Part 1

PHYTOCHEMICAL INVESTIGATION OF SOME MEDICINAL PLANTS AND STUDIES ON THE BIOLOGICAL ACTIVITIES OF THE ISOLATED COMPOUNDS

Chapter 1

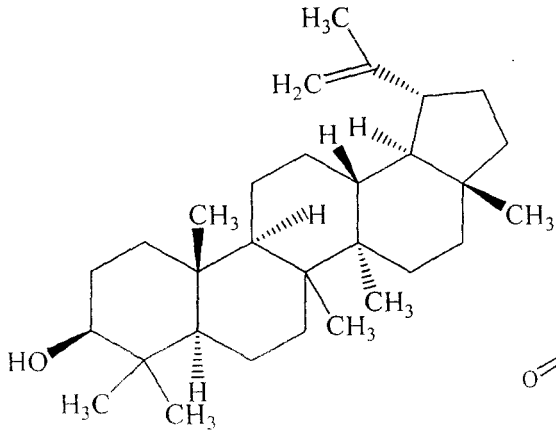
A SHORT REVIEW ON THE PHYTOCHEMICAL INVESTIGATION OF MEDICINAL PLANTS AND BIOLOGICAL ACTIVITY OF THE ISOLATED PHYTOCONSTITUENTS.

This chapter is divided into two sections, Section A and Section B

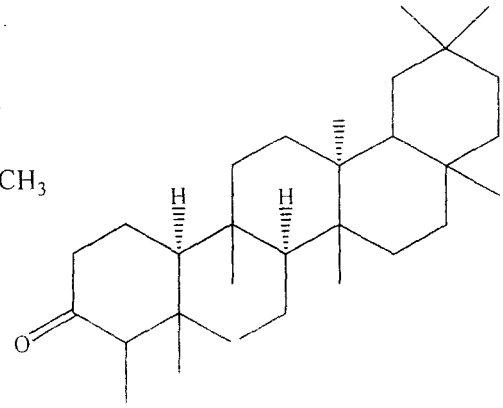
Section A: A short review on the Phytochemical Investigation of medicinal plants

The northern part of West Bengal commonly called as North Bengal is endowed with diverse natural resources. Darjeeling hills and Tarai region of West Bengal are full of flora and more than 6000 different plant species having medicinal value. The tribal medicinal practice in the above region provides the evidence of the utilization of medicinal plants by the local people as a folk lore. Our laboratory is actively involved in chemical investigation on Medicinal plants of Darjeeling hill and Tarai region. As a result a number of new di and triterpenoids have been isolated and chracterised so far. Potent pharmacological activity of such type of compounds has been documented recently by some group of workers. The observation of the previous workers in concord with the present line of investigation is being presented, in a selective manner, in the following paragraphs.

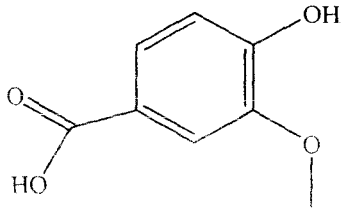
Rahaman *et al.* [1] reported that two new triterpenoids, 18 alpha, 19 beta-20(30)taraxasten-3 beta, 21 alpha-diol (cichoridol) (1) and 17-epi-methyl-6-hydroxyangolensate (intybusoloid) (2) obtained from the methanolic extract of seeds Cichorium intybus (Asteracae) along with eleven known compounds, lupeol (3), friedelin (4), betunaldehyde (9), syrginic acid (10), vanillic acid (11), 6,7-dihydroxycoumarin (12) and methyl-alpha-D-galactopyranoside (13). Compound 1 and 11 reported to exhibit a good alpha-glucosidase inhibitory activity.



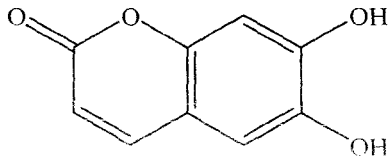
1(lupeol)



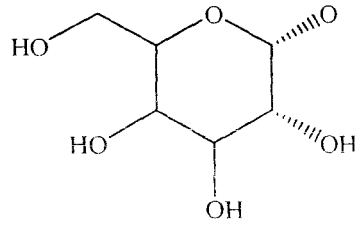
friedelin



Vanillic acid

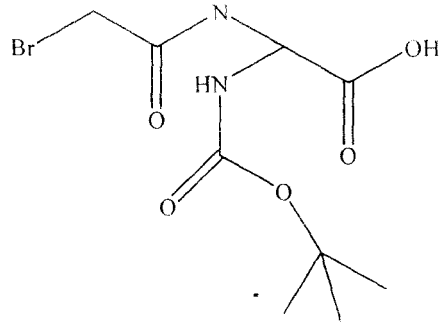


6, 7-dihydroxycoumarin

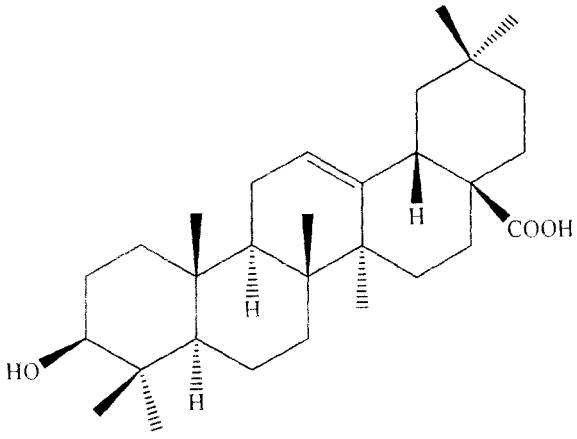


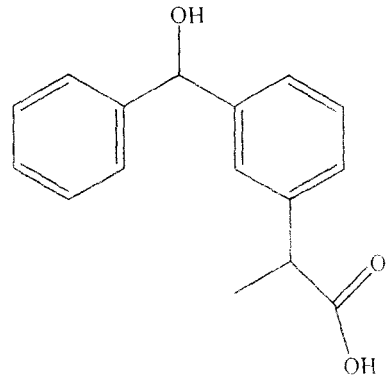
Methyl-α--D-galactose

Choudhury *et al.* [2] reported that biotransformation of a pentacyclic triterpene, oleanolic acid (1), with *Fusarium lini* afforded two oxidative metabolities, 2α, 3β–dihydroxy-olean-12-en-28-oicacid (2), and 2α, 3β, 11β–trihydroxyolean-12-en-28-oicacid(3). They also found that metabolities 3 is a new compound. The structures were characterized on the basis of spectroscopic studies. These metabolities exhibited a potent inhibition of α–glucosidase enzyme and thus are effective in diabetes by delaying the glucose absorption.



2α, 3β, 11β–trihydroxyolean-12-en-28-oic acid

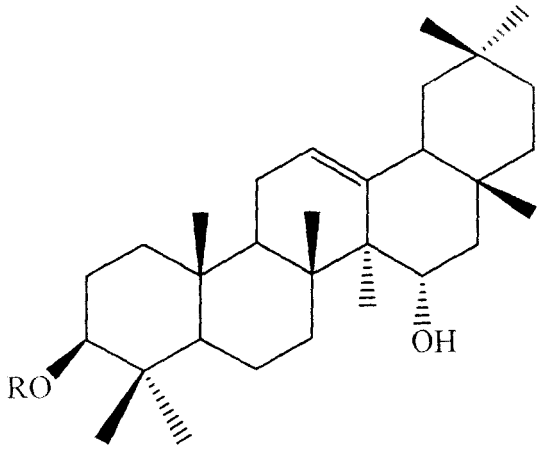


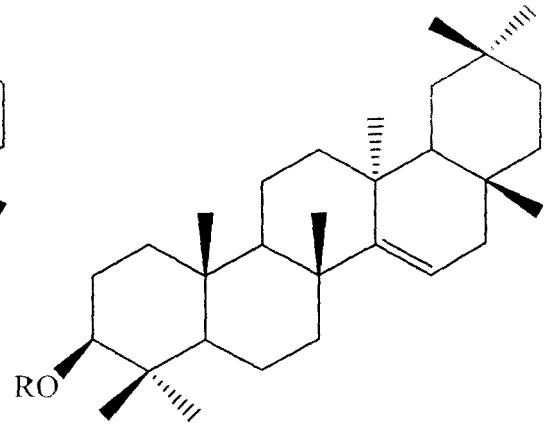


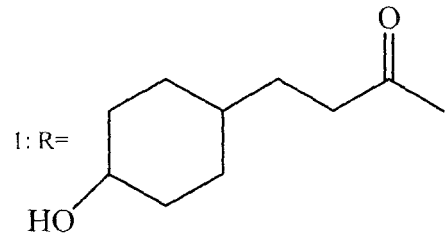
2 (Oleanolic acid)

2α, 3β–dihydroxy-olean-12-en-28-oicacid

Li *et al.* [3] isolated seven pentacyclic triterpenoids including 3β–o–coumaryl (1) 15α–hydroxy, β–amyrin (2), 3β–taraxerol (3), 3β–taraxerol formate (4), 3β–taraxerol acetate (5), 3β–o–(E)–coumaryl-taraxerol (6) and 3-β-o-(Z)-coumaroyl-taraxerol (7) from the stems and twigs of the mangrove plant *Rhizophora stylosa* (Rhizophorace). The structures of the isolated compounds were determined by extensive analysis of their spectroscopic data. They found that among these metabolites, compound 1 is a new olenane type terpenoid coumaroyl ester, while compound 4 is a new natural product obtained for the first time.







1. R = 3β–o–coumaryl

2. R=H=15α–hydroxy, β–amyrin

3. R=H=3β–taraxerol

4. R=Formyl=3β–taraxerol formate

5. R=Acetyl=3β–taraxerol acetate

6. R=E-coumaryl=3β–o–(E)–coumaryl-taraxerol

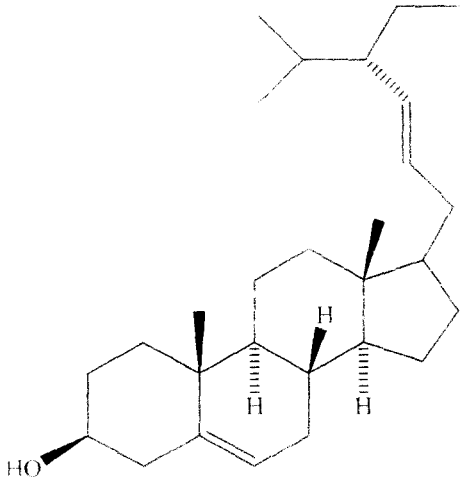
7. R=Z-coumaryl=3–β-o- (Z)-coumaroyl-taraxerol

He *et al.* [4] isolated the chemical constituents of the roots of *Aconitum taipaicum* (Ranunlaceae) and purified using silica gel column chromatography. They found new norditerpenoid alkaloids, isodelelatine along with five known alkaloids. The structure of the new compound was elucidated on the basis of spectral data.

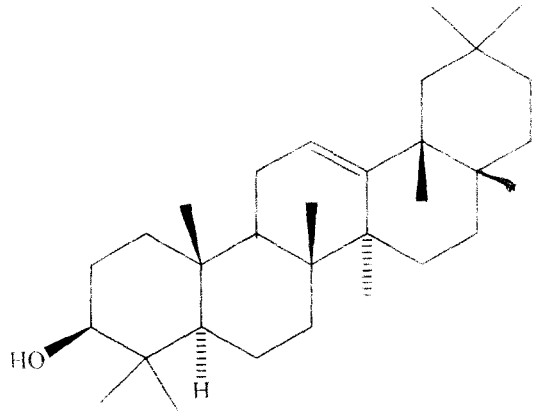
Srikrishna *et al.* [5] carried out antibacterial activity using cup plat method by pet. ether, chloroform, methanol and water extract of the bark of *Aporosa lindleyana* (Euphorbiaceous). They observed that the compounds showed moderate to very good activity against *Bacillus subtilis*, *Escherichia coli* and compared with the standard drug tetracycline. They studied the antifungal activity against *Penicillium chrysogenous*, *Candida albicans*, *Aspergilus niger* and *Trichoderma vridar* and compared with the standard drug fluconazole. The pet.ether extract showed considerable activity towards all the four fungal organism. Analgesic activity has been carried out on Swiss albino male mice by abdominal constriction method. All the extracts showed moderate analgesic activity while methanol extract showed very good activity.

Ohtsu *et al.* [6] isolated four known and four abietane diterpenes from the $CHCl_3$ extract of the *Larix kaempferi*. A known compound 13, 14-seco-13, 14-et-13-en-18-oic acid was isolated from natural sources for the first time. Their structure was determined by chemical and spectropic methods and crystallographic analysis. They studied the inhibitory effects of these compounds on EBV-EA activation induced by tumor promoter and results are reported.

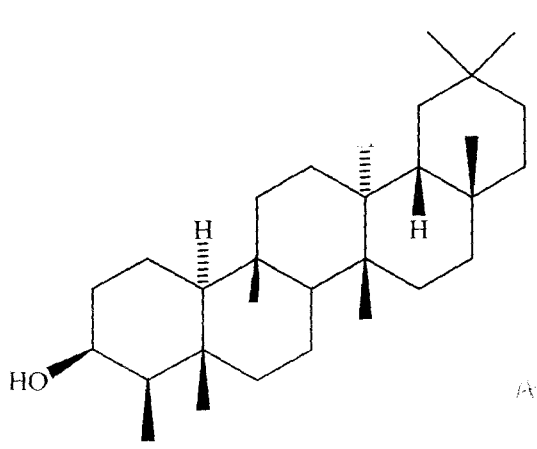
Singh *et al.* [7] extracted a mixture of triterpenoids: β–sitosterol, stigmasterol, β–amyrin, friedelan-3β–ol (epifridelenol), cycloartenone, β–amyrin acetate, friedelin and epi-friedenyl acetate *Heliotropium marifolium* using hexane as a solvent. They tested the isolated triterpenoids against selected pathogenic bacteria and fungi, e.g. *Escherichia coli, Staphylococcus aureus, Aspergillus niger and Penicillium chrysogenum.* They also discussed the quantification and assessment of their growth inhibitory potency and found that cycloartenone was the major triterpenoids in both in *vivo* and *vitro* cell culture.



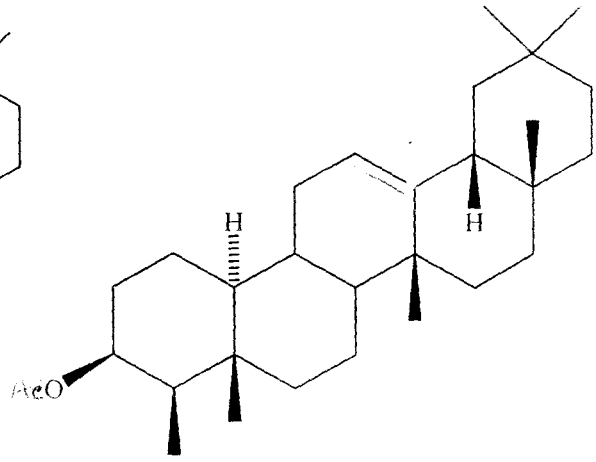
Stigmasterol



Betaamyrin



Friedelan-3β-ol



β–amyrin acetate

Larshini *et al.* [8] extracted 12 plants selected on the basis of the folk-medicine reports and examined their anti bacterial effects against eight pathogenic bacteria. They found that the n-butanol extract of *Calotropis procera* flowers and the aqueous extract of *Eugenia caryophyllata* were the most effective against the bacteria tested.

Shrimali *et al.* [9] extracted compounds from the dried stem bark of *Ailanthus excelsa* using different solvent and studied their antibacterial activity against different bacterial strains. The ethyl acetate (EA) fraction inhibited the growth of all test bacteria. The MIC of the EA fraction was found to be 6 mg/disc.

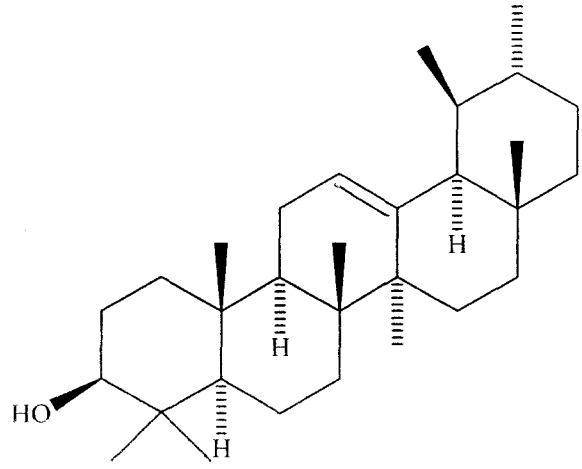
Pichai *et al.* [10] extracted the powdered material of "Vidattali" equated to *Dichrostachys cinerea* and separated n-octacosanol, β–sitosterol, friedelin, epifridelinol, α-amyrin and β–sitosterol-3-β-D-glucopyranoside in the aerial part. They were studied antibacterial and antifungal activities of n-hexane and chloroform extracts on four bacteria *Escherichia coli*, *Pseudomonas aeruginasa*, *Staphylococcus aureus* and two fungi *Aspergillus flavus* and *Mucor* sp. at 1.25, 2.5, 5.0 and 10 mg/ml concentration levels in nutrient agar and SDA mediums respectively by steak method. They observed that the chloroform extract showed moderate inhibition of *E.coli* and *Staphylococcus* at higher concentrations of 5-10 mg/ml. Antifungal activities of these extracts against *Aspergillus* and *Mucor* were observed at higher concentration.

Xu *et al.* [11] isolated Geumonoid, a new triterpene from *Geum japonicum* and its structure was elucidated on the basis of 1D, 2D NMR and MS spectroscopic analysis. They observed that Geumonoid showed inhibitory activity against HIV-1 protease.

Sukul and Chaudhuri [12] extracted the leaves of *Lantana canara* using different solvents. They observed that four fractions of petroleum ether extract showing significant antibacterial activity against some human pathogens under in *vitro* conditions. The MIC of the methanol fraction, containing triterpenoids, active against these pathogens was found to be comparable with those of some therapeutically used antibiotics.

Panizzi *et al.* [13] isolated some constituents from the flowering aerial parts of *Geum rivale* and studied their antimicrobial activity on bacteria and fungi. The activity was concentrated in the triterpenes fraction and, for Gram-positive and Gram-negative bacteria, and also in the flavonoids fraction.

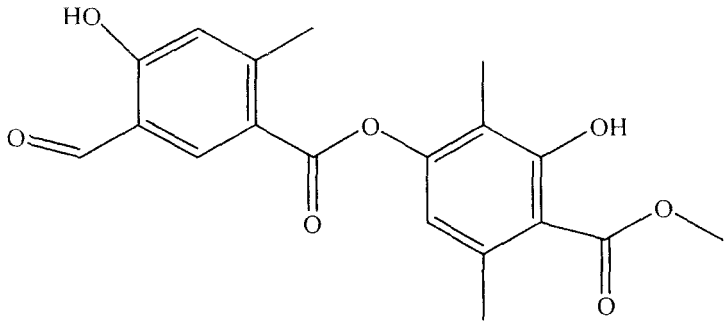
Pitchai and Saraswath [14] extracted n-octacosanol, β–sitosterol, friedelin, epifridelinol, α-amyrin and β–sitosterol-3-β-D-glucopyranoside from the aerial part of *Dichrostachys cinerea* using different solvents. They studied the antibacterial and antifungal activities of n-hexane and chloroform extracts in four bacteria at 1.25, 2.5, 5.0 and 10 mg/ml concentration in nutrient agar medium by streak method. They observed that n-hexane showed 100 percent inhibition to the growth of *Escherichia coli* and *Pseudomonas aeruginosa* in all concentrations whereas *Staphylococcus aureus* and *S.albus* were moderately affected at 5.0 and 10 mg levels. The $CHCl_3$ extract showed moderate inhibition at higher concentration to *S.albus* and *E.coli.* Antifungal activities of these extracts against *Aspergillus flavus* and *Mucor* sp. were found at higher concentration.



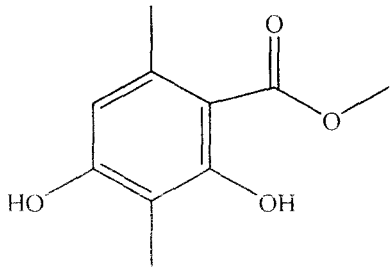
α-amyrin

Chizozem *et al.* [15] isolated two new friedelane-type triterpenes named 12alpha-hydroxyfridelane-3,15-dione and 3beta-hydroxyfriedelan-25-al, together with six known compounds from the stems of *Drypetes paxii* Hutch.(Euphorbiaceae) and established their structures. They also tested the antimicrobial activity of the five friedelane-type triterpenes, one olean-12-ene triterpene saponin against some Gram-positive and Gram-negative bacteria and they appeared to be modestly active.

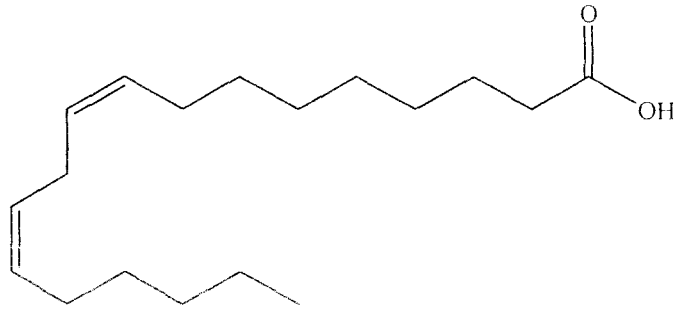
Mutai *et al.* [16] isolated three new pentacyclic triterpenoids : (20R)-3-oxolupan-30-al (1), (20*S*)-3-oxolupan-30-al (2) and (20*R*)-28-hydroxylupen-30-al-3-one (3), along with (20*S*)-3β-hydroxylupan-30-al (4) and the known metabolites 30-hydroxylup-20-(29)-en-3-one (5), 30-hydroxylup-20-(29)-en-3β-ol (6), atranorin, methyl 2,4-dihydroxy-3,6-dimethylbenzoate, sitosterol-3β-O-glucoside and linoleic acid from *Acacia mellifera* . The structures of the new metabolites were elucidated by extensive spectroscopic analyses and their relative stereochemistry was determined by NOESY experiments. They observed that the new metabolite 3 exhibited significant cytotoxic activity against the NSCLC-N6 cell line, derived from a human non-small-cell bronchopulmonary carcinoma.



Atranorin

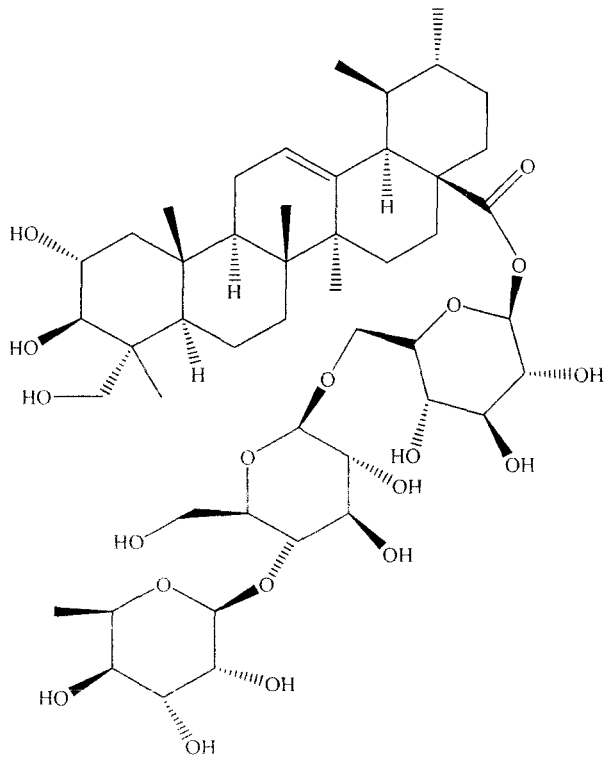


Methyl2,4-dihydroxy-3,6-dimethylbenzoate

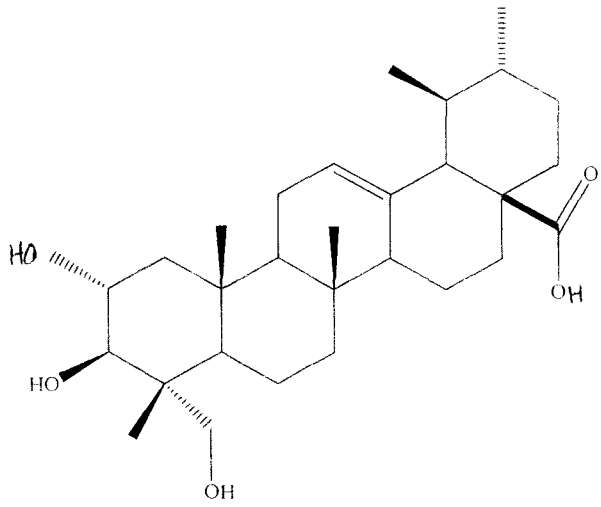


Linoleic acid

James and Dubery [17] accumulated large quantities of pentacyclic triterpenoid saponins collectively known as centelloids from Centella asiatica. These terpenoids include asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiatic-, brahmic-, centellic- and madecassic acids. They studied biological activity of these compounds, the Centella triterpenoids can be regarded as phytoanticipins due to their antimicrobial activities and protective role against attempted pathogen infections. They reported that these plant-derived pharmacologically active compounds have complex structures, the production of secondary metabolities by cultured cell provides a particularly important benefit of manipulate and improve the production of the desired compounds.



Asiaticoside



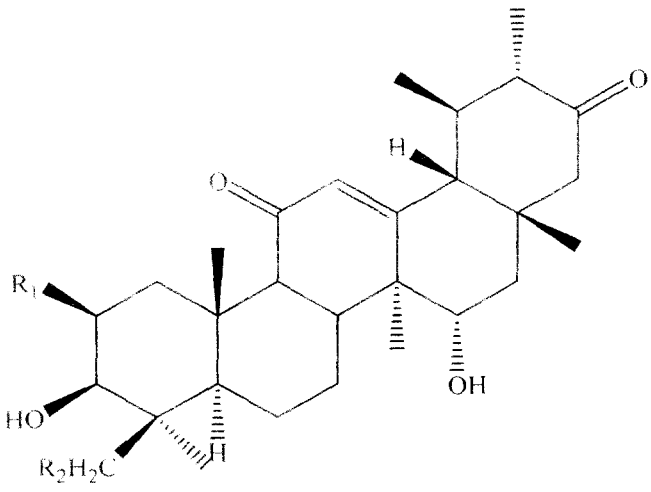
Asiatic acid

Antonia *et al.* [18] extracted lupane triterpenoid lupeol, the ursane triterpenoid α–amyrin and esters of these compounds from the bark of roots of *Alstonia boonei* and observed that these compounds have anti-inflammatory properties. They found that □amyrin is a competitive inhibitor of bovine trypsin and chymotrypsin, lupeol linoleate, lupeol palmitate and α–amyrin linoleateare non-competitive inhibitor of chymotrypsin. They also found that lupeol, α–amyrin, palmitic and linoleic acid esters of these compounds are very weak inhibitors of porcine pancreatic elastase and of *Lucilia cuprina* and *Helicoverpa punctigera* leucine aminopeptidases.

Ghosh *et al.* [19] extracted seeds of *Swietenia mahagoni* using methanol as a solvent and reported pharmacological activity including anti-inflammatory activity of the extract. They evaluated the anti-inflammatory activity using acute, sub-chronic, and chronic models of inflammation in rodents. The anti pyretic and analgesic activities were evaluated in mice models. They studied the acute toxicity of the extract using different doses and the effect was compared with the standard drug, ibuprofen. The results revealed

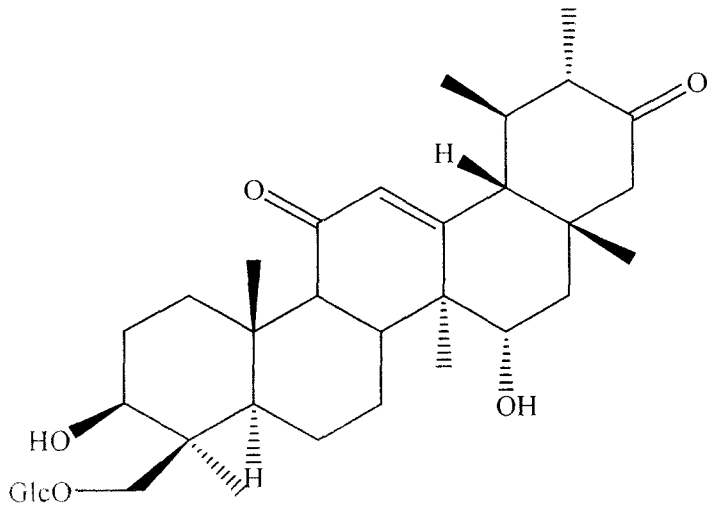
that the extract produces anti-inflammatory activity through dual inhibition of the cyclo-oxygenase and lipo-oxygenase pathways of archidonic acid metabolism.

Zhou *et al.* [20] isolated three new triterpenoids, 11,21-dioxo-2beta,3beta,15alpha-trihydroxyurs-12-ene-2-O-beta-D-glucopyranoside (1), 11,21-dioxo-3beta,15alpha,24-trihydroxyurs-12-ene-24-O-beta-D-glucopyranoside (2), and 11,21-dioxo 3beta, 15alpha,24-trihydroxyolean-12-ene-24-O-beta-D-glucopyranoside (3), and two new flavonoids, apigenin-7-O-[2''-O-(5'''-O-feruloyl)-beta-D-apiofuranosyl]-beta-D-glucopyranoside (4) and chrysoeriol-7-O-[2''-O-(5'''-O-feruloyl)-beta-D-apiofuranosyl]-beta-d-glucopyranoside (5) from the whole plant of fresh *Apium graveolens* together with 10 known flavonoids. The structures of the new compounds were elucidated by analysis of spectroscopic data. The evaluated the inhibitory effects of the compounds isolated on nitric oxide production in lipopolysaccaride-activated macrophages.

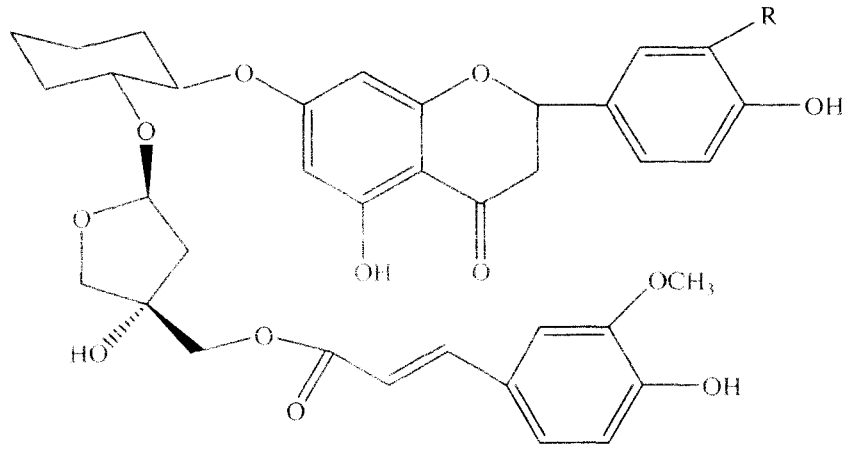


1 R_1=OGlc, R_2=H ; 11,21-dioxo-2beta,3beta,15alpha-trihydroxyurs-12-ene-2-O-beta-D-glucopyranoside

2R_1=H R_2=OGlc ; 11,21-dioxo-3beta,15alpha,24-trihydroxyurs-12-ene-24-O-beta-D-glucopyranoside



3 = 11,21-dioxo-3beta,15alpha,24-trihydroxyolean-12-ene-24-O-beta-D-glucopyranoside



4 R=H; apigenin-7-O-[2"-O-(5"'-O-feruloyl)-beta-D-apiofuranosyl]-beta-D-glucopyranoside

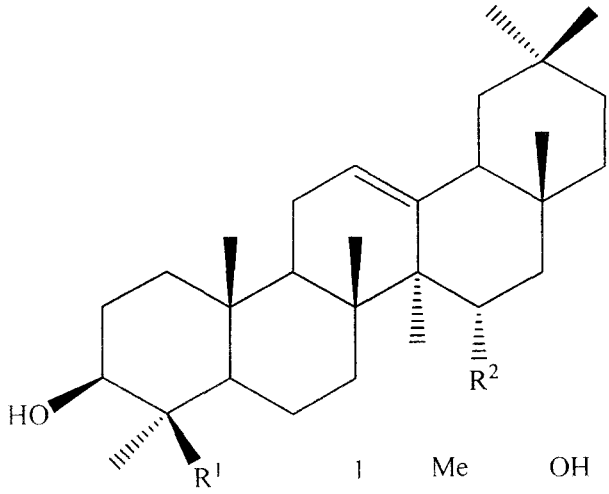
5 R=OCH_3; chrysoeriol-7-O-[2"-O-(5"'-O-feruloyl)-beta-D-apiofuranosyl]-beta-d-glucopyranoside

Angeh *et al.* [21] isolated four known triterpenoids, 1alpha,3beta-dihydroxy-12-oleanen-29-oic (1), 1-hydroxy-12-olean-30-oic acid (2), 3,30-dihydroxyl-12-oleanen-22-one (3), and 1,3,24-trihydroxyl-12-olean-29-oic acid (4) along with a new pentacyclic triterpenoid (1alpha,23-dihydroxy-12-oleanen-29-oic acid-3beta-O-2,4-di-acetyl-L-rhamnopyranoside) 5 through a bioassay-guided procedure from the leaves of *Combretum imberbe*. The structures of the compounds were elucidated on the basis of 1D and 2D

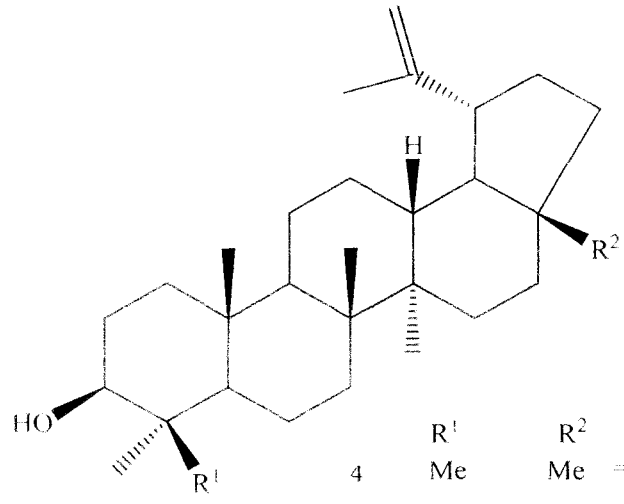
NMR experiments, as well as mass spectrometric data. They observed that all the isolated compounds have moderate (62 microg/ml) to strong (16 microg/ml) antibacterial activity (MIC values) against *Staphylococcus aureus* and *Escherichia coli*, with 1 and 5 being most active. The results of this study give credence to the ethnomedicinal use of Combretum imberbe and expand our knowledge on the biological activity of its metabolites.

Mathabe *et al.*[22] extracted four known compounds, two triterpenoids, compound 1 [d-friedoolean-14-en-oic acid (3-acetyl aleuritolic acid)] and compound 2 (lupeol), and two diterpenes, compound 3 [ent-2,6alpha-dihydroxy-norbeyer-1,4,15-trien-3-one (diosphenol 2)] and compound 4 (ent-3beta-hydroxy-beyer-15-ene-2-one) from the bark of *Spirostachys africana* using ethanol as a solvent . They were tested the antibacterial activity of the isolated compounds using micro-dilution method and observed that Compound 1, exhibited minimum inhibitory concentration (MIC) of 50 microg/ml against *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Escherichia coli* and *Shigella dysentery*.

Wada *et al.* [23] isolated lupane- and oleanane- type triterpenoids from the bark of *Phyllanthus flexusus* and screened inhibitory activity on human Topos (topoisomerases) I and II. They found that olean-12-en-3β, 15α-diol (1), olean-12-en-3β, 15α, 24-triol (3), lupeol (4), and betulin (6) were selective catalytic inhibitors of human Topo II activity with IC_{50} values in the range of 10-39 μM.



		1	Me	OH	= olean-12-en-3 15 -diol
R^1	R^2	3	CH_2OH	OH	= olean-12-en-3 , 15 , 24-triol

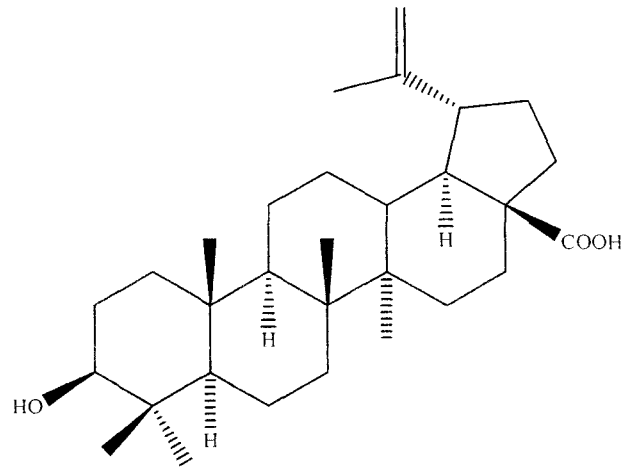


	R^1	R^2	
4	Me	Me	= Lupeol
6	Me	CH_2OH	= Betulin

Li *et al.* [24] extracted a new lupane type triterpenoid, 3β, 11α–dihydroxy-30-norlupan-20-one and six known lupane triterpenoids from the whole plant of Salvia roborowskii Maxim using petroleum ether as a solvent. They elucidated their structures by means of spectral methods including NMR and MS techniques.

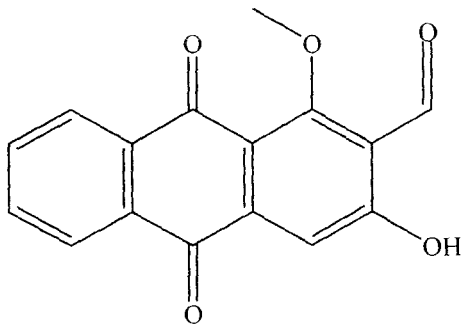
Tostikoya *et al.* [25] studied the biological activity of natural and semi synthetic lupane triterpenoid and discussed in two-part review. The first part was devoted to the pharmacological properties of natural lupane triterpenoids. They reported that betulinic

acid proven to be the most effective antitumor agent among more than fifty natural lupanes.

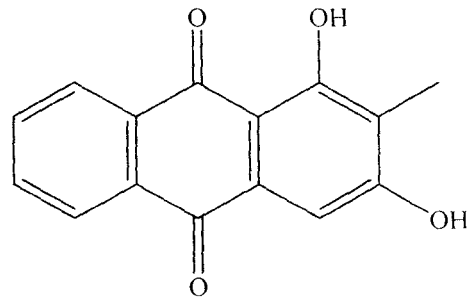


betulinic acid

Kanokmedhakul *et al.* [26] isolated a new 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone **(1)**; six known anthraquinones, nordamnacanthal **(2)**, damnacanthal **(3)**, rubiadin **(4)**, rubiadin-1-methyl ether **(5)**, lucidin-ω-methyl ether **(6)**, and 1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone **(7)**; a β-sitosterol **(8)**; together with two known triterpenoids, β-acetylolean-12-en-28-olic acid **(9)**, and 3β-O-acetyl-11α,12α-epoxyolean-28,13-olide **(10)** from the roots and stems of *Prismatomeris fragrans*. Their structures were established on the basis of spectral data. This was the first isolation of compounds **2, 6, 7, 9** and **10** from *Prismatomeris* genus. They studied the antiplasmodial, antituberculosis, antifungal and anticancer cell lines tests of the isolated compounds and the bioactivity assays showed that only **9** exhibited moderate antimalarial activity, **2** and **3** exhibited antifungal activity while **2, 3, 4, 7** and **9** showed antituberculosis activity. In addition, compounds **2, 3** and **7** exhibited cytotoxicity to BC cell line while **1, 1a** (the methyl ether derivative of **1**), **2, 3, 4, 5,** and **9** exhibited cytotoxicity to NCI-H187 cell line.

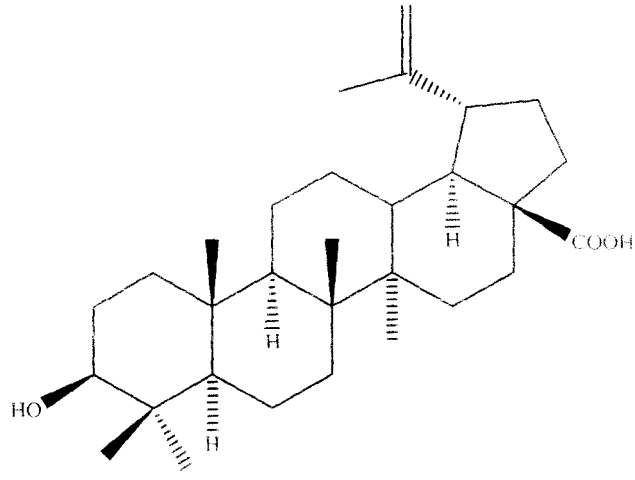


Damnacanthal



Rubiadin

Setzer *et al.* [27] extracted the crude from the bark of Syncarpia glomulifera using chloroform as a solvent and reported antibacterial and cytotoxic activity. They isolated oleanolic acid-3-acetate, ursolic acid-3-acetate and betulinic acid from the bark. They observed that the relatively large abundance (10 % of the crude extract) and high degree of activity of betulinic acid were responsible for the bioactivity of the crude bark extract.

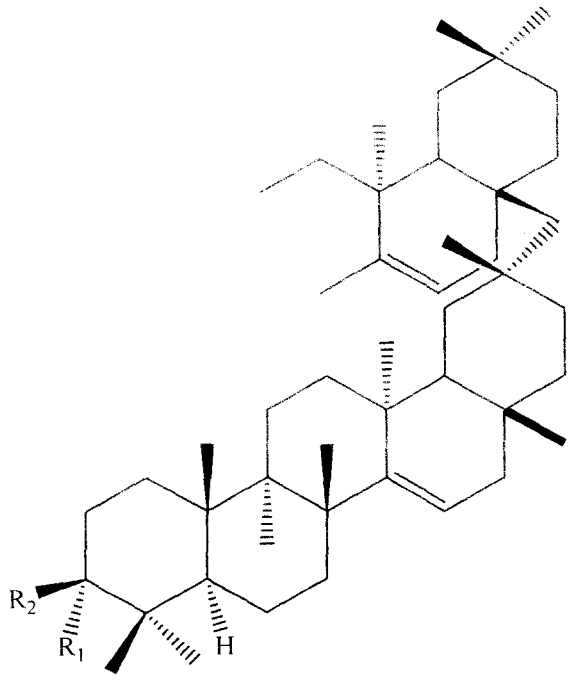


betulinic acid

Lutskii *et al.* [28] isolated triterpenoids from the plants of the *Thalictrum genus* and the structural, chemical and spectral properties were systematized for the first time. They discussed the features of the ^{13}C NMR spectra of cycloartane triterpenoids and also gave the data for the biological activities of certain cycloartane and oleanane triterpenoids.

Li *et al.* [29] isolated seven pentacyclic triterpenoids including 3β-*O*-(*E*)-coumaroyl-15α-hydroxy-β-amyrin (**1**), 15α-hydroxy-β-amyrin (**2**), 3β-taraxerol (**3**), 3β-taraxerol formate (**4**), 3β-taraxerol acetate (**5**), 3β-*O*-(*E*)-coumaroyl-taraxerol (**6**), and 3β-*O*-(*Z*)-coumaroyl-taraxerol (**7**) from the stems and twigs of the mangrove plant *Rhizophora stylosa*. The structures of the isolated compounds were determined by extensive analysis of their spectroscopic data. They reported that among these metabolites, compound **1** is a new oleanane-type triterpenoid coumaroyl ester, while compound **4** is a new natural product obtained here as an isolated substance for the first time.

Laphookhieo *et al.* [30] isolated six new pentacyclic triterpenoids esters (1-6) together with 3α- and 3β-tarexerol from the fruits of *Bruguiera cylindrica*. The structures of the new compounds were characterized as 3α-E-feruloyltaraxerol (1), 3α-Z-feruloyltaraxerol (2), 3 β-E-feruloyltaraxerol (3), 3 β–Z-feruloyltaraxerol (4), 3α-E-coumaroyltararaxerol (5), and 3α-Z-coumaroyltaraxerol (6). They reported that compounds 2 and 6 exhibited weak cytotoxicity against the NCl-H187 cell line.



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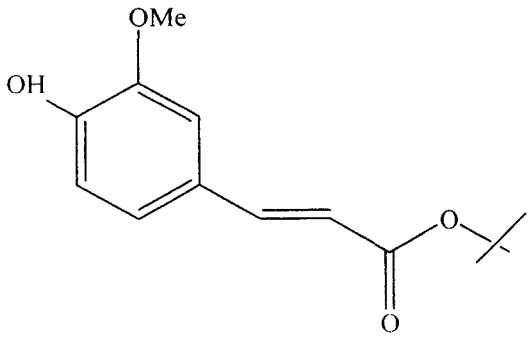
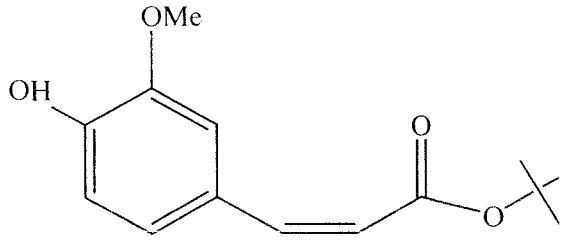
R_1 R_2

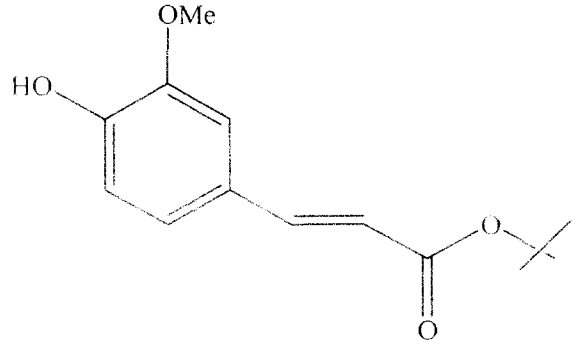


H = 3α–E-feruloyltaraxerol



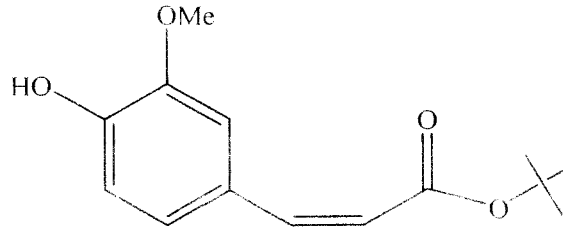
H =3α–Z-feruloyltaraxerol

H

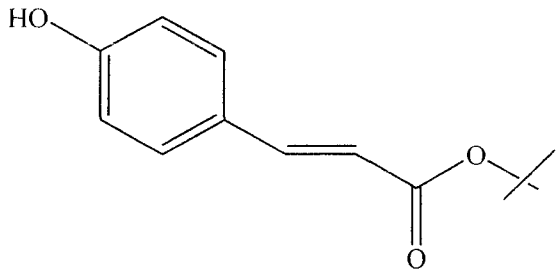


= 3β–E-feruloyltaraxerol

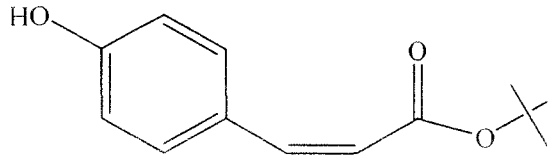
H



=3β–Z-feruloyltaraxerol

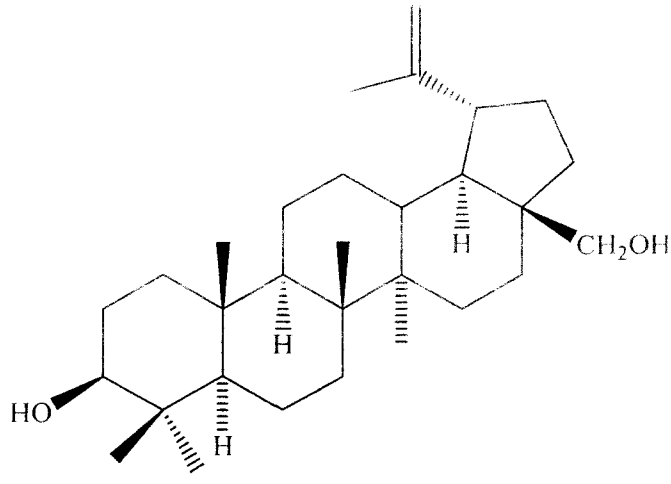


H = 3α–E–coumaroyltaraxerol



H = 3β–Z–coumaroyltaraxerol

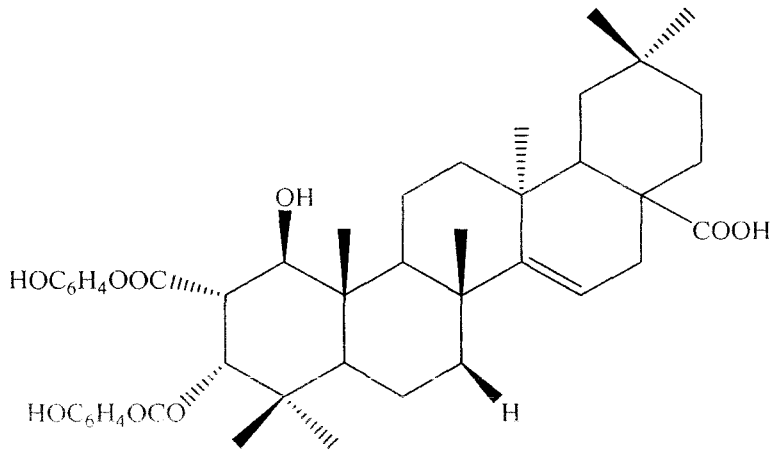
Araujo and Chaves [31] isolated eleven oleanane, ursane and lupane-type triterpenes daturadiol (3β,6β–dihydroxy-olean-12-ene), 3β-hydroxy-30-norlupan-20-one, lupenone, β-amyrenone, α–amyrenone, lupeol, β-amyrin, α–amyrin, betulin, erythrodiol and uvaol, in addition to squalene, sitosterol and α–tocopherol from the leaves of *Terminalia brasiliensis* Camb. They identified the structures of these compounds by ^{1}H and ^{13}C NMR spectral analysis.



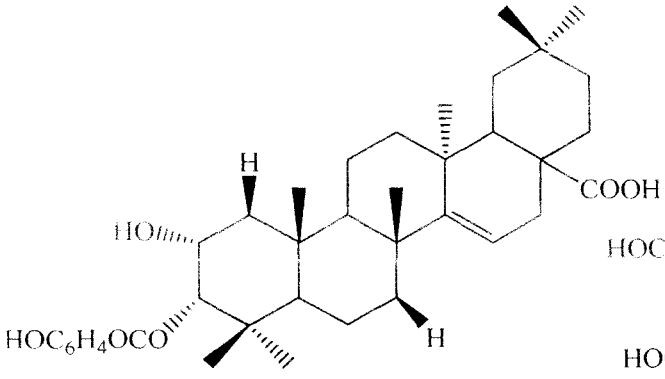
4 (Betulin)

Chaudhuri *et al.* [32] isolated pentacyclic triterpenoids based on the taraxer-14-ene skeleton with a C-28 attached carboxylic acid group from the roots of *Maprounea*

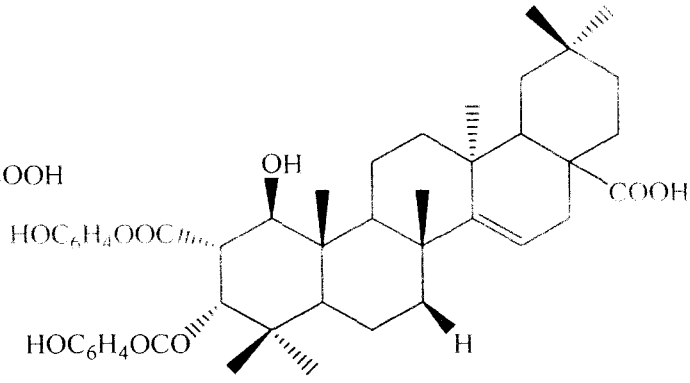
africana. They identified these compounds as 1β, 2α-dihydroxyaleuritolic acid 2,3-bis-hydroxybenzoate[1], 2□-hydroxyaleuritolic acid 3-p-hydroxybenzoate [2], 2α-hydroxyaleuritolic acid 2,3-bis-p-hydroxybenzoate[4], aleuritolic acid 3-p-hydroxybenzoate[5], aleuritolic acid [6], and aleuritolic acid 3-acetate [7]. They reported that compounds 1 and 2 are new triterpene esters.



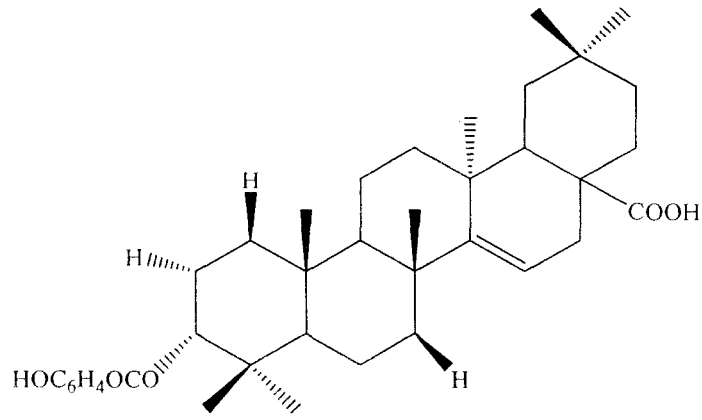
1β, 2α-dihydroxyaleuritolic acid 2,3-bis-hydroxybenzoate



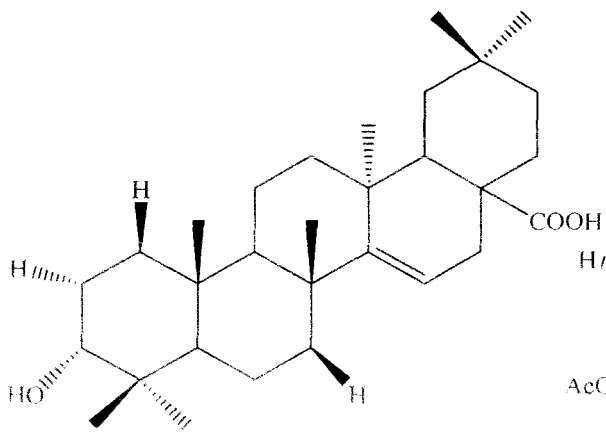
2α-hydroxyaleuritolic acid 3-p-hydroxybenzoate



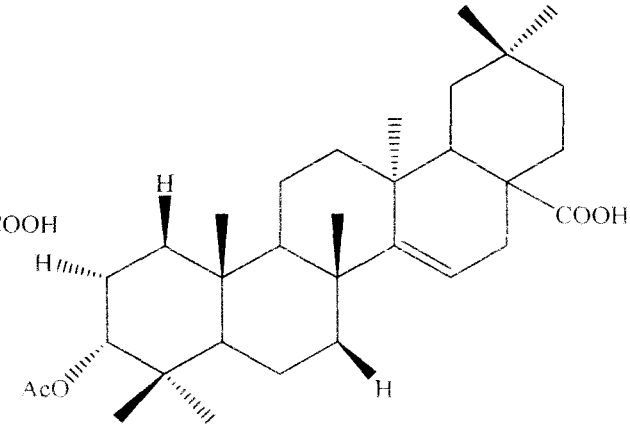
2α-hydroxyaleuritolic acid 2,3-bis-p-hydroxy benzoate



Aleuritolic acid 3-p-hydroxybenzoate

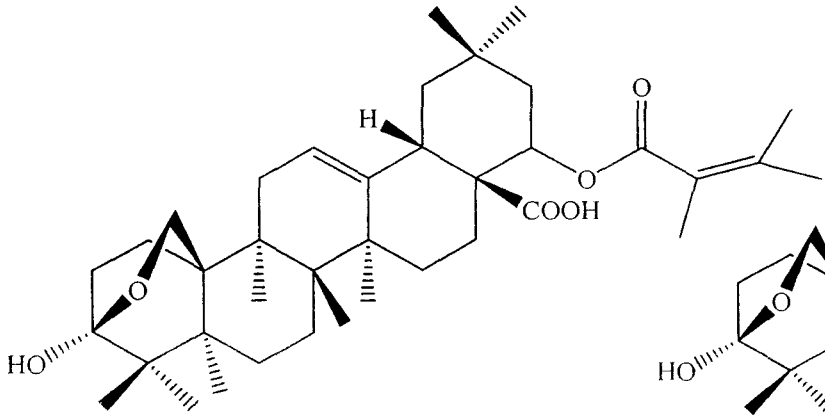


Aleuritolic acid

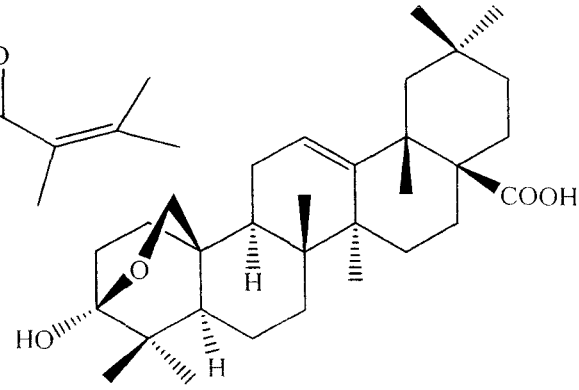


Aleuritolic acid 3-acetate

Begum *et al.* [33] isolated three new pentacyclic triterpenoids, camaryoloic acid (1), methylcamaralate (2) and camangeloyl acid (3) with six known compounds, □-sitosterol 3-O-□-D-glucopyranoside (4), octadecanoic acid (5), docosanic acid (6), palmitic acid (7), camaric acid (8) and lantanolic acid (9) from the aerial parts of *Lantana camara*. They elucidated the structures of the new compounds by spectroscopic and chemical methods.



Camaric acid



lantanolic acid

Machocho *et al.* [34] isolated five oleanane-type pentacyclic triterpenoids by chromate graphic separation from the stem bark of Embelia schimperi using chloroform solvent. They reported that three compounds have a methyleneoxy bridge and two compounds ,embelinone and schimperinone were first time extracted from natural source. Their structures were determined by spectroscopic techniques, among which 2-D NMR were useful for complete characterization. They observed that three of the triterpenoids exhibited mild antibacterial properties against the Gram-positive bacterial strain Rhodococcus sp.

Siddiqui *et al* [35] isolated nine pentacyclic triterpenoids along with a coumarin from a fresh, undried and uncrushed spring leaves of *Plumeria obtuse*. They characterized the new triterpenes obtusin and obtusilic acid as the 24-E and 27-Z p-coumaric esters of the novel 3β, 24-dihydroxyrus-12-en-28-oic acid and 3β,27-dihydroxyrus-12-en-30-oic acid respectively through chemical and spectral studies while the other eight compounds identified were known kaneroside, oleandrin, α-amyrin, neriucoumaric acid, isoneriucoumaric acid, alphitolic acid, oleanonic acid, methyl p-E-coumarate and scopoletin.

Karalai and Laphookhieo [36] isolated three new pentacyclic triterpenoid esters 1-3 together with six known lupane-type triterpenoids from *Bruguiera cylindrica*. They elucidated the structures of the new compounds by spectroscopic methods and were

characterized as 3α-E-coumaroyllupeol 1, 3α-Z-coumaroyllupeol 2 and 3α-E-caffeoyltaraxerol 3.

Begum *et al.* [37] isolated three pentacyclic triterpenoids including one new guajavanoic acid (2) and two known obtusinin (1) and goreishic acid I (3) from the leaves of *Psidium guajava* . They characterized the new constituent 2 as 2 α-hydroxy-3 β-p-E -coumaroyloxyurs-12, 18-dien-28-oic acid through 1 H-NMR and 13 C-NMR. They isolated compound 1 and 3 first time from the genus *Psidium.*

Laphookhieo *et al.* [38] isolated a new sesquiterpene (1) and two new pentacyclic triterpenoid esters (2,3) together with three known compounds (4-6) from the fruits of *Rhizophora mucronta.* They elucidated the structures of the isolated compounds and characterized as 3-hydroxy-3, 7, 11-trimethyl-9-oxododeca-1,10-diene(1),3β-E-caffeoyltaraxerol (2) and 3β-Z-caffeoyltaraxerol (3).

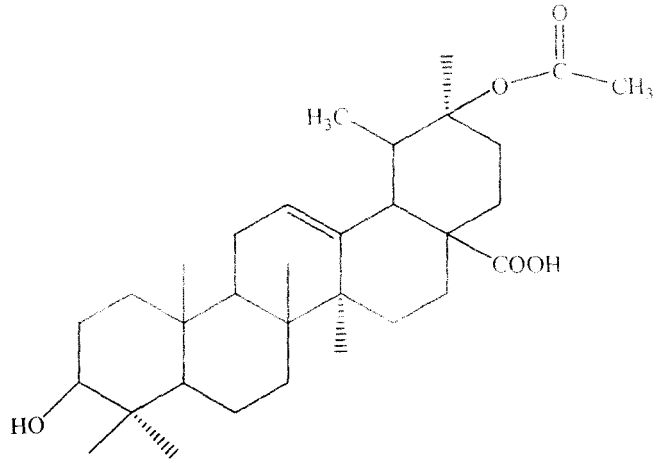
Ibrahim and Ali [39] isolated a long chain ketone, a pentacyclic triterpenoid coupled with fatty acid moiety, and an acyclic diterpenoid from the ethanol soluble part of *Nepeta crassifolia* collected from Kangavar, Iran. They elucidated the structures of all the metabolities with the aid of spectroscopic techniques, including 2D NMR experiments.

Tabopda *et al.* [40] isolated four new triterpene glucosides (1-4) using methanol as a solvent from the stem bark of *Terminalia superba.* The structures of the new compounds were established by spectroscopic method and characterized as alpha,3 beta-dihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (1), 2 alpha,3 beta, 21 beta-trihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (2), 2 alpha,3 beta, 29-trihydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (3) and 2 alpha,3 beta,23,27-tetrahydroxyolean-12-en-28-oic acid 28-O-beta-D-glucopyranoside (4) together with the known triterpene 2 alpha,3 beta,23-trihydroxyolean-12-en-28-oic acid (5). They investigated the antibacterial activity of 1-5 against two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), and four Gram-negative (*Escherichia coli, Shigella flexenari, Pseudomonas aeruginosa, Salmonella typhi*) bacterial strains.

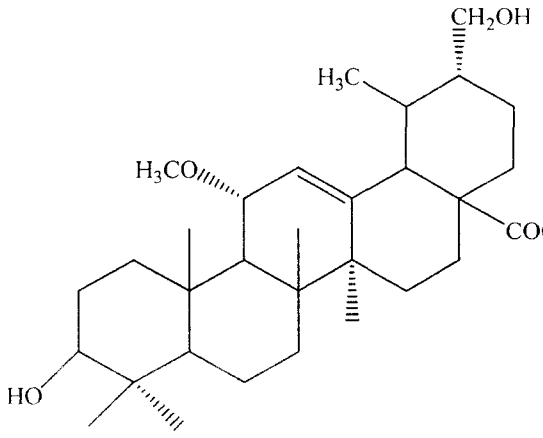
Siddiqui *et al.* [41] investigated the constituents of the fresh, uncrushed leaves of *Eucalyptus camaldulensis* var. obtusa and isolated a triterpenoid amirinic acid with four known triterpenoids ursolic acid lactone, betulinic acid, oleanolic acid and ursolic acid. They transformed amirinic acid into amirolide in deuterated chloroform at room temperature. The new products were characterized by exhaustive spectroscopic studies.

Siddiqui *et al.* [42] studied the fresh leaves of *Carissa carandas* collected from the Karachi Region in Pakistan and isolated four pentacyclic triterpenoids (1-4) including one new constituent carissin (1) and two hitherto unreported compounds 2 and 3. They elucidated the structure of the new compounds as 3beta-hydroxy-27-E-feruloyloxyurs-12-en-28-oic acid.

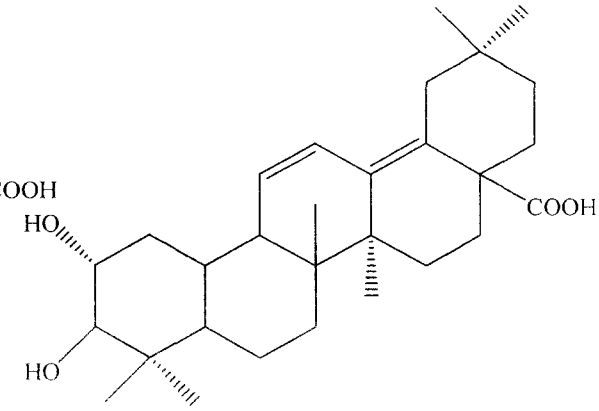
Begum and Farhat [43] investigated the constituents of fresh, uncrushed leaves of *E. camaldulensis* var. obtuse and isolated a known and 3 new triterpenoids. They characterized the new compounds by chemical and spectroscopic studies as camaldulic acid (20 beta-acetoxy-3 beta-hydroxyrus-12-en-28-oic acid), camaldulensic acid (3 beta, 30-dihydroxy-11 alpha-methoxyrus-12 en-28-oic acid) and camaldulenic acid (2 alpha, 3 beta-dihydroxyolean-11,13(18)-dien-28-oic acid)



Camaldulic acid



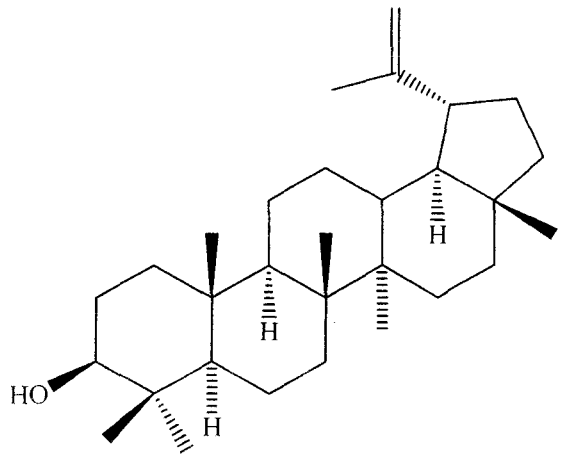
Camaldulensic acid



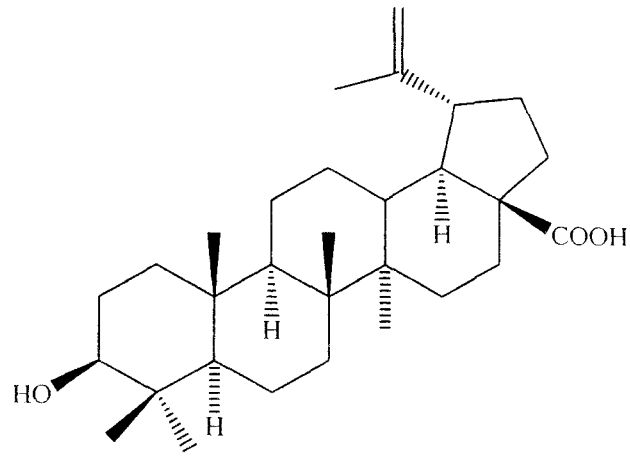
Camaldulenic acid

Begum *et al.* [44] isolated two triterpenoids, 20 beta-acetoxy-2 alpha,3 beta-dihydroxyrus-12-en-28-oic acid (guavanoic acid, 3) and 2 alpha,3 beta-dihydroxy-24-p-z-coumaroyloxyrus-12-en-28-oic acid (guavacoumaric acid,7) along with six known compounds 2 alpha-hydroxyursolic acid (1), jacoumaric acid (2), isoneriucoumaric acid (4), asiatic acid (5), ilelatifol D and (6) beta-sitosterol-3-O-beta-D-glucopyranoside (8) from the leaves of *Psidium guajava*. They determined the structures of the isolated compounds through spectroscopic methods.

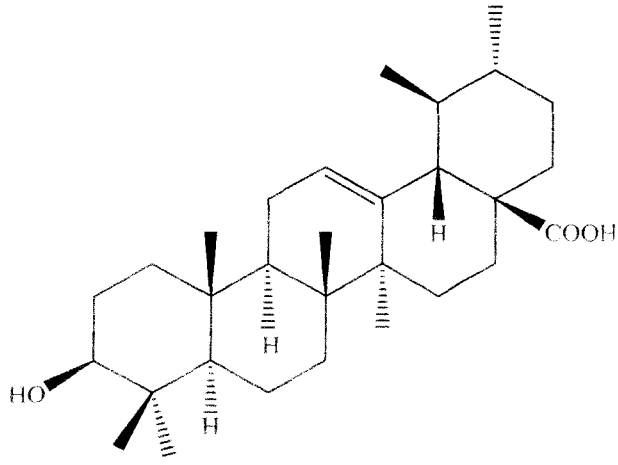
Shai *et al.* [45] isolated four compounds lupeol (1), betulinic acid (2), ursolic acid (3) and 2 alpha-hydroxyursolic acid (4) from the leaves of *Curtisia dentate*. They studied the antibacterial and antifungal activity using broth microdilution assay and bioautography method and found that betulinic acid, ursolic acid and 2 alpha-hydroxyursolic acid appreciably inhibited fungal growth with MIC values ranging from 8 to 63μg/ml.



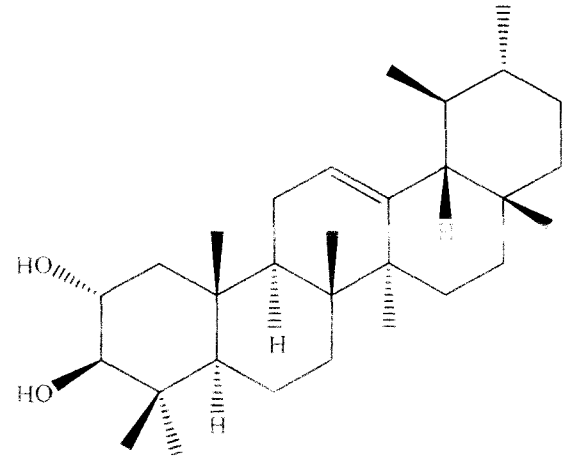
Lupeol



Betulinic acid

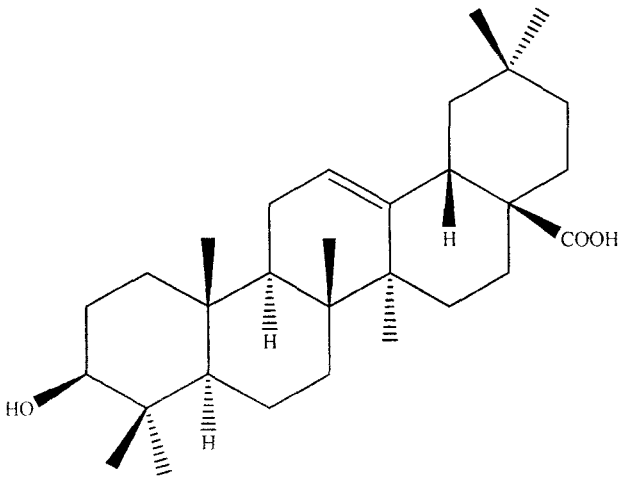


Ursolic acid

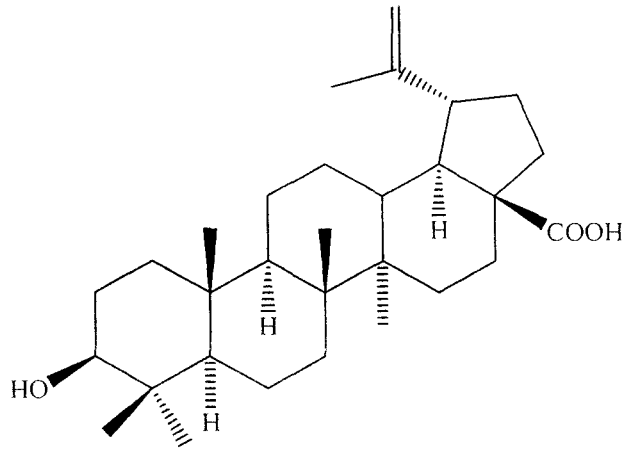


2-Alpha-hydroxyursolic acid

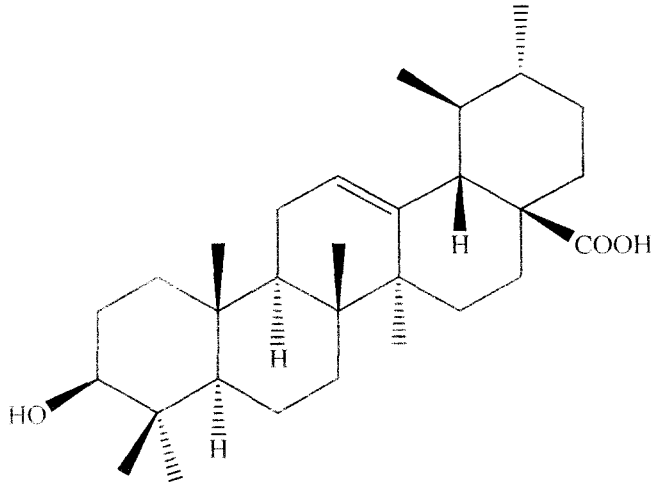
Gu *et al.* [46] derived three pentacyclic triterpenoids from plant as oleanolic acid (**1**), betulinic acid (**2**) and ursolic acid (**3**) and found that this triterpenoids exhibit moderate anti-tubercular activity in a microplate alamar blue assay.



Oleanolic acid

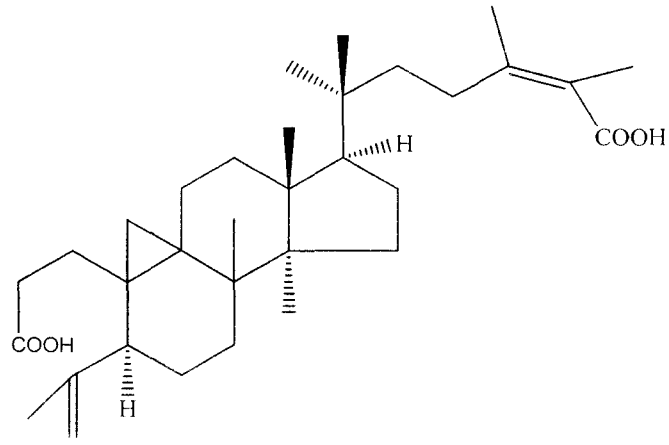


Betulinic acid



Ursolic acid

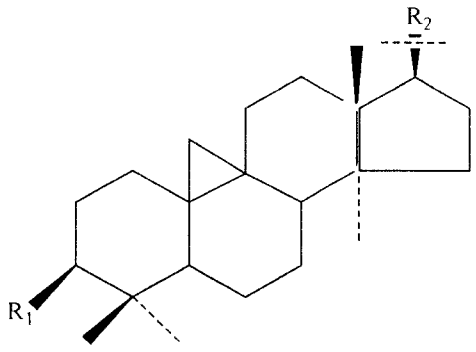
Sun *et al.* [47] isolated a ring-secocycloartene triterpenoid, nigranoic acid (3,4-secocycloarta-4(28),24-(Z)-diene-3,26-dioic acid from the stem of Schisandra sphaerandra, a Chinese traditional medicinal plant and its structure elucidation and unambiguous NMR spectral assignment were achieved by the combination of 1D- and 2D-NMR techniques with the aid of computer modeling. They found that nigranoic acid showed activity in several anti-HIV reverse transcriptase and polymerase assays.



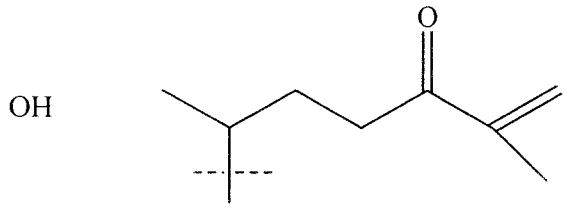
Nigranoic acid (3,4-secocycloarta-4(28),24-(Z)-diene-3,26-dioic acid

Takeoka *et al.* [48] isolated three triterpenoids betulinic acid, oleanolic acid and ursolic acid as their methyl esters from diethyl ether extracts of almond hulls using flash chromatography and preparative high performance liquid chromatography. They were characterized triterpenoids using chromatographic and spectroscopic methods and these studies demonstrated that almonds hills are a rich source of triterpenoids. They reported anti-inflammatory, anti-HIV and anti-cancer activities of these triterpenoids.

Madureira *et al.* [49] carried out phytochemical reinvestigation of the whole plant of *Euphorbia segetalis* and isolated five tetracyclic triterpenes: 3β-hydroxy-cycloart-25-en-24-one (**1**), cycloart-25-ene-3β,24-diol (**2**), cycloart-23-ene-3β,25-diol (**3**), lanosta-7,9(11),24-trien-3β-ol (**4**) and lanosta-7,9(11),24(31)-trien-3β-ol (**5**). 3β-acetoxy-cycloart-25-en-24-one (**1a**) and glutinol (**6**), lupenone (**7**), friedelin (8) dammaranodienol (**9**), cycloartenol acetate (**10**), 24-methylenecycloartanol acetate (**11**) and β-sitosterol (**12**). They were studied for their antiviral activities against Herpes simplex virus (HSV) and African swine fever virus (ASFV) and observed that lupenone exhibited strong viral plaque inhibitory effect against HSV-1 and HSV-2. The *in vitro* antifungal and antibacterial activities of **1a**, cycloart-23-ene-3β,25-diol, 3-acetate (**3a**) and **6-12** were also investigated.

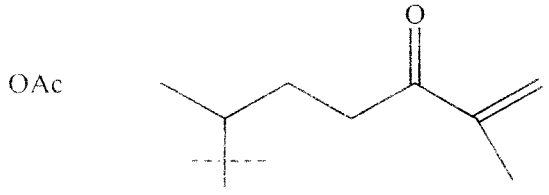


R1 R2

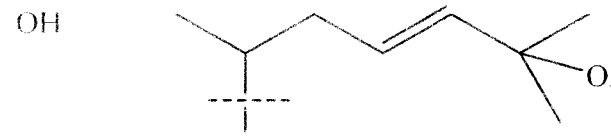


= 3 β-hydroxy-cycloart-25-en-24-one

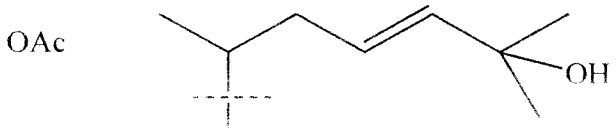
R1 R2



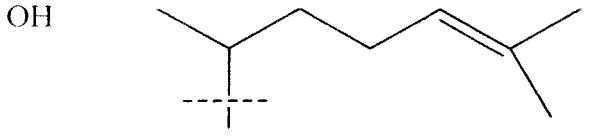
= 3β-acetoxy-cycloart-25-en-24-one



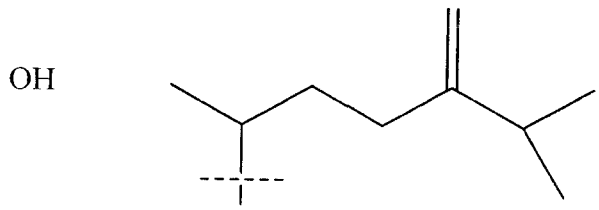
= cycloart-25-ene-3β,24-diol



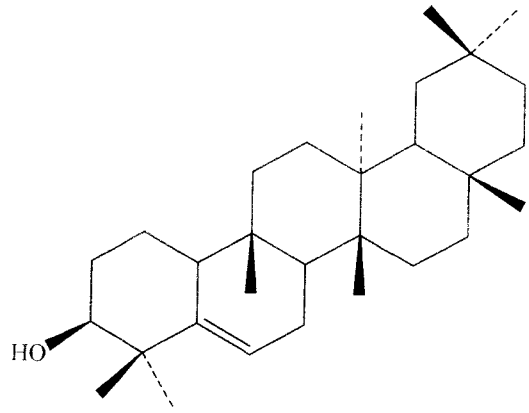
= cycloart-23-ene-3β,25-diol



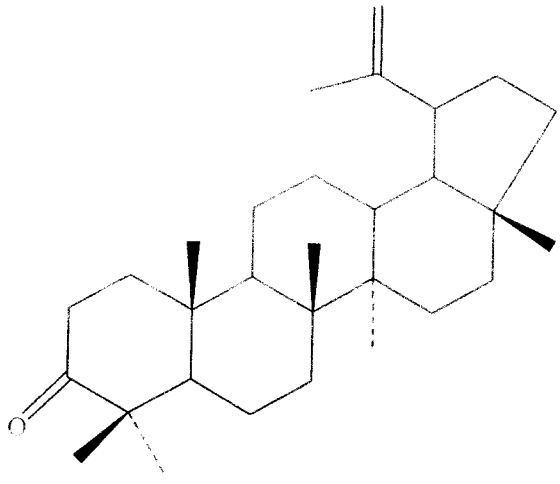
= cycloartenol



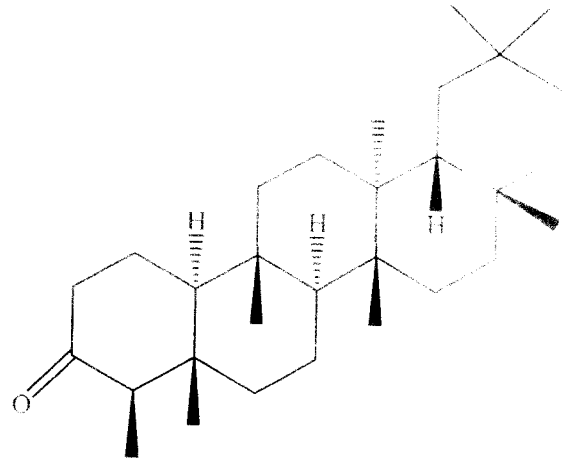
= 24-methylenecycloartanol



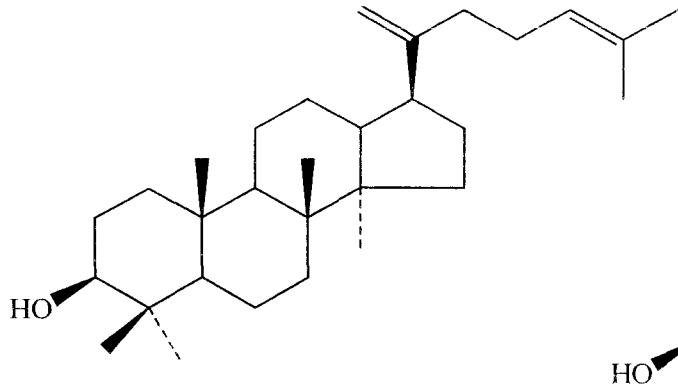
Glutinol



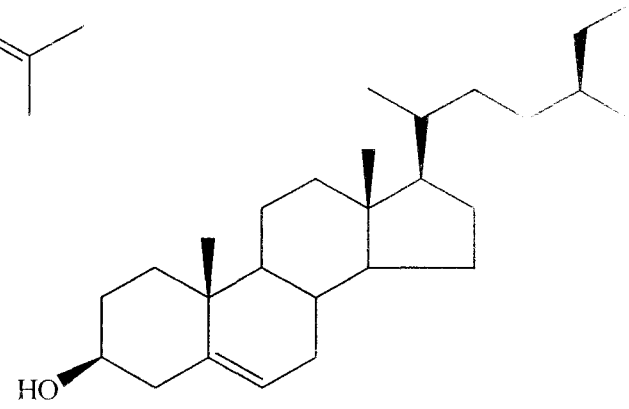
lupenone



friedelin

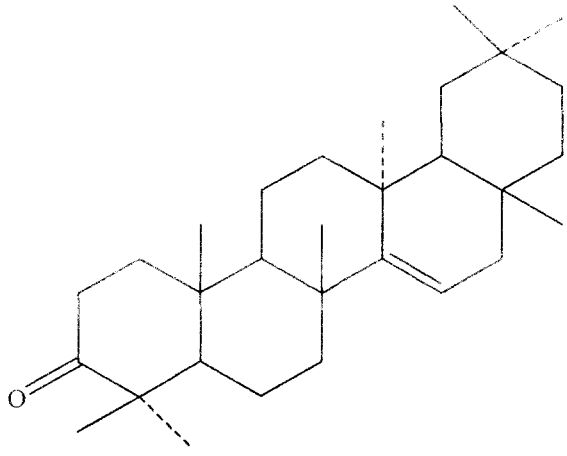


Dammaranodienol

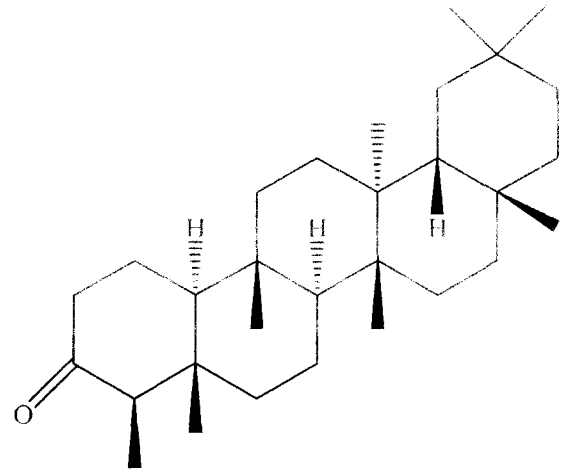


β-sitosterol

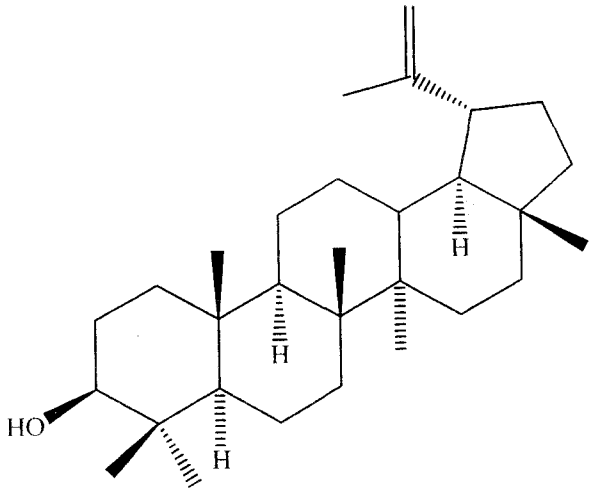
Gunaskera et al. [50] isolated new lupine derivative 3β–hydroxy-28-p-coumaroyloxy-lup20(29)-27-oic acid from *Camipa densifolia* and whose structure was deduced by chemical correlation with betulin (6) Simiarenol (l) taraxerone (2) friedelin (3) lupeol (4) betulinic acid (5) betulin (6) and β-sitosterol-g-D-glucoside.



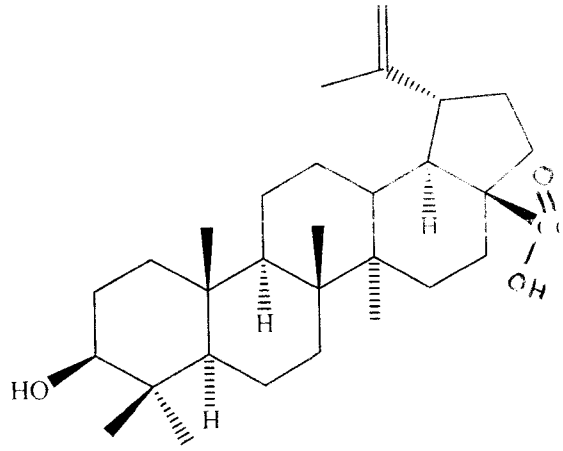
Taraxerone



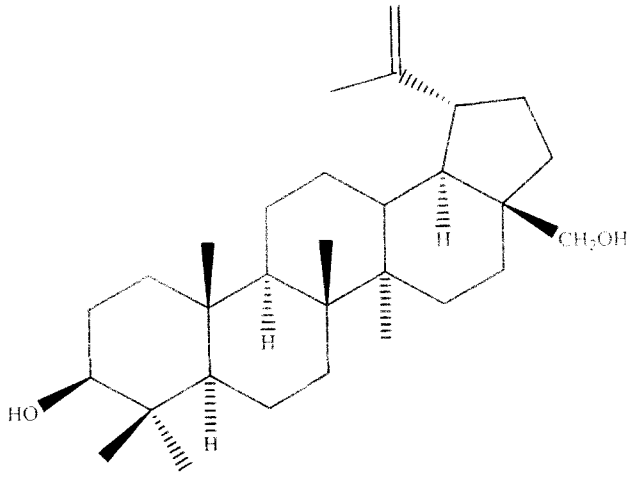
Friedelin



Lupeol



Betulinic acid



Betulin

Section B: A Short Review on the Biological activity of isolated materials from medicinal plants

Throughout history, mankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. Various extracts of different parts of plants have been widely used in folk medicines and perfumes as well as in food flavor and preservatives and are more commonly utilized in chronic diseases like cancer, diabetes and asthma [104,105].

The ancient Egyptians have described several useful preparations such as opium and castor oil. They also used "rotten bread" for treating infections which resembles our use of antibiotics from moulds and fungi.

The Chinese are considered as leaders in using natural products for healing. The oldest compilation of Chinese herbs is Shen Nung Pen Ts'ao. Which lists 385 materials. 5267 medicinal herbs were used in China in 1967. One of the most famous herbs among them is the ginseng root, *Panax ginseng* is used for health maintenance and for the treatment of various diseases. Another popular folk drug is the extract of the Ginkgo tree, *Ginkgo biloba* which can improve memory and mental alertness.

During the 17th century, the Jesuite brought with them from South America the bark of the China tree for the treatment of malaria. In 1820, Pelletia and Caventou isolated from the China tree the active compound, quinine. American Indians used the powerful hallucinogen, mescaline for a long period. The Indian hemp plant, *Cannabis sativa*, has been used since 3000 BC, and it is used as marijuana or hashish.

At the onset of the present study it was considered to review the reports presented by the earlier workers regarding the biocidal activity of various plant extracts tested on different organisms, especially on them selected for the present investigation. The observation of the previous workers in concord with the present line of investigation is being presented, in a selective manner, in the following paragraphs.

Mansouri [51] found new antibacterial agents from ethanolic extracts of ten plants. The agents were effective against *Staphylococcus aureus*. Several samples (489 samples) of *S.aureus* were isolated from healthy carriers (nose and throat) or clinical samples. Out of 489 isolates 98.6% were sensitive to trimethoprim-sulfamethoxazole. The extracted compounds from the plants were screened for antibacterial activity. *Myrtus communis* L. (leaves) showed the greatest activity, inhibiting the growth of 99% of the isolates. *Glycyrrhiza glabra* L., *Eucalyptus globolus Labill* and *Menta vividis L.* were also active against the isolates and inhibited the growth of 90, 59.5 and 48.7% of the isolates respectively.

Reddy *et al.* [52] studied the antibacterial activity of the pure isolates from *Piper longum* (L) (black pepper) and *Taxus bacata* (L) (yew). Three isolates of black pepper were active against Gram positive bacteria and moderately active against Gram negative bacteria. They reported that each isolates was highly active against at least one particular species of bacteria: Piperlongumine against *Bacillus subtilis*, Piperine against *Staphylococcus aureus*, and Pellitorine against *Bacillus sphaericus*. 3-(3'-4'-5'-Trimethoxyphenyl). Propionic acid did not show any bacterial activity. From the results they showed that most of the isolates of *piper longum* had antibacterial activity.

Samy and Ignacimuthu [53] reported the antifungal activity of crude drug from the tree bark of *T. arjuna* which was tested against bacteria using the hole-plate diffusion method with concentrations of 5-25 mg/mL. The effective results of bacteria were confirmed by the dilution method (1.25-2.0 mg/ml) in MIC. The results were supported by pathochemical analysis. The specific activity against pathogenic bacterium, *Bacillus subtilis* and *Staphylococcus aureus* showed the traditional usage of bark of *T. arjuna*.

Khan *et al.* [54] fractionated extracts of leaves, stem bark and root bark of *Eupomatia laurina* and performed test against 13 Gram-positive and 12 Gram-negative bacteria, a protozoan and four fungi. They found that all the extracts were active against most of the bacteria and fungi and the dichloromethane and ethyl acetate extracts of the stem bark and the dichloromethane extract of the root bark exhibited superior levels of antibacterial activity.

Ramesh *et al.* [55] isolated Friedelin, epi-Friedelin, n-Octacosanol, α–Amyrin, Sitosterol, Sitosterol-3- -D-glucopyranoside and luteoforol from Bridelia crenulaa Roxb. The aqueous and methanolic extracts and their fractions were tested against ten human pathogenic bacteria and four fungal strains. They observed that inhibitory activities were maximum in the chloroform-methanol (1:1) fraction of the methanolic extract against *E.coli, K.pneumoniae* and *P.aeruginasa*, which were responsible for the pathogenesis of urinary tract infection. The above study provided scientific evidence for the efficacy of the use.

Murillo-Alvarez *et al.* [56] extracted compounds from plants used in the traditional medicine of Baja California sur (Mexico) using ethanol as a solvent. They tested antimicrobial activities of the isolated compounds. The antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Candida albicans* and *Escherichia coli* was determined and *Aristolochia monticola, A .brevipes, Hymenoclea sp.* were found to be the most active.

Smith *et al.* [57] performed influence of medium type inoculum density and a cold incubation on antimicrobial assay sensitivity test. The largest and most distinct zones were produced using nutrient agar and the $1/10^4$ inoculum density for *Pseudomonas aeruginasa* and *Escherichia coli*. The greatest number of zones was detected without cold incubation. Using this method eight plants from Belize were screened for antibacterial activity. They reported that six plants showed activity against the four organisms tested. Both inoculum density and medium type played important roles in assay sensitivity.

Srikrishna *et al.* [58] carried out antibacterial activity using cup plate method. They observed that pet. ether, chloroform, methanol and water extract of the bark of *Aporosa lindleyana* (Euphorbiaceae) showed moderate to very good activity against bacteria such as *Bacillus subtilis, Escherichia coli.* They studied antifungal activity such as *Penicillium chrysozenous, Candida albicans, Aspergilus niger* and *Trichoderma vridar* and compared with the standard drug fluconazole. The pet. ether extract showed considerable activity towards all the four fungal organisms.

Akinpelu [59] observed that *Anacardium occidentale* bark 60 percent methanolic extract exhibited antimicrobial activity against 13 out of 15 bacterial isolates at a concentration of 20 mg/ml.

Audu *et al.* [60] extracted components from *Annona senegalensis* (root), *Nauclea latifolia* (stem bark) and *Ziziphus abyssinica* (root bark) using methanol, diethyl ether and cold water as solvent. They studied their activity on *Candida albicans*, *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* at different concentrations and found that all these components inhibited the growth of microbes.

Kamalakanman *et al.* [61] extracted 20 plant leaf and screened their inhibitory effect against the rice blast pathogen. They reported that *Prosopis juliflora* followed by *Zizyphus jujube* and *Abutilon indicum* significantly inhibited the mycelial growth and biomass as well as toxin production and spore germination under laboratory conditions.

Mehmood *et al.* [62] studied the antimicrobial potential of some Indian medicinal plants and their formulations. They tested twenty five different formulations based on five alcoholic extracts against several pathogenic micro-organisms. They observed that ten formulations showed higher potency compared to their constituents and good synergistic activity leading to significant reduction in the MIC values.

Ragasa *et al.* [63] extracted the air dried leaves of *Vitex negundo* which afforded vitexilactone and casticin by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR spectroscopy. They studied their activity and found to inhibit the growth of the fungi: *Candida albicans* and *Aspergillus niger* and the bacteria: Staphyllococcus aureus and Pseudomonas aeruginasa, but inactive against *Escherichia coli* and *Bacillus subtilis*.

Habtemariam and Macpherson [64] investigated the cytotoxic and antibacterial activity of an ethanol extract of leaves of a herbal drug *Eupatorium perfoliatum*. They observed that the extract showed a potentcytotoxicity and weak antibacterial activity against gram positive test organisms *Staphylococcus aureus* and *Bacillus megaterium*.

Mackeen *et al.* [65] reported that the crude ethanol extracts exhibited predominantly antibacterial activity with the root extract showing the strongest inhibition against the test bacteria at a minimum inhibitory dose (MID) of 15.6 microg/disc. They observed that most of the extracts failed to inhibit the growth of fungi but the root, leaf, trunk and stem bark extracts showed strong antioxidant activity. Antitumour-promoting activity was shown by the fruit, leaf, stem, and trunk bark extracts.

Lall and Meyer [66] observed that the water and acetone extracts of roots of *Euclea natalensis* inhibited the growth of *Bacillus cerus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus* at concentration ranging between 0.1 and 6.0 mg/ml. They found that the water extract did not exert any inhibitory action on Gram-negative bacteria while the acetone extract showed inhibitory activity at a concentration of 5.0 mg/ml against all the Gram-negative bacteria investigated. The antibacterial activity of acetone extract was also investigated by a direct bioassay on TLC plates against *S. aureus*.

Pichai *et al.* [67] extracted the leaves of *Tabebuia rosea* using n-hexane, chloroform and aqua as a solvents and screened the antibacterial activities against the pathogens Escherichia coli, Salmonella typhi and Staphylococcus aureus by agar dilution method. They observed that the aqueous extract exhibited potential antibacterial activity against *E. coli, S. Typhi* and *S. aureus* the hexane extracts had no effect on the three bacteria.

Ahmad and Beg [68] extracted 45 Indian plants traditionally used in medicine using ethanol as a solvent and studied their antimicrobial activity against certain drug-resistant bacteria and a yeast *Candida albicans*. They observed that of these 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts.

Savikin *et al.* [69] investigated the antimicrobial activity of the methanolic extracts of flowers and leaves of *Gentiana lutea* L together with the isolated compounds mangiferin, isogentisin and gentiopicrin. They studied the activity against a Gram-positive and a Gram-negative bacteria as well as the yeast *Candida albicans* and observed that both

extracts and isolated compounds showed antimicrobial activity with MIC values ranging from 0.12-0.31 mg/ml.

Al-Hussaini and Mahasneh [70] studied the antimicrobial and antiqurorum sensing activities of fourteen ethanolic extracts of different parts of eight plants against four Gram-positive, five Gram-negative bacteria and four fungi. They were recorded variable activities depending on the plant part extract and microorganism at 3 μg/disc. They found that among the Gram-positive bacteria tested, the activities of *Laurus nobilis* bark extract ranged between a 9.5 mm inhibition zone against Bacillus subtilis up to a 25 mm one against methicillin resistant *Staphylococcus aureus*. They also found that *Staphylococcus aureus* and *Aspergillus fumigatus* were the most susceptible among bacteria and fungi tested towards plants parts. However, minimum inhibitory concentrations (MIC's) for both bacteria and fungi were relatively high (0.5-3.0mg).

Ettebong and Nwafor [71] studied the antimicrobial activities of n-hexane, chloroform, ethyl acetate and methanol extract of *Carpolobia lutea* root which were used as a folk medicine in southern Nigeria against four typed cultures of bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and two clinical strains of fungi, namely *Candida albicans* and *Tinea capitis* using agar well diffusion method. They reported that the ethyl acetate extract gave the widest zone of inhibition (21.0 mm) followed by chloroform when tested on *E.coli*. They also reported that none of the extracts showed any inhibitory effect against *Pseudomonas aeruginosa* and the fungal strains of *Candida albicans* and *Tinea capitis* and the most potent of these extracts was chloroform extract with Minimum Inhibitory Concentration (MIC) of 25 mg/ml for bacteria. The Phytochemical screening of the root of *C. lutea* revealed the presence of saponins, anthraquinones, flavonoids, cardiac glycosides, simple sugar and terpenes.

Alves *et al.* [72] evaluated the antimicrobial, antifungal and antiadherent activity of aroeira-do-sertao, mallow and guava tree on oral biofilm microorganisms and oral *candidiasis* in vitro. They found that the extracts were shown to be effective the inhibition the growth of bacteria of the oral biofilm and fungi of oral *candidiasis*.

Qadrie *et al.* [73] studied the antibacterial activity of the ethanolic extract of *Indoneesiella echioides* (L) nees by filter paper disc method, this method was based on the diffusion of an antibiotic from a filter paper disc through the solidified culture media of a Petri dish. They observed that the growth was inhibited entirely in a circular area "zone around the filter" paper disc containing a solution of antibiotic and the plant extract. The used microorganisms were: *Staphylococcus aureus* and *Escherichia coli* and the organisms were maintained on nutrient agar slants. They tested the organisms using nutrient broth, one loop full of the respective cultures was taken in slants which were inoculated below 40 degree C and incubated at 37 degrees C for 24 hrs and observed the growth with naked eye for their turbid nature and compared with that of sterile broth.

Duraipandiyan *et al.* [74] studied the antimicrobial activity of 18 ethnomedicinal plant collected from Palni hills of Southern Western Ghats against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp, Proteus vulgaris*) and one fungal strain(*Candida albicans*) using paper disc diffusion method. They reported that out of 18 plants, 10 plants exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5and 5 mg/disc. The study evaluated the antimicrobial activity of the some ethnomedicinal plants used in folkloric medicine.

Gangoue-Pieboii *et al.* [75] investigated the antimicrobial activities in Vitro of 10 plant species (*Voacanga africana, Crepis cameroonica, Plagiostyles africana, Crotalaria retusa, Mammea africana, Lophira lanceolata, Ochna afzelii, Ouratea elongate, Ou. flava* and *Ou. sulcata*) each of which used in the traditional medicine in Cameroon. They studied the activities of methanol extract of each plant in disc diffusion assays against 37 species or laboratory strains of seven species of microorganism (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus hirae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans).* They observed that each of the 10 methanol extracts displayed some degree of antimicrobial activity against at least one species of microorganisms and no activity was found against the Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*

Pseudomonas aeruginosa) and Plagiostyles africana showed greatest antimicrobial activity.

Yasunaka *et al.* [76] studied the antibacterial activity of the thirty two extracts from 22 Mexican medicinal plants of 15 different families against *Escherichia coli and Staphylococcus aureus.* They reported that seventeen plants showed antibacterial, while five plants showed no activity against both bacteria and all of the extracts showed higher activity against *Staphylococcus aureus* than *Escherichia coli* except one.

Kumar *et al.* [77] carried out antimicrobial properties of a series of 61 medicinal plants belonging to 33 different families used in various infectious disorders at 1000 and 500 microg/ml concentration by agar dilution method against *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa , Streptococcus faecali, Candida albicans, Aspergillus niger and Saccharomyces cerevisiae.* They found that 28 plant extracts showed activity against at least one of the test organisms used. The crude extracts of *Dorema ammoniacum, Sphaeranthus indicus, Dracaena cinnabari, Mallotus philippinessis, Jatropha gossypifolia, Aristolochia indica, Lantana camara, Nardostachys jatamansi, Randia dumetorum* and *Cassia fistula* exhibited significant antimicrobial activity and property that support the folkloric use in the treatment of as broad-spectrum antimicrobial agents.

Adamu *et al.* [78] carried out a survey of medicinal plants used locally in the treatment of various diseases in Bauchi State-Nigeria and total 84 medicinal plants were listed. They investigated the antimicrobial activity of the aqueous extracts of the plants and found that out of 84 plants, 75 exhibited antimicrobial activity against one or more of the test organisms at a concentration of 200 mg/ml. They found that the extracts showed potentially interesting activity against *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.*

Bonjar [79] studied the antibacterial activities of the 45 species of 29 plant families used in the traditional medicine by Iranian people against *Bacillus cereus, Bacillus*

pumilus, Bordetella bronchiseptica, Escherichia coli, Klebsiella pneumoniae Micrococcus luteus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis. He found that no plant showed activity against *Serratia marcescens and Bordetella bronchiseptica* was the most susceptible species. All the extracts showed the same activity 18 months later.

Saleh *et al.* [80] isolated the known triterpenoids lantic acid, camarinic acid and lantanilic acid from *Lantana camara* (L) cultivated in Egypt and carried out the antibacterial activity of lantic acid using bioautography assays for Gram-positive and Gram-negative bacteria. They found that lantic acid possess strong antibacterial activity against *Escherichia coli* and *Bacillus cereus* in which 0.08 and 0.1 μg were the minimum inhibition doses compared to 0.05 and 0.005 μg for chloramphenicol. The results showed that lantic acid has broad spectrum antibacterial activity and may hold potential as a non-selective antimicrobial agent.

Mathabe *et al.* [81] isolated four known compounds from the stem bark of *Spirostachys africana* using ethanol as a solvent which is used traditionally for the treatment of diarrhoea and dysentery in Limpopo province of South Africa. The isolated compounds were, two triterpenoids, compound 1 [d-Friedoolean-14-en-oic acid (3-acetyl aleuritolic acid)] and compound 2 (lupeol), and two diterpenes, compound 3[ent-2, 6alpha-hydroxy-norbeyer-1,4,15-trien-3-one (diosphenol2)] and compound 4 (ent-3beta-hydroxy-beyer-15-ene-2-one). They tested the antibacterial activity using micro dilution method and found that compound 1, exhibited MIC of 50 microg/ml against *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Escherichia coli* and *Shigella dysentery*, compound 2 was not active against all tested microorganisms at 200 microg/ml and at 200 microg/ml all four compounds were not active against *Shigella sonnei.*

Angeh *et al.* [82] isolated four known triterpenoids, 1alpha,3beta-dihydroxy-12-oleanen-29-oic acid (1), 1-hydroxy-2-olean-30-oic acid (2), 3,30-dihydroxyl-12-oleanen-22-one (3), and 1,3,24-trihydroxyl-12-olean-29-oic acid (4) along with a new pentacyclic

triterpenoids (1alpha,23-dihydroxy-12-oleanen-29-oicacid-3beta-O-2,4-di-acetyl-L-rhamnopyranoside) 5 through a bioassay-guided procedure from the leaves of *Combretum imberbe*. They found that all the isolated compounds had moderate (62 μγ/ml) to strong (16 μg/ml) antimicrobial activity (MIC values) against *Staphylococcus aureus*, *Escherichia coli*, and compound 1 and 5 was most active. The results of this study gave credence to the ethnomedicinal use of *Combretum imberbe* and biological activity of its metabolities.

Mothana *et al.* [83] studied the antiproliferative activity against three human cancer cells, antimicrobial activity against antibiotic susceptible three Gram-positive, three Gram-negative bacterial and one fungal stains and three multiresistant Staphylococcus strains by the agar diffusion method and the determination of MIC against three Gram-positive bacteria with the broth micro-dilution assay, as well as for their antioxidant activity using the DPPH radical scavenging method of sixty four methanolic and aqueous extracts of thirty Yemeni plants used in traditional medicine. They found that 12 plants showed growth inhibitory effect against all cancer cells with IC50 values< 50 μg/ml, 9 plants showed pronounced antimicrobial activity against Gram-positive bacteria among them multiresistant bacteria with inhibition zones>15 mm and MIC values < 500 μg.

Shai *et al.* [84] isolated four compounds lupeol (1), betulinic acid (2), ursolic acid (3) and 2-alpha-hydroxyursolic acid (4) from the leaves of *Curtisia dentate*. They studied the antibacterial and antifungal activity using broth microdilution assay and bioautography method and found that betulinic acid, ursolic acid and 2-alpha-hydroxyursolic acid appreciably inhibited fungal growth with MIC values ranging from 8 to 63μg/ml.

Khan *et al.* [85] extracted the leaves, seeds, stem and root barks, stem and root heart-woods of *Michelia champaca* using methanol, petrol, dichloromethane, ethyl acetate, butanol as a solvent and observed that different fractions exhibited antibacterial activity. They also observed that fractionation drastically enhanced the level of activity particularly in all fractions of the stem bark and dichloromethane fraction of the root bark and some fractions of the leaves, stem and root barks demonstrated activity against some of the tested moulds. They found that among all the fractions liriodenine was the active

constituent of the root bark, with a broader and in some cases, better level of activity as compared to the standard.

Khan and Omoloso [86] extracted different fractions from the leaves, stem and root barks of *Dracantomelon dao* using methanol, petrol, dichloromethane, ethyl acetate, butanol as a solvent and found that they demonstrated a very good level of broad spectrum antibacterial activity. They reported that the dichloromethane and butanol fractions of the leaf were the most active and they had antifungal activity.

Khan and Omoloso [87] reported that the methanolic extracts and the fractions (petrol, dichloromethane, ethyl acetate, butanol) obtained from the leaves, seeds, stem and root barks of *Sarcocephalus coadunatus* exhibited a high level of broad spectrum antibacterial activity. They found that the activity was more pronounced in the dichloromethane, ethyl acetate fractions of the leaves, ethyl acetate and butanol fractions of the seeds, dichloromethane fractions of the stem bark and the ethyl acetate fractions of the root bark and none of the fractions showed any antifungal activity.

Dulger *et al* [88] extracted compounds from three *Verbascum* L. species (*Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss.) and *Klebsiella pneumonia Klebsiella pneumonia Klebsiella pneumonia* investigated their antimicrobial activity using the agar disc diffusion assay against *Escherichia coli* ATCC 11230, *Micrococcus luteus* La 2971, *Staphylococcus aureus* ATCC 6538P, *Salmonella thyphi* ATCC 19430, *Klebsiella pneumonia* UC57, *Pseudomonas aeroginosa* ATCC 27893, *Corynebacterium xerosis* CCM 2824, *Bacillus cereus* ATCC 7064, *Bacillus megaterium* DSM 32, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427,*Candida albicans* ATCC 10231, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* ATCC 9763. They found that *Verbascum* L. species showed antimicrobial activity against the Gr (+) bacteria and yeasts, but no activity was seen against the Gr (-) bacteria used in this study.

Kirmizigul *et al.* [89] reported antimicrobial and antifungal activities of the MeOH extract from the flowers of Cephalaria transsylvanica and three triterpenic acid glycosides, transsylvanoside A-C by MeOH using an agar-disc diffusion method. They observed that

both the MeOH extract and the glycosides possess antimicrobial and antifungal activities against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Corynebacterium xerosis*, *Klebsiella pneumonia*, *Candida utilis*, *Kluyveromyces fragilis*, *Aspergillus oryzae* and *Aspergillus flavus* respectively.

Sudharmini and Ashalatha [90] isolated triterpenoids from *Myxopyrum smilacifolium* leaf and found the presence of ursolic acid (0.175mg/g). They reported that the tritrpenoids showed antimicrobial activity in gram positive bacteria and *Candida sps.*

Horiuchi *et al.* [91] isolated the effective compound and identified it as oleanolic acid, a triterpenoid from *Salvia officinalis* (Sage) leaves and tested antimicrobial activity against vancomycin-resistant enterococci (VRE). They also tested the antimicrobial activity of similar triterpenoids, ursolic acid, uvaol, betulinic acid and betulin and found that ursolic acid also showed antimicrobial activity against VRE. The minimum inhibitory concentrations (MICs) of oleanolic acid and ursolic acid were 8 and 4 μg/ml, respectively and these two compounds also showed antimicrobial activity against *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). They found that these compounds also showed bactericidal activity against VRE at least for 48 h when added at concentrations that were two-times higher than their MICs.

Khan *et al.* [92] isolated amblyone, a triterpenoid from *Amorphophallus campanulatus* and studied in vitro antibacterial, antifungal and cytotoxic activities using disc diffusion technique and minimum inhibitory concentration was determined using serial dilution technique. They observed large zones of inhibition in disc diffusion antibacterial screening against four Gram-positive bacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and six Gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and the MIC values against these bacteria ranged from 8 to 64 μg/ml. In antifungal screening, the compound showed small zones of inhibition against *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus aryzae*, and *Candida albicans* was resistant against the compound.

Leite *et al.* [93] obtained various organic and aqueous extracts from the leaves of *Indigofera suffruticosa* Mill (Fabaceae) by infusion and maceration and screened their

antibacterial and antifungal activities. They were tested the extracts against 5 different species of humanpathogenic bacteria and 17 fungal strains by the agar-solid diffusion method. They observed that most of the extracts were devoid of antifungal and antibacterial activities, except the aqueous extract of leaves of *I. suffruticosa* obtained by infusion, which showed strong inhibitory activity against the Gram-positive bacteria *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) of 5000 μg ml^{-1}. The MIC values to dermatophyte strains were 2500 μg ml^{-1} against *Trichophyton rubrum* (LM-09, LM-13) and *Microsporum canis*.

Mbwambo *et al.* [94] extracted compounds from stem bark, wood and whole roots of *Ternimalia brownii* using solvents of increasing polarity, namely, pet ether, dichloromethane, dichloromethane: methanol (1:1), methanol and aqua, respectively and the extracts were tested for antifungal and antibacterial activity. They observed that the extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi* and *Bacillus anthracis* and the fungi, *Candida albicans* and *Cryptococcus neoformans*. They found that aqueous extracts exhibited the strongest activity against both bacteria and fungi.

Escalante *et al.* [95] isolated three monodesmosidic triterpenoid saponins from the butanolic extract of *Phytolacca tetramera* and established their structures. They reported that the three saponins belong to the olean-type triterpenoid saponins, with 28,30 dicarboxylic groups and an olefinic double bond on C-12. They observed that phytolaccosides B and E showed antifungal activities against a panel of human pathogenic opportunistic fungi but phytolaccoside F did not show any activity. The most sensitive fungus was *Trichophyton mentagrophytes*.

Ofodile *et al.* [96] extracted compounds from the four species of *Ganoderma* available in Nigeria using n-hexane: diethyl ether, chloroform:acetone and methanol as a solvent and tested their antimicrobial activity. They found that all the three solvent extracts of all the species of *Ganoderma* were active against Pseudomonas syringae and *Bacillus subtilis*, whereas none of the extracts were active against Cladosporium herbarum.

Bouzada *et al.* [97] isolated 44 methanol extracts from 37 Brazilian traditional medicinal plants and evaluated for their antibacterial activity and toxicity to brine shrimp using agar-well diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Shigella sonnei* and *Bacillus cereus.* They were subjected to serial dilution assay by the extracts for determination of the minimal inhibitory concentration (MIC) and reported that extracts of Baccharis dracunculifolia, Cajanus cajan, Eugenia uniflora, Solanum palinacanthum and Solanum concinnum presented strong antibacterial activity with MIC values below 10 μg/ml for some bacterial strains. The phytochemical analysis showed extracts of the major groups were phytoconstituents.

Khan *et al.* [98] extracted crude from the leaves, stem bark, stem heart wood, root bark and root heart wood of *Euroschinus papuanus* and isolated fractions on partitioning with petrol, dichloromethane (D), ethyl acetate (E) and butanol (B) and studied antibacterial and antifungal activity. They observed that E fractions of the stem heart wood, D of root bark and E of root heart wood demonstrated excellent antibacterial activity and B fractions of leaves; stem heartwood and root bark demonstrated antifungal activity.

Ramesh *et al.* [99] tested the antimicrobial efficiency of aqueous, methanol, chloroform and hexane extracts of *Swertia corymbosa* and noticed maximum inhibitory activity against *Staphylococcus aureus* and *Salmonella typhi.*

Khan and Omoloso [100] extracted the *Breynia cernua* leaves, stem and root barks and heart-woods with petrol, dichloromethane, ethyl acetate, butanol and methanol which gave various fractions. They studied antimicrobial activity of these fractions and found that the best activity was exhibited by the methanol extract of the root bark followed by its butanol fraction and the dichloromethane fraction of the stem bark also demonstrated good activity.

Lauk *et al.* [101] investigated antifungal activity of methanolic extract and alkaloidal fraction of Berberis aetnensis against Candida species. They observed that the crude extracts were active against Candida species and this activity was higher than that of the alkaloidal fraction and berberine.

Aqueveque *et al.* [102] isolated a new biologically active triterpenoid favolon B from fermentation broths of *Mycena* sp. Strain 96180. They found that favolon B showed antifungal activities against *Botrytis cinerea, Mucor miehei, Paecilomyces variotii* and *Penicillium notatum*. No activities were observed against bacteria and yeast.

Ragasa *et al.* [103] extracted the essential oil of *Cymbopogon citrates* Stapf. by the supercritical fluid extraction process and fractionation of the oil afforded cymbopogonol(1) and citral(2). Antimicrobial tests on 1 and 2 indicated that they had moderate activity against *C. albicans* and low activity against *P. aeruginosa, E. coli, S. aureus* and *T. mentagrophytes* and both compounds were inactive against *B. subtilis* and *A. niger*.

From the above literature work the author has found some discrete work on phytochemical investigation of the plants particularly which are available in this region and no systematic study regarding their biocidal activity has so far been carried out. Thus it was felt necessary to undertake thorough study towards the phytochemical investigation of medicinal plants available in this region of West Bengal and also to make a systematic study of the biocidal activity of the isolated plant materials.