ejpmr, 2021, 8(3), 446-452

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL CONSTITUENTS OF THE LEAVES OF *BAUHINIA RACEMOSA* LAM. AND MYCELIA OF *CORDYCEPS SINENSIS* (BERK.) SACC.

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Article Received on 08/01/2021 **Article Revised on 29/01/2021** **Article Accepted on 19/02/2021**

ABSTRACT

Bauhinia racemosa Lam. (Caesalpiniaceae), distributed in India and other regions of south eastern Asia, is used to cure diarrhoea, dysentery, epilepsy, liver ailments, blood diseases, fever, headache, inflammation, malaria, skin diseases, tumors and ulcers. *Cordyceps sinensis* (Berk.) Sacc. (family Cordycipitaceae) is an entomopathogenic fungus found on Tibetan Plateau in south-western China and Himalayan regions of Bhutan and Nepal. It is used to treat bronchitis, cancer, high cholesterol, colds, coughs, depression, diabetes, erectile dysfunction, fatigue, jaundice, alcoholic hepatitis, male sexual problems, kidney and liver disorders, and tuberculosis. Phytochemical investigation of a methanolic extract of the leaves of *B. racemosa* led to isolate a dimethoxy-di-α-D-xyloside characterized as *α*-D-4-methoxyxylopyranosyl-(2→1′)-O-α-D-4′-methoxyxylopyranoside (**1**) and a quercetin derivative identified as 2′-hydroxyquercetin 3-O-*α*-D-xylopyranosyl-(2″→1‴)-O-α-D-xylopyranoside (**2**). Column chromatography of a methanolic extract of the mycelia of *Cordyceps sinensis* afforded *n*-octacosanoic acid (**3**), an aliphatic alcohol formulated as *n*-nonacosan-13β-ol (**4**), a di-β-D-glycoside elucidated as β-D-glucopyranosyl-(6→1′)-O-β-D-glucopyranoside (**5**) and an acyl tri-α-D-glucoside having a structure established as arachidyl α-*O*-D-glucopyranosyl-(6′→1″)-α-*O*-D-glucopyranosyl-(6″→1‴)-α-*O*-D-glucopyranoside (**6**). The structures of these phytoconstituents have been established by spectral data analysis and chemical reactions.

KEYWORDS: *Bauhinia racemosa* leaves, *Cordyceps sinensis* mycelia, chemical constituents, isolation, characterization.

INTRODUCTION

Bauhinia racemosa Lam. (family Caesalpiniaceae), called as bidi leaf tree, Burmese silk orchid, katmauli, kachnal and kachnaar, is a native of India, Myanmar, Cambodia, Caribbean, Sri Lanka, Thialand, Vietnam and Yunnan up to 1000 m. It is a small crooked deciduous tree with spreading crown, bark dark brown, rough with vertical cracks; leaves bilobed, glabrous above, hairy below, base cordate. Its tender shoot juice is mixed with mother's milk and used to clean and cool the eye. The stem juice is taken with cumin and milk to cure dysentery. A root decoction is drunk to prevent obesity. A root bark decoction with black peppers is given to epileptic patients. The leaves are eaten as an anthelmintic and to treat diarrhoea and liver ailments. In Ayurveda, the bark is taken as an acrid, astringent, refrigerant and to alleviate blood diseases, diarrhoea, dysentery, fever, headache, inflammation, malaria, skin diseases, tumors and ulcers. A stem bark decoction with that of *Terminalia arjuna* is useful against throat diseases. The flowers are beneficial to relieve cough, haemorrhage and piles.[1-4] The stem bark contained ß-sitosterol, β-amyrin and linoleiyl arabinoside[5-7], acyl glycosides and di-, tri- and tetragalactosides.[8] Kaempferol, quercetin, scopoletin and scopolin were isolated from the leaves.[9] Pacharin and resveratrol were reported from the heart wood.[10,11] The seed coats yielded flavonoids.[12] The roots produced a tetracyclic phenol and de-o-methylracemosol.[13-15]

Cordyceps sinensis (Berk.) Sacc., syn. *Ophiocordyceps sinensis* (Berk.) G.H. Sung, (family Cordycipitaceae), known as caterpillar fungus, yartsa gunbu, dōng chóng xià cǎo and Yarsha-gumba, is an entomo-pathogenic fungus mainly found in the meadows above 3,500 meters on Tibetan Plateau in south-western China and Himalayan regions of Bhutan and Nepal.[16] Cordyceps is used to treat bronchial asthma, bronchitis, cancer, high cholesterol, colds, coughs, depression, diabetes, erectile dysfunction, fatigue, jaundice, alcoholic hepatitis, male sexual problems, upper respiratory tract infections, kidney and liver disorders, and tuberculosis. It is regarded as having an excellent balance of yin and yang. It is taken after a kidney transplant. It improves energy, appetite,

stamina, libido, endurance and sleeping patterns, enhances sexual potency and desire, increases longevity, cures erectile dysfunction, boosts athletic performance and is utilized as a tonic.[17-20]

Cordyceps is reported to contain cordycepic acid, glutamic acid, amino acids (phenylalnine, proline, histidine, valine, oxyvaline, arginine), polyamines (1,3-diamino propane, cadaverine, spermidine, spermine, homospermidine, and purtescine), cyclic dipeptides [cyclo-(gly-pro), cyclo-(leu-pro), cyclo-(val-pro), cyclo-(ala-leu), cyclo-(alaval) and cyclo-(thr-leu)], d-mannitol, oligosaccharides, and polysaccharides, nucleotides and nucleosides (adenine, adenosine, uracil, uridine, guanine, guanosine themidine, and deoxyuridine and cordycepia), fatty acids and other organic acids (oleic, linoleic, palmitic and stearic acids),[21-27] cordycepin, cordycepic acid, amino acids, vitamins mainly E, K, B1, B2, B12, carbohydrates, proteins, sterols, nucleosides,[20] sterols (ergosterol, delta-3 ergosterol, ergosterol peroxide, β-sistosterol, daucosterol and campasterol)[28] cordyceamides A and B,[29] glutamic acid and aspartic acid,[30] mannitol, polysaccharide composed of glucose, mannose and galactose, glutamic acid, aspartic acid and phenylalanine, octadecdienoic acid and octadecenoic acid,[31] adenosine and cordycepin, mannitol and polysaccharides,[32] aromatic amino acids,[33] cordycepin, adenosine, cordycepic acid, ergosterol, linoleic acid, oleic acid, palmitic acid, stearic acid, *N*-acetyl muramic acid, hypoxanthine, nephthaquinone, dipliconic acid, myriocin, cordyheptapeptide A and cicadapeptin,[34,35] polysaccharide PS-A,[36] galactomannan[37] and mannoglucan.[38] 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, variation aspects of chemical constituents of the plants and development of ecofriendly, biodegradable and safer herbal preparations the leaves of *Bauhinia racemosa* and mycelia of *Cordyceps sinensis* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

All chemicals were procured from Sigma-Aldrich unless otherwise stated. Melting points were determined on a thermoelectrically heated Perfit apparatus without correction. UV spectra were obtained in methanol with a Lambda Bio 20 spectrometer. IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX 400 MHz spectrometer with TMS as an internal standard. Mass spectra were scanned on a Jeol D-300 (EI/CI) system. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size. The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F_{254} (0.25 mm, Merck, Mumbai, India). The spots were visualized by exposure to iodine vapors and under UV radiations at 254 and 366 nm and spraying with ceric sulphate solution.

Collection of Plant materials

The leaves of *B. racemosa* were collected from Gulbarga, Karnataka. The plant material was identified by Prof. M. P. Sharma, Department of Botany, Jamia Hamdard. The specimen vouchers of the drug was deposited in the herbarium of the Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard for future reference.

Cordyceps sinensis was collected during the rainy season (May-June) from wood logs and tree stumps from the hilly regions (at an altitude of over 4000 m) of the Northwest Himalayas at different locations in Pithoragarh, Uttarakhand, India, where the plant grows widely under natural conditions. It was characterized by the Ethno-Botany Division of Defence Institute of Bio Energy Research (DIBER), Haldwani, where the voucher specimen of the plant is preserved in the herbarium. The mature mycelia were selected, removed, and washed thoroughly with water, re-washed with distilled water, dried under shade in a clean, dust-free environment, and crushed using a laboratory blender.

Extraction and Isolation

The leaves of *B. racemosa* were coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark greenish mass, 132.5 g. The pulverized mycelia of *Cordyceps sinensis* were extracted with methanol in the Soxhlet apparatus. The extract was concentrated by removing the solvent to obtain a dark brown mass, 116.3 g. The extract (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 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 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.

Phytoconstituents isolated from the leaves of *Bauhinia racemosa*

α-D-4-Methoxyxylopyranosyl-(2→1′)-O-*α*-D-4′-methoxyxylopyranoside (1)

Elution of the column with chloroform-methanol (9:1) gave colourless crystals of **1**, yield 128 mg, m. p. 133 – 135 ºC; UV λmax (MeOH): 242 nm; IR υmax (KBr): 3386, 3241, 3169, 2927, 2846, 1448, 1337, 1272, 1211, 1124, 1017, 821 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.14 (1H, d, J = 3.1 Hz, H-1α), 3.79 (1H, m, H-2), 3.71 (1H, m, H-3), 3.57 (1H, m, H-4), 3.21 (2H, d, J = 3.8 Hz, H_2-5),

4.68 (1H, d, J = 5.2 Hz, H-1′α), 3.76 (1H, m, H-2′), 3.61 (1H, m, H-3′), 3.45 (1H, m, H-4′), 3.15 (1H, d, J = 5.2 Hz, H_2-5′a), 3.12 (1H, d, J = 4.5 Hz, H_2-5′b), 3.08 (6H, brs, 2 x OMe); ^{13}C NMR (DMSO-d_6): δ 98.52 (C-1), 77.39 (C-2), 73.41 (C-3), 70.62 (C-4), 61.49 (C-5), 93.63 (C-1′), 72.51 (C-2′), 71.01 (C-3′), 70.73 (C-4′), 61.20 (C-5′), 56.83 (OMe), 55.39 (OMe); ESI MS *m/*z (rel. int.): 310 $[M]^+$ ($C_{12}H_{22}O_9$) (4.7).

2′-Hydroxyquercetin 3-O-*α*-D-xylopyranosyl-(2′′→1′′′)-O-*α*-D-xylopyranoside (2)

Further elution of column with chloroform–methanol (9:1) gave creamy amorphous mass of compound **2**, 159 mg, m. p. 237-239 °C; UV λmax (MeOH): 269, 353 nm; IR υmax (KBr): 3431, 3361, 3279, 2982, 2843, 1669, 1591, 1509, 1452, 1365, 1289, 1211, 1135, 1087, 1018, 971 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.74 (1H, d, J = 6.8 Hz, H-5′), 6.95 (1H, d, J = 6.8 Hz, H-6′), 6.81 (1H, d, J = 1.8 Hz, H-8), 6.48 (1H, d, J = 1.8 Hz, H-6), 5.51 (1H, d, J = 2.1 Hz, H-1′′α), 4.89 (1H, m, H-2′′), 4.69 (1H, m, H-3′′), 3.97 (1H, m, H-4′′), 3.18 (2H, m, H_2-5′′), 5.21 (1H, d, J = 2.8 Hz, H-1′′′α), 4.78 (1H, m, H-2′′′), 4.65 (1H, m, H-3′′′), 3.81 (1H, m, H-4′′′), 3.14 (2H, m, H_2-5′′′), ^{13}C NMR (DMSO-d_6): δ 156.29 (C-2), 133.71 (C-3), 178.58 (C-4), 161.16 (C-5), 98.58 (C-6), 162.92 (C-7), 94.87 (C-8), 159.12 (C-9), 105.95 (C-10), 129.39 (C-1′), 133.71 (C-2′), 134.79 (C-3′), 148.51 (C-4′), 115.74 (C-5′), 120.41 (C-6′), 103.78 (C-1′′), 71.49 (C-2′′), 70.72 (C-3′′), 70.19 (C-4′′), 70.12 (C-5′′); 99.43 (C-1′′′), 71.15 (C-2′′), 70.31 (C-3′′′), 69.92 (C-4′′′), 69.76 (C-5′′′); ESI MS *m/*z (rel. int.): 582 $[M]^+$ ($C_{25}H_{26}O_{16}$) (3.6).

Phytoconstituents isolated from the mycelia of *Cordyceps sinensis*

***n*-Octacosanoic acid (3)**

Elution of the column with petroleum ether - chloroform (1:1) furnished a colourless amorphous powder of **3**, recrystallized from acetone-methanol (1:1), 221 mg, m. p. 90 - 91 ^{0}C; IR υ_{max} (KBr): 3227, 2921, 2841, 1701, 1640, 1406, 1258, 1081, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.72 (2H, t, J = 7.6 Hz, H_2- 2), 2.62 (2 H, m, H_2-3), 2.48 (2 H, m, H_2-4), 2.34 (4 H, m, H_2-5, H_2-6), 2.06 (4 H, m, H_2-7, H_2-8), 1.89 (2 H, m, H_2-9), 1.22 (2 H, m, H_2-10), 1.28 (34 H, brs, 17 × CH_2), 0.84 (3 H, t, J = 6.5 Hz, Me-28); ^{13}C NMR ($CDCl_3$): δ 180.42 (C-1), 33.81 (C-2), 30.12 (C-3), 29.36 (20 x CH_2), 28.88 (C-24), 25.92 (C-25), 23.56 (C-26), 22.68 (C-27), 14.11 (C-28); +ve ESI MS *m/z* (rel. int.): 424 $[M]^+$ ($C_{28}H_{56}O_2$) (55.4).

Nonacosan-13β-ol (4)

Elution of the column with chloroform produced colourless crystals of **4**, 108 mg, m. p. 107- 108 ºC; IR υmax (KBr): 3154, 2928, 2841, 1645, 1401, 1231, 1025, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.12 (1H, m, $w_{1/2}$ = 16.6 Hz, H-13α), 2.32 (4H, m, H_2-12, H_2-14), 2.24 (2H, m, H_2-11), 1.72 (4H, m, H_2-10, H_2-15), 1.56 (2H, m, H_2-16), 1.30 (2H, m, H_2-9), 1.26 (36H, s, H_2-17, 18 x CH_2), 1.12 (2H, m, H_2-2), 1.01 (3H, t, J = 6.5 Hz, Me-1), 0.68 (3H, t, J = 6.9 Hz, Me-29); ^{13}C NMR ($CDCl_3$): δ 71.27 (C-13), 48.30 (C-12), 38.72 (C-14), 30.27 (C-11), 24.39 (5 × CH_2), 20.79 (18 x CH_2), 15.21 (C-1), 11.64 (Me-29); ESI MS *m/z* (rel. int.): 424 $[M]^+$($C_{29}H_{60}O$) (68.5), 255 (11.2), 225 (6.3), 199 (21.2), 169 (4.8).

β-D-Glucopyranosyl-(6→1′)-O- β-D-glucopyranoside (5)

Elution of the column with chloroform – methanol (19:1) afforded a colourless powder of **5**, recrystallized from methanol, 121 mg, m. p. 160 - 162 °C, IR υ_{max} (KBr): 3437, 3217, 2927, 2843, 1437, 1389, 1263, 854 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.24 (1H, d, J = 7.3 Hz, H-1β), 4.78 (1H, m, H-5), 4.43 (1H, m, H-2), 3.79 (1H, m, H-3), 3.51 (1H, m, H-4), 3.34 (2H, d, J = 8.5 Hz, H_2-6), 5.03 (1H, d, J = 7.5 Hz, H-1′), 4.71 (1H, m, H-5′), 3.93 (1H, d, m, Hz, H-2′), 3.56 (1H, m, H-3′), 3.42 (1H, m, H-4′), 3.14 (2H, d, J = 9.1 Hz, H_2-6′); ^{13}C NMR ($CDCl_3$): δ 102.12 (C-1), 74.47 (C-2), 73.11 (C-3), 71.78 (C-4), 81.75 (C-5), 62.91 (C-6), 98.49 (C-1′), 73.76 (C-2′), 72.37 (C-3′), 70.21 (C-4′), 78.76 (C-5′), 60.23 (C-6′); +ve ESI MS *m/z* (rel. int.): 342 $[M]^+$ ($C_{12}H_{22}O_{11}$) (100), 179 (99.7), 163 (12.4).

Arachidyl tri-α-D-glucoside (6)

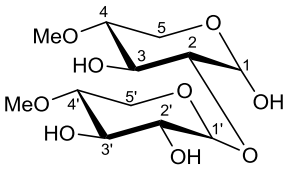
Elution of the column with chloroform - methanol (17 : 3) provided colourless crystals of **6**, recrystallized from acetone - methanol (1:1), 258 g, m. p. 211-213 ^{0}C; IR υ_{max} (KBr): 3510, 3483, 3225, 2928, 2848, 1713, 1645, 1404, 1233, 1079, 725 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.48 (2H, t, J = 7.3 Hz, H_2-2), 2.06 (2H, m, H_2-3), 1.84 (2H, m, H_2-4), 1.79 (2H, m, H_2-5), 1.22 (28H, brs, 14 x CH_2), 0.87 (3H, t, J = 7.0 Hz, Me-20), 4.86 (1H, d, J = 3.5 Hz, H-1′), 4.23 (1H, m, H-5′), 3.72 (1H, m, H-2′), 3.45 (1H, m, H-3′), 3.37 (1H, m, H-4′), 3.21 (2H, d, J = 6.5 Hz, H_2-6′), 4.83 (1H, d, J = 3.7 Hz, H-1′′), 4.18 (1H, m, H-5′′), 3.63 (2H, m, H-2′′), 3.42 (1H, m, H-3′′), 3.32 (2H, m, H-4′′), 3.14 (2H, d, J = 7.0 Hz, H_2-6′′), 4.79 (1H, d, J = 6.2 Hz, H-1′′′), 4.12 (1H, m, H-5′′′), 3.55 (1H, m, H-2′′′), 3.40 (1H, m, H-3′′′), 3.28 (1H, m, H-4′′′), 3.07 (2H, d, J = 7.4 Hz, H_2-6′′′); ^{13}C NMR (DMSO-d_6): δ 172.35 (C-1), 51.17 (C-2), 40.06 (C-3), 38.12 (C-4), 31.16 (C-5), 29.53 (13 x CH_2), 19.32 (C-19), 18.12 (C-20), 98.26 (C-1′), 72.96 (C-2′), 71.80 (C-3′), 64.33 (C-4′), 77.22 (C-5′), 61.71 (C-6′), 93.57 (C-1′′), 72.87 (C-2′′), 71.08 (C-3′′), 63.56 (C-4′′), 76.83 (C-5′′), 61.22 (C-6′′), 93.05 (C-1′′′), 72.07 (C-2′′′), 70.01 (C-3′′′), 63.31 (C-4′′′), 76.78 (C-5′′′), 60.02 (C-6′′′); ESI MS *m/*z (rel. int.): 798 $[M]^+$ ($C_{38}H_{70}O_{17}$) (2.3), 523 (7.4), 341 (12.6), 295 (100), 179 (9.3), 163 (23.1).

RESULTS AND DISCUSSION

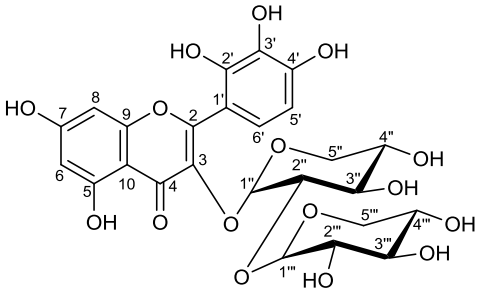
Compound **1**, a dimethoxy-di-α-D-xyloside, $[M]^+$ at *m/z* 310 ($C_{12}H_{22}O_9$), responded positively for glycoside tests and showed IR absorption bands for hydroxyl groups (3386, 3241 cm^{-1}). The ^{1}H NMR spectrum of **1** exhibited two one-proton doublets at δ 5.14 (J = 3.1 Hz) and 4.68 (J = 5.2 Hz) assigned to anomeric H-1 and H-1′ protons, respectively, supported the existence of α-glycosidic linkage of the disaccharide unit. The other sugar protons resonated as one-proton multiplets between δ 3.79 - 3.45, as a two-proton doublet at δ 3.21 (J = 3.8 Hz) and as one proton doublets at δ 3.15 (J = 5.2 Hz, H_2-5′a) and 3.12 (J

= 4.5 Hz, H_2-5′b) ascribed to oxymethylene H_2-5 and H_2-5' of the pentose units, respectively. A six-proton singlet at δ 3.08 was accounted to two methoxy group protons. The ^{13}C NMR spectrum of **1** displayed signals for anomeric carbons at δ 98.52 (C-1) and 93.63 (C-1′), other sugar carbons from δ 77.39 to 61.20 and methoxy carbons at δ 56.83 and 55.39. The presence of the sugar H-2 signal in the deshielded region as a one-proton multiplet at δ 3.79 in the ^{1}H NMR spectrum and C-2 carbon signal at δ 77.39 in the ^{13}C NMR spectrum suggested (2→1′) linkage of the sugar units. Acid hydrolysis of **1** yielded one of the monosaccharide as D-xylose, R_f 0.81 (*n*-butonal – pyridine – water, 6 : 4 : 3, *v/v*). On the basis of these evidences the structure of **1** has been formulated as *α*-D-4-methoxyxylopyranosyl-(2→1′)-O-α-D-4′-methoxyxylopyranoside, a new dixyloside from the leaves of *Bauhinia racemosa* (Fig. 1).

Compound **2** responded positively for glycoside tests and Shinoda test for flavonoids and showed UV absorption maxima at 269 and 353 nm typical of substituted flavones[39, 40]. It had IR absorption bands for hydroxyl groups (3431, 3361, 3279 cm^{-1}), carbonyl function (1669 cm^{-1}) and aromatic rings (1591, 1509, 1018 cm^{-1}). Its molecular ion peak was determined at *m/z* 582 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a flavonol diglycoside, $C_{25}H_{26}O_{16}$. The ^{1}H NMR spectrum of **2** exhibited four one-proton doublets at δ 7.74 (J = 6.8 Hz), 6.95 (J = 6.8 Hz), 6.81 (J = 1.8 Hz) and 6.48 (J = 1.8 Hz) assigned to flavone ortho-coupled H-5′ and H-6′, and meta-coupled H-8 and H -6 protons, respectively, supporting C-2′, C-3′ and C-4′ substitution of B ring. Two one-proton doublets at δ 5.51 (J = 2.1 Hz) and 5.21 (J = 2.8 Hz) were ascribed correspondingly to α-oriented anomeric H-1″ and H-1‴ protons. The other sugar protons resonated as one-proton multiplets from δ 4.89 – 3.81 and as two-proton multiplets at δ 3.18 (H_2-5″) and 3.14 (H_2-5‴). The ^{13}C NMR spectrum of **2** displayed carbon signals at δ 156.29 (C-2), 133.71 (C-3) and 178.58 (C-4) suggesting a flavonol-type molecule. Two carbon signals at δ 103.78 and 99.43 were accounted to the anomeric C-1″ and C-1‴ carbons, respectively. The other sugar carbons appeared from 71.49 to 69.76. The presence of the sugar H-2″ signal in the deshielded region as a one-proton multiplet at δ 4.89 in the ^{1}H NMR spectrum and C-2 carbon signal at δ 71.49 in the ^{13}C NMR spectrum suggested (2″ →1‴) linkage of the sugar units. Acid hydrolysis of **2** yielded D-xylose, R_f 0.81 (*n*-butonal – pyridine – water, 6 : 4 : 3, *v/v*). These data led to establish the structure of **2** as 2′-hydroxyquercetin 3-O-*α*-D-xylopyranosyl-(2″→1‴)-O-α-D-xylopyranoside, a new quercetin derivative (Fig. 1).



α-D-4-Methoxy xylopyranosyl-(2→1′)-*O*-α-D-4′-Methoxy xylopyranoside **(1)**



2′-Hydroxyquercetin 3-*O*-α-D-xylopyranosyl-(2″→1‴)-*O*-α-D-xylopyranoside **(2)**

Fig. 1: Chemical constituents of 1 and 2 isolated from the leaves of *Bauhinia racemosa*.

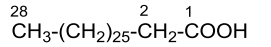
Compound **3** was a known fatty acid characterized as *n*-octacosanoic acid.[40,41] (Fig. 2).

The compound **4** displayed IR absorption bands for a hydroxyl group (3154 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 424 consequent to a molecular formula of a saturated aliphatic alcohol, $C_{29}H_{60}O$. The prominent ion peaks generating at *m/z* 199 [C_{14} – C_{13} fission, CH_3-$(CH_2)_{11}$-$CH(OH)]^+$, 225 [M – 199]$^+$, 169 [C_{13} – C_{12} fission, CH_3-$(CH_2)_{11}]^+$ and 255 [M – 169]$^+$ indicated the occurrence of the hydroxyl group at C-13 position. The ^{1}H NMR spectrum of **4** showed a one-proton multiplet at δ 3.12 with half width of 16.6 Hz associated with α-oriented carbinol H-13 proton. Two three - proton triplets at δ 1.01 (J = 6.5 Hz) and 0.68 (J = 6.9 Hz) were accounted to terminal C-1 and C-29 primary methyl protons, respectively. Six multiplets between δ 2.32 - 1.12 and a broad singlet at δ 1.26 (36H) were ascribed to the remaining methylene protons. The ^{13}C NMR spectrum of **4** exhibited the signals for a carbinol carbon at δ 71.27 (C-13), methyl carbons at δ 15.21 (C-1) and 11.64 (C-29) and methylene carbons in the range of δ 48.30 to 20.79. The deficiency of any signal further δ 3.13 in the ^{1}H NMR and 71.27 in the ^{13}C NMR suggested the saturated nature of the molecule. On the basis of the foregoing discussion the structure of the **4** has been formulated as *n*-nonacosan-13β-ol (Fig. 2), an unknown aliphatic alcohol.

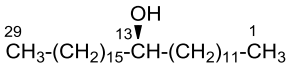
Compound **5**, a di-β-D-glycoside, [M]$^+$ at *m/z* 342 ($C_{12}H_{22}O_{11}$), gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3437, 3217 cm^{-1}). The ion peaks arising at *m/z* 179 [C_6 - $C_{1'}$ fission, $C_6H_{12}O_6]^+$ and 163 [M – 179]$^+$ in the mass spectrum indicated that two hexose units were linked to each other. The ^{1}H NMR spectrum of **5** exhibited two one-proton doublets at δ 5.24 (J = 7.3 Hz) and 5.03 (J = 7.5 Hz) assigned to anomeric H-1 and H-1′ protons, respectively, supported the existence of β-glycosidic linkage of the disaccharide unit. The other sugar protons resonated as one-proton multiplets between δ 4.78 - 3.42 due to oxymethine protons and as two-proton doublets at

δ 3.34 (J = 8.5 Hz) and 3.14 (J = 9.1 Hz) ascribed correspondingly to oxymethylene H_2-6 and H_2-6' of the hexose units. The ^{13}C NMR spectrum of **5** displayed signals for anomeric carbons at δ 102.12 (C-1) and 98.49 (C-1′) and other sugar carbons from δ 81.7.5 to 60.23. The presence of the sugar oxymethylene H_2-6 signal in the deshielded region as a two-proton doublet at δ 3.34 in the ^{1}H NMR spectrum and C-6 carbon signal at δ 62.91 in the ^{13}C NMR spectrum suggested (6→1′) linkage of the sugar units. Acid hydrolysis of **5** yielded D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of these evidences the structure of **5** has been elucidated as β-D-glucopyranosyl-(6→1′)-O-β-D-glucopyranoside (Fig. 2).

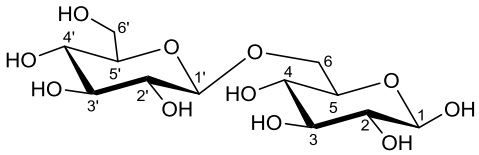
Compound **6**, named arachidyl tri-α-D-glucoside, $[M]^+$ at *m/*z 798 ($C_{38}H_{70}O_{17}$), responded positively to glycosidic tests and had IR absorption bands for hydroxyl groups (3510, 3483, 3225 cm^{-1}), ester function (1713 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The ion peaks produced at *m/*z 295 [C_1 – O fission, CH_3-$(CH_2)_{18}$-$CO]^+$, 523 [M – 295]$^+$, 163 [$C_{1'''}$ – O fission, $C_6H_{11}O_5$]$^+$, 179 [$C_{6''}$ - O fission, $C_6H_{11}O_6]^+$and 341 [$C_{6'}$ – O fission, $C_6H_{10}O_5$-$C_6H_{11}O_6]^+$ supported that three hexose units were linked to a C_{20}- fatty acid unit. The ^{1}H NMR spectrum of **6** showed three one-proton doublets at δ 4.86 (J = 3.5 Hz), 4.83 (J = 3.7 Hz) and 4.79 (J = 6.2 Hz) assigned to α-oriented anomeric H-1′, H-1″ and H-1‴ protons, respectively. The other sugar protons resonated as one-proton multiplets from δ 4.23 to 3.28 due to oxymethine protons and as two-proton doublets at δ 3.21 (J = 6.5 Hz), 3.14 (J = 7.0 Hz) and 3.07 (J = 7.4 Hz) associated correspondingly oxymethylene H_2-6′, H_2-6″ and H_2-6‴ protons. A two-proton triplet at δ 2.48 (J = 7.3 Hz) was ascribed to methylene H_2-2 protons adjacent to the ester group. The other methylene protons appeared as two-proton multiplets at δ 2.06 (H_2-3), 1.84 (H_2-4) and 1.79 (H_2-5) and as a broad singlet at δ 1.22 (28 Hz). A three-proton triplet at δ 0.87 (J = 7.0 Hz) was accounted to C-20 primary methyl protons. The ^{13}C NMR spectrum of **6** displayed signals for the ester carbon at δ 172.35 (C-1), anomeric carbons at δ 98.26 (C-1′), 93.57 (C-1″) and 93.05 (C-1‴), other sugar carbons in the range of 77.22 – 60.02, methylene carbons from δ 51.17 to 19.32 and methyl carbon at δ 18.12 (C-20). The appearance of H_2-6′ and H_2-6″ as two-proton doublets at δ 3.21 and 3.14 in the deshielded region in the ^{1}H NMR spectrum and C-6′ and C-6″ at δ 61.71 and 61.22 in the ^{13}C NMR spectrum, respectively, suggested (6′→1″) and (6″→1‴) attachment of the sugar units. The absence of any signal beyond δ 4.86 in the ^{1}H NMR spectrum and between δ 172.35 - 98.28 in the ^{13}C NMR spectrum supported saturated nature of the fatty ester unit. Acid hydrolysis of **6** yielded arachidic acid, m. p. 74 – 75 ^oC and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of the foregoing account, the structure of **6** has been characterized as arachidyl α-*O*-D-glucopyranosyl-(6′→1″)-α-*O*-D-glucopyranosyl-(6″→1‴)-α-*O*-D-glucopyranoside, a new acyl triglucoside (Fig. 2).



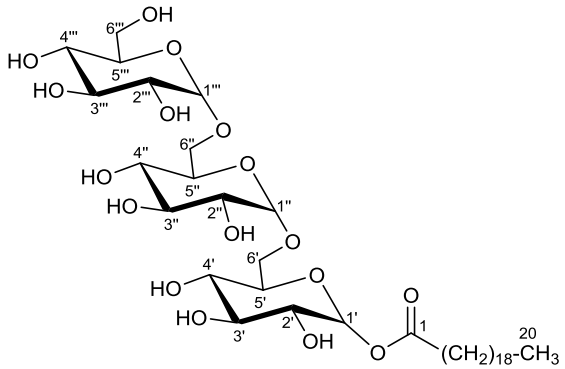
n-Octacosanoic acid **(3)**



Nonacosa-13β-ol **(4)**



β-D-Diglucoside **(5)**



Arachidyl-tri-α-D-glucoside **(6)**

Fig. 2: Chemical constituents of 3 - 6 isolated from the mycelia of *Cordyceps sinensis*.

CONCLUSION

Phytochemical investigation of a methanolic extract of the leaves of *B. racemosa* led to isolate *α*-D-4-methoxyxylopyranosyl-(2→1′)-O-α-D-4′-methoxyxylopyranoside (**1**) and 2′-hydroxyquercetin 3-O-*α*-D-xylopyranosyl-(2″→1‴)-O-α-D-xylopyranoside (**2**). Column chromatography of a methanolic extract of the mycelia of *Cordyceps sinensis* afforded *n*-octacosanoic acid (**3**), *n*-nonacosan-13β-ol (**4**), β-D-glucopyranosyl-(6→1′)-O-β-D-glucopyranoside (**5**) and arachidyl α-*O*-D-glucopyranosyl-(6′→1″)-α-*O*-D-glucopyranosyl-(6″→1‴)-α-*O*-D-glucopyranoside (**6**). Except compound **3**, all chemical constituents are reported for the first time. This work has enhanced understanding about the chemical constituents of the plant. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

ACKNOWLEDGEMENT

The authors are thankful to the Heads, Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi and Sophisticated Instrumentation Analytical Facility, Central Drug Research Institute, Lucknow for recording spectral data of the compounds.

REFERENCES

1. Kirtikar KR, Basu BD. Indian medicinal plants, Dehradun, India: Bishen Mahendra Pal Singh, 1975; 842-844.
2. Jain, S.K. Contribution to Indian ethnobotany. Scientific Publishers. India, 1997; 29-312.
3. Sahu T, Sahu J. *Buahinia racemosa* (kachnar): a review of its medicinal properties. World Journal of Pharmaceutical Research, 2015; 4(5): 686-696.
4. Panda P, Das D, Dash P, Ghosh G. Therapeutic Potential of *Bauhinia racemosa* - A Mini Review. International Journal of Pharmaceutical Sciences Review and Research, 2015; 32(2): 169-179.
5. Prakash A, Khosa RL. Chemical studies on *Bauhinia racemosa*. Current Science, 1976; 45(19): 705- 705.
6. Kumar RS, Sunderam RS, Sivakumar T, Sivakumar P, Sureshkumar R, Kanagasabi R, Vijaya M, Perumal BP, Gupta M, Mazumdar UK, Kumar MS, Kumar KA. Effect of *Bauhinia racemosa* stem bark on N–nitrosodiethylamine-induced hepatocarcinogenesis in rats. The American Journal of Chinese Medicine, 2007; 35: 103–114.
7. Rahman MA, Akhtar J. A new linoleiyl arabinopyranoside from the bark of *Bauhinia racemosa* Lam and a new flavonoidal glycoside from the leaves of *Cordia dichotoma* Linn. Natural Product Research, 2016; 30(20): 2265-2273.
8. Sultana S, Zaman K, Ali M and Mir SR. Chemical constituents from the stem bark of *Bauhinia racemosa* Lam. and leaves of *Machilus bombycina* King ex Hook. F. European Journal of Biomedical and Pharmaceutical Sciences, 2018; 5(1): 714-721.
9. El–Hossary GA, Selim MA, Sayed AE, Khaleel AE. Study of the flavonoid Content of *Bassia muricata* and *Bauhinia recemosa*. Bulletin of Faculty of Pharmacy, Cairo University, 2000; 38: 93-97.
10. Anjaneyulu ASR, Reddy AVR, Reddy DSK, Ward RS, Adhikesavalu D, Cameron TS. Pacharin - a new dibenzo (2,3–6,7) oxepin from *Bauhinia racemosa*. Tetrahedron, 1984; 40(21): 4245–4252.
11. Anjaneyulu ASR, Reddy AVB, Reddy DSK, et al. Rasveratrol - a novel tetracyclic phenol from *Bauhinia racemosa* Lamk. Tetrahedron, 1986; 42(9): 2417-2420.
12. Swantijian K, Pathak V, Srivastava BK. Characterisation of flavonoids from seed coat of *Bauhinia racemosa*. Asian Journal of Chemistry. 2002; 14(2): 1067-1068.
13. Prabhakar P, Gandhidasan R, Raman PV et al. De-o-methylracemosol – a tetracyclic 2,2-dimethylchroman from the roots of *Bauhinia racemosa.* Phytochemistry, 1994; 36(3): 817-818.
14. Jain R, Alam S, Saxena U. A new tetracyclic phenol and other constituents from the roots of *Bauhinia racemosa*. Indian Journal of Chemistry, 2002; 41B(6): 1321-1322.
15. Jain R, Saxena U, Rathore K, et al. Bioactivities of polyphenolics from the roots of *Bauhinia racemosa*. Archives Pharmacal Research, 2008; 31(12): 1525-1529.
16. Shrestha B, Zhang W, Zhang Y, Liu X. What is the Chinese caterpillar fungus *Ophiocordyceps sinensis* (Ophiocordycipitaceae)?, Mycology, 2010; 1(4): 228-236. DOI: 10.1080/21501203.2010.536791.
17. Panda AK. Tracing the historical prospective of *Cordyceps sinensis* –an aphrodisiac in Sikkim Himalaya. Indian Journal of History of Science, 2010; 45: 189-98.
18. Lin, B., Li, S. Cordyceps as an herbal drug. In Herbal Medicine: Biomolecular and Clinical Aspects, 2nd ed.; Benzie, I.F.F., Wachtel-Galor, S., Eds.; Taylor and Francis: Abingdon, UK; CRC Press: Boca Raton, FL, USA, 2011.
19. Panda AK, Swain KC. Traditional uses and medicinal potential of *Cordyceps sinensis* of Sikkim. Journal of Ayurveda and Integrative Medicine, 2011; 2(1): 9–13.
20. Choda U. Medicinal value of *Cordyceps sinensis*. Translational Biomedicine, 2017; 8(4): 132 1–5, DOI: 10.21767/2172-0479.100132.
21. Xiao YQ, Liu JM, Tu YY. Studies on chemical constituents in *Cordyceps sinensis*. Bulletin of Chinese Material Medica, 1983; 8: 32-3.
22. Tsuno A, Taketomo N, Hiroyuki I. Healthful composition obtained from the hot water extract of *Cordyceps sinensis* mycelia. Journal of Laboratory and Clinical Medicine, 1995; 8: 134-137.
23. Huang, L.F., Y.Z. Liang, F.Q. Guo, Z.F. Zhou and B.M. Cheng. Simultaneous separation and determination of active components of *Cordyceps sinensis* and *Cordyceps militarris* by LC/ESI-MS. Journal of Pharmaceutical and Biomedical Analysis, 2003; 33: 1155-1162.
24. Dong C, Yao Y. Isolation, characterization of melanin derived from *Ophiocordyceps sinensis*, an entomogenous fungus endemic to the Tibetan plateau. Journal of Bioscience and Bioengineering, 2012; 113: 474-479.
25. Shrestha, S., Shrestha, B., Park, J.-H., Lee, D.-Y., Cho, J.-G., Baek, N.-I. Chemical constituents of Yarsagumba (*Ophiocordyceps sinensis* (Berk.) Sung et al.), a valued traditional Himalayan medicine. Nepal Journal of Science and Technology, 2013; 13(1): 43-58. https://doi.org/10.3126/njst.v13i1.7398.
26. Liu, Y., Wang, J., Wang, W., Zhang, H., Zhang, X., Han, C. The chemical constituents and pharmacological actions of Cordyceps sinensis. Evidence-Based Complementary and Alternative Medicine, 2015, 2015, 575063, http://dx.doi.org/10.1155/2015/575063.
27. Mamta, Mehrotra S, Amitabh, Kirar V, Vats P, Nandi SP, Negi PS, Misra K. Phytochemical and antimicrobial activities of Himalayan *Cordyceps sinensis* (Berk.) Sacc. Indian Journal of Experimental Biology, 2015; 53(1): 36–43. http://hdl.handle.net/123456789/30279.

28. Bok, J.W., L. Lermer, J. Chilton, H.G. Klingeman and G.H.N. Towers. Antitumor sterols from the mycelia of *Cordyceps sinensis*. Phytochemistry, 1999; 51: 891-898.
29. Jia, J.M., H.H. Tao and B.M. Feng. Cordyceamides A and B from the culture liquid of *Cordyceps sinensis* (Berk.) Sacc. Chemical and Pharmaceutical Bulletin, 2009; 57: 99-101.
30. Hsu T.-H., Shiao L.-H, Hsieh C, Chang D.-M. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom Dong Chong Xia Cao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis.* Food Chemistry, 2002; 8(4): 463-469. https://doi.org/10.1016/S0308-8146(02)00158-9.
31. Zunsheng W, Yuxiang G, Li Z, Qinsheng Y, Yongxin Y. Chemical constituents of *Cordyceps sinensis* mycelial fermentation preparations in solid media. Natural Product Research and Development, 2005; 17(3): 331-336.
32. Zhou Y, Wang M, Zhang H, Huang Z, Ma J. Comparative study of the composition of cultivated, naturally grown *Cordyceps sinensis*, and stiff worms across different sampling years. PLoS ONE, 2019; 14(12): e0225750. https://doi.org/10.1371/journal.pone.0225750.
33. Wei X, Su Y, Hu H, Li X, Xu R, Liu Y. Quantification of aromatic amino acids in *Cordyceps* fungi by micellar electrokinetic capillary chromatography. Wuhan University Journal of Natural Sciences, 2019; 24(3): 245-250. DOI: 10.1007/s11859-019-1393-7.
34. Jia, J.M., X.C. Ma, C.F. Wu, L.J. Wu and G.S. Hu. Cordycedipeptide A, a new cyclodipeptide from the culture liquid of *Cordyceps sinensis* (Berk.) Sacc. Chemical & Pharmaceutical Bulletin, 2005; 53: 582-583.
35. Ashraf SA, Elkhalifa AEO, Siddiqui AJ, Patel M, Awadelkareem AM, Snoussi M, Ashraf MS, Adnan M, Hadi S. Cordycepin for health and well being: A potent bioactive metabolite of an entomopathogenic medicinal fungus *Cordyceps* with its nutraceutical and therapeutic potential. Molecules, 2020; *25*(12): 2735; https://doi.org/10.3390/molecules25122735.
36. Kim, S.D. Isolation, structure and cholesterol esterase inhibitory activity of a polysaccharide, PS-A, from *Cordyceps sinensis*. Korean Society for Applied Biological Chemistry, 2010; 53: 784–789.
37. Kiho, T H, Tabata, Ukai S. A minor, protein containing galactomannan from a sodium carbonate extract of *Cordyceps sinensis*. Carbohydrate Research, 1986; 156: 189-197.
38. Wu Y., Hu N, Pan Y, Zhou L, Zhou X. Isolation and characterization of a mannoglucan from edible *Cordyceps sinensis* mycelium. Carbohydrate Research, 2007; 342: 870-875.
39. Mabry TJ, Markham KR, Thomas MB. The systemic identification of flavonoids. New York, Springer- Verlag, 1970.
40. Harborne JB, Williams CA. Flavone and flavonol glycosides, In: The Flavonoids, Harborne JB, Mabry TJ, Mabry H. London, Chapman and Hall. 1975.
41. Mohamad H, Jamil WANWA, Abas F, Mohamad KS and Ali AM. Octacosanoic acid, long chains saturated fatty acid from the marine sponges Xestospongia sp. Pertanika Journal of Tropical Agricultural Science, 2009; 32(1): 51-55.
42. Aeri V., Alam P., Ali M., Ilyas UK. Isolation of new aliphatic ester linked with δ-lactone cos-11-enylpentan-1-oic-1,5-olide from the roots of *Streblus asper* Lour. Indo Global Journal of Pharmaceutical Sciences, 2012; 2(2): 114-120.