Chapter-1

TERMINALIA ARJUNA
Plates 1 : Terminalia arjuna
**Terminalia arjuna**  
(Combretaceae)  

**INTRODUCTION**

Terminalia is a large genus of mostly deciduous trees, fairly equally distributed between tropical Asia, tropical Africa, and tropical America. About 16 species occur in India. The genus is of importance in India for timber (Kirtikar & Basu, 2000).

**Vernacular names**

Hindi: Arjuna  
Bengali: Arjhan  
Marathi: Sanmadat, Sadaru, Vellamarda  
Gujarati: Sadado  
Telugu: Yeramaddi  
Tamil: Velamatta  
Kannad: Maddi  
Assam: Orjun  
Trade: Arjun

**Habitat**

A large, evergreen tree, with a spreading crown and drooping branches, common in most parts of India and also planted in many parts for shade and ornament. The tree is common throughout the greater part of the Indian Peninsula along rivers, streams, ravines and dry watercourses, reaching a large size on fertile alluvial loam. It is rare in the Karnataka, but
is fairly plentiful in Tirunelveli and on the West coast. It extends northwards to the sub-Himalayan tract, where it is distributed along the banks of streams; in Punjab, it is a cultivated tree. It is common in Chota Nagpur, Orissa and in Northern Circars (Rastogi & Mahrotra, 1998).

Morphology

It is a large, evergreen tree, with spreading branches and white bark. Stems rarely long or straight, generally always buttressed and often fluted; bark very thick, grey or pinkish green, smooth, exfoliating in large, thin irregular sheets; leaves sub opposite, oblong or elliptic, coriaceous, usually 10-15 cm long, occasionally 25 cm, cordate, shortly acute or obtuse at the apex; flowers sessile yellowish in panicled spikes; fruits 2.5-5.0 cm long, nearly glabrous, ovoid or ovoid-oblong, with 5-7 hard winged angles.

Chemical constituents

The bark contains sugar, tannin (20 – 24%), colouring matter and carbonates of calcium and sodium with traces of chlorides of alkali metals. The benzene extract leads to the isolation of crystalline substances arjunine and arjunetein. Arjuna bark also contains arjunolic acid, a triterpene saponin that is responsible for the diuretic property. The triterpene glycosides are Arjunoside I, II, III and IV. The flavone Arjunolone is also found. A triterpene glycoside-arjunolutin was isolated and its structure was established as 3-O-β-D-glucopyranosyl-2α, 3β, 23-trihydroxyolean-12-en-28-oic acid-28-O-β-D-glucoside (Rastogi, 1993).
The wood contains over three percent of a triterpene, arjunolic acid (C_{30}H_{48}O_{6}; m.p. 332-34°C). From the acetone extract of heartwood, a complex saponin of arjunolic acid and (+)-leucodelphinidin (C_{15}H_{14}O_{6}.H_{2}O; m.p. 245-48°C decomp.) have been isolated. The structure of a new sapogenin named arjungenin was shown to be 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oic acid. The structures of the two new triterpene glucosides, arjunglucoside I and arjunglucoside II were reported from the same plant (Tadashi, 1976). A glucoside, arjunglucoside III was isolated and characterized as β-D-glucopyranosyl 2α, 3β, 19α-trihydroxy-11-oxoolean-12-en-28-oate (Takahiko, 1979).

The dry bark from the stem contains 20-24 % tannin and the dry bark from the lower branches 15-18 %. The bark of secondary growth is also as rich in tannin as the old. The tannin is of a mixed type, containing both pyrogallol and catechol tannin, similar to the English Oak-bark. The chemical examination of a sample of the bark showed: tannin, 15.8; soluble non-tannin, 8.2; and water 7.5%. Both hydrolysable and condensed tannins were present. Ta-Chen et al. (1996) isolated and characterized a rare complex-type tannin, in addition to 9 well known hydrolysable tannins: 2,3-(S)-HHDP-D-glucose, 2,3-(S)-HHDP 6-O-galloyl-D-glucose, punicalin, punicalagin, terchebulin, terflavin C, castalagin, casuarin and casuarinin. The bark contains β-sitosterol, ellagic acid and a new trihydroxytriterpenemonocarboxylic acid, named arjunic acid (C_{30}H_{48}O_{6}.H_{2}O; m.p. 335°C). From the alcoholic extract of the bark, a
glucoside arjinetin (C_{36}H_{58}O_{15.3}H_{2}O; m.p. 238-40°), yielding glucose and arjunic acid on hydrolysis, has been isolated. The presence of friedelin in the bark has been reported in another investigation. The digestion of water-extracted bark of *Terminalia arjuna* with 30 % sulphuric acid at 100°C resulted in the isolation of oxalic acid in 12-21 % yield (Bhatia, 1980).

Terminalic acid, a trihydroxytriterpene monocarboxylic acid belonging to the oleanolic acid series was isolated from the bark of *Terminalia arjuna* and its structure was assigned as 2α, 3β, 19α-trihydroxyolean-12-ene-30-oic acid (Ahmad, 1983). A new flavone arjunolone (6, 4’- dihydroxy-7-methoxyflavone) was isolated from stem bark along with baicalein (Sharma, 1982).

The fruits contain 7-20 % tannin. The tannin content in the fallen fruits is much lower. The fruits on extraction with petroleum- ether gave an ester (C_{56}H_{122}O_{2}; m.p. 87-89°) together with β-sitosterol. Further extraction of the residual fruit-pulp with rectified spirit gave an extractive partly soluble in water; the water-insoluble part on recrystallization gave an acid (C_{30}H_{50}O_{5}; m.p. 275-76°), which has been named arjunic acid. The water-soluble part was a mixture of mannitol, tannin and a considerable amount of potassium chloride. Chromatographic resolution of the ethyl acetate extract of the fruits of *Terminalia arjuna* resulted in the isolation of sitosterol, sitosterol glucoside, afrormosin and quercetin-7-O-rhamnoside (Singh, 1995).

A new flavone 5,7,2’,4’-tetramethoxy flavone was isolated and its structure determined (Gujral, 1979a). Another new flavonone – arjunone was
reported from fruits along with cerasidin, β-sitosterol, friedelin, methyl oleanolate and gallic, ellagic and arjunic acids (Gujral, 1979b). The extraction of *Terminalia arjuna* fruits with petroleum ether yielded hentriacontane, arachidic stearate (C38H76O2) and myristyl oleate (Ahmad, 1982).

The root bark of *Terminalia arjuna* yielded two new triterpene glycosides (Arjunoside I and II) and a new triterpene carboxylic acid (terminic acid) besides 8-hydroxyhexadecanoic acid, β-sitosterol, oleanolic acid, arjunic acid and arjunolic acid along with saponins. The structure of Arjunoside I was established as arjunic acid 3-o-β-D-galactoside and stucture of Arjunoside II as arjunic acid 3-o-(β-D-glucopyranosyl-2-deoxyrhamnopyranoside). (Anjaneyulu, 1982a). The nonphenolic fraction of the alcoholic extract of root bark of *Terminalia arjuna* yielded Arjunoside III and IV, along with arjunglucoside I and arjunitin. The structure of Arjunoside III was established as 28-β-D (+)-glucuronopyronoside of arjunic acid and Arjunoside IV as 3-o-α-L (-)-rhamnoside of arjunic acid (Anjaneyulu, 1982b). A triterpene glycoside, arjunetoside (3-O-β-D-glucopyranosyl-2α, 3β, 19α-trihydroxyolean-12-en-28-oic acid, 28-O-β-D-glucopyranoside), together with oleanolic acid and arjunic acids were reported from the root bark of *Terminalia arjuna* (Upadhyay, 2001). A new cardenolide, 16,17-dihydroridienone-3-O-β-D-glucopyranosyl (1→6)-O-β-D-galactopyranoside, was isolated from the roots of *Terminalia arjuna* (Yadav, 2001). Evidences were reported for the presence of new
compound $2\alpha$, $19\alpha$-dihydroxy-3-oxo-olean-12-en-28-oic acid-28-O-glucopyranoside, which showed antifungal activity, from the roots of *Terminalia arjuna* (Chouksey, 2001).

A cardiac orid oxide, 14,16-diantiydrogitoxigenin-3-β-D-xylopyranosyl (1→2)-O-β-D-galactopyranoside, was isolated from the seeds of *Terminalia arjuna* by various color reactions, chemical degradations and spectral analysis (Yadav, 2000).

Chauhan et al (1997) reported β-amyrr, β-sitosterol and oleanolic acid along with maslinic acid, arjunolic acid, arjunetin and arjunoglucoside II. Later on Apigenin-7-O-nehesperidoside, gallic and ellagic acids were isolated from the leaves of *Terminalia arjuna* (Chauhan, 1998).

A tannin anti-cancer promoter, arjunin (an ellagitannin) along with four known tannins nad two phenolic acids were isolated from *Terminalia arjuna* (Kandii, 1998).

**Pharmacological Activity**

*Terminalia arjuna* has been reported to possess varied pharmacological activities which are attributed to the presence of different active constituents.
Hypocholesterolaemic action

The hypocholesterolaemic activity of different fractions from *Terminalia arjuna* were reported in rats fed on atherogenic diet. The possible mechanism suggested for this effect was rapid excretion of bile acids (Shaila, 2000). Shaila *et al.* (1998) also reported *Terminalia arjuna* to be most potent hypolipidemic agents amongst different species. The orally administered drug induced partial inhibition of rabbit atheroma. These reports were further supported by findings of Ram *et al.* (1997) where *Terminalia arjuna* tree bark exhibited hypocholesterolaemic activity without adversely affecting biochemical tests of liver and renal function in diet-induced hyperlipidemic rabbits (Ram, 1997).

The hypocholesterolaemic effect of *Terminalia arjuna* was later on corroborated in humans in a randomized placebo-controlled trial (Gupta, 2001) where the bark powder was reported to exhibit significant antioxidant action that was comparable to Vitamin E along with strong hypocholesterolaemic effect.

Cardioprotective action

The bark of the *Terminalia arjuna* tree has a long history of use as a cardiac tonic as well, and has been indicated in the treatment of coronary artery disease, heart failure, hypercholesterolaemia, and for relief of anginal pain (Miller, 1998).
The bark stem powder of the plant was studied in patients of myocardial infarction with angina and/or cardiomyopathy, where it provided symptomatic relief without any adverse effects on renal, hepatic and hematological parameters after prolonged administration (Dwivedi, 1997).

In another clinical study, monotherapy with *Terminalia arjuna* was suggested to be fairly effective in patients with symptoms of stable angina pectoris but had a limited role in unstable angina (Dwivedi, 1994).

Recently, Bharani et al (2002) reported comparative study of *Terminalia arjuna* with Isosorbide mononitrate in patients of chronic stable angina. They found that *Terminalia arjuna* bark extract, 500 mg 8 hourly, when given to patients with stable angina with provocabile ischemia on treadmill exercise, led to improvement in clinical and treadmill exercise parameters as compared to placebo therapy. These benefits were similar to those observed with isosorbide mononitrate (40 mg/day) therapy and the extract was well tolerated.

In an attempt to establish the mechanism involved in cardioprotection offered by *Terminalia arjuna*, Munasinghe et al (2001) studied the antiradical and antilipoperoxidative effects of T.arjuna. They reported *Terminalia arjuna* to exhibit strong antioxidant activity and the cardiovascular benefits were attributed to this activity of the plant. In accordance with this Gautham et al (2001) reported that crude bark of *Terminalia arjuna* augmented endogenous antioxidant components of rat
heart and also prevented oxidative stress associated with ischemic reperfusion injury of the heart.

In another study *Terminalia arjuna* was reported to protect against isoproterenol induced myocardial necrosis in rats, that suggested the potential usefulness of this plant in the prevention of ischemic heart disease (Seth, 1998).

Bhatia *et al* (1998) studied the antianginal effect of *Terminalia arjuna* and this effect was attributed to its ability to significantly enhance the coronary blood flow.

In another report, a proprietary herbal product Hartone (containing *Terminalia arjuna*) was studied in comparison with isosorbide mononitrate in stable angina pectoris patients, and it was reported to be safe and effective anti-anginal agent comparable to isosorbide mononitrate and was better tolerated (Kumar, 1999).

Recently Arjunolic acid, a new triterpene and a potent principle from the bark of *Terminalia arjuna* was reported to provide significant cardioprotection in isoproterenol induced myocardial necrosis in rats. This study suggested that the cardioprotection of arjunolic acid pre and post treatment could possibly be due to the protective effect against the damage caused by myocardial necrosis (Sumitra, 2001).

Clinical study with *Terminalia arjuna* in patients with refractory congestive heart failure, mostly related to idiopathic dilated cardiomyopathy, appeared
safe and provided long lasting improvement in signs and symptoms of heart failure along with improvement in left ventricular ejection phase indices with definite improvement in quality of life (Bharani, 1995).

Anticancer action

*Terminalia arjuna* in various studies has been suggested to possess anticarcinogenic potential. Various active constituents of *Terminalia arjuna*-gallic acid, ethyl gallate and luteolin, were found to inhibit various cancer cell lines (Petit, 1996).

Similarly, in another study, pretreatment with bark extracts from *Terminalia arjuna* inhibited the growth of human normal fibroblasts, osteosarcoma and glioblastoma cells in vitro, without affecting the growth of normal cells. The growth arrest of transformed cells was induced by p53-dependent and independent pathways (Nagpal, 2000).

Kandil and Soliman (1998) reported a new tannin anti-cancer promoter-arjunin-1, from *Terminalia arjuna*. The biological activity examination of the ethanolic extract of the leaves of *Terminalia arjuna* and the isolated new compound showed that they have a moderate cytotoxic activity against BT-20 human breast carcinoma cells and the growth inhibition effected by arjunin-1 was higher than that of the extract.
Antimutagenic and antigenotoxic action

There are growing body of scientific evidences suggesting strong antimutagenic and anticarcinogenic potential of *Terminalia arjuna*. In one such study, in-vitro protective effects of *Terminalia arjuna* bark extracts against the 4-nitroquinoline-N-oxide genotoxicity was reported and it was suggested that arjuna bark contains some nonpolar as well as polar compounds with antimutagenic activity (Pasquini, 2002).

Recently, Kaur *et al* (2002) reported antimutagenicity of phenolic fractions of *Terminalia arjuna* against two directly acting mutagens, 4-nitro-o-phenylenediamine and sodium azide, and against the S9 dependent mutagen 2-aminofluorene, in TA98 and TA tester strains of *Salmonella typhimurium*. In different studies, antimutagenic activities of acetone and methanolic fractions of *Terminalia arjuna* was reported (Kaur, 2001). In continuation with their work Kaur *et al* (2000) reported modulatory effects of a tannin fraction isolated from *Terminalia arjuna* on the genotoxicity of mutagens in *Salmonella typhimurium*.

Antiviral and antibacterial action

Different studies have indicated antiviral and antibacterial property of *Terminalia arjuna*. In one such report Casuarinin, a hydrolysable tannin isolated from the bark of *Terminalia arjuna* was investigated for its antiviral activity on herpes simplex type 2 in vitro. This study concluded that casuarinin possessed anti-herpes virus activity in inhibiting viral attachment
and penetration, and also disturbing the late event of infection (Cheng, 2002). In another study, stem bark of *Terminalia arjuna* was reported to inhibit the HIV-1 protease activity (Kusumoto, 1995). Perumal et al (1998) reported the antibacterial action of *Terminalia arjuna* against *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas vulgaris* and *Pseudomonas aerogenes*.

**Medicinal and therapeutic uses**

*Terminalia arjuna*’s traditional use as a cardiotonic has been confirmed by modern research. Arjuna bark has been used in Indian medicine for at least 3000 years as a remedy for heart ailments. Ayurvedic medicine employs *Terminalia arjuna* to restore balance of 3 humors. It has also been used as an aphrodisiac, diuretic and for earaches. This species is also used to reduce cholesterol levels. The bark is useful in allaying thirst and relieving pain. The bark is acrid, and credited with styptic, tonic, febrifugal and antidysenteric properties. In fractures and contusions, with excessive ecchymosis, the powdered bark is taken with milk. The powdered bark seemed to give relief in symptomatic complaint in hypertension; it apparently had a diuretic and a general tonic effect in case of cirrhosis of the liver. A decoction of the bark is used as a wash in ulcers. The fruit of *Terminalia arjuna* is tonic and deobstruent. The juice of the fresh leaves is used in earache. The twigs are used by tribals of bastar to cure blisters and ulcers of the mouth.
EXPERIMENTAL

Apparatus, Chemicals and Instruments

1. All the chemicals and reagents were obtained from s.d. fine chemicals and were of Analytical Reagent (AR) grade.

2. Silica gel (60-120 mesh) and silica gel-G, obtained from s. d. fine chemicals, were used for the column chromatography and thin layer chromatography (TLC), respectively.

3. Anhydrous sodium sulphate was used for drying organic solvents petroleum ether (60-80°C) and chloroform.

4. Melting points were determined on Perfit melting point apparatus and are uncorrected.

5. All the weighing was done on single pan Mettler Balance.

6. Optical Rotation $[\alpha]^{22}_D$ was recorded on an Abes Polarimeter.

7. Ultra-violet (UV) spectra were recorded on Lambda Bio 20 Spectrophotometer in methanol.

8. Fourier Transforms Infra Red (FTIR) spectra were recorded on Bio-Rad FT Spectrophotometer using KBr pellets; $\nu_{max}$ values are given in cm$^{-1}$. 
9. $^1$H-NMR spectra were screened on Advance DRY 400, Bruker Spectrospin 400-MHz instrument using CDCl$_3$ as solvent and TMS as an internal standard. Chemical shifts are given in $\delta$ (ppm) scale with tetramethyl silane (TMS) as internal standard. Coupling constants (J values) are expressed in Hz. Notation used throughout as s=singlet, d=doublets, dd=double doublets, t=triplet, m=multiplet, brs=unresolved broad singlet.

10. $^{13}$C FT-NMR Spectra were recorded on Advance DRY 400, Bruker Spectrospin 100-MHz with TMS as an internal standard in 5 mm spinning tubes at 27°C.

11. Mass Spectra were scanned by effecting Electron Impact (EI MS) ionization at 70 eV on a JEOL-JMS - DX 303 instrument, equipped with direct inlet probe system. The $m/z$ values of the more intense peaks were mentioned and the figures in brackets attached to each $m/z$ indicated relative intensity with respect to the base peak.

12. The plant material *Terminalia arjuna*, *Borago officinalis* seeds and *Bombyx mori* cocoons were purchased from the local drug market, Khari Baoli, Delhi and were identified by Dr. M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard.

**Packing of Column**

The lower end of column was plugged with absorbent cotton, over which a piece of filter paper was placed. The column was then half filled with
petroleum ether, silica gel was added in small portions and allowed to settle down gently until the necessary length of column was attained. All the air bubbles were allowed to escape by running the column continuously with solvent. The silica gel slurry of the extract was packed in the column and then eluted successively in order of increasing polarity with different solvents. The development and elution of the column was carried with successive series of solvents in various combinations, viz., petroleum ether, petroleum ether-chloroform (9:1, 3:1, 1:1, 1:3 v/v), chloroform, chloroform-methanol (99.5:0.5, 99:1, 98:2, 4:1, 3:1 and 1:1 v/v) and methanol. The completion of elution of the component(s) was confirmed via evaporating a small portion of eluent.

Homogeneity of the Fractions

The fractions collected were subjected to thin layer chromatography to check homogeneity of various fractions. Chromatographically identical fractions were combined and concentrated.

Preparation of Plates

Silica gel-G (35 g) was mixed with 80 ml of distilled water by trituration method in a glass pestle and mortar to form a fine thin cream. Thickness of 0.25 mm was achieved by means of an applicator. The plates were allowed to dry in air at room temperature.
Activation of Plates

The dried plates were kept in an oven at 110°C for an hour and were stored in a desiccator.

Equilibration of the Chromatographic Chamber

About 1 cm height of the solvent was taken in a clean dry chamber, after the walls of chamber were lined with a strip of filter paper impregnated with the solvent system. The chamber was closed and allowed to saturate with vapours of solvent.

Application of Spots

The base line was marked at about 1.5 cm above from the lower edge. The dissolved fractions were spotted on the plates with the fine capillary tubes and then allowed to dry in air.

Development of Chromatogram

The spotted plates were kept in the chromatographic chambers containing the solvent mixtures. The chambers were covered with greased glass plates. The solvent system was allowed to ascend up to 3/4 th the length of the plate.

Solvent Systems for TLC Plates

Petroleum ether: Chloroform: Methanol (1:4:0.1 v/v)
Petroleum ether: Chloroform: Methanol (1:4:1 v/v)

Chloroform: Methanol (95:5 v/v)

Chloroform: Methanol (9:1 v/v)

Chloroform: Methanol (3:1 v/v)

Benzene: Ethyl acetate: Diethyl amine (6:3:1 v/v)

Detection of TLC Spots

The air-dried plates were viewed in ultra-violet light chamber to look for the coloured fluorescent spots if any. Freshly prepared ceric-ammonium sulphate (CAS) was used as a spray reagent for location of spots. The reagent was carefully sprayed on the plates and was subsequently heated in an oven at 110°C for 10-15 minutes.

Preparation of Ceric-Ammonium Sulphate Solution

Saturated solution of CAS was prepared in 65% sulphuric acid. The solution was kept on steam bath for 15 minutes and left overnight. The clear supernatant solution was decanted and used for spraying.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out using the extracts of all the three drugs for different types of chemical constituents. The qualitative chemical tests give the general idea regarding the nature of
chemical constituents of crude drugs. The extracts were subjected to preliminary phytochemical investigation for detection of:

1. Alkaloids
2. Carbohydrates
3. Glycosides
4. Phenolic compounds
5. Flavonoids
6. Proteins and amino-acids
7. Saponins
8. Sterols
9. Acidic compounds
10. Mucilages

Tests for Alkaloids

Each of the extract residues was taken separately in 5 ml of 1.5 % hydrochloric acid and filtered. The filtrate was then tested with following reagents:

**Dragendorff's Reagent**

Few drops of Dragendorff's reagent were added in each of the extract and observed for formation of orange-yellow precipitate.

**Hager's Reagent**

Few drops of Hager's reagent were added in each of the extract and observed for formation of yellow precipitate.
Wagner's Reagent

Few drops of Wagner's reagent were added in each of the extract and observed for formation of precipitate.

Mayer's Reagent

Few drops of Mayer's reagent were added in each of the extract and observed for formation of white or cream coloured precipitate.

2. Tests for Carbohydrates

Molisch Test

Small quantities of alcoholic and aqueous extracts were dissolved separately in 5 ml of distilled water and filtered. To this solution 2-3 drops of α-naphthol were added. Then about 1 ml of concentrated sulphuric acid was added along the sides of tube so as to form two layers and observed for formation of violet coloured ring at the interface.

Fehling's Reagent (Detection of reducing sugar)

Few drops of Fehling's solution A and B in equal volume were added in dilute extracts and heated for 30 minutes and observed for the formation of brick red coloured precipitate.

3. Tests for Glycosides

About 2 ml alcoholic extract were taken and subjected to the following tests:
**Keller-Killiani Test**

One ml of glacial acetic acid containing traces of ferric chloride and one ml of concentrated H₂SO₄ were added to extract and observed for the formation of reddish brown colour at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

**Borntrager's Test**

One ml of benzene and 0.5 ml of dilute ammonia solution were added to the ethanolic extract and observed for the formation of reddish pink colour.

**Legal Test**

Concentrated ethanolic extracts were made alkaline with few drops of 10% sodium hydroxide solution and then freshly prepared sodium nitroprusside solution was added to the solution and observed for formation of blue colour.

**Bajje Test**

To the concentrated ethanolic extracts sodium picrate reagent was added and observed for formation of orange or yellow colour.

4. **Tests for Phenolic Compounds**

**Ferric chloride solution**

The extracts were taken in water and warmed. To this 2 ml of ferric chloride solution was added and observed for formation of green or blue colour.


**Lead acetate solution**

2 ml of the extracts were taken and lead acetate solution was added and observed for formation of precipitate.

**Gelatin solution**

A few ml of gelatin solution was added to the aqueous extract and observed for formation of precipitate or turbidity.

5. **Tests for Flavonoids**

**Ammonia Test**

Filter paper strips were dipped in alcoholic solution of the extract, ammoniated and observed for colour change from white to yellow.

**Shinoda/Pew Test**

A small quantity of residue was dissolved in 5 ml of ethanol and treated with few drops concentrated hydrochloric acid and 0.5 gm of magnesium turnings and observed for formation of pink colour.

6. **Tests for Proteins and Amino acids**

**Millon's Test**

To a few ml of alcoholic extract, five ml distilled water was added and filtered. To two ml of this filtrate 5-6 drops of Millon's reagent (solution of
mercury nitrate and nitrous acid) were added and observed for formation of red precipitate.

**Biuret Test**

To the ammoniated alkaline filtrate of the extract 2-4 drops of 0.02 % copper sulphate solution were added and observed for formation of red or violet colour.

**Ninhydrin Test**

To the extract, lead acetate solution was added to precipitate tannins. The precipitate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and heated at 110°C for 5 minutes and observed for formation of red or violet colour.

7. Tests for Saponins

i) **Foam Test**: Few mg of residue was taken in a test tube with a small amount of water and shaken vigorously for one minute and observed for formation of rich lather which was stable for more than ten minutes.

ii) To the alcoholic extract few drops of sodium bicarbonate were added, shaken well, and observed for the formation of Honeycomb like frothing.
8. Tests for Sterols

The alcoholic extract was evaporated to dryness and the residue was extracted with petroleum ether and acetone. The insoluble residue left after extraction with petroleum ether and acetone was tested for sterols as:

Liebermann-Buchard Test

The insoluble residue left after extraction was dissolved in chloroform and few drops of acetic anhydride were added along with a few drops of concentrated sulphuric acid from the sides of the test tube and observed for the formation of blue to blood red colour.

Salkowski Reaction

To the extract two ml of concentrated sulphuric acid were added and observed for the formation of yellow ring at the junction, which turn red after one minute.

9. Tests for Acidic compounds

i) To the alcoholic extract sodium bicarbonate solution was added and observed for the production of effervescence.

ii) A small amount of alcoholic extract was taken in warm water and filtered. The filtrate was then tested with litmus paper and methyl orange and observed for the appearance of blue colour.
10. Tests for Mucilage

The extract was treated with ruthenium red solution in lead acetate and observed for the formation of pink colour.

Extraction of *Terminalia arjuna* bark

The stem bark of *Terminalia arjuna* was dried in an oven at a temperature below 45°C for 2-3 days and coarsely powdered. The powdered bark (3 kg) was extracted exhaustively with methanol (95%). The ethanolic extract was concentrated under reduced pressure to yield (700 g, 23.3%) dark brown, viscous syrupy mass. The extract was analyzed chemically for determining the presence of different chemical constituents.

Isolation

The viscous dark brown mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. It was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in the order of increasing polarity to isolate following compounds:

**TA-1**

Elution of the column with petroleum ether furnished colourless crystals of TA-1, recrystallized from benzene, 190 mg (0.0052% yield).

Rf: 0.75 (Hexane: petroleum ether; 1:9).
m.p.: 221-222°C.

IR $\nu_{\text{max}}$ (KBr): 3445, 3410, 3390, 2960, 2877, 1685, 1635, 1470, 1365, 1210, 1120 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): Table 1.1

$^{13}$C NMR (CDCl$_3$): Table 1.2

$^{+ve}$ FAB-MS $m/z$ (rel. int.): 488 [M]$^+$ (C$_{30}$H$_{48}$O$_6$) (20.1), 470 (22.3), 452 (20.8), 434 (16.8), 264(13.5), 249 (18.2), 246 (20.6), 231 (18.9), 224 (18.7), 219 (19.6), 206 (12.2), 205 (47.5), 201 (60.3), 190 (30.8), 187 (51.3).

Acetylation of TA-1: Compound TA-1 (15 mg), dissolved in a 1:1 mixture of pyridine-acetic anhydride, was warmed slightly. On standing overnight water (50 ml) was added and the reaction mixture extracted with chloroform (3 x 10 ml). The organic phase was washed with water (2 x 10 ml), dried sodium sulphate and evaporated to get a triacetate. $\nu_{\text{max}}$ 3300, 1735, 1730, 1725 cm$^{-1}$.

Jones oxidation of TA-1: Jones reagent (2 ml) was added to a solution of TA-1 (10 mg) in acetone (5 ml) at 4°C. Water (10 ml) was added and the reaction mixture extracted with chloroform (3 x 10 ml). The organic phase was washed with H$_2$O (2 x 10 ml), dried with sodium sulphate and concentrated to yield 3-oxo-derivative. IR$\nu_{\text{max}}$ 3300, 1710, 1705, 1690 cm$^{-1}$.

Methylation of TA-1: The compound TA-1 (10 ml) was dissolved in solvent ether (15 ml), an ethereal solution of diazomethane added and the reaction
mixture kept overnight at room temperature. Evaporation of the solvent yielded a monomethoxy derivative. TLC comparable.

TA-2

Elution of the column with petroleum ether: chloroform (3:1) afforded colourless crystals of TA-2, recrystallized from benzene, 210 mg (0.0058 % yield).

Rf: 0.6 (Benzene: acetone; 9:1).

t.p.: 152-153°C.

IR $\nu_{max}$ (KBr): 3520, 2855, 2877, 795 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 3.87 (1H, brs, H$_2$-15a), 3.66 (1H, brs, H$_2$-15b), 2.57 (1H, m, $\omega_{1/2}$=15.2Hz, H-6), 2.31 (1H, m, $\omega_{1/2}$=15.5Hz, H-10a), 1.60 (4H, brs, H$_2$-5, H$_2$-7), 1.25 (16H, brs, 8 x CH$_2$), 1.01 (3H, brs, Me-16), 0.88 (6H, brs, Me-14, Me-17).

$^{13}$C NMR (CDCl$_3$): $\delta$ 63.2 (C-15), 48.9 (C-4), 47.6 (C-6), 45.1 (C-10), 63.11 (C-1'), 41.3 (C-5), 40.2 (C-1), 38.3(C-3), 35.4 (C-2), 30.7 (C-7), 29.6 (C-8, C-9, C-11, C-12, C-13), 24.8 (C-16), 17.3 (C-17), 14.5 (C-14).

$\text{+ve FAB-MS m/z (rel. int.) : 254 [M]$^+$ (C$_{17}$H$_{34}$O) (12.8), 239 (11.3), 225 (10.6), 211 (11.2), 197 (12.3), 169 (23.6), 155 (53.6), 141 (29.3), 127 (42.8).}$

Acetylation of TA-2: Compound 2 with pyridine-acetic anhydride yielded monoacetyl derivative.
Elution of the column with petroleum ether; chloroform (1:1) afforded colourless crystals of TA-3, recrystallized from chloroform: acetone (1:1), 335 mg (0.0093 % yield).

Rf: 0.55 (chloroform: acetone; 8:2).

m.p.: 199- 201°C.

IR \( \nu_{\text{max}} \) (KBr): 3500, 3450, 3300, 2920, 2845, 1699, 1638, 1472, 1395, 1260, 1120 cm\(^{-1}\).

\(^1\)H NMR (CDCl\(_3\)): Table 1.1

\(^{13}\)C NMR (CDCl\(_3\)): Table 1.2

\(+ve \ FAB-MS\) \(m/z\) (rel. int.): 650 [M]\(^+\) \((C_{36}H_{58}O_{10}) (6.3), 470 (12.6), 452 (8.2), 425 (7.9), 410 (5.3), 392 (8.7), 264 (11.3), 246 (10.3), 219 (9.1), 206 (11.2), 204 (8.7), 201 (8.8), 188 (9.1), 186 (8.7).

Acid hydrolysis of TA-3: Compound TA-3 (25 mg) was dissolved in ethanol (10 ml), dilute hydrochloric acid added and heated for 2 hr on a steam bath. The reaction mixture was extracted with chloroform (3 x 10 ml), the organic phase washed with water (2x 10 ml), dried sodium sulphate and evaporated to get aglycone. The residue was concentrated and chromatographed over TLC. The sugar was identified as D-glucose.
TA-4

Elution of the column with chloroform gave colourless crystals of TA-4, recrystallized from benzene: acetone (1:1), 236 mg (0.0065 % yield).

Rf: 0.66 (chloroform: methanol; 9:1).

m.p.: 188–189°C.

IR $\nu_{\text{max}}$ (KBr): 3510, 3460, 3300, 2956, 2860, 1697, 1636, 1474, 1360, 1210, 1120 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): Table 1.1

$^{13}$C NMR (CDCl$_3$): Table 1.2

$^+$ve FAB-MS m/z (rel. int.): 486 [M]$^+$ (C$_{30}$H$_{46}$O$_5$) (22.3), 441 (12.3), 423 (12.1), 408 (21.9), 390 (25.6), 248 (31.6), 238 (10.3), 220 (18.1), 203 (46.5), 202 (48.3), 188 (42.6), 187 (50.6), 172 (31.8).

TA-5

Elution of the column with chloroform: methanol (99:1) furnished colourless crystals of TA-5, recrystallized from benzene: chloroform (1:1), 330 mg (0.0091 % yield).

Rf: 0.7 (chloroform: methanol; 9:1).

m.p.: 252–253°C.
UV $\lambda_{\text{max}}$ (MeOH):

IR $\nu_{\text{max}}$ (KBr): 3510, 3460, 3300, 2950, 2860, 1640, 1375, 1210, 1120 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): Table 1.1

$^{13}$C NMR (CDCl$_3$): Table 1.2

$^+ve$ FAB-MS $m/z$ (rel. int.): 720 [M]$^+$ (C$_{41}$H$_{68}$O$_{10}$) (11.0), 557 (11.2), 411 (10.1), 396 (9.8), 394 (9.2), 273 (22.6).

TA-6

Elution of the column with chloroform: methanol (98:2) yielded colourless crystals of AK-6, recrystallized from chloroform: methanol (1:1), 235 mg (0.0065 % yield).

$R_f$: 0.57 (chloroform: methanol; 6:4).

m.p.: 138-139°C.

IR $\nu_{\text{max}}$ (KBr): 3510, 3450, 3300, 3280, 2950, 2840, 1699, 1640, 1473, 1370, 1210, 1120 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): Table 1.1

$^{13}$C NMR (CDCl$_3$): Table 1.2

$^+ve$ FAB-MS $m/z$ (rel. int.): 488 [M]$^+$ (C$_{30}$H$_{48}$O$_2$) (5.6), 264 (13.5), 249 (9.3), 240 (8.9), 222 (48.5), 207 (56.3), 205 (13.6), 192 (18.5).
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Coupling constants are given in parentheses in Hertz.
Table 1.2: $^{13}$C NMR spectral data of TA-1, TA-3 to TA-6

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Chapter-1

TERMINALIA ARJUNA

DISCUSSION

TA-1

Compound TA-1, named terminaloic acid, was obtained as colourless crystalline mass from petroleum ether eluants. It responded positively to Liebermann-Burchard test, yielded effervescences with NaHCO₃ solution and exhibited characteristic IR absorption bands for hydroxyl group (3445, 3410 cm⁻¹), carboxylic group (3390, 1685 cm⁻¹) and for unsaturation (1635 cm⁻¹). It had a molecular ion peak at m/z 488 corresponding to a triterpenic molecule C₃₀H₄₀O₅. It indicated seven double bond equivalents; five of them were adjusted in a pentacyclic triterpenic skeleton and remaining two in carboxylic function and olefinic linkages. The important ion peaks appearing at m/z 470 [M-H₂O]⁺, 452 [470-H₂O]⁺ and 434 [452-H₂O]⁺, suggested the presence of three removable hydroxyl groups in the molecule. The prominent ion fragments appearing at m/z 224 and 264, generated due to retro-Diels-Alder fragmentation pattern and the subsequent ion peaks at m/z 206[224-H₂O]⁺, 246[264-H₂O]⁺, 231[246-Me]⁺, 187[231- CO₂]⁺, 249[264 -Me]⁺, 205[249- CO₂]⁺, 190[205-Me]⁺, 219[264-CO₂H]⁺ and 201[219-H₂O]⁺ supported the presence of two hydroxyl groups in rings A/B and one hydroxyl and one carboxylic groups in rings D and E (Scheme 1.1).

The ¹H NMR spectrum of TA-1 displayed a one-proton double doublet for carbinol proton at δ 3.64, placed at C-3 on the basis of biogenetic analogy,
and its coupling interactions of 4.1 and 9.1 Hz indicated its β orientation. A one-proton multiplet at δ 3.27 with half width of 8.1 Hz and a one-proton signal at 3.06 with half width of 7.9 Hz were ascribed to β-oriented H-6 and H-22 hydroxymethine protons, respectively. A one-proton broad deshielded signal at δ 5.38 was accounted vinylc H-12. A six-proton signal at δ 0.94 was associated with C-23 and C-30 tertiary methyl protons. Five three-proton broad signals at δ 0.86, 0.79, 0.73, 1.00 and 1.27 were attributed to C-24, C-25, C-26, C-27 and C-29 tertiary methyl protons. The appearance of all the methyl protons in the region δ 1.27-0.73 suggested that these functionalities were attached on saturated carbons. The remaining methylene and methine protons resonated in the region δ 2.93-0.85 (Table 1.1).

The 13C NMR spectrum of TA-1 showed the presence of 30 carbon atoms in the molecule. The C-12 and C-13 vinylic carbons resonated at δ 123.0 and 142.5. The carbon signals at δ 80.4, 76.6 and 82.5 were accounted to C-3, C-6 and C-22 carbinol carbons, respectively. A deshielded signal at δ 179.6 as ascribed to C-28 carboxylic carbon. The remaining carbon signal appeared in the range δ 54.5-22.8. The absence of any carbon signal near δ 18.5 indicated the location of one of the hydroxyl group at C-6. The assignments of carbon chemical shifts were made by comparison with the δ values of the corresponding carbon atoms in the structurally similar compounds, viz., oleanolic acid, cincholic acid and gypsogenic acid (Mahato et al., 1994). Treatment of TA-1 with acetic anhydride and pyridine afforded a triacetyl derivative (1a), IRvmax 3300, 1735, 1730, 1725 cm⁻¹.
indicating the presence of three acetylable secondary hydroxyl groups. Jones oxidation of TA-1 furnished 3-oxo derivative, which responded positively to Zimernmann test for 3-oxo group. Methylation of TA-1 with diazomethane yielded a methoxy derivative. On the basis of these evidences, the structure of the natural product TA-1 has been formulated as olean-12-en-3β, 6α, 22α-triol-28-oic acids. This is a new triterpenic acid isolated from a natural or synthetic source for the first time.
Scheme 1.1: Mass fragmentation of terminaloic acid (TA-1).
Compound TA-2, named arjunasesquiterpenol, was obtained as colourless crystalline product from petroleum ether: chloroform (3:1) eluants. Its IR spectrum demonstrated the presence of a characteristic absorption band for hydroxyl group (3520 cm⁻¹). Its mass spectrum displayed a molecular ion peak at m/z 254 corresponding to a structural formula of a homosesquiterpene molecule C₁₇H₃₄O. It had one double bond equivalent adjusted in a monocyclic carbon skeleton. The prominent ion peaks at m/z 239 [M-Me]⁺, 225 [M-C₂H₅]⁺, 211 [M-C₃H₇]⁺, 197 [M-C₄H₇]⁺, 169 [M-C₅H₁₃]⁺, 155 [M-C₆H₁₅]⁺, 141 [M-C₇H₁₇]⁺ and 127 [M-C₈H₁₉]⁺ suggested that the molecule possessed a C₉-side chain attached to a hydroxy substituted dimethyl cyclohexane ring (Scheme 1.2).

The ¹H NMR spectrum of TA-2 showed two one-proton broad signals at δ 3.87 and 3.66 assigned to oxygenated C-15 methylene protons. A three-proton broad signal at δ 1.01 was attributed C-16 tertiary methyl protons. A six-proton broad signal at δ 0.88 was associated with C-14 and C-17 methyl protons. Two one-proton multiplets at δ 2.57 with half-width of 15.2 Hz and 2.31 with half-width of 15.5 Hz were accounted to α-oriented C-6 and C-10 methine protons. Two broad signals at δ 1.60 (4H) and 1.25 (16H) were due to the methylene protons.

The ¹³C NMR spectrum of TA-2 exhibited a oxygenated methylene carbon signal at δ 63.2. The carbon signals at δ 24.8, 17.3 and 14.5 were assigned
to C-16, C-17 and C-14 methyl groups, respectively. The C-6 and C-10 methine carbons resonated at δ 47.6 and 45.1, respectively. The remaining methylene carbons appeared in the range δ 41.3-29.6. The absence of any signal beyond δ 3.87 in the ¹H NMR spectrum and beyond δ 63.2 in the ¹³C NMR ruled out the existence of an olefinic linkage in the molecule.

Acetylation of TA-2 yielded a monoacetyl derivative indicating the presence of one acetylable hydroxyl group in the molecule. On the basis of the foregoing account, the structure of TA-2 has been established as 4-methyl-4-hydroxy-methylene-6β-(10-methyl octanyl)-cyclohexane. This is an unreported homosesquiterpene isolated from a natural source for the first time.

![Scheme 1.2: Mass fragmentation pattern of arjunasesesquiterpenol (TA-2).](attachment:image.png)

Scheme 1.2: Mass fragmentation pattern of arjunasesesquiterpenol (TA-2).

**TA-3**

Compound TA-3, named arjuna terminaloide, was obtained as a colourless crystalline mass from petroleum ether: chloroform (1:1). It responded
positively to glycoside tests and yielded effervescences with sodium bicarbonate solution. It showed characteristic IR absorption bands for hydroxyl group (3500, 3450 cm⁻¹), carboxylic group (3300, 1699 cm⁻¹) and unsaturation (1636 cm⁻¹). It had a molecular ion peak at m/z 650 in the mass spectrum corresponding to the molecular formula C₃₆H₃₅O₁₀. It indicated eight double bond equivalents, five of which fully adjusted to the pentacyclic rings of the triterpenic skeleton and the remaining one each to the vinylic linkage, carboxylic group and sugar moiety. The mass spectrum of TA-3 exhibited diagnostically important fragmentations at m/z 470[M-C₆H₁₂O₆]+, 452[470–H₂O]+, 425[470–COOH]+, 410[425–Me]+ and 392[410–H₂O]+ supporting the presence of two removable hydroxyl groups and one carboxylic function in the molecule. The retro-Diels-Alder fragmentation pattern of AK-3 generated typical ion peaks at m/z 264[C₁₆H₂₄O₃]+, 246[264–H₂O]+, 201[246–COOH]+, 186[201–Me]⁺, 219[264–COOH]+, 204[219–Me]⁺, 206[M–C₁₆H₂₄O₃–C₆H₁₂O₆]+ and 188[206–H₂O]+ indicating the existence of the glycosidic linkage in ring A/B, placed at C-3 on the basis of biogenetic considerations, one hydroxyl group in ring A/B, vinylic linkage in ring C and one hydroxyl and one carboxylic functions in ring D/E (Scheme 1.3).

The ¹H NMR spectrum of TA-3 displayed a one-proton double doublet at δ 5.41 (J=7.8, 8.1 Hz) assigned to vinylic H-12, two carbinol one proton double doublets at δ 3.64 (J=3.9, 9.6 Hz), and 3.45 (J=6.3, 8.7 Hz) ascribed to α-oriented H-3 and H-22, a one-proton multiplet at δ 3.57 (w = 15Hz) associated with α-oriented H-6, three one proton broad signal at δ 4.81 accounted to anomic H-1' and at δ 3.28 and 3.21 attributed to
oxygenated H₂-6' methylene protons and four one-proton multiplets at δ 3.27, 3.52, 3.39 and 4.78 due to H-2', H-3', H-4' and H-5' carbinol protons of glucose moiety. Five three-proton broad signals at δ 0.94, 0.90, 0.75, 0.70 and 1.26 and a six-proton broad signal at δ 1.26 were assigned to tertiary methyl signals, all attached to saturated carbon atoms. The remaining methine and methylene proton signals resonated between δ 2.90-1.03 (Table 1.1).

The ¹³C NMR spectrum of TA-3 exhibited the existence of 36 carbon signals of the molecule. A deshielded signal at δ 175.8 was accounted to C-29 carboxylic group. Signals at δ 123.2 and 142.1 were assigned to vinylic C-12 and C-13 carbons. A signal at δ 93.5 was due to anomeric C-1'. The carbinol carbon signals appeared between δ 82.5-60.9 (Table 1.2). The degree of protonation of each carbon was determined by DEPT experiments. The absence of carbon signals near δ 18.5 and 33.1 indicated the existence of one of the hydroxyl group at C-6 and carboxylic function C-29. The assignments of carbon chemical shifts were made by comparison with δ values in the corresponding carbon atoms in the structurally similar compounds (Mahato and Kundu, 1994).

The ¹³C DEPT NMR spectrum showed the presence of seven methyl, nine methylene, 12 methine and 8 quaternary carbons. Acid hydrolysis of TA-3 yielded aglycone and glucose. On the basis of these findings compound TA-3 was identified as olean-12-en-3β,6β,22β-triol-29-oic acid-3β-D-glucopyranoside. This is an unknown triterpenic glycoside isolated from a natural or synthetic origin for the first time.
Scheme 1.3: Mass fragmentation pattern of arjuna terminaloide (TA-3).
Compound TA-4, designated as terminaloic acid B, was obtained as colourless crystals from chloroform eluants. It gave a positive Liebermann-Burchard test, yielded effervescence with sodium bicarbonate solution and exhibited characteristic IR absorption bands for hydroxyl group (3510, 3460 cm\(^{-1}\)), carboxylic group (3300, 1697 cm\(^{-1}\)) and unsaturation (1636 cm\(^{-1}\)). Its mass spectrum displayed a molecular ion peak at m/z 486 consistent to a triterpenic carboxylic acid C\(_{30}\)H\(_{46}\)O\(_5\). It indicated a eight double bond equivalents; five of them were adjusted in a pentacyclic triterpenic carbon framework, two in vinylic linkages and one in carboxylic function. The prominent ion peaks in the mass spectrum at m/z 441 [M–COOH]^+, 423 [441– H\(_2\)O]^+, 408 [423–Me]^+ and 390 [408– H\(_2\)O]^+ supported the existence of one carboxylic group in the molecule. The retro-Diels-Alder fragmentation pattern of TA-4 yielded ion fragments at m/z 238 and 248 and subsequent ion peaks at m/z 220 [238 – H\(_2\)O]^+, 202 [220 – H\(_2\)O]^+, 187 [202 – Me]^+, 172 [187 – Me]^+, 203 [248 – COOH]^+ and 188 [203 – Me]^+ suggested the location of three hydroxyl group in ring A/B, one each vinylic linkage in ring B and C and carboxylic function in ring D/E [Scheme 1.4] (Ali, 2001).

The \(^1\)H NMR spectrum of TA-4 showed a one-proton multiplet at \(\delta\) 5.37 and a one proton broad signal at \(\delta\) 5.23 assigned to vinylic H-6 and H-12, respectively. Two one-proton doublets at \(\delta\) 3.52 (J=12 Hz) and 3.61 (J=9.9 Hz) and a one-proton double doublet at \(\delta\) 3.36 (J=9.9, 12 Hz) were
accounted correspondingly to hydroxymethine H-1, H-3 and H-2. Five three-proton broad signals at δ 0.99, 0.89, 0.75, 1.00 and 1.27 and a six-proton broad signal at δ 0.94 were attributed to seven tertiary methyl protons all attached to saturated carbons. The remaining methine and methylene protons resonated in the range of δ 2.93-0.96 (Table 1.1).

The $^{13}$C NMR spectrum of TA-4 showed the presence of 30 carbon signals. The important carbon signals appeared at δ 179.5 (C-28), 143.2 (C-5), 142.5 (C-13), 122.9 (C-12) and 121.0 (C-6). The hydroxymethine carbons appeared at δ 77.0 (C-1), 67.2 (C-2) and 80.4 (C-3). The assignment of carbon chemical shifts was made by comparison with the δ values of the corresponding carbon atoms in the structurally similar compounds (Mahato and Kundu, 1994). Methylation of TA-4 with diazomethane yielded a mono-methoxy derivative.

On the basis of the above mentioned evidence the structure of TA-4 has been elucidated as olean-5, 12-dien-1β, 2β, 3β-triol-28-oic acid.
Scheme 1.4: Mass fragmentation pattern of terminaloic acid B (TA-4).
Compound TA-5, a sterol glycoside, was obtained as colourless crystals from chloroform: methanol (99:1) eluants. It gave positive test for a steroidal glycoside. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3510, 3460, 3300 cm⁻¹) and unsaturation (1640 cm⁻¹). Its mass spectrum exhibited important ion peaks at m/z 557[M-C₆H₁₁O₆]⁺, 411[M-C₁₂H₂₁O₉]⁺, 396[411-Me]⁺, 394[M-C₁₂H₂₂O₁₀]⁺ and 273 [411-C₁₀H₁₉, side chain]⁺ suggesting that compound is a diglycoside of stigmasterol.

The ¹H NMR spectrum of TA-5 showed three one-proton deshielded vinylic proton signals as a one-proton broad signal at δ 5.31 assigned to H-6 and as a two-proton broad signal at δ 5.29 ascribed to H-22 and H-23. A one-proton broad multiplet at δ 3.58 with half-width of 16.5 Hz was attributed to α-oriented alpha H-3 carbinol protons. Two three-proton broad signals at δ 0.67 and 1.09 were associated with C-18 and C-19 tertiary methyl protons, respectively. The C-21, C-26, C-27 secondary and C-29 primary methyl proton signals appeared as doublets at δ 0.91 (J=6.5 Hz), 0.84 (J=6.2 Hz), 0.82 (J=6.3 Hz) and 0.80 (J=6.3 Hz), respectively. The H-1' and H-1'' anomeric protons resonated as one-proton broad signals at δ 4.83 and 4.80. The carbinol protons between δ 4.28 – 3.17 were accounted to two glucosyl residue present in the molecule. The remaining methine and methylene protons appeared in the range δ 2.23 – 1.13 (Table 1.1).
The $^{13}$C NMR spectrum of TA-5 showed vinylic carbon signals at δ 148.6 (C-5), 126.4 (C-6), 127.6 (C-12) and 145.3 (C-13), carbinol carbon at δ 81.8 (C-3), anomeric carbons at δ 106.0 and glucose carbons in the range δ 83.6 – 60.61.

The acid hydrolysis of TA-5 afforded glucose and stigmasterol. On the basis of the foregoing account the structure of TA-5 has been identified as stigmasterol-3β-D-glucopyranosido-1',4'-glucopyranoside.

TA-6

Compound TA-6, named terminaloic acid C., was obtained as a colourless crystals from chloroform; methanol (98:2) eluants. It gave positive Liebermann-Burchard test and effervescence with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3510, 3450, 3300 cm$^{-1}$), carboxylic group (3280, 1699 cm$^{-1}$) and unsaturation (1640 cm$^{-1}$). Its mass spectrum exhibited a molecular ion peak at m/z 488 corresponding to a pentacyclic triterpenic acid C$_{30}$H$_{48}$O$_{5}$. It had seven double bond equivalents; five of them were adjusted in a pentacyclic carbon framework and one each in vinylic linkage and carboxylic function. The retro-Diels-Alder ion fragments appearing at m/z 240 and 264 and subsequent ions 222[240- H$_2$O]$^+$, 207[222- Me]$^+$, 192[207- Me]$^+$, 249[264- Me]$^+$ and 205[249- CO$_2$]$^+$ suggested the location of the three hydroxyl groups in rings A and B and one each hydroxyl and carboxylic group in ring D/E (Scheme 1.5) (Ali, 2001).
The \(^1\)H NMR spectrum of TA-6 showed a one-proton broad signal at \(\delta 5.26\) assigned to vinylic H-12. Two one-proton double doublets at \(\delta 3.48\) (J = 5.5,
9.1 Hz) and 3.35 (J= 5.6, 9.6 Hz) and a one proton multiplets at δ 3.68 with half-width are attributed to the carbino! H-3α, H-22α and H-6β, respectively. A nine proton broad at δ 0.96 was accounted to tertiary methyls Me-23, Me-24 and Me-30. Four three proton broad signals at δ 0.76, 0.74, 1.01 and 1.26 were ascribed to tertiary methyls Me-25, Me-26, Me-27 and Me-29, respectively.

The 13C NMR spectrum of TA-6 showed 30 carbon signals in the molecules. Three deshielded carbon signals at δ 180.2, 123.9 and 142.7 were assigned corresponding to carboxylic C-28 and vinylic C-12 and C-13. The hydroxy methine carbon signals appeared at δ 79.2 (C-3), 68.1 (C-6), 83.0 (C-9) and 81.0 (C-22). The remaining methylene and methine carbons resonated in the range δ 54.9-16.0. The absence of any carbon signal near δ 18.1 and 47.0 suggested the location of the hydroxyl groups at C-6 and C-9. The degree of protonation of each carbon was determined by DEPT experiments and the assignment of carbon chemical shifts were made by comparison with δ values in the corresponding carbon atoms in the structurally similar triterpenes (Mahato and Kundu, 1994; Ali, 2001). The DEPT NMR spectra showed the presence of seven methyls, eight methylene, six methine and nine quaternary carbons. Acetylation of TA-6 yielded a triacetyl derivative suggesting the existence of three acetylatable and one quaternary hydroxyl groups in the molecules. Methylation of TA-6 with diazomethane gave monomethyl derivative 6a.

On the basis of these evidences the structure of TA-6 has been established as olean-12-en-3β, 6α, 9α, 22β-tetraol-28-oic acid. This is a new oleanane-type triterpene of plant origin.
Compounds isolated from *Terminalia arjuna*

**TA-1**

**TA-2**

**TA-3**
F2 - Acquisition Parameters
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Time: 12.07
NMR Spectrometer: 200 MHz
Sample: 5.0 mm Muli
Diluent: CD2Cl2
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TR/TE: 0.09123 ms
AQ: 1.0000000 ns
BG: 1.0000000 Hz
SN: 0.0000000 sec

--------------- CHANNEL 1 --------------
MCH: 100.00 us
CS: 0.00 us
SF: 75.4576 Hz

--------------- CHANNEL 2 --------------
MCH: 100.00 us
CS: 0.00 us
SF: 75.4576 Hz

F3 - Processing parameters
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TR: 3.00 ms
TE: 0
PD: 0.00 Hz

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REFERENCES


