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FLAVONOIDS FROM THE AERIAL PARTS OF *CUBA SPECIOSA* SPRENG., FRUITS OF *MALLOTUS PHILIPPENSIS* (LAM.) MÜLL. ARG. AND FLOWERS OF *PUNICA GRANATUM* L.

Mohammed Ali[1]*, Shahnaz Sultana[1,2] and Showkat Rassol Mir[1]

[1]Phytopharmaceuticals Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, P.O. Hamdard Nagar, New Delhi – 110062, India.

[2]College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

***Corresponding Author: Prof. Mohammed Ali**

Phytopharmaceuticals Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, P.O. Hamdard Nagar, New Delhi – 110062, India.

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ABSTRACT

Cuba speciosa Spreng. (Leguminosae) is a Leguminous plant and these species are used for improving human health, play an important role in nitrogen fixation in atmosphere and are taken as a source of fat, oil and proteins. *Mallotus philippensis* (Lam.) Müll. Arg. (family Euphorbiaceae) is useful to treat abdominal diseases, bronchitis, dysentery, fever, hepatic diseases, malaria, skin diseases, spleen enlargement and as an oral contraceptive. *Punica granatum* L. (Lythraceae, Punicaceae) is a deciduous, multi-stemmed, small tree and its flowers are used to treat diarrhoea, dysentery, haematuria, haemoptysis, nasal haemorrhage, leucorrhoea, sore throat and ulcers of the uterus and rectum. Phytochemical investigation of the aerial parts of *C. speciosa* gave apigenin-4′-rutinoside (**1**). The fruits of *M. philippensis* afforded 7,4′-dihydroxy-3′′, 3′′-dimethyl-(5,6-pyrano-1′′-one)-8- (3′′′, 3′′′-dimethyl allyl)-flavanone (mallotusflavanone, **2**). The flowers of *P. granatum* led to the isolation of four new chemical constituents identified as 4′β-hydroxynon-6′(*Z*)-enyl benzoate (**3**), 13-(15,19,19- trimethylcyclohex-16- en)-yl-2, 6,10-trimethyl-tridec-10-en-6α,13β-diol (punicasesterterpene diol, **4**), 3,7,8,4′- tetrahydroxy- 3′- myrt-1′′- en- yl flavone (punicaflavonyl 3′-myrt-1′′-ene, **5**) and α-D-galactopyranosyl-(6→1′)-O-α-D-galactopyranosyl- (6′→1′′)-O-α-D-galactopyranosyl-(6′′→1′′′)-O-α-D- galactopyranosyl-(6′′′→1′′′′)-O-α-D-rhamnopyranoside (tetragalactosidic rhamnoside, **6**). The structures of all these compounds have been established on the basis of spectral data analyses and chemical reactions.

KEYWORDS: *Cuba speciosa* aerial parts, *Mallotus philippensis* fruits, *Punica granatum* flowers, phytoconstituents, isolation, characterization.

INTRODUCTION

Cuba speciosa Spreng. is a Leguminous plants belonging to Leguminosae family. The leguminous plants grow as trees, shrubs or small plants, produce pods, have tap roots and compound leaves with several small leaflets; flowers like the bean or groundnut flower in shape. Legumes are widely distributed as the third-largest plant. India is the largest producer of pulses and also largest importer of pulses in the world. Farmed legumes include forage, grain, blooms, pharmaceutical, industrial, fallow, green manure, and timber species. The commonly used legumes include alfalfa, chick peas, clovers, cow peas, kidney beans, lentils, mung beans, peanuts, peas, pigeon peas, soy beans, and vetches.[1,2] Leguminous plants are natural biopharmaceutics used for improving human health. These plants play an important role in nitrogen fixation in atmosphere and produce mainly fat, oil (groundnuts and soya beans) and proteins (peas and beans). Plant therapeutics comprises pharmaceuticals, multi-purpose drugs, functional foods, recombinant proteins and vaccines. These modern products support conventional pharmaceuticals for disease diagnosis and treatment. The legumes are used as analgesic, hematinic, tonic (black bean, soy bean), lactagogue, diuretic (Abuki and black beans, pea), laxative (pea) and to treat arthritis, ascites, backache, belching, boils, burns, conjunctivitis, constipation, cough, diabetes, diarrhoea, dysentery, food stagnation, hiccups, hypertension, intestinal ulcers, jaundice, knee pain, leucorrhoea, mumps, oedema of lower extremities, skin eruptions, spasms, painful urination, toxemia during pregnancy, food toxicity and vomiting. Soybean and soyfood phytoestrogens are suggested as possible alternative to hormone replacement therapy for menopausal women. Rotenone from various Lonchocarpus and Derris species is one of the example of insecticide and molluscicide drug.[3] These plants usually contain phytoestrogens, flavonoids, saponins, coumestans and lignans. Peas, soybeans, and lucerne may be used to generate inexpensive monoclonal antibodies or plantibodies as therapeutics for human and

livestock.[4] The isolated proteins, starch and fibers from legume seeds have good physico-chemical and health protecting properties.[2] Stylosanthes, Pueraria, Lablab, Desmodium and other tropical pasture crops are important in tropical and sub-tropical regions as live stock fodder.[5] Legumes accelerate transit of digested food in the intestinal tract and decrease re-absorption of cholesterol, incomplete starch digestion and lowering fermentation processes.[6]

Mallotus philippensis (Lam.) Müll.Arg., syn. *Croton philippensis* Lam., *Echinus philippensis* (Lam.) Baill., *Rottlera tinctoria* Roxb. (family Euphorbiaceae), known as Kamala, Kapila, kumkum tree and Monkey face tree, occurs in China, India, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Philippines to Australia and the Solomon Island. It is a dioecious, up to 25 m tall tree, with pale brown bark; leaves simple, alternate, leathery, ovate-lanceolate, base acute or round, apex acuminate or acute, margin entire or sparsely serrate, glabrous above, greyish pubescent to fulvous tomentose with minute red glands beneath; flowers unisexual, brick red, puberulous; fruit a capsule, globose, 3-lobed, red-glandular, pubescent; seeds 1-4, globose, glabrous, black. All plant parts are used as an alexiteric, anthelmintic, antibacterial, antifilarial, anti-inflammatory, carminative, detergent, immune-regulatory, maturant, purgative, vulnerary, and to treat abdominal diseases, bronchitis, dysentery, fever, hepatic diseases, malaria, skin diseases and spleen enlargement.[7, 8] The plant powder is ingested as an oral contraceptive; the plant mixed with flowers and bark of *Pterospermum philippensis* is applied for suppurating smallpox; and plant juice is lapped and taken orally to subside skin diseases. The flower decoction is given as an anthelmintic. The bark is astringent, anthelmintic and blood purifier; a bark paste is applied to calm down toothache and muscular pain. A stem bark decoction is taken to relieve stomachache. The bark decoction together with *Cuscuta reflexa*, stem bark of *Mangifera indica* and leaves of *Dendrocalamus strictus* is used in baths to cure jaundice. The bark powder pounded with cumin seeds, asafetida and seeds of *Trachyspermum ammi* is given to cure hypocalcemia and Dower cow syndrome in veterinary medicine.[8] A seed decoction is given to expel intestinal worms; seed powder is taken with curd as a vermifuge; seed powder mixed with *Argemone mexicana* latex is layered to treat skin diseases. The seed powder is given as an astringent and anthelmintic and to treat diarrhoea and dysentery in veterinary medicine.[8] The leaves are appetizer, bitter and refrigerant. A leaf decoction is used to treat arthritis, diarrhoea, inflammation, rheumatism and osteoporosis. The bark decoction is taken to relieve abdominal pain and diarrhoea . The red powder of fruits is anthelmintic and administered internally to overcome constipation, dysentery, intestinal worms and tapeworm; it is mixed with a coconut oil and applied to cure ear blisters, skin diseases and ulcers; glands and hair present on the fruits are used as an anthelmintic and purgative; fruit powder is given to children with milk and curd to expel intestinal worms.[8] The fruits and bark are ingested to prevent stomach ulcers and tapeworm. Root scrapings are chewed with a betel mixture as a contraceptive for women, the roots are given as a postpartum remedy; fresh root juice is dropped into the ear to calm down earache.[8,9]

The *M. philippensis* plant contained acetyl aleuritolic acid, cortotoxigenin, its rhamnoside, α-amyrin, coroglaucigenin, its rhamnoside, octacosanol, β–sitosterol, its glycoside, bergenin, rottlerin, isoallorottlerin, isorottlerin, kamalins, wax, homorottlerin, phorbic acid, gum, bergenin, citric and oxalic acids, tannins, volatile oil, betulin-3-acetate, lupeol, its acetate, kamaladiol-3-acetate, kamalachalcones A, B and E, mallotophilippens C, D, and E and kamlolenic acid.[10-17] The fruits afforded 7,11-diketo-lanost-3-ol acetate, lanosta-8-ene-3*β*-ol acetate, pregnenolone acetate, *trans*-chalcone, kamalachalcone E, oleanolic acid, gallic acid, flavonoids, 4′-hydroxyisorottlerin, rottlerin and shikimic acid.[18,19]

Punica granatum L. (Lythraceae, Punicaceae), known as anar and pomegranate, is distributed from Iran to northern India, Mediterranean region, Middle East, tropical Africa, Arizona and California. It is a deciduous, multi-stemmed, small tree, 5 - 8 m in height; stem woody and spiny; bark smooth and dark grey; leaves simple, oblong or obovate, glabrous, oppositely placed, short-petioled, entire, shining; flowers regular, solitary or in fascicles at apices, petals lanceolate, 5-7, wrinkled and brilliant orange-red; fruit a round berry, attractive, reddish scarlet and edible, pericarp leathery, with persistent callipe and a coriaceous woody rind, interiorly compartmentalized with many pink-red sections of pulp-like tissue; seeds numerous, angular with fleshy testa.

The *P. granatum* flowers, known as Gulnar, are efficacious to treat cough, diarrhoea, dysentery, haematuria, haemoptysis, nasal haemorrhage, leucorrhoea, sore throat, and ulcers of the uterus and rectum.[8,20,21] Plant extract is used to cure ciguatera fish toxicity. A decoction of the tender shoots is given to relieve oliguria, typhoid fever and urinary troubles. A pomegranate bark decoction with whole plant of *Oxalis corniculata* is taken in loss of appetite. Fresh bark juice is given against diabetes. The root bark and wood are used as a vermifuge for tapeworm and against diarrhoea and dysentery. The fruits are taken as a laxative and to comfort brain diseases, chest troubles, dysentery, sore throat, stomach diseases and dysentery. Unripe fruits are ingested as an emetic and against diarrhoea and dysentery. The fruit rind is administered orally to treat ascariasis, diarrhoea, dysentery, prolapse of the rectum, irregular menstruation, excessive bleeding and white discharge. The seeds are eaten to improve digestion, stomach troubles and to increase sexual vigour. The seeds are reputed as a stomachic and to increase fertility. The leaf and root decoctions are recommended for

irregular menses. The leaf and fruit decoctions are astringent in dysentery and as an antiemetic in cholera. Tender leaves are ingested as an emetic and to prevent diarrhoea. The green leaf paste is applied to relieve conjunctivitis.[8,21,22]

The fruits of *P. granatum* contained phenolic compounds, organic acids, lignans, gallic and ellagic acids, punicalagins A and B,[23-25], anthocyanins, gallo- and ellagitannins, galloyl esters, hydroxybenzoic and cinnamic acids and dihydroflavonol.[26] The fruit rind afforded phenolic acids, punicalin, punicalagin, caffeic acid, flavonoids, ellagitannins, and pelletierine alkaloids.[26-28] Pomegranate juice possessed simple sugars, aliphatic organic acids, phenolic acids, quinic acid, flavonols, anthocyanins, amino acids, minerals, epigallocatechin gallate and ascorbic acid.[29-39] Dried pomegranate raisins (*anardana*) have substantial amounts of anthocyanins, ellagitannins and a neolignan.[40,41] The stem bark yielded ellagitannins, piperidine, pyrrolidine, pelletierine alkaloids , quercetin, flavonoid glycosides, pelargonidine-3,5- diglucoside and ellagic acid.[42-46] The flowers possessed gallic acid, triterpenic acids, tricin, catechin, rutin, apigenin, apigenin-7-O-glucoside, β-stitosterol, daucosterol, 2, 3, 4-trihydroxypentanoic acid, ellagitannins , flavonoids, phenolics, punicanolic acid and fatty acids.[47-53] The leaves furnished carbohydrates, reducing sugars, sterols, saponins, flavonoids, piperidine alkaloids, flavone glycoside and ellagitannins.[54-58] The heartwood produced ellagic- and gallotannins and ellagic acid rhamnosides.[59-61] The seeds afforded 3,3′-di- and 3,3′,4′-tri-O-methylellagic acids, punicic, oleic, palmitic, stearic and linoleic acids, tocopherols , steroids oestrogen, cerebroside and a fixed oil composed of mono- and triacyl glycerols. The seed cover of the fruits contained anthocyanin glycosides of delphinidin, cyanidin, and pelargonidin, punicic acid and linolenic acid.[62-73] Punicic, linolenic and phenolic acids, quercetin, naringenin, γ-tocopherol, carotenoids were present in the seed oil. The seed volatile fraction was composed chiefly of alcohols, aldehydes, ketones, esters and carboxylic acids. [74] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations, aerial parts of *Cuba speciosa*, fruits of *Mallotus philippensis* and flowers of *Punica granatum* 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 and ^{13}C NMR spectra were scanned on a Bruker DRX (400 MHz) instrument 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). Purity of the compounds was checked by TLC over 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 material

The aerial parts of *Cuba speciosa* were collected from the cultivated plants grown in Lucknow. The fruits of *Mallotus philippensis* and the flowers of *Punica granatum* were procured from the local market of Khari Baoli, Delhi. These plant materials were identified by Prof. M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard. The voucher specimens of these drugs are preserved in the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

Extraction and isolation

The pulverized materials (1.0 kg each) were extracted exhaustively in a Soxhlet apparatus with ethanol (95%). The combined extracts of each drug were dried under reduced pressure separately to secure a viscous dark brown residues (136 g, 118g and 121 g, respectively). A small portion of the each extract was analyzed chemically to determine the presence of different types of chemical constituents. The dried residues (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain slurries. The slurries were air-dried and chromatographed individually over a silica gel column loaded in petroleum ether (b. p. 60 - 80°C). 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 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 a chemical constituent from *Cuba speciosa* aerial parts

Apigenin-4′-rutinoside (1)

Elution of the column of *C. speciosa* extract with chloroform - methanol (4:1) produced yellow crystals of **1**, yield 204 mg, m. p. 292 - 294 °C; UV λmax (MeOH): 272, 325 nm (log ε 3.2, 3.6); IR (KBr) γ_{max} : 3424, 3372, 3218, 2973, 2916, 1657, 1607, 1528, 1494, 1450, 1369, 1298, 1178, 1074, 1043, 828 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.58 (1H, d, *J* = 1.8 Hz, H-8), 7.85 (2H, d, *J* = 9.8 Hz,

H-2′, H-6′), 6.92 (2H, d, *J* = 9.8 Hz, H-3′, H-5′), 6.72 (1H, s, H-3), 6.41 (1H, d, *J* = 1.8 Hz, H-6), 5.28 (1H, d, J = 2.2 Hz, H-1″α), 3.98 (1H, m, H-5″), 3.74 (1H, m, H-2″), 3.66 (1H, m, H-3″), 3.42 (1H, m, H-4″), 3.31 (2H, d, J = 6.8 Hz, H_2-6″), 5.19 (1H, d, J = 4.1 Hz, H-1‴α), 3.94 (1H, m, H-5‴), 3.68 (1H, m, H-2‴), 3.57 (1H, m, H-3‴), 3.39 (1H, m, H-4‴), 1.31 (3H, d, J = 6.3 Hz, Me-6‴); ^{13}C-NMR (DMSO-d_6): δ 162.91 (C-2), 103.84 (C-3), 183.64 (C-4), 164.33 (C-5), 100.05 (C-6), 166.67 (C-7), 95.86 (C-8), 158.85 (C-9), 105.57 (C-10), 122.94 (C-1′), 129.57 (C-2′), 117.07 (C-3′), 162.85 (C-4′), 117.05 (C-5′), 129.63 (C-6′), 102.56 (C-1″), 78.28 (C-2″), 72.15 (C-3″), 70.04 (C-4″), 79.07 (C-5″), 62.47 (C-6″), 99.75 (C-1‴), 73.99 (C-2‴), 71.39 (C-3‴), 69.86 (C-4‴), 79.04 (C-5‴), 18.28 (C-6‴); +ve FAB MS *m/z* (rel. int.): 578 $[M]^+$ ($C_{27}H_{30}O_{14}$) (28.9), 309 (12.6), 269 (9.8), 163 (13.6), 147 (16.1).

Isolation of a chemical constituent from *Mallatus philipinensis* fruits

Mallotusflavanone (**2**)

Elution of the column of *M. philipinensis* fruit extract with chloroform - methanol (9:1) fraction yielded yellow semisolid mass of **2**, yield 184 mg, UV λmax (MeOH): 291, 323 nm; IR (KBr) γ_{max} : 3414, 3327, 2913, 2843, 1681, 1640, 1623, 1582, 1443, 1367, 1298, 1127, 1109, 981, 887 cm^{-1}; ^{1}H NMR (DMSO-d_6): *δ* 7.53 (2H, d, J = 9.8 Hz, H-2′, H-6′), 6.69 (2H, d, J = 9.8 Hz, H-3′, H-5′), 5.59 (1H, dd, J = 2.6, 13.6 Hz, H-2), 2.88 (1H, dd, J = 13.6, 17.2 Hz, H-3a), 2.83 (1H, dd, J = 14.8, 2.7 Hz, H-3b), 3.65 (2H, s, H_2-2″), 1.59 (3H, s, Me-4″), 1.57 (3H, s, Me-5″), 5.58 (1H, dd, J = 2.7, 12.8 Hz, H-2‴), 3.37 (1H, d, J = 2.7 Hz, H_2-1‴a), 3.28 (1H, d, J = 12.7 Hz, H_2-1‴b), 1.96 (3H, brs, Me-4‴), 1.98 (3H, brs, Me-5‴); ^{13}C NMR (DMSO-d_6): δ 81.66 (C-2), 42.94 (C-3), 195.92 (C-4), 159.51 (C-5), 103.15 (C-6), 162.49 (C-7), 104.56 (C-8), 157.93 (C-9), 106.24 (C-10), 136.19 (C-1′), 115.67 (C-2′), 129.27 (C-3′), 157.56 (C-4′), 127.15 (C-5′), 126.53 (C-6′), 203.87 (C-1″), 33.23 (C-2″), 81.54 (C-3″), 16.63 (C-4″), 7.62 (C-5″), 29.13 (C-1‴), 130.16 (C-2‴), 136.18 (C-3‴), 27.91 (C-4‴), 27.86 (C-5‴); +ve FAB MS *m/z* (rel. int.): 422 $[M]^+$ ($C_{25}H_{26}O_6$) (1.8).

Isolation of chemical constituents from *Punica granatum* flowers

4′-Hydroxynon-6′(Z)-enyl benzoate (**3**)

Elution of the column of *P. granatum* flowers extract with petroleum ether - chloroform (1:1) afforded pale yellow crystals of **3**, recrytallized from chloroform-methanol (1:1), 112 mg, m. p.110-111 °C, IR γmax: 3413, 2924, 2825, 1725, 1631, 1550, 1402, 1362, 1253, 925, 1094, 794 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.30 (2H, m, H-2, H-6), 7.21 (2H, m, H-3, H-5), 6.63 (1H, m, H-4), 5.31 (1H, m, $w_{1/2}$ = 10.7 Hz, H-6′), 5.13 (1H, m, $w_{1/2}$ = 9.8 Hz, H-7′), 4.03 (2H, t, J = 6.3 Hz, H_2-1′), 3.86 (1H, brm, $w_{1/2}$ = 15.7 Hz, H-4′), 2.43 (2H, m, H_2-5″), 2.39 (2H, m, H_2-8′) 2.01 (2H, m, H_2-2′), 1.23 (2H, m, CH_2-3′), 0.83 (3H, t, J=6.1 Hz, Me-9′); ^{13}C NMR ($CDCl_3$): δ 136.3 (C-1), 129.7 (C-2), 121.9 (C-3), 115.6 (C-4), 118.9 (C-5), 128.6 (C-6), 171.3 (C-7), 64.2 (C-1′), 29.3 (C-2′), 28.9 (C-3′), 81.1 (C-4′), 33.6 (C-5′), 124.3 (C-6′), 123.7 (C-7′), 23.6 (C-8)′, 19.1 (C-9′); EIMS *m/z* (rel. int.): 262 $[M]^+$ ($C_{16}H_{22}O_3$) (1:1), 157 (8.5), 141 (3.5), 121 (26.3), 109 (30.9), 105 (28.7), 99 (12.1), 81 (45.11), 77 (10.3), 69 (84.5), 55 (100).

Punicasesterterpene diol (4)

Elution of the column with chloroform yielded pale yellow crystals of **4**, recrystallized from methanol, 427 mg, m. p. 121-122°C, IR γmax: 3390, 3365, 2923, 2853, 1631, 1453, 1377, 1258, 1109 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.99 (1H, dd, J = 9.9, 6.9 Hz, H-16), 5.64 (1H, m, $w_{1/2}$ = 10.8 Hz, H-17), 5.30 (1H, m, H-11), 3.65 (1H, brm, $w_{1/2}$=15.5 Hz, H-13α), 2.77 (1H, m, H-15α), 2.47 (3H, brs, Me-22), 2.23 (2H, m, H_2- 9), 2.09 (1H, d, J = 7.43 Hz, H-18a), 2.06 (IH, d, J = 16.4 Hz, H-18b), 1.98 (2H, m, H_2 -12), 1.44 (2H, m, H_2 –5), 1.39 (1H, dd, J = 14.9, 7.6 Hz, H-14), 1.28 (2H, m, H_2 -8), 1.24 (2H, m, H_2 -7), 1.33 (1H, m, H-2), 1.29 (2H, m, H_2 -3), 1.22 (2H, m, H_2 -4), 1.20 (3H, brs, Me-21), 1.18 (3H, d, J = 6.9 Hz, Me-23), 1.15 (3H, brs, Me-24), 1.13 (3H, brs, Me-25), 0.87 (3H, d, J = 6.6 Hz, Me-20), 0.83 (3H, d, J = 6.1 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 14.6 (C-1), 28.6 (C-2), 26.9 (C-3), 28.5 (C-4), 29.1 (C-5), 81.1 (C-6), 28.9 (C-7), 28.9 (C-8), 31.8 (C-9), 144.3 (C-10), 129.1 (C-11), 29.3 (C-12), 76.2 (C-13), 34.1 (C-14), 31.3 (C-15), 118.3 (C-16), 115.1 (C-17), 29.0 (C-18), 49.6 (C-19), 18.6 (C-20), 22.1 (C-21), 24.4 (C-22), 22.3 (C-23), 21.6 (C-24), 25.3 (C-25); EIMS *m/z* (rel. int): 378 $[M]^+$ ($C_{25}H_{46}O_2$) (12.3), 378 (5.1), 262 (21.7), 259 (22.3), 249 (100), 207 (21.6), 192 (11.3), 189 (17.6), 171 (13.2), 153 (32.6), 135 (33.1), 129 (26.5), 123 (92.6), 108 (30.3), 95 (32.6), 85 (11.9).

Punicaflavonyl 3′-myrt-1″-ene (5)

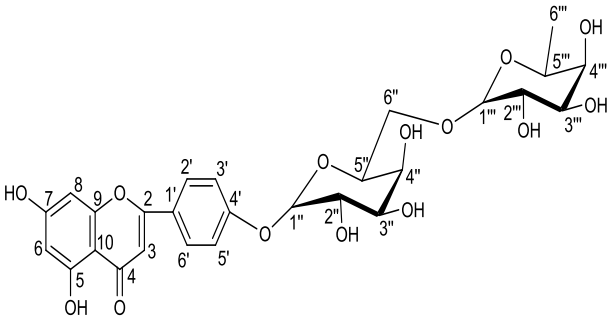
Elution of the column with chloroform - methanol (19:1) furnished light red coloured crystals of **5**, recrystallized from acetone, 135 mg, m. p. 294- 295°C; UVλ_{max} (MeOH) 271, 325 nm (log ε 7.1, 3.2), UVλ_{max} (MeOH + NaOMe) 279, 370 nm; UVλ_{max} (MeOH + NaOAc) 280, 326 nm; UVλ_{max} (MeOH + NaOAc + H_3BO_3) 283, 330 nm; UV (MeOH + $AlCl_3$) 270, 331 nm; IR γmax (KBr): 3439, 2922, 2851, 1680, 1620, 1555, 1408, 1361, 1315, 1098, 794 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.68 (1H, d, J = 8.5 Hz, H-5), 7.43 (1H, d, J = 3.0 Hz, H-2′), 7.38 (1H, m, H-6′), 6.81 (IH, d, J = 8.7 Hz, H-5′), 6.62 (1H, d, J=8.5 Hz, H-6), 5.37 (1H, m, H-2″), 2.48 (2H, brs, H_2-10″), 2.26 (1H, dd, J = 7.1 Hz, 4.4 Hz, H-6″), 1.97 (2H, m, H_2-3″), 1.60 (2H, m, H-4″), 1.23 (2H, m, H_2-7″), 1.21 (3H, brs, Me-8″), 0.83 (3H, brs, Me-9″); ^{13}C NMR (DMSO-d_6): δ 146.3 (C-2), 138.2 (C-3), 176.6 (C-4), 127.1 (C-5), 98.8 (C-6), 159.1 (C-7), 130.8 (C-8), 145.1 (C-9), 113.9 (C-10), 125.2 (C-1′), 113.8 (C-2′), 128.6 (C-3′), 151.3 (C-4′), 114.3 (C-5′), 127.0 (C-6′), 139.2 (C-1″), 130.8 (C-2″), 29.1 (C-3″), 33.7 (C-4″), 37.3 (C-5″), 31.3 (C-6″), 26.2 (C-7″), 21.6 (C-8″), 16.3 (C-9″), 33.9 (C-10′); EIMS *m/z* (rel. int.): 420 $[M]^+$ ($C_{25}H_{24}O_6$) (6.1), 285 (4.3), 284 (4.8), 256 (5.5), 227 (9.4), 212 (11.8), 121 (18.2), 136 (14.5), 135 (12.6), 108 (14.2), 106 (23.7), 83 (48.3), 68 (74.1), 55 (100).

Tetragalactosidic rhamnoside (6)

Elution of the column with chloroform - methanol (3:1) gave colourless crystals of **6**, recrystallized from methanol, 275 mg, m. p. 295 – 296 °C, IR γmax: 3550, 3430, 3360, 3240, 2957, 2848, 1640, 1457, 1393, 1239, 1064, 1006, 922, 774 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.33 (1H, d, J = 4.1 Hz, H-1), 4.83 (1H, m, H-5), 4.53 (1H, m, H-2), 3.80 (1H, m, H-3), 3.38 (1H, m, H-4), 3.27 (2H, d, J = 8.2 Hz, H_2-6), 5.21 (1H, d, J=5.4 Hz, H-1′), 4.80 (1H, m, H-5′), 4.48 (1H, m, H-2′), 3.76 (1H, m, H-3′), 3.36 (1H, m, H-4′), 3.25 (2H, d, J = 8.9 Hz, H_2-6′), 5.06 (1H, d, J = 3.9 Hz, H-1″), 4.78 (1H, m, H-5″), 4.43 (1H, m, H-2″), 3.73 (1H, m, H-3″), 3.33 (1H, m, H-4″), 3.21 (2H, d, J = 9.3 Hz, H_2-6″), 5.02 (1H, d, J = 4.8 Hz, H-1‴), 4.75 (1H, m, H-5‴), 4.39 (1H, m, H-2‴), 3.69 (1H, m, H-3‴), 3.31 (1H, m, H-4‴), 3.19 (2H, d, J = 8.9 Hz, H_2-6‴), 4.90 (1H, d, J =3.7 Hz, H-1⁗), 4.71 (1H, m, H-5⁗), 4.32 (1H, m, H-2⁗), 3.64 (1H, m, H-3⁗), 3.29 (1H, m, H-4⁗), 1.09 (3H, d, J=7.1 Hz, Me-6⁗); ^{13}C NMR (DMSO-d_6): δ 106.6 (C-1), 76.8 (C-2), 72.68 (C-3), 69.4 (C-4), 82.84 (C-5), 63.5 (C-6), 104.0 (C-1′), 75.89 (C-2′), 72.2 (C-3′), 69.0 (C-4′), 81.63 (C-5′), 63.0 (C-6′), 99.7 (C-1″), 75.46 (C-2″), 72.0 (C-3″), 68.4 (C-4″), 80.48 (C-5″), 62.6 (C-6″), 96.98 (C-1‴), 74.99 (C-2‴), 70.5 (C-3‴), 64.3 (C-4‴), 78.3 (C-5‴), 62.3 (C-6⁗), 102.7 (C-1⁗), 73.58 (C-2⁗), 70.35 (C-3⁗), 64.8 (C-4⁗), 77.3 (C-5⁗), 15.3 (C-6⁗); FAB MS *m/z* (rel.int.): 812 $[M]^+$ ($C_{30}H_{52}O_{25}$) (3.2), 503 (12.7), 341 (9.1), 325 (14.6), 179 (23.6), 163 (8.6).

RESULTS AND DISCUSSION

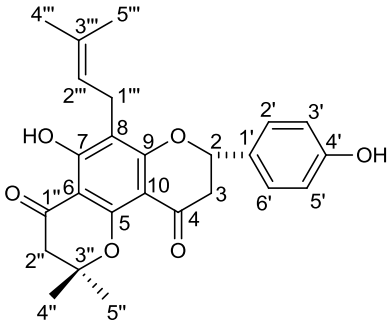
Compound **1** was a known flavone identified as apigenin 4′-O-*α*-L-glucopyranosyl-(6″→1‴)-*α*- L-rhamnopyranoside (apigenin-4′-rutinoside).[75] (Fig 1).



Apigenin-4′-rutinoside (1)

Fig. 1: Structural formula of chemical constituent 1 isolated from the *Cuba speciosa* aerial parts.

Compound **2**, named mallotusflavanone, was isolated as a yellow semisolid mass. Its UV spectrum had absorption maxima at 291 and 323 nm characteristic to a flavanone derivative.[76] A shift of + 40 nm of band I with increased intensity on addition of sodium methoxide supported a free C-7 hydroxy group and no free C-5 hydroxy group. There was a shift of 60 nm of band I on addition of sodium acetate indicating a free C-7 hydroxy group without C-5 hydroxy group. There was no change of band I on addition of aluminum chloride solution suggesting the absence of dihydroxy groups in the molecule.[77] It gave positive ferric chloride test for a phenolic compound. The IR spectrum of **2** disclosed the existence of distinctive absorption bands for the conjugated carbonyl groups (1681 cm^{-1}) and hydroxy functions (3414, 3327 cm^{-1}) in the molecule. The carbonyl groups were supported by carbon signals at δ 195.92 (C-4) and 203.87 (C-1″) in its ^{13}C NMR spectrum. The molecular ion peak of **2** was determined at *m/*z 422 on the basis of mass and ^{13}C NMR spectrum consistent with a molecular formula of an isopentenylated flavanone with pyranone ring, $C_{25}H_{26}O_6$. The ^1H NMR spectrum of **2** showed an ABX system of resonances as one-proton double doublets at δ 5.59 (J = 3.2, 12.9 Hz, H-2), 2.88 (J = 13.6, 17.2 Hz, H_2-3a) and 2.83 (J = 2.7, 14.8 Hz, H_2-3b) characteristic of oxymethine H-2 and methylene H_2-2 eq and H_2-2 ax, respectively, of a flavanone moiety.[78] Two two-proton doublets at δ 7.53 and 6.69 with coupling interactions of 9.8 Hz each were assigned to B-ring H-2′, H-6′ and H-3′ and H-5′ protons, respectively. The double doublet at δ 5.58 was due to vicinal coupling of H-2 proton separately with the axial and equatorial protons at positions C-3, whereas the double – doublets at δ 2.88 and 2.83 were due to both vicinal and germinal couplings as evident from the J values. A two-proton singlet at δ 3.65 was due to methylene H_2-2″ protons. Two three-proton singlets at δ 1.59 and 1.57 were associated to tertiary methyl H_3-4″ and H_3-5″ protons, respectively, of the pyranone ring. A one-proton double doublet at δ 5.58 (J = 2.7, 12.8 Hz) was accounted to vinylic H-2‴ proton linked a methylene group. Two one-proton doublets at δ 3.37 (J = 2.7 Hz) and 3.28 (J = 12.7 Hz) were accounted to methylene H_2-1‴a and H_2-1‴b protons present between vinylic and aromatic carbons. Two three-proton singlets at δ 1.96 and 1.98 were attributed correspondingly to methyl H_3-4‴ and H_3-5‴ protons linked to vinylic C-3‴ carbon. The ^{13}C NMR spectrum of **2** exhibited signals for oxymethine carbon at δ 81.66 (C-2), methylene carbon at δ 42.94 (C-3) and carbonyl carbon at δ 195.92 (C-4) supporting the flavanone-type carbon framework of the molecule[79, 80], other flavanone carbons between δ 162.49 - 103.15, dimethyl pyranone carbons at δ 203.87 (C-1″), 33.23 (C-2″), 81.54 (C-3″), 16.63 (C-4″), 7.62 (C-5″), vinylic carbons of isopentenyl unit at δ 130.16 (C-2‴) and 136.18 (C-3‴), methylene carbon at δ 29.13 (C-1‴) and methyl carbons at δ 27.91 (C-4‴) and 27.86 (C-5‴). The presence of C-6 carbon signal in the deshielded region at δ 103.15 and C-8 104.56 suggested substitutions at these carbons.[81] These evidences led to established the structure of **2** as 7,4′-dihydroxy-(5,6-pyrano-1″-one)-8- (3‴, 3‴-dimethyl-2‴-en allyl)- flavanone, a new flavanone derivative from a plant source (Fig 2).



Mallotusflavanone (2)

Fig. 2: Structural formula of chemical constituent 2 isolated from the *Mallatus philipinensis* fruits.

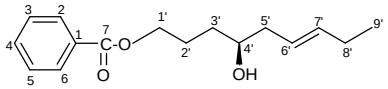
Compound **3** decolourized bromine water indicating its unsaturated nature. Its IR spectrum demonstrated the presence of a hydroxyl group (3413 cm-1), an ester function (1725 cm-1), unsaturation (1631 cm-1) and aromatic ring (1550, 1094 cm^{-1}). The mass spectrum of **3** exhibited a molecular ion peak at *m/z* 262 corresponding to an aromatic acid ester, $C_{16}H_{22}O_3$. The prominent ion peaks generating at *m/z* 77 $[C_6H_5]^+$, 105 $[C_6H_5CO]^+$, 121 $[C_6H_5COO]^+$, 157 [M-105, $C_9H_{17}O_2]^+$ and 141 [M-121, $C_9H_{17}O]^+$ indicated the involvement benzoic acid in the ester formation. The ion fragments arising at *m/z* 99 [C_3' - C_4' fission, $C_6H_{11}O]^+$, 69 [C_4'- C_5' fission, $C_5H_9]^+$ and 55 [C_5'-C_6' fission, $C_4H_7]^+$ suggested the existence of the hydroxyl group at C-4′ and vinylic linkage at $\Delta^{6'}$. The ^{1}H NMR spectrum of **3** displayed two multiplets at δ 7.30 and 7.21 integrating for two protons each and a one-proton multiplet at δ 6.63, all assigned to aromatic H-2 and H-6, H-3 and H-5 and H-4 protons, respectively. Two one- proton multiplets at δ 5.31 ($w_{1/2}$ = 10.7 Hz) and 5.13 ($w_{1/2}$ = 9.8 Hz) were accounted to cis- oriented (Z) vinylic H-6′ and H-7′ protons, respectively. A two-proton triplet at δ 4.03 (J=6.3 Hz) and a one- proton broad multiplet at δ 3.86 with $w_{1/2}$ 15.7 Hz were attributed to oxygenated H_2-1′ methylene and carbinol H-4′α protons, respectively. Four two-proton multiplets between δ 2.43 – 1.23 were ascribed to the methylene protons. A three-proton triplet at δ 0.83 (J = 6.1 Hz) was associated with C-9′ primary methyl protons. The ^{13}C NMR spectrum of **3** showed signals for ester carbon at δ 171.3 (C-7), aromatic and vinylic carbons between δ 136.3- 115.6, oxymethylene carbon at δ 64.2 (C-1′), carbinol carbon at δ 81.1 (C-4′), methylene carbons from δ 29.3 to 23.6 and methyl carbon at δ 19.1 (C-9′). On the basis of foregoing account, the structure of **3** has been established as 4′β-hydroxynon-6′(Z)-enyl benzoate, a new benzoic acid ester (Fig 3).

Compound **4**, named punicasesterterpene diol, decolourized bromine water and showed IR absorption bands for hydroxyl groups (3390, 3365 cm-1) and unsaturation (1631 cm^{-1}). Its mass spectrum displayed a molecular peak at *m/z* 378 corresponding to a dihydroxy-monocyclic sesterterpene, $C_{25}H_{46}O_2$. The important ion fragments produced at *m/z* 123 [C_{13}- C_{14} fission, $C_9H_{15}]^+$, 108 [123 – Me]$^+$, 153 [C_{12}- C_{13} fission, $C_{10}H_{17}O]^+$, 135 [153 – $H_2O]^+$, 207 [C_9- C_{10} fission, $C_{14}H_{23}O]^+$, 192 [207 – Me]$^+$ and 189 [207 – $H_2O]^+$ indicated the presence of an unsaturated cyclic ring at one of the terminal of an aliphatic chain, one of the hydroxy group at C-13 and the vinylic bond at C-10. The ion fragments arising at *m/z* 171 [M – 207]$^+$, 249 [C_6 – C_7 fission, $C_{17}H_{29}O]^+$, 129 [M – 249, $C_8H_{17}O]^+$, 85 [C_5 – C_6 fission, $C_6H_{13}]^+$, 259 [M – 85 –$H_2O]^+$ and 262 [M – 85 – Me]$^+$ supported the existence of another hydroxyl group at C_6 carbon. The ^{1}H NMR spectrum of **4** exhibited a one-proton triple doublet at δ 5.99 (J = 9.9, 6.9 Hz) and two one-proton multiplets at δ 5.64 ($w_{1/2}$ = 10.8 Hz) and 5.30 assigned to vinylic H-16, H-17 and H-11 protons, respectively. A one-proton broad multiplet at δ 3.65 with half-width of 15.5 Hz was ascribed to α-oriented carbinol H-13 proton. Four three-proton broad singlets at δ 2.47, 1.20, 1.15 and 1.13 were associated with tertiary C-22 methyl protons linked to vinylic C-10 carbon, C-21 methyl protons located on carbinol C-6 carbon and C-24 and C-25 methyl carbons present on C-19 quaternary carbon, respectively. Three doublets at δ 1.18 (J = 6.9 Hz), 0.87 (J = 6.6 Hz) and 0.83 (J = 6.1 Hz) integrating for three protons each were attributed to secondary C-23, C-20 and C-1 methyl protons, respectively. The remaining methine and methylene protons resonated as a one-proton double doublet at δ 1.39 (J = 14.9, 7.6 Hz, H-14), as one-proton doublets at δ 2.09 (J = 7.43 Hz, H-18a), 2.06 (J = 16.4 Hz, H-18b) and as multiplets from δ 2.23 to 1.22. The ^{13}C NMR spectrum of **4** showed signals for vinylic carbons at δ 144.3 (C-10), 129.1 (C-11), 118.3 (C-16) and 115.1 (C-17), carbinol carbons at δ 81.1 (C-6) and 76.2 (C-13) and methyl carbons between δ 25.3 – 14.6. The ^{1}H and ^{13}C NMR values of **4** were compared with the reported spectral data of other sesterterpene molecules.[82] On the basis of foregoing account, the structure of **4** has been established as 13-(15,19,19-trimethylcyclohex-16- en)-yl-2, 6,10-trimethyl-tridec-10-en-6α,13β-diol (Fig 3).

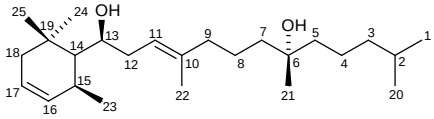
Compound **5**, named punicaflavonyl 3′-myrt-1′′-ene, was obtained as a light red coloured crystalline mass from the chloroform - methanol (19:1) eluants. It responded positively to flavonoid tests and displayed UV absorption maxima at 271 and 325 nm for a flavonol- type molecule. There was a shift of band I with sodium methoxide suggesting the presence of free hydroxy groups, a shift of bands with sodium acetate solution indicating free nature of 7-hydroxyl group, and a shift of band I with boric acid supporting the existence of free ortho-7,8-dihydroxyhydroxyl groups. There was no shift of band I with aluminum chloride and hydrochloric acid excluding the occurrence of 5-hydroxyl group and B-ring *o*-dihydroxy functions.[77,83] Its IR spectrum showed absorption bands for hydroxyl groups (3439 cm^{-1}), carbonyl function (1680 cm^{-1}), unsaturation (1620 cm^{-1}) and aromatic ring (1555, 1098 cm^{-1}). The mass spectrum of **5** exhibited a molecular ion peak at *m/z* 420 consistent with a molecular formula of a bicyclic monoterpene substituted flavonol, $C_{25}H_{24}O_6$. The important ion

fragments arose at *m/z* 108 [$C_{4,10}$ -$C_{9,0}$ fission]$^+$, and 136, 284 [$C_{3,4}$ -$C_{9,O}$ fission]$^+$ suggesting the existence of two hydroxyl groups in ring A and a monoterpenic moiety in ring B. The ion peaks formed at *m/z* 227 [C_2- C_1' fission]$^+$, 212 [227- Me] $^+$, 135, 285 [C_3'-C_{10}'' fission] $^+$, 121 [C_1''-C_{10}''fission] $^+$ and 106 [121- Me] $^+$ indicated location of another hydroxyl group and the monoterpenic moiety in ring B. The intense ion peaks produced at *m/z* 83 [C_1'', $_6''$- C_3'',$_4''$ fission]$^+$ and 68 [83- Me]$^+$ supported the presence of the tetracyclic ring in a myrtene- type monoterpene. The ^{1}H NMR spectrum of **5** displayed three one-proton *ortho*- coupled doublets at δ 7.68 (J=8.5 Hz), 6.62 (J=8.5 Hz) and 6.81 (J=8.5 Hz) assigned to H5, H-6 and H-5′ protons, respectively. A one- proton *meta*-coupled doublet at δ 7.43 (J=3.0 Hz) and a one-proton multiplet at δ 7.38 were accounted to H-2′ and H-6′ protons, respectively. Another one proton multiplet at δ 5.37 was ascribed to vinylic H-2″. Two three- proton singlets at δ 1.21 and 0.83 were associated correspondingly with C_8'' and C_9'' tertiary methyl protons. A singlet at δ 2.48 and a multiplet at δ 1.23 integrating for two protons each, a one-proton double doublet at δ 2.26 (J = 4.4, 7.1 Hz) and a one-proton multiplet at δ 1.60 were attributed to methylene H_2-10″ and H_2-7″ and methine H-6″ and H-4″ protons of the myrtene unit, respectively. The ^{13}C NMR spectrum of **5** exhibited carbon signals for the flavonol moiety between δ 176.6- 113.8. The vinylic carbons resonated at δ 139.2 (C-1″) and 130.8 (C-2″). The upfield carbon signals in the range δ 37.3 - 16.3 were assigned to the remaining monoterpenic carbons. The absence of any ^{1}H NMR signal between δ 5.37- 2.48 and ^{13}C NMR signal in the range of δ 113.8- 37.3 ruled out the existence of any carbinol signal in the molecule and supported the carbon linkage of the monoterpene moiety with the flavone between C-3′ and C-10″. The ^{1}H and ^{13}C NMR spectral data of **5** were compared with the reported spectral values of flavonoids.[84-86] Based on these evidences, the structure of **5** has been established as 3,7,8,4′-tetrahydroxy- 3′- myrt-1″- en- yl flavone (Fig 3).

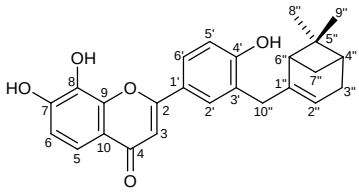
Compound **6**, named tetragalactosidic rhamnoside, was obtained as a colourless crystalline mass from chloroform -methanol (3:1) eluants. It responded glycosidic tests positively and exhibited characteristics IR absorption bands for hydroxyl groups (3550, 3430, 3360, and 3240 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **6** was determined at *m/z* 812 consistent with a molecular formula of a pentaglycoside, $C_{30}H_{52}O_{25}$. The ion peaks produced at *m/z* 179 [$C_6H_{11}O_6$]$^+$, 341 [$C_6H_{11}O_6$-$C_6H_{11}O_5$, $C_{12}H_{21}O_{11}$]$^+$ and 503 [$C_6H_{11}O_6$-$C_6H_{10}O_5$-$C_6H_{10}O_5$, $C_{18}H_{31}O_{16}$]$^+$ indicated that a hexoside type sugar unit was attached at one terminal of the pentaglycoside moiety. The ion peaks arose at *m/z* 163 [$C_6H_{11}O_5$]$^+$ and 325 [$C_6H_{11}O_5$-$C_6H_{10}O_5$]$^+$ supported the existence of rhamnose at another end of the pentaglycoside unit. The ^{1}H NMR spectrum of compound **6** exhibited five one – proton doublets at δ 5.33 (J = 4.1 Hz), 5.21 (J = 5.4 Hz), 5.06 (J = 3.9 Hz), 5.02 (J = 4.8 Hz) and 4.90 (J =3.7 Hz) assigned to α-oriented anomeric H-1, H-1′, H-1″, H-1‴ and H-1⁗ protons, respectively. A three-proton doublet at δ 1.09 (J = 7.1 Hz) was accounted to secondary C-6⁗ methyl protons of rhamnose unit. The other sugar protons resonated as multiplets between δ 4.83 - 3.29 due to oxymethine protons and as two - proton doublets at δ 3.27 (J = 8.2 Hz), 3.25 (J = 8.9 Hz), 3.21 (J = 9.3 Hz) and 3.19 (J = 8.9 Hz) associated with oxymethylene H_2-6, H_2-6′, H_2-6″ and H_2-6‴ protons, respectively.[84-86] The ^{13}C NMR spectrum of compound **6** displayed signals for anomeric carbons from δ 106.6 to 96.9, methyl carbon at δ 18.3 (C-6‴′) and other sugar carbons between 82.8 – 62.3. The presence of the oxymethylene of sugar protons in the deshielded region from δ 3.27 to 3.19 in the ^{1}H NMR spectrum and carbon signals C-6 to C-6‴ between δ 63.5 – 62.3 in the ^{13}C NMR spectrum suggested (6→1) linkages of the galactose units. Acid hydrolysis of **6** yielded D-galactose, R_f 0.38 and D-rhamnose, R_f 0.74 (*n*-butanol-acetone-pyridine-water, 1:1:0.5:0.5, v/v). On the basis of the foregoing discussion the structure of **6** has been established as α-D-galactopyranosyl-(6→1′)-O-α-D-galactopyranosyl- (6′→1″)-O-α-D-galactopyranosyl - (6″→1‴)-O-α-D- galactopyranosyl-(6‴→1⁗)-O-α-D-rhamnopyranoside, a new pentaglycoside derivative (Fig 3).



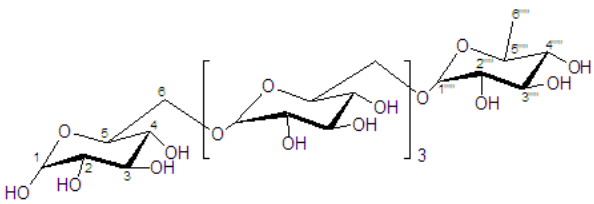
4′β-Hydroxynon-6′-(*Z*)-enyl benzoate (3)



Punicasesterterpene diol (4)



Punicaflavonyl myrt-1″-ene (5)



Tetra-α-D-galactosyl-α-D-rhamnoside (6)

Fig. 3: Structural formulae of chemical constituents 3 – 6 isolated from the *Punica granatum* flowers.

CONCLUSION

Phytochemical investigation of a methanolic extract of the aerial parts of *Cuba speciosa* gave apigenin-4′-rutinoside (**1**). The fruits of *M. philippensis* afforded a flavanone (**2**). The flowers of *P. granatum* yielded four new chemical constituents identified as 4′β-hydroxynon-6′(Z)-enyl benzoate (**3**), a sesterterpene diol (**4**), a flavone (punicaflavonyl 3′-myrt-1″-ene, (**5**) and a pentaglycoside (tetragalactosidic rhamnoside, (**6**). 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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