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CHEMICAL CONSTITUENTS FROM THE *ALSTONIA SCHOLARIS* STEM BARK, *ECLIPTA PROSTRATA* AERIAL PARTS AND *MORUS ALBA* STEM BARK

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ABSTRACT

Alstonia scholaris (L.) R. Br. (family Apocynaceae) is a large, evergreen, tropical tree and its stem bark is used to treat abdominal and respiratory complaints, fevers, gonorrhoea, headache, hypertension, influenza, irregular menstruation, malaria, skin diseases, snakebites and urticaria. *Eclipta prostrata* L. (family Asteraceae) is a small, erect, annual herb and used to relieve acidity, allergy, alopecia, asthma, baldness, cuts, dandruff, gingivitis, hypertension, insomnia, jaundice, leucoderma, lice infection, menorrhagia, piles, pimples, pneumonia, ringworm, scorpion sting, skin diseases, snake bites, sores, wounds and wrinkles. *Morus alba* L. (family Moraceae) is a fast-growing, deciduous, evergreen tree and its bark is taken orally to expel tape worms and to cure toothache. This research work was undertaken to characterize structures of chemical constituents isolated from these plants. The air-dried plant materials were exhaustively extracted with methanol individually in a Soxhlet apparatus. The concentrated methanol extracts were adsorbed on silica gel for column and chromatographed over silica gel columns separately. Each column was eluted with petroleum ether, chloroform and methanol successively to isolate the phytoconstituents. Phytochemical investigation of the stem bark of *A. scholaris* afforded two lupene-type pentacyclic triterpenoids identified as lup-5, 12, 20(29)-trien- 3β-acetoxy-22α-ol (alstrinine acetate, **1**) and lup-5, 12, 20(29)-trien- 3β, 22α-diol (alstrinine, **2**) along with 3-epi-stigmasterol (**3**), β-sitosterol (**4**) and β-sitosterol-3β-O-glucoside (**5**). The aerial parts of *E. prostrata* furnished a new tetracyclic triterpenoid characterized as lanost-5, 24-dien-7β-ol -18, 21-olide -3β-olyl palmitate (**6**). The stem bark of *M. alba* yielded two known pentacyclic triterpenoids marked as α-amyrin acetate (urs-12-en-3β-yl acetate, **7**) and 3-epi-betulinic acid (**8**). The structures of these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Alstonia scholaris* bark, *Eclipta prostrata* aerial parts, *Morus alba* bark, phytoconstituents, isolation, characterization.

INTRODUCTION

Alstonia scholaris (L.) R. Br., syn. *Echites scholaris* L. (family Apocynaceae), commonly called blackboard tree, devil's tree, scholar tree, dita bark, saptaparni, chitvan and satvin, is an evergreen tropical tree. It is distributed in southern China, Indian subcontinent, south-eastern Asia and Australia. It is a large, evergreen, tropical tree, bark grey-brown, irregularly cracked and shallowly fissured, subverrucose, lenticellate, outer layer thin, corky, inner layer brittle; latex milky white; branchlets whorled; leaves simple, oblanceolate, whorled ; petiole stout, glabrous; base cuneate or attenuate; apex obtuse or emarginate; margin entire, glabrous, subcoriaceous; flowers bisexual, greenish-white in terminal umbellate cymes; fruits with two linear, narrow, pendulous follicular mericarps, green; seeds flat, commate at both ends. Its bark is regarded as an alterative, anthelmintic, anticholeretic, antidysenteric, antiperiodic, antiseptic, antispasmodic, astringent, bitter, bronchodilator, cytotoxic, emmenagogue, febrifuge, molluscicidal, tonic and vulnerary; used to treat abdominal pains, asthma, bowel complaints, bronchitis, chest pain, colds, diarrhoea, dysentery, fevers, genital problems, gonorrhoea, headache, hypertension, influenza, intestinal worms, irregular menstruation, malaria, skin diseases, snakebites and urticaria.[1,2] The bark latex is considered as a tonic and vermifuge; useful to relieve malaria, neuralgia, rheumatic pains, sores, toothache and ulcers.[1,2] Bark sap is taken to induce abortion. Gum is ingested with sugar to overcome dysentery.[1,2] The leaves are administered as an oral contraceptive and to cure beriberi, dropsy, dysentery and congested liver. A leaf poultice is a good remedy for skin diseases. Leaf juice is applied to kill head lice and to subside sores and wounds of animals.[1,2]

The flowers of *A. scholaris* contained pentacyclic triterpenic acetates, *n*-hexacosane, lupeol, palmitic acid, β-amyrin, ursolic acid, picrinine and strictamine,[3] a volatile oil composed mainly of linalool, *cis* - and *trans* - linalool oxides, α-terpineol, 2-phenylethyl acetate and terpinen-4-ol,[4] 2-dodecyloxirane, 1,2-dimethoxy-4-(2-propenyl)-benzene, spinacene, 1,54-dibromotetrapentacontane, 2,6,10,15-tetramethyl heptadecane, terpenyl acetate, linalool and tritetracontane.[5] The root bark yielded α-amyrin acetate, lupeol and β-sitosterol[6] and indole alkaloids.[7] The stem bark afforded indole alkaloids akuammiginone, echitamidine N -oxide 19-O -β-D-glucoside, echitaminic acid, echitamidine N -oxide, N -demethylalstogustine N -oxide, akuammicine N -oxide and N -demethylalstogustine,[8] scholarisines B–G together with known analogues,[9] 11-noriridoids, namely, scholareins A–D, isoboonein, alyxialactone and loganin,[10] 17-O-acetylechitamine and echitamine[11] and scholarisines I and II.[12] The leaves yielded akuammidine, its N -oxide,[13] kaempferol, quercetin, isorhamnetin and their glycosides,[14] lagunamine, angustilobin B acid, losbanine, tubotaiwine, its oxide and 6,7-seco-angustilobine B,[11] 19-epischolaricine, N-methylscholaricine, N-methyl burnamine and vallesamine N-oxide,[15] *n* -alkanes C-17 to C-33,[16] alstonic acids A and B and N-methoxymethyl picrinine,[17] megastigmane-3β, 4α, 9-triol and 7-megastigmene-3,6,9-triol,[18] scholarisine A, 5β-methoxyaspidophylline, picrinine, picralinal and 5-methoxy- strictamine,[19,20] cycloeucalenol, α-amyrin acetate, β-amyrin-3-palmitate, lupen-3-ol, lupen-3-palmitate, β-sitosterol, squalene, α-tocopherol, α-tocopherol quinone, bis(2-ethylhexyl) phthalate, dibutyl phthalate, 1-hydoxy-3,5-dimethoxy- xanthone, and flavones,[21] E- and Z -alstoscholarine,[22] quercetin 3 -O and (−)-lyoniresinol 3 -O glycosides,[23] seco-uleine and indole alkaloids,[24-26] triterpenoids and sterols,[27] erythrodiol, uvaol, betulin, oleanolic and ursolic acids, α- and β- amyrin acetates, β-sitosterol, stigmasterol, squalene, β-sitosteryl-3β-glucoside-6 ′-O-fatty acid esters and chlorophyll a.[28] The fruits furnished indole alkaloids.[29,30] The seed oil was composed of oleic acid, linoleic acid, palmitic acid and stearic acid.[31]

Eclipta prostrata L., syn. *Acmella lanceolata* Link ex Spreng., *Anthemis cotula-foetida* Crantz, *Anthemis viridis* Blanco, *Cotula alba* (L.) L., *Eclipta alba* (L.) Hassk., *Eclipta erecta* L. and *Verbesina alba* L. (Asteraceae), known as bhringraj, false daisy, trailing eclipta, yerba de tago and karisalankanni, is distributed in India, China, Pakistan, Nepal, Brazil and the United States. It is a small annual herb with erect, flat or round, blackish green stem, profusely branched and pubescent; leaves are opposite, ovate, dentate, blackish green in colour; inflorescence is a head; flower is solitary, white; achene is compressed, narrowly winged; seeds are black, many.[32] The plant is regarded as an anticatarrhal, antihepatotoxic, deobstruent, emetic, febrifuge, hair tonic, refrigerant, rejuvenative, restorative and stimulant, used to treat acidity, allergy, alopecia, asthma, athlete's foot, baldness, body pain, bronchitis, burns, constipation, coughs, cuts, dandruff, debility, diabetes, diarrhoea, dysentery, edema, elephantiasis, eye sight weakness, fever, gingivitis, hair loss, graying of hair, hypertension, insomnia, jaundice, leucoderma, lice infection, liver and spleen enlargement, menorrhagia, palpitation of the heart, piles, pimples, pneumonia, ringworm, scorpion sting, skin diseases, snake bites, sores, ulcers, urinary tract infections, wounds and wrinkles.[1, 33,34] *E. prostrata* plant contained coumestans,[35,36] *α*-amyrin, oleanolic acid, ursolic acid, eclalbasaponins A – D and I – X, eclalbatin,[37-39] dasyscyphin C, phytosterols, steroidal alkaloids, verazine and its derivatives, ecliptalbine, luteolin, its 7-glucoside, apigenin, orobol, sesquiterpene lactones, fatty alcohols, volatile oil components, polyacetylenes, ecliptal,[40,41] protocatechuic and 4-hydroxy benzoic acids and a lanostenoid.[42-44]

Morus alba L., syn. *M. atropurpurea* Roxb., *M. chinensis* Lodd. ex Loudon, *M. intermedia* Perr., *M. latifolia* Poir., *M. multicaulis* (Perr.) Perr., *M. tatarica* L. (family Moraceae), known as shahtoot, white mulberry, Russian mulberry, shilkworm mulberry, is a native to northern China and India, and widely cultivated in the United States, Mexico, Australia, Kyrgyzstan, Argentina, Turkey and Iran.[45] It is a fast-growing, small to medium-sized deciduous, evergreen tree, up to 20 m in height, leaves are deeply and intricately lobed, with the lobes rounded, cordate at the base and rounded to acuminate at the tip, serrated on the margins; bark of the large stem is brown, rough with vertical fissures; flowers are single-sex catkins; fruits white, pink or deep purple, sweet.[46] The leaves are antibacterial, astringent, diaphoretic, expectorant, hypoglycaemic, odontalgic and ophthalmic; used to treat colds, coughs, dizziness, fever, influenza, elephantiasis, eye infections, nosebleeds, purulent fistulae, sore throat and tetanus. Mulberry foliage are fed the silkworm.[46,47] The stems are antirheumatic, antispasmodic, diuretic, hypotensive and pectoral; useful to relieve edema, hypertension, rheumatic pains and spasms in the upper limbs. The fruit has a tonic effect on kidney energy. It is used to prevent constipation, diabetes, dizziness, hypertension, insomnia due to anaemia, neurasthenia, premature greying of the hair, tinnitus and urinary incontinence. Mulberry fruit is a promising nature's functional tonic and has a unique nutritional profile containing a variety of chemical constituents which enhance its significance. The root bark is antiasthmatic, antitussive, diuretic, expectorant, hypotensive and sedative; taken orally to comfort asthma, bronchitis, coughs, diabetes, hypertension and oedema.[46-48] The stem bark is anthelmintic and purgative, administered internally to expel tape worms. A bark tincture is beneficial to cure toothache.

Mulberry leaves contained ascorbic acid, carotene, vitamin B1, folic acid, folinic acid, vitamin D, volatile oil composed of aliphatic alcohols, aldehydes, methyl ethyl acetaldehyde, methyl ethyl ketone, methyl hexyl

ketone, butylamine, and acetic, propionic and isobutyric acids; succinic, and tartaric acids, xanthophyll, isoquercitrin and tannins,[46] 1-deoxynojirimycin, mulberroside F, flavanes, chalcones, flavones, benzofurans, and coumarin, prenylflavanes, isoquercitrin, astragalin, scopolin, skimmin, roseoside II and benzyl D-glucoside, flavonoids kaempferol-3-, quercetin 3-, and isofraxidin-7-glycosides and (±) -3,5,6,7,8,4 ′-hexahydroxyflavane,[49-55] linoleic acid, beta-carotene, alpha-tocopherol and phenolic acids.[56,57] The fruits yielded 4-O-α-D-galactopyranosyl-calystegine B2 and 3β,6β-dihydroxynortropane,[58] polyhydroxylated alkaloids, anthocyanins which were cyanidin glycosides,[59] phenolic, flavonoids, anthocyanins, tocopherols, carotenes and fatty acids,[60-65] nortropane alkaloids amino acids morusimic acids A – F,[66] pyrrole alkaloids morrole B – F,[67, 68] phenolic, amino and organic acids,[57,69] essential oil composed of aldehydes, organic acids, esters, limonene, cis-α-ocimene, terpinonene, terpinen-4-ol and 6-methyl-5-hepten-2-one,[70] phytol, β-sitosterol, lanost-7-en-3-one, α- and β-amyrins, lupeol, mulberrin, cyclomulberrin, mulberrochomene, cyclomulberro-chromene, mulberranol, albanol A and B.[1] The root bark afforded prenylated flavonoids, moralbanone, mulberroside C, cyclomorusin, eudraflavone B hydroperoxide, oxydihydromorusin, leachianone G and alpha-acetyl-amyrin,[71,72] glycoprotein Moran 20K,[73] mulberrofuran G and albanol B,[74] polyhydroxylated alkaloids, 2-hydroxymethyl-3,4-dihydroxy-pyrrolidine-N-propionamide,[58] and kuwanon G.[59] The root possessed a prenylated flavonoid.[75] The wood produced 7, 2', 4', 6'-tetrahydoroxy-6-geranylflavanone.[75] The stem bark yielded albanols,[76] *n*-triacontan-7-ol-13-oxo-yl 3,4-dioxymethylene benzene, stearyl triarabinoferulate and methoxygallic acid diglucoside,[77] lupeols moruslupenoic acids A and B, lanst-5, 24-dien-3β-yl acetate, α-amyrin acetate, β-amyrin-β-D-glucopyranoside and betulinic acid,[78] fatty acid glycoside and flavone derivatives.[79,80] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations the stem bark of *A. scholaris*, aerial parts of *E. prostrata* and stem bark of *M. alba* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were scanned on a Bruker DRX instruments using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/z* values of the more intense peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant materials

The stem bark of *A. scholaris*, aerial parts of *E. prostrata* and stem bark of *M. alba* were collected locally from Delhi and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of these plant parts are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The stem bark of *A. scholaris*, aerial parts of *E. prostrata* and stem bark of *M. alba* (1 kg each) were coarsely powdered and extracted separately and exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 113.7 g, 135.2 g and 120.4 g, respectively. The dried residue (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) separately to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 – 80 °C) individually. Each column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, *v/v*). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

Isolation of phytoconstituents from the stem bark of *Alstonia scholaris*

Alstrinine acetate (1)

Elution of the column with petroleum ether gave colourless crystals of **1,** recrystallized from chloroform – methanol (1:1), 4.9 g, R_f : 0.82 (*n*-hexane-ethyl acetate, 9:1); m. p. 139-141 °C, UV λmax (MeOH): 214 nm (log Ɛ 4.9); IR γmax (KBr): 3463, 2959, 2841, 1730, 1612, 1462, 1380, 1275, 1121, 1076 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.17 (1H, dd, J = 3.4, 4.9 Hz, H-12), 5.12 (1H, dd, J = 3.9, 3.4 Hz, H-6), 4.68 (1H, s, H_2-29a), 4.56 (1H, s, H_2-29b), 4.45 (1H, dd, J = 5.8, 9.7 Hz, H-3α), 4.09 (1H, dd, J = 5.3, 5.8 Hz, H-22β), 2.36 (1H, d, J = 5.8 Hz, H-18β), 1.59 (1H, dd, J = 3.8, 8.9 Hz, H-9α), 1.52 (1H, ddd, J = 6.2, 5.8, 8.3 Hz, H-19β), 2.33 – 1.15 (14 H, m, 2 x CH_2), 1.68 (3H, s, Me-30), 0.99 (3H, s, Me-24), 0.96 (3H, s, Me-23), 0.93 (3H, s, Me-27), 0.87 (3H, s, Me-25), 0.83 (3H, s, Me-26), 0.79 (3H, s, Me-28), 2.04 (3H, brs,

$OCOCH_3$); ^{13}C NMR ($CDCl_3$): δ 39.61 (C-1), 26.62 (C-2), 80.96 (C-3), 33.79 (C-4), 145.18 (C-5), 124.33 (C-6), 33.73 (C-7), 40.05 (C-8), 55.41 (C-9), 36.80 (C-10), 20.97 (C-11), 121.65 (C-12), 139.62 (C-13), 42.83 (C-14), 27.45 (C-15), 39.99 (C-16), 42.98 (C-17), 47.68 (C-18), 47.99 (C-19), 150.85 (C-20), 29.86 (C-21), 76.35 (C-22), 17.47 (C-23), 17.99 (C-24), 15.98 (C-25), 16.47 (C-26), 14.50 (C-27), 19.29 (C-28), 109.33 (C-29), 23.70 (C-30), 21.23, 170.90 ($OCOCH_3$); +ve FAB MS *m/z* (rel. int.): 480 $[M]^+$ ($C_{32}H_{48}O_3$) (6.9), 465 (39.8), 462 (11.3), 450 (22.7), 437 (18.2), 420 (31.6), 248 (24.4), 232 (5.8), 214 (5.3), 205 (18.7), 191 (11.6), 188 (26.3).

Alstrinine (**2**)

Further elution of the column with petroleum ether furnished colourless crystals of **2,** recrystallized from chloroform –methanol (1:1), 4.7 g, R_f : 0.54 (*n*-hexane-ethyl acetate, 9:1); m. p. 129-130 °C, UV λmax (MeOH): 211 nm (log Ɛ 4.9); IR γmax (KBr): 3441, 2961, 2873, 1639, 1464, 1362, 1273, 1124, 1072, 1040 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.15 (1H, m, H-12), 5.11 (1H, m, H-6), 4.71 (1H, s, H_2-29a), 4.68 (1H, s, H_2-29b), 3.26 (1H, dd, J = 5.3, 9.2 Hz, H-3α), 3.22 (1H, dd, J = 5.1, 5.7 Hz, H-22β), 2.23 (1H, d, J = 6.1 Hz, H-18β), 1.53 (1H, dd, J = 4.9, 8.7 Hz, H-9α), 1.69 (1H, ddd, J = 6.8, 6.3, 8.7 Hz, H-19β), 2.03 – 1.08 (14 H, m, 2 x CH_2), 1.68 (3H, s, Me-30), 1.01 (3H, s, Me-24), 0.97 (3H, s, Me-23), 0.94 (3H, s, Me-27), 0.86 (3H, s, Me-25), 0.82 (3H, s, Me-27), 0.79 (3H, s, Me-28); ^{13}C NMR ($CDCl_3$): δ 39.31 (C-1), 26.69 (C-2), 77.91 (C-3), 33.38 (C-4), 145.90 (C-5), 125.14 (C-6), 34.52 (C-7), 40.91 (C-8), 55.92 (C-9), 36.28 (C-10), 20.02 (C-11), 122.45 (C-12), 140.36 (C-13), 42.44 (C-14), 27.65 (C-15), 39.57 (C-16), 43.70 (C-17), 47.96 (C-18), 47.58 (C-19), 151.64 (C-20), 30.57 (C-21), 79.73 (C-22), 32.67 (C-23), 17.53 (C-24), 15.26 (C-25), 16.20 (C-26), 14.75 (C-27), 19.09 (C-28), 106.65 (C-29), 23.27 (C-30); +ve FAB MS *m/z* (rel. int.): 438 $[M]^+$ ($C_{30}H_{46}O_2$) (22.6), 423 (31.7), 420 (8.3), 408 (12.1), 393 (11.2), 378 (11.1), 232 (18.6), 217 (22.8), 214 (13.5), 206 (21.7), 202 (8.5), 191 (21.4), 188 (32.5), 173 (29.1), 158 (31.7).

3-Epi-stigmasterol (3)

Elution of the column with petroleum ether – chloroform (3:1) yielded a colourless crystalline mass of **3**, recrystallized from chloroform-methanol (1:1), yield 218 mg; m. p. 108-110 °C; UV λmax (MeOH): 214 nm (log ε 5.8); IR γmax (KBr): 3452, 2960, 2823, 1640, 1462, 1381, 1273, 1123, 872 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, H-6), 5.18 (1H, m, H-22), 5.14 (1H, m, H-23), 3.52 (1H, m, $w_{1/2}$ = 10.9 Hz, H-3β), 2.26 (1H, m, H-24), 2.20 (1H, m, H-20), 1.53 (1H, m, H-25), 1.51 (1H, m, H-9), 1.36 (1H, m, H-17), 1.16 (1H, m, H-8), 1.13 (1H, m, H-14), 1.01 (3H, brs, Me-19), 0.96 (3H, d, J = 7.3 Hz, Me-21), 0.84 (3H, d, J = 7.2 Hz, Me-26), 0.82 (3H, d, J = 7.3 Hz, Me-29), 0.78 (3H, d, J = 6.8 Hz, Me-27), 0.69 (3H, brs, Me-18), 2.30 to 1.13 (18 H, m, 9 x CH_2); ^{13}C NMR ($CDCl_3$): δ 37.67 (C-1), 32.09 (C-2), 72.20 (C-3), 42.73 (C-4), 141.18 (C-5), 122.08 (C-6), 30.08 (C-7), 36.53 (C-8), 51.62 (C-9), 36.57 (C-10), 21.46 (C11), 40.19 (C-12), 40.83 (C-13), 57.18 (C-14), 24.68 (C-15), 28.61 (C-16), 56.49 (C-17), 12.38 (C-18), 19.16 (C19), 36.92 (C-20), 19.36 (C-21), 138.67 (C-22), 129.71 (C-23), 50.56 (C-24), 29.27 (C-25), 19.29 (C-26), 19.43 (C27), 23.48 (C-28), 12.25 (C-29); +ve FAB MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (27.6), 397 (41.2), 394 (24.3), 381 (41.6), 379 (16.2), 273 (5.2), 271 (74.9), 258 (4.9), 255 (16.8), 240 (11.9), 213 (17.8), 192 (10.1), 178 (18.5), 164 (24.8), 138 (7.8), 124 (26.1).

β-Sitosterol (4)

Elution of the column with petroleum ether - chloroform (1:4) afforded a colourless amorphous powder of **4**, R_f 0.35 (chloroform – methanol, 9: 1); m. p. 136-138 ° C; UV λ max (MeOH): 209 nm (log ε 4.3); IR γmax (KBr): 3432, 2927, 2847, 1620, 1463, 1381, 1266, 1158, 1086, 958 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, m, H- 6), 3.59 (1H, brs, w ½ = 18.3 Hz, H- 3α), 1.01 (3H, brs, Me-19), 0.95 (3H, d, J = 6.3 Hz, Me-21), 0.86 (3H, d, J = 6.7 Hz, Me-27), 0.83 (3H, J = 6.4 Hz, Me-26), 0.78 (3H, t, J = 6.5 Hz, Me-29), 0.68 (3H, brs, Me-18), 2.31 - 1.09 (29H, 11 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.31 (C- 1), 31.62 (C- 2), 71.78 (C- 3), 42.11 (C- 4), 140.71 (C- 5), 121.69 (C- 6), 31.98 (C- 7), 31.89 (C- 8), 51.21 (C- 9), 36.42 (C- 10), 21.12 (C- 11), 39.81 (C- 12), 42.34 (C-13), 56.23 (C- 14), 24.19 (C- 15), 28.73 (C- 16), 56.07 (C- 17), 11.89 (C- 18), 19.48 (C- 19), 36.11 (C- 20), 18.72 (C- 21), 23.13 (C- 22), 26.18 (C- 23), 45.81 (C-24), 29.59 (C- 25), 19.78 (C- 26), 19.23 (C- 27), 23.38 (C- 28), 11.79 (C- 29); +ve FAB MS *m/z* (rel. int.): 414 (14.2), $[M]^+$ ($C_{29}H_{50}O$) (47.5), 399 (6.1), 396 (13.8), 381 (13.9), 303 (21.1), 273 (14.2), 213 (14.5).

β-Sitosterol-3β-O-glucoside (**5**)

Elution of the column with chloroform- methanol (19: 1) produced a colorless amorphous powder of **5**, recrystallized from chloroform: methanol (1: 1); 269 mg, R_f : 0.72 (chloroform: methanol, 9.3:0.7); m. p. 275-277 ° C; UV λmax (MeOH): 241 nm (log ε 2.9); IR γmax (KBr): 3441, 3323, 2937, 2842 1651, 1375, 1265, 1176, 1072 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.35 (1H, m, H-6), 3.54 (1H, brs, $w_{1/2}$ = 18.6 Hz, H-3), 1.03 (3H, brs, Me-19), 0.96 (3H, d, J = 6.6 Hz, Me-21), 0.88 (3H, d, J = 6.7 Hz, Me-26), 0.83 (3H, d, J = 6.5 Hz, Me-27), 0.79 (3H, t, J = 6.8 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.68 - 1.09 (29H, m, 11 × CH_2, 7 × CH), 5.18 (1H, d, J = 7.3 Hz, H-1′), 4.79 (1H, m, H-5′), 3.79 (1H, m, H-2′), 3.57 (1H, m, H-3′), 3.46 (1H, m, H-4′), 3.15 (2H, d, J = 8.0 Hz, H_2-6′); ^{13}C NMR (DMSO-d_6): δ 38.61 (C-1), 33.89 (C-2), 73.49 (C-3), 42.31 (C-4), 140.23 (C-5), 122.27 (C-6), 36.22 (C-7), 31.91 (C-8), 50.23 (C-9), 37.21 (C-10), 25.29 (C-11), 39.69 (C-12), 42.32 (C-13), 56.76 (C-14), 24.31 (C-15), 29.38 (C-16), 55.93 (C-17), 11.73 (C-18), 19.78 (C-19), 36.72 (C-20), 21.23 (C-21), 29.24 (C-22), 28.28 (C-23), 45.86 (C-24), 29.69 (C-25), 18.97 (C-26), 19.41 (C-27), 22.97 (C-28), 11.89 (C-29), 101.94 (C-1'), 76.36 (C-2'), 75.67 (C-3'), 69.94 (C-4'), 79.29 (C-5'), 61.67 (C- 6'); +ve FAB MS *m/z* (rel. int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (24.4), 413 (29.3), 397 (31.8), 255 (6.9), 179 (10.3), 163 (8.1).

Isolation of a phytoconstituent from the aerial parts of *Eclipta prostrata*

Lanost-5, 24-dien-7 β-ol -18, 21-olide -3β-olyl palmitate (6)

Elution of the column with chloroform furnished a pale yellow amorphous powder of **6**, recrystallized from chloroform-methanol (1:1), yield 189 mg, m. p. 130-131 °C, R_f 0.86 **(c**hloroform-methanol-petroleum ether, 9: 0.5: 0.5); IR γ_{max} (KBr): 3429, 2921, 2852, 1736, 1723, 1642, 1464, 1365, 1216, 1045, 726 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.38 (1H, d, J = 7.2 Hz, H - 6), 5.20 (1H, m, H -24), 4.49 (1H, dd, J = 5.7, 10.4 Hz, H -3α), 4.13 (2H, d, J = 11.3 Hz, H_2 – 21), 3.68 (1H, dd, J =5.3, 8.5 Hz, H-7α), 2.27 (2H, t, J = 7.6 Hz, H_2 – 2′), 2.06 – 1.78 (9H, m, 3 CH, 6 x CH_2), 1.73 (3H, brs, Me -26), 1.70 (3H, brs, Me -27), 1.53 (2H, m, CH_2), 1.38 (1H, m, H-17), 1.29 (18H, brs, 9 x CH_2), 1.25 (20H, brs, 10 x CH_2), 1.13 (3H, brs, Me -19), 0.91 (3H, brs, Me –29), 0.84 (3H, t, J = 6.1 Hz, Me - 14′), 0.81 (3H, brs, Me - 28), 0.79 (3H, brs, Me -30); ^{13}C NMR ($CDCI_3$): δ 35.83 (C-1), 26.12 (C-2), 81.69 (C-3), 38.49 (C-4), 138.48 (C-5), 123.64 (C-6), 67.28 (C-7), 46.39 (C-8), 54.84 (C-9), 36.74 (C-10), 22.57 (C-11), 23.48 (C-12), 54.37 (C-13), 49.15 (C-14), 32.79 (C-15), 31.85 (C-16), 55.38 (C-17), 173.16 (C-18), 20.59 (C-19), 33.04 (C-20), 61.89 (C-21), 36.45 (C-22), 28.91 (C-23), 112.35 (C-24), 136.81 (C-25), 23.74 (C-26), 22.89 (C-27), 23.38 (C-28), 18.67 (C-29), 16.31 (C-30), 171.88 (C-1′), 50.09 (C-2′), 34.14 (C-3′), 29.52 (C-4′ to C-9′), 29.31 (C-10′), 29.25 (C-11′), 28.72 (C-12′), 26.07 (C-13′), 25.28 (C-14′), 22.69 (C-15′), 14.13 (C-16′); +ve FAB MS *m/z* (rel. int,): 708 $[M]^+$ ($C_{46}H_{76}O_5$) (3.8), 469 (8.1), 255 (34.7), 239 (21.4), 234 (10.3), 181 (14.2), 151 (12.6).

Isolation of phytoconstituents from the stem bark of *Morus alba*

α-Amyrin acetate (Urs-12-en-3β-yl acetate) (7)

Elution of the column with petroleum ether - chloroform (1:1) afforded colourless crystals of **7,** recrystallized from chloroform - methanol (1 : 1), 1.21 g, m. p. 239-240 ºC; R_f: 0.39 (petroleum ether); UV λmax (MeOH): 210 nm (log ε 4.8); IR γmax (KBr): 2958, 2864, 1731, 1635, 1451, 1379, 1274, 1193, 1089, 748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.14 (1H, dd, J = 5.31, 5.41 Hz, H-12), 4.48 (1H, dd, J = 3.75, 9.83 Hz, H-3α), 2.05 (3H, s, OCO-Me), 1.07 (3H, s, Me-25), 1.03 (3H, s, Me-23), 0.98 (3H, s, Me-27), 0.92 (3H, s, Me-24), 0.88 (3H, s, Me-26), 0.83 (3H, d, J = 6.1 Hz, Me-29), 0.77 (3H, d, J = 6.3 Hz, Me-30), 2.03- 1.19 (23 H, 9 x CH_2, 5 x CH); ^{13}C NMR ($CDCl_3$): δ 40.8 (C-1), 36.83 (C-2), 80.99 (C-3), 39.71 (C-4), 55.31 (C-5), 18.27 (C-6), 31.26 (C-7), 38.51 (C-8), 47.69 (C-9), 37.73 (C-10), 23.24 (C-11), 124.36 (C12), 139.66 (C-13), 42.12 (C-14), 28.75 (C-15), 28.08 (C-16), 47.49 (C-17), 59.12 (C-18), 39.68 (C-19), 38.49 (C-20), 32.91 (C-21), 41.56 (C-22), 28.11 (C-23), 16.89 (C-24), 16.73 (C-25), 15.73 (C-26), 23.63 (C-27), 26,63 (C-28), 17.49 (C-29), 21.32 (C-30), 171.96, 21.37 (OCO-Me); +ve FAB MS *m/z* (rel.int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (32.1), 408 (54.3), 250 (13.4), 218 (100), 203 (32.5), 190 (17.8), 188 (30.9), 175 (22.6), 173 (20.5), 160 (23.1), 145 (33.2), 135 (46.5), 119 (53.2), 105 (52.8).

3-Epi-betulinic acid (8)

Elution of the column with petroleum ether - chloroform (1:3) gave colourless crystals of **8,** recrystallized from chloroform –methanol (1:1), 412 mg, R_f : 0.50 (chloroform: methanol, 99:1); m.p: 220-223 °C, UV λmax (MeOH): 276 nm (log e 5.3). IR γmax (KBr): 3321, 2960, 2931, 2839, 1701, 1635, 1463, 1381, 1271, 1234, 11233, 1072 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (1H, s, H_2 -29a), 4.60 (1H, s, H_2 -29b), 3.47 (1H, dd, J = 5.1, 5.4 Hz, H-3β), 1.68 (1H, brs, Me-30), 1.25 (3H, brs, Me-23), 1.17 (3H, brs, Me-25), 1.01 (3H, brs, Me-27), 0.85 (3H, brs, Me-24), 0.77 (3H, brs, Me-26), 2.67-1.49 (25H, m, 10 x CH_2, 5 x CH); ^{13}C NMR ($CDCl_3$): δ 39.03 (C-1), 27.39 (C-2), 79.31 (C-3), 42.90 (C-4), 55.34 (C-5), 18.28 (C-6), 34.37 (C-7), 40.69 (C-8), 50.52 (C-9), 37.21 (C10), 20.85 (C-11), 27.99 (C-12), 38.72 (C-13), 42.47 (C-14), 27.21 (C-15), 29.69 (C-16), 56.35 (C-17), 49.28 (C-18), 46.90 (C-19), 150.52 (C-20), 30.57 (C-21), 32.90 (C-22), 29.14 (C-23), 15.56 (C-24), 16.58 (C-25), 16.98 (C-26), 15.33 (C-27), 181.11 (C-28), 109.74 (C-29), 19.37 (C-30); +ve FAB MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (6.9), 441 (22.3), 438 (11.3), 329 (13.3), 288 (15.6), 207 (8.9), 203 (19.5), 190 (7.8), 189 (17.5), 153 (100), 136 (73.6), 122 (13.7), 108 (21.9).

RESULTS AND DISCUSSION

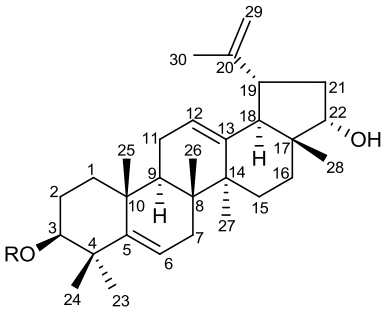
Compound **1**, named alstrinine acetate, showed distinctive IR absorption bands for hydroxyl group (3463 cm^{-1}), ester function (1730 cm^{-1}) and unsaturation (1612 cm^{-1}). Its molecular ion peak was determined on the basis of mass and ^{13}C NMR spectra at *m/z* 480 consistent with a molecular formula of a triterpenic ester, $C_{32}H_{48}O_3$. The ion peaks arising at *m/z* 437 $[M - COCH_3]^+$, 420 $[M - OCOCH_3]^+$, 462 $[M – H_2O]^+$, 465 $[M - CH_3]$+ and 450 $[465 - CH_3]$+ indicated the existence of acetoxy and hydroxy groups in the molecule. The ion peaks generated at *m/z* 248 $[C_{16}H_{24}O_2]^+$ and 232 $[C_{16}H_{24}O]^+$ due to retro-Diels Alder fragmentation of ring C, 205 $[248 – COCH_3]^+$, 188 $[248 – OCOCH_3]^+$, 214 $[232 – H_2O]^+$, 199 $[232 – Me]^+$ and 191 $[232 – C\text{-}(CH_2)CH_3]^+$ supported the existence of the acetoxy group in ring A placed at C-3 on the basis of biogenetic consideration, vinylic linkages in rings A/B and C, isopropenyl group in a lupene-type triterpene at C-19 and hydroxy group in ring D/E. The ^1H NMR spectrum of **1** exhibited two one-proton deshielded double doublets at δ 5.17 (J = 3.4, 4.9 Hz) and 5.12 (J = 3.9, 3.4 Hz) assigned to vinylic H-12 and H-6 protons, respectively, two one-proton singlets at δ 4.68 and 4.56 accounted to exocyclic vinylic methylene H_2-29 of a lupene - type triterpene, two one-proton double doublets at δ 4.45 (J = 5.8, 9.7 Hz) and 4.09 (J = 5.3, 5.8 Hz) ascribed correspondingly to α-oriented oxymethine H-3 and β-oriented carbinol H-22 protons, two three-proton singlets in the downfield region at δ 2.04 and 1.68 and six three - proton singlets between δ 0.99 - 0.79 associated with the tertiary C-23 to C-28 methyl protons. A one-proton doublet at δ 2.36 (J = 5.8

Hz), a one-proton double doublet at δ 1.59 (J = 3.8, 8.9 Hz) and a one-proton triple doublet at δ 1.52 (J = 6.2, 5.8, 8.3 Hz) were due to methine H-18α, H-9α and H-19β protons, respectively. The signals from δ 2.33 to 1.15 were designated to the remaining methylene protons. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 170.90 ($OCOCH_3$), vinylic carbons at δ 145.18 (C-5), 124.33 (C-6), 121.65 (C-12), 139.62 (C-13), 150.85 (C-20) and 109.33 (C-29), oxymethine carbons at δ 80.96 (C-3) and 76.35 (C-22), acetyl carbons at δ 21.23 and 170.90 ($OCOCH_3$) and methyl carbons between δ 22.70 -14.50. The ^{1}H and ^{13}C NMR spectral values of the triterpenic unit were compared with related lupene-type molecules.[81,82] On the basis of spectral data analysis and chemical reactions, the structure of **1** was formulated as lup-5, 12, 20(29)-trien-3β-acetoxy-22α-ol, a new lupene-type triterpenic ester (Fig. 1).

Compound **2**, named alstrinine, gave positive Liebermann-Burckhardt tests for triterpenoids, exhibited characteristic IR absorption bands for hydroxyl groups (3441 cm^{-1}) and unsaturation (1639 cm^{-1}) and its molecular ion peak was determined at *m/z* 438 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a triterpenol, $C_{30}H_{46}O_2$. The ion peaks arising at *m/z* 437 $[M - COCH_3]^+$, 420 $[M - OCOCH_3]^+$, 462 $[M - H_2O]^+$, 465 $[M - CH_3]^+$ and 450 $[465 - CH_3]^+$ indicated the existence of acetoxy and hydroxy groups in the molecule. The ion peaks at *m/z* 206 $[C_{14}H_{22}O]^+$ and 232 $[C_{16}H_{24}O]^+$ were produced due to retro-Diels Alder fragmentation of ring C along with the ion peaks formed at *m/z* 191 $[206 - CH_3]^+$, 188 $[206 - H_2O]^+$, 158 $[188 - 2\ Me]^+$, 214 $[232 - H_2O]^+$, 217 $[232 - Me]^+$, 202 $[217 - Me]^+$ and 191 $[232 - C\text{-}(CH_2)CH_3]^+$ supported the existence of the hydroxy groups in ring A placed at C-3 on the basis of biogenetic consideration, vinylic linkages in rings A/B and C, isopropenyl group in a lupene-type triterpene at C-19 and another hydroxy group in ring D/E. The other ion fragments appeared at *m/z* 420 $[M - H_2O]^+$, 423 $[M - Me]^+$, 408 $[423 - Me]^+$, 393 $[408 - Me]^+$ and 378 $[393 - Me]^+$ supporting triterpenic nature of the molecule. The ^{1}H NMR spectrum of **2** showed two one-proton deshielded multiplets at δ 5.15 and 5.11 assigned to vinylic H-12 and H-6 protons, respectively, two one-proton singlets at δ 4.71 and 4.68 ascribed to exocyclic vinylic methylene H_2 -29 protons of a lupene - type triterpene, two one-proton double doublets at δ 3.26 (J = 5.3, 9.2 Hz) and 3.22 (J = 5.1, 5.7 Hz) ascribed correspondingly to α-oriented carbinol H-3 and β-oriented H-22 protons, a three - proton singlet in the downfield region at δ 1.68 attributed to C-30 methyl protons located on C-20 vinylic carbon and six three - proton singlets between δ 1.01 - 0.79 associated with the tertiary C-23 to C-28 methyl protons. A one-proton doublet at δ 2.23 (J = 6.1 Hz), a one-proton double doublet at δ 1.53 (J = 4.9, 8.7 Hz) and a one-proton triple doublet at δ 1.71 (J = 6.8, 6.3, 8.7 Hz) were due to methine H-18β, H-9α and H-19β protons, respectively. The ^{13}C NMR spectrum of **2** displayed signals for vinylic carbons at δ 145.90 (C-5), 124.14 (C-6), 122.45 (C-12), 140.36 (C-13), 151.64 (C-20) and 106.65 (C-29), carbinol carbons at δ 77.91 (C-3) and 79.73 (C-22), and methyl carbons between δ 23.27 - 14.75. The ^{1}H and ^{13}C NMR spectral values of the triterpenic unit were compared with related lupene-type molecules.[81,82] On the basis of spectral data analysis and chemical reactions, the structure of **2** was formulated as lup-5, 12, 20(29)-trien- 3β, 22α-diol, a new lupene-type triterpenic ester (Fig. 1).

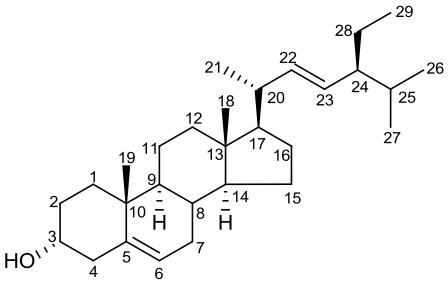
Compound **3** had IR distinctive absorption bands for hydroxyl function (3452 cm^{-1}) and unsaturation (1640 cm^{-1}). Its molecular ion peak was established at *m/z* 412 on the basis of mass and ^{13}C NMR spectra corresponding to the molecular formula of a sterol $C_{29}H_{48}O$. The ion peaks arising at *m/z* 394 $[M - H_2O]^+$, 397 $[M - Me]^+$, 382 $[397 - Me]^+$ and 379 $[397 - H_2O]^+$ indicated the presence of one hydroxy group in the molecule. The ion fragments produced at *m/z* 273 [$C_{17} - C_{20}$ fission, $M - C_{10}H_{19}$ (side chain)]$^+$, 255 $[273 - H_2O]^+$, 240 $[255 - Me]^+$ and 258 $[273 - Me]^+$ suggested the existence of an unsaturated side chain at C_{17} position. The ion peaks formed at *m/z* 124 [$C_{6,7} - C_{9,10}$ fission, $C_8H_{12}O]^+$, 138 [$C_{7,8} - C_{9,10}$ fission, $C_9H_{14}O]^+$, 164 [$C_{8,14} - C_{9,11}$ fission, $C_{11}H_{16}O]^+$, 178 [$C_{8,14} - C_{11,12}$ fission, $C_{12}H_{18}O]^+$ and 192 [$C_{8,14} - C_{12,13}$ fission, $C_{10}H_{20}O]^+$ supported the location of the hydroxy function in ring A placed at C-3 on the basis biogenetic consideration, the vinylic linkage in ring B at C-5 and saturated nature of the ring C. The ^{1}H NMR spectrum of **3** exhibited three multiplets integrating each for one proton at δ 5.35, 5.18 and 5.14 assigned correspondingly to vinylic H-6, H-22 and H-23 protons of a stigmasterol-type molecule, a one-proton multiplet at δ 3.52 with half-width of 10.9 Hz ascribed to β-oriented carbinol H-3 proton, two three-proton singlets at δ 1.01 and 0.69 due to tertiary C-19 and C-18 methyl protons, respectively, three doublets integrating each for three protons at δ 0.96 (J = 7.3 Hz), 0.84 (J = 7.2 Hz) and 0.78 (J = 6.8 Hz) attributed correspondingly to secondary C-21, C-26 and C-27 methyl protons and a three-proton doublet at δ 0.82 (J = 7.3 Hz, Me-29) accounted to primary C-29 methyl protons. The remaining methylene and methine protons resonated from δ 2.30 to 1.13. The ^{13}C NMR spectrum of **3** displayed 29 carbon signals including vinylic carbons at δ 141.73 (C-5), 122.08 (C-6), 138.67 (C22) and 129.71 (C-23), carbinol carbon at δ 72.20 (C-3) and methyl carbons at δ 12.38 (C-18), 19.16 (C-19), 18.49 (C-21), 19.36 (C-21), 19.29 (C-26), 19.43 (C27) and 12.25 (C-29). The ^{1}H and ^{13}C NMR spectral data of the steroidal nucleus were compared with the reported spectral values of similar compounds.[83,84] On the basis these evidences the structure of **3** has been elucidated as 3-epi-stigmasterol, a rare phytosterol (Fig. 1).

The structure of **4** was elucidated as β-sitosterol.[85] The compound **5** was a known steroidal glycoside characterized as β-sitosterol-3β-O-glucoside(Fig. 1).[86]

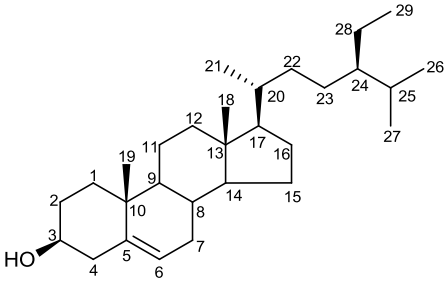


Alstrinine acetate, R =Ac **(1)**

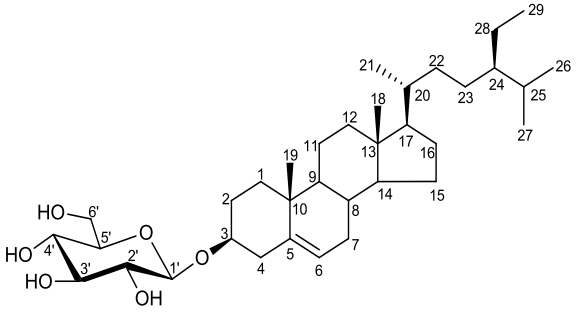
Alstrinine, R = H **(2)**



3-Epistigmasterol **(3)**



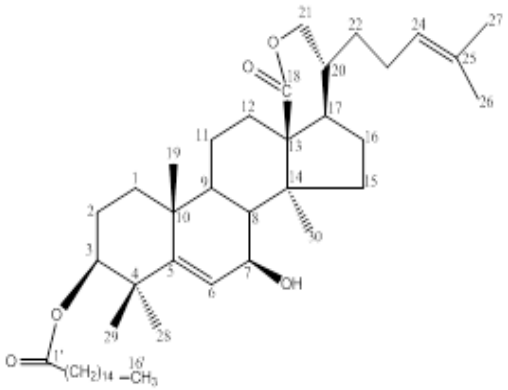
β-Sitosterol **(4)**



β-Sitosterol glucoside **(5)**

Fig 1: Structural formulae of the chemical constituents 1 – 5 isolated from the stem bark of *Alstonia scholaris*.

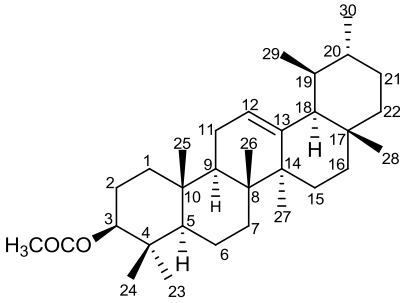
Compound **6** showed IR absorption bands for hydroxyl group (3429 cm^{-1}), ester and lactone groups (1723 cm^{-1}), unsaturation (1642 cm^{-1}), and long aliphatic chain (726 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 708, supported by ^{13}C NMR spectrum, corresponding to a tetracyclic triterpenic ester with δ – lactone function, $C_{46}H_{76}O_5$. The ion peaks arising at *m/z* 239 $[O - C_{1'}$ fission, $CH_3\text{-}(CH_2)_{14}\text{-}CO]^+$, 469 $[M - 239]^+$ and 255 $[O - C_3$ fission, $CH_3\text{-}(CH_2)_{14}\text{-}COO]^+$ indicated that palmitic acid was esterified with the triterpenol. The ion fragments generated at *m/z* 151 $[C_{6,7} - C_{9,10}$ fission, $C_{10}H_{15}O]^+$, 181 $[C_{7,8} - C_{9,10}$ fission, $C_{11}H_{15}O_2]^+$ and 234 $[C_{8,14} - C_{12,13}$ fission, $C_{15}H_{22}O_2]^+$ suggested the existence of the hydroxyl group at C-7 and olide ring at C-18 and C-21 position. The ^1H NMR spectrum of **6** displayed a one-proton doublet at δ 5.38 (J= 7.2 Hz) and a one -proton multiplet at δ 5.20 assigned to vinylic H-6 and H-24 protons, respectively. Two one-proton double doublets at δ 4.49 (J= 5.7, 10.4 Hz) and 3.68 (J= 5.3, 8.5 Hz) were attributed correspondingly to α– oriented carbinol H -3 and H-7 protons. A two-proton doublet at δ 4.13 (J = 11.3 Hz) was ascribed to oxygenated methylene H_2 – 21 protons. Two three-proton broad singlets at δ 1.73 and 1.70 were accounted to C -26 and C- 27 methyl protons, respectively, attached to a vinylic C-25 carbon. Four broad singlets at δ 1.13, 0.91, 0.81 and 0.79 were resultantly associated with tertiary C-19, C -29, C- 28 and C-30 methyl protons. A three- proton triplet at δ 0.84 (J = 6.1 Hz) was assigned to primary C- 14′ methyl protons. A two–proton triplet at δ 2.27 (J = 7.6 Hz) was due to methylene H_2- 2′ protons adjacent to the ester function. The remaining methine and methylene protons appeared as multiplets between δ 2.06 – 1.78 (9H), at δ 1.53 (2H) and 1.38 (1H, H-17) and as broad singlets at δ 1.29 (18H) and 1.25 (20H). The ^{13}C NMR spectrum of **6** exhibited signals for the ester carbon at δ 171.88 (C- 1′), lactone carbon at δ 173.16 (C- 18), oxymethine carbon at δ 81.69 (C - 3), carbinol carbon at δ 67.28 (C - 7), oxymethylene carbon at δ 61.89 (C - 21), vinylic carbons at δ 138.48 (C - 5), 123.64 (C -6), 112.35 (C - 24) and 136.81 (C -25) and methyl carbons between δ 23.74 – 14.13. Alkaline hydrolysis of compound **6** yielded triterpenic moiety and palmitic acid, m. p. 62 – 63 ° C; R_r 0.30 (85% glacial acid). On the basis of the spectral data analysis and chemical reactions, the structure of compound **6** has been formulated as lanost-5, 24-dien-7β-ol -18, 21-olide -3β-olyl palmitate, a new tetracyclic triterpenic olidic ester (Fig. 2).



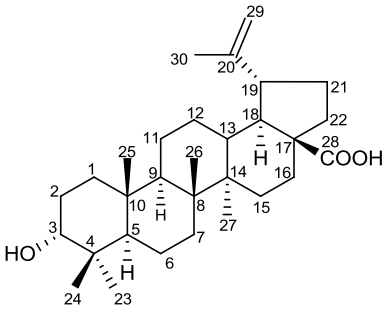
Lanost-5,24-dien-7β-ol-18,21-olide-3β-olyl palmitate (**6**)

Fig 2: Structural formula of the chemical constituent 6 isolated from the aerial parts of *Eclipta prostrata*.

Compound **7** was a known pentacyclic triterpenic ester characterized as α-amyrin acetate (urs-12-en-3β-yl acetate).[87,88] Compound **8** was a known lupeol – type triterpenol identified as 3-epi-betulinic acid (Fig. 3).[89]



α-Amyrin acetate **(7)**



3-*epi*-betulinic acid (8)

Fig 3: Structural formulae of the chemical constituents 7 and 8 isolated from the stem bark of *Morus alba*.

CONCLUSION

Phytochemical investigation of a methanolic extract of the stem bark of *A. scholaris* afforded two lupine-type pentacyclic triterpenoids identified as lup-5, 12, 20(29)-trien- 3β-acetoxy-22α-ol (**1**) and lup-5, 12, 20(29)-trien-3β, 22α-diol (**2**) along with 3-epi-stigmasterol (**3**), β-sitosterol (**4**) and β-sitosterol-3β-O-glucoside (**5**). The aerial parts of *E. prostrata* furnished a new tetracyclic triterpenoid lanost-5, 24-dien-7β-ol -18, 21-olide -3β-olyl palmitate (**6**). The stem bark of *M. alba* yielded two known pentacyclic triterpenoids characterized as α-amyrin acetate (**7**) and 3-epi-betulinic acid (**8**). This work has enhanced understanding about the phytoconstituents of these plants. These secondary metabolites can be used as analytical markers for quality control of these herbal drugs.

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