ABSTRACT

The thesis deals with the isolation and structural studies of the various constituents from the following Indian medicinal plants.

(1) Stem of *Angelo marmelos* Corr.
(2) Stem bark of *Sapium euphitolium* Ham.
(3) Roots of *Norinda citrifolia* Linna.

Antimicrobial activity of the ethyl acetate extract of the stem of *Angelo marmelos* and stem bark of *Sapium euphitolium* and ethanolic extract of roots of *Norinda citrifolia* has also been done. Further, the pharmacological activity of the ethyl acetate extract of the stem bark of *Sapium euphitolium* and ethanolic extract of roots of *Norinda citrifolia* has also been done and included in this thesis. In addition to these biological activities, the anticancerous activity of the new anthraquinone isolated from the stem bark of *Sapium euphitolium* has been also given in this thesis. All the biological and anticancerous activity have been described in the separate chapter of the present thesis.

CHAPTER - 1

The first chapter is introductory. It deals with the importance and development of phytochemistry with particular emphasis on the chemistry coumarin, anthraquinone and terpene.
Some of the important compounds isolated from various plant sources have also been described with their pharmacological uses in medicines.

CHAPTER - II

This chapter deals with the isolation, purification, crystallization and structural study of the various constituents isolated by the authoress from the stem of Aegle marmelos. A survey of literature available on the chemistry of Aegle marmelos has also been incorporated in this chapter. Three compounds - A, B and C have been isolated and characterized from the stem of this plant by the authoress and their structural study have been done by the application of chemical and spectral methods. The compound-A was already reported compound while compound B and C have reported for the first time in nature. The detailed investigation of these compounds have been discussed separately in three different sections- I, II and III respectively. A brief description of the compounds- A, B and C is mentioned herein.

Compound - A

Molecular formula, C_{19}H_{24}O_5 (M^+ at m/z 332), m.p. 122-23°, [α]_D + 28° (EtOH), gave negative Molisch's test showing the absence of glycosidic nature of the compound-A. The detailed study of infra-red spectrum, ¹H-NMR spectrum, mass spectrum, melting point and preparation of various
derivatives of the compound-A showed its identity as marmin which was finally confirmed by co-chromatography with an authentic sample.

Hence the structure of this compound-A has been represented by the following structure

\[
\begin{align*}
\text{CH}_2-\text{CH} &= \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{C} \quad \text{CH}_3 \\
\text{OH} & \quad \text{OH} \\
\text{O} & \quad \text{O}
\end{align*}
\]

**STRUCTURE OF THE COMPOUND-A**

(MARMIN)

**Compound - B**

Molecular formula, \( \text{C}_{16}\text{H}_{12}\text{O}_4 \) \( (\text{M}^+ \text{ at } \text{m/z } 268), \text{ m.p. } 266-268^\circ \), gave negative Molisch's test showing the absence of glycosidic nature of the compound-B. The compound-B was found to be an anthraquinone by its specific colour reactions and spectral studies.

The compound-B was found to contain one hydroxyl group (by the formation of its monoacetate) and one methoxyl group (by Zeisel's method of methoxyl group estimation). The compound-B on zinc dust distillation gave 2-methyl anthracene showing the presence of a methyl group at position-2 in the compound-B. The compound-B was identified as 1-hydroxy-6-methoxy-2-methyl-anthraquinone on the basis of its various specific colour
reactions, acetylation, methylation, demethylation, chromic acid oxidation and also by spectral studies.

Hence the compound-B has been assigned by the following structure.

\[
\text{Structure of the Compound - B}
\]

1-hydroxy-6-methoxy-2-methyl-anthraquinone

**Compound - C**

Molecular formula, \( \text{C}_{15}\text{H}_{16}\text{O}_{7} \), m.p. 185-187° was found to be an coumarin glycoside by its various specific colour reactions and spectral studies. Acid hydrolysis of the compound-C with 1% \( \text{H}_{2}\text{SO}_{4} \) yielded an aglycone and a sugar which was identified as L-rhamnose by its Co-paper chromatography and osazone formation. The aglycone, molecular formula, \( \text{C}_{9}\text{H}_{6}\text{O}_{3} \) (\( M^+ \) at m/z 162), m.p. 222-223° was found to contain one hydroxyl group. The aglycone did not contain any methoxy, methylenedioxy, ketone and aldehyde functions and was found to contain a coumarin nucleus. The presence of an epoxide groups was also excluded. On the basis of various specific colour reactions, ultraviolet-visible spectra, methylation, acetylation and also by spectral
studies, the aglycone was identified as 7-hydroxy coumarin (umbelliferone) which was finally confirmed by co-thin layer chromatography with an authentic sample.

The compound-C could be hydrolysed with takadistase solution to give L-rhamnose (Co-paper chromatography) and aglycone, umbelliferone (m.m.p. and co-thin layer chromatography) indicating the presence of $\alpha$-linkage between the aglycone and L-rhamnose.

The periodate oxidation of the compound-C showed that one molecule of compound-C was built up of one molecule of L-rhamnose and aglycone.

Hence the compound-C has been assigned by the following structure.

\[ \text{STRUCTURE OF THE COMPOUND-C} \]

\[ \text{UMBELLIFERONE-7-O-} \alpha \text{-L-RHAMNO PYRANOSIDE} \]

\[ \text{CHAPTER-III} \]

The third chapter deals with the isolation, purification, crystallization and structural studies of the three compound isolated from the stem bark of
Sapium eugnolium. A survey of literature available on the chemistry of Sapium eugnolium has also been incorporated in this chapter. One reported compound—A and two unreported compounds—B and C have been isolated from the stem bark of Sapium eugnolium by the authoress and their structural studies have been done by the application of chemical and spectral methods. The detailed investigations of these compounds have been discussed separately in three different sections— I, II and III respectively. A brief description of these compounds is mentioned herein.

Compound—A

Molecular formula, C_{30}H_{48}O \ (M^+ \text{ at } m/z \ 424), \text{ m.p. } 202-204^\circ \text{ was found to be identical with moretenone on the basis of its m.p., infra-red spectrum, mass spectral studies and also by the preparation their various derivatives. The compound—A was finally confirmed as moretenone by its m.m.p. and Co-thin layer chromatography with an authentic sample.}

Hence the compound—A can be assigned by the following structure.

![STRUCTURE OF THE COMPOUND—A](image_url)

(MORETENONE)
Compound - B

Molecular formula, \( C_{32}H_{50}O_4 \) (M⁺ at m/z 498), m.p. 220–222° gave negative Molisch's test showing the absence of glycosidic nature of the compound-B. The infra-red spectrum of the compound-B showed that the compound-B belongs to ursane series of the triterpene having acetate and carboxylic groups. One carboxylic group (by monoester formation with diazomethane) and an acetate group in the compound-B was confirmed by its spectral and analytical data. From the detailed study of its chemical and spectral data, the compound-C was found to be urs-3-O-acetyl-20(30)-ene-28-oic-acid.

Hence the compound-B has been assigned by the following structure.

![Structure of Compound-B](image)

**STRUCTURE OF THE COMPOUND - B**

**URS-3-O-ACETYL-20(30)-ENE-28-OIC-ACID**

Compound - C

Molecular formula, \( C_{18}H_{16}O_6 \) (M⁺ at m/z 328), m.p. 278–280°, gave negative Molisch’s test showing the absence
of glycosidic nature of the compound- C. The compound- C was found to be an anthraquinone by its specific colour reactions and spectral studies.

The compound- C was found to contain one hydroxyl group (by the formation of its monoacetate) and three methoxyls group (by Zeisel's method of methoxyl group estimation). The compound- C on zinc dust distillation gave 2-methyl anthracene showing the presence of a methyl group at position- 2 in the compound- C. The compound- C was identified as 3-hydroxy- 1,6,7-trimethoxy-2-methyl anthraquinone (named as eugnnone) on the basis of its various specific colour reactions, acetylation, methylation, demethylation, chromic acid oxidation and also by spectral studies.

Hence the compound- C has been assigned by the following structure.

![Structure of the Compound-C]

**STRUCTURE OF THE COMPOUND- C**

3-hydroxy-1,6,7-trimethoxy-2-methyl-anthraquinone
CHAPTER - IV

The fourth chapter deals with the isolation, purification, crystallization and structural studies of only one new compound isolated from roots of *Morinda citrifolia* by the authoress. A survey of literature available on the chemistry of *Morinda citrifolia* has also been incorporated in this chapter. The structural study has been done by the application of chemical and spectral methods. The detailed investigation of this compound is mentioned herein.

The compound, molecular formula, $C_{16}H_{12}O_{4}(M^+\text{at m/s } 268)$, m.p. 240-241°C, gave negative Molièse's test showing the absence of glycosidic nature of the compound. The compound was found to be an anthraquinone by its specific colour reactions and spectral studies.

The compound was found to contain one hydroxyl group (by the formation of its monoacetate) and one methoxyl group (by Zeisel's method of methoxyl group estimation). The compound on zinc dust distillation gave 2-methyl anthracene showing the presence of a methyl group at position-2 in the compound. The compound was identified as 7-hydroxy-8-methoxy-2-methyl anthraquinone on the basis of its various specific colour reactions, acetylation, methylation, demethylation, chromic and oxidation and also by spectral studies.
Hence the compound has been assigned by the following structure.

\[
\text{STRUCTURE OF THE COMPOUND}
\]
\[7\text{-HYDROXY}-8\text{-METHOXY}-2\text{-METHYL-ANTHRAQUINONE}\]

CHAPTER - V

This chapter has been divided into three parts - A, B and C respectively.

PART - A: This part deals with the importance and development of the antimicrobial activity along with some recent idea about the use of drug's as antimicrobial agents. The experimental technique has also been included in this part.

This part has been further divided into six sections-I, II, III, IV, V and VI to describe the antibacterial and antifungal activity of the ethyl acetate extract of the stem of Agole marmelos; stem bark of Sapium sebiferum and ethanolic extract of roots of Morinda citrifolia. For the antibacterial and antifungal activities, the following bacteria and fungi were selected during the study.
NAME OF BACTERIA

1. *Vibrio cholerae* (+)
2. *Salmonella typhimurium* (-)
3. *Staphylococcus aureus* (+)
4. *Pseudomonas putida* (-)
5. *Bacillus anthracis* (+)

NAME OF FUNGI

1. *Alternaria alternata*
2. *Curvularia lunata*
3. *Aspergillus fumigatus*
4. *Aspergillus niger*
5. *Rhizopus nodulans*
6. *Aspergillus nodulans*

All these extracts were found to be more or less effective against the tested bacteria and fungi and can be used as antibacterial and antifungal agents for therapeutic purpose against the diseases caused by the above bacteria and fungi.

PART - B

This part has been further divided into three section - I, II and III respectively.

Section - I : This section included the analgesic activity of a new anthraquinone; 3-hydroxy-1,6,7-trimethoxy-2-methyl-anthraquinone (ethyl acetate extract of stem bark of *Sapium sebiferum*)and ethanolic extract of roots of *Morinda citrifolia*. 
Section - II: This section included the local anaesthetic effect of ethanolic extract of roots of *Morinda citrifolia*.

Section - III: This section included the effect on perfused frog's heart of ethyl acetate extract of stem bark of *Sapium eugnolium* and ethanolic extract of roots of *Morinda citrifolia* respectively.

From the above pharmacological activities, it has been found that these extracts and compound showed the positive activity and could be used as drug.

PART - C:

The anticancerous activity of the eugnolone (isolated from stem bark of *Sapium eugnolium*) has been given in this part. The activity was done with the help of National Cancer Institute, Bethesda (USA).

**ANTICANCER ACTIVITY OF THE COMPOUND-EUGNOLONE**

(3-hydroxy-1, 6, 7-trimethoxy-2-methyl-anthraquinone).

The anticancerous activity of the eugnolone (a new anthraquinone) on various type of cancerous cells has been performed with the help of National Cancer Institute, Bethesda (USA). The activity was tested in vitro. The concentration of this compound that was found to cause 50% inhibition of the growth of each cell line listed under "Cell" is reported in the graph under "Log" ($IC_{50}$). The concentration and units of concentration were molar/microgram/ml.