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Original article

Chemical Constituents from the Stem Barks of *Plumeria rubra* L.

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Abstract

Background and objectives: *Plumeria rubra* L. (Apocynaceae) is a deciduous shrub or small tree cultivated in subtropical and tropical countries. Its bark is prescribed to treat amoebic dysentery, dropsy, jaundice, scabies, gonorrhea, venereal affections and wounds. In the present research, we aimed to isolate and identify the chemical constituents of the bark of *P. rubra*. **Method:** An air-dried bark powder of *P. rubra* was exhaustively extracted with methanol. Methanol was removed afterwards under vacuum to get a dark brown mass. The extract was dissolved and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry was loaded over silica gel column packed in petroleum ether. The column was eluted with organic solvents successively in order of increasing polarity to isolate the chemical constituents. The structures of the phytoconstituents, isolated from the plant for the first time, have been elucidated by analyzing the spectral data and on the basis of chemical reactions. **Results:** Phytochemical investigation of a methanol extract of the stem bark led to isolate *n*-octyl *n*-octadecanoate (*n*-octyl stearate, **1**), lauryl-O-α-D-glucopyranosyl–(2′→1″)-O-β-D-glucopyranoside (lauryl diglucoside, **2**), stearyl-α-L-xylopyranosyl–(2′→1″)-α-L-xlyopyranosyl-2″-(3″-oxy-4‴-hydroxy-5‴-methoxy)-benzoic acid (stearyl dixylosyl methoxygallic acid, **3**), vanillic acid 4-O-β-D-arabinopyranosyl-(2a→1b)-O-β-D-arabinopyranosyl- (2b→1c)-O-β-D-arabinopyranosyl-(2c→1d)-O-β-D-arabinopyranosyl-2d-stearate (vanillic acid 4-O-tetra-arabinosyl stearate, **4**), vanillic acid 4-O- β-D-arabinopyranosyl-(2a→1b)-β-D-arabinopyranosyl-(2b→1c)-β-D-arabinopyranosyl-(2c→1d)-β-D-arabinopyranosyl-(2d→1e)-β-D-arabinopyranosyl-(2e→1f)-β-D-arabinopyranosyl-2f-stearate (vanillic acid 4-O-hexa-arabinosyl stearate, **5**), β-D-glucopyranosyl-(2a→1b)-O-β-D-glucopyranosyl-(2b→1c)-O-β-D-glucopyranosyl- (2c→1d)-O-β-D- glucopyranosyl-(2d→1e)-O-β-D-glucopyranosyl-(2e→1f)-O-β-D- glucopyranoside (β-D-hexaglucoside , **6**) and β-D-glucopyranosyl-(2a→1b)-O-β-D-glucopyranosyl-(2b→1c)-O-β-D- glucopyranosyl-(2c→1d)-O-β-D-glucopyranosyl-(2d→1e)-O-D- glucopyranosyl-(2e→1f)-O-β-D- glucopyranosyl-(2f→1g)-O-β-D-glucopyranosyl-(2g→1h)-O-β-D-rhamnopyranoside (β-D-heptaglucosyl-β-D-rhamnoside, **7**). **Conclusion:** The stem bark of *P. rubra* contained a variety of chemical constituents like a fatty ester, acyl glycosides, vanillic acid glycosides and polyglycosides.

Keywords: characterization; chemical constituents; isolation; *Plumeria rubra;* stem bark

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Introduction

Plumeria rubra L., syn. *P. acutifolia* Poir; *P. acuminata* Ait. (Apocynaceae), known as frangipani, red-jasmine, or plumeria, is a deciduous spreading shrub or small tree native

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to Mexico, Central America, Colombia and Venezuela. It is cultivated in subtropical and tropical climates worldwide including Cambodia, Hawaii, Malaysia and India as a popular garden plant with thick stout branches; milky latex; large, leathery, obovate, oblong and acute leaves; flowers showy, large, waxy red or reddish white and very fragrant in terminal or lateral stalked clusters [1]. The whole plant is used to relieve cholera and indigestion. A plant decoction is taken to treat asthma, constipation and fever and to promote menstruation [2-4]. The bark is emmenagogue, febrifuge, purgative and stimulant, prescribed to alleviate amoebic dysentery, dropsy, jaundice, scabies, gonorrhea, venereal affections and wounds. The bark paste is applied to disperse tumors [5, 6]. The root bark is astringent, carminative, emmenagogue, febrifuge, purgative, stimulant and thermogenic; useful to cure gastric disorders, gonorrhea, inflammation, leprosy, pruritus, ulcers and venereal sores [3,5]. The leaves are used as rubefacient and to treat inflammation, leprosy, muscular swellings, sore and ulcers. The flowers are aromatic and beneficial to subside ague, bacillary dysentery, diabetes and whooping cough. The latex is purgative and rubefacient, effective to allay blennorrhea, boils, centipede bites, diarrhea, fevers, inflammation, insect stings, itches, piles, rheumatism, swellings, toothaches and wounds [3-6].

The bark contained bitter glycosides, plumieride, plumeric acid, β-sitosterol, lupeol, amyrin, fulvoplumierin, flavan-3- ol glycoside, iridoids plumeridoids A-C and epiplumeridoid C [7 - 10]. The heartwood possessed plumericin, amyrin, lupeol, isoplumericin, 4-hydroxy-acetophenone, plumieride, coumaryl plumieride and protoplumericine [11]. The roots yielded fulvoplumierin, plumericin, isoplumericin, β-dihydroplumericin, β-dihydroplumericinic acid, 3α-cycloart-22-ene-3,25-diol, plumerin R, allamcin, allamandin, fulvoplumierin, 2,5dimethoxy-p-benzoquinone, rubrinol, teraxasteryl acetate, lupeol, stigmasterol, oleanolic acid, 13-O-caffeoyl plumeride, 13-deoxyplumieride, pluminoside, 1α-protoplumericin A, 8-isoplumeride , plumeric acid and methyl plumerate [1,8, 9-18]. The flower essential oil consisted of palmitic, lauric and myristic acids, benzyl salicylate, benzyl benzoate, 2-phenylethyl benzoate, nerolidol, geraniol, non-2-en-1-ol, limonene, phenyl acetaldehyde, *n*-tetradecanal, γ-elemene and α-farnesene, (E)-non-2-en-1-ol, limonene , phenyl acetaldehyde, *n*-tetradecanal, γ-elemene and α-farnesene [19- 25]. The attractive colors of the flowers were due to the presence of cyanidin 3-O-β-(2″-glucopyranosyl-O-β-galactopyranoside) and cyanidin-3-O-β-galactopyranoside [26]. The major leaves oil constituents were β-farnesene, α-patchoulene, limonene, α- copaene and phytol [27]. This manuscript has described isolation and characterization of three phenolic glucosides together with two acyl picraldehydes and a capryl vanillic acid from the stem barks of *P. rubra.*

Material and Methods

General procedures

A Perfit melting apparatus (Ambala, Haryana, India) was used to determin the melting points; a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) to measure UV spectra in methanol; Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer to record Infra-red spectra using KBr pellets; γ_{max} values have been given in cm^{-1}; Advance DRX 400, Brukerspectrospin 400 and 100 MHz instrument (Karlesruthe, Germany) to screen ^{1}H and ^{13}C NMR spectra, respectively, in 5 mm spinning tubes at 27 ºC using TMS as the internal standard; and Q-TOF-ESI instrument (Waters Corp., UK) for Mass-spectrometric detection with a +ve ESI technique. Column chromatography was performed on silica gel (Qualigens, India), 60-120 mesh and solvents used were purchased from Merck Specialties (E. Merck, India). Precoated TLC plates with silica gel $60F_{254}$ (0.25 mm, Merck) were used to check purity of the isolated compounds and the spots were visualized by exposure to iodine vapors and under UV radiations and spraying with ceric sulfate solution.

Plant material

The stem barks of *P. rubra* were collected from Hisar (Haryana, India) in June 2014 and identified by Dr. H. B. Singh, Scientist, Raw Material Herbarium NISCAIR (CSIR), New Delhi, India. A specimen was preserved at the herbarium of the Department of Pharmaceutical Sciences, GJ University of Science and Technology, Hisar (Haryana) (Haryana, India).

Extraction and isolation

The dried bark powder (1.0 kg) was exhaustively extracted with methanol in a Soxhlet apparatus. The methanol extract was evaporated under

reduced pressure to get a brown viscous mass The dried extract was dissolved in minimum quantity of methanol and added to silica gel (60-120 mesh) to prepare slurry. It was air-dried, powdered and loaded on a silica gel column prepared in petroleum ether. The column was run with petroleum ether (b. p. 60 - 80°C), petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3 and 1:1, v/v) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds.

Results and Discussion

The following compounds were isolated from the methanol extract (95 g, 9.5 % yield).

n-Octyl stearate (**1**)

Elution of the column with petroleum ether-chloroform (3:1) gave colorless crystals of **1**, recrystallized from acetone - methanol (1:1), 205 mg, m. p. 85 - 87 ºC, UV λ_{max} (MeOH): 205 nm (log ε 4.5); IR ν_{max} (KBr): 2922, 2849, 1723, 1641, 1456, 1378, 1265, 1027, 883, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.02 (2H, t, J = 8.6 Hz, H_2-1′), 2.33 (2H, t, J = 7.3 Hz, H_2-2), 1.84 (2H, m, H_2-8), 1.63 (2H, m, CH_2), 1.55 (2H, m, CH_2), 1.48 (2H, m, CH_2), 1.32 (4H, m, 2 x CH_2), 1.28 (4H, brs, 2 x CH_2), 1.23 (26H, brs, 13 x CH_2), 0.89 (3H, t, J = 6.7 Hz, Me-8′), 0.85 (3H, t, J = 6.3 Hz, Me-18); ^{13}C NMR ($CDCl_3$): δ 171.37 (C-1), 65.27 (C-1′), 36,38 (CH_2), 33.63 (CH_2), 29.37 (CH_2), 29.29 (CH_2), 29.13 (CH_2), 29.11 (CH_2), 29.08 (CH_2), 29.03 (CH_2), 28.68 (7 x CH_2), 28.62 (CH_2), 28.56 (CH_2), 28.53 (CH_2), 26.53 (CH_2), 24.46 (CH_2), 22.08 (CH_2), 13.91 (Me-8′), 13.88 (Me-18); ESI MS *m/z* (rel. int.): 396 $[M]^+$ ($C_{26}H_{52}O_2$) (4.5), 283 (6.1).

Lauryl diglucoside (**2**)

Elution of the column with chloroform - methanol (19 : 1) mixture provided colorless mass of **2**; recrystallized from methanol; 214 mg; m. p. 179 - 180 °C; UV λ_{max} (MeOH): 208 nm (log ε 2.9); IR γ_{max} (KBr): 3417, 3387, 3266, 2924, 2853, 1725, 1636, 1462, 1374, 1265, 1175, 1071, 886, 723 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.63 (2H, t, J = 6.5 Hz, H_2-2), 2.32 (2H, m, CH_2), 1.55 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.29 (12H, m, 6 x CH_2), 0.89 (3H, t, J = 6.3 Hz, Me-12), 4.89 (1H, d, J = 3.7 Hz, H-1′α), 4.10 (1H, m, H-5′), 3.81 (1H, m, H-2′), 3.40 (1H, m, H-3'), 3.18 (1H, m, H-4′), 3.09 (2H, d, J = 11.6 Hz, H_2-6'), 4.85 (1H, d, J = 7.6 Hz, H-1"β), 4.13 (1H, m, H-5″), 3.55 (1H, m, H-2″), 3.51 (1H, dd, J = 5.3, 7.6 Hz, H-2″), 3.37 (1H, m, H-3"), 3.15 (1H, m, H-4″), 3.03 (2H, d, J = 9.2 Hz, H_2-6"); ^{13}C NMR (DMSO-d_6): δ 170.14 (C-1), 43.25 (C-2), 32.05 (C-3), 31.87 (C-4), 29.30 (C-5), 29.30 (C-6), 29.30 (C-7), 28.64 (C-8), 27.66 (C-9), 25.38 (C-10), 22.21 (C-11), 14.17 (C-12), 103.43 (C-1′), 85.59 (C-2′), 7175 (C-3′), 67.16 (C-4′), 76.58 (C-5′), 62.15 (C-6′), 97.76 (C-1″), 72.75 (C-2″), 70.18 (C-3″), 66.85 (C-4″), 74.75 (C-5″), 61.09 (C-6″); HRMS : found 524.5977 (calcd. 524.5989 for $C_{24}H_{44}O_{12}$); ESI MS *m/z* (rel. int.): 524 $[M]^+$ ($C_{24}H_{44}O_{12}$) (59.6), 341 (3.2), 325 (4.5), 199 (5.2), 183 (48.6), 179 (8.3), 163 (11.5), 155 (8.2).

Stearyl dixylosyl methoxygallic acid (**3**)

Elution of the column with chloroform- methanol (9:1) mixture yielded colorless mass of **3**; recrystallized from methanol; 197 mg; m. p.: 117 - 119 °C; UV λ_{max} (MeOH): 211, 268 nm (log ε 3.9, 2.9); IR γ_{max} (KBr): 3427, 3378, 3262, 2932, 2849, 1723, 1696, 1637, 1514, 1466, 1267, 1073, 721 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.81 (2H, t, J = 5.6 Hz, H_2-2), 1.52 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.31 (2H, m, CH_2), 1.29 (16H, brs, 8 x CH_2), 1.24 (8H, brs, 4 x CH_2), 0.85 (3H, t, J = 6.2 Hz, Me-18); 5.53 (1H, d, J = 5.5 Hz, H-1′α), 4.13 (1H, m, H-2′), 3.80 (1H, m, H-3'), 3.69 (2H, J = 8.1 Hz, H_2-5′), 3.07 (2H, m, H-4'), 4.52 (1H, d, J = 4.7 Hz, H-1"β), 4.38 (1H, m, H-2″), 3.76 (1H, m, H-3″), 3.65 (2H, d, J = 8.3 Hz, H-5″), 3.05 (1H, m, H-4″), 7.25 (1H, d, J = 2.7 Hz, H-6‴), 6.38 (1H, d, J = 2.7 Hz, H-2‴), 3.15 (3H, s, OMe); ^{13}C NMR (DMSO-d_6): δ 167.89 (C-1), 48.35 (C-2), 32.55 (C-3), 31.67 (C-4), 29.33 – 25.58 (12 x CH_2), 22.25 (C-17), 14.22 (C-18), 103.15 (C-1′), 82.47 (C-2′), 71.51 (C-3′), 70.38 (C-4′), 61.38 (C-5′), 95.76 (C-1″), 78.41(C-2″), 71.48 (C-3″), 69.78 (C-4″), 62.47 (C-5″), 138.13 (C-1‴), 129.84 (C-2‴), 150.71 (C-3‴), 148.28 (C-4‴), 140.38 (C-5‴), 121.78 (C-6‴), 179.27 (C-7‴), 51.31 (OMe); HRMS : found 730.8371 (calcd. 730.8344 for $C_{36}H_{58}O_{15}$); ESI MS *m/z* (rel. int.): 730 $[M]^+$ ($C_{36}H_{58}O_{15}$) (2.9), 315 (5.3), 183 (49.7).

Vanillic acid 4-O-tetra-arabinosyl stearate (**4**)

Further elution of the column with chloroform - methanol (9: 1) mixture afforded pale yellow

mass of **4**; recrystallized from methanol; 197 mg; m. p. 117 - 119 °C; UV λ_{max} (MeOH): 211, 268 nm (log ε 3.9, 2.9); IR γ_{max} (KBr): 3415, 3378, 3251, 2923, 2853, 1735, 1692, 1633, 1522, 1437, 1288, 1038, 725 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.24 (1 H, d, J = 1.9 Hz, H-2), 7.03 (1H, dd, J = 1.9, 7.9 Hz, H-6), 6.17 (1H, d, J = 7.9 Hz, H-5), 3.68 (3H, s, OMe), 5.33 (1H, d, J = 7.3 Hz, H-1a), 5.08 (1H, d, J = 7.5 Hz, H-1b), 4.95 (1H, d, J = 7.1 Hz, H-1c), 487 (1H, d, J = 7.3 Hz, H-1d), 4.28 - 4.15 (4H, m, H-2a, H-2b, H-2c, H-2d), 3.58 - 3.35 (4H, m, H-3a, H-3b, H-3c, H-3d), 3.26 - 3.22 (4H, m, H-4a, H-4b, H-4c, H-4d), 3.18 - 3.05 (8H, brs, H_2-5a, H_2–5b, H_2–5c, H_2–5d), 2.23 (2H, t, J = 7.2 Hz, H_2-2′), 1.72 (2H, m,CH_2), 1.54 (2H, m, CH_2), 1.29 (4H, brs, 4 x CH_2), 1.25 (18H, brs, 9 x CH_2), 0.86 (3H, t, J = 6.6 Hz, Me-18′); ^{13}C NMR (DMSO-d_6): δ 148.36 (C-1), 139.58 (C-2), 151.05 (C-3), 166.19 (C-4), 137.37 (C-5), 129.28 (C-6), 181.29 (C-7), 51.32 (OMe); 109.05 (C-1a), 88.17 (C-2a), 77.25 (C-3a), 69.78 (C-4a), 63.08 (C-5a), 98.25 (C-1b), 84.77 (C-2b), 76.35 (C-3b), 67.58 (C-4b), 61.18 (C-5b), 95.79 (C-1c), 83.57 (C-2c), 72.95 (C-3c), 67.35 (C-4c), 60.93 (C-5c), 91.94 (C-1d), 88.79 (C-2d), 72.45 (C-3d), 67.26 (C-4d), 63.04 (C-5d), 170.49 (C-1′), 48.49 (C-2′), 32.45 22.08 (C-3′ to C-17′), 13.94 (C-18′); HRMS : found 962.9997 (calcd. 963.0671 for $C_{46}H_{74}O_{21}$); ESI MS *m/z* (rel. int.): 962 $[M]^+$ ($C_{46}H_{74}O_{21}$) (2.1), 283 (5.8), 267 (2.7).

Vanillic acid 4-O-hexa-arabinosyl stearate (**5**)

Elution of the column with chloroform- methanol (17:3) mixture afforded pale yellow mass of **5**; recrystallized from methanol; 119 mg; m. p. 132 - 134 °C; UV λ_{max} (MeOH): 211, 268 nm (log ε 3.9, 2.9); IR γ_{max} (KBr): 3445, 3385, 3264, 2928, 2850, 1737, 1698, 1636, 1518, 1439, 1372, 1285, 1183, 1035, 728 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.48 (1 H, d, J = 2.2 Hz, H-2), 7.26 (1H, dd, J = 2.2, 8.6 Hz, H-6), 6.37 (1H, d, J = 8.6 Hz, H-5), 3.58 (3H, s, OMe), 5.53 (1H, d, J = 7.1 Hz, H-1a), 5.18 (1H, d, J = 7.3 Hz, H-1b), 4.92 (1H, d, J = 7.3 Hz, H-1c), 4.53 (1H, d, J = 7.4 Hz, H-1d), 4.50 (2H, brs, H-1e, H-1f), 4.21 (1H, m, H-2f), 3.79 (1H, m, H-1a), 3.75 - 3. 71 (4H, m, H-2b, H-2c, H-2d, H-2e), 3.58 – 3.35 (6H, m, H-3a, H-3b, H-3c, H-3d, H-3e, H-3f), 3.28 - 3.21 (6H, m, H-4a, H-4b, H-4c, H-4d, H-3e, H-3f), 3.14 - 3.02 (12H, brs, H_2-5a, H_2–5b, H_2–5c, H_2–5d, H_2–5e, H_2–5f), 2.32 (2H, t, J = 6.9 Hz, H_2-2′), 2.17 (2H, m,CH_2), 1.96 (2H, m, CH_2), 1.66 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.27 (12H, brs, 6 x CH_2), 1.24 (10H, brs, 5 x CH_2), 0.84 (3H, t, J = 6.3 Hz, Me-18′); ^{13}C NMR (DMSO- d_6): δ 148.37 (C-1), 139.53 (C-2), 150.89 (C-3), 166.29 (C-4), 137.31(C-5), 129.21 (C-6), 179.35 (C-7), 51.34 (OMe), 108.96 (C-1a), 82.82 (C-2a), 75.48 (C-3a), 69.81 (C-4a), 62.97 (C-5a), 103.97 (C-1b), 80.77 (C-2b), 75.25 (C-3b), 69.18 (C-4b), 62.86 (C-5b), 102.03 (C-1c), 77.21 (C-2c), 73.05 (C-3c), 69.42 (C-4c), 61.19 (C-5c), 98.19 (C-1d), 77.67 (C-2d), 72.07 (C-3d), 66.28 (C-4d), 62.89 (C-5d), 95.74 (C-1e), 76.37 (C-2e), 72.41 (C-3e), 69.12 (C-4e), 60.93 (C-5e), 91.94 (C-1f), 76.77 (C-2f), 72.48 (C-3f), 64.29 (C-4f), 60.84 (C-5f), 170.54 (C-1′), 48.54 (C-2′), 32.82 - 22.25 (C-3′ to C - 17′), 12.84 (C-18′); HRMS : found 1226.9989 (calcd. 1227.2964 for $C_{56}H_{90}O_{29}$); ESI MS *m/z* (rel. int.): 1226 $[M]^+$ ($C_{56}H_{90}O_{29}$) (5.2), 431 (33.1), 399 (11.5), 267 (55.9), 167 (8.7).

β-D-Hexaglucoside (**6**)

Elution of the column with chloroform - methanol (3: 1) mixture produced colorless crystals of **6**; recrystallized from methanol; 273 mg; m. p. 126 - 128 °C; UV λ_{max} (MeOH): 205 nm (log ε 4.1); IR γ_{max} (KBr): 3453, 3395, 3276, 2932, 2845, 1641, 1423, 1268, 1079 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.23 (1H, d, J = 7.1 Hz, H-1a), 5.09 (1H, d, J = 7.2 Hz, H-1b), 4.91 (1H, d, J = 7.3 Hz, H-1c), 4.85 (1H, d, J = 7.6 Hz, H-1d), 4.71 (1H, d, J = 7.2 Hz, H-1e), 4.67 (1H, d, J = 7.5 Hz, H-1f), 3.82 –3. 75 (5H, m, H-2a to H-2e), 3.68 - 3.60 (6H, m, H-5a to H-3f), 3.58 (1H, m, H-2f), 3.54 - 3.38 (6H, m, H-3a to H-3f), 3.30 - 3.21 (6H, m, H-4a to H-4f), 3.12 (2H, d, J = 6.7 Hz, H_2-6a), 3.10 (2H, d, J = 11.2 Hz, H_2-6b), 3.07 (2H, d, J = 9.2 Hz, H_2-6c), 3.04 (4H, m, H_2-6d, H_2-6e), 3.02 (2H, d, J = 9.6 Hz, H_2-6f), ^{13}C NMR (DMSO-d_6): δ 104.97 (C-1a), 83.36 (C-2a), 74.72 (C-3a), 70..97 (C-4a), 77.46 (C-5a), 61.38 (H_2-6a), 102.47 (C-1b), 82.97 (C-2b), 73.55 (C-3b), 70.79 (C-4b), 77.16 (C-5b), 61.23 (H_2-6b), 98.53 (C-1c), 82.27 (C-2c), 73.25 (C-3c), 70.36 (C-4c), 77.86 (C-5c), 61.24 (H_2-6c), 97.39 (C-1d), 82.07 (C-2d), 73,22 (C-3d), 69.62 (C-4d), 76.28 (C-5d), 61.18 (H_2-6d), 92.67 (C-1e), 81.27 (C-2e), 72.94 (C-3e), 70.29 (C-4e), 76.13 (C-5e), 61.09 (H_2-6e), 91.17 (C-1f), 75.27 (C-2f), 72.38 (C-3f), 68.21 (C-4f), 75.82 (C-5f), 61.05 (H_2-6f); HRMS : found 990.8574 (calcd. 990.8588 for $C_{36}H_{62}O_{31}$); ESI MS *m/z* (rel. int.): 990 $[M]^+$ ($C_{36}H_{62}O_{31}$) (2.3).

β-D-Heptaglucosyl-β-D-rhamnoside (**7**)

Elution of the column with chloroform- methanol (1: 1) mixture gave colorless crystals of **7**; recrystallized from methanol; 194 mg; m. p. 166 - 168 ^{o}C; UV λ_{max} (MeOH): 209 nm (log ε 3.2); IR γ_{max} (KBr): 3563, 3431, 3378, 3283, 2925, 2853, 1634, 1433, 1309, 1285, 1161, 1039, 1006, 861 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.53 (1H, d, J = 7.6 Hz, H-1a), 5.19 (2H, m, H-1b, H-1c), 5.13 (1H, d, J = 7.6 Hz, H-1d), 4.89 (3H, m, H-1e, H-1f, H-1g), 4.67 (1H, d, J = 8.1 Hz, H-1h), 4.39 - 4.25 (8H, m, H-5a to H-5h), 3.92 - 3.71 (7H, m, H-2a to H-2g), 3.67 - 3.58 (8H, m, H-3a to H-3h), 3.50 (1H, m, H-2h), 3.41 – 3.28 (8H, m, H-4a to H-4h), 3.11 (2H, d, J = 6.9 Hz, H_2-6a), 3.09 (2H, d, J = 18.7 Hz, H_2-6b), 3.07 (2H, d, J = 6.6 Hz, H_2-6c), 3.05 (4H, m, H_2-6d, H_2-6e), 3.03 (2H, d, J = 9.3 Hz, H_2-6f), 3.01 (2H, d, J = 8.5 Hz, H_2-6g), 1.27 (3H, d, J = 6.2 Hz, Me-6h), ^{13}C NMR (DMSO-d_6): δ 104.57 (C-1a), 83.34 (C-2a), 74.75 (C-3a), 70.71 (C-4a), 77.42 (C-5a), 61.68 (H_2-6a), 102.44 (C-1b), 83.09 (C-2b), 73.57 (C-3b), 70.79 (C-4b), 68.24.16 (C-5b), 61.53 (H_2-6b), 98.58 (C-1c), 82.37 (C-2c), 72.95 (C-3c), 70.31 (C-4c), 76.28 (C-5c), 61.19 (H_2-6c), 97.35 (C-1d), 82.17 (C-2d), 72,48 (C-3d), 69.65 (C-4d), 76.24 (C-5d), 61.11 (H_2-6d), 96.47 (C-1e), 81.37 (C-2e), 72.45 (C-3e), 70.29 (C-4e), 76.15 (C-5e), 61.07 (H_2-6e), 92.71 (C-1f), 79.09 (C-2f), 72.09 (C-3f), 69.68 (C-4f), 77.17 (C-5f), 60.95 (H_2-6f), 92.22 (C-1g), 77.76 (C-2g), 71.07 (C-3g), 68.67 (C-4g), 75.17 (C-5g), 60.81 (H_2-6g), 102.47 (C-1h), 75.29 (C-2h), 73.27 (C-3h), 73.29 (C-4h), 64.87 (C-5h), 21.21 (H_2-6h); HRMS : found 1299.1367 (calcd. 1299.1374 for $C_{48}H_{82}O_{40}$); ESI MS *m/z* (rel. int.): 1298 $[M]^+$ ($C_{48}H_{82}O_{40}$) (1.8).

The compound **1** exhibited distinct IR absorption bands for ester function (1723 cm^{-1}) and aliphatic chain (725 cm^{-1}). Its molecular ions peak was determined at *m/z* 396 consistent to a fatty ester, $C_{26}H_{52}O_2$. An ion peak arising at *m/z* 283 indicated that stearic acid was esterified with octanol. The ^{1}H NMR spectrum of **1** displayed two triplets at δ 4.02 (J = 8.6 Hz) and 2.33 (J = 7.3 Hz), integrating for two protons each, assigned to oxymethylene H_2-1′ and methylene H_2-2 adjacent to the ester function, respectively, other methylene protons between δ 1.84 - 1.23, and two three - proton triplets at δ 0.89 (J = 6.7 Hz) and 0.85 (J = 6.3 Hz) ascribed correspondingly to terminal C-8′ and C-18 primary methyl protons. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 171.37 (C-1), oxymethylene C-1′ carbon at δ 65.27, other methylene carbons between δ 36.38 - 22.08 and methyl carbons at δ 13.91 (C-8′) and 13.85 (C-18). On the basis of these evidences the structure of **1** has been formulated as *n*-octyl *n*-octadecanoate (*n*-octyl stearate) (figure 1)**.**

Compound **2,** named lauryl diglucoside, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3417, 3387, 3266 cm^{-1}), ester function (1725 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its mass displayed a molecular ion peak at *m/z* 524 consistent to the molecular formula of an acyl diglycoside, $C_{24}H_{44}O_{12}$. The ions peaks arising at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 325 $[C_6H_{11}O_6$- $C_6H_{10}O_4]^+$, 341 $[C_6H_{11}O_6$- $C_6H_{10}O_5]^+$, 199 [M – 325, CH_3-$(CH_2)_{10}$-$CO]^+$ and 183 [M – 325, CH_3-$(CH_2)_{10}$-$COO]^+$ indicated that two hexose units were linked to C-12 fatty acid. The ^{1}H NMR spectrum of compound **2** exhibited two one-proton doublets at δ 4.89 (J = 3.7 Hz) and 4.85 (J = 7.6 Hz), assigned to anomeric H-1′α and H-1″β protons, respectively, other sugar protons from δ 4.13 to 3.03, a two - proton triplet at δ 2.63 (J = 6.5 Hz) ascribed to methylene H_2-2 protons, a three - proton triplet at δ 0.89 (J = 6.3 Hz) accounted to terminal primary C-12 methyl protons and the remaining methylene proton signals between δ 2.32 - 1.29. The ^{13}C NMR spectrum of **2** displayed signals for ester carbon at δ 170.14 (C-1), methyl carbon at δ 14.17 (C-12), anomeric carbons at δ 103.43 (C-1′) and 97.76 (C-1″) and other sugar carbons from δ 85.59 to 61.09. The presence of proton H-2′ signal in the deshielded region at δ 3.81 and carbon C-2′ signal at δ 85.59 indicated the attachment of another sugar unit by (2′→1″) linkage. The HMBC spectrum of **2** showed correlations of H_2-2 and H-1′ with C-1; H-2′ and H-5′ with C-1′; H-4′, H-3′, H-1′ and H-1″ with C-2′; and H-4″ and H_2-6″ with C-5″.

Acid hydrolysis of **2** yielded lauric acid and glucose, R_f 0.55 (*n*-butanol - acetic acid - water, 2:1:1). On the basis of these studies the structure of **2** has been elucidated as lauryl-O-α-D-glucopyranosyl–(2′→1″)-O-β-D-glucopyranoside, a new acyl diglucoside (figure 1).

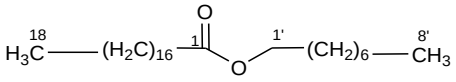
Compound **3,** named stearyl dixylosyl methoxygallic acid, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3427, 3378, 3262 cm^{-1}), ester function (1723 cm^{-1}), carboxylic group (1696 cm^{-1}), aromatic ring (1637, 1514, 1073 cm^{-1}) and

long aliphatic chain (721 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ions peaks of **3** was determined at *m/z* 730 consistent to the molecular formula of a phenyl substituted xylosyl gallic acid, $C_{36}H_{58}O_{15}$. The ions peaks arising at *m/z* 283 $[CH_3\text{-}(CH_2)_{16}\text{-}COO]^+$, 183 $[C_6H_2(OMe)(OH)(O)\text{-}COOH]^+$ and 315 $[C_5H_8O_4\text{-}C_6H_2(OMe)(OH)(O)\text{-}COOH]^+$ indicated the attachment of stearyl group with sugar unit and location of methoxygallic acid with a pentose moiety. The ^1H NMR spectrum of **3** exhibited two one - proton doublets at δ 7.25 (J = 2.7 Hz) and 6.38 (J = 2.7 Hz) assigned to aromatic *meta*-coupled H-6‴ and H-2‴ protons, respectively, two one-proton doublets at δ 5.53 (J = 5.5 Hz) and 4.52 (J = 4.7 Hz) ascribed correspondingly to anomeric H-1′α and H-1"β protons, other sugar protons between δ 4.38 - 3.05, a three - proton triplet at δ 0.85 (J = 6.2 Hz) accounted to terminal C-18 primary methyl protons, methoxy protons as a three-proton singlet at δ 3.15 and methylene protons between δ 2.81 - 1.24. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon 167.89 (C-1), carboxylic carbon at δ 179.27 (C-7‴), aromatic carbons between δ 150.71 - 121.78, methoxy carbon at δ 51.31, methylene carbons from δ 48.35 to 22.25, anomeric carbon at δ 103.15 (C-1′) and 95.76 (C-1″) and other sugar carbons in the range of δ 82.47 - 61.38. The presence of the sugar protons signals at δ 4.13 (H-2′) and 4.38 (H-2″) in the deshielded region and carbon signals at δ 78.41 (C-2′) and 82.47 (C-2″) supported the attachment of sugar units by (2′→1″) linkage and aromatic ring at C-2″. The HMBC spectrum of **3** exhibited correlations of H_2-2 and H-1′ with C-1; H-2′ and H_2-5′ with C-1′; H-4′, H-3′, H-1′ and H-1″ with C-2′; H-3″ and H-4″ with C-5″; and H-2″ and H-2‴ with C-3‴. Acid hydrolysis of 3 yielded stearic acid, L-xylose, R_f 0.27 (butanol-ethanol-water 4:1:2.2) and gallic acid, m. p. 258 - 260 °C; R_f 0.22 (toluene - ethyl acetate - formic acid 6:4:0.8). These spectral and chemical studies led to established the structure of **3** as stearyl-α-L-xylopyranosyl–(2′→1″)-α-L-xlyopyranosyl-2″-(3″-oxy-4‴-hydroxy-5‴-methoxy)-benzoic acid, a new stearyl dixylosyl gallic acid derivative (figure 1).

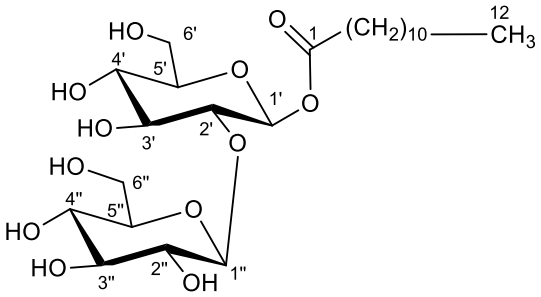
Compound **4,** named vanillic acid 4-O-tetra-arabinosyl stearate, gave positive tests for glucosides and showed IR absorption bands for hydroxyl groups (3415, 3378, 3251 cm^{-1}), ester function (3251 cm^{-1}), carboxylic group (3251 cm^{-1}), aromatic ring (1633, 1522, 1038 cm^{-1}) and long aliphatic chain (725 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 962 consistent to a molecular formula of an aromatic acid substituted tetra-pentosyl ester, $C_{46}H_{74}O_{21}$. The ions peaks arising at *m/z* 283 $[CH_3\text{-}(CH_2)_{16}\text{-}COO]^+$ and *m/z* 267 $[CH_3\text{-}(CH_2)_{16}\text{-}CO]^+$ indicated that stearic acid was esterified with the sugar unit. The ion peak generated at *m/z* 167 $[C_6H_3(OMe)(COOH)(O)]^+$ suggested the existence of vanillic acid moiety at one terminal of the sugar chain. The ^1H NMR spectrum of **4** exhibited aromatic signals as one - proton doublets at δ 7.24 (J = 1.9 Hz) and 6.17 (J = 7.9 Hz), as a one - proton double doublet at δ 7.03 (J = 1.9, 7.9 Hz) assigned to H-2, H-5 and H-6 protons, respectively, a three - proton singlet at δ 3.68 due to methoxy protons, four one - proton doublets at δ 5.33 (J = 7.3 Hz), 5.08 (J = 7.5 Hz), 4.95 (J = 7.1 Hz), 487 (J = 7.3 Hz) with coupling interactions between 7.5 - 7.1 Hz ascribed correspondingly to anomeric H-1a, H-1b, H-1c and H-1d protons, other sugar protons between δ 4.28 - 3.05, a three - proton triplet at δ 0.86 (J = 6.6 Hz) accounted to terminal C-18′ primary methyl protons and methylene protons in the range of δ 2.23 - 1.25. The ^{13}C NMR spectrum of **4** displayed signals for carboxylic carbon at δ 181.32 (C-7), aromatic carbons in the range of δ 151.05 -129.28, methoxy carbon at δ 51.29, anomeric carbons from δ 109 to 91.94, other sugar carbons in the range of δ 88.79 – 63.04, methyl carbon at δ 13.94 (C-18′) and methylene carbons from δ 48.49 to 22.08. The presence of the sugar proton signals at δ 4.28 (H-2a, H-2b and H-1c) and carbon signals at δ 88.17 (C-2a), 84.77 (C-2b), 83.57 (C-2c) and 88.79 (C-2d) in the deshielded regions supported the attachment of sugar units by (2a→1b) and similar linkages and ester linkage at C-4d. The HMBC spectrum of **4** exhibited interactions of H-2, H-5, H-6 and H-1a with C-4; H-1a, H-3a, H-4a and H-1b with C-2a; H-2b, H-2c and H_2-5c with C-1c; H-2c, H-2d and H-3d with C-1d; and H-2d and H_2-2′ with C-1′. Acid hydrolysis of **4** yielded vanillic acid, m. p. 81 - 83° C, R_f 0.56 (toluene - 1, 4- dioxin - acetic acid 9 : 2.5 : 0.4), D-arabinose, R_f 0.31 (butanol-acetic acid-water, 4 : 1 : 5) and stearic acid, m. p. 67 – 69 °C. On the basis of these evidences the structure of **4** has been established as vanillic acid 4-O-β-D-arabinopyranosyl-(2a→1b)-O-β-D-

arabinopyranosyl- (2b→1c)-O-β-D-arabinopyranosyl-(2c→1d)-O-β-D-arabinopyranosyl-2d-stearate, a new aromatic acid tetra-arabinosyl ester (figure 1).

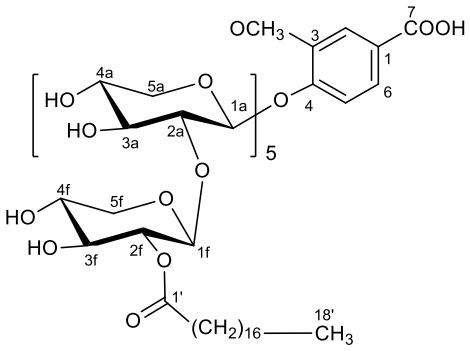
Compound **5**, designated as vanillic acid 4-O-hexa-arabinosyl stearate, $[M]^+$ at *m/z* 1226 ($C_{56}H_{90}O_{29}$), was a homologous compound of **4** having two extra arabinose units. On the basis of spectral data analysis, HMBC correlations and chemical reactions the structure of **5** has been formulated as vanillic acid 4-O- β-D-arabinopyranosyl-(2a→1b)-β-D-arabinopyranosyl- (2b→1c)-β-D-arabinopyranosyl-(2c→1d)-β-D-arabinopyranosyl-(2d→1e)-β-D-arabinopyranosyl-(2e→1f)-β-D-arabino- pyranosyl-2f-stearate, a new vanillic acid hexa-arabinosyl ester (figure 1).



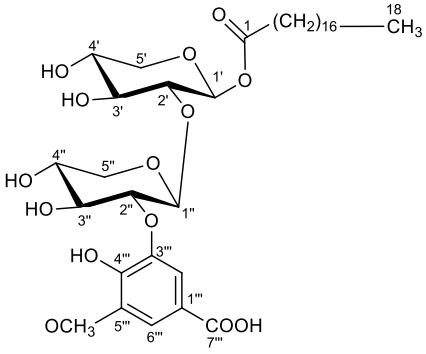
n-Octyl stearate **(1)**



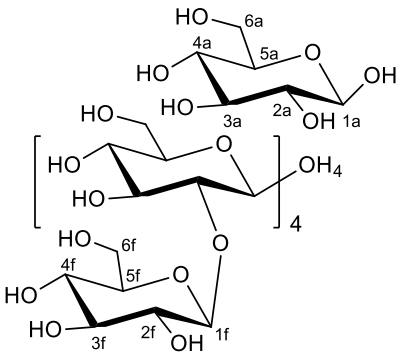
Lauryl diglucoside **(2)**



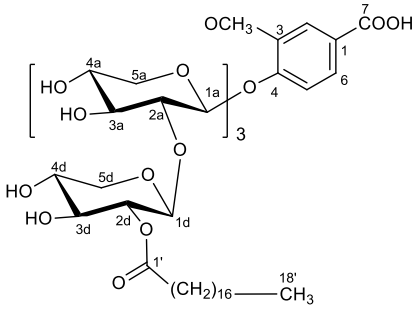
Vanillic acid-4-*O*-hexa arabinosyl stearate (**5**)



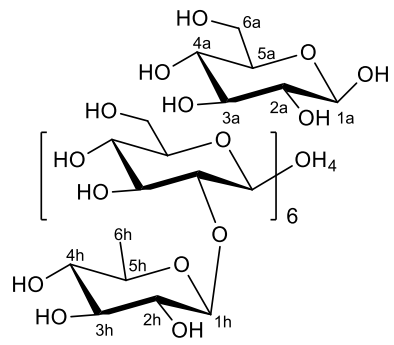
Stearyl dixylosyl methoxygallic acid **(3)**



β-D-Hexaglucoside **(6)**



Vanillic acid-4-*O*-tetra arabinosyl stearate **(4)**



β-D-Heptaglucosyl- β-D-rhamnoside **(7)**

Figure 1. Chemical constituents 1-7 from the stem barks of *Plumeria rubra* L.

Compound **6**, named β-D-hexaglucoside, $[M]^+$ at *m/z* 990 ($C_{36}H_{62}O_{31}$), gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3453, 3395, 3276 cm^{-1}). The ^{1}HNMR spectrum of **6** exhibited six one - proton doublets at δ 5.23 (J = 7.1 Hz), 5.09 (J = 7.2 Hz), 4.91 (J = 7.3 Hz), 4.85 (J = 7.6 Hz), 4.71 (J = 7.2 Hz) and 4.67 (J = 7.5 Hz) assigned to anomeric H-1a to H-1f protons, respectively, and other sugar protons as protons as multiplets from δ 3.82 to 3.21, as two - proton doublets at δ 3.12 (J = 6.7 Hz, H_2-6a), 3.10 (J = 11.2 Hz, H_2-6b), 3.07 (J = 9.2 Hz, H_2-6c) and 3.02 (J = 9.6 Hz, H_2-6f) and as a four - proton multiplet at δ 3.04 (H_2-6d, H_2-6e). The ^{13}C NMR spectrum of **6** displayed signals for six anomeric carbons from δ 104.97 to 91.17 and other sugar carbons between δ 83.36 – 61.05. The presence of C-2a to C-2e carbon signals in the deshielded range from δ 83.36 to 81.27 suggested the attachment of the sugar units by (2a→1b) and similar linkages. The HMBC spectrum of **6** exhibited interactions of H-1a, H-4a, and H_2-6a with C-5a; H-2a, H-2b, H-3b and H-5b with C-1b; H-2b, H-2c and H-5c with C-1c; H-2c, H-2d and H-3d with C-1d; H-2d and H-2e with C-1e; and H-2e and H-2f with C-1f. Acid hydrolysis of 6 yielded D-glucose, R_f 0.55 (*n*-butanol - acetic acid - water, 2 : 1 : 1). On the basis of these evidences the structure of **6** has been established as β-D-glucopyranosyl-(2a→1b)-O-β-D-glucopyranosyl-(2b→1c)-O-β-D-glucopyranosyl- (2c→1d)-O-β-D- glucopyranosyl-(2d→1e)-O-β-D-glucopyranosyl-(2e→1f)-O-β-D-glucopyranoside, a new hexaglucoside (figure 1).

Compound **7**, named β-D-heptaglucosyl-β-D-rhamnoside, $[M]^+$ at *m/z* 1298 ($C_{48}H_{82}O_{40}$), was analyzed for a polyglycoside. It had IR absorption bands for hydroxyl groups (3563, 3431, 3378, 3283 cm^{-1}). The ^{1}HNMR spectrum of **7** exhibited signals for anomeric protons H-1a to H-1h from δ 5.53 to 4.67 with coupling interactions between 8.1 - 7.6 Hz, a three - proton doublet at δ 1.27 (J = 6.2 Hz) ascribed to secondary C-6h methyl protons of rhamnose and other sugar protons from δ 4.39 to 3.01. The ^{13}C NMR spectrum of **6** displayed signals for eight anomeric carbons from δ 104.57 to 92.22, methyl carbon at δ 21.23 (C-6h) and other sugar carbons between δ 83.34 - 60.81. The presence of C-2a to C-2g carbon signals in the deshielded range from δ 83.34 to 77.76 suggested the attachment of the sugar units by (2a→1b) and similar linkages. The HMBC spectrum of **7** showed correlations of H-1a, H-4a, and H_2-6a with C-5a; H-2a, H-2b, H-3b and H-5b with C-1b; H-2b, H-2c and H-5c with C-1c; H-2c, H-2d and H-3d with C-1d; H-2d and H-2e with C-1e; H-2e and H-2f with C-1f; H-2f and H-2g with C-1g; H-2g and H-2h with C-1h; and H-1h, H-4h and H_3 -6h with C-5h. Acid hydrolysis of **7** yielded D-glucose, R_f 0.55 (*n*-butanol - acetic acid - water, 2 : 1 : 1) and D-rhamnose, R_f 0.57 (methanol). On the basis of these spectral studies and chemical reactions the structure of **7** has been established as β-D-glucopyranosyl-(2a→1b)-O-β-D-glucopyranosyl- (2b→1c)-O-β-D-glucopyranosyl- (2c→1d)-O-β-D-glucopyranosyl-(2d→1e)-O-D- glucopyranosyl-(2e→1f)-O-β-D- glucopyranosyl-(2f→1g)-O-β-D-glucopyranosyl-(2g→1h)-O-β-D-rhamnopyranoside, a new octaglycoside (figure 1). Based on our findings, phytochemical investigation of the methanol extract of the stem bark of *Plumeria rubra* led to isolate n-octyl stearate, lauryl diglucoside, stearyl dixylosyl methoxygallic acid, vanillic acid 4-O-tetra- and hexa-arabinosyl stearates, β-D-hexaglucoside and β-D-heptaglucosyl-β-D-rhamnoside. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can be utilized as effective analytical markers for identity purity and quality control of this plant in future.

Acknowledgments

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Author contributions

Surendra Kumar Sharma supervised the experimental work; Mohammed Ali analyzed the spectral data of the compounds; Naresh Kumar performed extraction and isolation work; Shahnaz Sultana surveyed literature of the plant and complied the manuscript; Showkat Rasool Mir helped in data analysis and revised the manuscript critically.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

b.p.: boiling point; TLC: Thin Layer Chromatography; m.p.: melting point; UV: ultra violet; IR: infrared; $CDCl_3$: deuterated chloroform; s: singlet; brs: broad singlet; m: multiplet; d: doublet; dd: double doublet; t: triplet; ESI MS: electrospray ionization mass spectrum; rel. int.: relative intensity; $[M]^+$: molecular ion peak; Me: methyl; HRMS: high resolution mass spectrometry