INTRODUCTION

Science is the reduction of the bewildering diversity of unique events to manageable uniformity within one of a number of symbol systems so as to control and organize unique events.

- ALDOUS HUXLEY

Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source based on their use in traditional medicine\(^1\). Plants have found a basis for traditional medicine system that has been used for thousands of years in countries with ancient civilization such as China (Chang & But 1986), India (Kapoor 1990) and Thailand (Subcharon 1998a).

The study of natural products has added immensely to the knowledge of pure chemistry by investing structure frequently of the type previously unknown. It provided variety of materials, such as alkaloids of useful biological activity or materials which can be used in the synthesis of more useful compounds. The investigation of natural products has also thrown considerable light on mechanism of biosynthesis and may be of increasing importance in taxonomy.

In the past traditional peoples or ancient civilizations depended greatly on local flora and fauna for their survival. They would experiment with various berries, leaves, roots, animal parts or minerals to find out what effect they had. As a result many crude drugs were observed by local healer or shaman to have some medical use. Although some preparations may have been dangerous or worked by a ceremonial or placebo effect, traditional healing system usually had a substantial active pharmacopoeia and in fact
most western medicines up until the 1920s were developed this way. Some systems like traditional Chinese medicine (TCM) or Ayurveda were fully as sophisticated and as documented systems as western medicines, although they might use different paradigms. Many of these aqueous, ethanolic, distilled, condensed or dried extract do indeed have a real and beneficial effect and a study of Ethanobotany can give clues as to which plants might be worth studying in more detail. Rhubarb has been used as purgative for many centuries.

Epidemiological evidence suggests that diets rich in fruit and vegetables decrease the risk of premature mortality from major clinical conditions, including cancer and heart disease. However, it is not yet clear which components or combination of components in fruit and vegetables are protective and what is their mechanism of action. Such scientific uncertainty does not seem to inhibit the marketing of a huge range of plant-based concoctions, promoted as ‘magic bullets’ for optimum health. For example, the purported health-giving properties of plant polyphenols represent a case in which enthusiastic marketing claims may far exceed the current scientific evidence. Even when good experimental evidence exists, results need to be interpreted with caution in relation to human health benefits, as polyphenols may have limited bioavailability and may also be extensively metabolized. In addition, some polyphenols can be toxic and mutagenic in some cell culture systems. Until more is known about the activity and metabolic fate of polyphenols in the body, it would be better for the consumer to increase fruit and vegetable intake, and also to be wary of claims that these compounds are a panacea for good health.

The application of increasingly sophisticated methods of structural analysis by spectroscopy, isotopic labeling, automated quantitative analysis and separation by chromatography and other partition procedures opened avenues for study of minute amounts of biochemical. Early advances in medicinal chemistry were concerned principally with the estimation, isolation, structural determination and synthesis of medicinal agents of natural origin.
The work embodied in this thesis, includes detailed chemical investigation of the plant Rhinacanthus species i.e. the roots of R. nasutus, stem of R. communis and chemical investigation of roots of plant Vernonia cinerea. A brief review of the commercial importance of these plants and past work reported from these plants is described as below:

**Rhinacanthus**

Rhinacanthus plant belongs to the family Acanthaceae, This genus known to have about 22 species in all over the world. It is a native from Africa to Asia. Some species of this genus are also found in bundelkhand region. It is a perennial herb. Leaves of this genus are opposite and petiolate. A leaf like part (bract) just below is invisible, lanceolate. Arrangement of flowers (inflorescence) is pedunculate, that is growing on a stalk, this is usually terminal, but sometimes axillary. Fruits of the Rhinacanthus genus are shaped in the form of a club (clavate) that is larger at one end. Seeds are black, rough and tuberculate that is covered with tubercles.

**Rhinacanthus nasutus**

- **Hindi** Palakjuhi
- **Sanskrit** Yuthikaparni
- **English** Snake jasmine

The plant Rhinacanthus nasutus belong to the family Acanthaceae. It is found throughout India on road sides.

It is a small shrub, 2-3 ft. tall. Its trunk is edge shape. The twigs and young leaves are covered with hair. The shape looks like egg. The end of leaves are sharp and long 6” to 8”. The twigs are short. The blossoms bloom in bunch at the lane of twigs. The white blossom bloom is 1” with hair covered. Its taste is toxic. Its leaf is used in making of herbal tea which lowers the blood pressure. In India some species of this genus are
used as skin affections. Rhinacanthus scoparius is used in the treatment of skin disease, Oedema⁹,¹⁰. The roots of Rhinacanthus gracilis are believed in some parts of India to be an antidote to the bites of poisonous snakes¹¹.

**Rhinacanthus communis**

- **Hindi** Palakyuti
- **Sanskrit** Nagathien

The plant Rhinacanthus communis belong to the family Acanthaceae, It is an undershrub and about 2-4 ft in height with spreading stem and branches. Its leaves are simple, opposite, elliptic and nearly glabrous. Flowers are purple, solitary or 2-3 together on the divaricated branches. Fruits are narrow, pointed, velvety pubescent, 4-seeded capsules. Seeds are tuberculate, glabrous, and black colored. Its seeds give strength to hair of the eye – brow¹².

**Anthraquinone in Rhinacanthus genus**

Anthraquinone are the largest group of naturally occurring quinones. Both natural and synthetic anthraquinone have been widely used as colorants in food, drugs, cosmetics, hair dyes and textiles¹³,¹⁴. 1-hydroxy anthraquinone can be used as an intermediate in the production of dyes and drugs¹⁵.

Many species of Rhinacanthus plant have been reported to contain triterpenoids, steroids, coumarin, anthraquinone, glycoside, rhinacanthine & rhinacanthone.

**Past work**

A literature survey shows that some species of genus Rhinacanthus have been chemically examined and various types of compounds of structural significance and medicinal importance isolated from different parts of genus Rhinacanthus¹⁶.
Some species of Rhinacanthus have been used in Thai Folk remedy for treatment of various diseases including cancer. It was found that as ethanolic extract of R. nasuta exhibited potent dose dependent anti-fungal activity against Candida albicans and Trichophyton mentagrophytes along with substantial anti-fungal activity against all the fungal strains tested. An anti-bacterial activity of the plant is also observed against gram-positive bacteria, however, was ineffective against gram-negative bacteria used in this study\textsuperscript{17}.

**Present work**

In the present work four new compounds were isolated from the roots of Rhinacanthus nasutus and two compounds were isolated from stem of Rhinacanthus communis and all these have been characterized as-

**Rhinacanthus nasutus**

1) 1, 4-dihydroxy-6-methyl anthraquinone.

2) (2(6′-hydro-7′hydroxygeranyl)-6-methoxyl-1, 3, 8 -trihydroxy anthraquinone.

3) (1-hydroxy-2-formyl-anthraquinone-3-o-\(\alpha\)-L- rhamnosyl(1→2)-0-\(\beta\)-D- glucopyranoside.

4) [{\(\alpha\)-L-arabinopyranosyl(1→3)} –{ \(\beta\)-D-galactopyranosyl(1→6}) – \(\beta\)-D- galactopyranosyl (1→3)]-3\(\beta\)-19\(\alpha\)-hydroxy-Urs -12 ene 24, 28 - dioate.

**Rhinacanthus communis**

1) Lupene - 20 (29) ene - 3 - ol

2) (19\(\alpha\), 24 - dihydroxy – urs – 12 - ene, 28 - oic acid – 3 – 0 – \(\beta\) - D-xylopyranoside
Importance of quinones

Quinones as oxidants and dehydrating agents:

Quinones have found extensive use as dehydrating agents in steroid chemistry.

The functional significance of quinones

The most important reaction of quinone as far as biology is concerned is their reversible reduction to the corresponding hydroquinone.

(a) The quinones that are primary metabolites:

1) In the photosynthesis of Eucaryotes.

2) In the photosynthesis of Procaryotes.

3) In the respiration of Eucaryotes.

4) In the respiration of Procaryotes.

(b) The quinones that are secondary metabolites:

It is apparent that quinones play a variety of roles in our overall life cycle and that interest in their biological function has stimulated basic chemical research in several areas. The use of quinones, in fact, dates to antiquity and the recorded and verifiable history of these compounds is perhaps longer than any other group of naturally occurring compounds. Quinones come to man's attention in two ways - firstly as pigments and secondly as drugs.

As drugs, Rhubarb, which contains various anthraquinones, is described in the Chinese herbal, pen-king, believed to date from 2700 B.C. The use of senna was introduced by the Arabs, who described its properties as early as the 9th century.

As pigments two materials stand out, henna and madder. The active principle in henna is 2-hydroxynaphthquinone (Lawsone).
Madder contains the anthraquinone alizarin. Madder was also as a drug (to treat amenorrhea) by the ancient and in the middle ages. Arab still make a Sherbat drink of madder which protect against the “evil eye”. Madder was a major commercial dye stuff of considerable importance until the present century.

Vernonia

Vernonia is a genus of about 1000 species of forbs and shrubs in the family Asteraceae. Species of this genus are found in South America, Africa, s.e. Asia, and North America. Some species are known as Ironweed. Some species are edible and of economic value. They are known for having intense purple flowers. The genus is named for English botanist William Vernon. There are numerous distinct subgenera and subsections in this genus. This has led some botanists to divide this large genus into smaller groups which separate the species into distinct genera\(^{18}\).

Vernonia cineria

- **Common name** Little ironweed, Purple feabane

- **Hindi** Sahadevi

Vernonia cineria belongs to the subfamily Cichorioideae of family Asteraceae. Although vernonia cineria is under cultivation in many parts of India but in Chhattisgarh fortunately it occurs as wasteland herb. Little ironweed is an annual or short-lived perennial to 50cm with ovate leaves. Purple floweres are locate at the top of the stem branch. Flowers appear throughout the year. This species of Vernonia also found on roadsides throughout India\(^{20}\).

Vernonia species are used as food plants by the larvae of some Lepidoptera species. V. amygdalina is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDS (an infusion of the plant is taken as needed).
TRITERPENOIDS

The term “triterpenoids” refers to a group of natural products containing thirty carbon atoms based on six isoprene units. Since several substances which contain more or less than thirty carbon atoms and also those which do not strictly follow the isoprene rule have been isolated and characterized as triterpenoids during the recent years.

The invention of highly sophisticated instrumental techniques and the contemporary developments in the biogenetic theories have been mainly responsible for the isolation and identification of new unconventional type of triterpenoids.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves and ginger, and the color of yellow flowers.

They form a large group of natural substances which includes steroids and consequently sterols. Squalene is the immediate biological precursor of all triterpenoids.

![Squalene](image)

The large group of steroids including sterols is present in very small amounts in bacteria but at larger amounts in plant and animals while the hopanoids are very abundant in prokaryotes where they replace cholesterol.
Among the large number of triterpenoid structures, some of them are shown below.

Lanosterol

Cycloartenol

Cephalosporin

Cyclohexine
Structure – Activity Correlation

Relationship between chemical structure and homolytic action and also surface activity of five oleanolic acid glucoside (1 Chart -1) has been established$^{24}$.

It was shown that monodesmosidic saponins were more homolytic than bidessmosidic ones. The homolytic activity of monodesmosidic side chain decreases with the length and the branching of glucoside chain. Surface activity has been found to be major in bidessmosides and it decreases with the length of the glucoside chain.
Anticancer activity of nine triterpenoid derivatives of human cancer cells were reported by Ling et al. Among the pentacyclic triterpenoids epimaniladiol (2, Scheme -1) was cytotoxic at 100 jig/ml. The presence of a 16-\(\alpha\)- and free 3 - OH has been found to be essential for cytotoxicity.

Structure activity studies of some anti-inflammatory oleanane triterpenoid glycosides and related compounds (obtained from the leaves of Tetrapanox papyriferum) reported by Ogihara et al\textsuperscript{25} deserves special mention here as they have identified the conformational requirement for anti-inflammatory activity in such compound.

Two of the compounds tested, viz.; papyriogenin A and C (3 & 4 Chart-1) manifested in these tests almost the same potency as prednisolone. The structure-activity correlation was explored by taking into account the variation in the oxygen function and the molecular conformation. It was found that anti-inflammatory activity of these compounds was favored when the molecules tended to take a planer conformation.
BIOACTIVE OLEAN - 12 - ENE DERIVATIVE

1. $R_1$ or $R_2 = H$ or $\beta-D$-glucopyranosyl
   $R = H$ or $\alpha-D$-glucopyranosyl

2. 

3. $R = O$

4. $R = OH$
Physical techniques used for structure elucidation of Triterpenoids

Infrared Spectroscopy (IR)

IR spectroscopy is an important technique which is frequently used for the structure determination of triterpenes. IR spectroscopy has been used as an important tool for the identification of functional groups –OH, O acetyl, carboxyl, carbomethoxy1 and carbonyl with conjugated unsaturation present in different classes of triterpenes \(^{26-32}\).

Tschesche et al.\(^{33}\) have utilized IR spectroscopy to differentiate ursane and oleanane series by talking the spectra of triterpenic acid and their derivative in pyridine. Differentiable spectra were observed in two characteristic regions:

1) Region A - have bands between 1392-1335 cm\(^{-1}\)

2) Region B - have bands between 1330-1245 cm\(^{-1}\)

Number of oleanane series has got two bands in 1392-1379 and 1370-1335 cm\(^{-1}\) in region A and three bands of increasing intensity at 1330-1315; 1306-1299 and 1269-1250 cm\(^{-1}\) in region B. On the other hand the number of ursane series have three bands in both of the two regions in between 1392-1386, 1383-1370, 1364-1359 cm\(^{-1}\) in region A the increasing intensity in region 1312-1308, 1276-1270 and 1250-1245 cm\(^{-1}\).

Tetracyclic triterpenic acids and their esters show only two strong absorptions in these two regions. Acids which do not belong to the type mentioned above possess their intensive bands outside the described limit.

In case of neutral triterpenes, the characteristic absorption in region -B have been found missing and the assignments are based on the absorption in region –A for differentiating \(\alpha\)-amyrrin from \(\beta\)-amyrrin and lupeol. IR spectroscopy has been used for differentiating primary, secondary and tertiary axial and equatorial groups.
\textbf{\textsuperscript{1}H NMR}

NMR spectroscopy technique is frequently used for the structure elucidation of triterpene sapogenins\textsuperscript{26-35}.

In the \textsuperscript{1}H NMR spectra of pentacyclic triterpenes sharp absorptions are found due to methyl esters and acetoxy groups. Well defined absorption due to the presence of angular methyl groups are seen in the $\delta$ 0.82 – 1.13 ppm region. Certain other functional groups such as olefinic protons have low and diffused absorptions in the region $\delta$ 5.66 – 5.44 ppm, $\delta$ 3.8 – 4.52 ppm respectively. Even so their absorptions are helpful for determining certain structural features of the triterpenes\textsuperscript{35,36}.

\textsuperscript{1}H NMR spectra series of pentacyclic triterpene were studied and some important correlations between the spectra and their structures were made by Shmna\textsuperscript{37}. It was noted that the chemical shift of highest (most shielded) methyl group is partially indicative of the position of carbomethoxy group in triterpenes of ursane or olenane series, when carbomethoxy is present at C-8 position. The position peaks appear up field from $\delta$ 0.775 ppm. The proton of the normal trisubstituted double bond in $\alpha$ – and $\beta$ – amyrin series absorbs in the region between $\delta$ 4.93 and 5.93 ppm. If the double bond is conjugated with carbonyl function at C – 11, the vinylic proton is found to absorb at low field at $\delta$ – 5.55 ppm and well defined absorption vinyl group appears from $\delta$ 0.625 to 1.5 ppm Acetoxyl protons appear between $\delta$ 1.82 – 2.09 ppm\textsuperscript{80}. Other protons present at position C – 3 , C – 12 , C – 15 , C – 16 , C – 22 , C – 28 appears in the range of $\delta$ 3.75 to 5.62 ppm The shifting of the peak in downfield region indicates the presence of some electronegative group surrounding it. \textsuperscript{1}H NMR has also been used for the study of conformational equilibrium at varying temperatures. The \textsuperscript{1}H NMR spectra of acetic acid\textsuperscript{38} and polygallic acid\textsuperscript{39} assisted in the assignment of the conformation of ring and conformation of hydroxyl group.

\textbf{\textsuperscript{13}C NMR Spectra}
$^{13}$C NMR spectroscopy has solved many problems of structure elucidation and biosynthesis of triterpenoids$^{40-43}$. Identification of saponins which consist of the acid unstable dammarane-type sapogenin has been done excellently with the help of $^{13}$C NMR$^{44}$. The $^{13}$C NMR signals of olean – 12 – ene in CDCl$_3$ have been reported$^{45}$. The hydroxylations effects on 13C chemical shift reflect have been found useful in structural studies of all triterpenoids.

The $^{13}$C signals of oleanane type triterpenes were assigned using known chemical shift rule$^{46}$ such as hydroxyl substituent shift$^{47}$ acetylation shift$^{48}$, steric$^{49}$ γ and δ$^{50}$ effect by chemical shift comparison with previously reported data$^{40-43}$.

**Mass spectroscopy**

A detailed study of the mass spectral fragmentation patterns of pentacyclic triterpene by noting peak shift in various derivatives has been described$^{51,52}$. This has led to important generalizations, particularly in Δ$^{12}$ compounds and has been extremely useful in structural elucidation studies of Δ$^{12}$-olenane and Δ$^{12}$ ursane derivative$^{52,53}$. The mass spectrum of Δ$^{12}$-olenane and Δ$^{12}$-ursane are very similar, giving rise to fragments at identical m/z values. The only difference is that in mass spectrum of Δ$^{12}$-olenane, the fragment ion at m/z 203 is more intense than the peak at m/z 191 while reverse is true in mass of Δ$^{12}$-ursane.

**Peak M-153**

This peak represents the most abundant fragment ion above m/z 218. The mechanism involves homolytic cleavage of 9-10 bond in molecular ion (a) to afford (b), followed by hydrogen transfer from C-26 to C-27 with concomitant homolysis of the 7-8 bond to afford resonance stabilized species (C) (m/z 257(Chart : 2)).
Peak M-192

The intense and diagnostically important m/z peak (M-192) has been discussed in considerable detail. The ion at m/z 218 is formed by retro - Diel's - Alder decomposition (a → d).

Peak M-207

The fragment ion at m/z 203 in the mass spectra of Δ^{12}-olenane and Δ^{12}-ursane proved to be more interesting peak from a mechanistic stand point. This moiety resulted from further loss of 15 mass units from the retro – Diels – Alder fragment (d). It was found that m/z 203 ion result from the loss of the methyl, substituents at C-17 or C-20 from (d) (d → f and d → g) (Chart: 2).
CHART 2

MASS FRAGMENTATION PATTERN OF Δ^{12} OLEINANES

a \rightarrow b \rightarrow c \quad m/z \ 257

a \rightarrow d \quad m/z \ 218

-dCH_{3}\rightarrow i \quad m/z \ 203

-dCH_{3}\rightarrow a \quad m/z \ 203
Lupane derivatives

This series is characterized by contraction of ring E to a five membered to which an isopropyl or isopropenyl group is attached. The loss of 43 mass units C₃H₇ is very pronounced in certain members, but becomes minimal in highly substituted derivatives or in the presence of an isopropenyl function.

Saturated Lupane

Lupane-3-one (1a) exhibit loss of methyl (m/z 411) and isopropyl (m/z 383). The most abundant fragment occurs at m/z 205 corresponding to species hopane observed to some extent in the spectra of all the pentacyclic triterpenes. A peak at m/z 191 and 189 is also observed in Lupane-3-one.

\[
\begin{align*}
  \text{a } & R_1 = O \quad R_2 = \text{CH}_3 \\
  \text{b } & R_1 = \text{H} \quad R_2 = \text{CH}_3 \\
  \text{c } & R_1 = (\text{H})\text{OAc} \quad R_2 = \text{CH}_2\text{OAc}
\end{align*}
\]
\textbf{A^{12} Lupanes}

Member of this series (2) exhibited mass fragments at m/z 216 due to retro – Diels – Alder decomposition of ring C. The most characteristic peaks occur at m/z 187, 189, 201, and both 12, 13- dihydro-lupenone (2a) and the corresponding 3-acetate (2b) while in 12, 13 – dihydro-20, 30 dehydro-lupenol (2c) peaks at m/z 189, 191 and 204 are noticed.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
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<tbody>
<tr>
<td>a O</td>
<td>Me</td>
<td>C₃H₅</td>
</tr>
<tr>
<td>b (H) OAc</td>
<td>Me</td>
<td>C₃H₅</td>
</tr>
<tr>
<td>c (H) OH</td>
<td>Me</td>
<td>C₂H₅</td>
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</tbody>
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\textbf{An important tool for the Structure Elucidation of the Glycone part – Physical Techniques}

\textbf{1H NMR}

\textsuperscript{1}H NMR clearly establishes the nature (α, β) of linkage of the glycosidic point of the saponins. The anomeric protons of various sugars show peak in the region between 4.0-6.30 ppm. D-sugars generally have β-linkages with high coupling constants (J=6-9
H₂) whereas rarely occurring α-linkages of D-monosaccharides have low values (J=2-9 H₂). The coupling constants of the commonly α-linked L-rhamnose (J=2 H₂) and α-arabinose (J=6-8 H₂) distinguishes these sugars from each other as well as other sugars.

13C NMR

13C NMR spectra have been found to be useful in the structure elucidation of glycone part of saponins in two ways.

a. The type of linkage at glycosidic points can be confirmed.

b. The exact position of linkages in the glycone part is established.

The chemical shifts values of some methyl monosaccharides are known. The type of linkage at glycosidic points can be determined by comparing the chemical shift values observed, with the reported values for the methyl pyranoside. It has been found that 13C NMR signals shift in the change from aglycone alcohol and pyranose into glycopyranoside, that is glycosidation shift.

There are characteristic of chemical and steric environments of the hydroxyl group in which the glycosidation takes place. This discovery has become important and useful for determining the glycosidation position in an aglycone moiety and the kinds and sequence of sugar moiety present in a natural glycoside without chemical degradation as well as assigning 13C NMR signals of glycoside.
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