CHAPTER - 1

INTRODUCTION

AN ACCOUNT OF ANTIVIRAL ACTIVITY, THE PLANT UNDER INVESTIGATION, SOME RECENTLY ISOLATED AND CHARACTERIZED STEROIDAL AND TRITERPENOIDAL SAPONINS, BIOLOGICAL IMPORTANCE AND STRUCTURE RELATIONSHIP OF SAPONINS WITH ANTIVIRAL ACTIVITY. MODERN SEPARATION AND SPECTRAL TECHNIQUES AND THEIR APPLICATIONS IN IDENTIFICATION. ACTUAL WORKDONE AND PLAN AND REFERENCES.
Self-reliance is the key word in all spheres of national endeavour. Our country suffers from a grave shortage of primary health care centers. Medical benefits are still lacking to our vast unmanageable population, due to infrastructure constraints which are costly. As we are richly endowed with flora and fauna, it is fortunate that an increasing number of people even in urban areas are reverting to traditional medicines like Ayurveda with beneficial results. The old materia medica was rich in drugs of vegetable origin. The active principles extracted from roots, leaves and bark of various plants are used even today in modern medicine.

Diseases known to be caused by virus were recognised for about thousand of years. Small pox are in records dated about 2500 B.C. in the Chinese medicinal descriptions Paracelsus (1493-1541 A.D.) introduced mixed vegetable preparation for the treatment of syphilis.

In 1892 Dimitrii Ivanowski\(^1\) first conclusively proved that the causative agent of tobacco mosaic disease was a viral entity that was filterable through pores of filters which do not permit the passage of bacteria. He named it as Contagium vivum fluidum. Another milestone in virology was the use of electron microscope after 1930 that revealed the shape, size as well as some of the internal structure of virion. One of the most important scientific contribution to the field of virology was of Wendell Stanley (1935)\(^2\) when he crystallised tobacco mosaic virus (TMV) for which he shared the 1946 Noble prize in Chemistry.

By means of electron microscope, X-ray diffraction, and other studies we have learned that the virion is composed of nucleic acid, which gives it infective capability. It is a known established fact that viruses
have no capacity for independent metabolism or motility. They reproduce by replication in a host cell and are capable of mutation.

A remarkable development in the understanding of viruses was the cultivation of viruses in the laboratory. The first method was the Chick-embryo technique. The embryo technique has been used for the study purpose and for the production of virus for vaccines against small pox, yellow fever and other diseases. A significant advancement in this field was the Tissue culture technique. This technique began with the cultivation of a minced chick embryo serum. Tissue culture techniques are most widely used methods for isolating and propagating viruses from clinical material and an extension to this approach made it possible to culture it for study and for commercial production of vaccines.

Virology as a distinct branch of pathology evolved with the growth of knowledge about bacterial viruses, also called bacteriophages. Many recent advances in virology have come from research on this parasitism of the single celled bacterial host. Knowledge of bacteriophages provide a convenient route to the study of viral structure, metabolism, genetics, dynamics, Infectivity and other activities.

The evolution of molecular biology as a disciple then further boosted the advances in the field of virology. Its study is concerned with the form, structure, reproduction, physiology, metabolism of microorganism. Virology thus became an integral part of molecular biology as viruses are morphologically placed with the domain of subcellular entities.

Antiviral chemotherapy presents special and difficult problems in chemotherapy. Major problems confronting effective antiviral therapy are-
1. First, since viral replication is intracellular, antiviral drugs must be highly selective, and should not interfere in the metabolic process of the host cells.

2. Second, most viral diseases are of a short duration and the predormal phase of viral growth is asymptomatic.

3. Third, relative lack of intrinsic virus enzyme system that may serve as specific target for drugs.

Plant virus inhibitors\(^3\) has been reported in various parts of several plants. Ragetli and Weinturb in 1962 isolated, purified and characterised first inhibitor from carnation and thereafter enormous plants have been screened for their antiviral activity and it was recognised that the occurrence of potent and active ingredients in them may serve as antiviral principles. At present no drugs are active against the free virus particle. The remedies for the clinical spectrum of viral infection are vaccines. Viral nucleic acid polymerase are the main target of antiviral drugs.

Spondias pinnata Kurz. syn. Spondias mangifera Willd\(^4\) is commonly known as Amra in Hindi, Wild Mango, Indian Hog Plum in English and belongs to natural order Anacardiaceae.

It is glabrous deciduous tree\(^5,6\) of 10m height with straight trunk. The leaves are imparipinnate, smooth, 25-50 cm in length with leaflets in pairs and a single occupying terminal position, generally oblong, acuminate with numerous straight nerves connected by intramarginal nerve. The flowers are whitish, 1-2 sexual, numerous in sparingly branched panicles of 30 cm length with ovate-oblong petals, disk 10 crenate, 10 stamens. The drupes are yellow-light green, ovoid of 4-5 cm length. The seed is one, oblong-elliptic. The bark is gray, smooth and thick. The stone is woody with cavities and furrows, hard. The sapwood is
grayish-white, the heartwood is gray. The wood is straight-grained, light
with coarse-texture, used for match splints, packing cases, canoes and as a
fuel with calorific value 4,067 cal. The fruit has nutritive value and used
as vegetable.

The plant is found almost throughout in India\(^6\) and extends upto
Sub- Himalayan valleys\(^4\) (3,000 ft) from Chenab-Eastwards. It is also
found in Andamans. The conditions in shade for its survival are 38 to 48\(^0\)
(maximum temp), -1 to 16\(^0\) (absolute minimum temp), 75-400 cm rainfall.
The stone germinates in rains. It could be artificially propagated via
cuttings or seeds (sowed in September). It takes about five years for fruit
to ripen (It occurs in October-November).

The whole plant is precious for inherent medicinal\(^4,5,6\) properties. It
has been known by grand-parents/ancestors to be in use for various
diseases and sufferings from the era of Ayurvedas.

The plant possess antitubercular properties. The fruit is astringent,
refrigerant, antiscorbutic, tonic and is used in bilious dyspepsia, against
poisonous arrows (as an antidote). The fruit is also used in ulcers,
burning sensations, phthisis and blood complaints (Ayurvedas).

The leaves are, astringent and acidic. Juice of leaves are applied in
earache. The bark is astringent, refrigerant and is used as constituent in
remedies against the snake-bite (Bapat) also used in gonorrhoea, vomiting,
dysentery, diarrhoea. The bark-paste is applied in rheumatism. The root is
useful in regulating menstruation.

Out of Ninety three plants checked by Rajendra Singh and Raghuraj
Singh for anativiral properties\(^7\) of their bark extracts, eleven plants were
found to inhibit Potato virus X growth fully. Among eleven plants viz.
Acacia arabica Willd, Aegle marmelos (L) Corr., Cassia fistula L.,
Casurina aquisetifolia Forst, Delonix regia Raf., Spondias pinnata (L) Kurz is also one of them which inhibited PVX multiplication 100%.

Reports are also available in Journals dealing with Organic Natural products whereby the extracts from leaves, flowers, foliage, barks of numerous plants produces virus inhibitory agents.

All this therefore initiated interest to authorese to investigate roots of Spondias mangifera Willd syn. Spondias pinnata(L) Kurz as a potent antiviral agent source.

Hederagenin, 18-β glycyrrhetic acid, oleanolic acid, Ursolic acid are triterpenoids which are most actively studied triterpenoidal saponin in terms of R.N.A. and D.N.A viruses abolition and so for no direct relationship could be established between number and nature of sugar moiety with respect to viruses abolition but it was found that triterpenoidal glycosides generally show higher antiviral activity in comparison to their sapogenins.

On common structure basis of aglycones, groups which are responsible for antiviral activity among triterpenoidal saponins are given here in table.

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tr>
<td><strong>Aglycones</strong></td>
</tr>
<tr>
<td>Hederagenin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>18 β glycyrrhetic acid</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Oleanolic acid</td>
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<td></td>
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<tr>
<td>Ursolic acid</td>
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</table>
Studies made by Simos et al. showed tormentic acid ester glucoside and oleanolic acid glucoside possess strong action against HSV-1 and poliovirus.

Various carbohydrates and phytosteroidal components which have been isolated by previous workers from the plant Spondias mangifera Willd includes 24 methylene cyclo artanone, stigma-4-en-3-one, β-sitosterol, β-sitosterol-β-D-glucoside, β-amyrin, glucose, sucrose, glucouranic acid, xylose etc. from various parts of plant.

Thus studies initiated to reveal saponins from plant Spondias mangifera Willd which may potentially possess antiviral properties.

Saponins generally occur naturally as plant glycosides and is difficult to purify because of their high molecular weight, characterized by their ability to form foams in water. These are highly toxic as their aqueous solutions have the property to destroy RBCs when injected intravenously into animals but when ingested are comparatively harmless.

Saponin possess property of marked activity in lowering surface tension and their toxicity appears to be related to this property and therefore are highly toxic to cold blooded animals also.

Saponins are powerful emulsifier, used as detergents and they have derived their name because of this particular property. Some natural products may produce lather with water but exhibit other properties which separate them from saponins. (e.g. cardiac glycosides; having different property and “specific biological activity”)

An important characteristic of saponins is formation of molecular compounds with cholesterol and other 3-β hydroxy steroids and therefore can be used for isolation of saponins as well as for separation and purification of 3-β hydroxy steroids, mostly digitonin is used for forming
steroid complexes of high insolubility, for purification its 1:1 mol compound with amyl alcohol may be utilised, and its complexes with ($\pm$)-ac-tetrahydro-\(\beta\)-naphthol and ($\pm$) terpineol\(^{14}\) have been utilised for resolution of these substances.

The aglycone part of saponins are known as sapogenin. By hydrolysis with acid or enzymes saponins yield \(C_{27}\) sapogenins and various sugars. Sugars have been considered as united in a chain with the terminal unit attached to a -OH group of the sapogenin. e.g. 
\[
\text{dioscorea sapotoxin} = \text{diosgenin- glucoside rhamnoside}
\]
and this type of view has been confirmed\(^{15}\).

In general saponins have been reviewed in certain published works\(^{16-22}\). Saponins are of three types described as follows:

1. **STEROIDAL SAPONIN [S s]**:

   These are saponins in which aglycone is generally 5\(\alpha\) or 5\(\beta\) spirostanol or its modification. The first indication of the steroid structure of the sapogenins was the isolation of 3'-methyl 1,2 cyclopenteno phenanthrene from sarasapogenin and gitogenin by dehydrogenation with selenium\(^{23}\).

   The steroid nucleus present in plant kingdom as glycosides in which normally the sugars are attached to 3 hydroxy groups are called steroidal saponins. These saponins mostly possess spiroketal side chain. Some of the better characterised known natural saponins are amol unin, digitonin, dioscorea sapotoxin, gitonin, kammonin, sarasaponin, tigonin and yuccin.

   In brief steroid saponins have been described by Hibnum\(^{24}\), Elks\(^{25,26}\), Takeda\(^{27}\) in their reviews.
Example of few well established steroid saponins\textsuperscript{28-43} have been presented in Table II. Some recently studied saponins of this class are described in several standard works\textsuperscript{44-49} as given in a Table III.
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<th>Plant</th>
<th>Structure</th>
<th>Figure</th>
<th>Reference</th>
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<tr>
<td>Funkioside B</td>
<td>Funkia ovata</td>
<td>Diosgenin glc-(3β-OH); glc-(26-OH)</td>
<td>I</td>
<td>28</td>
</tr>
<tr>
<td>258-266°, -135°(MeOH)</td>
<td></td>
<td></td>
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<tr>
<td>Ophiopogonin C</td>
<td>Ophiopogon japonicus</td>
<td>Disogenin (4-O-acetyl)rha&lt;sup&gt;2&lt;/sup&gt;glc-(3β-OH) xyl&lt;sup&gt;2&lt;/sup&gt;glc-(3β-OH)&lt;br&gt;</td>
<td>II</td>
<td>29</td>
</tr>
<tr>
<td>238-240°(dec), -99° (pyridine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epiruscogenin glucoside</td>
<td>Senshokushichiken</td>
<td>Epiruscogenin glc-(3α-OH)</td>
<td>III</td>
<td>30</td>
</tr>
<tr>
<td>Floribumcasaponin B</td>
<td>Dioscorea floribunda</td>
<td>Pennogenin rha&lt;sup&gt;4&lt;/sup&gt;glc-(3β-OH)</td>
<td>IV</td>
<td>31</td>
</tr>
<tr>
<td>251-253°(dec), -86.5°(pyridine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lanatigonin I,</td>
<td>Digitalis lanata</td>
<td>Tigogenin glc&lt;sup&gt;3&lt;/sup&gt;gal&lt;sup&gt;2&lt;/sup&gt;glc&lt;sup&gt;-3&lt;/sup&gt;gal-(3β-OH) xyl&lt;sup&gt;2&lt;/sup&gt;glc&lt;sup&gt;-4&lt;/sup&gt;gal-(3β-OH)&lt;br&gt;</td>
<td>V</td>
<td>32</td>
</tr>
<tr>
<td>275-285°(dec), +42°(pyridine)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Paniculonin A,</td>
<td>Solanum paniculatum</td>
<td>Paniculogenin xyl-3qui-(6α-OH) Gitogenin, glc&lt;sup&gt;-3&lt;/sup&gt;gal&lt;sup&gt;2&lt;/sup&gt;glc&lt;sup&gt;-4&lt;/sup&gt;gal-(3β-OH); glc-(26-OH) xyl&lt;sup&gt;2&lt;/sup&gt;glc&lt;sup&gt;-4&lt;/sup&gt;gal-(3β-OH); glc-(26-OH)&lt;br&gt;</td>
<td>VI</td>
<td>33</td>
</tr>
<tr>
<td>262-264°,-61° (pyridine)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hispinin C</td>
<td>Solanum hispidum</td>
<td>Neochlorogenin rha&lt;sup&gt;-3&lt;/sup&gt;rha-(6α-OH)</td>
<td>VIII</td>
<td>35</td>
</tr>
<tr>
<td>285-288°,-59°(pyridine)</td>
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</tr>
<tr>
<td>Saponin (mp[α]D)</td>
<td>Plant</td>
<td>Structure</td>
<td>Figure</td>
<td>Reference</td>
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<tr>
<td>Desgluco-deshrhamnoparillin 250-265⁰, -65.5⁰ (CHCl₃ + MeOH)</td>
<td>Smilax aristolochiaefolia</td>
<td>Sarsasapogenin glc-⁶glc-(3β-OH)</td>
<td>X</td>
<td>37</td>
</tr>
<tr>
<td>Convallasaponin B 273-274⁰, -56⁰(CHCl₃+MeOH)</td>
<td>Convallaris keisukei</td>
<td>Convallagenin B ara-(5β-OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prototokorin 177-178⁰, -3.8⁰ (MeOH)</td>
<td>Dioscorea tokoro</td>
<td>Tokorogenin glc-(1β-OH); glc-(26-OH)</td>
<td>XI</td>
<td>38</td>
</tr>
<tr>
<td>Glycoside I, 300-303⁰</td>
<td>Methanartheicum luteo-viride</td>
<td>Epimetagenin ara-(11α-OH)</td>
<td>XII</td>
<td>39</td>
</tr>
<tr>
<td>Avenacoside A</td>
<td>Avena sativa</td>
<td>Nuatigenin rha</td>
<td>XIII</td>
<td>40</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Convalamaronin 215-218°, -30°(MeOH)</td>
<td>Convallaria majalis</td>
<td>Convalamaronogenin rha-(3β-OH); rha-²qui-(1β-OH)</td>
<td>XIV</td>
<td>41</td>
</tr>
<tr>
<td>Cryptogenin glycoside 238-241⁰ (dec), -125⁰ (EtOH)</td>
<td>Paris tetraphylla</td>
<td>Cryptogenin glc-(3β-OH)</td>
<td>XV</td>
<td>42</td>
</tr>
<tr>
<td>Trillenoside A 209-220⁰ (dec), -142⁰</td>
<td>Trillium kamtschaticum</td>
<td>Trillenogenin apio(fur)-³rha</td>
<td>XVI</td>
<td>43</td>
</tr>
</tbody>
</table>
I 25R, 3β-O-Glu, 26-O-Glu  
II 25R, 3β-O-Sugars  
III 25R, 1β, 3α-O-Glu  
IV 25R, 3β-O-Sugars, 17α-OH  

V 25R, H₁ = Sugars  
VI 25S, 23-B-OH, 6α-O-Sugars  
VII 25R, 2α-OH, H₁ = Sugars, 26-O-Glu  
VIII 25S, 6α-O-Sugars  

IX 25S, 3β-O-Sugars  
X 25S, 1β, 3β, 4β-OH, 5β-O-Ara  
XI 25R, 2β, 3α-OH, 1β-O-Glu, 26-O-Glu  
XII 25R, 2β, 3α-OH, 11α-O-Ara  

XIII $R₁ = $ Sugars, $R₂ = $ -Glu  
XIV $R₁ = $ 3β-Rha, $R₂ = $ Sugars  

XV $R = $ 3β-Glu  
XVI $R = $ Sugars
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant And Family</th>
<th>Saponins</th>
<th>Isolated Compounds</th>
<th>Figure</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Polygonatum sibiricum</td>
<td>Sibiricoside A</td>
<td>26-O-β-D-glu-22-O-methyl-25(s)furost-5-ene-3β,26 diol 3-O-β-lycotetraside</td>
<td>XVII</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>(N.O.Liliaceae)</td>
<td>Sibiricoside B</td>
<td>(23S,25R)-Spirost-5-ene-3β,14α,23-triol 3-O-β-lycotetraside</td>
<td>XVIII</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Balanites aegyptica</td>
<td>Yamogenin 1</td>
<td>Yamogenin 3β-O-β-D glu-(1→3)- β-D glu-(1→4)-[α-L-rha- (1→2)]-β-D-glucopyranoside</td>
<td>XIX</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>(N.O. Balanitaceae)</td>
<td>Yamogenin 2</td>
<td>Yamogenin 3-β-O-α-L rha-(1→3)-β-D glu-(1→4)-[α-L-rha-(1→2)]-β-D-glucopyranoside</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yamogenin 3</td>
<td>Yamogenin 3-β-O-β-D-glu-(1→4)-[α-L rha-(1→2)]-β-D glucopyranoside</td>
<td>XXI</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Yucca aloifolia</td>
<td>Gitogenin glycoside</td>
<td>3-O-[β-D-xyl(1→3)-β-D-glu-(1→3)-β-D-xyl(1→3)-β-D-glu-(1→3)-β-D-glu- (1→3)]β-D-glu-25 R, 5α-spirostan-2α,3β-diol</td>
<td>XXII</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>(N.O. Agavaceae)</td>
<td></td>
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<tr>
<td>4.</td>
<td>Anemarrhena asphodeloides</td>
<td>pseudoprototimo-saponin A III</td>
<td>a furostenol glycoside</td>
<td>XXIII</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(N.O. Liliaceae)</td>
<td></td>
<td></td>
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<tr>
<td>5.</td>
<td>Chamaedorea linearis</td>
<td>glycoside 1</td>
<td>1-O-[β-L fucopyranosyl-(4’ sulfate)]-25 R, 5α-spirostan-1β, 3β-diol.</td>
<td>XXIV</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glycoside 2</td>
<td>1-O-[β-L fucopyranosyl-(4’ sulfate)]-25 R, 5α-spirostan-1α, 3β-diol.</td>
<td>XXV</td>
<td></td>
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<tr>
<td>6.</td>
<td>Vernonia amygdalina</td>
<td>Vernonoioside D</td>
<td>Stigmastane type steroid glycosides</td>
<td>XXVI</td>
<td>49</td>
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<tr>
<td></td>
<td>(N.O. Compositae)</td>
<td>Vernonoioside E</td>
<td></td>
<td>XXVII</td>
<td></td>
</tr>
</tbody>
</table>
XVII  \( R = \text{Sugars} \)

XVIII  \( R = \text{Sugars} \)

XIX  \( R = \text{Glu - Glu - Glu} \)

XX  \( R = \text{Glu - Glu - Rha} \)

XXI  \( R = \text{Glu - Glu - Rha} \)

XXII  \( R = \text{Sugars} \)

XXIII  \( \text{Gal-O-Glu} \)

XXIV  \( 1B; \ R_2^-B-L-Fuc(4'-\text{Sulphate}) \)

XXV  \( 1\alpha; \ R_2^-B-L-Fuc(4'-\text{Sulphate}) \)

XXVI  \( R = \beta^-D-Glu \)

XXVII  \( R = \beta^-D-Glu \)
2. TRITERPENOIDS SAPONINS [T.Ts]:

These are widely distributed in nature, for the most part in the plant kingdom. These are divided into groups according to basic structure of triterpenes which constitute their aglycone part. Generally these aglycones have C\textsubscript{30} carbon system\textsuperscript{50} and occur as free triterpenes or combined with sugars in glycosidic linkage to form saponins.

These are classified into three groups:

(a) ambrein and squalene

(b) the tetracyclic triterpenoids,

(c) the pentacyclic triterpenoids.

As mentioned, first group consists of only two substances squalene; which was acyclic, contained six double bonds and ambrein; which was tricyclic tertiary alcohol, contained two double bonds.

The second group includes methylated steroids. Two main families in this group of compounds to which all others are related are represented by lanosterol and euphol.

The third group is largest and subdivided into \(\alpha\)-amyrin group (ursolic acid and \(\alpha\) boswellic acid group), \(\beta\)-amyrin group (oleanolic acid and \(\beta\)-boswellic acid group) and lupeol group (monohydric terpene group).

Triterpenoidal saponins have been reviewed by Basu\textsuperscript{51}, Agrawal\textsuperscript{52}, Rastogi, R. P\textsuperscript{53}.

Example of few well established triterpenoidal saponins\textsuperscript{54-67} have been presented in Table IV. A few recently investigated saponins of this class are described in several works\textsuperscript{68-73} as given in Table V.
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<th>S. No.</th>
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<th>Structure</th>
<th>Figure</th>
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<tbody>
<tr>
<td>1.</td>
<td>Schima mertensiana</td>
<td>Desacetyl bonin-saponin</td>
<td>Barrigenol A&lt;sub&gt;1&lt;/sub&gt; (\alpha)-L-rha-(1→2)-(\beta)-D-gal-(1→4)-[(\beta)-D-glc-(1→2)]-(\beta)-D-glur-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>I</td>
<td>54</td>
</tr>
<tr>
<td>2.</td>
<td>Agrostemma githago</td>
<td>Githagoside</td>
<td>Gypsogenin (\beta)-D-glc-(1→3)-(\alpha)-L-rha-(1→2)-[(\beta)-D-xyl-(1→4)]-(\beta)-D-fuc-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>II</td>
<td>55</td>
</tr>
<tr>
<td>3.</td>
<td>Panax japonicus</td>
<td>Chiksetsusaponin L9a</td>
<td>24 Hydroxy-20(s) protopanaxatriol-(\beta)-D-glc-C&lt;sub&gt;12&lt;/sub&gt;</td>
<td>III</td>
<td>56</td>
</tr>
<tr>
<td>4.</td>
<td>Panax japonicus</td>
<td>Chiksetsusaponin LT5</td>
<td>3(\beta),20(s)-OH, 12-oxo-dammar-24-ene,(\beta)-D-glc-C&lt;sub&gt;3&lt;/sub&gt; ; (\beta)-D-glc-(1→6)-(\beta)-D-glc-C&lt;sub&gt;20&lt;/sub&gt;</td>
<td>IV</td>
<td>57</td>
</tr>
<tr>
<td>6.</td>
<td>Cordia obliqua</td>
<td></td>
<td>Lupeol, (\beta)-D-glc-(1→4)-(\beta)-D-glc-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>V</td>
<td>58</td>
</tr>
<tr>
<td>7.</td>
<td>Panax japonicus</td>
<td>Chiksetsusaponin II</td>
<td>Panaxdiol, (\beta)-D-xyl-(1→6)-(\beta)-D-glc-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>VI</td>
<td>59</td>
</tr>
<tr>
<td>8.</td>
<td>Combretum molle</td>
<td>Mollic acid glucoside</td>
<td>Mollic acid, (\beta)-D-glc-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>VII</td>
<td>60</td>
</tr>
<tr>
<td>9.</td>
<td>Tetrapanax papyrifera</td>
<td>Papyrioxide L-lia</td>
<td>Papyriogenin A; (\alpha)-L-rha-(1→4)-(\beta)-D-glc-(1→6)-(\beta)-D-glc-C&lt;sub&gt;28&lt;/sub&gt;</td>
<td>VIII</td>
<td>61</td>
</tr>
<tr>
<td>10.</td>
<td>Clematis songarica</td>
<td>Songaroside A</td>
<td>Hederagenin, (\alpha)-L-rha-(1→4)-(\alpha)-L-rha-(1→2)(\alpha)-L-ara-C&lt;sub&gt;3&lt;/sub&gt; ; (\alpha)-L-rha-(1→4)-(\beta)-D-glc-(1→6)-(\beta)-D-glc-[1(→2)-(\alpha)-L-ara(1→2)(\beta)-D-glc]-C&lt;sub&gt;28&lt;/sub&gt;</td>
<td>IX</td>
<td>62</td>
</tr>
<tr>
<td>11.</td>
<td>Putranjiva roxburghii</td>
<td>Putranoside A</td>
<td>Oleanolic acid, (\alpha)-L-rha-(1→3)-(\beta)-D-glur-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>X</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Putranoside B</td>
<td>Oleanolic acid, (\alpha)-L-rha-(1→3)((\beta)-D-xyl-(1→4))-(\beta)-D-glur-C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>XI</td>
<td></td>
</tr>
<tr>
<td>S. No.</td>
<td>Source</td>
<td>Saponin</td>
<td>Structure</td>
<td>Figure</td>
<td>Reference</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------</td>
<td>-------------</td>
<td>---------------------------------------------------------------------------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td>12.</td>
<td>Polygala senega var. latifolia</td>
<td>SenIGIN II</td>
<td>Presenegenin $\beta$-D-gluc-C$<em>3$; $\beta$-D-gal-(1$\rightarrow$4)$\beta$-D-xyl-(1$\rightarrow$4)-$\alpha$-L-rha-(1$\rightarrow$2)[$4$-dimethoxy(3',4')cinnamoyl]-$\beta$-D-fuc-C$</em>{28}$</td>
<td>XII</td>
<td>64</td>
</tr>
<tr>
<td>13.</td>
<td>P. pseudoginseng subsp. Himalaicus var. angustifolius</td>
<td>Saponin D</td>
<td>$20(s)$Protopanaxdiol $\beta$-D-gluc-(1$\rightarrow$2)$\beta$-D-gluc-C$<em>3$; $\beta$-D-gluc-(1$\rightarrow$6)$\beta$-D-gluc-C$</em>{20}$</td>
<td>XIII</td>
<td>65</td>
</tr>
<tr>
<td>14.</td>
<td>Panax ginseng (roots)</td>
<td>Ginsenoside Rd</td>
<td>$20(s)$Protopanaxatriol $\alpha$-L-rha-(1$\rightarrow$2)$\beta$-D-gluc-C$<em>6$; $\beta$-D-gluc-C$</em>{20}$</td>
<td>XIV</td>
<td>66</td>
</tr>
<tr>
<td>15.</td>
<td>Pulsatilla cernua</td>
<td>Saponin III</td>
<td>Hederagenin $\alpha$-L-rha-(1$\rightarrow$2)-[$\beta$-D-gluc-(1$\rightarrow$4)]$\alpha$-L-ara-C$<em>5$; $\alpha$-L-rha-(1$\rightarrow$4)$\beta$-D-gluc-(1$\rightarrow$6)$\alpha$-L-ara-C$</em>{28}$</td>
<td>XV</td>
<td>67</td>
</tr>
</tbody>
</table>
I 15α, 16α, 28-OH; R = Sugars
II 4-CHO, 28-COOH, R = Sugars
X 28-COOH, R = (-Rha-Glu-C_3)
XI 28-COOH, R = Sugars
XII 2β, 27-OH, 23-COOH, 28-COOR,
    R = Sugar, R_1 = Sugars

III R_1 = R = OH, R_2 = -Glu-C_{12},
    R' = R'' = H
XIII R_1 = Δ^{24}, R' = Sugars,
    R_2 = R = H, R'' = Sugars
XIV R_1 = Δ^{24}, R = -O-Rha-Glu-C_6,
    R' = R_2 = H, R'' = Sugars

IV R = -Glu-C_3
    R_2 = -Glu-Glu-C_{20}

V R = -Glu-Glu-C_3

VI R = -Xyl-Glu-C_3

VII R = -Glu-C_3

IX R = Rha-Rha-Ara-C_3,
    R_2 = Rha-Glu-Glu(Ara-Glu)-C_{28}

XV R = Rha-(Glu')-Ara-C_3
    R_2 = Rha-Glu-Ara-C_{28}

VIII R = -Rha-Glu-Glu-C_{28}
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant and family</th>
<th>Saponins</th>
<th>Isolated Compounds</th>
<th>Figure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stauntonia chinensis</td>
<td>Yemoside YM₈</td>
<td>3-O-β-D-glu-(1→3)-α-L-rha-(1→2)-α-L-ara-30-noroleana-12, 20(29)-dien-28-oic acid 28-O-α-L-rham-(1→4)-β-D-glu-(1→6)-β-D-glucopyranoside</td>
<td>XVI</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Lardizabalaceae</td>
<td>Yemoside YM₉</td>
<td>3-O-β-D-glu-(1→3)-α-L-rha-(1→2)-α-L-ara-30-noroleana-12, 20(29)-dien-28-oic acid 28-O-β-D-glu-(1→6)-β-D-glucopyranoside</td>
<td>XVII</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Schefflera octophylla</td>
<td>An acetylated bidesmosidic saponin</td>
<td>3-epi-betulinic acid 3-O-β-D-6'-acetylglucopyranoside 28-[α-L-rha-(1→4)-O-β-D-glu-(1→6)]=[β-D-glucopyranoside</td>
<td>XVIII</td>
<td>69</td>
</tr>
<tr>
<td>(Lour) N.O. Araliaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Gouania lupuloides</td>
<td>Gouanoside A</td>
<td>Gouanogenin A; α-L-rham-(1→6)-β-D-glucose</td>
<td>XIX</td>
<td>70</td>
</tr>
<tr>
<td>N.O.</td>
<td></td>
<td>Gouanoside B</td>
<td>Gouanogenin B; α-L-rham-(1→6)-β-D-glucose</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>Araliaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Rhannaceae</td>
<td>Dipsacus saponin B</td>
<td>Hederagenin-3-O-β-D-glu-(1→4)-[α-L-rha-(1→6)]-β-D-glu-(1→3)-α-L-rha-(1→2)-α-L-arabinopyranoside</td>
<td>XXI</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Dipsacus</td>
<td>Dipsacus saponin C</td>
<td>Hederagenin-3-O-β-D-xyl-(1→4)-β-D-glc-(1→4)-β-D-glu-(1→3)-[α-L-rha-(1→4)]-α-L-rha-(1→2)-α-L-arabinopyranoside</td>
<td>XXII</td>
<td></td>
</tr>
<tr>
<td>Asper N.O. Dipsacaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Clinopodium chinensis</td>
<td>Clinopodiside D</td>
<td>3β, 16β, 23-trihydroxy-12-keto-13-β-28 epoxyolean-9(11)-en-3-yl-[β-D-glu(1→2)]-[β-D-glu(1→3)]-[β-D-glu(1→3)]-[β-D-fucopyranoside</td>
<td>XXIII</td>
<td>72</td>
</tr>
<tr>
<td>N.O.</td>
<td></td>
<td>Clinopodiside E</td>
<td>16β-propionyl-3β,23 dihydroxyolean a-11, 21-diene-3-yl-[β-D-glu(1→2)]-[β-D-glu(1→3)]-[β-D-fucopyranoside</td>
<td>XXIV</td>
<td></td>
</tr>
<tr>
<td>Labiatae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Clinopodium chinensis</td>
<td>Clinopodiside F</td>
<td>3β, 16β, 21 α 23, 28-pentahydroxy-11 methoxyolean-12-en-3-yl-[β-D-glu(1→2)]-[β-D-glu(1→3)]-[β-D-glu(1→3)]-[β-D-fucopyranoside</td>
<td>XXV</td>
<td>73</td>
</tr>
<tr>
<td>N.O.</td>
<td></td>
<td>Clinopodiside G</td>
<td>3β, 16β, 21 β 23, 28-pentahydroxy-11, 13(18)-dien-3-yl-[β-D-glu-(1→2)]-[β-D-glu-(1→3)]-[β-D-fucopyranoside</td>
<td>XXVI</td>
<td></td>
</tr>
</tbody>
</table>
XVI $R_1 = \text{Glu-Rha-Ara}$  
$R_2 = \text{Glu-Glu}$

XVII $R_1 = \text{Glu-Rha-Ara}$  
$R_2 = \text{Rha-Glu-Glu}$

XVIII $R_1 = \text{6'Acetyl Glu}$,  
$R_2 = \text{Glu-Glu-Rha}$

XIX $R = \text{Glu-Rha}$,  
$R' = \text{-CH=CH-CH=CH}_2 \text{Me}_2$

XX $R = \text{Glu-Rha}$,  
$R' = \text{-CH}_2\text{-CH(OH)-CH=CH}_2 \text{Me}_2$

XXI $R = \text{Glu-(-Rha)-Glu-Rha-Ara}$

XXII $R = \text{-Xyl-Glu-Glu-Rha-Rha-Ara}$

XXIII $R = \text{Sugars}$

XXIV $R = \text{Sugars}$

XXV $R = \text{Sugars}$

XXVI $R = \text{Sugars}$
3. BASIC STEROIDAL SAPONIN:

The group of compounds which are having nitrogen analogues of steroid sapogenins as their aglycone come in this category of saponins.

Several reviews on saponins of this class have been published by Schrciber\textsuperscript{74} Herbert\textsuperscript{75} and Harrison\textsuperscript{76}.

Only a few of this type are isolated and characterized\textsuperscript{77-78}.

ISOLATION:

The physiochemical methods used for isolation, detection, purifications and structural determination of both types of saponins are same with certain modifications.

Liebermann Burchard reaction is generally applied for the detection of triterpenoid\textsuperscript{79} and steroid nucleus\textsuperscript{80}, and also differentiating triterpenoidal saponin (pink colour was produced) from steroidal saponin (blue green colour was produced).

Newer methods have also been developed while haemolysis of blood is most common method for the determination of saponin content.

Development of recent techniques [techniques like DEAE Sephadex, L-H-20 Sephadex, DCCC HPLC on a reversed phase column] for isolation, purification of saponin in addition to paper chromatography\textsuperscript{81} (purification of lupene based saponin), thin layer chromatography\textsuperscript{82} (purification of sapogenin) and column chromatography\textsuperscript{82} have been of immense help for isolation of complex mixtures e.g. for separation of complex mixture of steroidal glycosides from the starfish "halitry regualaris" Iorizzi et al.\textsuperscript{83} used following successive chromatographic steps:
(a) recovery of polar material from aqueous extracts on a column of Amberlite XAD-2,

(b) chromatography of the methanol eluate on a column of Sephadex L H-60 to separate the more polar sulfated "astero- saponins" from the less polar steroids,

(c) dccc to fractionate the less polar components, and

(d) hplc on a preparative C_{18\mu} Bondapak column for further purification and separation.

STRUCTURAL ELUCIDATIONS:

Structure elucidation of saponins are effectively brought about by acid hydrolysis normally by 2-4 N mineral acids, use of perchloric acid has also been observed, which yields the aglycone and sugar moieties which are analysed separately.

In addition to hydrolytic methods more effective cleavage methods are also in use, as such few are given. Ultraviolet photolysis of a methanolic solution of oleanone based saponins. Ultraviolet photolysis of 2'Keto derivatives of arabinob and galactosidio saponins (prepared by using DMSO/ acetic anhydride), acetic anhydride/pyridine treatment for glycosidic linkage in saponins selectively at the glucuronide moiety, degradation being different from ordinary acetolysis.

Enzymatic hydrolysis has also been used commonly, as has been shown in a work published on hydrolysis with snail enzyme, holotherin-A gave holotherin-B and its prosapogenols 3 (in major quantity) and 4 (in traces) while with naringinase enzyme, holotherin-A gave holotherin-B and its prosapagenol 4.
SUGARS:

Commonly found sugars in sugar moiety of saponins includes D glucose, D galactose, L- arabinose, L- rhamnose, D-xylose, D-ribose, L- fucose. In few cases quiniovose, apiose have also been reported. Sugar moiety or oligosaccharides are identified as monosaccharides obtained on hydrolysis of saponins (by PC, GLC). Sugar units and their attachment are determined by saponin- methylation followed by hydrolysis and their identification as methylated derivatives by GLC or PC, their linkages as α, β generally being determined by enzymatic hydrolysis or by Klyne's rule on molecular rotation difference.

Colorimetric methods are also in use for sugars estimation.

Physical, Analytical, Spectroscopic methods and studies are utmost important for arriving at valuable conclusions during phytochemical investigations. These are cited below:

X-RAY CRYSTALLOGRAPHY:

There has been reports of X-ray crystallographic studies in full structural determination of saponins. e.g lyofoligenic acid.

On the basis of structure of triacetate of soyasapogenol B, revision of soyasapogenols structure by X-ray crystallography has been shown.

U. V.

U. V spectroscopy has been found to be useful and reliable to recognise a characteristic groups in molecules of widely varying complexities.

Generally its use has been limited to conjugated systems.
It showed absorption at 253, 243, 237 nm to characterize a transoid heteroannular diene system\textsuperscript{95} which helped in determining diene in case of structure elucidation of a C\textsubscript{31} lanostane type triterpene.

I.R.

A number of reference material, text\textsuperscript{96,97} and manuals\textsuperscript{98,99} are available covering every aspect of infra red spectroscopy which permits the chemist to obtain important structural knowledge about entire molecule, groups or atoms through characteristic group frequencies.

The position of cyclopropane ring in cycloartenol\textsuperscript{100} terpenoid has been fixed as it had a bond at 3045 cm\textsuperscript{-1} characteristic of a methylene group in a cyclopropane ring as shown by Cole.

It shows characteristic absorption in between 657-58 cm\textsuperscript{-1} for the presence of double bond in sterols.

Characteristic bands appear in the IR spectra at 657 cm\textsuperscript{-1} and 863 cm\textsuperscript{-1} for 25 S and 25 R sapogenins\textsuperscript{101}.

I.R spectra helps in deciding furostanol and spirostanol nucleus as the former exhibit no spiroketal side chain absorptions in its IR spectrum which were characteristic of later. A published work\textsuperscript{44} is there as an evidence where sibiricoside A and B, a furostanol and spirostanol glycoside respectively being isolated and identified.

\textbf{\textsuperscript{1}H NMR :}

There is abundance of valuable references\textsuperscript{102,103,104} which are convenient sources of concise information which are helpful in structural determination.
\(^1\text{H} \text{NMR}\) is the most important and surpasses all other spectral studies in structural elucidation.

On the basis of \(^1\text{H} \text{NMR}\) spectra the stereochemistry of terminal conjugated double bond has been assigned for various cis-trans isomers of \(\alpha\text{-}\beta\text{-unsaturated esters.}\)

It is well established that \(\beta\text{-olefinic proton cis to carbomethoxy group}\) is more deshielded than when trans. Thus in a work reported\(^{105}\) for structure determination of triterpenic acids with cyclopropane ring (structurally related compounds with sapogenin type), it has been possible to assign trans structure to mangiferolic acid (\(\Delta_\text{cis} - \delta_\text{trans} = .75\)) and methyl masticadienolate (\(\Delta_\text{cis} - \delta_\text{trans} = .72\)) by comparing chemical shift differences of \(\beta\text{-olefinic protons of,}\)

\[
\text{me tiglolate, } \beta H = 6.73 \quad \text{and me mangiferolate, } \beta H = 6.63 \\
\text{me anglelate, } \beta H = 5.98 \quad \text{me masticadienolate, } \beta H = 0.59
\]

respectively.

\(^1\text{H} \text{NMR}\) Spectrum is found to be useful in the determination of mode of sugar linkages by estimation of saponin peracetates. It was observed that when anomeric proton signal appeared as doublet, \(J\sim7\text{Hz}\) generally the D-glucopyranose and L-arabinopyranose was shown to have \(\beta\) and \(\alpha\) configuration respectively in \(4\text{C}_1\) conformation.

It has been also seen that \(\beta\) anomeric protons of D-glucosides, D-mannosides, D-rhamnosides were observed at higher field (\(\delta 4.5 - 5.0\)) in comparision to \(\alpha\) anomeric protons (\(\delta 5.0 - 6.0\)).

\(^{13}\text{C} \text{NMR}\):
The increased emphasis on $^{13}$C NMR as a tool of the organic chemist is readily apparent from the high resolution NMR Spectrometers$^{92}$ and text available$^{106,107}$.

It has been employed successfully in case of triterpenic glycosides$^{108}$, steroidal sapogenins$^{109}$ for configurational and conformational studies.

The type of linkages at the glycosidic bonds in case of oleonane based$^{90}$ triterpenoid saponin were determined by $^1$H and $^{13}$C NMR spectra (by deshielding for carbon resonances).

In case of suberosol (a lanostane triterpene) the position of exomethylene group has been confirmed by $^1$H-$^{13}$C long-range correlation$^{95}$ between exomethylene proton signals [$\delta$ 4.66(s), 4.72(s)] and C-22, C-25 carbon resonance [δ31.21,31.81] respectively.

**MASS SPECTROSCOPY:**

Molecular weight determination of saponin has now become easier by development of mass spectrometry. Some reported evidences are EIMS for identification, purity estimation and structural characterisation of volatile derivatives$^{110}$. FIMS applied for permethylated oligosaccharides$^{111}$, FDMS applied for underivatized steroid and triterpenoid saponins$^{112,113}$. Fabms (-ve ion mode) in case of various other saponins$^{114,115}$ successfully lead to identify sugar pentoses, hexoses and their sequences and hence important in identification of saponins.

In a published report to elucidate sequence of sugar units$^{116}$, the mass spectra of permethylated derivatives of oleanane type saponins containing one to five sugar units have been studied.

**BIOLOGICAL ACTIVITY:**
Saponins occur abundantly in nature with wide variety of biological properties.

**METABOLIC ACTIVITIES AND APPLICATIONS**

Saikosaponins (T.T s) from Bupleurum falcatum, used in oriental medicine as a crude drug for curing hepatobiliary diseases was observed to show various metabolic actions in rat viz. cholesterol lowering effect, hepatic protein synthesis enhancing effect etc. Their activity seems to being related to presence of sugar moiety or CH$_2$OH at C$_6$.$^{117}$

Incorporation rate (x) of labeled leucine into rat serum protein was found to be affected by ginsenoside saponins, (T.T s) where Rb$_1$ was ineffective while Rb$_2$ Rc,Rc$_2$, Re, Rg1,Rd stimulates and increases, Rd being most active and it was observed that (X ~ dose given)$^{118}$.

Saponins (T.T s) are found to improve functions of kidney in rat via protein and RNA synthesis in rat.$^{119}$

Ginseng saponins(T.T s) in general stimulates the central nervous system but inhibits it at higher doses.

It was shown that low concentration of digitonin(a S s) inhibited stimulation of glucose uptake by insulin when this metabolic activity was observed on isolated fat cell.$^{120}$

Tribulus terrestris(a S s) when given orally at a rate of 10-15mg/kg/day with cholesterol (200mg/kg/day) for about 3 months to rabbits prevents protein, carbohydrate, lipid deficiency in their liver.$^{121}$

**HAEMOLYTIC AND PROTECTIVE ACTIONS**

Ginseng radix saponin (a T.T s) and its related compounds from Ginseng radix showed haemolytic activity (by certain fractions having
20(s) panaxtriol as aglycone part) and also protective activity against haemolysis (by other fractions having 20(s) panaxdiol as aglycone part\textsuperscript{122}). It was also found that their aglycones alone did not exhibited these activities. The haemolytic action\textsuperscript{123} on human is caused by following saponins (S s) in the order shown below:

"digitonin>tomatine> parillin" where digitonin showed higher activity and parillin was comparatively very resistant.

**CARDIO-VASCULAR ACTIVITIES**

Both type of saponins are found to show effect on cardio-vascular activities. ruscoside A and B\textsuperscript{124} (S s) slow down cardiac rhythm and respiration of human suffering from arteriosclerosis. T.T saponin\textsuperscript{125} has been observed to decrease cardiac contraction frequency.

**ANTIFERTILITY FACTORS**

(T.T s) from Gleditschia horrida and (S s) from C. speciosus has antifertility effect on goats, rats and cows\textsuperscript{128,129}. Antispermatozoic effect in rats and humen was observed by s-captita and p-saman (T.T s).

**SOME OTHER BIOLOGICAL ACTIVITIES**

They are also shown to possess antinflammatory\textsuperscript{128} antirheumatic, antiulcerogenic, hypotensive\textsuperscript{124}, molluscicidal\textsuperscript{129}, pescicial activities and inhibitory activity against induced sleep as an example; A dose of 1:25 - 5mg/kg s.c of Aralia modshusica saponins\textsuperscript{130} lowers the chloral hydrate induced sleep in mice.

**ANTI MICROBIAL AGENTS**

Steroidal saponins; spinasaponins A and B, cyclamin showed antibiotic activities while hederasaponin C, gypsoside may be observed to
show antibiotic activities when sugars attached to \(-\text{COOH}\) group, was removed. Saponins are effective antibacterial\textsuperscript{133} antiviral and antifungal agents\textsuperscript{132,133} where the no. of monosaccharides and their sequence showed marked effect on activities\textsuperscript{135}, generally sapogenins alone and acetylatable sapogenins found to lower the effect.

A few reports where saponins and related compounds were found to be effective antiviral agents are as follows:

A C\textsubscript{31} lanostane type triterpene, suberosol\textsuperscript{95} has been isolated from Polyalthia suberosa as an anti HIV principle (1993).

Triterpenoid saponins were isolated as Anti HIV principles\textsuperscript{134} from fruits of Gledutsia japonica and Gynocladus chinesis, 3,16, di-O-acetyl echinocystic acid was also shown to be HIV agent (1995).

Hepatic keratitis induced in rabbit eyes was treated with antiviral drugs and saponins from A. arovensis and the effect was found to be relative\textsuperscript{135} (1988).

Different saponins are observed to differ in various biological activities, some was found to show higher effect while few are very resistant.

Some biological activities more or less are found to be affected by nature of aglycones\textsuperscript{136}.

Thus we arrived at the state of knowledge that the plant has enough importance in medicinal field and there is enough scope for carrying out further phytochemical researches on saponins of Spondias mangifera Willd for the discovery of antiviral agents.
ACTUAL WORK DONE AND PLAN

India is quite rich in flora and fauna of medicinal value. It is known from time immemorial to medicinal scientists and chemist all over the world.

With the increase in dreadful diseases and to obviate the drain created by various kind of pollutions, disturbances caused by technological advancements on environment by men, development of new diseases, ailments, economic condition of common men and high cost of therapeutic drugs, there is imperative need to unreveal indigenous sources and remedies from nature which could be within the reach of mass to overcome all these problems related with medicinal sufferings.

The authoress was fascinated because of the so important antiviral activity associated with Spondias mangifera Willd and triterpenoidal compounds mostly saponins and therefore decided that it is worthwhile to carry out investigations on plant by the help of proper and systematic pharmacological studies so as to illucidate their hidden therapeutic values which could be utilised for the production of important potent antiviral moiety and her findings are presented below.

This knowledge when used judiciously, properly in well organised manner coupled with characterisation and quantitative analysis of phytomedical compounds by spectrophotometric method will be of immense help for health of mankind.
ISOLATION AND STUDY OF A NEW SAPONIN; 28-O-α-L-ARABINOPYRANOSYL-(1→6)-O-β-D-GLUCOPYRANOSYL-3, 21 DIHYDROXY-OLEAN-12-EN-28-OATE FROM THE STEMS OF SPONDIAS MANGIFERA WILLD.

This chapter deals with the study of a new triterpenoidal saponin extracted from the actone methanol: ethyl acetate (4:1:2) fractions of the methanol soluble fractions of concentrated 95% ethanolic extract of stems of Spondias mangifera Willd (yield 0.383%), molecular formula C_{41}H_{66}O_{13}; m.p. 260°, [α]_{D}^{27}-15 and [M]_{1}^{+} 766 (EIMS).

It was identified by colour reactions, chemical degradations and IR, ^1HNMR, ^13CNMR and Mass spectroscopy 28-O-α-L-arabinopyranosyl-(1→6)-O-β-D-glucopyranosyl-3,21-dihydroxy-olean-12-en-28-oate (I).
II. ISOLATION AND STUDY OF A NOVAL TRITERPENOIDAL SAPONIN; ECHINOSYSTIC ACID-3-O-β-D-GALACTOPYRANO-\text{-SYL-}(1→5)-O-β-D-XYLOFURANOSIDE FROM THE ROOTS OF SPONDIAS MANGIFERA WILLD.

The concentrated ethyl acetate extract of the concentrated 95% ethanolic extract of roots of Spondias mangifera Willd when worked up, by column chromatography yielded from the benzene : chloroform (4:6) fractions of a novel triterpenoidal saponin.(yield .0275%), molecular formula C_{41}H_{66}O_{13} ; m.p. 238^0 and [M]^+ 766 (EIMS) and was identified as ;

Echinocystic acid -3-O-β-D-galactopyranosyl-(1→5)-β-Dxylofurano-side (II) on the basis of colour reactions, chemical degradations and U.V., $^1$HNMR, $^{13}$CNMR and Mass spectroscopy.
III. ISOLATION AND STUDY OF A NOVEL STEROIDAL SAPONIN, STIGMA-4-EN-3-0-β-D-GLUCOPYRANOSIDE FROM THE ROOTS OF SPONDIAS MANGIFERA WILDL.

This chapter involves the study of a novel steroidal saponin extracted from the chloroform : methanol (9:1) fractions of the ethyl acetate soluble fraction of concentrated ethanolic extract of roots of Spondias mangifera Willd. (yield .0292%), molecular formula C$_{35}$H$_{60}$O$_{69}$; m.p. 218$^0$ and [M]$^+$ 576 (EIMS).

It was identified on the basis of usual chemical degradations, specific colour reactions and UV, IR, $^1$HNMR, $^{13}$CNMR and Mass spectroscopy as; Stigma-4-en-3,β-D-glucopyranoside (III).
IV ISOLATION AND STUDY OF THE STEROIDAL SAPONIN; β-SITOSTEROL-3-O-β-D-XYLOPYRANOSYL-(1→2)-α-L-ARABINOPYRANOSYL-(1→6)-β-D-GLUCOPYRANOSIDE FROM THE STEMS OF SPONDIAS MANGIFERA WILLLD.

This chapter incorporates structure elucidation of a steroidal saponin (yield 0.293%), molecular formula $C_{45}H_{76}O_{14}$; m.p. 206° and $[M]^+ 840$ (EIMS) extracted from the acetone : methanol (5:1) fractions of concentrated 95% ethanolic extract of stems of Spondias mangifera Willd and was identified as; β-sitosterol-3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (IV) on the basis of systematic chemical degradations, colour reactions and spectroscopic studies.
REFERENCES


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