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CHEMICAL CONSTITUENTS FROM THE WHOLE PLANTS OF *CRESSA CRETICA* L., *KYLLINGA TRICEPS* ROTTB. AND *VERNONIA AMYGDALINA* DELILE

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ABSTRACT

Cressa cretica L. (Convolvulaceae) is a densely branching subshrub used to treat anaemia, anorexia, asthma, bronchitis, colic, constipation, general debility, diabetes, dyspepsia, flatulence, leprosy, skin disease, urinary discharges and uterine tumours. *Kyllinga triceps* Rottb. is a perennial, clustered herb and its rhizomes and roots are effective to relieve arthritis, bronchitis, common colds, cough, dermatitis, diabetes, fever, fistula, hepatopathy, injuries, malaria, thirst in fevers, splenopathy and tumours. *Vernonia amygdalina* Delile (Asteraceae) is a much branched, spreading tree and its leaves are utilized to cure amoebic dysentery, diabetes, liver diseases, malaria and other fevers, menstruation pain, skin, sexual and viral diseases and wound healing. Our study was planned to isolate chemical constituents from the methanolic extracts of these plants and to characterize their structures. Phytochemical investigation of the whole plant of *C. cretica* gave two flavonols characterized as quercetin (**1**) and luteolin -7-O-α-D-rhamnopyranoside (**2**). The plant methanolic extract of *K. triceps* afforded two new acyclic monoterpenic esters identified as 1-hydroxygeranilan -10-olyl benzoate (**3**) and 7α- salicyloxygeranilane **(4)** and a steroidal ester recognized as stigmast-5,22E-dien-3beta-yl octadecenoate (stigmasterol 3-stearate, **5**). The methanolic extract of *V. amygdalina* plant furnished a new monoterpenic xyloside and its structure was elucidated as geranilanyl-1-O-β-D-xylopyranoside (**6**).

KEYWORDS: *Cressa cretica*, *Kyllinga triceps*, *Vernonia amygdalina*, whole plants, phytoconstituents, isolation, characterization.

INTRODUCTION

Cressa cretica L. (Convolvulaceae), known as Rudravanti and Rudanti, is found in northern and central Africa, southern Europe, western and south-eastern Asia, Australia, the Arabian Peninsula, the Middle East, India and Sri Lanka. It is a densely branching subshrub, up to 38 cm, leaves are small, stubby, obtuse and clad in silky hairs, flowers white, grow in groups in the axils of the upper leaves; fruits are ovoid, pointed capsules, usually containing a single seed, glabrous, smooth, and shining to reticulate, dark brown. This plant is alterative, anthelmintic, antibilious, antitubercular, aphrodisiac, blood purifier, digestive, emetic, expectorant, stomachic and tonic.[1] The plant has a sour unpleasant taste, used to treat anaemia, anorexia, asthma, bronchitis, colic, constipation, general debility, diabetes, dyspepsia, flatulence, leprosy, skin disease, urinary discharges and as a suppository for uterine tumours.[2,3] The whole plant contained quercetin glycoside,[4] *n*-octacosanol, β-sitosterol, umbelliferone, scopoletin, isopimpinellin, β-sitosterol D-glucoside and quercetin,[5] acyclic terpenoids cressanyl ester A - G, and cressatriterpenic acid,[6] creticane, cressatetracosanoate, cressanonacontanoic acid, cressatetratriacontanoic acid, cressatriacontanone and cressanaphthacenone,[7] syringaresinol-β-D-glucoside, scopoletin, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid,[8] quercetin, quercetin-3-O-glucoside, kampferol-3 O- glucoside, kampferol-3-O-rhamnoglucoside and rutin,[9] triacontanoic acid, 24-hydroxy-4-octacosanone, 24-nor-12-ursene, β-amyrin, stigmasterol, ursolic acid, and stigmasterol 3-O-β-D-glucoside,[10] umbelliferone, daphnetin, flavonoids, phytosterols,[11] phenolic contents,[12] β-sitosterol, stigmasterol, avenasterol, β-tocopherol, tannins and saponins.[13] The fruits afforded a coumaranochromone glycoside (cresoside).[14] The seeds possessed fatty acids composed of C-12 to C-26 saturated fatty acids, palmitoleic, linoleic and gadoleic acids, [15] phytosterols, hydrocarbons (C12, C18, C21, C22, C26 and C32), and tocopherols.[16] The leaves provided mainly lactose, 3-deoxy-L-ribose-2,5-dibenzoate, octadecadiynoic acid methyl ester, D-mannose, 3-hydroxy-dodecanoic acid,

geranyl isovalerate, tetradecanoic acid , 6-epishyobunol and cis-9-hexadecenoic acid.[17]

Kyllinga triceps Rottb., syn. *Cyperus dubius* Rottb. (family Cyperacea), known as nirvishi and soft sedge, is distributed throughout Australia, tropical Africa, China, India, Indonesia, Nepal, Pakistan, Philippines, Sri Lanka, Thailand and Vietnam. It is a perennial, 5 to 35 cm long, clustered herb, stem smooth, trigonous, base bulbous; leaves veined, linear, basal, flat, greyish green, margins smooth, apex long-attenuate, acute, trigonous, scabrous; inflorescence sessile, compact, globose, greenish-white, ovoid head; nuts oblong-ellipsoid, trigonous, light brownish. The plant rhizomes and roots are anthelmintic, antidiarrheal, antidote to toxins, aromatic, astringent, bitter, demulcent, diuretic, expectorant, febrifuge, refrigerant, stomachic and tonic, used to treat arthritis, bronchitis, common colds, cough, dermatitis, diabetes, fever, fistula, hepatopathy, injuries, malaria, thirst in fevers, vitiated states of pitta and vata, splenopathy and tumors. The root essential oil is applied to relieve pruritus of the skin; it is given orally in torpor of the liver. The fresh plant juice is used to wash wounds and taken internally against indigestion. Kyllinga is effective to cure diarrhoea in Malaysia, dysentery, eye and skin diseases in China, and joint pain and rheumatic problems in Polynesia.[18-20] The crushed roots are boiled in mustard oil and the oil is lapped to subside skin diseases.[21] The whole plant contained quercetin, rutin, β-sitosterol and stigmasterol.[22] The entire plant showed the presence of phytosterols, guanosine, ergost-5-en-3β-ol, olivetol dimethyl ether, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, 2-methyl-2-[2-(2,6,6-trimethyl-3-methylene- cyclohex-1-enyl)- vinyl]-[1,3] dioxolane, oleic acid, methyl commate C, 2,5-dimethoxybenzylamine, 4-formyl-2-methoxyphenyl acetate and γ-tocopherol.[23] The rhizomes afforded cis- and trans-forms of ferruginol.[20] The rhizome essential oil was composed mainly of β-pinene, 1, 8-cineole, cyperene, linalool, terpin-4-ol, α-terpineol, myrtenol, thymol methyl ether, carvacrol methyl ether, thymoquinone, isomethyl acetate, β-caryophyllene, Z-β-farnesene, ferruginol and α-eudesmol.[24]

Vernonia amygdalina Delile (Astraceae), known as Congo bololo, bitter leaf and South African leaf, grows in tropical Africa. It is a much branched, up to 10 m tall, spreading tree; bark light grey or brown, smooth, fissured; leaves petiolate, elliptical, lanceolate oblong, apex and base tapering, margin entire or finely dentate; flower heads thistle like, small, creamy white, axillary and terminal; fruit a 10-ribbed achene, pubescent, glandular, brown to black, crowned with long pappus bristles; seedling with epigeal germination. The leaves are antihelminth, antimicrobial, antiparasitic, antiscorbutic, appetizer, laxative, molluscicide, purgative, used to cure amoebic dysentery, anaemia, bacterial infection, boils, burns, cancer, candidiasis, constipation, convulsions, coughs, diabetes, diarrhoea, dysentery, eczema, emesis, gastrointestinal disorders, gingivitis, haematuria, helminthiasis, post-parturn hemorrhage, hepatitis, hiccups, hypercholestrolaemia, hypertension, inflammatory diseases, intestinal parasites, jaundice, joint pain, kidney problems, measles, pile, liver diseases, malaria, typhoid and yellow fevers, menstruation pain, nausea, scabies, schistosomiasis, skin depigmentation, stomach-ache, sexually transmitted diseases, toothache, urinary tract inflammation, induction of uterine mobility , vagina itching, viral diseases and wound healing. The leaves are substituted for quinine and used to enhance breast milk in nursing mothers, to treat fever in poultry , helminthiasis in livestock, mastitis and de-ticking in cattle. The bitter leaves are eaten as raw vegetables and cooked in soups.[25-27] The roots and twigs chewed as an appetizer, laxative and to treat convulsion, fever, hepatitis, malaria, stomach ache, venereal diseases, worms, and wounds.[26] The plant contained sesquiterpene lactones vernolide, vernodalol, vernodalin, hydroxyvernolide, vernodalinol, vernomygdin, epivernodalol, 11,13-dihydrovernodalin and 3′-deoxyvernodalol,[28-32] flavonoids – luteolin, luteolin 7-O-β-glucuronoside and luteolin 7-O-β-glucoside,[33] steroid glucosides vernoniosides A_1 to A_4, B_1, B_2, B_3 and the aglycone of A_4, steroid saponins- Vernoamyosides A – E and vernoniosides D and E,[37, 38] 4α-hydroxy-*n*-pentadecanoic acid, 11α-hydroxyurs-5,12-dien- 28-oic acid-3α, 25-olide, 1-heneicosanol-O-β-D-glucopyranoside, 10-geranilanyl-O-β-D-xyloside, 6β,10β,14β- trimethyl heptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide and glucuronolactone.[39]

The presence of herbal chemical constituents vary due to many factors such as soil, geographic regions, seasonal changes, plant species and application of fertilizers. Keeping in views the various therapeutic values and variation aspects of chemical constituents of the plants and development of ecofriendly, biodegradable and safer herbal preparations the whole plants of *Cressa cretica*, *Kyllinga triceps* and *Vernonia amygdalina* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[40-42]

Collection and authentication of plant materials

Fresh plants of *Cressa cretica* and *Kyllinga triceps* were collected from the campus of Venkateswara University, Tirupati, and Andhra Pradesh, India. These plants were identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, (Andhra Pradesh), India. The voucher specimens of the plants have been deposited under reference No. TMU/Consult/2011-12/204 for *C. cretica* and No. TMU/Consult/2011-12/206 for *K. triceps* in the College of Pharmacy, TMU, Moradabad.

The *Vernonia amygdalina* plant was collected from Umuono community, Ngodo village, Nise, Anambra state, Southeast Nigeria and authenticated by a taxonomist in the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria.[39] The plant species was deposited there under voucher No. Intercedo/041.

Extraction and isolation

The whole plants of *C. cretica*, *K. triceps* and *V. amygdalina* (1 kg each) were dried in air, 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, 121.6 g, 114.9 g and 128.1 g, respectively. Each 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, 19:1, 9:1, *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 whole plant of *Cressa cretica*

Quercetin (1)

Elution of the column with chloroform – methanol (9:1) gave yellow crystals of **1**, recrystallized from acetone – methanol (4:1), yield 197 mg, m. p. 313 - 315 °C; R_f : 0.38 (toluene : ethyl acetate : formic acid, 5 : 4 : 0.1 (v/v/v); UV λmax (MeOH): 273, 307, 339 nm (log ε 5.7, 1.3, 0.9); IR υmax (KBr): 3510, 3481, 2941, 2837, 1670, 1635, 1525, 1450, 1345, 1262, 1139, 1015 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.84 (1H, d, J = 2.5 Hz, H-2′), 7.73 (1H, dd, J = 2.5, 8.1 Hz, H-6′), 7.02 (1H, d, J = 8.1 Hz, H-5′), 6.54 (1H, d, J = 2.1 Hz, H-8), 6.28 (1H, d, J = 2.1 Hz, H-6); ^{13}C NMR (DMSO-d_6): δ 147.44 (C-2), 135.85 (C-3), 175.67 (C-4), 161.42 (C-5), 98.27 (C-6), 164.09 (C-7), 93.56 (C-8), 156.87 (C-9), 103.24 (C-10), 122.87 (C-1′), 115.31 (C-2′), 144.92 (C-3′), 146.06 (C-4′), 114.86 (C-5′), 120.57 (C-6′); ESI MS *m/z* (rel. int.): 302 $[M]^+$ ($C_{15}H_{10}O_7$) (15.2).

Quercetin 3-O-β -D-rhamnosyl-2-O- β -D-xylopyranoside (2)

Elution of the column with chloroform – methanol (9:1) produced yellow crystals of **2**, recrystallized from acetone – methanol (4:1), yield 197 mg, m. p. 241 - 243 °C; R_f : 0.62 (methanol – water – glacial acetic acid, 5:5:6); UV λmax (MeOH): 278, 311, 336 nm (log ε 5.4, 1.4, 1.1); IR υmax (KBr): 3527, 3406, 3324, 2928, 2842, 1663, 1611, 1561, 1521, 1463, 1321, 1264, 1196, 1013, 823 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.56 (1H, d, J = 1.8 Hz, H-2′), 7.53 (1H, dd, J = 1.8, 7.5 Hz, H-6′), 6.82 (1H, d, J = 7.5 Hz, H-5′), 6.59 (1H, d, J = 2.1 Hz, H-8), 6.31 (1H, d, J = 2.1 Hz, H-6), 5.33 (1H, d, J = 7.2 Hz, H-1″), 4.54 (1H, m, H-5″), 4.41 (1H, m, H-2″), 3.72 (1H, m, H-3″), 3.35 (1H, m, H-4″), 1.12 (3H, d, J = 6.8 Hz, Me-6″), 5.09 (1H, d, J = 7.2 Hz, H-1‴), 4.38 (1H, m, H-2‴), 3.38 (1H, m, H-3‴), 3.29 (1H, m, H-4‴), 3.22 (1H, d, J = 6.5 Hz, H_2-5‴a), 3.10 (1H, d, J = 5.6 Hz, H_2-5‴b); ^{13}C NMR (DMSO-d_6): δ 148.06 (C-2), 133.78 (C-3), 177.83 (C-4), 161.69 (C-5), 99.12 (C-6), 164.52 (C-7), 94.63 (C-8), 157.06 (C-9), 104.44 (C-10), 122.04 (C-1′), 116.73 (C-2′), 145.23 (C-3′), 145.21 (C-4′), 115.69 (C-5′), 121.65 (C-6′), 101.66 (C-1″), 76.38 (C-2″), 73.22 (C-3″), 67.45 (C-4″), 76.93 (C-5″), 18.16 (C-6″), 101.28 (C-1‴), 74.54 (C-2‴), 71.83 (C-3‴), 70.48 (C-4‴), 68.69 (C-5‴); ESI MS *m/z* (rel. int.): 580 $[M]^+$ ($C_{26}H_{28}O_{15}$) (1.8), 301 (2.1).

Isolation of phytoconstituents from the whole plant of *Kyliinga triceps*

1-Hydroxygeranilan -10-olyl benzoate (3)

Elution of the column with petroleum ether - chloroform (4:1) furnished a brown solid mass of compound **3**, yield 217 mg , m. p. 82-85 °C; UV λ_{max} (MeOH): 212, 275 nm; IR υmax (KBr): 3360, 2925, 2853, 1723,1632, 1515, 1449, 1373, 1260, 1166, 1086, 1033, 909, 801 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.08 (2H, d, J = 10.5 Hz, H_2-10), 3.34 (2H, dd, J = 11.1, 13.8 Hz, H_2-1), 2.16 (1H, m, H-3), 1.98 (1H, m, H-7), 1.47 (2H, m, H_2-2), 1.32 (2H, m, H_2-4), 1.21 (4H, m, H_2-5, H_2-6),), 0.92 (3H, d, J = 6.9 Hz, Me-8), 0.83 (3H, d, J = 6.7 Hz, Me-9), 7.32 (1H, m, H-2', H-6'), 7.25 (1H, m, H-4'), 6.77 (2H, m, H-3', H-5'); ^{13}C NMR ($CDCl_3$): δ 66.17 (C-1), 34.08 (C-2), 38.75 (C-3), 31.43 (C-4), 23.81 (C-5), 22.97 (C-6), 34.48 (C-7), 18.92 (C-8), 19.15 (C-9), 71.78 (C-10), 138.24 (C-1′), 132.37 (C-2′), 130.91 (C-3′), 130.85 (C-4′), 130.91 (C-5′), 132.35 (C-6′), 172.21 (C-7′); ESI MS *m/z* (rel. int.): 278 $[M]^+$ ($C_{17}H_{26}O_3$) (2.1).

7α- Salicyloxygeranilane (4)

Elution of the column with petroleum ether - chloroform (1:1) afforded a light brown solid mass of **4**, yield 308 mg , m. p. 146 - 148 °C; UV λ_{max} (MeOH): 215, 279 nm; IR υmax (KBr): 3432, 2924, 2855, 1721, 1636, 1551, 1495, 1392, 1319, 1273, 1235, 1138, 1028, 975 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.65 (1H, m, H-3), 2.31 (2H, m, H_2-6), 1.66 (2H, m, H_2-5), 1.38 (2H, m, H_2-2), 1.30 (2H, m, H_2-4), 1.25 (6H, brs, Me-8, Me-9), 0.98 (3H, d, J = 6.6 Hz, Me-10), 0.89 (3H, t, J = 6.8 Hz, Me-1), 7.72 (1H, m, H-3'), 7.54 (1H, m, H-4'), 7.51 (1H, m, H-5'), 6.90 (1H, m, H-6'); ^{13}C NMR ($CDCl_3$): δ 10.93 (C-1), 23.71 (C-2), 38.69 (C-3), 33.59 (C-4), 29.64 (C-5), 27.95 (C-6), 71.78 (C-7), 19.12 (C-8), 22.94 (C-9), 14.05 (C-10), 141.42 (C-1′), 160.08 (C-2′), 133.71 (C-3′), 130.87 (C-4′), 128.79 (C-5′), 132.33 (C-6′), 167.11 (C-7′); ESI MS *m/z* (rel. int.): 278 $[M]^+$ ($C_{17}H_{26}O_3$) (1.3).

Stigmasterol 3-stearate (5)

Elution of the column with chloroform yielded a colourless amorphous powder of **5**, recrystallized from chloroform, 241 mg, R_f : 0.65 (chloroform – ethyl acetate – formic acid, 5: 4 : 1); m. p. 158 -160 ^{o} C; UV

λmax (MeOH): 209, 231 (log ε 4.3, 2.9); IR υmax (KBr): 2919, 2850, 1728, 1637, 1467, 1381, 1260, 1176, 1105, 1011, 982, 805, 719 cm^{-1} ; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 5.12 (1H, m, H-22), 5.01 (1H, m, H-23), 4.47 (1H, brm, $w_{1/2}$ = 18.2 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.96 (3H, d, J = 6.3 Hz, Me-21), 0.90 (3H, d, J = 6.1 Hz, Me-26), 0.86 (3H, d, J = 6.5 Hz, Me-27), 0.82 (3H, t, J = 6.1 Hz, Me-29), 0.73 (3H, brs, Me-18), 2.28 (2H, t, J = 7.5 Hz, H_2 -2′), 2.02 to 1.35 (25H, m, 9 x CH_2, 7 x CH), 1.25 (26H, brs, 13 × CH_2), 1.07 (2H, m, CH_2), 0.84 (3H, t, J = 6.2 Hz, Me-18′); ^{13}C NMR ($CDCl_3$): δ 37.17 (C-1), 31.94 (C-2), 80.61 (C-3), 43.36 (C-4), 142.71 (C-5), 121.68 (C-6), 31.34 (C-7), 33.35 (C-8), 51.16 (C-9), 37.40 (C-10), 21.17 (C-11), 38.64 (C-12), 39.15 (C-13), 55.61 (C-14), 25.20 (C-15), 23.71 (C-16), 55.28 (C-17), 11.31 (C-18), 18.17 (C-19), 37.73 (C-20), 16.77 (C-21), 140.03 (C-22), 129.79 (C-23), 45.18 (C-24), 26.19 (C-25), 16.62 (C-26), 16.11 (C-27), 27.96 (C-28), 12.14 (C-29), 173.70 (C-1′), 38.42 (C-2′), 34.89 (C-3′), 34.37 (C-4′), 32.35 (C-5′), 29.76 (C-6′), 29.71 (C-7′), 29.69 (C-8′), 29.69 (C-9′), 29.69 (C-10′), 29.69 (C-11′), 29.20 (C-12′), 29.20 (C-13′), 28.35 (C-14′), 28.30 (C-15′), 25.13 (C-16′), 22.71 (C-17′), 14.57 (C-18′); ESI MS *m/z* (rel. int.): 678 $[M]^+$ ($C_{47}H_{82}O_2$) (1.1), 411 (1.3), 283 (24.2), 267 (4.3).

Isolation of a phytoconstituent from the whole plant of *Vernonia amygdalina*

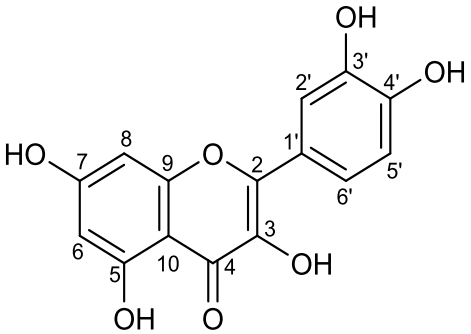
Geranilanyl-1-O-β-D-xylopyranoside (6)

Elution of the column with chloroform - methanol (19:1) provided a colourless crystalline product of **6**, yield 112 mg, m. p. 127 -129 ^0C; UV λ_{max} (MeOH) 117 nm (log ε 3.8); IR υ_{max} (KBr): 3518, 3278, 2921, 2883, 1442, 1375, 1327, 1138, 1045, 1003, 945, 838, 794 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.06 (2H, t, J = 5.6 Hz, H_2-1), 2.90 (1H, m, H-3), 1.82 (1H, m, H-7), 1.73 (2H, m, H_2-2), 1.62 (2H, m, H_2-4), 1.24 (2H, m, H_2-5), 1.16 (2H, m, H_2-6), 1.10 (3H, d, J = 6.5 Hz, Me-10), 0.72 (3H, d, J = 6.3 Hz, Me-8), 0.68 (3H, d, J = 6.6 Hz, Me-9), 5.19 (1H, d, J = 7.6 Hz, H-1′), 4.16 (1H, m, H-2′), 3.62 (1H, m, H-3′), 3.42 (1H, m, H-4′), 3.11 (2H, d, J = 8.8 Hz, H_2-5′); ^{13}C NMR ($CDCl_3$): δ 62.79 (C-1), 31.02 (C-2), 40.44 (C-3), 37.03 (C-4), 36.01 (C-5), 35.04 (C-6), 37.10 (C-7), 19.87 (C-8), 19.85 (C-9), 18.77 (C-10), 102.34 (C-1′), 78.09 (C-2′), 77.86 (C-3′), 75.14 (C-4′), 71.67 (C-5′); +ve ESI MS *m/z* (rel. int.): 290 $[M]^+$ ($C_{15}H_{30}O_5$) (3.5), 157 (28.4), 149 (12.3), 141 (20.6).

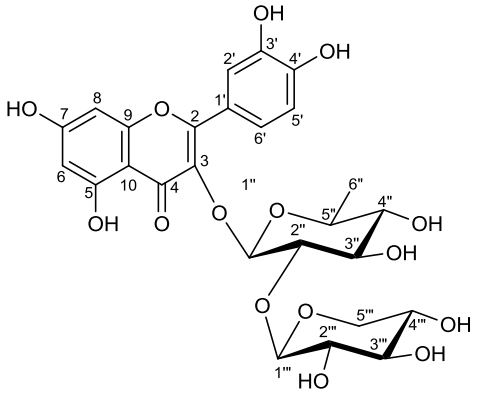
RESULTS AND DISCUSSION

Compound **1** was a known flavonol identified as quercetin (Fig. 1).[43,44]

Compound **2** responded positive tests for flavonoid glycosides, exhibited UV absorption maxima at 278, 311 and 336 nm for flavones and IR absorption bands for hydroxyl groups (3527, 3406, 3324 cm^{-1}), carbonyl function (1663 cm^{-1}), unsaturation (1611 cm^{-1}) and aromaticity (1561, 1521, 1013 cm^{-1}). There was a shift of band I with sodium methoxide suggesting the presence of free phenolic groups, shift of bands with sodium acetate solution indicating free nature of 7-hydroxyl group and a shift of band I with aluminium chloride supporting the presence of free 5-hydroxyl group. There was a shift of band I with aluminium chloride and hydrochloric acid supporting the existence of B-ring *o*-dihydroxy functions. [45-47] On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **2** was established at *m/z* 580 consistent with a molecular formula of flavonol glycoside, $C_{26}H_{28}O_{15}$. An ion fragments arising at *m/z* 301 [$C_{1''}$ - O fission, $C_{15}H_9O_7$]$^+$ suggested that a rhamno-pentoside unit was linked with the flavonol moiety. The ^1H NMR spectrum of **2** displayed four one-proton doublets at δ 7.56 (J = 1.8 Hz), 6.82 (J = 7.5 Hz), 6.59 (J = 2.1 Hz) and 6.31 (J = 2.1 Hz) and a one-proton double doublet at δ 7.53 (J = 1.8, 7.5 Hz) assigned to the aromatic B-ring H-2′ and H-5′, A-ring H-8 and H-6 and meta-, ortho-coupled H-6′ protons, respectively. Two one-proton doublets at δ 5.33 (J = 7.2 Hz) and 5.09 (J = 7.2 Hz) were accounted correspondingly to β-oriented anomeric H-1″ and H-1‴ protons. The other sugar protons resonated as one-proton multiplets from δ 4.54 to 3.29. A three-proton doublet at δ 1.12 (J = 6.8 Hz) was due to C-6″ secondary methyl protons of the rhamnose unit. Two one-proton doublets at δ 3.22 (J = 6.5 Hz, H_2-5‴a) and 3.10 (J = 5.6 Hz, H_2-5‴b) were attributed to oxymethylene H_2-5‴ protons of the pentose unit. The ^{13}C NMR spectrum of **2** exhibited signals for carbonyl carbon δ at 177.83 (C-4) and signals at δ 148.06 (C-2) and 133.78 (C-3) supporting the flavonol-type carbon framework of the molecule, other flavonol carbon signals between δ 164.52 – 94.63, anomeric carbons at δ 101.66 (C-1″) and 101.28 (C-1‴), methyl carbon of the rhamnose unit at δ 18.16 (C-6″) and remaining sugar carbons between δ 76.93 – 67.45. Acid hydrolysis of **2** yielded quercetin, m. p. 314 -316 ^oC, R_f 0.38 (toluene : ethyl acetate : formic acid, 5 : 4 : 0.1 (*v/v/v*), D-rhamnose, R_f 0.74 (*n*-butanol-acetone-pyridine-water, 1:1: 0.5: 0.5, *v/v*) and D-xylose, R_f 0.81 (*n*-butonal – pyridine – water, 6 : 4 : 3, *v/v*). On the basis of above mentioned discussion, the structure of compound **2** has been characterized as luteolin -7-O-α-D-rhamnopyranoside, a new flavone rhamnoside (Fig. 1).



Quercetin (**1**)



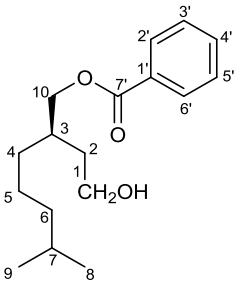
Quercetin-3-*O*-β-D-rhamnosyl-2″-*O*-β-D-xyloside. (**2**)

Fig. 1: Chemical constituents 1 and 2 isolated from the plant of *Cressa cretica* L.

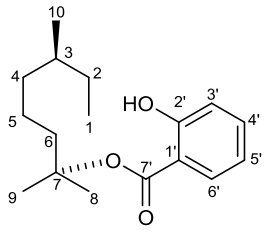
Compound **3** showed UV absorption maximum at 275 nm for an aromatic compound and characteristic IR absorption bands for a hydroxyl function (3360 cm^{-1}), ester group (1723 cm^{-1}) and aromatic ring (1632, 1515, 1086 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **5** was determined at *m/z* 278 consistent with the molecular formula of a monoterpenic aromatic ester, $C_{17}H_{26}O_3$. The ^{1}H NMR spectrum of **3** exhibited three deshielded aromatic proton multiplets at δ 7.32 (2H, H-2' and H-6'), 7.25 (1H, H-4') and 6.77 (2H, H-3' and H-5'). A two-proton doublet at δ 4.08 (J = 10.5 Hz) and a two-proton double doublet at δ 3.34 (J = 11.1, 13.8 Hz) were ascribed to oxymethylene H_2-10 and hydroxyl methylene H_2-1 protons, respectively. Two one-proton multiplets at δ 2.16 and 1.98 were attributed correspondingly to methine H-3 and H-7 protons. The methylene protons appeared as multiplets at δ 1.47 (H_2-2), 1.32 (H_2-4) and 1.21 (H_2-5 and H_2-6). Two three-proton doublets at δ 0.92 (J= 6.9 Hz) and 0.83 (J= 6.7 Hz) were accounted to secondary C-8 and C-9 methyl protons, respectively. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 172.21 (C-7'), aromatic carbons between δ 138.24 – 130.85, methyl carbons at δ 18.92 (C-8) and δ 19.15 (C-9), oxymethylene carbons at δ 66.17 (C-1) and δ 71.78 (C-10), methine carbons at δ 38.75 (C-3) and 34.48 (C-7) and methylene carbons from δ 34.08 to 22.97. Based on the above spectral data analysis, the structure of **3** was established as 1-hydroxygeranilan -10-olyl benzoate, a new monoterpenic ester (Fig. 2).

Compound **4**, $[M]^+$ at *m/z* 278 for $C_{17}H_{26}O_3$, gave positive tests of phenols and had a UV absorption maximum at 279 nm indicating aromatic nature of the compound, Its IR spectrum showed characteristic absorption bands for a hydroxyl function (3432 cm^{-1}), ester group (1721 cm^{-1}) and aromatic ring (1636, 1551 and 1028 cm^{-1}). The ^{1}H NMR spectrum of **4** displayed four one-proton multiplets between δ 7.72 – 6.90 assigned to aromatic H-3' to H-6' protons. A one-proton multiplet at δ 2.65 was ascribed to methine H-3 proton. Four two-proton multiplets at δ 2.31, 1.66, 1.38 and 1.30 were attributed to methylene H_2-6, H_2-5, H_2-2 and H_2-4 protons, respectively. A six-proton singlet at δ 1.25 was due to tertiary C-8 and C-9 methyl protons attached to the quaternary C-7 oxy-carbon. A doublet at δ 0.98 (J = 6.6 Hz) and a triplet at δ 0.98 (J = 6.8 Hz), both integrating for three protons each, were accounted to secondary C-10 and primary C-1 methyl protons, respectively. The ^{13}CNMR spectrum of **4** showed the presence of the ester carbon at δ 167.11 (C-7'), aromatic carbons between δ 160.08 – 128.87, quaternary oxycarbon at δ 71.78 (C-7), methyl carbons at δ 10.93 (C-1), 19.12 (C-8), 22.94 (C-9) and 14.05 (C-10), and methine and methylene carbons in the range of δ 38.69 - 27.95. The absence of any ^{1}H NMR signal between δ 6.90 – 265 and the existence ^{13}C NMR at δ 71.78 (C-7) supported the presence of oxy- quaternary carbon. On the basis of these evidences, the structure of **4** has been elucidated as 7α- salicyloxygeranilane, a new acyclic monoterpenic ester (Fig. 2).

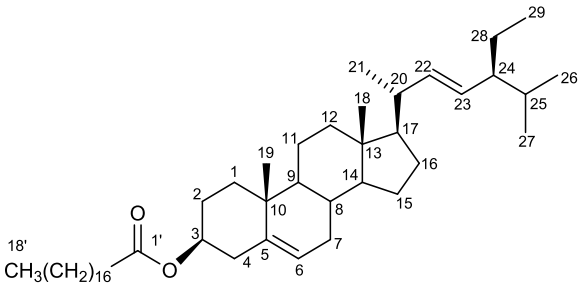
Compound **5** was a known steroidal ester characterized as stigmast-5,22E-dien-3beta-yl octadecenoate (stigmasterol 3-stearate) (Fig. 2).[48]



1-Hydroxygeranilan-10-olyl benzoate (**3**)



7- α-Salicyloxygeranilane (**4**)

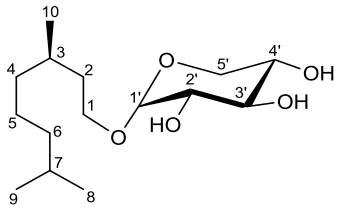


Stigmasterol-3-O-stearate (**5**)

Fig. 2. Chemical constituents 3, 4 and 5 isolated from the plant of *Kyllinga triceps* Rottb.

Compound **6** gave positive tests of glycosides and showed IR absorption bands for hydroxyl groups (3518, 3278 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **6** was established at *m/z*

290 consistent with the molecular formula of a monoterpenic glycoside, $C_{15}H_{30}O_5$. The ion peaks generating at *m/z* 141 [O - C_1 fission, $C_{10}H_{21}]^+$, 149 [M – 141]$^+$ and 157 [$C_{1'}$ - O fission, $C_{10}H_{21}O]^+$ indicated that an acyclic monoterpenic unit was linked with a pentose sugar moiety. The ^{1}H NMR spectrum of **6** displayed a two – proton triplet at δ 3.06 (J = 5.6 Hz) assigned to oxymethylene H_2-1 protons. Three three-proton doublets at δ 1.10 (J = 6.5 Hz), 0.72 (J = 6.3 Hz) and 0.68 (J = 6.6 Hz) were accounted to secondary C-10, C-8 and C-9 methyl protons, respectively. The other methine and methylene protons resonated from δ 2.90 to 1.16. A one – proton doublet at δ 5.19 (J = 7.6 Hz) was ascribed to the anomeric H-1′ proton. The other sugar protons appeared as one – proton multiplets at δ 4.16 (H-2′), 3.62 (H-3′) and 3.42 (H-4′) and as a two – proton doublet at δ 3.11 (J= 8.8 Hz, H_2-5′). The ^{13}C NMR spectrum of **6** displayed signals for anomeric carbon at δ 102.34 (C-1′), other sugar carbons between δ 78.09 – 71.67, oxymethylene carbon at δ 62.79 (C-1), methyl carbons at δ 19.87 (C-8), 19.85 (C-9) and 18.77 (C-10), and the remaining methylene and methine carbons from δ 40.44 to 31.02. The absence of any ^{1}H NMR signal beyond δ 5.19 and ^{13}C NMR signal further δ 102.34 (C-1′) suggested saturated nature of the molecule. Acid hydrolysis of **9** yielded D- xylose, R_f : 0.2 (ethyl acetate-pyridine- water; 8:2:1). On the basis of above discussion the structure of **6** was elucidated as geranilanyl-1-O-β-D-xylopyranoside, a new monoterpenic xyloside (Fig. 3).



Geranilanyl-1-*O*-β-D-xylopyranoside **(6)**

Fig. 3. Chemical constituent 6 isolated from the plant of *Vernonia amygdalina* Delile.

CONCLUSION

Phytochemical investigation of the whole plant of *Cressa cretica* gave two flavonols characterized as Quercetin (**1**) and luteolin -7-O-α-D-rhamnopyranoside (**2**). The plant methanolic extract of *Kyllinga triceps* afforded two new acyclic monoterpenic esters identified as 1-hydroxygeranilan -10-olyl benzoate (**3**) and 7α-salicyloxygeranilane (**4**) and a steroidal ester recognized as stigmast-5,22E-dien-3beta-yl octadecenoate (stigmasterol 3-stearate, **5**). The methanolic extract of *Vernonia amygdalina* plant furnished a new monoterpenic xyloside and its structure was elucidated as geranilanyl-1-O-β-D-xylopyranoside (**6**). This work has enhanced understanding about the chemical constituents of the undertaken plants. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

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