IR, $^1$H NMR and MS.

**XI-4 : Friedelin**

M. F. $C_{30}H_{50}O$

M. P. 262-63°C

M. W. 426

Crystallized with chloroform-methanol as white crystalline solid.

It has been characterized with the help of spectral studies, IR, $^1$H NMR and mass.
Summary

**XI-5: Olean-12-en-3α-ol-28-oic acid 3′α-D-glucopyranoside**

(new-compound)

M. F. $C_{36}H_{58}O_8$

M. P. 213-214°C

M. W. 618

Crystallized from CHCl₃ – MeOH to give colorless shining crystals (100 mg).

Structural elucidation is based on the basis of spectral studies, IR, UV, \(^1\)H NMR, \(^13\)C NMR and MS.

**XI-6: Kaempferol**

M. F. $C_{15}H_{10}O_6$

M. P. 276-278°C

M. W. 286

Crystallized from chloroform–methanol as yellow shining crystals.

Acetylated with Ac₂O/pyridine (mild)-tetra acetate

m.p. 180-182°C

Its structure has been elucidated with the help of spectral studies, IR, UV, \(^1\)H NMR and MS.
Summary

XI-7: Quercetin

M. F. C_{15}H_{10}O_{7}

M. P. 311-12\degree C

M.W. 302

Crystallized in chloroform–methanol as yellow shining crystals (120 mg).

Acetylated with Ac_{2}O/pyridine (mild)-Pentaacetate

m.p. 194-95\degree C

Crystallized from ethyl acetate as cream colored crystals

Structural elucidation is based on spectral studies, IR, UV, ^1H NMR and MS.

XI-8: Kaempferol-3-rhamnoside

M. F. C_{20}H_{20}O_{10}

M. P. 177-78\degree C.

M.W. 420

Crystallized in methanol–chloroform yield yellow granular crystals (100 mg).

Acetylated with Ac_{2}O/pyridine (mild)-hexa acetate

m. p. 159-60\degree C

Structural elucidation is based on spectral studies, IR, UV, ^1H NMR and MS.
**Summary**

**XI-9 : Quercetin-3-rhamnoside**

M. F. \( \text{C}_{21}\text{H}_{20}\text{O}_{11} \)

M. P. \( 186-87^\circ \text{C} \)

M. W. 448

XI-8 on crystallization with methanol gave yellow microscopic needles \( (110 \text{ mg}) \).

Structural elucidation is carried out on the basis of spectral studies, UV, \(^1\text{H} \) NMR and MS.

Acetylated with \( \text{Ac}_2\text{O}/\text{pyridine (mild)-hepta acetate} \)

m. p. 302-03^\circ \text{C}

Structural elucidation is based on spectral studies, IR, UV, \(^1\text{H} \) NMR and MS.

**XI-10 : kaempferol-3-β-xylopyranoside-4'-α-rhamnoside**

(new)

M. F. \( \text{C}_{26}\text{H}_{28}\text{O}_{14} \)

M. P. \( 265-67^\circ \text{C} \).

M. W. 568

\( \text{R}_1, \text{R}_2=\text{H}, \text{R}_3=\text{rham}, \text{R}_4=\text{xyl} \)

XI-10 could not be induced to crystallize. It gave pale yellow granules \( (220 \text{ mg}) \).

Structural elucidation is carried out on the basis of spectral studies, IR, UV, \(^1\text{H} \) NMR and MS.

Acetylated with \( \text{Ac}_2\text{O}/\text{pyridine (mild)-octa-acetate} \)

m.p. 126-28^\circ \text{C}. 
Chapter Fourth: Chemical constituents From the Leaves of Crataegus crenulata

Following seven compounds have been isolated and characterized from *Crataegus crenulata*

Cc-1: Lanost-5-en-2β,3β-diol-21-oic acid
(New Compound).

M. F. C_{30}H_{50}O_{4}

M. W. 474

M. P. 249-250°C

It was crystallized from chloroform-methanol as colorless crystals (100 mg). Structural elucidation is done on spectral studies, IR, \textsuperscript{1}H NMR and MS.

Cc-2: β-sitosterol-D-glucoside.

M. F. C_{35}H_{60}O_{6}

M. W. 576

M. P. 282°C

It was crystallized from chloroform-methanol as colourless crystals (150 mg).

Acetylated with Ac_{2}O/pyridine (mild)-tetra acetate.

Crystallization from methanol-chloroform afforded colorless crystals (30 mg), m.p. 166°C.

Structural elucidation is done on spectral studies \textsuperscript{1}H NMR and MS.
Summary

Cc-3: 5,7,3',5'-tetrahydroxy flavanone

M. F.  C₁₅H₁₂O₆

M.W.  288

M. P.  230°C

It gave cream color prisms colorless prisms (160 mg) with chloroform-acetone mixture. Structural elucidation is done on spectral studies, IR, UV, ¹H NMR, ¹³C NMR and MS.

Cc-4: Acacetin-7-O-neohesperidoside

M. F.  C₂₈H₃₂O₁₄

M.W.  592

M. P.  266-268°C

It was obtained from methanol-chloroform mixture as light yellow crystals (270).

Acetylated with Ac₂O/pyridine (mild)-hepta acetate.

It was crystallized from ethyl acetate-petroleum ether as white crystals

m.p. 216-20°C

Structural elucidation is done on spectral studies, IR, UV, ¹H NMR and MS.

Cc-5: Vitexin

M. F.  C₂₁H₂₀O₁₀

M.W.  432

M. P.  263-64°C

Structural elucidation is done on spectral studies, IR, UV, ¹H NMR and MS.
Summary

It was crystallized from methanol-chloroform mixture as yellow needles (250 mg).

Acetylated with Ac₂O/pyridine (mild)-hepta acetate

m.p. 154-56⁰C

Structural elucidation is based on spectral studies, IR, UV, ¹H NMR and MS.

Cc-6: Dalpanitin

M. F. C₂₂H₂₃O₁₁
M.W. 462
M. P. 213-214⁰C

It was crystallized from methanol mixture as yellow plates.

Acetylated with Ac₂O/pyridine (mild)-hepta acetate.

It was crystallized from chloroform-methanol mixture as colorless crystals, m.p. 134⁰C

Structural elucidation is done on spectral studies, IR, UV, ¹H NMR and MS.

Cc-7: 5,7,5'-trihydroxy-3'-C-β-D-glucopyranosyl flavanone (cranulatin)

M. F. C₂₁H₂₂O₁₀
M.W. 434
M. P. 278-79⁰C

It was crystallized with methanol-chloroform and afforded light yellow crystals (200 mg).

Structural elucidation is done on spectral studies, IR, UV, ¹H NMR, ¹³C NMR and MS.
Chapter Fifth: *Chemical constituents From the Leaves of Rhus alata.*

Following seven compounds have been isolated and characterized from *Rhus alata*:

**Ra-1: Dimethylester of terephthalic acid.**

- M. F. $\text{C}_{10}\text{H}_{10}\text{O}_4$
- M. P. 138°C
- M. W. 194

Crystallized from petroleumether-chloroform as white needles (100 mg). Its structure has been elucidated on the basis of spectral studies, IR, UV, $^1\text{H}$ NMR and MS.

**Ra-2: Lupeol**

- M. F. $\text{C}_{30}\text{H}_{50}\text{O}$
- M. W. 426
- M. P. 214°C

It was crystallized from chloroform-methanol as crystalline solid (170 mg).

Structural elucidation is done on spectral studies, IR, $^1\text{H}$ NMR and MS.

**Ra-3: Oleanolic acid**

- M. F. $\text{C}_{30}\text{H}_{48}\text{O}_3$
- M. W. 456
- M. P. 299-300°C

It was crystallized from chloroform-methanol as white needle-shaped crystals (225 mg). Structural elucidation is done on spectral studies, IR, $^1\text{H}$ NMR and MS.
Summary

Ra-4: Taraxerone

M. F. C_{30}H_{48}O

M.W. 424

M. P. 240°C.

It was crystallized from chloroform-ethanol as white needles (170 mg). Structural elucidation is done on spectral studies, IR, ^1H NMR and MS.

Ra-5: Ethyl gallate

M. F. C_{9}H_{10}O_{5}

M.W. 195

M. P. 155°C

It was crystallized from chloroform-methanol as reddish buff needles (120 mg). Structural elucidation is done on spectral studies, IR, ^1H NMR and MS.

Ra-6: (2E)-3-(4-hydroxy-5,7-dimethoxylbenzo[3,4-b]furan-6-yl oxy)-prop-2-enoic acid (new compound)

M. F. C_{13}H_{12}O_{5}

M.W. 248

M. P. 155°C

It was crystallized from chloroform-methanol as white crystals (190 mg). Structural elucidation is done on spectral studies, IR, ^1H NMR, ^13C-NMR and MS.
Ra-7: Arbutin

M. F. C₁₂H₁₆O₇
M.W. 272
M. P. 199-200°C

Crystallized from chloroform-acetone as colourless needles (120 mg). Structural elucidation is done on spectral studies, IR, UV, ¹H NMR, ¹³C-NMR and MS.

Chapter sixth: Chapter sixth deals with the following pharmacological activities.

A. Antinociceptive activity of extracts of Ficus comosa, Xylosma longifolium, Crataegus crenulata and Rhus alata.

B. Antibacterial and antifungal activity of Ficus comosa, Xylosma longifolium, Crataegus crenulata and Rhus alata and some of their compounds.

C. Insecticidal activity of extracts of Ficus comosa, Xylosma longifolium, Crataegus crenulata and Rhus alata.
Dedicated to
My
Worthy parents
Certificate

This is to certify that work embodied in this thesis entitled “Chemical and Biological Studies of the Extracts of Medicinal Plants”, is the original work carried out by Mr. Raza Murad Ghalib under my supervision. The thesis is suitable for submission for the award of Ph.D. degree in Chemistry.

Dr. Mehtab Parveen
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All the praise to Allah, the lord of the Universe, who says in his glorious book, Kalam –e-Majeed, “There has come to you from Allah a light and a plain book,” and peace and blessings of Allah be upon the noblest of the prophets and messengers, our prophet Mohammed who has said, “you should insist on acquiring knowledge even if you have to travel up to China.” And “seek knowledge from cradle to grave.”

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Chapter I
Introduction
Utilization of plants for medicinal purposes in India has been documented long back in ancient literature. Plants continue to be used world wide for the treatment of diseases and novel drug entities continue to be developed through research into their constituents. India has luminous past in following human health management services for using the drug derived from plants since the beginning of civilization. First documented record of medicinal literature is available in Rig-Veda (3500-1620 BC). Curing property of the medicinal plants is directly related with chemical substances deposited in various parts of the plant. The medicinal plants and their parts used as such on commercial scale for drug formulation are called as crude or raw materials. There is evidence that a large number of microbial pathogens have become drug resistant and herbal medicine may provide an alternative avenue in this regard. Out of about 2000 items recorded in Indian literature, less than 200 are of minerals and animals origin, the rest are derived from vegetable sources. Indian systems in different parts of India tried to utilize the locally growing plants as far as possible and accepted those which gave promising effects for various ailments. Systematic investigation of drugs used in indigenous medicine in India on modern scientific line was started more than thirty years ago. A number of medicinal plants prescribed by Kavirajas and Kakins have been investigated. The extracts have been examined for pharmacological activity. The active principles worked out by animal experimentation and preparations made from the drugs have been tested on patients in hospitals.

It is only by a thorough enquiry that the merit of these drugs can be proved and a demand created for them not only in India but also in other parts of the World. A large number of drugs examined have been shown to possess significant activity and can however be used as substitutes for official drugs. Plants have always been common source of medication either in the form of traditional preparations or as pure active principles. In a survey done by WHO it has been estimated that 80% of more than 4000 million inhabitants of the World rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles. In the developed countries too, plants derived drugs are important. Farnsworth et al
pointed out in their review article that there are at least 119 distinct chemical substances derived from plants that can be considered as important drugs currently in use. Today the importance of crude drugs in therapeutics is much more than it was 25 years ago. The preference to drugs, perfumes, cosmetics, dyes, flavors and food colors obtained from plants sources has been increasing day by day. To exemplify, one of the important plant products is artemisin which is obtained from the flowering tops of the Chinese plant *Quinghao* (*Artemisia annua*). Preliminary clinical trials of a derivative of artemisin have shown that it is effective against strain of *Plasmodium falciparum*, where synthetic anti-malarials have failed to cure the disease. It is useful for treating cerebral malaria. Taxol is proving to be a life saving drug against the cancer ailment of breast and ovary. In most developing countries where coverage by health services is limited, the treatment of the patient is largely based on medicinal plants. Early in 20th century the greater part of medicinal therapy in the industrialized countries was dependent on medicinal plants but with the growth of pharmaceutical industries, their use fell out of favour. However now the value of medicinal plants is receiving distinguished global attention. Due to the wide spread reports of toxicity and health hazards associated with indiscriminate use of synthetic drugs and antibiotics, accompanied by rising cost of organic chemicals and regarding, the basic thinking and attitude of the modern society has been changing since last 20 years. A large number of plants used for health care are rich in polyphenolic compounds. Various types of plant phenols have been shown to possess antioxidant activity based on free radical scavenging activity. The activity may under line various effects of polyphenols in plant tissues and also their medicinal effects. Such as those related to inhibition of lipid peroxidation and tumor promotion. Natural polyphenols are widely used to delay/prevent the oxidation of fats and oils in variety of food. Plant polyphenols comprises flavonoids, alkaloids, coumarins, terpenes and steroids. Biologically active flavonoids are distributed universally among vascular plants and found practically in all parts of plants. Flavonoids not only contribute to plant colour but also physiologically active. Gabor has received trends in research on the pharmacodynamic effects of flavonoids, mostly rutin and its derivatives. With the extensive screening programmes of plant products for anticancer drugs, it has been reported that flavonoids may contribute to or be effective in combating certain
Chapter - 1

types of cancer.\textsuperscript{12,13,17,18} The effect of flavonoids on cancer cells in various pharmacokinetics of a flavonolignan couple has also been studied.\textsuperscript{19} A recent survey of literature showed that the flavonoids field is still very important to chemists and their interest is increasing in screening the new flavonoids and their physiological activities. Flavonoids may also exhibit useful antibacterial activity. Preliminary studies with \textit{Combretum erythrophyllum} showed antimicrobial activity against gram-positive and gram-negative bacteria. Seven antimicrobial flavonoids were subsequently isolated by bioassay guided fractionated i.e. apigenin, genkwanin, 5-hydroxy-7, 4-dimethoxy flavone, rhamnocitrin, kaempferol, quercetin 5, 3’-dimethyl ether, rhamnatin. All the compounds have good activity against vibrio cholerae and anterococcus faucalis, with MIC values in the range of 25-50 mg/ml. Rhamnocitrin and quercetin-5, 3-dimethyl ether also inhibited \textit{Micro coccus luttus} and \textit{Shigella sorei} at 25 μg/ml, with the exception of 5-hydro-7,4-dimethoxy flavone the flavonoids were not toxic towards human lymphocytes. This compound is potentially toxic towards human cells and exhibited the poorest anti-oxidant activity whereas rhamnocitrin and rhamnazin exhibited strong antioxidant activity. Genkwanin, rhamnocitrin, quercetin-5,3’-dimethyl ether, rhamnazin had a higher anti-inflammatory activity than the positive control mefenamic acid.\textsuperscript{20} several flavonoids have been shown to have potential as hepatoprotective agents\textsuperscript{21}, such as dihydro kaempferol-3-rhamnoside and 5,7,3’,5’-tetrahydroxy flavononol-3-rhamnoside showed some hepatoprotective activity.\textsuperscript{22} The diprenylisoflavones, 6,8-diprenylgenistein, 6,3’-diprenylgenistein and derrisisoflavone were found to be active antifungal agents against the human pathogen, \textit{Trichophyton mentagrophytes}.\textsuperscript{23}

In the present study, we have tried to carryout systematic chemical investigation of important indigenous medicinal plants with a view to characterize their chemical components preferably flavonoids & terpenoids which could be starting point for the chemists who are mainly working on pharmaceutical and clinical aspects of these herbal drugs.

A recent survey of literature showed that the flavonoids and terpenoids field is still very popular to the chemists and their interest is increasing in isolating new flavonoids and their physiological activity. A large number of naturally occurring new
and novel flavonoids and terpenoids are added to literature every year. Few of the recently isolated flavonoids and terpenoids are listed for ready reference to the studies reported in the thesis.

1. **Flavones:**

   Kittisak Likhitwitayawuid *et al.*\(^{24}\) have isolated a new furano flavone named as 3,5'-dimethoxy-[2", 3": 7, 8]-furano-flavone (I) from *Millettia erythrocalyx*.

   \(\text{[Chemical structure of (I)]}\)

   Rakesh Maurya *et al.*\(^{25}\) have isolated 4',7-dihydroxy-3'-methylflavone (II) from *Boeerhavia diffusa*.

   \(\text{[Chemical structure of (II)]}\)
2. Flavone C-Glycosides:

X.A.Wu, et al.\textsuperscript{26} have isolated a new C-glycosyl flavone (III) from \textit{Tortollis ledebouri reichb}.

\begin{center}
\includegraphics[width=0.5\textwidth]{flavone_c_glycoside}
\end{center}

(Ill)

3. Prenylated flavones

Jose I.de Souza et al.\textsuperscript{27} have isolated three new prenylated flavones, identified as 5,7,5'-trimethoxy-6-(3''-hydroxy,3''-methyl-trans-but-1''-enyl)-3',4'-methylenedioxy flavone (IV), 5,4'-dihydroxy-3',5'-dimethyl-6-7(2'',2''-dimethyl pyran)-flavone (V), and 5,4'-dihydroxy-8,3',5'-trimethoxy-6,7-(2'',2''-dimethyl pyran)-flavone (VI) from the aerial parts of \textit{Neoraputia paraensis}.

\begin{center}
\includegraphics[width=0.5\textwidth]{prenylated_flavones}
\end{center}

(V): $R_1=H$, $R_2=H$, $R_3=H$

(VI): $R_1=\text{OMe}$, $R_2=H$, $R_3=H$
4. **Isoflavone:**

Chihiro Ito *et al.*\(^{28}\) have isolated new isoflavone (VII) from *Millettia pachycarpa*.

\[
\text{(VII)} \\
\]

5. **Serratone triterpene:**

H. Shi *et al.*\(^{29}\) have isolated a new serratone triterpene (VIII) from *Lycopodium phlegmaria*.

\[
\text{(VIII)} \\
\]
6. **Flavanone:**

N.P. Reddy *et al.*\(^{30}\) have isolated a new flavanone named as (2S)-5,7,3',4'-tetramethoxy flavanone (IX) from *Limnophila indica*.

![Diagram of IX](image)

(IX)

Bina S. Siddiqui *et al.*\(^{31}\) have isolated a new flavanone as azharone [5,7,4'-trihydroxy-3'-(3''-methyl-2''-3''-epoxybutyl) flavone-4-one] (X) from the flowers of *Azadirachta indica*.

![Diagram of X](image)

(X)

7. **Prenyl flavanones:**

Maria L. Uriburu, *et al.*\(^{32}\) have isolated three prenyl flavanones namely (2S)-8-(3''-methyl butyl-2''-enyl)-7,3',4'-trihydroxyflavanone (XI), (2S)-8-(3''-methyl-4''-hydroxy-but-2''-enyl)-7,3',4'-trihydroxyflavanone (XII) and (2S)-8-(3''-methyl-4''-hydroxy-but-2''-enyl)-5,3',4'n-trihydroxy-7-methoxyflavanone (XIII) from *Flouresia fiebrigii*. 
Zhongxiang Zhao et al.\textsuperscript{33} have isolated flavan-4-ol-glycosides compound (2S,4S) 6,8-dimethyl-7-hydroxy-4'-methoxy-4,2'-oxidoflavan-5-O-\(\beta\)-D-6-acetyl-glucopyranoside (XIV), (2S,4S)-6,8-dimethyl-7-hydroxy-4'-methoxy-4,2''-oxidoflavan-5-O-\(\beta\)-D-3-O-acetyl-glucopyranoside (XV), (2S,4S)-6,8-dimethyl-7-hydroxy-4'-methoxy-4,2''-oxidoflavan-5-O-\(\beta\)-glucopyranoside (XVI) from the rhizomes of \emph{Abacopteris penangiana}. 

\begin{itemize}
  \item \(\text{(XI)}: \ R_1 = \text{H}, \ R_2 = \text{OH}, \ R_3 = \text{OH}, \ R_4 = \text{H}\)
  \item \(\text{(XII)}: \ R_1 = \text{H}, \ R_2 = \text{OH}, \ R_3 = \text{OH}, \ R_4 = \text{H}\)
  \item \(\text{(XIII)}: \ R_1 = \text{OH}, \ R_2 = \text{OMe}, \ R_3 = \text{OH}, \ R_4 = \text{OH}\)
\end{itemize}
9. Isoflavone-O-Glycoside:

Ming Zhao et al.\textsuperscript{34} have isolated a new isoflavone-O-glycoside (XVII) from \textit{Desmodium styracifolium}.

\[
\text{(XVII)}
\]

10. Chromones:

R. Kumar \textit{et al.}\textsuperscript{35} have isolated a new chromone named as 5,4'-dihydroxy-7-methyl-3-benzylchromone (XVIII) from \textit{Cassia nodosa}.

\[
\text{(XVIII)}
\]

Jeong Seon Yoon \textit{et al.}\textsuperscript{36} have isolated neuroprotective, 5-hydroxy-2(2-phenyl ethyl) chromone (XIX) from \textit{Imperata cylindrica}.

\[
\text{(XIX)}
\]
11. **Chromenes:**

Rodrigo O. Saga Ritamura *et al.*[^1] have isolated two new chromenes [5-hydroxy-8-(3',7'-dimethylocta-2',6'-dienyl)-2,2,7-trimethyl-2H-1-chromene (XX) and 5-hydroxy-8-(3'-methyl-2'-butenyl)-2,2,7-trimethyl 2H-1-chromene-6-carboxylic acid (XXI) from the leaves of *Peperomia serpens.*

![Chemical structures](image)

(XX) (XXI)

12. **Chromone glycoside:**

Y.B. Wang *et al.*[^2] have reported four new chromone glycosides identified as 7-O-β-D-allopyranosyl-5-hydroxy-2-methylchromone (XXII) acetyl-glucopyranosyl-5-hydroxy-2-methyl chromone (XXIII), 7-O-[6-O-(4-O-trans-cafeoyl-β-D-allopyranosyl)]-β-D-glucopyranosyl-5-hydroxy-2-methyl chromone (XXIV), and 7-O-[6-O-(4-O-trans-feruloyl-β-D-allopyranosyl)]-β-D-glucopyranosyl-5-hydroxy-2-methylchromone (XXV) from the whole plant of *Knoxia corymbosa.*

![Chemical structures](image)

(XXII). R= H (XXIII). R= Ac
13. **Chalcone:**

Maria A. Ponce *et al.*\(^{39}\) have isolated a chalcone identified as 2',3',4', 5',6'-pentahydroxy chalcone (XXVI) from *Brassica alba*.

\[(XXVI)\]

14. **Benzofuran:**

S.Y. Shi *et al.*\(^{40}\) have isolated a new dibenzofuran named as 1,2,4-trimethyl-7,8-dimethoxy-dibenzofuran (XXVII) from a Chinese medicinal plant *Ligularia caloxantha*.

\[(XXVII)\]
15. **Flavonol:**

Maria A. Ponce *et al.*\(^{39}\) have isolated a flavonol named as 3,5,6,7,8 pentahydroxy-4'-methoxy flavone (XXVIII) from *Brassica alba*.

![](image)

(XXVIII)

16. **Flavonol glycoside:**

Ilina Krasteva *et al.*\(^{41}\) have isolated a new flavonol glycoside, 7-O-methyl kaempferol - 4'-β-D-galactopyranoside (rhamnocitrin - 4'-β-D-galactopyranoside) (XXIX) from *Astragalus hamosus*.

![](image)

(XXIX)

17. **Kaempferol Furanoside:**

N. Wang *et al.*\(^{42}\) have isolated a new kaempferol glycoside, kaempferol-3-0-β-D-glucopyranoside-7-O-α-L-arabinofuranoside (XXX) from *Pyrrosia petolosa (christ) ching*.
18. **Dihydroflavonols:**

M.T. Islam and S. Tahara\(^4\) have isolated (2R, 3S)-(+)-3',5-dihydroxy-4',7-dimethoxydihydroflavonol (XXXI) and (2R,3R)-(+)-4',5,7-trimethoxy dihydroflavonol (XXXII) from stem bark of *Lannea coromandelica*.

![Chemical Structures](image)

(XXXI)  
(XXXII)

19. **Flavonol tetruglicosides:**

Geoffrey C. Kite *et al.* \(^44\) have isolated two flavonol tetruglicosides (XXXIII, XXXIV) from the leaves of *Styphnolobium japonica*.
20. **Triterpenic acid:**

F. Wang *et al.* have reported triterpenic acid named as $3\beta,6\beta$-dihydroxy-11-oxo-olean-12-en-28-oic acid (XXXV) from *Styrax torkinensis.*
21. **Triterpene glycosides:**

A.A. Majid *et al.* have isolated two new triterpene glycosides identified as 3-O-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl gypsogenic acid (XXXVI) and 3-O-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl hederagenin (XXXVII) from *Caryocar glabrum*.

\[
\begin{align*}
R_1 & \quad R_2 & \quad R_3 & \quad R_4 \\
(XXXVI) & H & COOH & Gal-(1→3)-Glu & H \\
(XXXVII) & H & CH_2OH & Gal-(1→3)-Glu & H
\end{align*}
\]

22. **Triterpenes:**

A.A. El-Jamal isolated two new triterpenes named as 6β-hydroxy-3,20-dioxo-30 norlupane-28-oic acid (XXXVIII) and 3,4-secolup-4,20-dihydroxy-3,28-dioic acid-3-oic acid methyl ester (XXXIX) from *Viburnum awabuki*.

\[
(XXXVIII)
\]
23. **Triterpene lactone:**

Min-Chen and Dao-fengchen\(^{48}\) have isolated a new triterpenoid lactone (LX) named as renchanglactone from *Kadsura renchangiana*. 
Spectroscopic study:

Since mainly the spectroscopic techniques UV, IR, $^1$H-NMR, $^{13}$C-NMR and mass have been used in the identification and structure elucidation of the products isolated from different plants during the course of the present work, a brief review of each technique has been discussed below:

1. **Ultra Violet Spectroscopy:**

The Ultra-Violet spectra of different flavonoids are very characteristic and along with color reactions have been used extensively to distinguish the various groups of this class of the compounds.

U.V. spectroscopy has become a major technique for the structure analysis of flavonoids for two main reasons. The first is that only a small quantity of pure material is required. The second reason is that the structural information obtained about flavonoid from UV is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus. The addition of these reagents separately to an alcoholic solution of the flavonoid induced structurally significant shifts in the UV spectrum. The commonly used shift reagents are sodium methoxide (NaOMe), sodium acetate (NaOAc), sodium acetate/ boric acid (NaOAc / H$_3$BO$_3$), aluminium chloride (AlCl$_3$) and aluminium chloride/ hydrochloric acid (AlCl$_3$ / HCl).

The UV spectra of most flavonoids consist of two major absorption bands. Band I occurs at higher wavelength of 320-380 nm and is associated with ring-B cinnamoyl absorption (LXI). Band II occurs in the range of 240-285 nm and is associated with ring-A benzoyl system (LXII).

(LXI)  

(LXII)
Substitution in ring-B especially at 4'-position stabilizes the cinnamoyl chromophore resulting in a red shift of band I whereas, substitution in ring-A has a similar effect on the position of band II. The presence of a free hydroxyl group at C-5 and C-3 positions is established by measuring the spectra in the presence of AlCl₃. Compounds having a free 5-hydroxy group absorbs at higher wavelength and methylation of this hydroxyl group brings about a blue shift of 1-15 nm of both bands.

The hydroxyl groups at C-7 and 4' position are more acidic than others and their occurrence is established by red shifts of band I and band II on the addition of fused sodium acetate. The presence of a hydroxyl groups at 4’ position is also confirmed by a large red shift in band I without a decrease in intensity on the addition of sodium methoxide. The position of OH group at C-5 is confirmed by a large red shift in band-I on addition of AlCl₃.

In flavanones and isoflavones, due to the absence of cinnamoyl chromophore the high wavelength band (band-I) is either totally absent or present only as an inflection. Thus it is difficult to distinguish between flavanones and isoflavones with the help of UV spectrum alone. The UV spectra of biflavonoids with the only difference that the molecular extinction coefficient (ε) of the biflavone is approximately double as compared to the corresponding monoflavonoids. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonoids.

2. **Infra-red Spectroscopy:**

The IR spectrum in practice, play an important role and offers the first clue to nature of the compound. It provides valuable information of functional groups in a molecule. In the case of flavonoids, IR measurements are helpful in providing evidence for the presence of (a) pyrone ring (b) chelated hydroxyl groups and (c) the geminal dimethyl grouping.

The substitution pattern of the benzene ring can be inferred from bands in the 690-800 cm⁻¹ region. Such evidence is helpful in distinguishing between flavonoids
and coumarins. This spectroscopic method has mostly been used to adduce corroborative evidence.

The IR spectra of flavones show the carbonyl band at 1660 cm\(^{-1}\)\(^\dagger\) owing to the conjugation with olefinic double bond. Introduction of a hydroxyl group at 5-position does not alter the band position appreciably. Luteolin and apigenin show the carbonyl bands at 1655 and 1650 cm\(^{-1}\) respectively.\(^5^0\) The IR spectrum of unsubstituted flavanone shows the carbonyl absorption at 1680 cm\(^{-1}\), the standard value for aromatic ketones. The shift of carbonyl band at 1620 cm\(^{-1}\) in 5-OH flavanones is largely due to chelation. Consequently, methylation of the 5-OH produces only a small frequency shift. The existence of chelation is, however, clearly demonstrated by the absence of the hydroxyl bands at the usual position in 5-hydroxy compounds. Apparently it comes to lie in the \(-OH\) stretching region and is thus obliterated. A similar high frequency shift of 4'-substituted flavanone is attributed to intermolecular hydrogen bonding.\(^5^5\)

The IR spectra of isoflavones are similar to those of flavones. Another interesting feature of the IR spectra of flavones is that the carbonyl frequency is independent of the substitution pattern in ring A and B and is effected only by the introduction of a hydroxyl at 3-position.\(^5^6\)

3. **Nuclear Magnetic Resonance Spectroscopy:**

The application of NMR spectroscopy has proved to be most powerful tool in the structure determination of flavonoids. Since flavonoid compounds contain, in general very few protons, nuclear magnetic resonance spectroscopy is a useful tool in the structure elucidation of this class of compounds. By the use of \(^1\)H NMR studies of silyl derivatives\(^5^7\), double irradiation techniques\(^5^8\), solvent induced shift studies\(^5^8^a\), and lanthanide induced shift studies\(^6^1\) (LIS), one can come to the structure of flavonoids without tedious and time consuming chemical degradation and synthesis.

\(^1\)H-NMR signals in trimethyl silylated flavonoids\(^4^9\) normally occur between 0 and 9 ppm. The chemical shift of the protons of ring-A & B prove to be independent of each other but are affected by the nature of ring-C. The trimethyl silyl derivatives
can be conveniently prepared by treatment of the compound with hexamethyl disilazone and trimethyl silyl chloride in pyridine and the spectrum is measured in carbon tetrachloride with tetramethyl silane as external or internal reference. Table I gives the chemical shifts of different protons of certain representatives of flavones. The most detailed and systematic studies of the $^1$H-NMR spectra of flavonoids are due to Mabry,\textsuperscript{49,62} Batterham and Hightet,\textsuperscript{63} Clark Lewis,\textsuperscript{64} Massicot,\textsuperscript{65} Kawano,\textsuperscript{61,66} and Pelter.\textsuperscript{73} These studies have simplified the task of determining the substitution pattern of flavonoids with the help of NMR-spectroscopy. An obvious advantage of this technique in its application to flavonoids is that they can be readily distinguished not only from flavones and isoflavones but also from the isomeric chalcones which in view of the extreme ease of the isomerisation process is often not possible with other methods.
### TABLE- I

$^1$H- NMR spectral data of flavones and isoflavones, value on $\delta$ scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Apigenin</th>
<th>Luteolin</th>
<th>Robinetin</th>
<th>Genistein 5-methyl ether</th>
<th>Orobol</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3</td>
<td>6.35 (s)</td>
<td></td>
<td></td>
<td></td>
<td>7.7 (s)</td>
</tr>
<tr>
<td>H-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>6.15</td>
<td>6.14</td>
<td>6.75</td>
<td>6.20</td>
<td>6.18</td>
</tr>
<tr>
<td></td>
<td>(d, $J$ = 2.5 Hz)</td>
<td>(d, $J$ = 2.5 Hz)</td>
<td>(d, $J$=8.5, 2.5 Hz)</td>
<td>(d, $J$=2.5Hz)</td>
<td>(d, $J$=2.5Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>6.50</td>
<td>6.50</td>
<td>6.18</td>
<td>6.24</td>
<td>6.39</td>
</tr>
<tr>
<td></td>
<td>(d, $J$ = 2.5 Hz)</td>
<td>(d, $J$ = 2.5 Hz)</td>
<td>(d, $J$ = 2 Hz)</td>
<td>(d, $J$=2.2Hz)</td>
<td>(d, $J$=2.5Hz)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>7.70</td>
<td>7.35</td>
<td>7.20</td>
<td>7.30</td>
<td>7.01</td>
</tr>
<tr>
<td></td>
<td>(d, $J$ = 9.0 Hz)</td>
<td>(dd, $J$=8.5, 2.5 Hz)</td>
<td>(d, $J$ = 2 Hz)</td>
<td>(d, $J$=8.5Hz)</td>
<td>(dd, $J$=2.5, 9.0 Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td></td>
<td>6.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d, $J$ = 8.5 Hz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>6.85</td>
<td></td>
<td></td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d, $J$ = 9 Hz)</td>
<td></td>
<td></td>
<td>(d, $J$=2.5Hz)</td>
<td></td>
</tr>
<tr>
<td>5-OMe</td>
<td></td>
<td></td>
<td></td>
<td>3.80 (s)</td>
<td></td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, dd = double doublet
The most commonly occurring hydroxylation pattern in natural flavonoids is 4', 5, 7- trihydroxy (LXVIII) system. The signals arising from ring-A protons in most flavonoids occurring upfield from those of ring-B protons and are readily recognized.

Ring-A protons:

The two ring -A protons of flavonoids with 5, 7 dihydroxylation pattern give rise to two meta coupled doublets ($J=2.5$ Hz) between δ 6.0-6.66 from tetra methyl silane. There is, however, small but predictable variation in the chemical shifts of the C-6 and C-8 proton signals depending on the 5- and 7- substituents. In flavanones the 6, 8- protons give a signal near δ 5.95, with the addition of a 3- hydroxyl group (flavonols) the chemical shift of these protons are slightly altered and the pattern changes to very strongly coupled pair of doublets. The presence of double bond in ring-C of flavones and flavonols causes a marked downfield shift of these peaks again producing the two doublet pattern out of 6- and 8- protons, the latter appears downfield.
**Ring-B protons:**

All ring-B protons appear around $\delta 7.7-6.7$, a region separate from the usual ring-A protons. The signals from the aromatic protons of a substituted ring-B in a flavone appear as a broad peak centered at about $\delta 7.45$. The presence of ring-C double bond causes a shift of 2',6'-protons and the spectrum shows two broad peaks, one centered at $\delta 7.5$ (H-2', 6') and the other at $\delta 6.85$ (H-3',4',5').

With the introduction of 4'-hydroxyl group the ring-B protons appear effectively as a four peak pattern, A$_2$B$_2$ pattern. Introduction of one more substituent to ring-B gives the normal ABC pattern, the hydroxyl group increases the shielding on the adjacent 3',5'-protons and the signals moves substantially up field. The 2',6' protons of flavanones give signals centered at about $\delta 7.35$.

**Ring-C protons:**

Considerable variations are generally found for the chemical shifts of ring-C protons among the several flavonoid classes. For example, the C-3 proton in flavones gives a sharp singlet near $\delta 6.3$, the C-2 proton of isoflavones is normally observed at about $\delta 7.7$, while the C-2 protons in flavanones is split by C-3 proton into a doublet of $J_{\text{cis}}= 5.0$ Hz, $J_{\text{trans}}= 11.0$ Hz and occurs near $\delta 5.2$. The two C-3 protons occur as two quartets ($J_{\text{H3a,b}}= 17$ Hz) near $\delta 2.8$. However, they often appear as two doublets since two signals of each quartet are of low intensity. The C-2 proton in dihydroflavonols appears near $\delta 4.9$ as a doublet ($J= 11$ Hz) coupled to the C-3 proton which comes at about $\delta 4.2$ as doublet.

**Flavonoid monosaccharide (sugar protons):**

The chemical shift of the C-1" proton of a sugar directly attached to the flavonoid hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of attachment to it. The sugar's C-1 proton (H-1") signal is generally found well downfield from the bulk of the sugar protons signals, and the exact chemical shift can give some information regarding the site of glycosylation, and on occasions, the nature of the sugar. For example, the H-1" signal in spectra of
flavonol-3-O-glucosides at δ 5.7-6.0 ppm is well distinguished from those in flavonoid-7,5- and 4'-O-glucosides which occur in the region δ 4.8-5.2 ppm (as also do the H-1'' signal in 6- and 8-C-glycosides). Further, the 3-O-glucosides of flavonols are clearly distinguishable from the 3-O-rhamnosides, the spectra of which show the H-1'' signal at δ 5.0-5.1 ppm. 49

Glucose commonly forms a β-linkage in flavonoid glycosides and the C-1'' proton of the linked sugar has a diaxial coupling with the C-2'' proton. Thus the C-1'' proton usually appears as a doublet with a coupling constant of about 7.0 Hz, due to the restricted rotation of sugar moiety with respect to the flavonoid nucleus. In flavonoid 7-O-glucoside, however, the glycosyl C-1'' proton does not appear as a sharp doublet but instead gives a complex multiplet owing to more free rotation of sugar moiety with respect to flavonoid nucleus. 56 In the naturally occurring α-linked rhamnosides, the diequatorial coupling between H-1'' and H-2'' gives rise to a coupling constant of only 2 Hz. 78 Rhamnosides are characterized by the signal for the rhamnose C-methyl which appears as a doublet (J=6.5 Hz) or multiplet 79 in the region δ 0.8-1.2 ppm.

Flavonoid disaccharides:

The C-1''' proton of terminal sugar (H-1'''''), being relatively distant from the influence of the flavonoid nucleus, resonates up field from H-1''. The extent, however, can vary depending upon the position of attachment of the terminal sugar. This fact has been used to distinguish rutinosides (H-1'' at δ 4.2-4.4 ppm, J=2.0 Hz) from neohesperidosides (H-1'' at δ 4.9-5.0 ppm, J=2.0 Hz) in 3- and 7-O-glycosylated flavonoids. 59 The position of the C-methyl resonance (rutinosides 0.70-1.0 ppm, neohesperidosides, 1.1-1.3 ppm) may also be used to distinguish these glycosides. Methylated 60 and acetylated 80-82 derivatives have been used for disaccharide linkage determinations.

Acetyl and methoxyl protons:

In the NMR spectra of acetylated flavonoids (CDCl3) the position of methyl of acetyl group can also give a useful information about position of acetyl group by
which the position of a hydroxyl group can be confirmed. The methyl signals of 4'-
and 7-O- acetyl groups appear in the range of δ 2.30- 2.35 ppm. While the methyl
signal of a 5-O-acetyl groups appears at about δ 2.45 ppm. The aliphatic acetoxyl
signals of sugars generally appear in the range of δ1.65-2.10 ppm. The position of the
aliphatic acetoxyl groups of sugar also help in the location of sugar moiety in C-
glycosyl flavonoids. Within the aliphatic acetoxyl groups signals, the 2'''-O acetyl
signal appears at δ 1.70-1.75 ppm in 8-C- glycosylflavonoids and at 1.80-1.83 ppm in
6-C-glycosylflavonoids and 6''-O-acetyl in 8-C-glycosyl flavonoid appears at 1.9-
1.95 ppm and in 6-C-glycosyl flavonoids it appears between δ 1.98-2.04 ppm.
Methoxyl proton signals with a few exceptions appear in the range of 3.5-4.1
ppm.

4. \(^{13}\text{C}-\text{NMR spectroscopy:}\n
\(^{13}\text{C}-\text{NMR spectroscopy has been used in natural product chemistry in variety
of ways at various stages of the structure determination.}^{13}\text{C}-\text{NMR spectral data
furnish key information such as number of carbon atoms and establish if they are
primary, secondary, tertiary, aromatic, olefinic or part of fundamental groups.}

The \(^{13}\text{C}-\text{NMR spectra of flavonoids and their glycosides}^{67}\) are of some interest
in the context of compounds isolated during the course of this work. The spectra can
be analysed by reference to those of simple compounds such as acetophenones^{67,68}
and cinnamic acids^{68} which possess structural features characteristic of flavonoids.

It is worthwhile to see how introduction of oxygen at various positions of
these, affects the chemical shifts. In hydroxyacetophenone (LXIX) the nuclear carbon
linked directly to oxygen of hydroxyl group gives rise to a singlet at δ 161.5 and the
two adjacent carbons give to singlet at ≈ 118.0. The carbon para to the carbonyl is the
most deshielded and its singlet appears at δ 135.5. In 2,6-dihydroxy acetophenone (L)
the carbon bonded directly to oxygen give rise to singlet at δ 161.4 and the two
adjacent carbons produce singlet at δ 106.5. The meta carbon which is para to the
acetyl group is deshielded and its singlet appears at δ 134.0.
Thus, chemical shifts correlate to those for protons on these carbons, the protons ortho and para to hydroxyl being shielded more than the one at meta positions and protons para and ortho to carbonyl being the ones most exposed to the deshielding influence of the carbonyl group. In 2,4,6 trihydroxy acetophenone (LI) the oxygenated nuclear carbons show singlet at δ 165.10 ppm while the dihydroxy acetophenone (L) it is δ 161.4. This slight deshielding at δ 3.70 can be attributed to the hydroxyl group at meta position. The unsubstituted carbons 3 and 5 are shielded due to enhanced mesomeric effect and their signals appear at δ 94.5. These effects can be assumed to be general and are relied upon in making assignments in flavonoid spectra. The other structural unit of flavonoids is akin to cinnamic acid derivatives are, therefore, of interest. The chemical shifts of the parent cinnamic acid and its mono, di and tri substituted derivatives are indicated in the structure (LII, LIII, LIV, and LV).
The 3,4-type of substitution is the one most commonly encountered in flavones and chemical shifts of carbon 3 and 4 of 3-methoxyl 4-hydroxyl cinnamic acid (LIV) δ 149.11 and δ 149.78 respectively are substantially different from those of carbons under oxygen in acetophenone. This makes it possible to distinguish between oxygenated ring-A and ring-B carbons of flavones. The carbons ortho and para to phenolic hydroxyl are shielded, compared to unsubstituted benzene and appear at δ 112.28 and 116.89, the cinnamic acid double bond causing a further shift of C-2 resonance. Carbon-1 adjacent to olefinic double bond of cinnamic acid is almost at the same value as in substituted benzene but different in unsubstituted benzene. The α-carbon appears at δ 116.7 and the β carbon at δ 146.92. In trisubstituted benzene (LXIX), the carbons attached to oxygen are further shielded and in 3, 5-dimethoxy 4-hydroxyl cinnamic acid appear at δ 149.17 and δ 138.79 respectively. The carbon bearing the hydroxyl is shielded to a greater extent because of resonance contribution from the flanking methoxyl groups. The same type of resonance effect is responsible for the shielding of 2 and 6 carbons.

The chemical shifts of flavones, substituted flavones and isoflavones are reproduced in the following table.
## Chemical shifts (in δ downfield from TMS)

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>Flavone</th>
<th>7-methoxy flavone</th>
<th>5-hydroxyl flavone</th>
<th>5,7,3',4'-tetrahydroxy flavones</th>
<th>7-methoxy isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>163.2</td>
<td>162.6</td>
<td>164.07</td>
<td>165.07</td>
<td>152.4</td>
</tr>
<tr>
<td>3.</td>
<td>107.6</td>
<td>107.2</td>
<td>105.61</td>
<td>103.94</td>
<td>125.1</td>
</tr>
<tr>
<td>4.</td>
<td>178.4</td>
<td>177.4</td>
<td>182.90</td>
<td>182.63</td>
<td>175.3</td>
</tr>
<tr>
<td>5.</td>
<td>125.2</td>
<td>126.7</td>
<td>155.85</td>
<td>158.24</td>
<td>127.6</td>
</tr>
<tr>
<td>6.</td>
<td>125.2</td>
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<td>7.</td>
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<td>135.61</td>
<td>164.34</td>
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</tr>
<tr>
<td>8.</td>
<td>118.1</td>
<td>100.2</td>
<td>110.83</td>
<td>99.91</td>
<td>100.0</td>
</tr>
<tr>
<td>9.</td>
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<td>157.7</td>
<td>159.82</td>
<td>161.56</td>
<td>157.7</td>
</tr>
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<td>110.13</td>
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</tr>
<tr>
<td>1'</td>
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<td>131.6</td>
<td>130.54</td>
<td>123.06</td>
<td>127.9</td>
</tr>
<tr>
<td>2'</td>
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<td>125.8</td>
<td>126.39</td>
<td>114.38</td>
<td>128.2</td>
</tr>
<tr>
<td>3'</td>
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<td>128.7</td>
<td>128.91</td>
<td>145.95</td>
<td>128.8</td>
</tr>
<tr>
<td>4'</td>
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<td>131.1</td>
<td>131.97</td>
<td>149.84</td>
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<td>128.8</td>
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<tr>
<td>6'</td>
<td>126.3</td>
<td>125.8</td>
<td>126.39</td>
<td>120.14</td>
<td>127.9</td>
</tr>
</tbody>
</table>
5 Mass spectrometry:

Recently mass spectrometry has been successfully employed for the structure elucidation of flavonoids and their glycosides\(^{69-73}\) and a conclusive structure fragmentation pattern relationship established. Generally the fragmentation is related to the structure of the intact molecule. Electron impact mass spectrometry of both flavonoid aglycones and glycosides serves as a valuable aid in determining their structures, especially when only very small quantities (i.e. less than 1 mg) of the compound are available. The flavonoids aglycones and their glycosides have been subjected to GC-MS spectroscopy in the form of their permethylethers, perdeuteriomethyl ethers\(^{74-75}\) and trimethylsilyl ethers\(^{76-77}\).

Flavones:

Most flavonoids yield intense peak for the molecular ion (M\(^+\)) and indeed this is often the base peak. In addition to the molecular ion, flavonoids usually afford major peak for [M-1] and [M-15] when methoxylated. Perhaps the most useful fragmentation in terms of flavonoids identification are those which involve the cleavage of A and B-ring fragments. Kingstons\(^{77}\) had discussed in detail the mass spectra of large number of flavones, flavonols and their ether derivatives (Scheme I, II &III). The fragmentation pattern of mono flavones has been summarized as follows.

a): Flavones with fewer than four hydroxyl groups don’t readily fragment a consequence of the stability of their molecular ion.

b): Flavones with fewer than four hydroxyl groups tend to undergo decomposition predominantly by the retro-Diels-Alder (RDA) process. This and other common fragmentation process are shown in (Scheme-I) using apigenin\(^{73}\) as a typical example.

c): An [M-1] ion is often found in the mass spectra of flavones, its origin is however, obscure.
**Chapter - 1**

**d)**: The presence of ion m/z 137 (Scheme-II) frequently more intense when a 3-hydroxy group is present, is attributed to the alternative mode of retro-Diels-Alder fragmentation.

**e)**: Doubly charged ions are frequently present.

**f)**: When heavily substituted with hydroxyls and methoxyls, the flavones tend to fragment in a less predictable manner, retro-Diels-Alder process becomes insignificant and the spectrum is dominated by the molecular ion and ions at M-15, M-28, and M-43.\(^{74,76}\)
Flavonoid glycosides:

Mass spectrometry has been extensively used in the structure elucidation of flavonoid–O-glycosides as well as flavonoid–C-glycosides. Mass spectrometric sequencing of oligosaccharide derivatives has been much refined to furnish not only the sequence of sugars involved but also in many cases the position of their interglycosidic linkage and even information about the stereochemistry at the anomeric centre. The mass spectrometric study of glycosides can be done in two groups:

(I) Mass spectrometry of flavonoid O-glycosides.

(II) Mass spectrometry of flavonoid C-glycosides.

Flavonoid O-glycosides:

The position of a sugar residue in a flavonoid aglycone can be easily recognized from the mass spectrum of permethylated glycoside. The sugar attached to the position 5 and 3 splits more rapidly than those at position 7 and as a result the molecular ion peak is of low intensity or totally absent.

The 7-O-glycosides usually show an intense molecular ion peak amounting to 50% or higher of the base peak. The 4'- and 3-O-glycosides represent an intermediate case, having small but distinct molecular ion peak.

The sequencing of sugars in flavonoid oligosaccharide derivatives can be determined by mass spectrometry. The principal fragmentation pattern of predeuteriomethylated flavonoid disaccharide is illustrated in scheme (III).

Fission of glycosidic carbon-oxygen bond of the terminal sugar leads to the ion $T_1$ which successively looses CD$_3$ methanol to give $T_2$ and $T_3$. 

33
Rupture of the ethereal carbon oxygen bond between the terminal and second sugar give rise to the sequence ions. Fission of the carbon oxygen bond between the sugar and the aglycone is indicated by fragment A, invariably formed by transfer of hydrogen and followed by loss of CO. Retention of charge on the disaccharide residue after this type of rupture leads to the oligosaccharide ion OS. Peaks due to RDA-cleavage of the flavonoid aglycone are small or absent as is often observed in highly substituted compounds.

**Sequence of sugars and position of interglycosidic linkage:**

Information about the terminal sugar is obtained from the difference (M'-S) and from peaks due to ions of the T-series. The T-series is more reliable induction since the (M'-S) value can be charged by H-transfer or, in the case of apiosyl containing compounds, even more complicated reaction. In compounds containing glucose as the second sugar moiety, the OS-T1 rather than the (S-A) difference seems to be the more useful for mass identification.

Differences in hydrogen transfer to some peaks allow prediction of the position of interglycosidic linkage. The sequence peak is formed without hydrogen transfer (flavonols) or with single hydrogen transfer (flavone, flavanone) in the case of 1→6 linked flavonoid disaccharide derivatives, while transfer of two hydrogens take place in the case of 1→2 linked compounds. The aglycone and the
oligosaccharide fragments A and OS behave similarly. For 1→6 linked derivatives the A+H and OS peaks are prominent, while A+2H and OS+H peaks are observed for 1→2 linked compounds. Further (OS-CD$_3$-Methanol) differentiation as possible by a strong (OS-CD$_3$-Methanol) peaks present only in the 1→2 linked glycosides, and by the S+62 (flavones, flavanones) or S+63 (flavanonols) peak observed only in 1→6 linked compounds in Scheme IV.

Flavonoid C-glycosides:

The direct mass spectral analysis of a C-glycosyl flavone$^{95-97}$, rarely produces an observable molecular ion, the base peak is usually the aglycone fragment with only a CH$_2$ group remaining of the original C-glycosyl moiety. This ion is stabilized by rearrangement to a tropylium ion. The additional hydrogen is derived from a sugar hydroxyl group.$^{98}$ The other fragments come (Pathway-I and II processes) from the base peak ion giving the B$_2^+$ and A$^+$ ions. Besides these fragments, other fragments
corresponding to ion (M-18)^{+}, (M-36)^{+}, (M-54)^{+}, are formed by the successive loss of water molecules from the molecular ion.

Mass spectrometry also helps to distinguish 6- and 8-C- glucosylflavones as in the case of 6-C-glycosyl flavone (M-148)^{+}, peak (equivalent to aglycone + CH₂ + H) has 50% to 80% of intensity compare to (M-149)^{+} peak whereas with 8-C-glucosylflavones (M-148)^{-}, ion is usually only about 25% of the intensity of the (M-149)^{+} peak.

The mass fragmentation of 8-C-glycosyl-4'-methoxy apigenin is given in (Scheme V).
**Chapter 1**

**Structural determination of triterpenoids by spectroscopic methods:**

**Ultraviolet spectroscopy:**

The UV spectroscopy finds its main use in the terpene chemistry for the detection of conjugation. The UV spectrum of pentacyclic triterpenoids in sulphuric acid shows characteristic absorption maxima at 310 nm regardless of the substituents present. The hypsochromic shift in the UV spectrum associated with the 18 β to 18 α transformations, observed in the case of α-bosmellic acid has been reported as a diagnostic technique in conformational analysis.\(^{99a}\)

**Infrared spectroscopy:**

IR spectrum of terpenoids has two important regions. Absorption bands in the region 3500-3650 cm\(^{-1}\) and 2650-3350 cm\(^{-1}\) are due to OH stretching and -CH stretching respectively. Absorption in the region of 1640-1820 cm\(^{-1}\) is attributed to C=O stretching respectively and it is possible to recognize saturated and conjugated esters, aldehydes, ketones, acids, and lactone from the frequency of absorption maxima.

IR studies are also used to make a distinction between tertiary equatorial (3613 cm\(^{-1}\)) and axial (3617 cm\(^{-1}\)) hydroxyl group. For example secondary OH in methyl melaleucate absorbs at 3629 cm\(^{-1}\) while its 3-epimer (axial, secondary) absorbs at 3636 cm\(^{-1}\).

The IR spectra of 18α-H and 18β-H olean-12-ene derivatives shows that 18β-H compounds with an axial COOMe at C-20 absorbs at 1165 and 1090 cm\(^{-1}\), while the 18α-H epimers absorbs at 1117 cm\(^{-1}\). The 18β-H compounds with an axial CH\(_2\)OH at C-20 absorbs at 1210-1205 cm\(^{-1}\) whereas 18α-H epimers absorbs at 1193-1189 cm\(^{-1}\).

**\(^1\)H-NMR Spectroscopy:**

\(^1\)H-NMR spectroscopy is a great help in structural elucidation of triterpenoids. \(^1\)H-NMR not only gives chemical shift information that identifies the presence of methyl ester, OH group and vinyl protons but also provides valuable stereochemical
information via coupling constant. For example, in 3 β-OAc, H-3 is doublet of
doublet with J=8.0 Hz and H=5 Hz, while for 3 α-OAc it is a triplet with J=3.0 Hz. In
addition to, 1H-NMR is also used in determining the configuration of 2,3-dihydroxy,
2,3,23-trihydroxy and 2,3,24-trihydroxy substituent terpenoids. In 3β-23-diol the
primary carbinol proton resonates at δ 3.4 and δ 3.65 while in 3 β, 24-diol they appear
at δ 3.75 and 4.05.

**Heteronuclear Correlation spectroscopy [HETCOR] 13C-H**

Heteronuclear correlation spectroscopy is one of the most powerful 2D
experiments. It provides connectivity between 13C and 1H signals and also give
the number of hydrogen atoms attached to individual 13C atom. The long-range
HETCOR experiments help to detect connectivity mediated by two or three bonds
(frequently) and provides important information about the molecular structure. An
indirect 13C-1H shift correlated pulse sequence XCOREF (x- nucleus correlation with
fixed evolution time) has been proposed for unambiguous assignment of 13C spectra
of triterpenes. The ability of XCOREF to distinguish two and three- band connectivity
is particularly useful in completing the assignments of carbons which shows no
methyl 1H cross peaks. As terpenes have many methyls groups, long range 13C-1H
correlation spectra are very useful for assigning 13C NMR data of these compounds.

Reynolds et al introduced further improvements on the basis of shift correlation
sequence by incorporation two BLRD (Bilinear rotation decoupling). This techniques
leads to significant improvement in sensitivity over the basis sequence, outstanding
suppression of one bond connectivity and partial 1H-1H decoupling.

**Mass Spectroscopy:**

Mass spectroscopy is the most powerful tool for the establishment of the
skeleton and location of double bond in the pentacyclic terpenoids. The mass
spectroscopy is an excellent method for finding the molecular weights of organic
compounds. Along with other spectral information; the mass spectroscopy is a tool for
the determination of the structure of compounds. It has been unique advantages over
other techniques. The reports on the mass spectral studies of large number of
tetracyclic and pentacyclic triterpenoids are available. A detailed study of the systematic cracking pattern of pentacyclic triterpene by noting peak shifts in various derivatives was very well carried out by C. Djerassi and J.S Shannon. This led to important generalizations about triterpenic compounds particularly of \( \Delta^{12} \)-oleanene and \( \Delta^{12} \)-ursene derivatives. With the unsaturated triterpenes, one of the characteristic fragmentation is a retro Diel-Alder reaction i.e. abstraction of an electron from the olefinic \( \pi \) bond, followed by a R-D-A type opening of ring C.

The mass of fragments provide considerable information about the position of double bonds and substituents. Triterpenes have been examined by several workers C. D Jerassi, J.S Shannon, R.O. Donchaf have shown that there are important characteristics of the group as well as of the individuals members. Some of the most important fragmentation include dehydration of its equivalent \((M^+ - Sc)\). In \( \Delta^{12} \)-oleanene and \( \Delta^{12} \)-ursene derivatives, Oxygen containing substituents at C-17 or C-3 (COOH, CH\(_2\)OH, CH\(_2\)OAc or CHO) are lost in preference to methyl from R-D-A fragmentation owing to stabilization of the expelled radical by oxygen, \( \Delta^{12} \) oxygenated triterpene exhibited base peak at m/z 234.

The process of fragmentation of basic triterpene of \( \beta \)-amyrin and \( \alpha \)-amyrin series hydrocarbons was shown by taking example of \( \Delta^{12} \)-oleanene and \( \Delta^{12} \)-ursene. The mass spectra of these compounds it can be seen that they give rise to fragments at identical m/z values. But fragment ion m/z 203 is more intense than m/z 191 in case of \( \Delta^{12} \)-oleanene and reverse is seen in \( \Delta^{12} \)-ursene. This peak can be utilized to differentiate between two.

**The important peaks of triterpenes:**

Peak M-153 (m/z 257) results from the hemolytic cleavage of 9-10 bond in the molecular ion (a) to afford (b) followed by hydrogen transfer from C-26 to C-7 with concomitant homolysis of 7-8 bond to afford the resonance stabilized species (c) m/z 257 (Scheme-VI).
Peak M-192 (m/z 218) The intense and diagnostically important m/z 218 peak, base peak in $\Delta^{12}$-oleanene and $\Delta^{12}$-ursene. Mechanism of formation of m/z 218 species by R-D-A decomposition is already discussed and shown in scheme-VII.
Peak M-207 (m/z 203) This is the most interesting peak from mechanistic point of view and may result from fragmentation described in scheme-VIII.
References


34. M. Zhao, J. Duan, and C. Tao Che *Phytochemistry*, 68(10), 1471-1479, (2007).
51. Y. Asahina and M. Inubuse Ber, 61, 1646 (1928).
52. Y. Asahina and M. Inubuse Ber, 64, 1256 (1931).


Chapter-II

Chemical Constituents from the leaves of Ficus benjamina (Var. Comosa)
Discussion
CHEMICAL CONSTITUENTS FROM THE LEAVES OF FICUS BENJAMINA (VAR. COMOSA) (MORACEAE)

*Ficus benjamina* (Var. comosa) (Moraceae) is commonly known as 'Kabra', is a moderate sized, evergreen epiphytic tree.¹ *Ficus benjamina* is used as folk medicine for the treatment of certain skin diseases and respiratory disorders.²⁻³ Its fruit extract is also known to possess antitumor activity and significant antibacterial activity.⁴⁻⁷ Earlier reports mention the isolation of benjaminamide (a ceramide)⁸, α-amyrin, bergapten, and imperatorin⁹ from this plant. Medicinal importance and scarcity of work on this plant encourages us to carryout the comprehensive investigation of the leaves of *F. benjamina* (var. comosa). The present discussion deals with the isolation and characterization of the following compounds from the leaves of *Ficus benjamina*.

1. pentacontanyl decanoate
2. β-sitosterol
3. friedelin
4. (9, 11), (18, 19)-disecoolean-12-en-28-oic acid (new compound)
5. serrat-3-one (new compound)
6. β-amyrin

The leaves of *Ficus benjamina* (var. comosa) (3.0 kg) were collected from the botanical garden, AMU Aligarh India and identified by Prof. Wazahat Hussain, Taxonomist Department of Botany, AMU, Aligarh. The leaves of *F. benjamina* were dried under shade and crushed to make powder. The dried leaves were treated with light petroleum ether (60-80°C) to remove the fatty material and chlorophyll. The residue was exhaustively refluxed with chloroform. The solvent was removed by distillation. The chloroform extract was concentrated under reduced pressure to yield the greenish gummy mass. The concentrate was therefore, subjected to column chromatography over silica gel. Elution of the column with light petroleum ether-benzene in ratio 9:1-1:1 and benzene gave various fractions which upon repeated column chromatography and fractional crystallization afforded six compounds marked as Fe-1, Fe-2, Fe-3, Fe-4, Fe-5 and Fe-6.
Chapter II

Characterization of compound Fc-1

The compound Fc-1 was obtained as white granular crystals from light petroleum ether-benzene (9:1) eluants and crystallized with chloroform-acetone. Its IR spectrum (Fig-I) showed the characteristic band at 1725 cm\(^{-1}\) for ester carbonyl group. Its molecular formula was established as C\(_{60}\)H\(_{120}\)O\(_2\) on the basis of expected molecular ion peak at m/z 872 and elemental analysis. The \(^1\)H NMR spectrum (Fig. 2, Table-1) showed a pair of doublets integrating for one proton each at \(\delta\) 3.57 (\(J=6.5\) Hz) and 3.41 (\(J=6.5\) Hz) was assigned to (oxygenated methylene H\(_2\)-1) as in structure (I). Two independent broad singlets of one proton each at \(\delta\) 2.39 and 2.17 were ascribed to methylene H\(_2\)-2' protons adjacent to the ester group. A broad singlet at \(\delta\) 1.55 integrating for 106 protons corresponded to 53 methylene groups. Another broad singlet of four protons at \(\delta\) 1.25 was assigned to two methylene groups. The remaining two methyl groups i.e. Me-10' and Me-50 were resonating as two independent triplets of three protons each at \(\delta\) 0.86 (\(J=7.2\) Hz) and 0.82 (\(J=6.61\) Hz). The molecular ion peak at m/z 872 in the mass spectrum (Fig. 3) was not observed and the other fragments are rationalized from scheme-I. On the basis of above spectral data analysis the structure of Fc-1 has been established as pentacontanyl decanoate (I).

![Structure](Image)

(I)
<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Chemical shift of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me-50</td>
<td>3</td>
<td>0.82 (3H, t, J=6.61 Hz,)</td>
</tr>
<tr>
<td>Me-10'</td>
<td>3</td>
<td>0.86 (3H, t, J=7.2 Hz)</td>
</tr>
<tr>
<td>2 x CH₂</td>
<td>4</td>
<td>1.25 (4H, brs)</td>
</tr>
<tr>
<td>53 x CH₂</td>
<td>106</td>
<td>1.55 (106 H, brs)</td>
</tr>
<tr>
<td>H-2'b</td>
<td>1</td>
<td>2.17 (1H, brs)</td>
</tr>
<tr>
<td>H-2'a</td>
<td>1</td>
<td>2.39 (1H, brs)</td>
</tr>
<tr>
<td>H-1'b</td>
<td>1</td>
<td>3.41 (1H, d, J=6.5 Hz)</td>
</tr>
<tr>
<td>H-1'a</td>
<td>1</td>
<td>3.57 (1H, d, J=6.5 Hz)</td>
</tr>
</tbody>
</table>

brs = broad singlet, d = doublet, m=multiplet, spectrum run in CDCl₃ at 400 MHz, TMS as internal standard, values are recorded on δ scale.
Scheme I

\[
\begin{align*}
10' & \quad \text{CH}_3 - (\text{CH}_2)_8 - \text{C}^+ - \text{O} \quad 1^+ \quad \text{CH}_2 - \text{CH}_2 - (\text{CH}_2)_{47} - \text{CH}_3 \\
\text{[M+H]} & \quad \text{[absent]} \\
\downarrow & \\
\text{CH}_3 - (\text{CH}_2)_8 - \text{C}^+ & \quad \text{CH}_3 - (\text{CH}_2)_{9}^+ \\
\text{m/z 155} & \quad \text{m/z 141} \\
\downarrow & \\
\text{CH}_3 - (\text{CH}_2)_{4}^+ & \\
\text{m/z 71} & \\
\downarrow & -\text{CH}_2 \\
\text{m/z 57} & 
\end{align*}
\]
FC-1
1H IN CDCl3
AVANCE-300MHz
Characterization of compound Fc-2

It was obtained by elution of column with light petroleum ether- benzene (7:3) mixture followed by crystallization with chloroform-ethanol as white crystalline solid m.p. 136-37°C. Fc-2 gave an acetate with acetic anhydride and pyridine m.p.114-16°C, and benzoate with benzoyl chloride (1.0 ml) and pyridine (0.5 ml), m.p.145-46°C. It gave positive Leibermann-Burchard test\textsuperscript{10} and responded positively to the tetraniitromethane color test. The IR spectrum showed the presence of gem-dimethyl groups, hydroxyl group and olefinic double bond with bands at 3340 cm\textsuperscript{-1} (OH), 1055 cm\textsuperscript{-1}, 840 cm\textsuperscript{-1} 1460 cm\textsuperscript{-1} (C=C) and 1375 cm\textsuperscript{-1} (C-Me\textsubscript{2}). The \textsuperscript{1}H-NMR data is given in (Table 2). The mass spectrum showed the molecular ion peak at m/z 414.

From the above data and direct comparison with an authentic sample\textsuperscript{11}, Fc-2 was identified as $\beta$-Sitosterol (II).
### Table 2

**$^1$H-NMR spectral data of Fc-2**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Chemical shift of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-CH₃</td>
<td>3</td>
<td>0.70 (3H, s)</td>
</tr>
<tr>
<td>29-CH₃</td>
<td>3</td>
<td>0.80 (3H, d, J=6.8 Hz)</td>
</tr>
<tr>
<td>26, 27-CH₃</td>
<td>6</td>
<td>0.88 (6H, d, J=6.5 Hz.)</td>
</tr>
<tr>
<td>21-CH₃</td>
<td>3</td>
<td>0.92 (3H, d, J=6.5, Hz)</td>
</tr>
<tr>
<td>19-CH₃</td>
<td>3</td>
<td>1.02 (3H, s)</td>
</tr>
<tr>
<td>C3β-H</td>
<td>1</td>
<td>3.56 (1H, m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.36 (1H, m)</td>
</tr>
<tr>
<td>-CH₂ and -CH proton of cyclic system and side chain</td>
<td>30</td>
<td>1.07-2.34</td>
</tr>
<tr>
<td>OH</td>
<td>1</td>
<td>4.25 (m, 1H)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m=multiplet, spectrum run in CDCl₃ at 400 MHz, TMS as internal standard, values are recorded on δ scale.
Characterization of compound Fc-3:

It was eluted from the column with light petroleum ether-benzene (1:1) and crystallized from chloroform-methanol as white crystalline solid (150 mg) m.p. 262-64°C. It analysed for C_{30}H_{50}O (M^{+} m/z 426), It responded positively for the colour test of triterpene.\textsuperscript{10-12} The melting point agreed with that of friedelin. Its identity as friedelin (III) was established by comparison of its $^1$H NMR (Table 3), IR and mass spectra with an authentic sample of friedelin\textsuperscript{13-15} (III).
Table 3

$^1$H NMR spectral data of Fe-3

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.72 (3H, s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.87 (3H, s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.89 (3H, s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.92 (3H, s)</td>
</tr>
<tr>
<td>2 × CH$_3$</td>
<td>6</td>
<td>0.95 (6H, s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.05 (3H, s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.18 (3H, s)</td>
</tr>
<tr>
<td>-CH$_2$ protons and -CH protons</td>
<td>22</td>
<td>1.25, 1.34, 1.45, 1.52, 1.58 (22 H, m)</td>
</tr>
<tr>
<td>C$_2$-2H and C$_4$-1H</td>
<td>3</td>
<td>2.26-2.41 (3H, m)</td>
</tr>
</tbody>
</table>

s = singlet, m=multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard, values are recorded on δ scale.
Elution of the column with light petroleum ether-benzene (4:6) followed by crystallization with chloroform-methanol afforded Fc-4 as white crystalline solid m.p. 195°C. Elemental analysis along with molecular ion peak at m/z 444 agreed with the molecular formula C₃₀H₅₂O₂. Considering the carboxylic group and one double bond in the structure along with the fact that molecular composition indicates five double bond equivalents, it is considered to be a tricyclic compound. Its IR spectrum (Fig-4) showed characteristic absorption bands at 3459, 1719 and 1642 cm⁻¹ corresponded to carboxyl group which was further confirmed by the appearance of effervescences with NaHCO₃ and the fragment ion peak at m/z 427 corresponded the loss of -OH from the molecular ion 444 (characteristic of carboxylic group). The carboxylic group refused to undergo esterification under normal conditions showing the tertiary nature. A characteristic band at 1642 cm⁻¹ exhibited the presence of double bond. On the basis of composition and IR spectrum it was anticipated to be a triterpenic compound with two incomplete rings [ring C & E] and the structure 4 was tentatively proposed which satisfied the mass fragmentations and the NMR spectrum as given. The prominent ion peaks generated at m/z 55 [C₃,₄-C₅,₁₀-C₅,₆ fission]⁺, 69 [C₃,₄-C₅,₁₀-C₆,₇ fission]⁺, 83 [C₃,₅-C₅,₁₀-C₇,₈ fission]⁺, 97 [C₂,₃-C₅,₁₀-C₇,₈ fission]⁺, 111 [C₂,₃-C₅,₁₀-C₇,₈ fission]⁺, 124 [C₅,₆-C₉,₁₀ fission]⁺ suggested saturated nature of rings A and B and devoid of any functional group in these rings (Fig-6). The base peak at m/z 193 was formed due to cleavage of C₈-C₁₄ linkage supporting seco nature of ring C. The ion peaks at m/z 234 [C₁₃,₁₄-C₁₄,₁₅ fission]⁺, 219 [234-Me]⁺, 314 [C₁₆,₁₇-C₁₇,₁₈ fission]⁺ and 299 [314-Me]⁺ indicated saturated nature of ring D and seco pattern of ring E (Scheme-II, III, IV). The ¹HNMR spectrum of Fc-4 (Fig-5 & Table 4) exhibited one proton broad signal at δ 5.63 assigned to vinylic H-12. A three proton broad signal at δ 1.56 was ascribed to C-11 (methyl) protons attached to C-12 vinylic carbon. Eight broad signals at δ 1.36, 1.269, 1.17, 1.10, 1.06, 0.90, 0.89 and 0.87 all integrated for three protons each, can be ascribed to C-23, C-30, C-27, C-26, C-29, C-19, C-25 and C-24 tertiary methyl signals. In the light of the foregoing discussion the compound Fc-4 can best be characterized as (9, 11), (18, 19)-disecoolean-12-en-28-oic acid (IV), which is being reported for the first time.


<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Chemical shift of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-CH₃</td>
<td>3</td>
<td>0.87 (3H, brs)</td>
</tr>
<tr>
<td>25-CH₃</td>
<td>3</td>
<td>0.89 (3H, brs)</td>
</tr>
<tr>
<td>19-CH₃</td>
<td>3</td>
<td>0.90 (3H, brs)</td>
</tr>
<tr>
<td>29-CH₃</td>
<td>3</td>
<td>1.06 (3H, brs)</td>
</tr>
<tr>
<td>26-CH₃</td>
<td>3</td>
<td>1.10 (3H, brs)</td>
</tr>
<tr>
<td>27-CH₃</td>
<td>3</td>
<td>1.17 (3H, brs)</td>
</tr>
<tr>
<td>30-CH₃</td>
<td>3</td>
<td>1.269 (3H, brs)</td>
</tr>
<tr>
<td>23-CH₃</td>
<td>3</td>
<td>1.36 (3H, brs)</td>
</tr>
<tr>
<td>11-CH₃</td>
<td>3</td>
<td>1.56 (3H, brs)</td>
</tr>
<tr>
<td>H-12</td>
<td>1</td>
<td>5.63 (1H, brs)</td>
</tr>
</tbody>
</table>

brs = broad singlet, spectrum run in CDCl₃ at 400 MHz, TMS as internal standard, values are recorded on δ scale.
Scheme II

Scheme III
Scheme IV
Fig. 5
Fig. 6
Characterization of compound Fc-5

The compound Fc-5 was obtained from light petroleum ether-benzene (3:7) eluate and crystallized from chloroform-methanol as colorless needles m.p. 230°C. It responded positively to Leibermann-Buchard test for triterpene and a Zimmermann test for 3-oxo group in a triterpene.\(^{16,17}\) Its IR spectrum (Fig-7) showed characteristic absorption band for keto group at 1715 cm\(^{-1}\). Elemental analysis along with the positive FABMS and \(^{13}\)C NMR agreed with the molecular formula \(C_{30}H_{50}O\) [M\(^{+}\)426].

The compound did not show the presence of double bond as it responds negatively to tetraniromethane test / addition of Br\(_2\) and even in the spectral studies. It indicated six double bond equivalents. Five of them were adjusted in a pentacyclic carbon framework of a triterpene and one in the carbonyl group. The prominent ion peak generated in mass spectrum (Fig. 11, scheme IV, V, VI) at m/z 411 [M-Me\(^{+}\)], 396[411-Me\(^{+}\)], 111 [C\(_{2,3}\)-C\(_{5,10}\)-C\(_{7,8}\) fission\(^{+}\)], 125 [C\(_{2,1}-C_{5,10}-C_{7,8}\) fission\(^{+}\)], 139 [C\(_{1,10}-C_{5,10}-C_{7,8}\) fission\(^{+}\)], 138 [C\(_{5,6}-C_{9,10}\) fission\(^{+}\)], 152 [C\(_{6,7}-C_{9,10}\) fission\(^{+}\)], 166 [C\(_{7,8}-C_{9,10}\) fission\(^{+}\)], 274 [M-152\(^{-}\)] and 260 [M-166\(^{-}\)] suggested saturated nature of ring A and B and the existence of carbonyl group in ring A which was placed at C-3 on the basis of biogenetic consideration. The ion peak arose at m/z 206 [C\(_{8,27}-C_{9,11}\) fission\(^{-}\)], 192 [C\(_{12,13}-C_{8,27}\) fission\(^{-}\)] and 178 [C\(_{14,27}-C_{12,13}\) fission\(^{-}\)] indicated saturated nature of ring C and D. The \(^1\)H NMR spectrum of Fc-5 (Table-5, Fig. 8) showed two one proton double doublets at \(\delta\) 2.56 (J=3.6, 10.2 Hz) and 2.51 (J=3.6, 5.4 Hz) assigned to C-2 methylene protons adjacent to the carbonyl group. Seven three proton broad signals at \(\delta\) 1.32, 1.19, 1.14, 1.09, 1.009, 0.86 and 1.02 were attributed to tertiary C-23, C-24, C-25, C-26, C-28, C-29 and C-30 methyl protons all attached to saturated carbons.

The remaining methylene and methine protons resonated between \(\delta\) 2.48-1.35. The \(^{13}\)C NMR spectrum of Fc-5 (Fig. 9) displayed 30 carbon signals in the molecule. A signal at \(\delta\) 213.30 was ascribed to C-3 carbonyl carbon. The signals at \(\delta\) 14.73, 20.34, 32.17, 18.02, 18.32, 6.90 and 22.36 were accounted to the C-23, C-24, C-25, C-26, C-28, C-29 and C-30 methyl carbons. The remaining methylene methine and quaternary carbons appeared between \(\delta\) 59.47-28.25. The absence of any signal beyond \(\delta\) 2.56 in the \(^1\)H NMR spectrum and signal between \(\delta\) 202.61-59.47 in \(^{13}\)C NMR spectrum indicated the saturated nature of the molecule which was devoid of any carbinol group. Analysis of DEPT spectrum of Fc-5 (Fig. 10) indicated the presence of seven
methyls, twelve methylene, five methine & six quaternary carbons. The appearance of
C-27 methylene carbon in the deshielded region at δ 42.78 in the $^{13}$C NMR spectrum
supported serratone type carbon framework of the molecule. On the basis of
above spectral data analysis and chemical reactions, the structure of Fc-5 can best be
formulated as **serrat-3-one (V)**. This is a new phytoconstituent isolated from a plant
source for the first time.
### Table 5

**$^1$H NMR and $^{13}$C NMR spectral data of Fc-5**

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H NMR (δ)</th>
<th>$^1$H NMR (δ)</th>
<th>$^{13}$C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α H (ppm)</td>
<td>β H (ppm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.63 dd (3.6, 10.2)</td>
<td>1.81 dd (5.4, 3.3)</td>
<td>39.33</td>
</tr>
<tr>
<td>2</td>
<td>2.56 dd (3.6, 10.2)</td>
<td>2.51 (3.6, 5.4)</td>
<td>28.25</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>213.30</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>38.38</td>
</tr>
<tr>
<td>5</td>
<td>1.62 dd (5.4, 3.3)</td>
<td>-</td>
<td>53.18</td>
</tr>
<tr>
<td>6</td>
<td>1.76 m</td>
<td>1.59 m</td>
<td>18.74</td>
</tr>
<tr>
<td>7</td>
<td>1.79 m</td>
<td>1.55 m</td>
<td>42.23</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>39.78</td>
</tr>
<tr>
<td>9</td>
<td>1.44 dd (5.1, 8.4)</td>
<td>-</td>
<td>58.31</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>36.09</td>
</tr>
<tr>
<td>11</td>
<td>2.09 m</td>
<td>1.69 m</td>
<td>30.08</td>
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<tr>
<td>12</td>
<td>1.76 m</td>
<td>1.83 m</td>
<td>30.58</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>2.14 m</td>
<td>59.56</td>
</tr>
<tr>
<td>14</td>
<td>2.48 m</td>
<td>2.37 m</td>
<td>41.37</td>
</tr>
<tr>
<td>15</td>
<td>1.53 m</td>
<td>1.35</td>
<td>35.42</td>
</tr>
<tr>
<td>16</td>
<td>1.39 m</td>
<td>1.49 m</td>
<td>32.50</td>
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<tr>
<td>17</td>
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<td>1.83 dd (7.5, 5.7)</td>
<td>41.61</td>
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<tr>
<td>18</td>
<td>-</td>
<td>-</td>
<td>37.52</td>
</tr>
<tr>
<td>19</td>
<td>1.57 dd (2.1, 6.7)</td>
<td>1.65, dd (5.4, 3.3)</td>
<td>35.10</td>
</tr>
<tr>
<td>20</td>
<td>1.49 m</td>
<td>1.69 m</td>
<td>31.86</td>
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<tr>
<td>21</td>
<td>1.87 dd (5.7, 6.3)</td>
<td>2.07 m</td>
<td>32.85</td>
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<td>22</td>
<td>-</td>
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<td>35.70</td>
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<td>23</td>
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<td>14.73</td>
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<td>1.19 brs</td>
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<td>1.14 brs</td>
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<tr>
<td>26</td>
<td>1.09 brs</td>
<td>-</td>
<td>18.02</td>
</tr>
<tr>
<td>27</td>
<td>1.69 brs</td>
<td>1.43 brs</td>
<td>42.87</td>
</tr>
<tr>
<td>28</td>
<td>1.09 brs</td>
<td>-</td>
<td>18.32</td>
</tr>
<tr>
<td>29</td>
<td>0.86 brs</td>
<td>-</td>
<td>6.90</td>
</tr>
<tr>
<td>30</td>
<td>1.02 brs</td>
<td>-</td>
<td>22.36</td>
</tr>
</tbody>
</table>

Spectrum recorded in CDCl$_3$ at 400 MHz, using TMS as internal standard, coupling constant in Hertz are given in parentheses.
Scheme VI
Fig. 7
Fig. 10
Characterization of compound Fc-6

Fc-6 was eluted from the column with light petroleum ether: benzene (2:8) and crystallized from chloroform-methanol as white crystalline solid m.p.198°C. $[\alpha]^D_{D}+88.4^\circ$ (CDCl$_3$). It gives positive Leibermann-Burchard test.$^{10}$ The Infrared spectrum showed the bands at 3360 cm$^{-1}$ (OH), 2960 cm$^{-1}$, 2880 cm$^{-1}$, 1650 cm$^{-1}$, 1465 cm$^{-1}$, (C=C), 1040 and 980 cm$^{-1}$ indicating the presence of hydroxy and olefinic group.

The mass spectrum of Fc-6 gave molecular ion peak at m/z 426 and analyzed for C$_{30}$H$_{50}$O. Its $^1$H NMR data are given in (Table 6).

From the above data and their direct comparison with an authentic sample, Fc-6 was identified as $\beta$-amyrin (VI).$^{11}$
### Table 6

^1H NMR spectral data of Fe-6

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 x CH$_3$</td>
<td>24</td>
<td>0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H, s), 1.0 (3H, s), 1.14 (3H, s)</td>
</tr>
<tr>
<td>-CH$_2$ and -CH protons of cyclic system</td>
<td>23</td>
<td>1.08, 2.01, 3.01 (dd, J = 9.0 Hz &amp; 7.0 Hz)</td>
</tr>
<tr>
<td>α-to OH</td>
<td>1</td>
<td>4.88 (brs)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.21 (m)</td>
</tr>
</tbody>
</table>

s = singlet, brs = broad singlet, dd = double doublet, m = multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard, values are recorded on δ scale.
Experimental
The melting points were taken on a Kofler block and are uncorrected. $^1$H NMR and $^{13}$C NMR were recorded on Bruker DRX-300 and Bruker Avance 400 MHz with TMS as an internal standard. IR spectra were taken on Shimadzu IR -408 Perkin Elmer 1800 (FTIR). The mass spectra were measured in both E1 Mode and Jeol D-300 and FAB mode on Jeol SX 102/DA-6000 mass spectrometers.

The silica gel used for different chromatographic purposes, was obtained from E Merck (India) and E. Merck (Germany), SRL (India). TLC systems used were light petroleum ether-benzene (4:1), light petroleum ether–benzene (1:1), light petroleum ether - benzene (3:7), light petroleum ether - benzene (2:3), light petroleum ether-chloroform (1:1), benzene-chloroform (1:1).

**EXTRACTION OF THE LEAVES OF FICUS BENJAMINA. L**
**VAR. COMOSA** (MORACEAE)

The leaves of Ficus benjamina (var. comosa) (3.0 kg) procured from Botanical garden AMU, Aligarh were dried under shade and crushed to make powder then dried, powdered leaves were defatted with light petroleum ether were then extracted with chloroform. The chloroform extract, after complete removal of solvent under reduced pressure gave a greenish gummy mass (150.0 gm). This fraction responded to usual color test for triterpenes and revealed a complex mixture of several compounds on TLC plates coated with silica gel.

The extract was therefore, subjected to column chromatography over silica gel. The column was eluted with light petroleum ether-benzene mixture in different ratio (9:1-1:1), benzene and benzene-ethylacetate (9:1-1:1), as eluting solvents. The fraction showing similar behavior on TLC examination and same IR spectra were mixed together. Repeated column chromatography followed by fractional crystallization afforded six compounds, marked as Fe-1, Fe-2, Fe-3, Fe-4, Fe-5 and Fe-6.
The fraction obtained by the elution of the column with light petroleum ether-benzene (9:1) was crystallized with chloroform-acetone as white granular crystals (30 mg) mp. 265°C.

**Analysed for C_{60}H_{120}O_{2}**:

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, 84.49; H, 13.82%</td>
<td>C, 84.43; H, 13.76%</td>
</tr>
</tbody>
</table>

**IR, $\nu_{\text{max}}$ cm$^{-1}$**:

2931, 2873, 1725 (C=O of ester), 1461, 1372, 1265, 1168, 1068, 1024 cm$^{-1}$.

**$^1H$ NMR (400 MHz, CDC$_3$) on $\delta$ scale**:

0.82 (3H, t, J=6.61 Hz, CH$_3$-5O), 0.86 (3H, t, J=7.2 Hz, CH$_3$-10'), 1.25 (4H, brs, H$_2$-2, H$_2$-2'), 1.55 (106 H, brs, 53 x CH$_2$), 2.17 (1H, brs, H-2'b), 2.39 (1H, brs, H-2'a), 3.41 (1H, d, J=6.5 Hz, H-1b), 3.57 (1H, d, J=6.5 Hz, H-1a).

**Mass, m/z (rel. Intensity)**:

872 [M]$^+$ (not observed), 155 (16), 141 (38), 97 (12), 71(38), 57 (75).

**Fc-2**

Elution of column with light petroleum ether-benzene (7:3) gave a TLC homogeneous substance which on repeated crystallization from chloroform-ethanol afforded white needle shaped crystals (90 mg) m.p. 136-37°C.

**IR, $\nu_{\text{max}}^{KBr}$ cm$^{-1}$**:

3340 (OH), 1655, 1460 (C=O), 1375 (C-Me$_2$), 1055, 840.
\(^{1}\text{H NMR (400 MHz, CDCl}_3\text{) on } \delta \text{ scale:}\)

\begin{align*}
0.70 \text{ (3H, s, Me-18)}, & \quad 0.80 \text{ (3H, d, J=6.8 Hz, Me-28)}, \quad 0.88 \text{ (6H, d, J=6.5 Hz, 26, 27-Me)}, \quad 0.92 \text{ (3H, d, J=6.5 Hz, Me-21)}, \\
1.02 \text{ (3H, s, Me-19)}, & \quad 3.56 \text{ (1H, m, 3-ax-H),} \\
5.36 \text{ (1H, m, Olefinic proton)}, & \quad 1.07-2.34 \text{ (-CH}_2\text{ and -CH protons of cyclic system side chain).}
\end{align*}

Mass, m/z:

414 [M]\(^{++}\).

Acetylation of Fc-2:

Crystalline Fc-2 (30.0 mg) was treated with acetic anhydride (2.0 ml) and pyridine (1.0 ml) and allowed to stand over night at room temperature and then heated on a steam bath for 2 hours. After usual work up, the solid was washed well with water and dried. On several crystallization from chloroform-methanol, it gave colorless flakes (15mg), m.p. 114-15\(^0\) C, \([\alpha]\)\(^{17}_D\) - 48.5\(^0\).

Analysed for C\(_{31}\)H\(_{52}\)O\(_2\):

Calcd: C, 81.57; H, 11.40 %

Found: C, 81.52; H, 11.37%

Benzoate formation:

The Fc-2 (40 mg) was treated with benzoyl chloride (1ml) and pyridine (0.5 ml), the mixture was allowed to stand at room temperature overnight and then heated for about 6 hours on a water bath. The reaction mixture was cooled and ice cold water was added. The solid thus separated was filtered, washed with aqueous solution of potassium hydroxide (KOH) (2\%) and then with water. It was crystallized from methanol, m.p. 145-46\(^0\) C (25 mg), \([\alpha]\)\(^{17}_D\) -7.52.
Chapter - II

Fc-3

This compound was obtained on elution of the column with light petroleum ether-benzene (1:1). After crystallization with chloroform-methanol, it gave white crystalline solid, m.p. 262-64°C (140 mg).

Analysed for $C_{31}H_{52}O$:

Calcd: C, 84.50; H, 11.30%  
Found: C, 84.47; H, 11.2%

IR, $v_{\text{max}}$ cm$^{-1}$:

$2900$ (CH, str), $1705$ (>$C=O$), $1455$, $1385$, $1355$, $1170$, $1070$.

$^1$H NMR (400 MHz, CDCl$_3$) on $\delta$ scale:

0.72 (3H, s, CH$_3$), 0.87 (3H, s, CH$_3$), 0.89 (3H, s, CH$_3$), 0.92 (3H, s, CH$_3$), 0.95 (6H, s, 2CH$_3$), 1.05 (3H, s, CH$_3$), 1.18 (3H, s, CH$_3$), 1.25, 1.34, 1.45, 1.52, 1.58 (22 protons, m, -CH$_3$), 2.26-2.41 [3H, m, (C$_2$-2H and C$_4$-1H)].

Mass, m/z:

$426$ [M]$^+$ (92), 411 (40), 341 (32), 303 (70), 273 (100).

Fc-4

Elution of the column in light petroleum ether-benzene in ratio (4: 6) gave a fraction which on crystallization in chloroform–methanol yielded white crystals (40 mg) m.p.195°C.

Analysed for $C_{30}H_{51}O_2$:

Calcd: C, 81.02; H, 11.79%  
Observed: C, 81.0; H, 11.6%
IR, $v_{\text{max}}^{\text{KBr}}$ cm$^{-1}$:

3459, 2929, 2864 1719, 1642, 1461, 1383, 1202, 1111, 995, 878, 723 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) on $\delta$ scale:

0.87 (3H, brs, CH$_3$-24), 0.89 (3H, brs, CH$_3$-25), 0.90 (3H, brs, CH$_3$-19), 1.06 (3H, brs, CH$_3$-29), 1.10 (3H, brs, CH$_3$-26), 1.17 (3H, brs, CH$_3$-27), 1.269 (3H, brs, CH$_3$-30), 1.36 (3H, brs, CH$_3$-23), 1.56 (3H, brs, CH$_3$-11), 5.63 (1H, brs, H-12).

Mass, m/z (rel. Intensity):

m/z 444 [M]$^+$ C$_{30}$H$_{52}$O$_2$ (43.6), 427 (10.5), 414 (16.7), 314 (28.7) 299 (9.8), 274 (74.1), 234 (30.0), 219 (12), 193 (100), 150 (14.5), 136 (81.9), 124 (9.7), 123 (16.8), 121 (13.7), 111 (21.3), 109 (19.8), 97 (43.7), 83 (28.1), 69 (73.9), 55 (76.2), 43 (52.6).

Fc-5

The compound Fc-5 was obtained from column with light petroleum ether – benzene (3:7) eluate and crystallized from chloroform-methanol as colorless crystals (40 mg); m.p. 230°C. It gives positive Leibermann-Burchard test and Zimmermann test.

Analysed for C$_{30}$H$_{50}$O:

Calcd: C, 84.44; H, 11.81%

Observed: C 84.31; H, 11.63%

IR, $v_{\text{max}}^{\text{KBr}}$ cm$^{-1}$:

2926, 2870, 1715, 1640, 1540, 1459, 1365, 1260, 1110 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) on $\delta$ scale:

1.63 (1H, dd, J=3.6, 10.2, H-1 $\alpha$), 1.81 (1H, dd, J=3.3, 5.4, H-1 $\beta$), 2.56 (1H, dd, J=3.6, 10.2, H-2 $\alpha$), 2.51 (1H, dd, J=3.6, 5.4 H-2 $\beta$), 1.62 (1H, dd, J= 5.4, 3.3 H-5
α), 1.76 (1H, m, H-6α), 1.59 (1H, m, H-6β), 1.79 (1H, m H-7 α), 1.55 (1H, m H-7 β), 1.44 (1H, dd, J=5.1, 8.4,  H-9 α), 2.09 (1H, m H-11 α), 1.69 (1H, m H-11 β), 1.76 (1H, m H-12 α), 1.83 (1H, m, H-12 β), 2.48 (1H, m H-14 α), 2.37 (1H, m H-14 β), 1.53 (1H, m H-15 α), 1.35 (1H, H-15 β), 1.39 (1H, m H-16 α), 1.49 (1H, m, H-16 β), 1.83 (1H, dd, J=5.7, 7.5, H-17 β), 1.57 (1H, dd, J=2.1, 6.7, H-19 α), 1.65 (1H, dd, J=5.4, 3.3, H-19 β), 1.49 (1H, m, H-20 α), 1.69 (1H, m, H-20 β), 1.87 (1H, dd, J=5.7,6.3, H-21 α), 2.07 (1H, m, H-21 β), 1.32 (1H, brs, H-23 α), 1.19 (1H, brs H-24 α), 1.14 (1H, brs H-25 α), 1.09 (1H, brs H-26 α), 1.69 (1H, brs H-27 α), 1.43 (1H, brs H-27 β), 1.09 (1H, brs H-28 α), 0.86 (1H, brs H-29 α), 1.02 (1H, brs H-30 α).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) on \(\delta\) scale:

39.33 (C-1), 28.25 (C-2), 213.30 (C-3), 38.38 (C-4), 53.18 (C-5), 18.74 (C-6), 42.23 (C-7), 39.78 (C-8), 58.31 (C-9), 36.09 (C-10), 30.08 (C-11), 30.58 (C-12), 59.56 (C-13), 41.37 (C-14), 35.42 (C-15), 32.50 (C-16), 41.61 (C-17), 37.52 (C-18), 35.10 (C-19), 31.86 (C-20), 32.85 (C-21), 35.70 (C-22), 14.73 (C-23), 20.34 (C-24), 32.17 (C-25), 18.02 (C-26), 42.87 (C-27), 18.32 (C-28), 6.90 (C-29), 22.36 (C-30)

Mass, m/z (rel. Intensity):

m/z 426 [M]\(^{+}\) (53.8), 411 (9.7), 396 (3.5), 395 (47), 328 (6.8), 274 (18.3), 260 (52), 206 (41.8), 203 (22.9), 192 (16.5), 178 (12.3), 166 (27.1), 152 (35.5), 139 (47.2), 138 (65.8), 125 (46.9), 124 (41.2), 111 (13.1), 109 (62.2), 107 (63.3), 95 (63.8), 91 (61.2);

Fc-6

Fc-6 was obtained by elution of the column with light petroleum ether-benzene (2:8) and crystallized as white crystalline solid from chloroform-methanol, m.p. 198°C, R\(_f\) = 0.63 (benzene-chloroform, 8:2). It gave positive Leibermann-Burchard test.

\(\text{IR, } v_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}:\)

3360 (OH), 2960, 2880, 1650, 1465 (C=C), 1040 and 980 cm\(^{-1}\).
Chapter - II

$^1$H NMR (400 MHz, CDCl$_3$) on $\delta$ scale:

0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H, s), 1.0 (3H, s), 1.14 (3H, s), 1.08, 2.01 (-CH$_2$ and -CH protons of cyclic system and side chain), 3.01 (1H, dd, J=9.0 Hz and 7.0 Hz ), 4.88 (brs, 1H, OH proton), 5.21 (1H, m, olefinic proton).

Mass, m/z:

426 [M]**

Acetylation of Fc-6:

The compound Fc-6 (25 mg) was acetylated by heating it with acetic anhydride (1ml) and pyridine (0.5 ml) on a boiling water bath for 4 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was washed well with water and dried. On crystallization from chloroform-methanol, it gave colorless needles m.p. 241-42$^0$C, $[\alpha]_D^{23}$ + 68.9$^0$. 

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Chapter-III

Chemical Constituents from the leaves of Xylosma longifolium
Discussion
CHEMICAL CONSTITUENTS FROM THE LEAVES OF XYLOSMA LONGIFOLIUM

The genus Xylosma, a large genus of shrubs and trees is chiefly distributed in most of the tropical and subtropical regions. About four species occur in India. The Xylosma species are reported to possess medicinal properties such as Xylosma japonica is used to heal jaundice, scrofula, sore and tumours, as desulfurizing agent and as a deminozide agent. Earlier investigations on the sister species X. velutina and X. flexuosum of this plant reported the isolation of xylosmacin (phenolic glycoside), 2-(β-glucopyranosyloxy)-2,5-dihydroxybenzyl alcohol and velutinic acid. Xylosma longifolium commonly known as “Khandhara” is found to resemble opium in its action and is used with it for house pests and fences. Medical importance of X. longifolium prompted us to carry out the comprehensive investigation of the leaves of X. longifolium.

The present chapter deals with the isolation and characterization of following compounds from the leaves of Xylosma longifolium.

1. n-Hentriacontane
2. β-sitosterol
3. β-amyrin
4. Friedelin
5. Olean-12-en-3α-ol-28-oic acid 3α-D-glucopyranoside (new compound).
7. Quercetin.
8. Kaempferol-3-rhamnoside.
9. Quercetin-3-rhamnoside.
10. Kaempferol-3-β-xylopyranoside-4′-α-rhamnoside (new compound).

The leaves of Xylosma longifolium collected from Forest Research Institute, Dehradun in winter season and identified by Professor Wazahat Husain, were dried under shade and powdered. The air dried powdered leaves (2.5 kg) were thoroughly extracted with light petroleum ether (60-80°C), benzene and methanol successively.
The light petroleum ether and benzene extracts were found to be same on TLC and hence were mixed together. The mixed extract was then chromatographed over silica gel column loaded in light petroleum ether. The column was eluted successively with light petroleum ether, light petroleum ether-benzene (9:1-1:1), benzene, benzene-ethylacetate (9:1–1:1) and ethylacetate as eluting solvents. Light petroleum ether-benzene (9:1) eluate on crystallization furnished crystals of XI-1 and XI-2. The fractions obtained in light petroleum ether-benzene (8:2-1:1) gave two spots on TLC which were crystallized as XI-3 and XI-4.

The methanolic extract gave positive test of flavonoids. This extract was chromatographed over silica gel column using successively benzene, benzene-ethyl acetate (9:1–1:1) mixtures, ethyl acetate, ethyl acetate-methanol (9:1–1:1) mixtures and methanol as eluting solvents, appropriate fractions (IR spectra and TLC) were combined. The fractions obtained from benzene-ethyl acetate (9:1-8:2) solvent system gave three compounds coded as XI-5, XI-6 and XI-7. The eluants obtained in solvent system (7:3-1:1) showed three major spots on TLC, which were obtained in pure form on repeated column chromatography and fractional crystallization as XI-8, XI-9 and XI-10.

**Characterization of compound XI-1:**

Elution of the column with light petroleum ether-benzene (9:1) furnished a fraction which upon crystallization from chloroform-ethanol afforded XI-1 (410 mg), m.p. 68°C. Its molecular formula was established as C₃₁H₆₄ on the basis of expected molecular ion peak at m/z 436 and elemental analysis. Its identity as *n*-hentriacontane was further confirmed by comparison its spectral data ¹H-NMR (Table-1) and mass, (Scheme-1), melting point, mixed melting points and Co-TLC with those of authentic sample of *n*-hentriacontane.

\[
\text{CH}_3 (\text{CH}_2)_{29} \text{CH}_3
\]

\[\text{CH}_3 (\text{CH}_2)_{29} \text{CH}_3\]

(1)
Table I

$^1$H NMR spectral data of XI-I on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$-31</td>
<td>3</td>
<td>0.82 (3 H, t, $J=6.30$ Hz)</td>
</tr>
<tr>
<td>CH$_3$-1</td>
<td>3</td>
<td>0.91 (3 H, t, $J=6.9$ Hz)</td>
</tr>
<tr>
<td>5 x CH$_2$</td>
<td>10</td>
<td>1.25 (10 H, brs)</td>
</tr>
<tr>
<td>3 x CH$_2$</td>
<td>6</td>
<td>1.28 (6 H, brs)</td>
</tr>
<tr>
<td>2 x CH$_2$</td>
<td>4</td>
<td>1.37 (4 H, brs)</td>
</tr>
<tr>
<td>2 x CH$_2$</td>
<td>4</td>
<td>1.42 (4 H, brs)</td>
</tr>
<tr>
<td>15 x CH$_2$</td>
<td>30</td>
<td>1.53 (30 H, brs)</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>2</td>
<td>1.88 (2 H, m)</td>
</tr>
<tr>
<td>2 x CH$_2$</td>
<td>4</td>
<td>2.25 (4H, m)</td>
</tr>
</tbody>
</table>

brs = broad singlet, t = triplet, m = multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard.
C_{31}H_{64}, [M^+] m/z 436

Scheme-1
Characterization of compound XI-2:

It was obtained by elution of column with light petroleum ether-benzene (9:1) mixture followed by crystallization with chloroform-methanol as white crystalline solid (80 mg) m.p. 133-35°C. It gave positive Leibermann-Burchard test\textsuperscript{10a} and responded positively to the tetranitromethane color test. The mass spectrum showed the molecular ion peak at m/z 414.

It was characterized as β-Sitosterol (II) by direct comparison of spectral data (\textsuperscript{1}H NMR, MS, IR) and m.p with that of an authentic sample.\textsuperscript{11}

\begin{center}
\includegraphics[width=0.5\textwidth]{sitosterol.png}
\end{center}

(II)

Characterization of compound XI-3

XI-3 was eluted from the column with light petroleum ether-benzene (8:2) and crystallized from chloroform-methanol as white crystalline solid (80 mg) m.p. 197-98°C. It responded positively to Leibermann-Burchard test\textsuperscript{10a} The mass spectrum of XI-3 gave molecular ion peak at m/z 426 and analyzed for C\textsubscript{30}H\textsubscript{50}O.

From the above data and their direct comparison with an authentic sample, XI-3 was characterized as β-amyrin (III).\textsuperscript{11}
Characterization of compound XI-4:

XI-4 was eluted from the column with light petroleum ether-benzene (1:1) and crystallized from chloroform-methanol as shining needles (120 mg) m.p. 262-63°C. It was analysed for C_{30}H_{50}O (M^{+} m/z 426), It responded positively for the colour test of triterpene. The melting point agreed with that of friedelin. Its identity as friedelin (IV) was established by comparison of its $^1$H NMR, IR and mass spectra with an authentic sample of friedelin (IV).\textsuperscript{12-14}

Characterization of compound XI-5:

The compound XI-5 was eluted from the column of methanol extract with ethylacetate-methanol (9:1) and crystallized from chloroform-methanol as colorless shining crystals (100 mg) m.p. 278°C. Its elemental analysis agreed with the molecular formula C_{36}H_{58}O_{8}. It produced effervescence with sodium bicarbonate.
solution and responded positively with triterpenic glycosidic test. The IR spectrum of XI-5 (Fig. 1) showed characteristic absorption bands for hydroxyl groups at 3525, 3515, 3510, 3419, 2932, 2959, 2869, 1690 (COOH) and 1636 (C=C). Its mass spectrum (Fig. 3) displayed a molecular ion peak at m/z 618 consistent to a pentacyclic triterpenic glycoside, $C_{35}H_{59}O_{8}$. It indicated eight double bond equivalents. Five of them were adjusted in the pentacyclic framework of the triterpene and one each in vinylic linkage, carboxylic group and monosaccharide moiety. The characteristics ion fragments generated at m/z 248 and 206 due to retro Diels–Alder fragmentation pattern and subsequent ion fragments arose at m/z 203 [248 - COOH]$^+$, 191 [206 - Me]$^+$, 176 [191 - Me]$^+$, 233 [248 - Me]$^+$, 218 [233 - Me]$^+$, 600 [618 - H$_2$O]$^+$ and 557 (M – COOH – Me)$^+$ indicated the existence of vinylic linkage at C-12 in the oleanane type triterpenic nucleus. A fragment ion at m/z 601 indicated the loss of OH group from molecular ion at m/z 618, which further pointing out the presence of COOH group. Carbinol proton in ring A which was placed at C-3 on the basis of biogenetic consideration and carboxylic group at C-17.

The $^1$HNMR spectrum of XI-5 (Fig. 2, Table 2) showed six singlets at $\delta$ 0.85 (6H), 0.87 (3H), 0.91 (3H), 0.96 (3H), 0.98 (3H), 1.02 (3H) attributed to protons of seven tertiary methyl protons. The methylene protons appeared as multiplets in the range of $\delta$ 1.33-2.59. A broad singlet at $\delta$ 5.39 integrating for one proton was assigned to C-12 olefinic proton. The C-3 proton resonated as a double doublet $\delta$ 3.72 (J = 5.7, 4.5 Hz) supporting the $\beta$-position of the carbinol proton. The sugar protons appeared at $\delta$ 4.94, 4.30, 4.50, 3.45, 3.42, 3.18 accounted to H-1', 2', 3', 4', 5' and 6' respectively. The anomeric proton H-1' of glucose appeared as a doublet at $\delta$ 4.94 (J = 4.2 Hz). The chemical shift confirmed the direct attachment of the sugar moiety to the aglycone and the coupling constant (J = 4.2 Hz) between H-1' and H-2' suggested $\alpha$-configuration of glucose which was rarely seen.

Hydrolysis of the glycoside XI-5 with 0.2 N HCl gave an aglycone mp. 197 – 99°C and a sugar. The sugar was identified as glucose by comparison with authentic sugar on paper chromatography (n-butanol: acetic acid: water, 4:1:5) and aglycone was identified as oleanolic acid by comparison of m.p, m.m.p, R$_f$. value with that of authentic sample.
On the basis of above spectral data analysis and chemical reaction, the compound XI-5 has been characterized as olean-12-en-3α-ol-28-oic acid 3α-D-glucopyranoside (V).
Table 2

$^1$H NMR data of XI-5 (CDCl$_3$) 400 MHz

<table>
<thead>
<tr>
<th>Position</th>
<th>$\alpha$-Proton</th>
<th>$\beta$-Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.33 (ddd, $J$= 7.2, 7.5, 7.5 Hz)</td>
<td>1.71 (m)</td>
</tr>
<tr>
<td>2</td>
<td>1.88 (m)</td>
<td>1.85</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>3.72 (dd, $J$= 4.5, 5.7 Hz)</td>
</tr>
<tr>
<td>5</td>
<td>1.57 (m)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1.67 (m)</td>
<td>1.44 (m)</td>
</tr>
<tr>
<td>7</td>
<td>1.40 (m)</td>
<td>1.25 (m)</td>
</tr>
<tr>
<td>9</td>
<td>2.04 (dd, $J$= 5.5, 13.5 Hz)</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>2.45 (m)</td>
<td>2.42 (m)</td>
</tr>
<tr>
<td>12</td>
<td>5.39 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>1.33 (m)</td>
<td>1.28 (m)</td>
</tr>
<tr>
<td>16</td>
<td>1.96 (m)</td>
<td>1.23 (m)</td>
</tr>
<tr>
<td>18</td>
<td>2.59 (dd, $J$= 4.8, 8.1 Hz)</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>2.23 (d, $J$= 4.8 Hz)</td>
<td>2.19 (d, $J$= 8.1 Hz)</td>
</tr>
<tr>
<td>21</td>
<td>1.73 (m)</td>
<td>1.69 (m)</td>
</tr>
<tr>
<td>22</td>
<td>1.96 (m)</td>
<td>1.53 (m)</td>
</tr>
<tr>
<td>23</td>
<td>1.02 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>0.85 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>0.87 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>0.89 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>0.91 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29</td>
<td>0.96 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>0.98 (brs)</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>4.94 (d, $J$= 4.2 Hz)</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>4.30 (d, $J$= 7.8 Hz)</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>4.50 (dd, $J$=5.4, 5.7 Hz)</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>3.45 (m)</td>
<td>-</td>
</tr>
<tr>
<td>5'</td>
<td>3.42 (m)</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>3.18 (d, $J$= 9.0 Hz)</td>
<td>3.13 (d, $J$= 9.0 Hz)</td>
</tr>
</tbody>
</table>

The coupling constants are provided in paranthesis.
Fig. 3
**Chapter - III**

**Characterization of compound XI-6:**

The compound XI-6 was obtained by further eluting the column in solvent system ethylacetate-methanol (9:1) and crystallized from chloroform-methanol as yellow shining crystals (110 mg) m.p. 276-278°C. It gave greenish brown color with ferric chloride. The flavonoidic nucleus of XI-3 was evidenced by positive Shinoda test\(^{17,19}\) and Wilson–boric acid tests.\(^{16}\)

The U.V spectrum of the compound showed two maxima at 268 nm and 368 nm with inflexions at 294 and 322 nm. Addition of AlCl\(_3\) produced a very large bathochromic shift of band I to 421 nm, which was not affected by the addition of HCl. This indicated hydroxyls at 3 and 5 positions. Sodium acetate produced a bathochromic shift in band II of 7 nm (at 275 nm), indicating presence of 7-OH group. There was no appreciable shift in band I on addition of NaOAc/H\(_3\)BO\(_3\) indicating absence of ortho-dihydroxy grouping. These data can be rationalized in terms of Kaempferol structure for the compound.

A further support to the above structure was obtained by the mass spectral data of the compound. The mass spectrum showed the molecular ion peak at the m/z 286. The other important peaks were at m/z 258, 153, 134, and 121. The fragmentation pattern has been showed below in Scheme-II.

The above compound XI-6 on treatment with acetic anhydride and pyridine gave the acetyl derivative m.p. 180-182°C. The \(^1\)H-NMR spectrum of the acetate showed four acetoxyl functions, three of them were appeared as a singlet at \(\delta\) 2.3 and the fourth one also exists as a separate singlet at \(\delta\) 2.4. A pair of meta-coupled doublet at \(\delta\) 6.8 (J=2.0 Hz) and at \(\delta\) 7.0 (J=2.0 Hz) were attributed to H-6 & H-8 protons respectively. Another pair of ortho coupled doublets of two protons each at \(\delta\) 7.3 (J=9.0 Hz) and at \(\delta\) 7.7 (J=9.0 Hz) were assigned to H-3',5' & H-2',6' respectively. These data reconfirmed that the parent compound is Kaempferol.

Its identity as kaempferol was further confirmed by its melting, mixed melting points and Co-TLC with an authentic sample of kaempferol (VI).\(^{22}\)
Chapter - III

![Molecule Diagram](image)

(VI)

Table 3

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.7 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.3 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.0 (d, J=2.0 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.8 (d, J=2.0 Hz)</td>
</tr>
<tr>
<td>3 x OAc</td>
<td>9</td>
<td>2.3 (s)</td>
</tr>
<tr>
<td>1 x OAc</td>
<td>3</td>
<td>2.4 (s)</td>
</tr>
</tbody>
</table>

*s=singlet, d=doublet, spectrum run in CDCl₃ at 400 MHz, TMS as internal standard.*
Scheme-II
**Chapter III**

**Characterization of compound XI-7:**

The compound XI-7 was eluted from the column in solvent system ethylacetate-methanol (8:2) and crystallized from chloroform–methanol as yellow shining crystals m.p. 311-12°C. It was identified as quercetin (VII a) by co-chromatography with an authentic sample. It showed no depression in melting point on admixture with an authentic sample of quercetin. It gave an acetate (VII b), m. p. 194-95 °C and penta methyl ether (VII c), m. p 151-52°C; its identity as quercetin was further confirmed by comparison of its spectral data with those of authentic sample of quercetin (IVa).\(^{18}\)

The \(^1\)H NMR spectrum of its acetate XI-7Ac (VII b, Table-4) showed the signals due to five acetoxyl groups in the range of \(\delta 2.35-2.38\). The meta-coupled doublets at \(\delta 6.79\) and 7.21\((J=2.5\) Hz\) were assigned to C-6 & C-8 protons of A-ring respectively. The B-ring protons showed the ABX pattern, Two doublets at \(\delta 7.70\) \((J=2.5\) Hz\) for H-2' & 7.26 \((J=9.0\) Hz\) for H-5' and a quartet at \(\delta 7.62\) \((J_1=2.5\) Hz, \(J_2=9.0\) Hz\) for H-6'.

In the light of the above results, the compound XI-7 was assigned the structure as 5,7,3,3',4'-penta hydroxy flavone (quercetin, VIIa), supported by the mass spectrum which showed molecular ion peak at m/z 302.

![Diagram of quercetin structure]

(VII)
(VIIa) \(R=H\)
(VIIb) \(R=Ac\)
(VIIc) \(R=Me\)
Table 4

\[^{1}\text{H NMR spectral data of XI-7Ac on }\delta\text{-scale}\]

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.70 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>7.62 (q, J₁=2.5 Hz, J₂=9.0 Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>7.26 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.21 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.79 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>1 x OAc</td>
<td>3</td>
<td>2.38 (s)</td>
</tr>
<tr>
<td>4 x OAc</td>
<td>12</td>
<td>2.35 (m)</td>
</tr>
</tbody>
</table>

s=singlet, d=doublet, q=quartet, m=multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard.

**Characterization of compound XI-8:**

The compound XI-8 was eluted from column in solvent system ethylacetate-methanol (7:3), recovery of the solvent left a solid mass, which on several crystallisations from methanol-chloroform yielded light yellow granular crystals (100 mg) m.p. 177-78°C. It responded positively to Molisch test after hydrolysis and by the formation of osazone confirming the glycosidic nature. The glycoside gave red color with Mg/HCl$^{19a,19b}$ and pink color on reduction with sodium amalgam followed by acidification indicating it to be flavanone or 3-substituted flavonol.$^{20}$ The possibility of its being a flavanone glycoside was ruled out as it gave a yellow color with Wilson boric acid reagent.$^{21}$ The spot of the compound appeared deep purple under UV light which turned yellow on exposing to ammonia vapors, further supporting the 3-substituted nature of the flavonol.
The analysis of functional groups revealed the presence of \( \alpha, \beta \)-unsaturated \( >\text{C}=\text{O} \) (1650), phenolic OH (3420) and a complex aromatic substitution pattern 1620, 1570, 1370, 1140, 800 cm\(^{-1}\), besides a strong band at 2950 cm\(^{-1}\). The UV spectrum showed \( \lambda_{\text{max}}^{\text{acetonitrile}} \) at 244 sh, 265 and 350 nm. The bathochromic shifts of 15 nm in NaOAc in band II, 50 nm with AlCl\(_3\) and +42 nm with NaOMe in band I (without a decrease in intensity) indicated the presence of 5, 7 and 4'-hydrolysis.

The hydrolysis of the glycoside with 6% HCl gave an aglycone and a sugar. The aglycone was characterized as Kaempferol by m. p. 280-81°C and mixed melting point with an authentic sample. Further confirmation of the identity of the aglycone was furnished by co-TLC and spectral data. The sugar was identified as rhamnose by co-chromatography with an authentic sample.

Acetylation of XI-8 with Ac\(_2\)O/pyridine afforded hexa-acetate. The \(^1\)H NMR spectrum of the acetate (Table-5) exhibited two meta coupled doublets at \( \delta \) 6.38 (\( J=2.5 \) Hz) and \( \delta \) 6.20 (\( J=2.5 \) Hz), each integrating for one proton assigned to H-8 and H-6 protons respectively. It showed \( A_2B_2 \) pattern of B ring protons, Ortho coupled doublets at \( \delta \) 7.79 (\( J=9.0 \) Hz) and \( \delta \) 6.90 (\( J=9.0 \) Hz) were assignable to H-2', 6' and H-3', 5'. Total number of 18 protons was observed over the range of \( \delta \) 1.9 to 2.45 attributed to six acetoxyls, comparising three aromatic acetoxyls and three aliphatic acetoxyls. The sugar protons were observed in the range of \( \delta \) 3.24-5.09 (m) accounted for four protons. The anomic proton of rhamnose appeared at \( \delta \) 5.21 (d, \( J=2.0 \) Hz) and rhamnosyl methyl appeared at \( \delta \) 0.87 (d, \( J=6.0 \) Hz).

The partial methyl ether obtained on hydrolysis of the methylated glycoside was characterized by melting and mixed melting points as 5,7,4'-trimethoxyl kaempferol. The formation of the above trimethyl ether of kaempferol proved the attachment of the sugar residue at C-3 of the aglycone. The quantitative estimation of sugar by Somogyi’s copper micro method\(^{22}\) showed the presence of one mole of rhamnose per mole of aglycone. XI-8 was therefore characterized as kaempferol 3-O-rhamnoside (VIII).\(^{24}\)
Table 5

$^1$H NMR spectral data of XI-8Ac on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>7.79 (2H, d, $J$=9.0 Hz)</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>2</td>
<td>6.90 (2H, d, $J$=9.0 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.38 (1H, d, $J$=2.5 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.20 (1H, d, $J$=2.5 Hz)</td>
</tr>
<tr>
<td>H-1'' (rhamnosyl)</td>
<td>1</td>
<td>5.21 (1H, d, $J$=2.0 Hz)</td>
</tr>
<tr>
<td>sugar protons</td>
<td>4</td>
<td>3.24-5.09 (4H, m)</td>
</tr>
<tr>
<td>aliphatic acetoxyls</td>
<td>9</td>
<td>1.9-2.12 (9H, m)</td>
</tr>
<tr>
<td>aromatic acetoxyls</td>
<td>9</td>
<td>2.32, 2.34, 2.45 (9H, each singlet)</td>
</tr>
<tr>
<td>rhamnose methyl</td>
<td>3</td>
<td>0.87 (3H, d, $J$=6.0 Hz)</td>
</tr>
</tbody>
</table>

d = doublet, m=multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard.
Chapter III

Characterization of compound XI-9

Elution of the column with Ethylacetate-methanol (7:3) gave a fraction which upon crystallization with methanol-chloroform afforded XI-9 (110 mg) m.p 186-870°C. The glycosidic nature of the compound (XI-9) was evidenced by positive Molish test obtained after hydrolysis and by the formation of an osazone. The $^1$H NMR spectrum of its acetate (Table-6) showed four aromatic acetoyls at $\delta$ 2.52-2.90 (m) and three alcoholic acetoxyls at $\delta$-1.9 to 2.21 (m) and an anomeric proton at $\delta$ 5.40 (d, $J$=2.0 Hz) indicating it to be a rhamnoside. The glycoside gave positive tests with magnesium and hydrochloric acid and sodium amalgam followed by acidification, yellow color with Wilson boric acid reagent indicated it to be a 3-substituted flavonol. The XI-9 on hydrolysis gave an aglycone m.p. 312-14°C which was characterized as quercetin as described earlier. The sugar was found to be rhamnose by $R_f$ value, co-chromatography and by the formation of osazone.
Table 6

$^1\text{H NMR spectral data of XL-9Ac on } \delta\text{-scale}$

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-6'</td>
<td>1</td>
<td>7.40 (q, J=2.1 Hz and 9.0 Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.20 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>7.10 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.70 (d, J=2.1 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.51 (d, J=2.1 Hz)</td>
</tr>
<tr>
<td>H-1'' (anomeric protons)</td>
<td>1</td>
<td>5.40 (d, J=2.0 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td>4</td>
<td>3.30-5.70 (m)</td>
</tr>
<tr>
<td>Aromatic OAc (3',4',5,7)</td>
<td>12</td>
<td>2.52-2.90 (m)</td>
</tr>
<tr>
<td>Aliphatic OAc</td>
<td>9</td>
<td>1.90-2.21 (m)</td>
</tr>
<tr>
<td>Rhamnosyl methyls</td>
<td>3</td>
<td>0.86</td>
</tr>
</tbody>
</table>

q=quartet, d= doublet, m=multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard.
Chapter - III

The position of the sugar residue in the glycoside was determined by the hydrolysis of the methylated glycoside. The partial methyl ether obtained was characterized by melting and mixed melting points with an authentic sample (m.p. 193°C) as 3',4',5,7-tetramethoxy quercetin (IX). The formation of the tetramethyl ether of quercetin proved the attachment of sugar residue at C-3 position of the aglycone.

The quantitative estimation of sugar by Somogyi’s Copper micro method\(^{23}\) showed the presence of one mole of rhamnose per mole of the aglycone. XI-9 was therefore characterized as Quercetin-3-O-rhamnoside (X).\(^ {25}\)

\[
\begin{align*}
\text{CH}_3\text{O} & \\
\text{OCH}_3 & \\
\text{OCH}_3 & \end{align*}
\]

\[(\text{IX})\]

\[
\begin{align*}
\text{HO} & \\
\text{O} & \\
\text{O} \text{---rham} & \end{align*}
\]

\[(\text{X})\]

**Characterization of compound XI-10**

Elution of the column with ethylacetate-methanol (4:6-1:1) gave fractions which on crystallization with methanol-chloroform afforded pale yellow granules (220 mg) m.p. 265-67°C. The glycosidic nature of the compound (XI-10) was evidenced by the positive Molish test obtained after hydrolysis and by the formation of an osazone. The glycosidic nature was further confirmed by the \(^{1}\text{H}-\text{NMR} \) spectrum
of the acetate of XI-10 \textbf{(Table-7)} as it showed two aromatic acetoxyls at $\delta$ 2.45 (3H) and $\delta$ 2.34 (3H) and six aliphatic acetoxyls at $\delta$ 1.98-2.20 (18H, m, 6 x OAc ) indicating it to be a diglycoside.

The glycoside gave pink colour with Zn/HCl and red color on treatment with sodium amalgam followed by acidification$^{19}$ indicating its flavanone or flavone nature. A yellow color with Wilson boric acid reagent$^{26,27}$ and maxima at 240sh, 269, 306sh, 345 in the UV spectrum indicated it to be a flavonol glycoside. It gave brownish green color with FeCl$_3$ indicating the presence of hydroxyl group at C-5. The IR spectrum showed strong absorption bands at 3450 (OH), 1665 (C=O), 2950 (C-H), 1645 (C=C, aromatic) and a broad band at 1105-1000 cm$^{-1}$ indicating its glycosidic nature A bathochromic shift of +43 nm in band I with AlCl$_3$ further confirmed the presence of a free 5-OH group. The presence of free 7-OH group is confirmed by a bathochromic shift of +12 nm in band II with fused NaOAc.

Total acid hydrolysis of the glycoside with 2N HCl yielded equimolar mixture of L- rhamnose, D-xylose (PC and GLC) and an aglycone, m.p. 280.-81$^0$C characterized as kaempferol (VI) by spectral and chromatographic comparison with authentic sample.$^{28}$

Acetylation of XI-10 with AC$_2$O and pyridine afforded octa-acetate XI-10Ac m.p. 126-28$^0$C. The $^1$H-NMR spectrum of XI-10Ac \textbf{(Fig-4)}, showed two meta coupled doublets at $\delta$ 6.80 and 7.10 ($J=2.20$ Hz) were attributed to C-6 and C-8 protons respectively. Two ortho-coupled doublets at $\delta$ 7.89 ($J=8.5$ Hz) and $\delta$ 7.26 ($J=8.5$ Hz) which corresponded to A$_2$B$_2$ pattern were assigned to C-2', 6' and C-3', 5' protons of B-ring respectively. The anomeric protons at $\delta$ 5.15 ($J=9.5$) and 5.60 ($J=1.2$ Hz) were assigned to H-1'' xylose ($\beta$-configuration) and H-1'' rhamnose ($\alpha$-configuration) respectively. The rhamnosyl methyl appeared as a doublet at $\delta$ 1.20 ($J=6.0$ Hz). The remaining sugar protons were observed in the range $\delta$-3.77-5.60.
Table 7

$^1$H NMR spectral data of XI-10Ac on δ-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.89 (d, J=8.5 Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.26 (d, J=8.5 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.10 (d, J=2.2 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.80 (d, J=2.2 Hz)</td>
</tr>
<tr>
<td>2- aromatic acetoxyls</td>
<td>6</td>
<td>2.45 (s, OAc-5), 2.34 (s, OAc-7)</td>
</tr>
<tr>
<td>6- aliphatic acetoxyls</td>
<td>18</td>
<td>1.98 -2.20 (m)</td>
</tr>
<tr>
<td>H-1&quot;,2&quot;,3&quot;&quot;,4&quot;&quot;, (rhamnosyl)</td>
<td>11</td>
<td>3.77-5.60 (m)</td>
</tr>
<tr>
<td>H-1&quot;&quot;,2&quot;&quot;,3&quot;&quot;,4&quot;&quot;, (xylosyl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhamnosyl-CH$_3$</td>
<td>3</td>
<td>1.20 (d, J=6.0 Hz)</td>
</tr>
<tr>
<td>H-1&quot; (rhamnosyl)</td>
<td>1</td>
<td>5.60 (d, J=1.20 Hz)</td>
</tr>
<tr>
<td>H-1&quot;&quot; (xylosyl)</td>
<td>1</td>
<td>5.15 (d, J=9.50 Hz)</td>
</tr>
</tbody>
</table>

$s$=singlet, $d$=doublet, $m$=multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard.
The mass spectrum of the acetylated glycoside (Fig-5, scheme-III), was in agreement with the above assigned structure of the glycoside. The mass spectrum showed the presence of acetylated pentapyranoside, m/z 259 and acetylated hexapyranoside, m/z 273. The fragment ions observed at m/z 642 and m/z 628 accounted for the loss of acetylated pentopyranoside and hexopyranoside respectively from the molecular ion peak. The loss of both acetylated sugar moieties gave fragment at m/z 370. The aglycone fragment was observed at m/z 286. A retro Diels-Alder fragmentation pattern was observed at m/z 153 and 121 leading to fragments [A₁+H] and B₂⁺ which supported the presence of two hydroxyl groups in ring-A and one hydroxyl group in ring-B. Other prominent sugar fragments appeared at m/z 213, 171, 153, 111, 97, 96.

Enzymatic hydrolysis of the parent glycoside (XI-10) gave conclusive evidences of the position of attachment of two sugars and the nature of their linkages. Hydrolysis of XI-10 with α-rhamnosidase (containing α-pectinase) gave L-rhamnose indicating α-nature of sugar and a partial glycoside XI-10b, which gave a bathochromic shift of +60 nm with NaOMe in band I without a decrease in intensity (absent in glycoside). Thus showing that C-4' hydroxyl which was glycosylated in XI-10 had become free. The partial glycoside (XI-10b) was identified as kaempferol-3-xyloside, m.p. 224-26°C by UV diagnostic shift reagents and co-chromatography with authentic sample. Methylation of partial glycoside (XI-10b) followed by hydrolysis with 2N HCl gave a partial methyl ether (XI-10c), m.p. 134-35°C characterized as 3-OH, 5,7,4'-trimethoxyflavone (kaempferol 5,7,4'-trimethyl ether) by spectral and chromatographic comparison with authentic sample. The methylated sugars were identified as 2,3,4-tri-O-methyl-xylose by SiO₂ TLC according to Petek. This finally established that L-rhamnose was α-linked at C-4' while D-xylose was β-linked at 3-position. Quantitative estimation of sugar by Somogyi's copper micro method indicated the presence of 2 moles of sugar/mole of aglycone.
On the basis of these data, compound XI-10 was identified as kaempferol-3-\(\beta\)-xylopyranoside-4'-\(\alpha\)-rhamnoside (XI-10). To the best of our knowledge it is being reported for the first time.

![Chemical structure of XI-10]

- **XI-10**: \(R_1, R_2=H, R_3=rham, R_4=xyl\).
- **XI-10a**: \(R_1, R_2, R_3, R_4=H\)
- **XI-10b**: \(R_1, R_2, R_3=H, R_4=xyl\)
- **XI-10c**: \(R_1, R_2, R_3=CH_3, R_4=H\)
Scheme-III
Experimental
EXTRACTION OF THE LEAVES OF *XYLOSMA LONGIFOLIUM*

The fresh leaves of *Xylosma longifolium* collected from Forest Research Institute, Dehradun were dried under shade and crushed to make powder. The air-dried powdered leaves (2.5 kg) were thoroughly extracted by refluxing with light petroleum ether (60-80\(^0\)), benzene and methanol successively. The solvent was removed by distillation and each extract was concentrated under reduced pressure to gummy mass.

The light petroleum ether and benzene extracts were found to exhibit similar behavior on TLC examination in different solvent systems [petrol-benzene (1:1), benzene-Chloroform (3:1), benzene-acetone (5:1)] etc. Therefore, they were mixed together. The mixed extract was subjected to column chromatography over silica gel. The column was eluted successively with light petroleum ether, light petroleum ether-benzene (9:1-1:1), benzene, benzene-ethylacetate (9:1-1:1), ethylacetate, ethylacetate-methanol (9:1-1:1) eluants respectively. Light petroleum ether-benzene (9:1) eluant gave the different fractions which upon crystallization with CHCl\(_3\)-EtOH afforded the compounds XI-1 and XI-2. The fraction obtained in solvent system light petroleum ether-benzene (8:2) was crystallized from chloroform-methanol as fine crystals labeled XI-3. The eluates from column in solvent system Light petroleum ether-benzene (1:1) gave one major spot along with some minor impurities on TLC which was removed through crystallization with chloroform-methanol. The compound so obtained was labelled as XI-4.

The methanolic extract responded positively to color test for flavonoid. TLC examination of the methanolic extract in different solvent systems [benzene-pyridine-formic acid (36: 9: 5) and toluene-ethyl formate- formic acid (5: 4: 1)] showed it to be a complex mixture of six major compounds along with some minor impurities. They were tried to purify into individual components through column chromatography over silica gel. The column was eluted successively with benzene; benzene- ethyl acetate, ethyl acetate, ethyl acetate- methanol mixtures and finally with methanol. The purity of the fractions was monitored by TLC examination and infrared spectral studies. Appropriate fractions which showed similar behavior on TLC and same IR spectra
were mixed together. Repeated column chromatography of the fractions followed by fractional crystallization furnished six crystalline homogenous compounds, marked as XI-5, XI-6, XI-7, XI-8 XI-9 and XI-10.


Alcoholic ferric chloride, iodine vapors, aniline hydrogen phthalate and p-anisidine phosphate solutions were used as spray reagents for visualization of spots on TLC and paper chromatograms.

XI-1

The fraction eluted from the column with light petroleum ether-benzene- (9:1) was crystallized from CHCl₃-EtOH as colorless crystals (110 mg), m.p. 68⁰C.

**Analysed for C₃₁H₆₄:**

Calcd: C 85.23% H 14.77%

Found: C 85.20% H 14.73%

IR, ν_{max}^{KBr} cm⁻¹:

2931, 2852, 1453, 1372, 1169, 1078, 1017, 795 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) on δ-scale:

2.25 (4H, m, 2 x CH₂), 1.88 (2H, m, CH₂), 1.53 (30H, brs, 15 x CH₂), 1.42 (4H, brs, 2 x CH₂), 1.37 (4H, brs, 2 x CH₂), 1.28 (6H, brs, 3 x CH₂), 1.25 (10H, brs, 5 x CH₂), 0.91 (3H, t, J=6.9 Hz, Me-1), 0.82 (3H, t, J=6.3, Me-31).
Chapter - III

**Mass m/z:**

\[436 [M^+]\], 421, 407, 405, 85, 71, 57, 43.

**XI-2**

Elution of column with light petroleum ether-benzene (9:1) gave a substance which on repeated crystallization from chloroform-ethanol afforded white crystalline solid (80 mg) m.p. 133-35°C.

**IR, \(\nu_{\text{max}}^{KBr} \text{ cm}^{-1}\):**

3350 (OH), 1660, 1455 (C=C), 1370 (C-Me₂), 1055, 850.

**Mass, m/z:**

414 [M]⁺.

**XI-3**

XI-3 was obtained by elution of the column with light petroleum ether-benzene (8:2) and crystallized from chloroform-methanol as white crystalline solid (80 mg) m.p. 197-98°C.

**IR, \(\nu_{\text{max}}^{KBr} \text{ cm}^{-1}\):**

3350 (OH), 2955, 2885, 1660, 1460 (C=C), 1050 and 980 cm⁻¹.

**Mass, m/z:**

426 [M]⁺.

**XI-4**

Elution of the column with light petroleum ether-benzene (1:1) gave a fraction which upon crystallization with chloroform-methanol afforded white shining crystals of XI-4 (120 mg) m.p. 262-63°C.
Chapter - III

Analysed for $C_{31}H_{52}O_4$:

Calcd: C, 84.52; H, 11.36%  
Found: C, 84.48; H, 11.31%

$\text{IR, } v_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$:

2900 (CH, str), 1710 (>C=O), 1465, 1380, 1360, 1160, 1060.

Mass, m/z:

426 [M]$^+$ (92), 411 (40), 341(32), 303 (70), 273 (100).

$Xl-5$

The fraction eluted from the column of methanol extract with ethylacetate-methanol (9:1) yielded $Xl-5$, which was crystallized from $\text{CHCl}_3$-$\text{MeOH}$ to obtain colorless shining crystals (100 mg) of m.p. 278°C.

Analysed for $C_{36}H_{56}O_8$:

Calcd: C 69.9% H 9.38%  
Found: C 68.8% H 9.25%

$\text{IR, } v_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$:

3525, 3515, 3419 (OH groups), 2932, 2959, 2869, 1690 (COOH) 1636 (C=C), 1462, 1379, 1259, 1166, 1073, 1022, 1005, 800 cm$^{-1}$.

$^1$H NMR (400 MHz, CDC$_3$) on δ-scale:

1.33 (1H, ddd, J= 7.2, 7.5, 7.5 Hz, H-1α), 1.71 (1H, m, H-1β), 1.88 (1H, m, H-2α), 1.85 (1H, H-2β), 3.72 (dd, J= 4.5, 5.7 Hz, H-3β), 1.57 (1H, m, H-5α), 1.67 (1H, m, H-6α), 1.44 (1H, m, H-6β), 1.40 (1H, m, H-7α), 1.25 (1H, m, H-7β), 2.04 (dd, J= 5.5, 13.5 Hz, H-9α), 2.45 (1H, m, H-11α), 2.42 (1H, m, H-11β), 5.39 (1H, brs, H-12α), 1.33 (1H, m, H-15α), 1.28 (1H, m, H-15β), 1.96 (1H, m, H-16α), 1.23 (1H, m, H-16β), 1.25 (1H, m, H-17α), 1.25 (1H, m, H-17β), 2.04 (dd, J= 5.5, 13.5 Hz, H-9α), 2.45 (1H, m, H-11α), 2.42 (1H, m, H-11β), 5.39 (1H, brs, H-12α), 1.33 (1H, m, H-15α), 1.28 (1H, m, H-15β), 1.96 (1H, m, H-16α), 1.23 (1H, m, H-16β), 1.25 (1H, m, H-17α), 1.25 (1H, m, H-17β), 2.04 (dd, J= 5.5, 13.5 Hz, H-9α), 2.45 (1H, m, H-11α), 2.42 (1H, m, H-11β), 5.39 (1H, brs, H-12α), 1.33 (1H, m, H-15α), 1.28 (1H, m, H-15β), 1.96 (1H, m, H-16α), 1.23 (1H, m, H-16β), 1.25 (1H, m, H-17α), 1.25 (1H, m, H-17β), 2.04 (dd, J= 5.5, 13.5 Hz, H-9α), 2.45 (1H, m, H-11α), 2.42 (1H, m, H-11β), 5.39 (1H, brs, H-12α), 1.33 (1H, m, H-15α), 1.28 (1H, m, H-15β), 1.96 (1H, m, H-16α), 1.23 (1H, m,
Chapter - III

H-16β), 2.59 (dd, J= 4.8, 8.1 Hz, H-18α), 2.23(d, J= 4.8 Hz, H-19α), 2.19 (d, J= 8.1 HzH-19β), 1.73 (1H, m, H-21α), 1.69 (1H, m, H-21β), 1.96 (1H, m, H=22α), 1.53 (1H, m, H-22β), 1.02 (1H, brs, H=23α), 0.85 (1H, brs, H-24α), 0.87 (1H, brs, H-25α), 0.89 (1H, brs, H-26α), 0.91(1H, brs, H-27α), 0.96 (1H, brs, H-29α), 0.98 (1H, brs, H-30α), 4.94 (1H, d, J= 4.2 Hz, H-1'a), 4.30 (1H, d, J= 7.8 Hz, H-2'a), 4.50 (1H, dd, J=5.4, 5.7 Hz, H-3'a), 3.45 (1H, m, H-4'a), 3.42 (1H, m, H-5'a), 3.18 (1H, d, J= 9.0 Hz, H-6'a), 3.13 (1H,d, J= 9.0 Hz, H-6'β)

Mass m/z:


XI-6

It was eluted with ethylacetate-methanol (9:1) and crystallized from chloroform–methanol as yellow shining crystals (110 mg) m.p. 276-278°C.

Analysed for C_{15}H_{12}O_6

Calcd: C, 62.94; H, 3.52 %

Found: C, 62.44; H, 3.38 %

Mass m/z:

286[M⁺], 258, 153, 134, and 121.

UV data with shift reagents λ_{max} nm:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>254</td>
<td>268</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>260 sh</td>
<td>268</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>258 sh</td>
<td>267</td>
</tr>
<tr>
<td>NaOAc</td>
<td>275 sh</td>
<td>306</td>
</tr>
<tr>
<td>NaOAc /H₃BO₃</td>
<td>268</td>
<td>322</td>
</tr>
</tbody>
</table>

104
Acetylation of XI-6:

The crystalline compound XI-6 (20 mg) was acetylated with pyridine (2 ml) and acetic anhydride (4 ml) on water bath for about 4 hours. It was cooled to room temperature and poured to crushed ice. The separated solid was filtered, washed with distilled water and dried. On usual work up followed by crystallization with chloroform-methanol, it gave yellow colorless needles of XI-6Ac m.p. 180-82°C.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) on \(\delta\)-scale:

2.3 (9H, s, 3 x OAc), 2.4 (3H, m, 1 x OAc), 6.80 (1H, d, \(J=2.0\) Hz, H-6), 7.0 (1H, d, \(J=2.0\) Hz, H-8), 7.3 (1H, d, \(J=9.0\) Hz, H-3',5'), 7.7 (d, \(J=9.0\) Hz, H-2' H-6').

XI-7

Elution of the column with ethyl acetate-methanol in ratio (8:2) gave a fraction which on crystallization from chloroform-methanol yielded XI-7 as yellow shining crystals (120 mg) m.p. 311-12°C.

Analysed for \(C_{15}H_{10}O_7\):

Calcd: C, 59.62; H, 3.31 %
Found: C, 59.70; H, 3.33 %

UV data with shift reagents \(\lambda_{\text{max}}^{\text{MeOH}}\) nm:

- MeOH: 258, 272 sh, 300 sh, 371
- NaOMe: 248 sh, 321
- AlCl\(_3\): 273, 304 sh, 335, 457
- AlCl\(_3\)/HCl: 264, 358, 429
- NaOAc: 258 sh, 275, 390
- NaOAc/H\(_3\)BO\(_3\): 263, 301, 389
Acetylation of XI-7:

XI-7 (20 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on water bath for about 4 hours. On usual work up as described above followed by crystallization with ethanol, it gave yellow colored crystals of XI-7Ac, m.p. 194-95°C.

$^1$H NMR (400 MHz, CDCl$_3$) on δ-scale:

2.38 (3H, s, 1 x OAc), 2.35 (12H, m, 4 x OAc), 6.79 (1H, d, J=2.5 Hz, H-6), 7.21 (1H, d, J=2.5 Hz, H-8), 7.26 (1H, d, J=9.0 Hz, H-5'), 7.62 (q, J$_1$=2.5 Hz, J$_2$=9.0 Hz, H-6'), 7.70 (1H, d, J=2.5 Hz, H=2').

Methylation of XI-7:

The compound XI-7 (25 mg) in dry acetone (30 ml) was refluxed with (CH$_3$)$_2$SO$_4$ (1.0 ml) and sintered K$_2$CO$_3$ (0.5 g) for 20 hours. It was filtered and the residue washed several times with boiling acetone. After usual work up, the solid obtained was crystallized from chloroform-methanol to give (XI-7Me) as colorless needles, m. p. 151-52°C.

XI-8

Elution of the column with ethylacetate-methanol (7:3) gave fractions which on crystallization from chloroform-methanol yielded yellow granular crystals (100 mg) m.p.177-78°C. It gave positive Molish test and brown color with alcoholic ferric chloride.

UV data with shift reagents $\lambda_{max}^{MeOH}$ nm:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{max}^{MeOH}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>244 sh, 265, 350</td>
</tr>
<tr>
<td>NaOMe</td>
<td>279, 300, 392</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>265, 386 sh, 400</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>266, 386, 400</td>
</tr>
<tr>
<td>NaOAc</td>
<td>259, 369</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>255, 311sh, 368</td>
</tr>
</tbody>
</table>
IR, $v_{max}^{KBr}$ cm$^{-1}$:

3420, (OH), 2950, 1690, 1650 (C=O), 1620 (C=C), 1570, 1370, 1140, 800 cm$^{-1}$

1 complex aromatic substitution pattern

**Acetylation of XI-8:**

Acetic anhydride (3.0 ml) was added to pyridine (1.50 ml) solution of XI-8 (20 gm). The mixture was left for 24 hours at room temperature and then heated on a steam bath for two hours. After usual work up as described earlier, the solid was crystallized from chloroform-methanol mixture as colorless needles, m. p. 159-60°C.

$^1$H NMR (400 MHz, CDCl$_3$) on δ-scale:

7.79 (2H, d, J= 9.0 Hz, H-2', H-6'), 6.90 (2H, d, J= 9.0 Hz, H-3', H-5'), 6.38 (1H, d, J=2.5 Hz, H-8), 6.20 (1H, d, J=2.5 Hz, H-6), 5.21 (1H, d, J=2.0 Hz, H-1" rhamnosyl), 3.24-5.09 (4H, m, sugar protons), 2.32, 2.34, 2.45 (9H each singlet, aromatic acetoxyls), 1.9-2.12 (9H, m, aliphatic acetoxyls), 0.87 (3H, d, J=6.0 Hz, rhamnosyl methyl).

**Acid hydrolysis of XI-8:**

The glycoside XI-8 (30 mg) was hydrolyzed by heating it with 8% HCl, on water bath for 2 hours. The mixture was left overnight. The aglycone thus separated out was filtered, washed well with distilled water and dried. The crude product on crystallization from methanol gave kaempferol as yellow needles, m. p. 280-81°C.

**Analysed for C$_{15}$H$_{10}$O$_{6}$:**

Calcd: C, 62.93; H, 3.48 %

Found: C, 62.95; H, 3.49 %

**UV data with shift reagents $\lambda_{Max}^{MeOH}$ nm:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{Max}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>250sh, 266, 355</td>
</tr>
<tr>
<td>NaOMe</td>
<td>278, 320, 420</td>
</tr>
</tbody>
</table>
Acetylation of the aglycone:

The aglycone (30 mg) was heated with pyridine (1 ml) and Ac₂O (2 ml) on water bath for 2 hours. After usual workup it gave kaempferol tetra-acetate as cream colored needles, m. p. 180-82°C.

Analysed for C₂₃H₁₈O₁₀:

Calcd:  C, 60.79; H, 3.96 %
Found:  C, 60.75; H, 3.98 %

¹H NMR of acetate (400 MHz, CDCl₃) values on δ-scale

8.25 (2H, d, J= 9.0 Hz, H-2', H-6'), 7.19 (2H, d, J= 9.0 Hz, H-3', H-5'), 6.85 (1H, d, J=2.5 Hz, H-8), 6.62 (1H, d, J=2.5 Hz, H-6), 2.34-2.45, (12H, m, OAc-5, 7, 3, 4')

Identification of the sugar:

The acidic filtrate, left after filtering the aglycone was extracted with ether and then ethyl acetate to ensure the complete removal of any residual aglycone. The solution was concentrated to syrup in vacuum over NaOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on whatman No. 1 filter paper using n-butanol: water: ethanol (60:28:5:16:5) as solvent systems, employing descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying, were sprayed with aniline phthalate and p-anisidine phosphate solution. The chromatograms on drying at 100-105°C showed the presence of rhamnose only. The quantitative estimation of sugar by Somogyi’s copper micro method gave the value (0.44 ml) which corresponds to 1 mole of sugar per mole of aglycone.
Location of the sugar position:

Glycoside (40 ml) was dissolved in dry acetone and refluxed with dimethyl sulphate (1 ml) and ignited potassium carbonate (1 ml) for 36 hours on water bath. The mixture was cooled and filtered; the residue was washed with hot acetone. The washing and filtrate were combined and evaporated to dryness. The reddish brown oily residue left behind was washed by hot petroleum ether to remove the excess of dimethyl sulphate. Repeated attempts to crystallize the semi-solid mass proved fruitless. It was, therefore, directly hydrolyzed by heating with 7% H$_2$SO$_4$ for two hours on a steam bath. The reaction mixture was left over night. A fainty yellowish solid separated out. It was crystallized from methanol as very light yellow needles, m. p. 159°C. Methyl ether obtained on hydrolysis of the methylated glycoside was characterized by melting and mixed melting points as 5, 7, 4'-trimethoxyl kaempferol. The formation of the above trimethyl ether of kaempferol proved the attachment of the sugar residue at C-3 of the aglycone.

XI-9

XI-9 was eluted from the column with ethylacetate-methanol (7:3) and crystallized from methanol-chloroform to give yellow microscopic needles (110 mg) m.p. 186-87°C.

Analysed for $C_{21}H_{20}O_{11}$:

Calcd: C, 56.25; H, 4.46 %  
Found: C, 56.29; H, 4.43 %

UV data with shift reagents $\lambda_{\text{Max}}$ nm:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{\text{Max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>256, 264sh, 300sh, 355</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>279, 303sh, 335, 430</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>274, 309sh, 360</td>
</tr>
<tr>
<td>NaOAc</td>
<td>278, 305sh, 370</td>
</tr>
<tr>
<td>NaOAc /H$_3$BO$_3$</td>
<td>259 301sh, 367</td>
</tr>
</tbody>
</table>
Chapter - III

Acid hydrolysis of XI-9:

An alcoholic solution of XI-9 (30 mg) was hydrolyzed by heating it with 8% aq. HCl, which gave an aglycone, 312-14\(^0\)C and a sugar. Aglycone was identified as quercetin by direct comparison with authentic specimen. The sugar was identified as rhamnose (R\(_f\) 0.36) by co-paper chromatography using ethyl acetate-pyridine-water (12:5:4) as solvent system.

Analysed for C\(_{15}\)H\(_{10}\)O\(_{7}\):

Calcd: C, 59.60; H, 3.31 %
Found: C, 56.54; H, 3.029 %

Acetylation of XI-9:

The crystalline glycoside (40 mg) was acetylated with dry Ac\(_2\)O/py (1:1) at room temperature for 48 hr. After usual workup as described earlier, it was crystallized with methanol-chloroform as colorless needles, m.p. 302-03\(^0\)C.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) on \(\delta\)-scale:

7.40 (1H, q, J=2.1 Hz, and 9.0 Hz H-6'), 7.20 (1H,d, J=2.50 Hz, H-2'), 7.10 (1H, d, J = 9.0 Hz, H-5'), 6.70 (1H, d, J = 2.1 Hz, H-8), 6.51 (1H, d, J=2.1 Hz, H-6'), 5.40 (1H, d, J=2 Hz, H-1''), 3.30-5.70 (sugar protons, m, H-2'',3'',4'',5''), 2.52-2.90 (12H, m, OAc-3',4',5,7), 1.90-2.21 (9H, m, OAc-aliphatic), 0.86 (3H, d, J=6.5 Hz, rhamnosyl methyls).

Identification of sugars:

The neutral hydrolysate was concentrated and chromatographed on whatman No.1 filter paper using n-BuOH-acetic acid-water (4:1:5) and EtOAc- Pyridine-H\(_2\)O (2:1:2) as solvent systems, employing the descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature, they were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05\(^0\)C, showed the presence of only
rhamnose (0.36, 0.28) which was further confirmed by osazone formation, L-rhamnose, m.p. 184-85°C. Somogyi's copper micro method gave value (0.44 ml) which corresponded to one mole of sugar per mole of the aglycone.

**Location of the sugar position:**

Glycoside (30 ml) was dissolved in dry acetone and refluxed with dimethyl sulphate (1 ml) and ignited potassium carbonate (1 ml) for 48 hours on water bath. The mixture was cooled and filtered; the residue was washed with hot acetone. The washing and filtrate were combined and evaporated to dryness. After usual workup, a light yellow solid was obtained which could not be crystallized. It was, therefore, directly hydrolyzed by heating with 7% H₂SO₄ for two hours on a steam bath. The reaction mixture was left over night. A faintly yellowish solid separated out. It was crystallized from methanol as light yellow needles, m. p. 193°C. Methyl ether obtained on hydrolysis of the methylated glycoside was characterized by melting point and mixed melting points as 3',4',5,7-tetramethoxy quercetin (IX). The formation of the above tetramethyl ether of quercetin proved the attachment of the sugar residue at C-3 of the aglycone.

**XI-10**

It was obtained from ethylacetate-methanol (4:6-1:1) eluates and crystallized with methanol-chloroform as pale yellow granules (220 mg) m.p. 265-67°C.

**Analysed for C₂₆H₂₈O₁₄:**

Calcd: C, 56.11; H, 5.03 %  
Found: C, 56.14; H, 5.07 %

**UV data with shift reagents λ_{Max}^{MeOH} nm:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>λ_{Max}^{MeOH} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>240sh, 269, 306sh, 345</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>245sh, 312, 305sh, 340, 398</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>277, 304, 340, 396</td>
</tr>
<tr>
<td>NaOAc</td>
<td>281, 355, 370</td>
</tr>
</tbody>
</table>

111
Acetylation of XI-10:

The crystalline glycoside (45 mg) was acetylated with dry Ac₂O/py (1:1) at room temperature for 48 hr. After workup by the procedure described earlier, it was crystallized from chloroform-methanol as cream needles, m.p. 126-28°C.

\(^1\)H NMR (400 MHz, CDCl₃) on δ-scale:

\[6.80 \ (1H, \ d, \ J=2.2 \ Hz, \ H-6), \ 7.10 \ (1H,d, \ J=2.20 \ Hz, \ H-8), \ 7.89 \ (2H, \ d, \ J = 8.50 \ Hz, \ H-3', \ 5'), \ 5.15(1H, \ d, \ J=9.50 \ Hz, \ H-1'' \ xylose), \ 5.60 \ (1H, \ d, \ J=1.2 \ Hz, \ H-1'' \ rham), \ 1.20 \ (3H, \ d, \ J=6.1 \ Hz, \ rham-CH₃), \ 3.77-5.60 \ (1OH, \ m, \ gly-H), \ 2.45 \ (3H, \ s, \ OAc-5), \ 2.34 \ (3H, \ s, \ OAc-7), \ 1.98-2.20 \ (18H, \ m, \ aliphatic \ OAc).\]

Mass m/z:

\[642 \ [M^-acetylated \ pentose + H^+]^+, \ 628 \ [M^-acetylated \ hexose + H^+]^+, \ 370 \ [M^-acetylated \ pentose-acetylated \ hexose + 2H^+]^+, \ 286 \ [M^-614]^+, \ 273 \ [(rham) \ Ac₃]^+, \ 259 \ [(xyl) \ Ac₃]^+, \ 153 \ [A₁+H]^+, \ 121 \ [B₂]^+.\]

Acid hydrolysis of XI-10:

The glycoside XI-10 was hydrolyzed with 2N HCl-MeOH (5 ml), (100°C, refluxed for 2 hr). Water was added and the mixture was extracted with ethylacetate. The aqueous hydrolysate was neutralized with Ag₂CO₃, ppt filtered off and the filtrate evaporated in vacuo giving a residue.

Identification of aglycone:

The aglycone in EtOAc fraction was crystallized from CHCl₃-MeOH as yellow needles, m.p. 280-81°C and identified as kaempferol by spectral and chromatographic comparison with authentic sample.
Chapter - III

Analysed for $C_{15}H_{10}O_6$:

Calcd: C, 62.93; H, 3.49%
Found: C, 62.88; H, 3.45%

UV data with shift reagents $\lambda_{max}$ nm:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{max}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>249 sh, 267, 295 sh, 330 sh, 370</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>260 sh, 268, 300sh, 364, 421</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>260 sh, 267, 301sh, 365, 423</td>
</tr>
<tr>
<td>NaOMe</td>
<td>260 sh, 282, 316, 432</td>
</tr>
<tr>
<td>NaOAc</td>
<td>278, 301, 399.</td>
</tr>
</tbody>
</table>

Identification of sugars:

The neutral hydrolysate was concentrated and chromatographed on whatman No.1 filter paper using n-BuOH-acetic acid-water (4:1:5) and EtOAc- Pyridine-H$_2$O (2:1:2) as solvent systems, employing the descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature, they were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05$^\circ$C, showed the presence of only rhamnose (0.37, 0.28) and xylose (0.30, 0.22).

GLC of TMSi ethers of sugars:

The TMSi ethers of sugar was obtained by taking 15 mg of sugar in drying pyridine (0.5 ml) and hexamethyldisilazane (0.2 ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethyl chlorosilane was added and flask was stoppered and allowed to stand at room temperature for 45 minutes. The solution was then dried and taken in heptane. The heptane soluble TMSi ether derivatives of sugar were then subjected to GLC (2% OV-1, column temp.150-250$^\circ$C, 10 min. dect. temp.300$^\circ$C, N$_2$, 50 ml/min) along with silyl derivatives of standard sugars(R$_t$ 3.9, 3.8 min for rhamnose, 3.9, 4.5 min for xylose). The observed $R_t$ values were in agreement with those of an authentic sample of rhamnose and xylose.
Chapter - III

Enzymatic hydrolysis of XI-10:

A mixture of compound XI-10 (100 mg) and α-rhamnosidase (contain α-pectinase) (10 mg) was incubated in (NH₄)₂SO₄-NaOAc buffer (pH 5.0) at 250°C for 30 hours and then after addition of water, it was extracted with n-BuOH. The BuOH extract was chromatographed on silica gel column to give a partial glycoside, m.p. 254-56°C, identified as kaempferol-3-O xyloside. From the H₂O layer, L-rhamnose was identified by PC (n-butanol: acetic acid: water, 4:1:5).

Methylation of Partial glycoside:

CH₃I (1 ml) and Ag₂O (30 mg) were added to a solution of partial glycoside (30 mg) in DMF (3 ml). The mixture was stirred in dark at room temperature for 48 hours. The contents were filtered and the residue washed with little DMF. The filtrate was evaporated to dryness and the residue was treated with ethanol (25 ml). The alcohol was recovered and the syrupy residue was hydrolysed with 2N HCl. On usual workup it gave 3-OH, 5,7,4'- trimethoxy flavone (14 mg) m.p. 135-36°C. calcd. For C₁₈H₁₆O₆: C, 65.85; H, 4.87, Found: C, 65.91; H, 4.92%.

Quantitative estimation of sugars:

The anhydrous glycoside (25 mg) was hydrolyzed by refluxing with 0.2 N HCl. After cooling over night, the aglycone was dried and weighed (11.5 mg), the ratio of aglycone to glycoside is 44.2% indicating the presence of two moles of sugar/mole of aglycone.
Chapter - III


Chapter-IV

Chemical Constituents from the leaves of Crataegus crenulata
Discussion
Chapter IV

CHEMICAL CONSTITUENTS FROM THE LEAVES OF CRATAEGUS CRENULATA

Crataegus crenulata (Syn. Pyracantha crenulata, family Rosaceae) is an ornamental shrub or small tree, distributed in Himalayas in dry localities, from Kashmir to Bhutan (excluding Sikkim), alt-700-2400 m. Crataegus oxyantha is reported to be astringent, sedative, cardiotonic, diuretic, stomachic and antispasmodic. The extract of leaves, flowers and fruits containing flavonoids showed inhibitory effect on activity of guinea pig heart phosphodiesterase. The young leaves are used as a substitute for tobacco. The leaves contain vitexin-4'-rhamnoside, vitexin, quercetin, hyperoside, dimeric leucoanthocyanidide and epicatechin. Crataegus crenulata contains Pyracrenic acid named as 3β (3,4-dihydroxycinnamoyl)-oxygen-20(29)-en-oic acid. The acid exhibited anti-inflammatory activity by the formation of granulated tissue. Medicinal importance and scanty of work on C. crenulata accelerates our interest to carryout comprehensive investigation on its leaves.

The present thesis deals with the isolation and characterization of the following seven compounds from the leaves of Crataegus crenulata.

1. Lanost-5-en-2β,3β-diol-21-oic acid (new Compound).
2. β-sitosterol-D-glucoside.
3. 5,7,3',5'-tetrahydroxy flavanone (new Compound).
4. Acacetin-7-O-neohesperidoside.
5. Vitexin.
6. Dalpanitin.
7. 5,7,5'-trihydroxy-3'-C-β-D-glucopyranosyl flavanone (cranulatin) (new Compound).

The fresh leaves of Crataegus crenulata procured from Nainital valley were identified by professor Wazahat Husain, taxonomist, Department of Botany, AMU, Aligarh. Air dried and powdered leaves (2.0 kg) were successfully extracted with light petroleum ether, benzene, ethyl acetate, acetone and finally with methanol. The petrol and benzene extracts were found to contain mainly chlorophyll; waxy matter and resinous materials, hence were not analyzed further. The ethyl acetate and acetone
concentrates were found to contain same compounds in varying concentrations. The above concentrates were therefore, mixed together and subjected to column chromatography over silica gel. The column of mixed ethyl acetate and acetone concentrates was eluted with light petroleum ether, light petroleum ether-benzene mixture in different ratio (9:1-1:1), benzene and benzene: ethyl acetate (9:1-1:1), ethyl acetate and ethyl acetate: methanol (9:1-1:1) and finally with methanol as eluting solvents. Repeated column chromatography followed by fractional crystallization afforded seven compounds marked as Cc-1, Cc-2, Cc-3, Cc-4, Cc-5, Cc-6 and Cc-7.

**Characterization of compound Cc-1:**

Compound Cc-1, named lanostalysatoic acid, was eluted from the column in benzene-ethylacetate (9:1) and crystallized from chloroform-methanol as colorless crystals (100 mg), m.p. 249-250°C. It responded positively to Leibermann-Burchard test and yielded effervescences in sodium bicarbonate solution indicating triterpenic carboxylic acid nature of the compound. Its IR spectrum (Fig-1) displayed characteristic absorption bands for hydroxyl groups (3450 cm⁻¹), carboxylic group (1696 cm⁻¹) and unsaturation (1635 cm⁻¹).

The mass spectrum (Fig-4, Scheme I & II) of Cc-1 displayed a molecular ion peak at m/z 474 corresponding to dihydroxy substituted lanostanoic acid, C₃₀H₄₀O₄. It indicated six double bond equivalents. Four of them were adjusted in the lanostane-type triterpenic carbon framework, one each in the vinylic linkage and carboxylic function. The ion fragments generated at m/z 331 [M-C₈H₁₅O₂, side chain]⁺, 289 [331-ring D]⁺, 295 [331-2xH₂O]⁺ and 316 [331-Me]⁺ indicated that the carboxylic group was located in the side chain and two hydroxylic groups in the tetracyclic skeleton. The ion peaks arising at m/z 168 [C₇ₓHₛ-C₉₁₀ fission]⁺, 150 [168-H₂O]⁺, 163 [M-168-side chain]⁺, 182, 192 [C₇ₓHₛ-C₉₁₀ fission]⁺, 164 [182-H₂O]⁺, 149 [292-side chain]⁺, 238 [C₈₁₄-C₁₂,₁₃ fission]⁺, 95 [238-side chain]⁺, 218 [M-238-H₂O]⁺, 252 [C₈₁₄-C₁₁,₁₂ fission]⁺, 109 [252-side chain]⁺ and 184 [C₁₄,₁₅-C₁₃,₁₁ fission]⁺ supported saturated nature of ring C and D and presence of the vinylic linkage in ring B and hydroxyl
groups in ring A. One of the hydroxyl groups was placed at C-3 on the basis of biogenetic consideration.

The $^1$H-NMR spectrum (Fig-2, Table-1) exhibited a one proton broad singlet at $\delta$ 5.27 assigned to vinylic C-5 proton. A broad signal at $\delta$ 3.97 integrating for one proton was assigned to carbinol C-2β, equatorial proton. A one proton doublet at $\delta$ 3.23 was accounted to carbinol C-2β axial proton. Five three proton broad signals at 1.27, 1.10, 1.01, 0.88 and 0.79 were attributed to tertiary C-28 C-19, C-30, C-29, and C-18 methyl functionalities. A broad singlet integrating for six protons at $\delta$ 0.95 were ascribed to C-26 and C-27 secondary methyl protons. The location of all methyl signals in the range $\delta$1.27-0.79 indicated that all the methyl groups were located on the saturated carbons. An allylic C-7 proton was found to appear as a distorted doublet at $\delta$ 2.19 (J=9.0 Hz). A one proton multiplet at $\delta$ 2.14 was attributed to C-20H. It appeared at downfield due to presence of COOH group. A singlet at $\delta$ 3.5 was corresponded to two OH groups centered at C-2 & C-3 carbons respectively. It disappeared on addition of D$_2$O further confirming the existence of OH groups (Fig-3). The methylene protons were resonated in the range of $\delta$ 1.35-1.93. The $^1$H-NMR values were compared with the other lanostane-type triterpenes. On the basis of spectral data analysis the structure of Cc-I has been established as lanost-5-en-2β, 3β-diol-21-oic acid (I).
## Table 1

### $^1$H NMR spectral data of Cc-1

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-18</td>
<td>3</td>
<td>0.79 (3H, brs)</td>
</tr>
<tr>
<td>C-29</td>
<td>3</td>
<td>0.88 (3H, brs)</td>
</tr>
<tr>
<td>C-26, 27</td>
<td>3</td>
<td>0.95 (6H, brs)</td>
</tr>
<tr>
<td>C-30</td>
<td>3</td>
<td>1.01 (3H, brs)</td>
</tr>
<tr>
<td>C-19</td>
<td>3</td>
<td>1.10 (3H, brs)</td>
</tr>
<tr>
<td>C-28</td>
<td>3</td>
<td>1.27 (3H, brs)</td>
</tr>
<tr>
<td>C$_{20}$H</td>
<td>1</td>
<td>2.14 (1H, m)</td>
</tr>
<tr>
<td>C-3βH, eq</td>
<td>1</td>
<td>3.97 (1H, brs)</td>
</tr>
<tr>
<td>C-2αH, ax</td>
<td>1</td>
<td>3.24 (1H, brs, $W_2=18$ Hz)</td>
</tr>
<tr>
<td>C$_5$H</td>
<td>1</td>
<td>(1H, brs)</td>
</tr>
<tr>
<td>C-7H</td>
<td>1</td>
<td>2.19 (1H, distorted doublet $J=9.0$ Hz)</td>
</tr>
<tr>
<td>OH</td>
<td>2</td>
<td>3.51 (2H, s)</td>
</tr>
</tbody>
</table>

brs=broad singlet, $m$= multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard, chemical shifts are quoted on δ scale.
Scheme I
Characterization of compound Cc-2:

The compound Cc-2 was eluted from the column by benzene-ethylacetate (8:2) and crystallized from chloroform-methanol as colourless crystals (150 mg). m.p. 282°C. It gave positive Liebermann Burchard test and Molish test for glycosides. The elemental analysis agreed with molecular formula C_{35}H_{60}O_{6}.

The $^1$H NMR spectrum of Cc-2 (Table 2) shows the presence of six methyl groups in the range of $\delta$ 0.70-1.01. The signal at $\delta$ 5.2 indicated the characteristic olefinic proton of steroid. The protons appeared as multiplet in the range of $\delta$ 4.0-5.1 were assigned to sugar protons. An anomeric proton appeared at $\delta$ 4.2 as a doublet (J = 10 Hz). Hence, it was concluded that compound Cc-2 is the monoglycoside of a steroids.

Acetylation of Cc-2 with acetic anhydride and pyridine gave a colourless acetyl derivative Cc-2 (Ac), m.p. 166°C. $^1$H NMR spectrum of Cc-2Ac (Table 3) showed six methyl groups in the range of $\delta$ 0.71-1.04. The four independent singlets of three protons each at $\delta$ 1.95, 1.99, 2.02 and $\delta$ 2.04 supported the presence of four aliphatic acetoxyl groups of monoglycosidic acetate. There was a signal centered at $\delta$ 5.4 characteristic of an olefinic proton of steroid. It showed a group of multiplets from $\delta$ 4.2- 5.3 for a proton $\alpha$ to acetoxyl reminiscent of monoglycosidic acetate. From these data it could be concluded that the parent compound is monoglycoside of sterol. Similar conclusions were drawn from the mass spectral data too. The mass spectrum of glucoside acetate Cc-2Ac (Scheme III) shows the fragmentation pattern characteristic of $\beta$-sitosterol.

Hydrolysis of Cc-2 by Killani’s mixture yielded glucose and an aglycone Cc-2(ag). The aglycone having m.p. 137°C was identified as $\beta$-sitosterol by m.p, m.m.p, co-TLC and comparison of the spectral data (IR, $^1$H NMR and MS) with those of authentic sample.

On the basis of above results the compound Cc-2 was characterized as $\beta$-sitosterol-D-glucoside (II).
Table 2

$^1$H NMR spectral data of Ce-2

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$6 \times \text{CH}_3$</td>
<td>18</td>
<td>0.70 (s)- 1.01 (s)</td>
</tr>
<tr>
<td>CH$_2$-protons</td>
<td>2</td>
<td>1.10-1.23 (m)</td>
</tr>
<tr>
<td>H-1' (anomeric)</td>
<td>1</td>
<td>4.2 (d, J=10.0 Hz))</td>
</tr>
<tr>
<td>H-1',2',3',4',5',6'</td>
<td>7</td>
<td>4.0-5.1 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.2 (m)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m= multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard, chemical shifts are quoted on δ scale
Table 3

$^1$H NMR spectral data of Cc-2(Ac)

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 x CH₃</td>
<td>18</td>
<td>0.71-1.04</td>
</tr>
<tr>
<td>H-6 (olefinic proton)</td>
<td>1</td>
<td>5.4 (m)</td>
</tr>
<tr>
<td>Sugar acetoxyls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 x acetoxyl</td>
<td>12</td>
<td>1.95 (3H, s), 1.99 (3H, s), 2.02 (3H, s) and 2.04 (3H, s).</td>
</tr>
<tr>
<td>H-1',2',3',4',5',6'</td>
<td>7</td>
<td>4.2-5.3 (m)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m= multiplet, spectrum run in CDCl₃ at 400 MHz, TMS as internal standard, chemical shifts are quoted on δ scale
Scheme-III

$[M]^+_{absent}$

- glucose tetraacetate

$\text{m/z 396}$

$-C_{10}H_{21}$

$\text{m/z 255}$

$\text{m/z 214}$
Chapter - IV

Characterization of compound Cc-3:

Cc-3 was eluted from the column by benzene-ethyl acetate (1:1). On crystallization with chloroform-acetone mixture, it gave cream colour prisms (160 mg), m.p. 230°C. The molecular ion peak at m/z 288 [M]+ and the elemental analysis agreed with the molecular formula as C_{15}H_{12}O_{6}. A blue color with magnesium and hydrochloric acid and a red color with sodium amalgam followed by acidification and UV spectrum suggested a flavanone nucleus for the compound.²⁰,²³b Analysis of functional groups (Fig-5) revealed the presence of phenolic OH (3370 cm⁻¹), 2930 and α,β-unsaturated ketone (1643 cm⁻¹) and 1603 (C=C). The aromatic substitution pattern was exhibited by band at 964, 1014, 1078, 1163, 1273, 1348, 1478, 1533 cm⁻¹. Its UV spectrum showed the characteristic absorption at 314 nm & 283 nm. It gave a bathochromic shift of 26 nm with AlCl₃ in band-II suggesting the presence of free OH group at 5-position while a bathochromic shift of 32 nm with NaOAc indicated the presence of free OH at position at 7-position. In its ^1H-NMR spectrum (Fig-6, Table-4) two protons (C₃-2H) multiplet centered δ 2.87 and a one proton (C₂-H) multiplet centered around δ 5.36, clearly defines Cc-3 as a flavanone. The 5,7-disubstitution of ring-A was demonstrated by a solitary singlet at δ 5.86 integrating for two protons was attributed to H-6 and H-8 protons. Two independent singlets at δ 6.85 integrating for one proton and at δ 6.73 integrating for two protons were accounted for H-4' & H-2', 6' respectively.

In the ^13C-NMR spectrum of the Cc-3 (Fig-7) (Table-5) the signal at 78.81 ppm & 42.32 ppm were attributed to C-3 and C-2 carbons of flavanone nucleus. C-6 & C-8 carbons appeared at 96.22 ppm & 95.42 ppm respectively. The assignement were made on the basis of cited literature.²⁴

The above assignment was further supported by the mass spectrum (Fig-8, Scheme-IV) which showed the molecular ion peak at m/z 288. The retro-Diel's Alder cleavage was facile and lead to the fragment ion at m/z 153 [A]^+ & [B]^+ which were
indicative of two hydroxyl group in ring-A and two hydroxyl groups in ring-B. The other fragment ions were rationalised from the scheme-IV.

Therefore, the two hydroxyl groups in B ring of Cc-3 can be placed at position C-3' and C-5' respectively. On the basis of above results the compound Cc-3 was tentatively assigned as 5,7,3',5'-tetrahydroxy flavanone (III).
### Table 4

**1H NMR spectral data of Cc-3**

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-3</td>
<td>1</td>
<td>2.70 (dd, $J_1=17.0$ Hz, $J_2=3.0$ Hz)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>5.36 (dd, $J_1=15.0$ Hz, $J_2=3.0$ Hz)</td>
</tr>
<tr>
<td>H-6,8</td>
<td>2</td>
<td>5.86 (s)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>6.73 (s)</td>
</tr>
<tr>
<td>H-4'</td>
<td>1</td>
<td>6.85 (s)</td>
</tr>
<tr>
<td>7-OH</td>
<td>1</td>
<td>9.4142 (s)</td>
</tr>
<tr>
<td>5-OH</td>
<td>1</td>
<td>12.38 (s)</td>
</tr>
<tr>
<td>3'-OH</td>
<td>1</td>
<td>9.1407</td>
</tr>
<tr>
<td>5'-OH</td>
<td>1</td>
<td>9.199</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, t = triplet, q = quartet, spectrum run in CDCl$_3$ at 300 MHz, TMS as internal standard, chemical shifts are quoted on $\delta$ scale
Table 5

$^{13}$C NMR spectral data of Cc-3

<table>
<thead>
<tr>
<th>Assignments</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>78.81</td>
</tr>
<tr>
<td>C-3</td>
<td>42.32</td>
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<tr>
<td>C-4</td>
<td>196.69</td>
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<tr>
<td>C-5</td>
<td>167.09</td>
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<tr>
<td>C-6</td>
<td>96.22</td>
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<tr>
<td>C-7</td>
<td>163.81</td>
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<td>C-8</td>
<td>95.42</td>
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<td>C-9</td>
<td>146.03</td>
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<td>C-10</td>
<td>102.15</td>
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<tr>
<td>C-1'</td>
<td>129.86</td>
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<tr>
<td>C-2'</td>
<td>118.48</td>
</tr>
<tr>
<td>C-3'</td>
<td>115.77</td>
</tr>
<tr>
<td>C-4'</td>
<td>114.63</td>
</tr>
<tr>
<td>C-5'</td>
<td>145.52</td>
</tr>
<tr>
<td>C-6'</td>
<td>118.48</td>
</tr>
</tbody>
</table>
Scheme-IV

(Mass fragmentation of Cc-3)
Characterization of compound Cc-4

The compound Cc-4 was eluted from the column by ethylacetate and crystallized with methanol-chloroform as light yellow crystals (270 mg), m.p. 266-268°C. The glycosidic nature of Cc-4 was evidenced by the positive Molish test. It gave greenish brown color with ferric chloride. The flavonoidic nucleus was evidenced by positive Shinoda test and its UV spectrum which showed \( \lambda_{\text{max}} \) at 269 and 325 nm. Analysis with diagnostic reagents gave a bathochromic shift of 32 nm with \( \text{AlCl}_3 \) indicating the presence of free hydroxyl group at 5 position. The possibility of its being a flavanone glycoside was eliminated as it gave yellow color with Wilson boric acid reagent.

Its infrared spectrum displayed a carbonyl group at 1665 cm\(^{-1}\), phenolic hydroxyl at 3410 cm\(^{-1}\) and a complex aromatic substitution pattern at 1500, 1420, 1350, 1240, 1170 and 800 cm\(^{-1}\).

Total hydrolysis of Cc-4 with 6% HCl yielded equimolar mixture of rhamnose and glucose and an aglycone m.p. 230°C. The aglycone was characterized as acacetin (VIa) by spectral, m.m.p. and chromatographic comparison with an authentic sample. The sugars were identified by GLC of TMSi ether derivative and co-chromatography with authentic samples.

The UV spectra of the aglycone and the glycoside were almost similar except that the aglycone gave a shift of 17 nm in band II with NaOAc (absent in glycoside), thus suggesting that 7-position is involved in glycosylation. Partial hydrolysis of the glycoside (IIIa) with 1% H\(_2\)SO\(_4\) and \( \alpha \)-rhamnosidase yielded L-rhamnose (identified by PC, co-chromatography and GLC) and a partial glycoside (V) indicating rhamnose to be the terminal sugar. Partial glycoside on hydrolysis with almond emulsion yielded D-glucose. Quantitative estimation of the sugars by “Somogyi’s copper micro method” and periodate oxidation of the glycoside further confirmed that the sugar moiety is a disaccharide and both the sugars to be in pyranose form.

The \(^1\)H NMR spectrum of Cc-4 (Table 6) in DMSO-\(d_6\) showed the presence of seven aromatic protons. A sharp singlet at \( \delta \) 6.45 indicated the presence of H-3 proton, (\( \gamma \) pyrone ring). Two meta-coupled doublets at \( \delta \) 6.75 (\( J=2.5 \) Hz) and \( \delta \) 6.95 (\( J=2.5 \) Hz) integrating for one proton each were assigned to H-6 and H-8 protons.
respectively. Two ortho-coupled doublets at δ 7.15 (J = 9.0 Hz) and δ 8.05 (J = 9.0 Hz) integrating for two protons each corresponded to H-3', 5' and H-2', 6' protons respectively. A sharp three protons singlet at δ 3.85 was assigned to methoxyl group. A doublet at δ 1.07 (J = 6.0 Hz) was ascribed to rhamnosyl methyl. The anomic proton of rhamnose H-1'' (rham) appeared as doublet at δ 4.54 (J = 2.0 Hz) while the anomic proton of glucose H-1'' as a doublet was centered at δ 5.09 (J = 11.0 Hz). The other sugar protons appeared in the range of δ 3.15-5.21.

Acetylation of Cc-4 with Ac₂O/dry pyridine gave a hepta acetate Cc-4Ac (IVb) m.p. 216-220°C. The ¹H NMR spectrum of the acetate (Table-7) exhibited multiplet at δ 1.60-2.02 integrating for eighteen protons were assigned to aliphatic acetoxyis while the solitary aromatic acetoxy appeared as a singlet of a three protons at δ 2.45. A singlet at δ 3.90 integrating for three protons was assigned to methoxyl group. It established A₂B₂ pattern of B ring protons, 2', 6' and 3', 5' protons appeared as ortho-coupled doublets (J = 9.0 Hz each) at δ 7.81 and 7.09 respectively. The C-6 and C-8 protons of A-ring appeared as meta-coupled doublets (J = 2.5 Hz each) centered at δ 6.60 and 6.72. A singlet at δ 6.25 of one proton was assigned to H-3 proton. The anomic protons of rhamnose (H-1'') and glucose (H-1'') were centered at δ 4.98 (d, J = 2.0 Hz) and δ 5.25 (J = 11.0 Hz) respectively. The coupling constant of anomic protons hhamnose and glucose suggested α-L- rhamnose and β- D- glucose. The signals over the range of δ 3.35- 5.26 account for twelve protons of glucose and rhamnose residue. The positions of anomic protons of rhamnosyl, glucosyl moieties and that of rhamnosyl methyl¹¹b and the absence of characteristic signal for 2''- O-acetyl¹⁶a at δ 1.70 suggested 1→2 inter sugar linkage (attachment of rhamnosyl residue at 2''- position of glucose, neoheesperidoside). This was further confirmed by the identification of methylated sugars. Hydrolysis of the methylated glycoside (IVC) with 0.2 N HCl gave 2,3,4-tri-O- methyl rhamnose and 3,4,6-tri-O- methyl glucose (identified by SiO₂-TLC and paper chromatography according to Petek¹⁷) and partially methylated aglycone (VIIb) which was characterized as 7- hydroxyl-5, 4'-dimethoxy flavone.

The above assigned structure was further supported by the mass spectrum (Scheme-V) of acetylated glycoside (IVb). The molecular ion peak as expected was not observed.¹⁶b The presence of an acetylated deoxyhexopyranoside and a
hexopyranoside was evidenced by the presence of fragment ions at m/z 273 and 289. The fragment ion at m/z 284 corresponded to aglycone. The fragment ion at m/z 284 corresponded to aglycone. The retro-Dies-Alder cleavage resulted in the formation of ions at m/z 152 due to A-ring and at m/z 132 and 135 due to B-ring.

On the basis of the above results, the compound Cc-3 was assigned as *Acacetin-7-O-neohesperidoside*¹⁸ (IVA).
Table 6

$^1$H NMR spectral data of Cc-4

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$ (rhamnose)</td>
<td>3</td>
<td>1.07 (d, J = 6.0Hz)</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>3</td>
<td>3.85 (s)</td>
</tr>
<tr>
<td>H-1&quot; (rham)</td>
<td>1</td>
<td>4.54 (d, J = 2.0 Hz)</td>
</tr>
<tr>
<td>H-1&quot; (glu)</td>
<td>1</td>
<td>5.09 (d, J = 6.5 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.45 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.75 (d, J = 2.5 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.95 (d, J = 2.5 Hz)</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>2</td>
<td>7.15 (d, J = 9.0 Hz)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>8.05 (d, J = 9.0 Hz)</td>
</tr>
<tr>
<td>HO-5</td>
<td>1</td>
<td>12.91 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m = multiplet, spectrum run in DMSO-d$_6$ at 400 MHz, TMS as internal standard, chemical shifts are quoted on δ scale.
Table 7

$^1$H NMR spectral data of Cc-4Ac

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$ (rhm)</td>
<td>3</td>
<td>1.15 (d, J= 6.0 Hz)</td>
</tr>
<tr>
<td>4-OCH$_3$</td>
<td>3</td>
<td>3.90 (s)</td>
</tr>
<tr>
<td>6 x OAc</td>
<td>18</td>
<td>1.60-2.02 (m)</td>
</tr>
<tr>
<td>5-OAc</td>
<td>3</td>
<td>2.45 (s)</td>
</tr>
<tr>
<td>H-1&quot; (rham)</td>
<td>1</td>
<td>4.98 (d, J= 2.0 Hz)</td>
</tr>
<tr>
<td>H-1&quot; (glu)</td>
<td>1</td>
<td>5.25 (d, J= 11.0 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1&quot;, H-2&quot;, H-3&quot;, H-4&quot;, H-5&quot;.</td>
<td>12</td>
<td>3.35-5.26 (m)</td>
</tr>
<tr>
<td>Aromatic protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.25 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.60 (d, J= 2.5 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.72 (d, J= 2.5 Hz)</td>
</tr>
<tr>
<td>H-3'-5'</td>
<td>2</td>
<td>7.09 (d, J= 9.0 Hz)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>7.81 (d, J= 9.0 Hz)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m = multiplet, spectrum run in CDCl$_3$ at 400 MHz, TMS as internal standard, chemical shifts are quoted on $\delta$ scale.
Scheme 1
**Chapter IV**

**Characterization of compound Cc-5:**

Cc-5 was eluted from the column by ethyl acetate-methanol (9:1). On crystallization with methanol–chloroform, it gave yellow crystals m.p. 263-64°C. The molecular ion peak at m/z 432 and the elemental analysis agreed with the molecular formula as C_{21}H_{20}O_{10}. A dark reddish color with magnesium and hydrochloric acid and a red color on treatment with sodium amalgam, followed by acidification suggested a flavone nucleus for the compound.

The Cc-5 gave a positive Molish test\(^8\) and a dark brown color with ferric chloride. The analysis of functional groups revealed the presence of \(\alpha\), \(\beta\)-unsaturated \(\text{>C}=\text{O}\) (1650), phenolic OH (3420) and a complex aromatic substitution, besides a strong band at 2950 cm\(^{-1}\). The UV spectrum showed \(\lambda_{\text{Max}}\) at 268 and 335 nm. The red shift of 10 nm with NaOAc in band-II, 11 nm with AlCl\(_3\) and 38 nm with NaOMe in band I (without decrease in intensity) indicated the presence of 5, 7 and 4' hydroxyls groups.

Prolonged heating (5 hours) of the glycoside with 0.4 M HCl failed to hydrolyse the glycoside, suggestive of a C-glycosyl nature of the compound. This was further supported by two bands at 1010 and 1038 cm\(^{-1}\) in its IR spectrum.\(^9\) The compound (Cc-5) was oxidized with FeCl\(_3\), the sugar obtained was identified as glucose (by m.p, co-chromatography and GLC of trimethylsilyl derivative). Pyranose structure of glucoside was confirmed by periodate oxidation of methyl ether of the glucoside.

On boiling the glucoside (Cc-5) with hydroiodic acid in phenol, the sugar moiety was decomposed. The resulting product was identified as apigenin (Cc-5ag) by comparison of m.p. and spectral data (UV, IR, \(^1\)H NMR and mass) with those of an authentic samples.\(^20\) The UV spectrum of the aglycone was found to be identical with that of glucoside. The acetate of the glucoside, prepared by heating it with acetic anhydride and pyridine gave white crystals (Cc-5Ac), m.p. 154-156°C analyzed for C_{33}H_{32}O_{16}. 

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The $^1$H-NMR spectrum (Table 8) exhibited a pair of Ortho-coupled doublets integrating for two protons each at $\delta$ 7.40 ($I = 9.0$ Hz) and $\delta$ 8.10 ($I = 9.0$ Hz) corresponding to H-3', 5' and H-2', 6' protons respectively. Two independent singlets of one proton each at $\delta$ 6.80 and $\delta$ 6.91 were assigned to H-3 and H-6 protons. The anomeric protons H-1" (glu) appeared as a doublets at $\delta$ 4.64 ($I = 10.0$ Hz) showing trans diaxial relationship with H-2", while the sugar protons appeared in the range of $\delta$ 3.60-5.70. Three singlets at $\delta$ 2.32, 2.43 and 2.51 integrating for three protons each were attributed to three aromatic acetoxyls at 4, 7 and 5 positions. The four aliphatic acetoxyls appeared as a multiplet at $\delta$ 1.72-2.02. The presence of the signals at $\delta$ 1.72 for 2"-OAc and 2.02 for 6"-OAc indicated the presence of sugar moiety at C-8. This was supported by the negative Gibb's test. The location of the sugar at C-8 was further confirmed by the mass fragmentation, as the characteristic fragments at m/z 312 (aglycone attached to CH$_2$-CHO) was observed. The other fragments observed were at m/z 283 [aglycone attached with CH$_2$] and 354 [M$^{+}$-C-Glucosyl +H$^+$]. The fragment ion at m/z 270 seemed to be formed by the loss of 2 x CH$_2$=C=O groups from m/z 354. The RDA fragments representing ring-A and ring-B were observed at m/z 194 [A$_1]^+$ and at m/z 118 [B$_1]^+$ respectively.

On the basis of the above results, the compound (Cc-5) was characterized as Vitexin (VIIa).
Table 8

$^1$H NMR spectral data of Ce-5Ac on δ-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic acetoxyls</td>
<td>12</td>
<td>1.72-2.02 (m)</td>
</tr>
<tr>
<td>Aromatic acetoxyls:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAc</td>
<td>3</td>
<td>2.32 (s)</td>
</tr>
<tr>
<td>OAc</td>
<td>3</td>
<td>2.43 (s)</td>
</tr>
<tr>
<td>OAc</td>
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<td>2.51 (s)</td>
</tr>
<tr>
<td>H-1&quot;,2&quot;,3&quot;,4&quot;,5&quot;,6&quot;</td>
<td>7</td>
<td>3.65-5.70 (m)</td>
</tr>
<tr>
<td>H-1&quot;</td>
<td>1</td>
<td>4.64 (d, J=10.0 Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.8 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.91 (s)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.4 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>8.1 (d, J=9.0 Hz)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, m = multiplet, spectrum run in CDCl$_3$ at 400 MHz, using TMS as internal standard
Characterization of compound Cc-6:

Elution of the column with ethyl acetate-methanol (8:2) followed by crystallization afforded yellow plates (110 mg) m.p. 213-214°C (dec). Elemental analysis agreed with the molecular formula $C_{22}H_{22}O_{11}$. It gave positive Molish test and a green ferric color. Cc-6 on reduction with sodium amalgam followed by acidification with conc. hydrochloric acid, gave a pink color but no color on treatment with Mg/HCl. The color reactions and UV spectrum [$\lambda_{\text{max}}$ 211 nm, 266 and 290 (inf.) nm] indicated it to be an isoflavone glycoside.

The glycoside could not be hydrolyzed even on prolonged heating with 7% aq. HCl indicating it to be a C-glycoside, further supported by the presence of two bands at 1010- and 1038 cm$^{-1}$ in IR spectrum. Keeping in view, the C-glycosyl nature of Cc-6, it was oxidized with aq. FeCl$_3$. The sugar isolated was identified as glucose.

Cc-6 when boiled with hydroiodic acid in phenol, demethylation as well as decomposition of sugar moiety occurred, the resulting product was identified as orobol by m.p., m.m.p. co-chromatography and superimposable IR and UV spectra. The formation of orobol showed the presence of 3',4',5,7-tetraoxygenation pattern in Cc-6. The 3',4'-oxygenation pattern in B ring was further confirmed by the formation of veratric acid when Cc-6 methyl ether was subjected to alkaline hydrogen peroxide oxidation.

The UV spectrum of Cc-6 showed bathochromic shift with aluminium chloride and sodium acetate respectively, indicating the presence of free hydroxyls at 5 and 7 position. Thus OMe group could be located only in the side phenyl ring either at 3' or 4' position. The presence of OMe at 4' position was ruled out as it gave vanillic acid when hepta-acetate of Cc-6 (IXb) was oxidized by potassium permanganate. Thus OMe was placed at 3'-position. The $^1$H NMR spectrum of hepta-acetate (IX b) of Cc-6 (Table-9) indicated it to be a 8-C- glucoside. Two one proton singlets at $\delta$ 8.21 and 6.35 were assigned to the protons at 2 and 6 positions respectively. The three B ring protons showed a typical ABX splitting pattern, signals at $\delta$ 7.12 (d, J=2.0 Hz), $\delta$ 6.83 (d, J=8.5 Hz) and $\delta$ 8.0 (q, J=2.0 Hz and J=8.5 Hz) were assigned to 2, 5' and 6' positions respectively.
A three protons singlet at δ 3.81 was accounted for one OMe group. The singlet over the range of δ 3.25 - 5.30 integrated roughly for seven protons of the glucosyl residue. In this range, one proton doublet centered at δ 4.72 (J=10.0 Hz) was assigned to the proton at 1''-position. The aliphatic acetoxyls were observed in the region δ 1.72 - 2.08. The characteristic signal for 2''-OAc at δ 1.72 indicated the presence of glucosyl moiety at 8-position of aglycone, further supported by negative Gibbs test.\textsuperscript{24} The aromatic acetoxyls appeared over the range of δ 2.33 to 2.43.

In the mass spectrum of Cc-6 the M\textsuperscript{+} as expected, was not observed, the characteristic fragment ions at m/z 342 and 313 indicated the attachment of sugar moiety at 8-position. The other fragment ions are rationalized in (scheme VI). In the light of the above observation the Cc-6 was characterized as dalpanitin, (8-C-β-D-glucopyranosyl-3'-OMe-4',5,7-trihydroxy isoflavone) (IX a).\textsuperscript{25}
Table 9

\(^{1}H\) NMR spectral data of Cc-6Ac

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAc-2''</td>
<td>3</td>
<td>1.72</td>
</tr>
<tr>
<td>OAc-6''</td>
<td>3</td>
<td>2.04</td>
</tr>
<tr>
<td>OAc-4'' and 3''</td>
<td>6</td>
<td>2.08</td>
</tr>
<tr>
<td>OAc-4'</td>
<td>3</td>
<td>2.33</td>
</tr>
<tr>
<td>OAc-7</td>
<td>3</td>
<td>2.38</td>
</tr>
<tr>
<td>OAc-5</td>
<td>3</td>
<td>2.43</td>
</tr>
<tr>
<td>-OCH(_3)</td>
<td>3</td>
<td>3.82 (s)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td>7</td>
<td>3.25-5.30 (m)</td>
</tr>
<tr>
<td>Anomeric proton</td>
<td>1</td>
<td>4.72 (d, J= 10.0 Hz)</td>
</tr>
<tr>
<td>(H-1'' glu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.35 (s)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>6.83 (d, J= 8.5 Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.12 (d, J= 2.0 Hz)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>8.0 (q, J= 2.0 Hz and 8.5 Hz)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>8.21 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d= doublet, q=quartet, m=multiplet, spectrum run in CDCl\(_3\) at 400 MHz, using TMS as internal standard.
Scheme VI
Characterization of compound Cc-7:

Cc-7 Was eluted from the column by ethyl acetate-methanol (1:1). On crystallization with chloroform-methanol, it afforded light yellow crystals, m.p. 278-79°C (200 mg). The molecular ion peak at m/z 434 [M]+ along with the elemental analysis agreed to the molecular formula as C21H22O10. It responded positively to Molish test showing its glycosidic nature. It gave a blue color with Mg/HCl and red color with sodium amalgam by acidification pointing out the presence of flavanone nucleus. In its 1H-NMR spectrum two protons (C3-2H) double doublet centered around δ 2.75 and a one proton (C2-H) double doublet centered around δ 5.45, further defines Cc-7 as a flavanone.

Analysis of functional groups (Fig-9) revealed the presence of phenolic OH (3392 cm⁻¹), 2928 (C-H) and α,β-unsaturated ketone (1685 cm⁻¹) and 1640 (C=C). The aromatic substitution pattern was exhibited by band at 827, 1073, 1018, 1169, 1194, 1296, 1347, 1377, 1448, 1581 cm⁻¹. The ultraviolet spectrum showed characteristic absorption maxima at λmax 287 (band II) with a shoulder at λmax 330 nm. Analysis with shift reagent gave a bathochromic shift of 26 nm with AlCl₃ and 32 nm NaOAc confirmed the presence of free hydroxyl groups at 5 and 7- positions which was further supported by the +ve color test specific for 5,7-dihydroxyl system with Vanillin/hydrochloric acid reagent. The color reaction coupled with IR, UV data with diagnostic shift reagent indicated it to be a flavanone glycoside.

Prolonged heating (6 hours) of the glycoside (Cc-7) with 0.4 M HCl failed to hydrolyse the glycoside, suggestive of C-glycosyl nature of the compound. This was further supported by two bands at 1018 and 1073 cm⁻¹ in its IR spectrum. The compound (Cc-7) was oxidized with FeCl₃, the sugar obtained was identified as glucose (by m.p, co-chromatography and formation of osazone). Pyranose structure of glucose was confirmed by periodate oxidation of methyl ether of the glucoside which consumed 2 molecules of HIO₄ yielding 1 mole of formic acid.

On boiling the glucoside (Cc-7) with hydroiodic acid in phenol, the sugar moiety was decomposed. The resulting aglycone Cc-7ag was identified as of 5,7,3',5'-tetrahydroxy flavanone (Cc-7ag) on the basis of m.p. and spectral data
(UV, IR, $^1$H NMR and mass). The UV spectrum of the aglycone was found to be identical with that of glucoside. The aglycone Cc-7ag was identified in all respect with compound Cc-3 (III).

The $^1$H-NMR spectrum of Cc-7 (Fig-10, Table-10) showed a pair of double doublet at $\delta$ 2.75 ($J_1$ = 18.0 Hz & $J_2$=3.0 Hz) at $\delta$ 5.45 ($J_1$ = 12.0 Hz & $J_2$=3.0 Hz) were assigned to H-3 and H-2 proton respectively further supporting the presence of flavanone nucleus. The 5,7-disubstitution of ring-A was demonstrated by a two proton singlet at $\delta$ 6.14 assigned to H-6 & H-8 protons. Two independent singlets at $\delta$ 6.88 integrating for one proton and at $\delta$ 6.75 integrating for two protons were accounted for H-4' & H-2', 6' respectively. The signals over the range of $\delta$ 3.31-5.45 integrating for seven protons represented sugar protons while the anomic protons H-1" (glu) resonated at $\delta$ 5.32 ($J$=9.0 Hz). The large coupling constant for H-1" (glucose) was due to trans-diaxial coupling with the proton at 2"-position supported C-β-D-glucopyranosyl residue. The remaining three singlets of one proton each at $\delta$ 12.04, 9.02 & 9.07 were assigned to 5-OH, 7-OH and 3'/5'-OH. Thus the sugar may be placed either at 3' or 5' position. Since the B- ring is perpendicular to the rest of the molecule and is in free rotation. When the glucose is placed at 3'/5' position the rotation will be checked. In order to avoid crowding the glucose the preferably as per convention at position 3' rather than 5'-position.

In the $^{13}$C-NMR spectrum of the glycoside (Cc-7, Fig-11), (Table-11) the signal at 78.76 ppm & 42.18 ppm were attributed to C-3 and C-2 carbons of flavanone nucleus. C-6 & C-8 carbons appeared at 99.49 ppm & 95.46 ppm. The assignement were made on the basis of citated litraure.

The above assignment was further supported by the mass spectrum (Fig-12, scheme-VII) which showed the molecular ion peak at m/z 434. The retro-Diel's Alder cleavage was facile and lead to the fragment ion at m/z 153 [A]$^+$ & 282 [B]$^+$ which were indicative of two hydroxyl group in ring-A and one hydroxyl & one glucose moiety in ring-B. The other fragment ions were rationalised from the scheme-VII.
On the basis of above results the compound Cc-7 was tentatively assigned as 5,7,5'-trihydroxy-3'-C-β-D-glucopyranosyl flavanone named as cranulatin (X). To the best of our knowledge it has been reported for the first time and its aglycone also constitute the first report.

(X)
Table-10

$^1$H NMR spectral data of Cc-7

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>6.753 (s)</td>
</tr>
<tr>
<td>H-4'</td>
<td>1</td>
<td>6.885 (s)</td>
</tr>
<tr>
<td>H-6, 8</td>
<td>2</td>
<td>6.14 (s)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>5.45 (dd, $J_1$=12.0 Hz, $J_2$=3.0 Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>2.75 (dd, $J_1$=18.0 Hz, $J_2$=3.0 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1&quot;</td>
<td>1</td>
<td>5.31 ($J$=9.0 Hz)</td>
</tr>
<tr>
<td>H-1&quot;,2&quot;,3&quot;,4&quot;,5&quot;,6&quot;</td>
<td>7</td>
<td>3.31-5.45 (m)</td>
</tr>
<tr>
<td>5-OH</td>
<td>1</td>
<td>12.04 (s)</td>
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<tr>
<td>7-OH</td>
<td>1</td>
<td>9.02 (s)</td>
</tr>
<tr>
<td>5'-OH</td>
<td>1</td>
<td>9.07 (s)</td>
</tr>
</tbody>
</table>

$s =$ singlet, $br=$ broad, $dd=$ double doublet, $m=$ multiplet, spectrum run in CDCl$_3$ at 300 MHz, TMS as internal standard, chemical shifts are quoted on $\delta$ scale.
Table-11

$^{13}$C NMR spectral data of Cc-7

<table>
<thead>
<tr>
<th>Carbons</th>
<th>$^{13}$C NMR</th>
</tr>
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<tbody>
<tr>
<td>C-2</td>
<td>78.76</td>
</tr>
<tr>
<td>C-3</td>
<td>42.18</td>
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<td>C-4</td>
<td>197.21</td>
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<td>C-5</td>
<td>165</td>
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<tr>
<td>C-6</td>
<td>99.49</td>
</tr>
<tr>
<td>C-7</td>
<td>162.92</td>
</tr>
<tr>
<td>C-8</td>
<td>95.46</td>
</tr>
<tr>
<td>C-9</td>
<td>145.83</td>
</tr>
<tr>
<td>C-10</td>
<td>103.30</td>
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<tr>
<td>C-1'</td>
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<tr>
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<td>118.12</td>
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<tr>
<td>C-3'</td>
<td>115.37</td>
</tr>
<tr>
<td>C-4'</td>
<td>114.45</td>
</tr>
<tr>
<td>C-5'</td>
<td>145.22</td>
</tr>
<tr>
<td>C-6'</td>
<td>118.12</td>
</tr>
<tr>
<td>Sugar carbons</td>
<td></td>
</tr>
<tr>
<td>C-1&quot;</td>
<td>99.50</td>
</tr>
<tr>
<td>C-2&quot;</td>
<td>73.04</td>
</tr>
<tr>
<td>C-3&quot;</td>
<td>76.34</td>
</tr>
<tr>
<td>C-4&quot;</td>
<td>69.53</td>
</tr>
<tr>
<td>C-5&quot;</td>
<td>77.09</td>
</tr>
<tr>
<td>C-6&quot;</td>
<td>60.60</td>
</tr>
</tbody>
</table>
Scheme-VII

(Mass fragmentation of Cc-7)
CC-7

[ Mass Spectrum ]
Date: 0313PM1156 Date: 31-Jan-2007 15:28
Sample: CC-7 DR M PARVEEN AMU ALIGARH #1.134
Note:
Inlet: Direct
Ion Mode: FAB+
Spectrum Type: Normal Ion (MF-Linear)
RT: 0.49 min Scan#: 15.6
BP: m/z 154.0200 Int.: 86.35
Output m/z range: 29.0000 to 468.2433 Cut Level: 0.00%

![Graph of Mass Spectrum]

Fig. 12
Experimental
Extraction of the leaves of Crataegus crenulata

The leaves of *Crataegus crenulata* (3.0 kg) were collected from Nainital valley in the summer season and identified by Prof. Wazahat Hussain, taxonomist of Department of Botany, A.M.U., Aligarh. The leaves were dried under shade and crushed to make powder. The air dried and powdered leaves (2.0 kg) of *Crataegus crenulata* were successfully extracted by refluxing with light petroleum ether, benzene, ethyl acetate, acetone and finally with methanol. The solvent was removed by distillation and each extract was concentrated under reduced pressure. The petrol and benzene extracts were found to contain mainly chlorophyll and waxy matter, hence were not examined further. The ethyl acetate and acetone concentrates responded positively to color test for flavonoids and showed similar behavior on TLC examination in different solvent systems viz toluene: ethyl formate: formic acid (TEF, 5: 4: 1); benzene: pyridine: formic acid (BPF; 36: 9: 5); toluene: pyridine: acetic acid (TPA, 10: 1: 1); ethyl acetate: ethyl methyl ketone: acetic acid: water (20: 3: 1: 1). The above two extracts were, therefore, mixed together and subjected to column chromatography over silica gel.

The column was eluted with light petroleum ether, light petroleum ether-benzene mixture in different ratio (9: 1-1: 1), benzene and benzene: ethyl acetate (9:1-1: 1), ethyl acetate and ethyl acetate: methanol (9: 1-1:1) and finally with methanol as eluting solvents. The fractions showing the similar behavior on TLC examination and same IR spectra were mixed together. Repeated column chromatography followed by fractional crystallization gave seven crystalline compounds, labeled as Cc-1, Cc-2, Cc-3, Cc-4, Cc-5, Cc-6 and Cc-7. Homogeneity of each compound was checked by TLC examination and were characterized on the basis of chemical and physical evidences.

Cc-1:

Elution of the column Benzene-ethyl acetate (9:1), furnished colorless crystals of Cc-1, which recrystallized from chloroform-methanol (100 mg), mp.249-250°C.
Chapter IV

Analysed for $C_{30}H_{50}O_4$:

Calcd: C, 75.90; H, 10.62 %

Found: C, 75.87; H, 10.60 %

IR, $v_{\text{KBr}}$ cm$^{-1}$ (KBr):

3450, 2921, 2865, 1696, 1635, 1458, 1382, 1275, 1184, 1093, 1002 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) on $\delta$-scale:

5.27 (1H, brs, H-5), 3.24(1H, brs, $\delta_2$=18.0 Hz, H-2β ax), 3.97 (1H, brs. H-3β eq). 2.19 (1H, distorted doublet. J=9.0 Hz, H-7). 2.14 (1H, m, H-20). 1.27 (30 H. brs, Me-28), 1.10 (3H, brs, Me-19), 1.01 (3H, brs, Me-30), 0.95 (6H, brs, Me-26, 27). 0.88 (3H, brs, Me-29), 0.79 (3H, brs, Me-18).

$^{+ve}$ ion FABMS m/z (ret.int):

474 [M]$^+$ ($C_{30}H_{50}O_4$) (3.5), 331 (42.6), 316 (10.1), 295 (37.8), 292(41.2). 289 (13.9), 252 (16.3), 238 (18.5), 218 (17.8), 190 (22.6), 184 (16.9), 182 (21.3), 168 (28.1), 164 (28.3),163 (18.3), 150 (21.6), 149 (18.7) 149 (16.2). 143 (28.2). 123 (26.8). 109 (55.7), 108 (21.6). 95 (100).

Cc-2

The compound Cc-2 was obtained from the column by benzene-ethylacetate (8:2). It was crystallized from chloroform-methanol as colorless compound (150 mg). m.p. 282°C. It gave positive Leibermann-Burchard test and Molish test.

Analysed for $C_{35}H_{60}O_6$:

Calcd: C, 72.91; H, 10.41 %

Found: C, 72.87; H, 10.34 %
$^1$H NMR (400 MHz, CDCl\textsubscript{3}) on δ-scale:

0.70 (3H, s), 0.73 (3H, s), 0.75 (6H, s), 0.78 (3H, s), 1.01 (3H, s), 1.10-1.23 (2H, m, CH\textsubscript{2} protons), 4.2 (1H, d, J=10.0 Hz, H-1'), 4.0-5.1 (7H, m, H-1', 2', 3', 4', 5' and 6'), 5.20 (1H, m, olefinic proton).

**Killiani's hydrolysis of Cc-2:**

The compound Cc-2 (30 mg) was taken in a thick walled test tube and 2.0 ml of Killiani's mixture (glacial acetic acid-conc. hydrochloric acid-water 7:2:1) was added to it. The contents were heated on a boiling water bath for 3 hours, after tightly fixing a cork on the mouth of the test tube and tightening it further with the help of a thread. The reaction mixture on dilution with water gave a precipitate which was extracted with ether three times (3 x 25 ml) in a separating funnel. All the ethereal extract were combined together and washed well with water to remove acid. The ethereal extract was then dried over anhydrous sodium sulfate and filtered to remove inorganic salts. Recovery of ether gave a residue, which on crystallization with methanol gave a colorless compound (10.0 mg), m.p. 137\textdegree C. By co-TLC, petroleum ether (60-80\textdegree C): toluene: ethyacetate (10: 5: 3) with an authentic sample of β-sitosterol, it was found to be identical with it, m.p of Cc-2 when mixed with an authentic sample of β-sitosterol did not show any depression (m.m.p. 137\textdegree C).

**Analysed for C\textsubscript{29}H\textsubscript{50}O:**

<table>
<thead>
<tr>
<th></th>
<th>Calcd: C, 84.05; H, 12.07 %</th>
<th>Found: C, 84.02; H, 12.04 %</th>
</tr>
</thead>
</table>

**Identification Of sugar:**

The acidic filtrate left after removing the aglycone was extracted with ethylacetate to ensure the complete removal of any residual aglycone. The filtrate was neutralized by concentrating it to a syrup in vacuum dissicator over KOH pellets. The hydrolysate was chromatographed on Whatmann No.1 paper using n-BuOH-AcOH-H\textsubscript{2}O (5: 4: 1) and ethylacetate-pyridine-water (2: 1: 2) as developing solvents along
with authentic sugars as check. The chromatograms were then sprayed separately with aniline phthalate and p-anisidine phosphate solutions and dried at 100-05°C in an oven for fifteen minutes. One colored spot was developed on the chromatograms which showed the presence of one sugar. The Rf-value of sugar was identical with that of glucose.

**Acetylation of Cc-2:**

Crystalline Cc-2 (40 mg) was acetylated by heating it with acetic anhydride (2 ml) and dry pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on water bath for two hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid separated was collected, washed well with water and dried. On crystallization from methanol-chloroform it gave colorless crystals (30 mg), m.p. 166°C.

**H NMR (400 MHz, CDCl₃) on δ-scale:**

0.71-1.04 (18H, s, 6 x CH₃), 1.95 (3H, s, OCOCH₃), 1.99 (3H, s, OCOCH₃), 2.02 (3H, s, OCOCH₃), 2.04 (3H, s, OCOCH₃), 4.2-5.3 (7H, m, protons α-to acetoxyl), 5.4 (1H, m, H-6).

**Mass m/z:**


**Cc-3**

Cc-3 was eluted from the column by benzene-ethyl acetate (1:1). On crystallization with chloroform-acetone, it gave cream color prisms (160 mg) m.p. 230°C.

**Analysed for C₁₂H₁₂O₆:**

<table>
<thead>
<tr>
<th>Analyzed</th>
<th>Calcd</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, 62.50</td>
<td>H, 4.67 %</td>
<td>C, 62.42</td>
</tr>
</tbody>
</table>
IR, $\nu_{\text{max}}^\text{KBr}$ cm$^{-1}$:

3370 (OH), 2930 1643 (chelated C=O), 1603, 1533 (aromatic ring), 1478, 1348, 1273, 1163, 1078, 1014, 964

$^1$H NMR (300 MHz, CDCl$_3$) on $\delta$-scale:

2.70 (1H, dd, $J_1$=17.0 Hz, $J_2$=3.0 Hz, H-3), 5.36 (1H, dd, $J_1$=15.0 Hz, $J_2$=3.0 Hz, H-2), 5.86 (2H, s, H-6,8), 6.73 (2H, s, H-2',6'), 6.85 (1H, s H-4'), 9.41 (1H, 7-OH) 12.39(1H, s, 5-OH), 9.14 (1H, s, 3'-OH), 9.19 (1H, s, 5'-OH).

$^{13}$C NMR (75 MHz, DMSO-d$_6$), values on ppm scale:

78.81 (C-2), 42.32 (C-3), 196.69 (C-4), 167.09 (C-5), 96.22 (C-6), 163.81 (C-7), 95.42 (C-8), 146.03 (C-9), 102.15 (C-10), 129.860 (C-1'), 118.48 (C-2'), 115.77 (C-3'), 114.63 (C-4'), 145.52 (C-5'), 118.48 (C-6').

Mass m/z:


UV with shift reagent $A_{\text{MeOH}}^\text{MeOH}$ nm:

<table>
<thead>
<tr>
<th>MeOH</th>
<th>287, 335 sh</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl$_3$</td>
<td>314, 375</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>315, 374</td>
</tr>
<tr>
<td>NaOAc</td>
<td>283, 319</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>289, 318</td>
</tr>
</tbody>
</table>

Cc-4

Elution of the column with ethyl acetate gave a fraction which was crystallized from methanol-chloroform as light yellow crystals (270 mg), m.p. 266-68°C. The compound gave positive Molish test. It gave greenish brown color with FeCl$_3$ and +ve Shinoda's test.
Analyzed for C_{28}H_{32}O_{14}:

Calcd : C, 55.86; H, 5.51 %
Observed : C, 55.82; H, 5.47 %

**UV with shift reagents, \( \lambda_{\text{max}}^{\text{MeOH}} \) nm:**

- MeOH 269, 325
- NaOMe 245 (sh), 289, 360
- AlCl\(_3\) 278, 301, 344, 384
- AlCl\(_3\)/HCl 279, 302, 340, 380
- NaOAc 269, 326
- NaOAc/ H\(_3\)BO\(_4\) 270, 328

**IR \( \nu_{\text{max}}^{\text{EBr}} \) \text{ Cm}^{-1}:**

3410 (OH), 1665 (C=O), 1500, 1420, 1350, 1240, 1170, 800.

**\(^1H\) NMR (400 MHz, DMSO-\text{d}_6), values on \( \delta \) scale:**

12.91 (2H, s, HO-5), 8.05 (2H, d, J = 9.0 Hz, H-2',6'), 7.15 (2H, d, J = 9.0 Hz, H-3', 5'), 6.95 (1H, d, J = 2.5 Hz, H-8), 6.75 (1H, d, J = 2.50 Hz, H-6), 6.45 (1H, s, H-3), 5.09 (1H, d, J = 6.5 Hz, glu H-1\text{"}), 4.54 (1H, d, J = 2.0 Hz, rham H-1\text{"}), 3.85 (3H, s, OCH\(_3\)), 3.15-5.21 (12 H, m, sugar protons H-1\text{"}, 2\text{"}, 3\text{"}, 4\text{"}, 5\text{"}, 6\text{"}, H-1\text{""}, 2\text{""}, 3\text{""}, 4\text{""}, 5\text{""}, H-6\text{""}), 1.07 (3H, d, J = 6.0 Hz, CH\(_3\)-Rham).

**Hydrolysis of Cc-4:**

The glycoside Cc-4 (30 mg) was dissolved in EtOH and acidified with 6% HCl. The mixture was refluxed over water bath for 4 hours; and then left overnight at room temperature. The aglycone was filtered, washed well with distilled water and dried. The ratio of the aglycone to the glycoside was found to be 40.5% indicating the presence of two moles of sugar per mole of aglycone. The aglycone was crystallized from ethyl acetate as yellow crystals. Yield 10 mg, m.p. 230\(^0\)C.
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Analyzed for C_{16}H_{12}O_5:

Calcd : C, 67.60; H, 4.22 %

Observed : C, 67.59; H, 4.31 %

UV with shift reagents, $\lambda_{max}^{\text{MeOH}}$ nm:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>268, 304 sh, 328</td>
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<tr>
<td>NaOMe</td>
<td>277, 293 sh, 366</td>
</tr>
<tr>
<td>AlCl_3</td>
<td>258 sh, 279, 292 sh, 301, 344, 385</td>
</tr>
<tr>
<td>AlCl_3/ HCl</td>
<td>216 sh, 280, 295 sh, 303, 339, 380</td>
</tr>
<tr>
<td>NaOAc</td>
<td>277, 298 sh, 360</td>
</tr>
<tr>
<td>NaOAc/ H_3BO_4</td>
<td>270, 310 sh, 333</td>
</tr>
</tbody>
</table>

Identification of sugars:

The acidic filtrate left after removing the aglycone was extracted with ethyl acetate to ensure the complete removal of any residual aglycone. The filtrate was neutralized by concentrating it to a syrup in a vacuum dessicator over KOH pellets. The hydrolysate was chromatographed on Whatman No-1 paper using n-BuOH: AcOH: H_2O (5: 4: 1) and ethyl acetate: pyridine: water (2: 1: 2) as developing solvents along with authentic sugars as checks. The chromatograms were then sprayed separately with aniline phthalate and p-anisidine phosphate solutions and dried at 100-105° in an oven for 15 minutes. Two colored spots were developed on the chromatograms which showed the presence of two sugars. The R_f values of sugars were identical with those of rhamnose (R_f 0.37) and glucose (R_f 0.18).

GLC of TMSi ether of sugars of Cc-4:

The TMSi ether of sugars was obtained by taking 15 mg of sugar in dry pyridine (0.5 ml) and hexamethyl disilazane (0.2 ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethyl chlorosilane was added and the flask was stoppered and allowed to stand at room temperature for 45 minutes. The solution was then dried and taken in heptane. The heptane soluble TMSi ether derivatives of sugars were then subjected to GLC (2% OV-1, column temp. 150-250°, 10 min, dect. temp.
300⁰, N₂, 50 ml/min) alongwith silyl derivatives of standard sugars (Rt. Glucose 1.0 and rhamnose Rt 0.21). The observed Rt values were in agreement with those of an authentic sample of glucose and rhamnose.

Partial Hydrolysis:

Cc-4 (50 mg) was hydrolyzed by heating it with 1% H₂SO₄. Aliquots were taken at different intervals and examined by paper chromatography ethyl acetate: pyridine: water (EPW, 2:1:2). After one hour, it gave a partial glycoside labelled as Cc-4 pg and a sugar. The sugar was identified by usual method as rhamnose.

Enzymatic hydrolysis of Cc-4:

The glycoside (50 mg) in H₂O (10 ml) was incubated with α-rhamnosidase (which contains α-pectinase) at 18⁰C. The solvent was evaporated in vacuo and the residue treated with methanol. The methanol soluble portion was subjected to column chromatography over Sephadex LH-20. The column was eluted first with 60% aq. MeOH to afford a partial glycoside labelled as Cc-4 pg followed by water to yield a sugar. The sugar was identified as L-rhamnose by paper chromatography.

Enzymatic hydrolysis of Cc-4 pg:

The partial glucoside Cc-4 pg (10 mg) was treated with emulsion, prepared from almond at 30-40⁰C. After 80 hours, liberation of D-glucose in the hydrolysate was confirmed by paper chromatography by usual methods.

Acetylation of Cc-4:

Cc-4 (30 mg) was dissolved in pyridine (2 ml) and acetic anhydride (4 ml) was added. The reaction mixture was heated on a water bath for 4-5 hours and then left overnight at room temperature. The reaction mixture was worked up as procedure described earlier. It was crystallized from ethyl acetate-petroleum ether as white crystals (15 mg), m.p. 216-20⁰C.
\textbf{Chapter - IV}

$^1$H NMR (400 MHz, CDCl$_3$), on $\delta$ scale:

7.81 (2H, d, J = 9.0 Hz, H-2', 6'), 7.09 (2H, d, J = 9.0 Hz, H-3', 5'), 6.72 (1H, d, J = 2.5 Hz, H-8), 6.60 (1H, d, J = 2.5 Hz, H-6), 6.25 (1H, s, H-3), 5.25 (1H, d, J = 11.0 Hz, glu, H-1''), 4.98 (1H, d, J = 2.0 Hz, rham H-1''), 3.90 (3H, s, OCH$_3$), 3.35-5.26 (12H, m, sugar protons H-1'', 2'', 3'', 4'', 5'', 6'', H-1'', 2'', 3'', 4'', 5'', 6''), 2.45 (3H, s, 5 x aromatic acetoxyls), 1.60-2.02 (18 H, m, aliphatic acetoxyls), 1.15 (3H, d, J = 6.0 Hz, CH$_3$-Rham).

\textbf{Mass m/z (relative Intensity):}

$[M^+]$ absent, 289 [glu (Ac)$_3$] (33.2), 273 [rham (Ac)$_3$] (32.5), 284 [aglycone] (95.7) RDA fragments 152 [A$^+$] (18.0), 132 [B$_1$]$^+$ (36.1), 135 [B$_2$]$^+$ (5.3).

\textbf{Permethylatio of the glucoside followed by hydrolysis:}

Freshly ether washed, powdered sodium hydride in DMF (1 ml) was added to the glycoside (80 mg) in DMF (1 ml). To this solution 10-15 drops of CH$_3$I were added. The flask was sealed and left overnight. Excess of CH$_3$I was removed in vacuo and water was added. Permethyl ether formed as isolated by extraction with ether and purified by PTLC (benzene-acetone 4:1). The permethylated glycoside on hydrolysis with 2N HCl gave 2, 3, 4-tri-O-methyl rhamnose identified by TLC SiO$_2$, toluene-methanol 4:1 and PC) and permethylated partial glycoside. The permethylated partial glycoside on FeCl$_3$ (aq) oxidation yielded 3, 4, 6-tri-O-methyl-D-glucose and partially methylated aglycone characterized as 7-hydroxy-5, 4'-dimethoxy flavone, identified (by TLC SiO$_2$).

\textbf{Periodate oxidation of glycoside methyl ether:}

Glycoside methyl ether (15 mg) was dissolved in methanol (10 ml) and an aq solution of NaIO$_4$, .047 N (15 ml) was added to it. The mixture was allowed to stand at 20$^0$C for 24 hours. Solid NaHCO$_3$ (2 gm) was then added followed by addition of Na$_3$AsO$_3$ solution, 0.05 N (25 ml). The mixture was titrated against iodine using starch as indicator. One mole of methyl ether consumed 3.14 mole of periodate.
Cc-5 was crystallized from methanol-chloroform mixture as yellow needles m.p. 263-64°C (250 mg). It gave dark reddish colour with Mg-HCl, and positive Molish test (2 ml of aq. extract of the compound was added two drops of a freshly prepared 20% alcoholic solution of α-napthol. The mixture on treatment with 2 ml of conc. H₂SO₄ produced a red-violet ring which disappeared on the addition of an excess of alkali solution) and a dark brown color with alcoholic FeCl₃ solution.

Analysed for C₂₁H₂₀O₁₀:

\[
\begin{align*}
\text{Calcd:} & \quad \text{C}, 56.12; \text{H}, 4.67\% \\
\text{Found:} & \quad \text{C}, 56.09; \text{H}, 4.62\%
\end{align*}
\]

\[
\text{IR } v^{KBr}_{\text{max}} \text{ cm}^{-1}:
\]

3420 (OH), 2950, 1650 (C=O).

UV with shift reagent \( \lambda_{\text{Max}}^{\text{MeOH}} \) nm:

\[
\begin{align*}
\text{MeOH} & : 268, 298, 335 \\
\text{NaOMe} & : 278, 325, 393 \\
\text{AlCl₃} & : 279, 301, 353, 384 \\
\text{AlCl₃/HCl} & : 276, 300, 351, 385 \\
\text{NaOAc} & : 278, 300, 377 \\
\text{NaOAc/H₃BO₃} & : 270, 308 \text{ sh}, 342
\end{align*}
\]

Acetylation of Cc-5:

The crystalline glycoside Cc-5 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand over night at room temperature and then heated on a water bath for about four hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was separated, washed well with water and dried. On crystallization from ethyl acetate-petroleum ether, it gave white crystals (Cc-5Ac), m.p. 154-56°C.
Analysed for C$_{33}$H$_{32}$O$_{16}$:

Calcd: C, 57.89; H, 4.67%

Found: C, 57.79; H, 4.63%

$^1$H NMR (400 MHz, CDCl$_3$) on δ-scale:

1.72-2.02 (12 H, m, 4 x OAc, aliphatic acetoxyls), 2.32, 2.43, 2.51 (9H, s, 3 x OAc, aromatic acetoxyls), 3.65-5.70 (7H, m, H-1", 2", 3", 4", 5", 6", ), 4.64 (1H, d, J=10.0 Hz, H-1''), 6.80 (1H, s, H-3), 6.91 (1H, s, H-6), 7.4 (2H, d, J=9.0 Hz, H-3', 5'), 8.1 (2H, d, J=9.0 Hz, H-2', 6').

Hydroiodic acid oxidation:

A mixture of the glycoside (Cc-5) (30 mg), phenol (70 ml) and hydroiodic acid (0.3 ml) was refluxed for about 9 hours. The mixture was cooled and sodium bisulphate (NaHSO$_3$) was added to it with stirring. The separated brown substance was purified by passing it through a silica gel column.

Elution of the column with benzene-ethyl acetate (1:1) mixture afforded the substance, Cc-5Ag. It was crystallized from chloroform-ethyl acetate as light yellow crystals.

Yield (15 mg), m.p. 347-48°C.

Analysed for C$_{15}$H$_{10}$O$_5$:

Calcd: C, 66.66; H, 3.70%

Found: C, 66.64; H, 3.54%

UV with shift reagent $^\lambda_{MeOH}$ nm:

| MeOH | 266, 297, 336 |
| NaOMe | 277, 326, 395 |
| AlCl$_3$ | 280, 303, 351, 382 |
| AlCl$_3$/HCl | 278, 300, 352, 384 |
NaOAc : 276, 300, 378
NaOAc/H₃BO₃ : 270, 306 sh, 345

**Ferric chloride oxidation:**

The glycoside (Cc-5) (30 mg) was added to a solution of FeCl₃ (120 mg) in 3 ml water. The mixture was heated on an oil bath at 125°C for about 7 hours. The reaction mixture after cooling, was diluted with water (10 ml), a small amount of dark colored solid formed was filtered off. The filtrate was purified by passing through a column of silica gel using water as eluant. The initial fraction obtained were combined and concentrated to a syrup which was subjected to paper chromatography on Whatman No.1 filter sheet using n-BuOH -AcOH-H₂O (4:1:5) and n-BuOH-water-ethanol (60:25:8:18.5) as solvent systems and employing the descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hrs and after drying at room temperature were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-105°C revealed the presence of glucose only.

**GLC of Trimethyl silyl ether of sugar:**

The TMSi ether of sugar was prepared by taking 15 mg of sugar in dry pyridine (0.5ml) and hexamethyl disilazane (0.2ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethyl chlorosilane was added. The flask was stoppered and kept at room temperature for one hour. The solution after drying was taken in heptane. The heptane soluble TMSi ether derivative of standard sugar was then subjected to GLC (2% OV-1, column temp. 150-250°C, 10 min. dect. temp.300°C, N₂ 50 ml/min) along with the silyl derivative of standard sugar. The observed $R_t$ value was found to be in agreement with that of authentic sample of glucose ($R_t$ Glucose 1.0).

**Periodate oxidation of glycoside methyl ether:**

Glycoside methyl ether (15.0 mg) of Cc-5 was dissolved in methanol (10 ml) and an aqueous solution of NaIO₄ (0.47 N, 15 ml) was added to it. The mixture was kept at 20°C in dark for 24 hours. Solid NaHCO₃ (2.0 gm) was then added following...
by the addition of Na$_3$AsO$_3$ solution (0.05N, 25 ml). The resultant mixture was
titrated against iodine using starch as indicator. One mole of methyl ether consumed
1.2 mole of periodate, with the liberation of one mole of formic acid.

Cc-6:

It was eluted from the column in EtOH-MeOH (8:2) and crystallized with
methanol as light yellow plates (110 mg) m.p. 213-14°C.

**Analysed for C$_{22}$H$_{22}$O$_{11}$:**

Calcd: C, 57.14; H, 4.76 %

Found: C, 57.09; H, 4.65 %

**UV data with shift reagents** $\lambda_{\text{Max}}$ **nm:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{\text{Max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>211 sh, 266, 290</td>
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<tr>
<td>AlCl$_3$</td>
<td>220, 277, 386</td>
</tr>
<tr>
<td>NaOAc</td>
<td>230, 278, 330</td>
</tr>
<tr>
<td>NaOAc /H$_3$BO$_3$</td>
<td>268, 290</td>
</tr>
<tr>
<td>NaOMe</td>
<td>217, 281, 331</td>
</tr>
</tbody>
</table>

**IR, $\nu_{\text{Max}}$ cm$^{-1}$:**

3450 br (OH), 1665 (conjugated C=O), 1625, 1590, 1530 (aromatic), 1010, 1038 cm$^{-1}$.

**$^1$H NMR (400 MHz, DMSO-$d_6$) on $\delta$-scale:**

8.21 (1H, s, H-2), 8.00 (1H, q, J=2.0Hz and 8.5Hz, H-6'), 7.12 (1H, d, J=2.0 Hz, H-2'), 6.82 (1H, d, J=8.5 Hz, H-5'), 6.35 (1H, s, H-6), 4.72 (1H, d, J=10.0 Hz, H-1", glu), 3.25-5.30 (7H, m, sugar protons), 3.82 (3H, s, OCH$_3$), 9.16 (br, s, 4'-OH, exchangeable), 10.43 (br, s, 7-OH, exchangeable), 13.22 (s, 5-OH, exchangeable).
Chapter IV

Mass m/z:

M\(^+\) absent, 444 [M\(^+\)-H\(_2\)O] (100), 426 [M\(^+\)-2H\(_2\)O] (45), 408 [M\(^+\)-3H\(_2\)O] (80), 342 [aglycone + CH\(_2\)-CHO] \(^+\), 313 [aglycone + CH\(_2\)] (60), 300 (45), RDA fragments, 165 (7) and 148 (12.3)

Acetylation of Cc-6:

Acetic anhydride (3.0 ml) was added to pyridine (1.5 ml) solution of Cc-6 (10 mg), was heated on a steam bath for three hours. After usual work up, the solid was crystallized from chloroform-methanol mixture as colorless crystals, m.p. 134°C.

Analysed for C\(_{36}\)H\(_{56}\)O\(_{18}\)

Calcd: C, 57.14; H, 4.76 %

Found: C, 57.10; H, 4.71 %

IR, \(v_{\text{max}}\) cm\(^{-1}\):

1770 br, 1660, 1620, 1600, 1520, 1400, 1220 br, 1140, 1060, 1045, 1010, 910 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDC\(_3\)) on \(\delta\)-scale:

8.21 (1H, s, H-2), 8.00 (1H, q, J= 2.0 Hz and 8.5 Hz, H-6\(^\prime\)), 7.12 (d, J = 2.0 Hz, H-2\(^\prime\)), 6.83 (d, J = 8.5 Hz, H-5\(^\prime\)), 6.35 (1H, s, H-6\(^\prime\)), 4.72 (1H, d, J =10.0 Hz H-1\(^\prime\)), 3.25-5.30 (6H, m, sugar protons), 3.82 (3H, s, OCH\(_3\)), 2.43 (3H, s, OAc-5), 2.38 (3H, s, OAc-7\(^\prime\), 2.33 (3H, s, OAc-4\(^\prime\)), 2.08 (6H, s, OAc-3\(^\prime\),4\(^\prime\)), 2.04 (3H, s, OAc-6\(^\prime\)) 1.72 (3H, s, OAc-2\(^\prime\)).

Oxidation of glycoside with aq. FeCl\(_3\):

The glycoside Cc-6 (20 mg) was refluxed with FeCl\(_3\) (250 mg) and H\(_2\)O on sand bath for 6 hours. After usual work up, as described earlier the sugar was identified as glucose (R\(_f\) 0.18, n-butanol: AcOH: H\(_2\)O, 4:1:5).

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Chapter IV

Action of HI on Cc-6:

The glycoside (25 mg) was refluxed with phenol (30 mg) and hydrogen iodide (1 ml) for 6 hours at 140°C. On usual work up, a gummy mass was obtained. It was crystallized from aq. methanol as colorless plates (10 mg), m.p. 275°C and was identified as orobol by comparing its IR and UV spectra with authentic sample.

Oxidation of the glycoside-methyl ether (6c) with alkaline hydrogen peroxide:

The glycoside (20 mg) in dry acetone (50 ml) was refluxed with dimethyl sulphate (1 ml) and ignited potassium carbonate (10 ml) for 12 hours. After usual work up, the methylated product obtained was oxidized with alkaline hydrogen peroxide method in which a solution of Cc-7 (20 mg) in acetone (50 ml) was treated with 5% alc. KOH aqueous (10 ml) followed by 30% H₂O₂ (1 ml), the mixture was kept at 45°C for 2 hours. Then it was cooled, poured into ice-cold water (10 ml), acidified with cold con. HCl and extracted with ether. The product so obtained (m.p. 179-80°C) was identified as 3,4-dimethoxy benzoic acid by m.m.p. and co-chromatography with an authentic sample.

Cc-7

Cc-7 was crystallized from methanol-chloroform mixture as light yellow crystals m.p., m.p. 278-79°C (200 mg). It gave positive Molish test. The mixture on treatment with 2 ml of conc. H₂SO₄ produced a red-violet ring which disappeared on the addition of an excess of alkali solution) and a pink color with magnesium and hydrochloric acid and a blue color with sodium amalgam and HCl.

Analysed for C₂₁H₂₂O₁₀:

Calcd: C, 58.06; H, 5.10 %

Found: C, 58.01; H, 5.13 %

IR ν max cm⁻¹:

3392 (OH), 2928 (C-H), 1685 (C=O), 1640 (C=C), 1581, 1448, 1377, 1347, 1296, 1194, 1169, 1018, 1073, 827.
UV with shift reagent, $\lambda_{max}^{MeOH}$ nm:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td>MeOH</td>
<td>287, 330 sh</td>
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<tr>
<td>AlCl₃</td>
<td>313, 342</td>
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<td>AlCl₃/HCl</td>
<td>312, 341</td>
</tr>
<tr>
<td>NaOAc</td>
<td>284, 319</td>
</tr>
<tr>
<td>NaOAc/H₂BO₃</td>
<td>284, 318</td>
</tr>
</tbody>
</table>

¹H NMR (300 MHz, DMSO-d₆), values on δ scale:

12.04 (1H, s, 5-OH), 9.07 (1H, s, 5'-OH), 9.02 (1H, s, 7-OH), 6.88 (1H, s, H-4'), 6.75 (2H, s, H-2',6'), 6.14 (2H, s, H-6, 8), 5.45 (1H, dd, $J_1 = 12.0$ Hz, $J_2 = 3.0$ Hz, H-2'), 5.31 (1H, d, $J = 9.0$ Hz, H-1''), 3.10-5.454 (7 H, m, sugar protons H-1'', 2'', 3'', 4'', 5'', 6''), 2.75 (1H, dd, $J_1 = 18.0$ Hz & $J_2 = 3.0$ Hz, H-3).

¹³C NMR (75 MHz, DMSO-d₆), values on ppm scale:

78.76 (C-2), 42.18 (C-3), 197.21 (C-4), 165.21 (C-5), 99.49 (C-6), 162.92 (C-7), 95 (C-8), 145.83 (C-9), 103.30 (C-10), 129.22 (C-1''), 118.12 (C-2''), 115.37 (C-3''), 114.45 (C-4''), 145.22 (C-5''), 118.12 (C-6''), 99.50 (C-1''), 73.04 (C-2'''), 76.34 (C-3'''), 69.53 (C-4''), 77.09 (C-5''), 60.60 (C-6'').

+Ve ion FABMS m/z (ret.int):

m/z 434 [M]+ (C₂₁H₂₂O₁₀), 433, 417, 282, 179, 153, 152, 124.

Hydroiodic acid oxidation:

A mixture of the glycoside (Cc-7) (35 mg), phenol (60 ml) and hydroiodic acid (0.3 ml) was refluxed for about 10 hours. The mixture was allowed to cool and sodium bisulphate (NaHSO₃) was added to it with stirring. The brown colored substance so obtained was purified through column chromatography over silica gel.

Elution of the column with benzene-ethyl acetate (7:3) mixture gave a fraction which upon crystallization with n-hexane and ethyl acetate afforded cream colored crystals (Cc-7ag) m.p. 229-230°C. Yield (15 mg).
Analysed for $C_{15}H_{12}O_6$:

Calcd: C, 62.50; H, 4.67 %
Found: C, 62.46; H, 4.60 %

UV with shift reagent, $\lambda_{max}^{MeOH}$ nm:

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<thead>
<tr>
<th>Reagent</th>
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<tr>
<td>MeOH</td>
<td>286, 333 sh</td>
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<tr>
<td>AlCl$_3$</td>
<td>303, 374</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>314, 372</td>
</tr>
<tr>
<td>NaOAc</td>
<td>283, 318</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>286, 317</td>
</tr>
</tbody>
</table>

Ferric chloride oxidation of Cc-7:

The glycoside (Cc-7) (30 mg) was added to a solution of FeCl$_3$ (150 mg) in 3 ml water. The mixture was heated on an oil bath at 125°C for about 7 hours. After usual workup, as described earlier the sugar was identified as glucose ($R_f$ 0.19, n-butanol-AcOH-H$_2$O, 4:1:5).


Chapter-V

Chemical Constituents from the leaves of Rhus alata
Discussion
The family *Anacardiaceae* consists of 73 genera and about 600 species. The genus *Rhus* one of the largest genera (about 50 species) of trees, shrubs and climbers is chiefly distributed in the warm temperate region of both the hemispheres extending in the tropical and cold temperate region. About a dozen species occur in India, find extensive use in folk medicines for the treatment as spasmolytic, antiviral and anticancer. Some species may cause dermatitis in sensitive people. Earlier report from this plant is the isolation and characterization of biflavones and triterpenes from the leaves. Medicinal importance and scanty of work accelerates our interest to carry out comprehensive investigation of leaves of *Rhus alata*.

The present discussion deals with the isolation and characterization of the following compounds from the leaves of *Rhus alata*.

1. Dimethylester of terephthalic acid
2. Lupeol
3. Oleanolic acid
4. Taraxerone
5. Ethyl gallate
6. (2E)-3-(4-hydroxy-5,7-dimethoxybenzo[3,4-b]furan-6-yloxy)-prop-2-enoic acid (new compound)
7. Arbutin

The plant *Rhus alata* was collected from Aizawl, Mizoram and was identified by Professor Wazahat Husain. The leaves of this plant were defatted with light petroleum ether (40-60°C), and were refluxed exhaustively with benzene, chloroform and ethyl acetate respectively. Benzene and chloroform extracts showed similar spots on TLC plates and responded positively to terpenoid test hence, they were mixed together. The gummy mass obtained after removal of the solvents was chromatographed over silica gel column, using different solvent systems.
The fractions obtained from petrol-benzene (9:1-1:1) showed two major spots on TLC plates with some minor impurities. Both the compounds were further chromatographed and eluted from the column in light petroleum ether-benzene (9:1) and light petroleum ether-benzene (1:1) mixture and labeled as **Ra-1** and **Ra-2**.

The fraction obtained from benzene and benzene-chloroform (9:1) eluates were found to contain one major substance, which was separated by repeated column chromatography in benzene-chloroform (9:1) mixture and labeled as **Ra-3**.

Benzene-chloroform fraction (8:2-1:1) were found to be a mixture of four major compounds which were separated by repeated column chromatography and labeled as **Ra-4**, **Ra-5**, **Ra-6**, and **Ra-7**.

**Characterization of compound Ra-1:**

Ra-1 was obtained from the column with light petroleum ether-benzene (9:1) mixture. It was crystallized from chloroform-light petroleum ether as white needles (100 mg), m.p. 138°C. The elemental analysis agreed with molecular formula C\textsubscript{10}H\textsubscript{10}O\textsubscript{4}, further supported by the molecular ion peak at m/z 194 [M\textsuperscript{+}].

The IR spectrum of the compound Ra-1 showed a carbonyl band at 1720 cm\textsuperscript{-1} along with bands at 1500, 1435 cm\textsuperscript{-1} characteristic of an aromatic compound. The UV spectrum showed maximum absorption at 265 nm along with weak absorption at 340 nm. The \textsuperscript{1}H-NMR spectrum showed a sharp singlet at δ 3.97 integrating for six protons, corresponded to two methoxy groups. A sharp singlet at δ 8.13 integrated for four protons was assigned to H-2 H-3, H-5 and H-6 of phthalic acid.

The mass spectrum was quite typical for aromatic methyl ester. The base peak was observed at m/z 163 [M\textsuperscript{+}-OCH\textsubscript{3}] and other fragments were observed at m/z 179 [M\textsuperscript{+}-CH\textsubscript{3}], 164 [M\textsuperscript{+}-2 x CH\textsubscript{3}], 135 [M\textsuperscript{+}-COOCH\textsubscript{3}] and 76 [M\textsuperscript{+}-2 x COOCH\textsubscript{3}] respectively.
On the basis of above results, the Ra-1 was identified as dimethyl ester of terephthalic acid (I).

\[
\text{COOCH}_3 \\
\text{COOCH}_3
\]

(I)

**Characterization of compound Ra-2:**

Ra-2 was obtained from the column with light petroleum ether-benzene (1:1) mixture and crystallized from chloroform-methanol (170 mg), m.p. 214-15°C, [\(\alpha\)]\(_{D}^{20}\) +23.64°(CDCl\(_3\)). It gave positive Leibermann-Burchard test\(^7\), Noller test\(^9\) and gave yellow colour with tetranitromethane. The elemental analysis agreed with molecular formula C\(_{30}\)H\(_{50}\)O. Its IR showed bands at 3360 cm\(^{-1}\) and 1030 cm\(^{-1}\) (OH), 1645 cm\(^{-1}\) (C=C) and 1385 cm\(^{-1}\) (geminal dimethyl), 885 cm\(^{-1}\) (terminal methylene). Mass spectrum of the triterpene alcohol gave [M⁺] m/z 426 (11.0 %) with other principal ions at m/z 411 [M-CH\(_3\)] (6.0%), 207 (34.0%), 189 (77.0 %) and a base peak at m/z 95. It afforded an acetate m.p. 218-20°C. IR spectrum of the acetate revealed the presence of terminal methylene by a band at 875 cm\(^{-1}\). Some other important bands were observed at 1245 cm\(^{-1}\) (acetate), 1640 cm\(^{-1}\) (C=C) and 1730 cm\(^{-1}\) (C=O). \(^1\)H NMR spectrum gave the signals at \(\delta\) 0.82, 0.87, 0.94, 1.04 (CH\(_3\)-protons), \(\delta\) 1.27, 1.41, 1.46, 1.47 (CH\(_2\)-protons), \(\delta\) 2.03 (OCOCH\(_3\)) and multiplets in the range of \(\delta\) 4.28-4.77 (>CHOAc), \(\delta\) 4.59- \(\delta\) 4.67 (J=8.0 Hz, >C-CH\(_2\)).

On the basis of the above physico-chemical data for Ra-2 and its derivatives, Ra-2 was identified as *Lupeol*\(^8\) (II).
Characterization of compound Ra-3:

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot along with some minor impurities. It was purified by crystallization into pure component Ra-3 (225 mg) m.p. 299-300 °C. It responded positively to Leibermann-Burchard test.

Its IR spectrum showed the characteristic bands at 3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210 cm⁻¹. The ¹H-NMR spectrum of Ra-3 (Table 1) indicated the presence of seven tertiary methyl groups at δ 0.71 (3H), 0.82 (6H), 0.84 (3H), 0.89 (6H), and 1.10 (3H). The two unresolved multiplets at δ 4.39 and 5.20 were ascribed to one proton α-to hydroxyl group and olefinic proton. Acetylation of the Ra-3 with acetic anhydride and pyridine afforded a monoacetate Ra-3 (Ac). The IR spectrum of Ra-3 (Ac) displayed bands at 1724, 1686 and 1250 cm⁻¹ characteristic of the acetyl function. The ¹H-NMR spectrum of the acetate Ra-3(Ac) showed seven tertiary methyls as four independent singlets at δ 0.75 (3H), 0.85 (6H), 0.94 (9H) and 1.12 (3H) and one singlet for an acetyl function at δ 2.02, an unresolved multiplet at δ 4.50 for one proton α-to the acetoxy and another multiplet at δ 5.25 for olefinic proton. These data further support to the β-amyrin skeleton of the compound with one acetyl function present. Formation of its methyl ester (m.p. 198 °C) with diazomethane and not with methanolic hydrochloric acid, coupled with difficulty of its hydrolysis suggested the attachment of carboxyl group at tertiary carbon atom (C-17). Its ¹H-NMR spectrum showed one singlet of methyl ester at δ 3.58 and seven tertiary
methyls as singlets at δ 0.70 (3H), 0.84 (6H), 0.86 (3H), 0.90 (6H), and 1.10 (3H). Two unresolved multiplets centered at δ 4.5 and δ 5.30 can be attributed to one proton α-to methyl ether and one olefinic proton respectively.

On the basis of above spectral data and comparison of m.p, m.m.p. with an authentic sample confirmed the identity of Ra-3 as Oleanolic acid (III).

![Oleanolic acid](image)

(III)

**Table 1**

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.71 (s)</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>6</td>
<td>0.82 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.84 (s)</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>6</td>
<td>0.89 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.10 (s)</td>
</tr>
<tr>
<td>H-α to hydroxy group</td>
<td>1</td>
<td>4.39 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.20 (m)</td>
</tr>
</tbody>
</table>

s = singlet, m=multiplet, spectrum run in CDCl$_3$ at 300 MHz, TMSi as internal standard.
Characterization of compound Ra-4:

Ra-4 was eluted from the column with benzene-chloroform (8:2) mixture. It was crystallized from chloroform-ethanol as colourless needles (170mg), m.p. 240°C. The elemental analysis agreed with molecular formula C_{30}H_{48}O. It gave positive Leibermann-Burchard test. The IR spectrum showed a strong carbonyl bond at 1690-1700 cm\(^{-1}\).

The \(^1\)H NMR spectrum of Ra-4 (Table-2) showed five singlets attributed to the protons of eight methyl groups at \(\delta\) 0.83 (3H), 0.93 (6H), 0.97 (3H), 1.06 (9H), and 1.12 (3H). Methylene and methine protons appeared as multiplets at \(\delta\) 1.32 and \(\delta\) 1.50-1.90. The multiplets at \(\delta\) 2.28-2.52 integrating for two protons were assigned to methylene protons at C-2 which is adjacent to carbonyl group at C-3. A double doublet at \(\delta\) 5.56 integrating for one proton is attributed to olefinic protons.

These data compare well with Taraxerone, thereby support its structure (IV). The mass spectrum also supported the above structure, showed a molecular ion peak at m/z 424 and the base peak at m/z 300.
Table 2

\(^1\text{H} \text{ NMR spectral data of Ra-4 on } \delta\text{-scale}\)

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(_3)</td>
<td>3</td>
<td>0.83 (s)</td>
</tr>
<tr>
<td>2 x CH(_3)</td>
<td>6</td>
<td>0.93 (s)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>3</td>
<td>0.97 (s)</td>
</tr>
<tr>
<td>3 x CH(_3)</td>
<td>9</td>
<td>1.06 (s)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>3</td>
<td>1.12 (s)</td>
</tr>
<tr>
<td>-CH and CH(_2) protons</td>
<td>21</td>
<td>1.32 (m), 1.5-1.9 (m)</td>
</tr>
<tr>
<td>-CH(_2) protons at C-2</td>
<td>2</td>
<td>2.28-2.52 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.56 (dd)</td>
</tr>
</tbody>
</table>

s = singlet, dd = double doublet, m = multiplet, spectrum run in CDCl\(_3\) at 300 MHz, using TMS as internal standard.

Characterization of compound Ra-5:

Ra-5 was eluted from the column by benzene-chloroform (7:3) mixture. It was crystallized from methanol as reddish buff needles (120 mg), m.p. 155\(^\circ\)C. It gave blue colour with alc. ferric chloride solution and brown spot under UV light. It was characterized as ethyl gallate by m.p., m.m.p, spectral studies and co-chromatography with an authentic sample.

The \(^1\text{H} \text{ NMR spectrum of Ra-5 (Table 3), showed a triplet at } \delta 1.31\) integrating for three protons and a quartet at \( \delta 4.24 \) integrating for two protons corresponded to methyl and methylene protons respectively.
On the basis of above results, Ra-5 was characterized as **ethyl gallate** (V).

![Formula of ethyl gallate](image)

Table 3

**$^1$H NMR spectral data of Ra-5 on $\delta$-scale**

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.36 (t, $J=7.0$ Hz)</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>2</td>
<td>4.24 (q, $J=7.0$ Hz)</td>
</tr>
<tr>
<td>H-2, 6</td>
<td>2</td>
<td>7.11 (s)</td>
</tr>
</tbody>
</table>

$s =$ singlet, $t=$triplet, $q=$quartet, spectrum run in DMSO-d$_6$ at 300 using TMS as internal standard.

**Characterization of compound Ra-6:**

Ra-6 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg); m.p.160-162 °C. Homogeneity of the compound was established by TLC examination using different solvent systems indicating it to be a pure compound. It showed blue fluorescence under UV light. Elemental analysis and the molecular ion peak at m/z 248 agreed with the molecular formula as C$_{13}$H$_{12}$O$_5$. 

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The IR spectrum (Fig.1) was helpful in identifying two functional groups present as COOH and OH. In the hydroxyl regions the absorption was evident between 3500 cm\(^{-1}\) and 2100 cm\(^{-1}\) with prominent peaks appearing at 3496, 3429, 3110 and 2817 cm\(^{-1}\). The OH stretching of the carboxyl group is reported to appear as a broad band with a series of minor peaks in the range of 3000-2500 cm\(^{-1}\) with the main peak around 3000 cm\(^{-1}\) and a satellite peak near 2650 cm\(^{-1}\).\(^{10a}\) The low frequency region of the hydroxyl absorption can thus be attributed to the O-H stretching in the carboxyl group. The peaks with high frequency region 3496 and 3429 cm\(^{-1}\), therefore can be attributed to a hydroxyl function. A corresponding peak at 1669 cm\(^{-1}\) in carbonyl region can thus be attributed to the carbonyl group of the carboxyl, either aromatic or more likely of \(\alpha, \beta\)-unsaturated character.\(^{11}\) A band in the range 1440-1375 cm\(^{-1}\) has been said to arise out of C–O vibration of the carboxyl group.\(^{10b}\) A band of fairly high intensity appearing at 1394 cm\(^{-1}\) in this instance can be reasonably assumed to confirm the presence of a carboxyl function in Ra-6. Another band appearing between 1320-1210 cm\(^{-1}\)\(^{10c}\) and found to be the most intense and the band appearing between 1600-700 cm\(^{-1}\) has also been attributed to a carboxyl function. The band appearing at 1276 cm\(^{-1}\) in the IR spectrum of Ra-6, can thus be assumed to arise only due to the presence of a carboxyl function. Another band appearing at 910 cm\(^{-1}\) can now be attributed with O–H deformation vibration of the COOH group, which has been reported to appear at 935 ± 15 cm\(^{-1}\).\(^{10d}\) Another feature which stands out in the IR spectrum is a band appearing at 1606 cm\(^{-1}\) equal in intensity of the carbonyl of the carboxylic group appearing at 1669 cm\(^{-1}\). This band based on its frequency and intensity can be reasonably attributed to a conjugated \(-\text{C} = \text{C} -\) system or to an aromatic system.

Ra-6 can therefore be tentatively concluded to possess either the system VI or VII, preferably VII as it has already concluded to carry a carboxyl function.

\[
\text{O} \\
\text{C} = \text{C} \quad \text{C} = \text{C} > \text{C} = \text{C} \quad \text{OH}
\]

\(\text{(VI)}\) \quad \text{\quad (VII)}
Additional information can be had from the $^1$H NMR and the $^{13}$C NMR spectra of Ra-6. The $^1$H NMR spectra (Fig. 2 Table 4), shows in all, the presence of 12 protons. The spectrum was determined in a mixture of CDCl$_3$ and DMSO-d$_6$ and hence the peak appearing at $\delta$ 2.39 was excluded from consideration. The peak appearing at the highest field at $\delta$ 2.71 from its integral is found to represent six protons and from its sharp nature can be concluded to represent two methyl groups situated in identical environments. Two sets of signals integrating for two protons each appeared in the region of $\delta$ 6.80 and $\delta$ 7.40. These signals when integrated with the postulated partial structure (VII) for Ra-6, can lead to the conclusion that one proton each from these sets can represent hydrogen each attached to $\alpha$, $\beta$-carbons represented in (VIII), leading to the partial structure (VIII).

\[
\begin{array}{c}
\text{H} \\
\text{CH}_3
\end{array}
\begin{array}{c}
\text{H} \\
\text{C} = \text{C} - \text{COOH}
\end{array}
\]

(VIII)

Ra-6 has already been found to have the molecular formula $C_{13}H_{12}O_5$, out of which (VIII) accounts for three carbons, three hydrogens and two oxygens. Additionally two methyl groups and a hydroxyl group have been concluded to be present in Ra-6, on the basis of its $^1$H NMR and IR spectra. This information take together with (VIII), now accounts for five carbons, ten hydrogens and three oxygens (XI), $C_3H_2O_2 + 2 \times CH_3 + 1 \times OH = C_5H_{10}O_3$). The partial structure for Ra-6 can therefore now be expanded to (IX).

\[
\begin{bmatrix}
CH_3 \times 2 \\
OH \times 1 \\
C_8H_2O_2
\end{bmatrix}
\begin{array}{c}
\text{H} \\
\text{H}
\end{array}
\begin{array}{c}
\text{C} = \text{C} - \text{CO}_2\text{H}
\end{array}
\]

(IX)
The signals for two methyl groups in Ra-6 appear in the region of aromatic methyls. Assuming thus Ra-6 to be aromatic in character, six carbons are accounted for bearing two carbons, two hydrogens and two oxygens yet to be accounted for. The two sets of relatively deshielded signals representing two hydrogens each, appearing in the region of δ 6.8 and 7.4 came in handy in further expanding the partial structure (IX). One hydrogen each from these two signals at δ 6.81 (J=9.0 Hz) and δ 7.43 (J=9.0 Hz) have been assigned to hydrogens attached to the α, β-unsaturated carbons of the acid moiety (VIII) coupling constant of which indicate the hydrogens to be cis-oriented.

Chemical shifts of one proton each from the two sets, δ 7.41 (J=3.0Hz) and δ 6.83 (J=3.0 Hz) correspond to the well documented values of the α'- and β'- protons of a furan ring in benzofurans at δ 7.3 and δ 6.6 respectively.\textsuperscript{11}

Thus Ra-6 is concluded to have a benzofuran moiety; two carbons, two hydrogens and one oxygen are further accounted for, thus enabling the partial structure for Ra-6 to be expanded as (X).

\[ + 2 \times \text{CH}_3 + 1 \times \text{OH} + -\text{CH} = \text{CH-COOH} \]

(X)

It can be noted that all the thirteen carbons are accounted for in (X) and hence appendages have to be attached to the eight carbons of the benzofuran nucleus. However, the furan ring, as evidenced by the presence of both the α and β- hydrogens in the \textsuperscript{1}H NMR spectrum is unsubstituted. Hence the groups to be accounted further in formulation of the structure for Ra-6 have to be appended to four aryl carbons, leading to the partial structure (XI). However, one more oxygen is to be accounted for.
Considering that there is no further locations it can be attached to, or any other atom which it can carry, it has to be present as an ether function. This further limits the location of this oxygen as present between an aryl carbons and the acid unit. The other alternative is automatically excluded in the absence of an aromatic methoxyl signal in the $^1$H NMR spectrum. Ra-6 can therefore be represented by (XII) or any of its regioomers.

The $^1$H-NMR spectrum of Ra-6, still requires further assignments. The signal appearing at $\delta$ 6.06 and broadish in nature can be assigned to the phenolic proton considering that DMSO-$d_6$ is used as a co-solvent. The most deshielded of the signals which appears at $\delta$ 9.89 should arise only out of the carboxylic proton.

The final structure of Ra-6 has now to be one of the possible regioisomers (XIII→XXIV).
Of the above structures, the one that can be assigned to Ra-6 should have some features present to enable the methyl signals in both the $^1$H NMR and $^{13}$C NMR to coincide and have identical chemical shifts. Three oxygens are directly attached to the phenyl ring and these are the one which can cause shielding as well as deshielding around the ring and more particularly on the methyl groups. Analyzing all the possible structures (XIII→XXIV), the structures XIV, XV, XXI, XXI and XXII emerge as the possible structure for Ra-6.
Of the above (XV) appears to be the structure which can correspond to Ra-6, not only with respect to situations of oxygen, but also with respect to the side chain which is capable of having similar effect on both the methyl groups. Ra-6 can therefore be assigned as a novel benzofuranic acid named as (2E)-3-(4-hydroxy-5, 7-dimethyl-benzo[3,4-b]furan-6-yloxy)-prop-2-enoic acid (XV).

The signals in the $^{13}$C-NMR spectrum (Fig.3) can be assigned as below by employing additive factors.$^{12}$

The mass of Ra-6 is compatible with the proposed structure (XV) as has been detailed in the scheme (I).
On the basis of above discussion, Ra-6 has been characterized as (2E)-3(4-hydroxy-5,7-dimethylbenzo[3,4-b]furan-6-yloxy-prop-2-enoic acid named as alatic acid (XV) which is being reported for the first time.

\[
\begin{align*}
\text{2} & \alpha' \text{ CH}_3 \\
\text{3} & \beta' \text{ CH=CH-CO}_2\text{H} \\
\text{1} & \text{H}_3\text{C} \\
\text{7} & \text{H}_6\text{C} \\
\text{6} & \text{H}_5\text{C} \\
\text{5} & \alpha \text{ CO}_2\text{H} \\
\text{4} & \beta \text{ OH} \\
\text{OH} & \text{CH=CH-CO}_2\text{H} \\
\end{align*}
\]

\[(XV)\]

Table-4

\[^1\text{H} \text{ NMR data of Ra-6, values on } \delta\text{-scale}\]

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x CH(_3)</td>
<td>6</td>
<td>2.71 (s)</td>
</tr>
<tr>
<td>(\alpha)- H of the acid moiety</td>
<td>1</td>
<td>6.81 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>(\beta)- H of the acid moiety</td>
<td>1</td>
<td>7.43 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>4- OH</td>
<td>1</td>
<td>6.06 (br s)</td>
</tr>
<tr>
<td>OH of acid moiety</td>
<td>1</td>
<td>9.89 (br s)</td>
</tr>
<tr>
<td>H-2 ((\alpha')- H of furan ring)</td>
<td>1</td>
<td>7.41 (d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-3 ((\beta')- H of furan ring)</td>
<td>1</td>
<td>6.83 (d, J=3.0 Hz)</td>
</tr>
</tbody>
</table>

s=singlet, br s= broad singlet, d=doublet, spectrum run at 300 MHz using TMS as internal standard.
Fig. 3
Scan: 17  RT= 1.69  No. ions: 191  Base= 27.0ZF  TIC=126098

Fig. 4
Characterization of compound *Ra-7*

Ra-7 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-acetone as colorless needles (120 mg), m.p. 199-200°C. It gave blue colour with alc. ferric chloride solution. It was characterized as arbutin<sup>13,14</sup> (XXIII) by m.p., m.m.p, spectral studies UV, IR, <sup>1</sup>H-NMR (Table-5) <sup>13</sup>C-NMR (Table- 6) and co-chromatography with an authentic sample.

![Diagram](Image)

(XXIII)

**Table 5**

<sup>1</sup>H NMR spectral data of *Ra-7* on δ-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2,6</td>
<td>2</td>
<td>6.84 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-3,5</td>
<td>2</td>
<td>6.87 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>OH</td>
<td>1</td>
<td>8.99</td>
</tr>
<tr>
<td>H-1',2',3',4',5',6'</td>
<td>7</td>
<td>5.40-6.38 (m)</td>
</tr>
<tr>
<td>H-1'</td>
<td>1</td>
<td>6.35 (d, J=9.0 Hz)</td>
</tr>
</tbody>
</table>

d = doublet, s=singlet m=multiplet spectrum run in DMSO-d<sub>6</sub> at 400 using TMS as internal standard.
Table 6

$^{13}$C NMR spectral data of Ra-7on δ-scale

<table>
<thead>
<tr>
<th>Assignments</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>152.5</td>
</tr>
<tr>
<td>C-2,6</td>
<td>119.4</td>
</tr>
<tr>
<td>C-3,5</td>
<td>116.7</td>
</tr>
<tr>
<td>C-4</td>
<td>153.8</td>
</tr>
<tr>
<td>C-1'</td>
<td>103.1</td>
</tr>
<tr>
<td>C-2'</td>
<td>75.0</td>
</tr>
<tr>
<td>C-3'</td>
<td>78.1</td>
</tr>
<tr>
<td>C-4'</td>
<td>71.5</td>
</tr>
<tr>
<td>C-5'</td>
<td>78.0</td>
</tr>
<tr>
<td>C-6'</td>
<td>62.6</td>
</tr>
</tbody>
</table>
Experimental
The leaves of *Rhus alata* were collected from Puchchunga University College, Aizawl, Mizoram. After being defatted with light petroleum ether (40-60°C), they were refluxed exhaustively with benzene, chloroform and ethyl acetate respectively. Benzene and chloroform extracts responded positively to terpenoid test and showed similar spots on TLC plates. Hence, they were mixed together. After removal of the solvents under reduced pressure, a gummy mass was obtained. The gummy mass was chromatographed over silica gel column, using different solvent systems namely light petroleum ether, light petroleum ether-benzene (9:1-1:1), benzene, benzene-chloroform (9:1-1:1) and ethyl acetate respectively. The fractions obtained from petrol-benzene (9:1-1:1) showed two major spots on TLC plates with some minor impurities. Repeated column chromatography followed by fractional crystallization with chloroform-methanol gave two compounds labeled as Ra-1 and Ra-2. Benzene and benzene-chloroform (9:1) eluates were found to contain same substance, which was separated by repeated column chromatography followed by fractional crystallization and labeled as Ra-3. Benzene-chloroform elutes (8:2-1:1) were found to be a mixture of four compounds which were separated by repeated column chromatography and labeled as Ra-4, Ra-5 Ra-6 and Ra-7.

**Ra-1**

Elution of the column with light petroleum ether-benzene (9:1) mixture and crystallized from chloroform-petroleum ether as white needles (100 mg), m.p. 138°C.

**Analysed for C_{10}H_{10}O_{4}:**

Calcd: C, 61.85; H, 5.15 %  
Found: C, 61.79; H, 5.12 %

**UV data \( \lambda_{\text{max}} \) (CHCl₃) nm:**

265, 340

**IR, \( v_{\text{max}} \) KBr cm⁻¹:**

1720, 1500, 1435.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) on \(\delta\)-scale:

\[3.97 \,(6\,\text{H, s, 2 x OCH}_3), \, 8.13 \,(4\,\text{H, s, Ar-H})\]

**Mass m/z:**

\[194 \,[\text{M}^+]^+, \, 163 \,[\text{M}^+-\text{OCH}_3] \,(100), \, 179 \,[\text{M}^+-\text{CH}_3], \, 164 \,[\text{M}^+-2 \times \text{CH}_3], \, 135 \,[\text{M}^+-\text{COOCCH}_3], \, 76 \,[\text{M}^+-2 \times \text{COOCCH}_3].\]

**Ra-2**

Elution of the column with light petroleum ether-benzene (1:1) mixture and purified by recrystallization from chloroform-methanol afforded Ra-2 as crystalline solid (170 mg), m.p. 214-15\(^\circ\)C, \([\alpha]^{20}D + \, 23.64\,(\text{CDCl}_3).\) It gave positive Leibermann-Burchard test, Noller’s test and yellow color with tetranitromethane.

**IR, \(v_{\text{max}}^{\text{KBr}}\) cm\(^{-1}\):**

\[3360, \, 1030 \,(\text{OH}), \, 1645 \,(\text{C=C}), \, 1385 \,(\text{geminal dimethyl}), \, 885 \,(\text{terminal methylene}).\]

**Analysed for C\(_{30}\)H\(_{50}\)O:**

Calcd: C, 84.95; H, 11.50 %

Found: C, 84.93; H, 11.45 %

**Acetylation of Ra-2:**

Crystalline Ra-2 (30 mg) was treated with acetic anhydride (2.0 ml) and pyridine (0.5 ml) and allowed to stand over night at room temperature. After usual work-up the solid obtained was crystallized from chloroform-methanol as colourless flakes (25 mg), m.p. 218-20\(^\circ\)C.

**IR, \(v_{\text{max}}^{\text{KBr}}\) cm\(^{-1}\):**

\[1730 \,(\text{C=O}), \, 1640 \,(\text{C=C}), \, 1245 \,(\text{acetate}), \, 875 \,(\text{terminal methylene}).\]
**1H NMR (400 MHz, CDCl₃) values on δ-scale:**

0.82, 0.87, 0.94, 1.04 (21H, s, 7 x CH₃), 1.27, 1.41, 1.46, 1.47 (m, -CH and -CH₂ protons), 2.03 (OCOCH₃), 4.28-4.77 (1H, m, α-to acetate), 4.59-4.67 (2H, d, J=8.0 Hz, olefinic protons).

**Analyzed for C₃₂H₅₂O:**

Calcd: C, 82.05; H, 11.11 %
Found: C, 82.14; H, 11.17 %

**Ra-3**

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot along with some minor impurities. It was purified by crystallization into pure component Ra-3 (225 mg), 299-300°C. It responded positively to Leibermann-Burchard test.

**Analysed for C₃₀H₄₈O₃:**

Calcd: C, 78.9; H, 10.59 %
Found: C, 78.6; H, 10.48 %

**IR, v max cm⁻¹:**

3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210.

**1H NMR (300 MHz, CDCl₃) values on δ-scale:**

0.71 (3H, s, CH₃), 0.82 (6H, s, 2 x CH₃), 0.84 (3H, s, CH₃), 0.89 (6H, s, 2 x CH₃), 1.10 (3H, s, CH₃), 4.39 (1H, m, α-to hydroxy! group), 5.20 (1H, m, olefinic proton).

**Acetylation of Ra-3:**

Crystalline Ra-3 (100 mg) was treated with acetic anhydride (2.0 ml) and pyridine (2.0 ml) and allowed to stand over night at room temperature. After usual
workup the solid obtained was crystallized several times from methanol to give Ra-3(Ac) (80 mg), m.p. 258-60°C.

IR, ν max cm⁻¹:

1724, 1686, and 1250

¹H NMR (300 MHz, CDCl₃) values on δ-scale:

0.75 (3H, s, CH₃), 0.85 (6H, s, 2 x CH₃), 0.94 (9H, s, 3 x CH₃), 1.12 (3H, s, CH₃), 2.02 (3H, s, OCOCH₃), 4.50 (1H, m, H- α-to acetoxy), 5.25 (1H, m, olefinic proton).

Methylation of Ra-3(Ac):

The acetate Ra-3 (Ac) (40 mg) was dissolved in ether and treated with an excess of ethereal solution of diazomethane. After leaving the contents at room temperature over night, the ether was evaporated and the product crystallized from methanol-chloroform as colorless shining plates (30 mg), m.p. 198°C.

¹H NMR (300 MHz, CDCl₃) values on δ-scale:

0.70 (3H, s, CH₃), 0.84 (6H, s, 2 x CH₃), 0.86 (3H, s, CH₃), 0.90 (6H, s, 2 x CH₃), 1.10 (3H, s, CH₃), 3.58 (3H, s, methyl ester), 4.50 (1H, m, H-α to methyl ether), 5.30 (1H, m, olefinic proton).

Ra-4

Ra-4 was eluted from the column with benzene-chloroform (8:2) mixture. It was crystallized from chloroform-ethanol as white needles (170mg), m.p. 240°C. It gave positive Leibermann-Burchard test.

IR, ν max cm⁻¹:

1690-1700 (C=O), 1655, 1055, 840 (C=C).
\(^1\text{H} \text{NMR (300 MHz, CDCl}_3\) on \(\delta\)-scale:}

0.83 (3H, s, CH\(_3\)), 0.93 (6H, s, 2 x CH\(_3\)), 0.97 (3H, s, CH\(_3\)), 1.06 (9H, s, 3 x CH\(_3\)), 1.12 (3H, s, CH\(_3\)), 1.32, 1.50-1.90 (21 H, m, -CH and -CH\(_2\) protons), 2.28-2.52 (2H, m, -CH\(_2\) protons at C-2), 5.56 (1H, dd, olefinic proton)

\textbf{Mass m/z (relative intensity):}

424 [M]+ (37.0), 409 (18.3), 300 (100), 285 (45.4), 257 (9), 232 (7.3), 218 (16.5), 205 (62.6), 204 (95), 189 (10), 149 (10), 133 (25), 121 (12), 119 (10), 109 (15), 107 (10), 95 (17), 93 (10), 91 (8), 67 (15), 57 (15), 43 (12).

\textit{Ra-5}

\textit{Ra-5} was eluted from the column with benzene-chloroform (7:3) mixture. It was crystallized from chloroform-methanol as reddish buff needles (120 mg), m.p. 155\(^\circ\)C. It gave blue color with alc. ferric chloride solution and brown spot under UV light.

\textbf{Analyzed for C\(_9\)H\(_{10}\)O\(_5\):}

Calcd: \hspace{1cm} C, 54.9; H, 5.05 \\
Found: \hspace{1cm} C, 54.7; H, 5.0 \\

\(^1\text{H} \text{NMR (300 MHz, DMSO-\text{d}_6\) on \(\delta\)-scale:}

1.31 (3H, t, J=7.0 Hz, CH\(_3\)), 4.24 (2H, q, J=7.0 Hz, CH\(_2\)), 7.11 (2H, s, H-2,6).

\textbf{Mass m/z (relative intensity):}

195 [M]+ (45.0), 183 (8.0), 170 (20), 153 (100.0), 125 (20), 107 (4.0), 79 (9.0), 51 (6.0).

\textit{Ra-6}

\textit{Ra-6} was eluted from the column with benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg), m.p. 160-162\(^\circ\)C.
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Analyzed for C\textsubscript{13}H\textsubscript{12}O\textsubscript{5}:

Calcd: C, 62.90; H, 4.83 %

Found: C, 62.84; H, 4.79 %

\(\text{IR, } v^\text{Kbr} \text{ cm}^{-1}:\)

\begin{align*}
3496 & \text{(OH)}, 3429, 3110, 3000, 2817, 2100, 1669 \text{ (C=O), 1606, 1600, 1440-}
1375 \text{ (C-O vib. Of COOH), 1394, 1320-1210, 1276 \text{ (COOH), 910 (OH def. of}}
\text{COOH).}
\end{align*}

\(^1\text{H NMR (300 MHz, CDCl}_3+\text{DMSO-d}_6\text{) values on } \delta\text{-scale:}\)

\begin{align*}
2.71 \text{ (6H, s, 2 } \times \text{ CH}_3), & \quad 6.06 \text{ (1H, brs, 5-OH), 6.81 \text{ (1H, d, J=9.0 Hz, } \alpha\text{-H of}}
\text{acid moiety), 6.83 \text{ (1H, d, J=3.0 Hz, H-2 of furan ring), 7.41 \text{ (1H, d, J=3.0 Hz, H-3 of}}
\text{furan ring), 7.43 \text{ (1H, d, J=9.0 Hz, } \beta\text{-H of acid moiety), 9.89 \text{ (1H, brs, COOH).}}
\end{align*}

\(^1\text{C NMR (100 MHz, CDCl}_3+\text{DMSO-d}_6\text{) values on } \delta\text{-scale:}\)

\begin{align*}
161.57 \text{ (C}_{3\beta}), & \quad 161.02 \text{ (COOH), 155.07 \ (}\beta\text{-C), 152.87 \ (C-6), 152.85 \ (C-4),}
125.40 \ (C-2), & \quad 113.02 \ (C-5), 112.45 \ (C-7), 110.7 \ (C_{4\beta}), 102.97 \ (C-3), 162.97 \ (\alpha\text{-C),}
18.45 \ (2 \times \text{ CH}_3).
\end{align*}

\textbf{Mass m/z:}

\begin{align*}
248 & \ [M]^+\ (90.4), 233 \ [M-\text{CH}_3] \ (9.2), 219 \ [M-\text{CO-H}] \ (9.4), 217 \ [233-\text{CH}_3-H]
(1.5), 216 \ [217-H] \ (13.8), 204 \ [203+H] \ (17.5), 203 \ [M-\text{COOH}H] \ (100), 202 \ [M-\text{H}_2-O-CO]
(9.8), 201 \ [202-H] \ (17.2), 190 \ [2 \times \text{ CH}_3-\text{CO}] \ (15.8), 189 \ [204-\text{CH}_3] \ (23.6), 177
[M-\text{CH=CH-CO}_2\text{H}] \ (6.6), 176 \ [M-\text{CO-CO}_2] \ (22.6), 174 \ (189-\text{CH}_3) \ (8.3), 173 \ [174-H]
(16.4), 161 \ [M-\text{O-CH=CH-CO}_2] \ (7.0), 159 \ [177-\text{H}_2\text{O}] \ (5.6), 149 \ (10.5), 148 \ (26.9),
147 \ (23.5), 146 \ (7.1).
\end{align*}

\textbf{Ra-7}

Elution of the column with chloroform: benzene (1:1) mixture and crystallized from chloroform-acetone as colourless needles (120 mg), m.p. 199-200\degree C.
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Analyzed for $C_{12}H_{16}O_7$:

Calcd: C, 52.94; H, 5.92 %

Found: C, 52.91; H, 5.89 %

UV data $\lambda_{\text{max}}^\text{DCH}$ nm:

286 (3.42)

IR, $v_{\text{max}}^\text{KBr}$ cm$^{-1}$:

3334, 1514, 1375, 1212, 1101, 832.

$^1$H NMR (300 MHz, CDCl$_3$) on $\delta$-scale:

5.40-6.38 (7H, m, H-2',3',4',5',6'), 6.35 (1H, d, J=9.0 Hz, H-1'), 6.87 (2H, d, J=9.0 Hz, H-3, 5), 6.84 (2H, d, J=9.0 Hz, H-2, 6), 8.99 (1H, s, 4-OH).

$^{13}$C NMR (75 MHz, CDCl$_3$) on $\delta$-scale:

152.5 (C-1), 119.4 (C-2,6), 116.7 (C-3,5), 153.8 (C-4), 103.1 (C-1'), 75.0 (C-2'), 78.1 (C-3'), 71.5 (C-4'), 78.0 (C-5'), 62.6 (C-6').

Mass m/z:

272 [M]$^+$.
References


Chapter VI

Biological studies of Ficus benjamina (Var. Comosa), Xylosma longifolium, Crataegus crenulata and Rhus alata and some of their compounds.
Theoretical
The importance of medicinal plants in traditional healthcare, practices, providing clues to new area of research. India has luminous past in following human health management services for using the drug plants since the beginning of civilization. Out of the total 422,000 flowering plants reported from the world more than 50,000 are used for medicinal purpose. In India more than 43% of the total flowering plants are reported to be of medicinal importance.

The Vedas give the earliest written record about the science of healing. The reference to medicinal plants is also found in Ebbers Papyrus (16th Century B.C) which lists in detail over 7000 herbal remedies, for example-Poppy, Castor oil, Carraway etc. The Indians were the first to use Chaulmoogra fruits for its antileptic activity. The Brazilians employed ‘Ipecacuanha’ for the treatment of dysentery and diarrhea. The roots of Rauwolfia serpentine, an indigenous plant, has since time immemorial been widely used in India and Malaysia as an antidote for insect and snake bites and in mental derangement.

The utility of plants as therapeutic agent in traditional medicinal system is still prevalent today, for example, the middle eastern civilization developed the Greco-Arabic system of medicine (Unani system of medicine), which is practiced in the Indian subcontinent. Similarly Chinese race developed the Chinese system of medicine largely based on its unique system of theories including the concept of Yen and theory of influence impart from nature.

The Ayurveda and Sidha systems of medicine were contributed by Indians. All these systems procure more than 80% of their medicaments from plants. Till recently these medicines used to be prepared by the practicing physician himself for the use of his patients. In the absence of any scientific method of identification and standardization, these plants based medicines were more often than not, in a state of uncertainty about the identity, use and efficacy of the drug.

However, Natural Product Chemistry has under gone an explosive growing during the later half of the 20th century. This has been brought about by a number of factors. One of these has been drawing number of substances from natural sources which display interesting pharmacological activity. Another factor has been the
improvement made in the technology of isolation process which includes high performance liquid chromatography. The techniques have allowed rapid isolation of substances previously difficult to obtain by classical procedures. The most important factor has been developed of new spectroscopic techniques which have opened up whole new vistas in this exciting field. Prominent in these advances have been $^1$H-NMR, $^{13}$C-NMR, Two dimensional NMR spectroscopy, Mass spectroscopy and X-rays diffraction Studies etc.

At the present time the trend of use of crude extracts and compounds of plant as drug is increasing day by day for example, the different extracts of the plants Jasmonia Montana showed hypoglycemic, antidiabetic and antimicrobial activities, the petroleum ether and ethanolic extracts exhibited hypoglycemic and antidiabetic activity and the petroleum ether and chloroform extract showed antimicrobial activity.$^2$

Antioxidant activity of the methanolic extract of Hypericum perforatum L. showed that the calabrian samples were more active than those from Sardinia. The antimicrobial activity evidenced the best performance on gram positive bacteria with a MIC value of 50 µg/ml. Moreover antifungal activity of all the extracts was also tested which showed interesting results particularly on the phytopathogene fungus P-ultimum. The phytochemical analysis and the biological activity data suggested a possible use of H. perforatum extract in alimentary, cosmetic and pharmaceutical fields.$^3$

The leaves of Blumea balsamifera afforded ichtutherol acetate (1), cryptomeridiol (2), lutein and β-carotene. Antimicrobial tests indicated that (1) has moderate activity against the fungi Aspergillus niger, Trichophyton mentagrophytes and Candida albicans while (2) has low activity against the same. Both compounds have no antimicrobial activity against Psuedomonas aeruginosa, Staphylococcus aurens, Bacillus subtilis and Escherichia coli.$^4$

Sesquiterpene Pseudoneolinderane (1) and Linderalactone (2) isolated from the stem of Neolitsea parvigemma, were tested for anti-inflammatory activities. 1 and 2 showed significant inhibitory effects on Superoxide anion generation by human
neutrophils in response to MLP/CB. The IC$_{50}$ of 1 and 2 were found to be 3.21 and 8.48 u.g/ml respectively. $^5$ Rehmannia glutonosa (gaerth) Liliosch (Chinese name, Dihuang) is indigenous to China where it has been cultivated as medicinal plant. The roots of the plants have been utilized in China medicine as hematopoietics or tonic. A novel bis-furan derivative isolated from the CHCl$_3$ extracts of the dried roots of Rehmannia Glutinosa showed that it can promote immune activity and can inhibit blood platelet aggregation.$^6$

$^*$Poulsenia armata (Moraceae) known commonly as ‘Majagua’ or ‘Damagua’ is a hardwood species from the American tropical region. Crude petroleum ether extract from bark of Poulsenia armata showed antimicrobial activity.$^7$

It was found that flavones ovaliform pingol methyl ether and millettocayxin A and the Stilbene oxyresveratrol obtained from Millettia erythrophylla and Artocarpus lakoocha possessed moderate activity against both types of HSV. In addition Oxyresveratrol was evaluated for potential anti HIV activity against a wild type human Immunodeficiency virus type (HIV-I/LAI), isolate and was found to be a modest inhibitor of HIV (EC$_{50}$ 18.2 u.m), showing no toxicity in PBM, CEM and vero cells at 100 u.m. The heart wood of A. lakoocha which contains a large amount of oxyresveratrol, could be considered as a source of starting material for the development of new natural product based anti-HSV and anti-HIV agents.$^8$

Minimum erythremic dose (MED) of fruits of Ficus hispida show fair complexioned human volunteers using U.V. lamp studies. Oral administration of unripe fruit powder (12.0 g twice a day for three days) cause photosensitization.$^9$

The extract of Ficus racemosa at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models.$^{10}$

The extracts of new species of plants of genus Phyllanthus (Euphorbiaceae) exhibit antinociceptive properties.$^{11}$

Opium was being used in Egypt as far back as 2000 BC as a children’s sedative and teething remedy. According to the Roman writer Prosper Alpinus, the
Egyptian were practiced opium-esters and became faint and languid through want of it. They prepared and drank it in the form of 'creticwine' which they flavored by adding pepper and other aromatics. Interpretations of certain sections of the Old Testament suggest that opium was known to the ancient Hebrews. Their word rosh, meaning head, is believed to refer to the head of the poppy and the word merosh, to the juice of the poppy. Opium mentioned in Ebers Papyrus too, which happens to be the earliest record in medicine. This document was found between the legs of a mummy in a tomb near Luxor, a town on the east banks of the river Nile. It is dated about 1550 BC. This papyrus describes a mixture of Opium and another material, which was found effective in quietening crying children. Till some time ago children in Egypt, India and even Europe were being soothed with Opium. It is said that mothers often used Poppy juice to smear on their nipples so that the child would immediately stop crying on sucking this drugged milk. Opium was also used extensively by Arab Physicians, the most celebrated of whom was Ibn Sina (980-1037 AD).

Ibn Sina recommended opium especially for Diarrhoea and eye problems and it is said that he himself succumbed to an overdose of the drug. It was Arab traders who introduced opium to the East around this time. Prohibition of wine by Holy Quran made Muslims very vulnerable to the use of opium. The Mughal emperors Baber, Humanyun, and Akbar were inveterate opium esters (A. Agarwal, 1995). 

_Bauhinia microstachya_ (Leguminosae) a native plant widely distributed in south Brazil. The methanolic extracts (3-30 mg/kg, i.p) of the leaves of _Bauhinia microstachya_ exhibit antinociceptive action.

The anti-oxidant capacities of the soxhlet methanolic extracts are determined and indicate that _Tamarandus indica_ may be an important source of cancer chemotherapeutic natural products in tropical regions.

_Ficus benjamina_ is used as folk medicine for the treatment of certain skin disorders and respiratory disorders and fruit extract of _F. benjamina_ is also known to possess antitumor activity and significant antibacterial activity.
Crataegus crenulata contains pyracenic acid named as $3\beta$ (3,4-dihydroxycinnamoyl)-oxylup-20(29)-en-oic acid. The acid exhibited anti-inflammatory activity by the formation of granulated tissue.\textsuperscript{21}

About a dozen species of Rhus occur in India\textsuperscript{22}, find extensive use in folk medicines for the treatment as spasmolytic, antiviral and anticancer.\textsuperscript{23} Some species may cause dermatitis in sensitive people.\textsuperscript{24,25}

The \textit{Xylosma} species are reported to possess medicinal properties such as \textit{Xylosma japonica} is used to heal jaundice, serofula, sore and tumours\textsuperscript{26}, as desulfurizing agent\textsuperscript{27} and as a deminozide agent.\textsuperscript{28} \textit{Xylosma longifolium} is found to resemble opium in its action and is used with it for house pests and fences.\textsuperscript{29}

The present chapter deals with the Antinociceptive, Antimicrobial activity, and insecticidal activity of plants, \textit{Ficus benjamina (Var. comosa)}, \textit{Xylosma longifolium}, \textit{Crataegus renulata} and \textit{Rhus alata}. The new and some rare compounds from these plants have also been checked for antimicrobial activity.
Materials  
&  
Methods
The study was conducted on seven weeks old male albino rats of 100-200 gms of Charles foster strain. The rats were housed in groups of eight in standard breeding cages with free access to a solid diet (Gold Mohar, Lipton [India], Ltd.) and tap water except during the times of experiment. The “Animal Observation Room” was controlled so that the light dark cycle (light period 6.00 am to 6.00 pm, 12 hours) and temperature ($23\pm 2^\circ C$) were nearly constant.

The animals were subjected to the Screening of Analgesic activity on Analgesiometer.

**General method for preparation of extracts:**

The leaves of *Ficus benjamina* (Var. *Comosa*), *Xylosma longifolium*, *Crataegus crenulata* and *Rhus alata* were dried under shade and crushed to make powder separately. The air dried and powdered leaves (100 gm) of each plant were exhaustively extracted by refluxing them separately with alcohol and double distilled water using soxhlet apparatus for 72 hours. The extracts were used in doses of 50 and 100 mg/kg in each.

**Screening for analgesic activity:**

Analgesics activity of *Ficus benjamina* (Var. *comosa*), *Xylosma longifolium*, *crataegus crenulata* and *Rhus alata* were assessed by Analgesiometer test.
a. Analgesiometer Tests:

The test was carried out by the tail flick procedure described by D'Amour and Smith using analgesiometer. Rats were selected by preliminary screening. Those showing variation of more than one second between two reaction times at 15 minutes interval, or more than three seconds from the group mean were discarded. The time response curve was plotted. Six albino Charles foster rats weighing 150-200 gms of either sex were placed in restraining holder so that the tail between the hole and tail tip or single point 3-5 cm from the tip of tail are directly kept over a heated nichrome wire. The reaction due to thermal stimulus in the form of tail-flick in normal, untreated rats is adjusted within 5-6 seconds. Since the stimulus capable of producing tissue damage is stated to be about twice that required to produce pain, the system is preset to automatically cut off at 16 seconds, that is, the tails were exposed to heat for a maximum of 16 seconds.

The cutoff time was obtained after requiring the determination of the reaction time of each untreated rat at 0, 10, 20 minutes. The reading are averaged and multiplied by 1.5, thereby providing an arbitrary cutoff for each rat.

Tail flick latency was tested before and 2 hours after the drug administration at 20 minutes intervals for 3 hours. If the tail-flick reaction time equals or exceeds the cut off time a “+” is recorded. Two consecutive trials were taken as positive for the agent to be considered analgesic (all-or none response). The percentage response will then be the fraction of the group failing to react within the cutoff time.
Drugs:

*Ficus benjamina, Xylosma longifolium, Crataegus crenulata* alcoholic and aqueous extract were administered in doses of 50 and 100 mg/kg in a suspension using distilled water. Similarly, *Rhus alata* alcoholic extract was given in a dose 50 and 100 mg/kg respectively.

The test was done in fourteen groups of rats as follows:

i  Test group: (n = 8)- *Ficus benjamina* aqueous extract, 50 mg/kg, p.o.

ii Test group: (n = 8)- *Ficus benjamina* aqueous extract 100 mg/kg, p.o.

iii Test group: (n = 8)- *Ficus benjamina* alcoholic extract, 50 mg/kg, p.o.

iv Test group: (n = 8)- *Ficus benjamina* alcoholic extract, 100mg/kg, p.o.

v Test group: (n = 8)- *Xylosma longifolium* aqueous extract, 50 mg/kg, p.o.

vi Test group: (n = 8)- *Xylosma longifolium* aqueous extract 100 mg/kg, p.o.

vii Test group: (n = 8)- *Xylosma longifolium* alcoholic extract, 50 mg/kg, p.o.

viii Test group: (n = 8)- *Xylosma longifolium* alcoholic extract,100 mg/kg, p.o.

ix Test group: (n = 8)- *Crataegus crenulata* aqueous extract, 50 mg/kg, p.o.
Antimicrobial Activity

The *in vitro* antibacterial activity was carried out against *Escherichia coli*, *Staphylococcus aureus, Salmonella typhimurium, Bacillus subtilis* and *in vitro* antifungal activity was carried out against *Candida albicans*, *Fusarium oxysporum*, *Penicillium notatum*, *Aspergillus niger* and *Trichoderma viridae*. The agar well diffusion method was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Mueller Hinton Agar plates (Hi-Media Pvt. Ltd., Mumbai, India). The wells of 8 mm diameter were punched into the agar medium and filled with 100 µl of plant extracts and some plant products of 1 mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37°C overnight. The antibiotic (Chloramphenicol) and antifungal disc (Nystatin) of 30 mcg potency each were used in the test system as positive controls. Zone of inhibition of bacterial and fungal growth around each well was measured in mm.
Insecticidal activity

The noctuid *Helicoverpa armigera* is one of the most important constraints to crop production in Asia, Africa, Australia and Mediterranean Europe. It attacks over 200 crop species belonging to 45 families. Globally yield loss tune to US $ 2 billion annually. In India, the loss tune to 200 million US $ on Pigeon pea and chickpea. The pest status of *Helicoverpa* is well justified in its polyphagy on all economically important crops and the hurdles in its management. This necessitates the search for more potent insecticides which are safer to the user and consumer.

Due to high cost of protecting crops with chemical pesticides and due to increasing resistance and resurgence, there is growing interest in the use of botanical pesticides which differ considerably from conventional products and this would make the existing IPM programme more effective and sustainable while decreasing the reliance on synthetic insecticides.

Currently *Helicoverpa armigera* control is based on neurotoxic insecticides which are damaging the environment and public health via food, ground water contaminators or accidental exposure, these problems are getting amplified by the residual toxicities of the trophic levels. Plants based insecticides have the potential to play a minor role in pest management in sustainable agriculture production. They are the renewable, non-persistant in environment and relatively safe to natural enemies, non-target organisms and human beings. More than 1000 species of plants are known to possess insecticidal properties, 380 species antifeedent properties, 300 species repellent properties and 30 species each possessing attractant and insect growth regulator (IRG) properties. Plants produce a range of chemical substances to protect themselves from insect pests. They produce secondary metabolites which include alkaloids terpenoids, flavonoids and acetogenins. Biopesticides reduce the risk of environmental contamination, have narrow target range very specific mode of action, slow acting, and relatively critical application times, suppress rather than eliminate pest population, limited field persistence, short life and safe to man and animals.
In a view to develop an array of botanicals, this experiment was designed with two very promising plants *Crataegus crenulata* and *Xylosma longifolium*, against the gram pod bore on laboratory conditions with chickpea as its host plant for its insecticidal

The fresh leaves of *Xylosma longifolium* collected from Forest Research Institute, Dehradun were dried under shade and powdered. The air dried powdered leaves were thoroughly extracted with petroleum ether (60-80°C), Benzene and methanol successively. The petroleum ether and benzene extracts were found to the same on TLC and hence mixed together.

The leaves were identified by Prof. Wazahat Hussain, taxonomist Deparment of botany AMU, Aligarh.

The air dried powdered leaves (100 gms) of *X. longifolium* were extracted with alcohol and then distilled water using soxhlet apparatus for 72 hrs. The alcoholic extract was further successively refluxed with petrol, benzene, chloroform, ethylacetate and finally with methanol to give different fractions. The class of compounds that were fractionated from *Crataegus crenulata* include vitexin, triterpenoidic acid and isovitexin. Similarly, the compounds isolated from *Xylosma longifolium* were triterpene glycoside, kaempferol, quercetin and flavonol glycosides.

*Helicoverpa armigera* larvae were collected from the department of plant protection and maintained under controlled laboratory conditions. The larvae were provided fresh chickpea pods and leaves from the departmental field and reared till they reached the fifth instars for experimentation. Each treatment included 15 insects and was replicated thrice.

The late instars were starved for 12 hrs. The fresh weight of the larvae was noted and food (in the form of chickpea pods and leaves), Dipped in the different concentrations (0.2, 0.5, 1.0, and 2.0%) prepared for the different plant extracts in their respective solvents was provided. The exposed larvae were kept under observation and the duration for completing their larval period was marked. Once pupated, the pupal weight was taken, though very carefully. Again the duration of pupation was counted till the adults emerged. Any sort of abnormalities in any stage
was noted down. Thus, the insecticidal properties of the fractions via the leaf-dip method were analyzed through the activity shown by the exposed larvae, the resultant time and dose depended-mortality and the statistical analysis for significance of the incurred data.
Observations
&
Results
Study of *Ficus benjamina (Var. comosa)* for analgesic activity:

Six groups of eight animals each were taken and grouped as: Group 1: Control (double distilled water), Group 2: 50 mg/kg aqueous extract, Group 3: 100 mg/kg aqueous extract, Group 4: 50 mg/kg alcoholic extract, Group 5: 100 mg/kg alcoholic extract and Group 6: 30 mg/kg Standard drug (Pentazocine). Initially basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire of analgesiometer. After administration of drugs, reaction time was noted two hours later in groups 2, 3, 4, and 5 while 20 minutes later in groups 1 and 6. All readings were taken in an interval of 20 minutes each.

Oral administration of alcoholic and aqueous extracts of leaves at doses 50 and 100 mg/kg p.o. showed significant antinociceptive activity in analgesiometer test. The aqueous extract at doses 50 and 100 mg/kg in rats causes gradual increase in reaction time which reached to its maximum values $6.27\pm0.223$ and $6.15\pm0.197$ in 220 and 200 minutes respectively. The effect gradually decline within next 80 and 100 minutes. The aqueous extract has been found to less potent then the standard drug. The administration of alcoholic extract in doses 50 and 100 mg/kg p.o. cause gradual increase to its peak values $7.1\pm0.19$ and $7.5\pm0$ in 240 and 220 minutes after drug administration. The effect gradually decline within next 120 minutes in each dose. Table (1&2). The effect of *Ficus benjamina (Var. comosa)* was compared with pentazocine, which was given in dose of 30 mg/kg i.p. It was found that alcoholic extract of test drug has peak values same as standard drug than the aqueous extract. Alcoholic extract showed analgesic response in dose dependent manner (Fig.1).
Table 1

Reaction Time in Analgesiometer Test of *Ficus benjamina* (Var. comosa) extracts

<table>
<thead>
<tr>
<th>Time (min) After drug Administration</th>
<th>Fc(n=8) 50mg/kg (aqueous extract of leaves) P.O.</th>
<th>Fe(n=8) 100mg/kg (aqueous extract of leaves) P.O.</th>
<th>Fc(n=8) 50mg/kg (alcoholic extract of leaves) P.O.</th>
<th>Fc (n=8) 100mg/kg (alcoholic extract of leaves) P.O.</th>
<th>Pentazocine(n=8) 30 mg/kg, I.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.62±0.19</td>
<td>4.8±0.23</td>
<td>4.8±0.23</td>
<td>4.76±0.16</td>
<td>4.41±0.01</td>
</tr>
<tr>
<td>20</td>
<td>4.87±0.21</td>
<td>5.08±0.17</td>
<td>5.07±0.23</td>
<td>5.55±0.19</td>
<td>6.29±0.58</td>
</tr>
<tr>
<td>40</td>
<td>5.16±0.15</td>
<td>5.35±0.17</td>
<td>5.35±0.18</td>
<td>6.41±0.30</td>
<td>6.50±0.40</td>
</tr>
<tr>
<td>60</td>
<td>5.25±0.16</td>
<td>6.0±0.27</td>
<td>5.90±0.15</td>
<td>6.82±0.21</td>
<td>6.96±0.26</td>
</tr>
<tr>
<td>80</td>
<td>5.67±0.19</td>
<td>6.15±0.19</td>
<td>6.11±0.15</td>
<td>7.26±0.11</td>
<td>6.88±0.19</td>
</tr>
<tr>
<td>100</td>
<td>6.27±0.22</td>
<td>5.76±0.16</td>
<td>6.47±0.17</td>
<td>7.5±0</td>
<td>7.01±0.17</td>
</tr>
<tr>
<td>120</td>
<td>6.04±0.31</td>
<td>5.35±0.13</td>
<td>7.1±0.18</td>
<td>7.34±0.07</td>
<td>6.87±0.18</td>
</tr>
<tr>
<td>140</td>
<td>5.7±0.27</td>
<td>5.2±0.13</td>
<td>7.07±0.19</td>
<td>7.28±0.08</td>
<td>6.73±0.18</td>
</tr>
<tr>
<td>160</td>
<td>5.22±0.21</td>
<td>5.1±0.16</td>
<td>6.77±0.19</td>
<td>6.92±0.05</td>
<td>6.38±0.19</td>
</tr>
<tr>
<td>180</td>
<td>4.87±0.21</td>
<td>5.08±0.17</td>
<td>6.48±0.25</td>
<td>6.17±0.09</td>
<td>5.95±0.21</td>
</tr>
<tr>
<td>200</td>
<td>--</td>
<td>--</td>
<td>5.82±0.21</td>
<td>5.54±0.10</td>
<td>5.05±0.10</td>
</tr>
<tr>
<td>220</td>
<td>--</td>
<td>--</td>
<td>5.55±0.20</td>
<td>5.16±0.09</td>
<td>4.37±0.04</td>
</tr>
<tr>
<td>240</td>
<td>--</td>
<td>--</td>
<td>5.18±0.20</td>
<td>4.83±0.17</td>
<td>---</td>
</tr>
<tr>
<td>260</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>---</td>
</tr>
</tbody>
</table>

Fc - *Ficus comosa*
Table 2

Onset and Offset of *Ficus benjamina* (*Var. comosa*) aqueous and alcoholic extracts of leaves

<table>
<thead>
<tr>
<th>Group(n)</th>
<th>Treatment</th>
<th>Onset</th>
<th>Peak</th>
<th>Recovery</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)</td>
<td>N. Saline</td>
<td>4.12±0.21</td>
<td>4.23±0.11</td>
<td>4.02±0.22</td>
<td>0 Min</td>
</tr>
<tr>
<td>Standard(8)</td>
<td>Pentazocine (30mg/kg)</td>
<td>6.29±0.58 ●</td>
<td>7.01±0.17*</td>
<td>4.37±0.04●</td>
<td>200 Min</td>
</tr>
<tr>
<td>Test(8)</td>
<td>Aq. extract (20 Min)</td>
<td>5.08±0.17●</td>
<td>6.15±0.19*</td>
<td>5.08±0.17●</td>
<td>160 Min</td>
</tr>
<tr>
<td></td>
<td>Fc50 mg/kg</td>
<td>4.87±0.21●</td>
<td>6.27±0.22*</td>
<td>4.87±0.21△</td>
<td>160 Min</td>
</tr>
<tr>
<td></td>
<td>Aq. extract (20 Min)</td>
<td>5.07±0.24●</td>
<td>7.1±0.18*</td>
<td>5.18±0.20●</td>
<td>220 Min</td>
</tr>
<tr>
<td></td>
<td>Fc100 mg/kg</td>
<td>5.55±0.19*</td>
<td>7.5±0*</td>
<td>4.84±0.17△</td>
<td>240 Min</td>
</tr>
</tbody>
</table>

* P<0.001  ● P<0.01  ■ P<0.05  △ P<0.02  ◆ P<0.2
Study of Xylosma longifolium for analgesic activity:

Six groups of eight animals each were taken and grouped as . Group 1: Control (double distilled water), Group 2: 50 mg/kg, aqueous extract, Group 3: 100 mg/kg aqueous extract, Group 4: 50 mg/kg alcoholic extract, Group 5: 100 mg/kg alcoholic extract and Group 6: 30 mg/kg Standard drug (Pentazocine). Initially basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire.
of analgesiometer. After administration of drugs, reaction time was noted an hours later in groups 2, 3, 4, and 5 while 20 minutes later in groups 1 and 6. All readings were taken in an interval of 20 minutes each.

The oral administration of aqueous and alcoholic extracts of leaves at doses 50 mg/kg and 100 mg/kg p.o showed significant antinociceptive activity in analgesiometer test. The aqueous extract at doses 50 and 100 mg/kg in albino rats causes gradual increase in reaction time which reached to its maximum values (7.44±0.063) and (7.5±0) in 100 and 80 minutes respectively. The duration of maximum effect in both the doses is 20 and 60 minutes. The effect gradually decline in within next 100 and 140 minutes. The aqueous extract has been found to be more potent than the standard drug. The duration of analgesia for 50 mg/kg aq. extract is less than the standard but for 100 mg/kg aq. extract, it is same as that of the standard drug. The alcoholic extract at doses 50 and 100 mg/kg also causes gradual increase in reaction time which reached to its maximum values (7.5±0) in 80 minutes in each dose. The duration of maximum effect in both the doses is 40 and 20 minutes. The effect gradually decline within next 120 and 160 minutes (Table 3 & 4). Both the alcoholic drugs have been found to be more potent than the standard drug. The duration of analgesia for 50 mg/kg alcoholic extract is less than the standard drug, but for 100 mg/kg alcoholic extract it is more potent than the standard drug. It was found that alcoholic extract is more potent than the aqueous extract of *X. longifolium* leaves. Both the aqueous and alcoholic extracts show analgesic response in dose dependent manner. The high analgesic activity of different *X. longifolium* extracts add one more positive attribute to its known medicinal properties and hence its use in home remedies as a pain killer may be promoted (Fig. 2).
Table 3

Reaction Time in Analgesiometer Test of Xylosma longifolium leaves extracts

<table>
<thead>
<tr>
<th>Time (Min) After drug Administration</th>
<th>XI (n=8) 50 mg/kg (aqueous extract of leaves) P.O.</th>
<th>XI (n=8) 100 mg/kg (aqueous extract of leaves) P.O.</th>
<th>XI (n=8) 50 mg/kg (alcoholic extract of leaves) P.O.</th>
<th>XI (n=8) 100 mg/kg (alcoholic extract of leaves) P.O.</th>
<th>Pentazocine (n=8) 30 mg/kg, I.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.79±0.21</td>
<td>5.05±0.18</td>
<td>4.88±0.11</td>
<td>4.59±0.17</td>
<td>4.41±0.01</td>
</tr>
<tr>
<td>20</td>
<td><strong>5.25±0.21</strong></td>
<td><strong>5.4±0.20</strong></td>
<td><strong>5.36±0.15</strong></td>
<td>5.04±0.13</td>
<td>6.29±0.58</td>
</tr>
<tr>
<td>40</td>
<td>5.75±0.25</td>
<td>6.05±0.15</td>
<td>6.48±0.25</td>
<td>6.8±0.26</td>
<td>6.50±0.40</td>
</tr>
<tr>
<td>60</td>
<td>6.29±0.13</td>
<td>7.09±0.18</td>
<td>7.06±0.19</td>
<td>7.4±0.25</td>
<td>6.96±0.26</td>
</tr>
<tr>
<td>80</td>
<td>6.65±0.15</td>
<td><strong>7.5±0.0</strong></td>
<td>7.5±0.0</td>
<td>6.88±0.19</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td><strong>7.44±0.06</strong></td>
<td>7.5±0.0</td>
<td><strong>7.5±0.0</strong></td>
<td><strong>7.38±0.13</strong></td>
<td><strong>7.01±0.17</strong></td>
</tr>
<tr>
<td>120</td>
<td>7.3±0.14</td>
<td>7.5±0.0</td>
<td>7.15±0.23</td>
<td>7.35±0.12</td>
<td>6.87±0.18</td>
</tr>
<tr>
<td>140</td>
<td>6.81±0.20</td>
<td>7.24±0.11</td>
<td>6.84±0.25</td>
<td>7.23±0.18</td>
<td>6.73±0.18</td>
</tr>
<tr>
<td>160</td>
<td>6.2±0.10</td>
<td>6.84±0.25</td>
<td>6.2±0.28</td>
<td>6.8±0.22</td>
<td>6.38±0.19</td>
</tr>
<tr>
<td>180</td>
<td>5.8±0.16</td>
<td>6.24±0.25</td>
<td>5.51±0.19</td>
<td>6.35±0.25</td>
<td>5.95±0.21</td>
</tr>
<tr>
<td>200</td>
<td><strong>5.14±0.20</strong></td>
<td>5.55±0.24</td>
<td><strong>5.15±0.15</strong></td>
<td>5.71±0.14</td>
<td>5.05±0.10</td>
</tr>
<tr>
<td>220</td>
<td>--</td>
<td><strong>-5.11±0.15</strong></td>
<td></td>
<td></td>
<td>4.99±0.06</td>
</tr>
<tr>
<td>240</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
<td>4.65±0.14</td>
</tr>
<tr>
<td>260</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
<td>---</td>
</tr>
</tbody>
</table>

XI- Xylosma longifolium
Onset and Offset of *Xylosma longifolium* aqueous and alcoholic extracts of leaves

<table>
<thead>
<tr>
<th>Group(n)</th>
<th>Treatment</th>
<th>Onset</th>
<th>Peak</th>
<th>Recovery</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)</td>
<td>N. Saline</td>
<td>4.12±0.21</td>
<td>4.23±0.11</td>
<td>4.02±0.22</td>
<td>0 Min</td>
</tr>
<tr>
<td>Standard(8)</td>
<td>Pentazocine (30mg/kg)</td>
<td>6.29±0.58**</td>
<td>7.01±0.17*</td>
<td>4.37±0.04**</td>
<td>200 Min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 min)</td>
<td>(100 Min)</td>
<td>(220 Min)</td>
<td></td>
</tr>
<tr>
<td>Test(8)</td>
<td>XI. 50mg/kg</td>
<td>5.25±0.21**</td>
<td>7.44±0.063*</td>
<td>5.14±0.202**</td>
<td>180 min</td>
</tr>
<tr>
<td></td>
<td>Aq. extract</td>
<td>(20 min)</td>
<td>(100 min)</td>
<td>(200 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XI. 100 mg/kg</td>
<td>50.4±0.20*</td>
<td>7.5±0.0*</td>
<td>5.11±0.16**</td>
<td>200 min</td>
</tr>
<tr>
<td></td>
<td>Aq. extract</td>
<td>(20 min)</td>
<td>(80 min)</td>
<td>(220 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XI. 50 mg/kg</td>
<td>5.36±0.15*</td>
<td>7.5±0.0*</td>
<td>5.15±0.15*</td>
<td>180 min</td>
</tr>
<tr>
<td></td>
<td>Alc. extract</td>
<td>(20 min)</td>
<td>(80 min)</td>
<td>(200 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XI. 100 mg/kg</td>
<td>5.04±0.13**</td>
<td>7.5±0.0*</td>
<td>465±0.14■■</td>
<td>220 min</td>
</tr>
<tr>
<td></td>
<td>Alc. extract</td>
<td>(20 min)</td>
<td>(80 min)</td>
<td>(240 min)</td>
<td></td>
</tr>
</tbody>
</table>

*---p<0.001
**---p<0.01
■---p<0. 2
■■---p<0.05
Study of *Crataegus crenulata* for analgesic activity:

Six groups of eight animals each were taken and grouped as Group 1: Control (double distilled water), Group 2: 50 mg/kg, aqueous extract, Group 3: 100 mg/kg aqueous extract, Group 4: 50 mg/kg alcoholic extract, Group 5: 100 mg/kg alcoholic extract and Group 6: 30 mg/kg Standard drug (Pentazocine). Initially basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire of analgesiometer. After administration of drugs, reaction time was noted an hours
later in groups 2, 3, 4, and 5 while 20 minutes later in groups 1 and 6. All readings were taken in an interval of 20 minutes each.

The oral administration of aqueous and alcoholic extracts of leaves at doses 50 mg/kg and 100 mg/kg p.o showed significant antinociceptive activity in analgesiometer test. The aqueous extract at doses 50 and 100 mg/kg in albino rats causes gradual increase in reaction time which reached to its maximum values (7.04±0.22) and (7.4±0.1) in 80 minutes in each respectively. The duration of maximum effect in both the doses is 20 minutes. The effect gradually decline within next 100 minutes in both the doses. The aqueous extract has been found to be more potent than the standard drug, but the duration of analgesia (180 Min) is less than the standard drug (200 Min). The alcoholic extract at doses 50 and 100 mg/kg also causes gradual increase in reaction time which reached to its maximum values (6.34±0.19) and (7.50±0.0) in 100 and 120 minutes respectively. The duration of maximum effect in both the doses is 20 and 40 minutes. The effect gradually decline within next 80 and 120 minutes. Both the alcoholic drugs have been found to be more potent than the standard drug. The duration of analgesia for 50 mg/kg alcoholic extract is less than the standard drug, but for 100 mg/kg alcoholic extract it is more than the standard drug (Table 5&6). It was found that alcoholic extract at the dose 100 mg/kg is most effective among all the doses. Both the aqueous and alcoholic extracts show analgesic response in dose dependent manner. The high analgesic activity of different *Crataegus crenulata* extracts add one more positive attribute to its known medicinal properties and hence its use in home remedies as a pain killer may be promoted (Fig. 3).
Table 5

Reaction Time in Analgesiometer Test of *crataegus renulata* leaves extracts

<table>
<thead>
<tr>
<th>Time (Min) After drug Administration</th>
<th>CC (n=8) 50 mg/kg (aqueous extract of leaves) P.O.</th>
<th>CC (n=8) 100 mg/kg (aqueous extract of leaves) P.O.</th>
<th>CC(n=8) 50 mg/kg (alcoholic extract of leaves) P.O.</th>
<th>CC(n=8) 100 mg/kg (alcoholic extract of leaves) P.O</th>
<th>Pentazocine(n=8) 30 mg/kg, I.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.8±0.19</td>
<td>4.89±0.177</td>
<td>4.86±0.12</td>
<td>4.93±0.12</td>
<td>4.41±0.01</td>
</tr>
<tr>
<td>20</td>
<td><strong>5.39±0.14</strong></td>
<td>5.4±0.26</td>
<td>5.21±0.13</td>
<td>5.34±0.09</td>
<td>6.29±0.58</td>
</tr>
<tr>
<td>40</td>
<td>5.89±0.16</td>
<td>6.16±0.32</td>
<td>5.56±0.15</td>
<td>5.90±0.21</td>
<td>6.50±0.40</td>
</tr>
<tr>
<td>60</td>
<td>6.59±0.21</td>
<td>7.38±0.13</td>
<td>5.99±0.18</td>
<td>6.65±0.22</td>
<td>6.96±0.26</td>
</tr>
<tr>
<td>80</td>
<td>7.04±0.22</td>
<td><strong>7.4±0.1</strong></td>
<td>6.26±0.20</td>
<td>6.89±0.18</td>
<td>6.88±0.19</td>
</tr>
<tr>
<td>100</td>
<td><strong>6.98±0.23</strong></td>
<td>7.31±0.15</td>
<td>6.34±0.19</td>
<td><strong>7.43±0.06</strong></td>
<td><strong>7.01±0.17</strong></td>
</tr>
<tr>
<td>120</td>
<td>6.81±0.21</td>
<td>6.85±0.26</td>
<td>5.90±0.20</td>
<td>7.50±0.0</td>
<td>6.87±0.18</td>
</tr>
<tr>
<td>140</td>
<td><strong>6.49±0.19</strong></td>
<td><strong>6.01±0.21</strong></td>
<td>5.74±0.23</td>
<td>7.50±0.0</td>
<td>6.73±0.18</td>
</tr>
<tr>
<td>160</td>
<td>5.80±0.28</td>
<td>5.5±0.190</td>
<td>5.33±0.18</td>
<td>7.20±0.11</td>
<td>6.38±0.19</td>
</tr>
<tr>
<td>180</td>
<td>5.21±0.15</td>
<td>5.05±0.17</td>
<td>5.16±0.15</td>
<td>6.76±0.19</td>
<td>5.95±0.21</td>
</tr>
<tr>
<td>200</td>
<td><strong>4.86±0.17</strong></td>
<td>4.9±0.16</td>
<td>4.94±0.10</td>
<td>6.59±0.19</td>
<td>5.05±0.10</td>
</tr>
<tr>
<td>220</td>
<td></td>
<td></td>
<td></td>
<td>6.06±1.16</td>
<td>4.37±0.04</td>
</tr>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td>5.46±0.14</td>
<td>---</td>
</tr>
<tr>
<td>260</td>
<td></td>
<td></td>
<td></td>
<td>4.98±0.12</td>
<td>---</td>
</tr>
</tbody>
</table>
### Table 6

Onset and Offset of *Crataegus crenulata* aqueous and alcoholic extracts of leaves

<table>
<thead>
<tr>
<th>Group(n)</th>
<th>Treatment</th>
<th>Onset</th>
<th>Peak</th>
<th>Recovery</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)</td>
<td>N. Saline</td>
<td>4.12±0.21</td>
<td>4.23±0.11</td>
<td>4.02±0.22</td>
<td>0 Min</td>
</tr>
<tr>
<td>Standard(8)</td>
<td>Pentazocine (30mg/kg)</td>
<td>6.29±0.58**</td>
<td>7.01±0.17*</td>
<td>4.37±0.04■</td>
<td>200 Min</td>
</tr>
<tr>
<td></td>
<td>(20 min)</td>
<td>(100 Min)</td>
<td>(220 Min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test(8)</td>
<td>Cc.50 mg/kg</td>
<td>5.39±0.14*</td>
<td>7.04±0.22*</td>
<td>4.86±0.17**</td>
<td>180 Min</td>
</tr>
<tr>
<td></td>
<td>Aq. extract (20 Min)</td>
<td>(80 Min)</td>
<td>(200 Min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cc.100 mg/kg</td>
<td>5.4±0.26**</td>
<td>7.4±0.1*</td>
<td>4.9±0.16**</td>
<td>180 Min</td>
</tr>
<tr>
<td></td>
<td>Aq. extract (20 Min)</td>
<td>(80 Min)</td>
<td>(200 Min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cc.50 mg/kg</td>
<td>5.21±0.13*</td>
<td>6.34±0.19*</td>
<td>4.94±0.10**</td>
<td>240 Min</td>
</tr>
<tr>
<td></td>
<td>Alc. extract (20 Min)</td>
<td>(100 Min)</td>
<td>(200 Min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cc.100 mg/kg</td>
<td>5.34±0.09*</td>
<td>7.50±0.0*</td>
<td>4.98±0.12**</td>
<td>260 Min</td>
</tr>
<tr>
<td></td>
<td>Alc. extract (20 Min)</td>
<td>(120 Min)</td>
<td>(260 Min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.001
** p<0.01
■ p<0.2
Study of *Rhus alata* for analgesic activity:

Four groups of eight animals each were taken and grouped as Group 1: Control (double distilled water), Group 2: 50 mg/kg alcoholic extract, Group 3: 100 mg/kg alcoholic extract, Group 4: 30 mg/kg Standard drug (Pentazocine).

Initially basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire of analgesiometer. After administration of drugs, reaction time was noted 90 minutes later in groups 2, 3, while 20 minutes later in groups 1 and 4. All readings were taken in an interval. The oral administration of
alcoholic extracts of leaves at doses 50 mg/kg and 100 mg/kg p.o showed significant antinociceptive activity in analgesiometer test. The alcoholic extract at doses 50 and 100 mg/kg in albino rats causes gradual increase in reaction time which reached to its maximum values (5.48±0.12) and (6.09±0.064) in 80 minutes each. The duration of maximum effect in both the doses is 20 minutes. The effect gradually decline within next 60 minutes in both the doses. Both the doses has been found to be less potent than the standard drug. The duration of analgesia for 100 mg/kg and 50 mg/kg alcoholic extracts is less than the standard. It was found that 100 mg/kg alcoholic extract is more potent than the 50 mg/kg alcoholic extract of *Rhus alata* leaves (Table-7 & 8). Both the alcoholic extracts show analgesic response in dose dependent manner. The aqueous extract at the doses 50 mg/kg and 100 mg/kg has been found to be non significant in analgesiometer test. The analgesic activity of alcoholic extracts of *Rhus alata* add one more positive attribute to its known medicinal properties and hence its use in home remedies as a pain killer may be promoted (Fig. 4).
## Table 7

Reaction Time in Analgesiometer Test of *Rhus alata* (Ra) leaves extracts & Pentazocine

<table>
<thead>
<tr>
<th>Time (Min) After drug Administration P.O</th>
<th>Ra (50 mg/kg alcoholic extract of leaves)</th>
<th>Ra (100 mg/kg alcoholic extract of leaves)</th>
<th>Pentazocine 30 mg/kg, I.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.46±0.45</td>
<td>4.38±0.28</td>
<td>4.41±0.01</td>
</tr>
<tr>
<td>20</td>
<td>4.78±0.18</td>
<td>4.95±0.12</td>
<td>6.29±0.58</td>
</tr>
<tr>
<td>40</td>
<td>5.16±0.16</td>
<td>5.16±0.087</td>
<td>6.50±0.40</td>
</tr>
<tr>
<td>60</td>
<td>5.34±0.13</td>
<td>5.95±0.073</td>
<td>6.96±0.26</td>
</tr>
<tr>
<td>80</td>
<td>5.48±0.12</td>
<td>6.09±0.064</td>
<td>6.88±0.19</td>
</tr>
<tr>
<td>100</td>
<td>5.38±0.077</td>
<td>5.99±0.061</td>
<td>7.01±0.17</td>
</tr>
<tr>
<td>120</td>
<td>5.25±0.063</td>
<td>5.36±0.08</td>
<td>6.87±0.18</td>
</tr>
<tr>
<td>140</td>
<td>4.94±0.122</td>
<td>5.20±0.076</td>
<td>6.73±0.18</td>
</tr>
<tr>
<td>160</td>
<td>---</td>
<td>---</td>
<td>6.38±0.19</td>
</tr>
<tr>
<td>180</td>
<td>---</td>
<td>---</td>
<td>5.95±0.21</td>
</tr>
<tr>
<td>200</td>
<td>---</td>
<td>---</td>
<td>5.05±0.10</td>
</tr>
<tr>
<td>220</td>
<td>---</td>
<td>---</td>
<td>4.37±0.04</td>
</tr>
<tr>
<td>240</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>260</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
### Table 8

Onset and Offset of Antinociceptive activity of *Rhus alata* alcoholic extract of leaves

<table>
<thead>
<tr>
<th>Group(n)</th>
<th>Treatment</th>
<th>Onset</th>
<th>Peak</th>
<th>Recovery</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)</td>
<td>N. Saline</td>
<td>4.12±0.21</td>
<td>4.23±0.11</td>
<td>4.02±0.22</td>
<td>0 Min</td>
</tr>
<tr>
<td>Standard(8)</td>
<td>Pentazocine(30mg/kg)</td>
<td>6.29±0.58**</td>
<td>7.01±0.17*</td>
<td>4.37±0.04■</td>
<td>200 Min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 min)</td>
<td>(100 Min)</td>
<td>(220 Min)</td>
<td></td>
</tr>
<tr>
<td>Test(8)</td>
<td>Ra. 50 mg/kg alcoholic. extract</td>
<td>4.78±0.18■</td>
<td>5.48±0.12*</td>
<td>4.94±0.122*</td>
<td>120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 min)</td>
<td>(80 Min)</td>
<td>(140 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ra.100 mg/kg alcoholic. extract</td>
<td>4.95±0.12**</td>
<td>6.09±0.064*</td>
<td>5.20±0.076*</td>
<td>120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 min)</td>
<td>(80 min)</td>
<td>(140 min)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.001  
**p<0.01  
■■p<0.2  
■p<0.05
Ra- Rhus alata

Antimicrobial Activity of *F. Comosa, X. longifolium, C. crenulata* and *R. alata* extracts:

The antimicrobial activity of aqueous and alcoholic extracts of *Ficus comosa, X. longifolium, C. crenulata* and *R. alata* extracts was investigated against bacterial and fungal strains. It may be seen that all the extracts exhibited a low, moderate or high activity against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and the yeast (*Candida albicans*) (Table 9).
F. comosa aqueous extract showed high activity against S. aureus, Escherichia coli and Salmonella typhimurium and moderate activity against Bacillus subtilis and yeast Candida albicans.

Alcoholic extract of F. comosa was found highly active against S. aureus and moderately active against Bacillus subtilis.

X. longifolium aqueous extract showed high activity against Staphylococcus aureus (IAO-SA-22) and Candida albicans (IAO-109) and moderate activity against Bacillus subtilis (MTCC-121) and Trichoderma viridae (lab isolate (ICAR)). The alcoholic extract found showed high activity against Staphylococcus aureus (IAO-SA-22) and Candida albicans (IAO-109). And moderate activity against Bacillus subtilis (MTCC-121), Salmonella typhimurium (MTCC-98) and Aspergillus brassicola.

C. crenulata aqueous extract showed high activity against Staphylococcus aureus (IAO-SA-22), Bacillus subtilis (MTCC-121) and moderate activity against Escherichia coli (K-12), Candida albicans (IAO-109) and Fusarium oxysporum (lab isolate) but it show low activity against Trichoderma viridae (lab isolate (ICAR) and Aspergillus brassicola. The alcoholic extract found to show high activity against Staphylococcus aureus (IAO-SA-22) Escherichia coli (K-12), Candida albicans (IAO-109) and moderate activity against Bacillus subtilis (MTCC-121) and Trichoderma viridae (lab isolate (ICAR). but it show low activity against Fusarium oxysporum (lab isolate) and Aspergillus brassicola.

R. alata alcoholic extract showed high activity against Staphylococcus aureus (IAO-SA-22), Bacillus subtilis (MTCC-121) and moderate activity against Candida albicans (IAO-109) and Aspergillus brassicola.

The antimicrobial activity of new and rare compounds from F. comosa X. longifolium, C. crenulata and R. alata extracts was investigated against bacteria and fungal strains. It may be seen that the compounds exhibited a low, moderate or high and even no activity against gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and the yeast (Candida albicans) (Table 10).
<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em> (IAO-SA-22)</th>
<th><em>Escherichia coli</em> (K-12)</th>
<th><em>Salmonella typhimurium</em> (MTCC-98)</th>
<th><em>Bacillus subtilis</em> (MTCC-121)</th>
<th><em>Candida albicans</em> (IAO-109)</th>
<th><em>Fusarium oxysporum</em> (lab isolate)</th>
<th><em>Aspergillus niger</em> (lab isolate ICAR)</th>
<th><em>Trichoderma viridae</em> (lab isolate ICAR)</th>
<th><em>Aspergillus brassicola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>F.e. aq. extract</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>F.e. alc. extract</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>8</td>
<td>10</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>XI aq. extract</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>XI alc. Extract</td>
<td>14</td>
<td>-</td>
<td>7</td>
<td>09</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Cc. aq. extract</td>
<td>15</td>
<td>8</td>
<td>-</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>-</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Cc. alc. extract</td>
<td>15</td>
<td>13</td>
<td>-</td>
<td>09</td>
<td>12</td>
<td>4</td>
<td>-</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>
Antimicrobial Activity of *F. comosa* and *X. longifolium*, *C. crenulata* and *R. alata* Aqueous and Alcoholic extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em> (IAO-SA-22)</th>
<th><em>Escherichia coli</em> (K-12)</th>
<th><em>Salmonella typhimurium</em> (MTCC-98)</th>
<th><em>Bacillus subtilis</em> (MTCC-121)</th>
<th><em>Candida albicans</em> (IAO-109)</th>
<th><em>Fusarium oxysporum</em> (lab isolate)</th>
<th><em>Aspergillus niger</em> (lab isolate ICAR)</th>
<th><em>Trichoderma viridae</em> (lab isolate ICAR)</th>
<th><em>Aspergillus brassicola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra.alc. extract</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

Low activity (1-5mm), moderate activity (6-10mm), high activity (11-15mm), very high activity (16-20mm) no activity (-).

F.c-  *F. comosa* and

Xl-  *X. longifolium*

Cc-  *C. crenulata*

Ra-  *R. alata*
Table 10

Antimicrobial Activity of the compounds from the plants *F. comosa* and *X. longifolium*, *C. crenulata* and *R. alata*

<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em> (IAO-SA-22)</th>
<th><em>Escherichia coli</em> (K-12)</th>
<th><em>Salmonella typhimurium</em> (MTCC-98)</th>
<th><em>Bacillus subtilis</em> (MTCC-121)</th>
<th><em>Candida albicans</em> (IAO-109)</th>
<th><em>Fusarium oxysporum</em> (lab isolate)</th>
<th>Aspergillus niger (lab isolate ICAR)</th>
<th>Trichoderma viride (lab isolate ICAR)</th>
<th><em>Aspergillus brassicola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc-1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Fc-4</td>
<td>12</td>
<td>10</td>
<td>16</td>
<td>9</td>
<td>14</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Fc-5</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>5</td>
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<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Xi-1</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Xi-2</td>
<td>13</td>
<td>5</td>
<td>-</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Xi-7</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Ce-1</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ce-3</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Ce-8</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ra-6</td>
<td>15</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>15</td>
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<td>Ra-7</td>
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<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Low activity (1-5 mm), moderate activity (6-10 mm), high activity (11-15 mm), very high activity (16-20 mm) no activity (-).
Insecticidal Activity of Xylosma longifolium and Crataegus crenulata extracts:

*Crataegus crenulata* was evaluated in the two solvents of ethyl acetate and acetone and water. At the lowest conc. of 0.2%, the larvae were semi-active in both the solvents. No marked difference was noted in the larval and pupal weights or their developmental durations. But the pods were repelled by the starved larva with a slight increase in conc. (0.5%) in the former solvent only. 1% showed complete antifeedency as the larvae didn’t touched the diet in both the solvents. This resulted in 36.64% mortality in the aqueous fraction (*Table-12*) and 78.03% in ethylacetate and acetone extracts (*Table-11*). There was remarkable decrease in the larval and pupal weight (288.50 and 193.42, resp) and prolonged larval and pupal duration of 16.55 and 14.61 days, respectively (*Table-11*).

The *Table 13-15* depicted the contribution of *X. longifolium* in the various parameters of *Helicoverpa armigera* larvae. Among the three different solvents used, phytotoxicity was observed in the petrol fraction more than 1% conc. (*Table-12*) unlike the rest two. The larvae repelled its food at 0.5% conc. (*Table-13*) and higher doses (1% and 2%) showed complete antifeedent response. The chloroform solvent was at par with ethylacetate in terms of the activity shown by the treated larvae, but in terms of mortality, the chloroform fraction revealed 52.58% mortality as against 73.97% by the later one.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc.</th>
<th>Larval Weight</th>
<th>Pupal Weight</th>
<th>Larval Period</th>
<th>Pupal Period</th>
<th>% mortality</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Acetate + Acetone</td>
<td>0.2</td>
<td>461.25 ± 1.893d</td>
<td>234.80 ± 1.914d</td>
<td>12.89 ± 1.283a</td>
<td>11.83 ± 0.211a</td>
<td>7.025 ± 0.35a</td>
<td>Semi active</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>363.70 ± 6.645c</td>
<td>224.71 ± 1.537c</td>
<td>13.51 ± 1.320b</td>
<td>12.57 ± 0.244c</td>
<td>18.30 ± 0.35b</td>
<td>Repellency</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>347.30 ± 9.286b</td>
<td>218.64 ± 0.789b</td>
<td>15.27 ± 1.453c</td>
<td>13.72 ± 0.200d</td>
<td>42.12 ± 0.62c</td>
<td>Antifeedency</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>288.50 ± 8.266a</td>
<td>193.42 ± 0.913a</td>
<td>16.55 ± 1.485d</td>
<td>14.61 ± 0.220e</td>
<td>78.03 ± 0.42d</td>
<td>Antifeedency</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>459.51 ± 2.086d</td>
<td>336.14 ± 4.182e</td>
<td>16.78 ± 0.213d</td>
<td>12.23 ± 0.151b</td>
<td>--</td>
<td>Actively feeding</td>
</tr>
</tbody>
</table>

F = 1.01 F = 0.77 F = 0.77 F = 5.81 F = 0.43

d. f. = 4, 49 d. f. = 4, 49 d. f. = 4, 49 d. f. = 4, 49 d. f. = 3, 39
p < 0.01 p < 0.01 p < 0.01 p < 0.01 p < 0.01

LSD value

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.08</td>
<td>3.24</td>
<td>0.37</td>
<td>0.21</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Means within a column with the same letters are not significantly different (LSD test at 1% level).
Table 12. Efficacy of *Crategus crenulata* extract in aqueous solvent against late instar of *Helicoverpa armigera*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc.</th>
<th>Larval Weight</th>
<th>Pupal Weight</th>
<th>Larval Period</th>
<th>Pupal Period</th>
<th>% mortality</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>0.2</td>
<td>442.22 ± 1.265d</td>
<td>247.14 ± 1.444d</td>
<td>11.93 ± 1.190a</td>
<td>10.89 ± 0.108a</td>
<td>7.10 ± 0.20a</td>
<td>Semi active</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>350.16 ± 1.109c</td>
<td>233.72 ± 1.685c</td>
<td>12.15 ± 1.179a</td>
<td>11.24 ± 0.161b</td>
<td>9.15 ± 0.25b</td>
<td>Semi active</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>330.63 ± 1.642b</td>
<td>211.09 ± 0.846b</td>
<td>12.60 ± 1.185b</td>
<td>12.00 ± 0.218c</td>
<td>17.06 ± 0.18c</td>
<td>Antifeedency</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>295.14 ± 1.078a</td>
<td>200.80 ± 1.756a</td>
<td>13.43 ± 1.160c</td>
<td>12.38 ± 0.209d</td>
<td>36.64 ± 0.26d</td>
<td>Antifeedency</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>459.51 ± 2.086e</td>
<td>336.14 ± 4.182e</td>
<td>16.78 ± 0.213d</td>
<td>12.23 ± 0.151d</td>
<td>--</td>
<td>Actively feeding</td>
</tr>
</tbody>
</table>

F = 0.80  F = 1.10  F = 1.36  F = 2.50  F = 0.77

d. f. = 4, 49  d. f. = 4, 49  d. f. = 4, 49  d. f. = 4, 49  d. f. = 3, 39

p < 0.01  p < 0.01  p < 0.01  p < 0.01  p < 0.01

LSD value | 6.83 | 3.21 | 0.33 | 0.22 | 0.33

Means within a column with the same letters are not significantly different (LSD test at 1% level).
Table 13. Efficacy of *Xylosma longifolium* extract in ethyl acetate solvent against late instar of *Helicoverpa armigera*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc.</th>
<th>Larval Weight</th>
<th>Pupal Weight</th>
<th>Larval Period</th>
<th>Pupal Period</th>
<th>% mortality</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>0.2</td>
<td>290.93 ± 1.499d</td>
<td>235.10 ± 1.057c</td>
<td>13.96 ± 0.195a</td>
<td>12.68 ± 0.192b</td>
<td>6.60 ± 0.27a</td>
<td>Semi active</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>265.39 ± 2.220c</td>
<td>232.57 ± 1.657c</td>
<td>15.26 ± 0.191b</td>
<td>13.33 ± 0.196c</td>
<td>13.77 ± 0.19b</td>
<td>Repellency</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>220.71 ± 1.607b</td>
<td>191.82 ± 1.463b</td>
<td>16.76 ± 0.203c</td>
<td>15.51 ± 0.223d</td>
<td>33.48 ± 0.29c</td>
<td>Antifeedency</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>207.78 ± 1.473a</td>
<td>188.16 ± 1.418a</td>
<td>18.50 ± 0.252d</td>
<td>16.60 ± 0.212e</td>
<td>73.97 ± 0.23d</td>
<td>Antifeedency</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>459.51 ± 0.206e</td>
<td>336.14 ± 0.151d</td>
<td>16.78 ± 0.206c</td>
<td>12.23 ± 0.151a</td>
<td>--</td>
<td>Actively feeding</td>
</tr>
</tbody>
</table>

F and *p* value:
- F = 0.75  F = 2.28  F = 1.04  F = 0.84  F = 3.32
- d. f. = 4, 49  d. f. = 4, 49  d. f. = 4, 49  d. f. = 4, 49  d. f. = 3, 39
- *p* < 0.01  *p* < 0.01  *p* < 0.01  *p* < 0.01  *p* < 0.01

LSD value:
- 2.61  2.86  0.30  0.28  0.28

Means within a column with the same letters are not significantly different (LSD test at 1% level).
Table 14. Efficacy of *Xylosma longifolium* extract in chloroform solvant against late instar of *Helicoverpa armigera*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc.</th>
<th>Larval Weight</th>
<th>Pupal Weight</th>
<th>Larval Period</th>
<th>Pupal Period</th>
<th>% mortality</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.2</td>
<td>391.95 ± 1.813d</td>
<td>292.08 ± 1.396d</td>
<td>12.71 ± 0.231a</td>
<td>11.86 ± 0.142a</td>
<td>3.28 ± 0.05a</td>
<td>Actively feeding</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>380.52 ± 1.324c</td>
<td>274.27 ± 1.472c</td>
<td>13.23 ± 0.205b</td>
<td>12.38 ± 0.277b</td>
<td>10.06 ± 0.21b</td>
<td>Semi-active</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>372.11 ± 1.117b</td>
<td>268.99 ± 2.318b</td>
<td>14.48 ± 0.237c</td>
<td>13.69 ± 0.256c</td>
<td>12.10 ± 0.33c</td>
<td>Repellency</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>358.43 ± 1.521a</td>
<td>253.69 ± 1.899a</td>
<td>15.58 ± 0.175d</td>
<td>14.25 ± 0.247d</td>
<td>36.71 ± 0.16d</td>
<td>Antifeedency + phytotoxic</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>459.51 ± 0.206e</td>
<td>336.14 ± 0.151e</td>
<td>16.78 ± 0.206e</td>
<td>12.23 ± 0.151a</td>
<td>--</td>
<td>Actively feeding</td>
</tr>
</tbody>
</table>

F and p value

- F = 0.57  
- F = 1.28  
- F = 2.63  
- F = 2.30  
- F = 3.15  
- d. f. = 4, 49  
- d. f. = 4, 49  
- d. f. = 4, 49  
- d. f. = 4, 49  
- d. f. = 3, 39  
- p < 0.01  
- p < 0.01  
- p < 0.01  
- p < 0.01  
- p < 0.01

LSD value

- 2.38  
- 3.33  
- 0.26  
- 0.28  
- 0.24

Means within a column with the same letters are not significantly different (LSD test at 1% level).
Table 15. Efficacy of *Xylosma longifolium* extract in chloroform solvent against late instar of *Helicoverpa armigera*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc.</th>
<th>Larval Weight</th>
<th>Pupal Weight</th>
<th>Larval Period</th>
<th>Pupal Period</th>
<th>% mortality</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.2</td>
<td>391.87 ± 0.860d</td>
<td>329.17 ± 2.633d</td>
<td>12.80 ± 0.218a</td>
<td>12.35 ± 0.202a</td>
<td>5.64 ± 0.28a</td>
<td>Semi active</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>380.43 ± 0.915c</td>
<td>299.85 ± 3.092c</td>
<td>13.61 ± 0.278b</td>
<td>13.29 ± 0.255b</td>
<td>11.57 ± 0.22b</td>
<td>Repellency</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>372.36 ± 0.749b</td>
<td>274.44 ± 1.692b</td>
<td>14.42 ± 0.196c</td>
<td>13.83 ± 1.407c</td>
<td>22.00 ± 0.22c</td>
<td>Antifeedency</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>361.43 ± 0.580a</td>
<td>255.18 ± 1.450a</td>
<td>15.18 ± 0.205d</td>
<td>14.32 ± 0.247d</td>
<td>52.58 ± 0.23d</td>
<td>Antifeedency</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>459.51 ± 0.206e</td>
<td>336.14 ± 0.151e</td>
<td>16.78 ± 0.206e</td>
<td>12.23 ± 0.151a</td>
<td>--</td>
<td>Actively feeding</td>
</tr>
</tbody>
</table>

F and p value

\[
\begin{align*}
F \ &= \ 1.15 \\
F \ &= \ 1.30 \\
F \ &= \ 0.48 \\
F \ &= \ 1.63 \\
F \ &= \ 2.57 \\
d. f. \ &= \ 4, 49 \\
d. f. \ &= \ 4, 49 \\
d. f. \ &= \ 4, 49 \\
d. f. \ &= \ 4, 49 \\
d. f. \ &= \ 3, 39 \\
p < 0.01 \\
p < 0.01 \\
p < 0.01 \\
p < 0.01 \\
p < 0.01 \\
\end{align*}
\]

LSD value

\[
\begin{align*}
1.62 & \\
3.83 & \\
0.33 & \\
0.30 & \\
0.28 & \\
\end{align*}
\]

Means within a column with the same letters are not significantly different (LSD test at 1% level).
Chapter - VI


34. I. Ahmad, A.Z. Beg *J. Ethnopharmacol, 74*, 113-123 (2001).
List of Publications


2. “Chemical Constituents from the leaves of Xylosma lonifolium” Hamdard Medicus. (in press).


PAPERS PRESENTED AT CONFERENCES/SYMPOSIA


