ABSTRACT

The thesis entitled “Phytochemical Study of Some Plants of Combretaceae Family and to Evaluate Their Pharmacological and Antimicrobial Activities” deals with the isolation, structural study and biological activity of the various constituents from the roots of the following three combretaceous Indian medicinal plants:

1. *Terminalia alata*
2. *Terminalia arjuna* and
3. *Terminalia catappa*

The thesis has been divided into five chapters.

CHAPTER-1

The first chapter is introductory. It deals with the importance and development of the chemistry of natural products with particular emphasis on the chemistry of steroids, terpenoids, flavonoids and tannins. Some important compounds isolated from various plant sources have also been described with their pharmacological uses in medicines.

CHAPTER-2

This chapter deals with the isolation, purification, crystallization and structural study of the various constituents isolated from the roots of *Terminalia alata*. A survey of the available literature on the chemistry of *Terminalia alata* has also been incorporated in this chapter. Nine compounds viz. A, B, C, D, E, F, G, H and I have been isolated and characterised by the author and their structural study have been done by the application of chemical and spectral methods. The detailed investigation of these compounds has been discussed separately in nine different sections - I, II,
III, IV, V, VI, VII, VIII and IX respectively. A brief description of the compounds - A, B, C, D, E, F, G, H and I is mentioned herein.

**Compound-A**: Molecular formula C$_{30}$H$_{50}$O (M$^+$ 426), m.p. 268-269° was found to be identical with friedelin by its m.p., chemical reactions, IR spectrum, mass spectral data and also by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-A has been assigned as follows:

![Compound-A: Friedelin](image)

**COMPOUND-A**: FRIEDELIN

**Compound-B**: Molecular formula C$_{30}$H$_{50}$O (M$^+$ 426), m.p. 195-196° was found to be a triterpene by its specific chemical reactions. The detailed study of its IR, $^1$H-NMR and mass spectral data as well as preparation of various derivatives of the compound-B showed its identity as β-amyrin which was finally confirmed by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-B has been assigned as follows:

![Compound-B: β-Amyrin](image)

**COMPOUND-B**: β-AMYRIN

**Compound-C**: The compound-C, molecular formula C$_{31}$H$_{50}$O$_3$ (M$^+$ 470), m.p. 203-204° gave all the positive reactions of a triterpene. The compound-C was found to
contain hydroxyl and ester groups. The compound-C was confirmed as methyl oleanolate by its m.p., various chemical reactions, acetylation and hydrolytic study, IR, $^1$H-NMR and mass spectral data. It was finally confirmed by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound C has been assigned as follows:

![Structure of Compound-C: Methyl Oleanolate]

**COMPOUND-C : METHYL OLEANOLATE**

**Compound-D**: Molecular formula $C_{30}H_{50}O$ (M$^+$ 426), m.p. 212-214$^\circ$ was found to be a triterpenoid by its specific colour reactions. The compound-D was found to be identical with lupeol by its m.p., chemical reactions, spectral data and also by m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-D has been assigned by the following structure:

![Structure of Compound-D: Lupeol]

**COMPOUND-D : LUPEOL**

**Compound-E**: Molecular formula $C_{43}H_{76}O_{14}$, m.p. 160-162$^\circ$, was found to be a triterpenoid glycoside by its specific colour reactions and Molisch's test. Acid hydrolysis of the compound-E with 7% H$_2$SO$_4$ afforded a genin and sugars. The
sugars were identified as L-rhamnose and D-glucose by their Rf values and Co-paper chromatography with their authentic samples.

The genin, molecular formula C31H50O5 (M+ 502), m.p. 203-204° was responded colour reactions specific for a triterpenoid. The genin was found to contain three hydroxyl groups (by its triacetate formation). The detailed study of its IR, 1H-NMR, 13C-NMR and mass spectral data as well as preparation of various derivatives of the genin showed its identity as 2α, 3β,19α-trihydroxy-olean-12-en-28-oic acid methyl ester. Permethylation of compound-E followed by 5% HCl-MeOH treatment afforded 2, 3, 4-tri-O-methyl derivatives of D-glucose and L-rhamnose. Partial acid hydrolysis of the compound-E yielded L-rhamnose followed by D-glucose indicating the presence of L-rhamnose as the terminal sugar. The compound-E on takadiastase hydrolysis gave L-rhamnose and a prosaponin which on almond emulsin hydrolysis yielded D-glucose, and a genin showing the presence of an α-linkage between L-rhamnose and D-glucose and a β-linkage between D-glucose and the genin. The periodate oxidation of the compound-E led to the conclusion that the molecule of the compound-E is built up of one molecule of genin and L-rhamnose and D-glucose. Thus, the new compound-E was identified as 2α, 3β,19α-trihydroxy-olean-12-en-28-oic acid methyl ester-3β-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Hence the structure of the compound-E has been assigned as follows:

\[
\text{COMPOUND-E: } 2α, 3β, 19α-\text{TRIHYDROXY-OLEAN-12-EN-28-OIC ACID METHYL ESTER}
-3β-O-α-L-RHAMNOPYRANOSYL-(1→6)-β-D-GLUCOPYRANOSIDE}
\]
Compound-F: Molecular formula $C_{42}H_{68}O_{15}$, m.p. 304-305$^0$ was shown to be a triterpenoid glycoside by its specific colour reactions and Molisch’s test. Acid hydrolysis of the compound-F with 7% $H_2SO_4$ afforded a genin, D-glucose and D-galactose (Co-PC).

The genin, molecular formula $C_{30}H_{48}O_5 \ (M^+ 488)$, m.p. 330-332$^0$ responded to colour reactions specific for a triterpenoid. The genin was found to contain three hydroxyl groups (by its triacetate formation) and one carboxylic group (by its methyl ester formation). The detailed study of its spectral data and various chemical reactions confirmed its structure as $2\alpha, 3\beta, 19\alpha$-trihydroxy-olean-12-en-28-oic acid. Permethylolation of the compound-F followed by 5% HCl-MeOH treatment afforded 2, 3, 4, 6-tetra-O-methyl-D-galactose and 2, 4, 6-tri-O-methyl-D-glucose. Partial acid hydrolysis of the compound-F gave D-galactose first confirming the terminal sugar. The periodate oxidation and almond enzyme emulsin hydrolysis of the compound-F confirmed its structure as $2\alpha, 3\beta, 19\alpha$-trihydroxy-olean-12-en-28-oic acid-3-O-$\beta$-D-galactopyranosyl-(1→3)-$\beta$-D-glucopyranoside.

Hence the structure of the compound-F has been assigned as follows:

![Compound-F Structure](image.png)

**COMPOUND-F:** $2\alpha, 3\beta, 19\alpha$-TRIHYDROXY-OLEAN-12-EN-28-OIC ACID 3-O-$\beta$-D-GALACTOPYRANOSYL-(1→3)-$\beta$-D-GLUCOPYRANOSIDE
Compound-G: Molecular formula C_{20}H_{22}O_{8} (M^+ 358), m.p. 140-142° gave a negative Molisch’s test showing the absence of glycosidic nature of the compound-G. The compound-G gave all the positive colour tests for a flavanove. The compound-G was found to contain four methoxyl groups by Zeisel’s method of methoxyl group estimation. No bathochromic shift of longest wavelength was observed with AlCl_{3} and NaOAc indicating the presence of methoxyls at C-5 and C-7 positions. KOH degradation of the compound-G yielded 6-methyl-3,5-di-O-methylphloroglucinol and 2, 4-dimethoxybenzoic acid respectively. On demethylation, the compound-G afforded another new flavanone which on KOH degradation yielded 2, 4, 6-trihydroxytoluene and 2, 4-dihydroxybenzoic acid. The detailed study of its {^1}H-NMR, {^{13}C-NMR}, MS, UV shift study and various chemical reactions confirmed its structure as 8-methyl-5, 7, 2', 4'-tetramethoxy-flavanone.

Hence the compound G has been assigned as follows:

![Flavanone Structure]

**COMPOUND-G: 8-METHYL-5, 7, 2', 4'-TETRAMETHOXY-FLAVANONE**

Compound-H: Molecular formula C_{25}H_{30}O_{11}, m.p. 105-107° was found to be a chalcone glycoside by its UV spectrum, colour reactions, diagnostic shifts and Molisch’s test. The acid hydrolysis (7% H_{2}SO_{4}) of the compound-H afforded an aglycone and D-glucose (Co-PC and osazone formation).

The aglycone, molecular formula C_{16}H_{20}O_{6}, m.p. 123-124° gave all the positive reactions for a chalcone. The compound-H was found to contain four methoxyl groups (by Zeisel’s method of methoxyl group estimation). The aglycone was confirmed as 2'-hydroxy-2, 4, 4', 6'-tetramethoxy-chalcone (cerasidin) by its m.p.,
various chemical reactions, KOH degradation, demethylation, IR, UV, $^1$H-NMR and mass spectral data. The periodate oxidation and almond enzyme emulsin hydrolysis of the compound-H confirmed its structure as 2, 4, 4', 6'-tetramethoxy-chalcone-2'-O-β-D-glucopyranoside, a new chalcone glycoside.

Hence the structure of the compound-H has been assigned as follows:

![Structural formula of compound-H](image)

**COMPOUND-H: 2, 4, 4', 6'-TETRAMETHOXY-CHALCHONE -2'-O-β-D-GLUCOPYRANOSIDE**

**Compound-I:** Molecular formula C$_{15}$H$_{14}$O$_7$ (M$^+$ 306), m.p. 228-229$^0$ was found to be an anthocyanidin, by its specific colour reactions. The compound-I was found to be identical with leucoanthocyanidin by its m.p., chemical reactions, spectral data and also by m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-I has been assigned by the following structure.

![Structural formula of compound-I](image)

**COMPOUND-I: LEUCOANTHOCYANIDIN**

**CHAPTER-3**

This chapter describes with the isolation, purification, crystallization and structural study of the various constituents isolated from the roots of *Terminalia*
arjuna. A survey of literature available on the chemistry of *Terminalia arjuna* has also been incorporated in this chapter. Five compounds - A, B, C, D and E have been isolated from the roots of this plant and their structural studies have been done by the application of chemical and spectral methods. The detailed investigation of these compounds have been discussed separately in five different sections - I, II, III, IV and V respectively. A brief description of the compounds - A, B, C, D and E is mentioned herein.

**Compound-A**: Molecule formula $C_{30}H_{50}O(M^+ 426)$, m.p. 268-269º was found to be identical with friedelin by its m.p., chemical reactions, IR and mass spectral data and also by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-A has been assigned as follows:

![Compound-A: Friedelin](image)

**Compound-B**: Molecular formula $C_{30}H_{50}O(M^+ 426)$, m.p. 195-196º was found to be identical with β-amyrin on the basis of various chemical reactions, m.p., IR spectral study, preparation of their derivatives, m.m.p and Co-TLC respectively.

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

![Compound-B: β-Amyrin](image)
Compound-C: Molecular formula C_{29}H_{50}O (M^+ 414), m.p. 133-134^0 was found to be identical with β-sitosterol by m.p., chemical reactions, IR and mass spectral data and also by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-C has been assigned as follows:

![Compound-C: β-Sitosterol](image)

**COMPOUND-C: β-SITOSTEROL**

Compound-D: Molecular formula C_{36}H_{56}O_{10}, m.p. 147-148^0 was found to be a triterpenoid glycoside by its specific colour reactions and spectral studies. Alkaline hydrolysis (1N KOH) of the compound-D yielded a genin and a sugar which was identified as D-glucose (Co-paper chromatography and osazone formation). The genin, molecular formula C_{30}H_{46}O_{5}, m.p. 278-279^0 was shown to contain two hydroxyl groups (by its diacetate formation) and one carboxylic group (by methyl ester formation). The genin gave a positive response to Zimmermann test and on reduction with NaBH₄, the genin formed a triol (arjunic acid) showing the presence of keto group at position-3 in the genin. The genin was identified as 2α, 19α-dihydroxy-3-oxo-12-oleanen-28-oic acid by its various specific colour reactions, IR, ^1H-NMR, ^13C-NMR and mass spectral studies. The compound-D could be hydrolysed with almond emulsin solution to give D-glucose and a genin indicating the presence of β-glycoside linkage between the genin and D-glucose. The periodate oxidation of compound-D showed that one molecule of the compound-D was built up of one molecule of D-glucose and a genin. Thus, the new compound-D was identified as 2α, 19α-dihydroxy-3-oxo-12-oleanen-28-oic acid-28-O-β-D-glucopyranoside.
Hence the compounded-D has been assigned by the following structure:

**COMPOUND-D: 2α, 19α-DIHYDROXY-3-OXO-12-OLEANEN-28-OIC ACID-28-O-β-D-GLUCOPYRANOSIDE**

**Compound-E**: Molecular formula C_{16}H_{10}O_{6}, m.p. 272-273° was found to be a derivative of ellagic acid by its chemical test and UV spectrum. The compound-D contains two hydroxyls and two methoxyls (by the usual methods). The compound-D was confirmed as 3, 3'-di-O-methyl-ellagic acid by its m.p., various chemical reactions, their derivatives and spectral studies which was finally confirmed by its m.m.p. and Co-TLC with an authentic sample.

Hence the compound-E has been assigned as follows:

**3, 3'-DI-O-METHYL-ELLAGIC ACID**

**CHAPTER-4**

The fourth chapter deals with the isolation, purification, crystallization and structural study of the various constituents isolated from the roots of *Terminalia catappa*. A survey of literature available on the chemistry of *Terminalia catappa* also
been incorporated in this chapter. Four compounds - A, B, C and D have been isolated from the roots of *T. catappa*. The detailed investigation of these compounds have been discussed separately in four different sections - I, II, III and IV respectively. A brief description of these compounds is mentioned herein.

**Compound-A:** Molecular formula C\textsubscript{29}H\textsubscript{50}O (M\textsuperscript{+} 414), m.p. 133-134° was found to be identical with β-sitosterol by its m.p., chemical and spectral methods and also by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-A has been assigned as follows:

![Compound-A: β-Sitosterol](image)

**Compound-A:** β-SITOSTEROL

**Compound-B:** Molecular formula C\textsubscript{30}H\textsubscript{50}O (M\textsuperscript{+} 426), m.p. 212-214° was found to be a triterpenoid by its specific colour reactions. The compound-B was found to be identical with lupeol by its m.p., chemical reactions spectral data and also by m.m.p. and Co-TLC with an authentic sample.

Hence the structure at the compound-B has been assigned by the following structure:

![Compound-B: Lupeol](image)

**Compound-B:** LUPEOL
Compound-C: Molecular formula C_{41}H_{66}O_{9}, m.p. 190-92^0 was shown to be a triterpenoidal glycoside by its specific colour reactions, spectral studies and Molisch's test. Acid hydrolysis of the compound-C with 7% H_{2}SO_{4} afforded a genin, D-xylose and L-rhamnose (Co-PC).

The genin, molecular formula C_{30}H_{50}O, m.p. 212-214^0 responded to colour reactions specific for a triterpenoid. The genin was found to contain one hydroxyl group (by its monoacetate formation). The detailed study of its IR, ^1H-NMR, MS and various chemical reactions confirmed its structure as lup-20(29)-en-3β-ol. The identity of the genin was also finally confirmed by its m.m.p. and Co-TLC with an authentic sample. Permethylation of the compound-C followed by 5% HCl-MeOH treatment gave 2, 3, 4-tri-O-methyl-D-xylose and 2, 3-di-O-methyl-L-rhamnose. The sequence of the sugars in the compound-C has been established by partial acid hydrolysis which resulted in the formation of D-xylose first followed by a prosaponin identical to lup-20(29)-en-3-O-α-L-rhamnioside (m.p., m.m.p. and Co-TLC). The compound-C on alomond emulsin hydrolysis gave D-xylose and a prosaponin which on treatment with takadiastase afforded L-rhamnose showing the presence of β-linkage between D-xylose and L-rhamnose and α-linkage between L-rhamnose and the genin. The periodate oxidation of the compound-E led to the conclusion that the molecule of the compound-C is built up of one molecule of genin and L-rhamnose and D-xylose. Since only one hydroxyl group was available for sugars, thus sugars was found to be attached with C-3 hydroxyl group of the genin part. Thus, the new compound-C was identified as lup-20(29)-en-3-O-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranoside.

Hence the compound-C has been assigned as follows:
LUP-20 (29)-EN-3-O-β-D-XYLOPYRANOSYL-(1→4)-α-L-RHAMNOPYRANOSIDE

Compound-D: Molecular formula $C_{27}H_{30}O_{14}$, m.p. 198-199° was found to be a flavone glycoside by its UV spectrum, colour reactions and Molisch’s test. On acid hydrolysis, compound-D gave an aglycone, D-glucose and L-rhamnose (Co-PC). The aglycone molecular formula $C_{15}H_{10}O_{5}$, m.p. 345-346° was found to be identical with apigenin by its m.p., chemical oxidation and degradation reactions and also by spectral data. The identity of the aglycone as apigenin was finally confirmed by its m.m.p. and Co-TLC with an authentic sample. Permethylation followed by acid hydrolysis yielded 4', 5-di-O-methyl ether of apigenin and 3, 4, 6-tri-O-methyl-D-glucose and 2, 3, 4-tri-O-methyl-L-rhamnose indicating the presence of 2→1 linkages between glucose and rhamnose respectively. The enzymatic hydrolysis and periodate oxidation of the compound-D confirmed its structure as apigenin-7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside which was finally confirmed by its m.m.p. and Co-TLC with an authentic sample.

Hence the structure of the compound-D has been assigned as follows:
COMPOUND-D: APICENIN-7-O-α-L-RHAMNOPYRANOSYL-(1→2)-β-D-GLUCOPYRANOSIDE

CHAPTER-5

This chapter deals with the biological activity of that plants extracts and constituents which have showed positive results. For this purpose, the author has evaluated the antiinflammatory and antimicrobial activities of the extracts and the new isolated constituents from the roots of Terminalia alata and Terminalia arjuna. This chapter has been divided into three sections - I, II and III respectively.

Section-I: This section deals with the introduction, development and various methods which are used for the evaluations of the antiinflammatory activity.

The ethanolic extract and the new constituent, 2α,19α-dihydroxy-3-oxo-12-oleanen-28-oic acid 28-O-β-D-glucopyranoside (compound-D) isolated from the roots of Terminalia arjuna were shown to display the antiinflammatory activity by using the method as described by Winter, Risely and Nuss.

The ethanolic extract and the compound-D were found to exhibit 65.21% and 68.48% respectively inflammation in comparison with standard drug acetyl salicylic acid which showed 71.73% inhibition in the same condition on rats.
Section-II: This section describes the antibacterial activity of the ethanolic extract and new isolated constituents, 2α, 3β,19α-tri hydroxy-olean-12-en-28-oic acid methyl ester-3β-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (compound-E); 2α, 3β, 19α-t ri hydroxy-olean-12-en-28-oic acid-3-O-β-D-galactopyranosyl-(1→3)-β-D-glucopyranoside (compound-F); 8-methyl-5, 7, 2′, 4′-tetramethoxy flavanone (compound-G) and 2, 4, 4′, 6′-tetramethoxy-chalcone-2′-O-β-D-glucopyranoside (compound-H) from the roots of T. alata. The experimental technique has also been included in this section.

For the antibacterial, the following bacteria were selected during the study: Escherichia coli, Streptococcus aureus, Shigella dysenteriae and Shigella flexneri using streptomycin as a standard drug.

The result showed that the ethanolic extract and compound - E, F, G and H exhibited positive activity.

Section-III: This section describes the antifungal activity of the ethanolic extracts and the compounds - E, F, G and H isolated from the roots of T. alata and the compound-D isolated from the roots of T. arjuna. The experimental technique has also been included in this section.

The following fungi were selected for the study: Candida albicans, Cryosporium pannical, Aspergillus niger and Rizopus oryzae. The standard drug Griseofulvin was used for comparison.

The result indicated that EtOH extracts and the compounds - E, F, G and H of T. alata and the compound-D of T. arjuna showed antifungal activity against the above selected fungi.