IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
June 2017 Vol.:9, Issue:3

Chemical Constituents from the Roots of *Picrorhiza kurroa* Royle Ex Benth

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

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

[1]*Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi*

110 062, India.

[2]*Present address: College of Pharmacy, Jazan University, Jazan, Saudi Arabia.*

Submission: 2 June 2017
Accepted: 10 June 2017
Published: 25 June 2017

Keywords: *Picrorhiza kurroa*, Rhizomes, Chemical constituents, Isolation, Characterization.

ABSTRACT

Picrorhiza kurroa Royle ex Benth. (Scrophulariaceae) is a perennial herb found in the alpine Himalayas from Kashmir to Sikkim. Its rhizome is used to treat anemia, asthma, cold, cough, diabetes, digestive disorders, liver complaints, skin diseases, vitiligo and wounds. The rhizome powder was exhaustively extracted with methanol and the extract concentrated to yield a dark brown viscous mass. It was dissolved in the small quantity of methanol and adsorbed onto silica gel (60 - 120 mesh) for preparation of a slurry. The air dried slurry was subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the new phytoconstituents characterized as vanillin-α-D-glucopyranoside (α-glucovanillin) (**4**), picraldehyde 4-O-α-D-glucopyranosyl-(6′→1″)- O-α-D-glucopyranoside (picraldehyde α-D-diglucoside) (**5**) and 3-methoxy-4-hydroxyphenyl-*n*-butanyl-α-O-D-glucopyranosyl-(6a→1b)- α-O-D-glucopyranosyl-(6b→1c)- α-O-D-glucopyranosyl-(6c→1d)- α-O-D-glucopyranosyl-4d-3′-methoxy-4′-hydroxyphenyl *n*-pent-7′,9′-dien-11′-oate (picrortetra- glucoside) (**6**) along with the known compounds 3- methoxy-4-dodecanoxyphenyl- *n*-pent-7, 9-dien-11-al (lauryl picraldehyde) (**1**), 3-methoxy-4-tetradecanoxy-phenyl *n*-pent- 7, 9 –diene-11-al (myristyl picraldehyde) (**2**) and 3-methoxy-4-decanoxy benzoic acid (capryl vanillic acid) (**3**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

INTRODUCTION

Picrorhiza kurroa Royle ex Benth., syn. *P. lindleyana* (Wall.) Steud., *Veronica lindleyana* Wall. (Scrophulariaceae), commonly known as kutki, occurs in the alpine Himalayas from Kashmir to Sikkim between 3000 m and 4500 m altitudes[1,2]. It is a small perennial herb with small, weak, creeping, leafy and slightly hairy stems; jointed, zigzag, greyish-brown, cylindrical, irregularly curved rhizomes and radical, serrate leaves[3,4]. The rhizome is used as an antimalarial, anthelmintic, appetizer, carminative, stomachic, febrifuge, hepatoprotective, immunomodulant, laxative and to treat anemia, asthma, alcoholic toxicity, cold, cough, diabetes, diarrhea, dysentery, digestive disorders, high fever, liver complaints, jaundice, metabolic disorders, skin diseases, vitiligo and wounds. It is a substituent for Indian gentian (*Gentiana kurroo*)[3,5,6]. The plant contained kutkin, picrosides, pikuroside, kutakoside, D-mannitol, kutkiol, kutkisterol, apocynin, phenol glycosides, androsin, picein, kutkoside, minecoside, picrorhizin, arvenin, veronicoside, cucurbitacin glycosides and 4-hydroxy-3-methoxy acetophenone[7 - 13]. This manuscript describes isolation and characterization of three phenolic glucosides together with two acyl picraldehydes and a capryl vanillic acid from the rhizomes of *P. kurroa*.

MATERIALS AND METHODS

General procedures

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer-Rotkreuz, Switzerland) in methanol. Infrared spectra were recorded on Bio-Rad FTIR 5000 spectrophotometer (FTS 135, Kowloon, Hong Hong) using KBr pellets; γmax values are given in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were screened on Advance DRX Bruker spectroscopic 400 and 100 MHz, respectively, instruments (Karlsruhe, Germany) using $CDCl_3$ or DMSO-d_6 as a solvent and TMS as an internal standard. Mass spectra were scanned by effecting ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapors and UV radiation and spraying with ceric sulfate solution.

Plant material

The rhizomes of *P. kurroa* were procured from the Khari Baoli market, Delhi and identified by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen has been retained in the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

Preparation of extract

The dried rhizomes (1.5 kg) were coarsely powdered and exhaustively extracted in a Soxhlet apparatus with methanol. The methanolic extract was concentrated under reduced pressure to yield a dark brown viscous mass (130.8 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

Isolation of Phytoconstituents

The viscous dark brown extract (100 g) was dissolved in small quantity of methanol and adsorbed onto silica gel (60 - 120 mesh) for preparation of slurry. The slurry was air dried and subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3), chloroform and the mixture of chloroform - methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various fractions were collected separately and matched by TLC to check the homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

Lauryl picraldehyde (**1**)

Elution of the column with chloroform furnished colourless crystals of compound **1**, recrystallized from acetone : methanol (1:1) 184 mg, m. p. 101-102 °C; IR ν_{max} (KBr): 1725, 1704, 1631, 1516, 768 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 9.56 (1H, d, J = 8.3 Hz, H-11), 7.44 (1 H, d, J = 2.3 Hz, H-2), 7.37 (1H, dd, J = 2.3, 8.9 Hz, H-6), 6.67 (1H, d, J = 8.9 Hz, H-5), 5.21 (1H, d, J = 9.3 Hz, H-7), 4.95 (1H, dd, J = 9.3, 8.4 Hz, H-8), 4.86 (1H, dd, J = 8.4, 8.1 Hz, H-9), 4.82 (1H, dd, J = 8.1, 8.0 Hz, H-10) 3.59 (3H, brs, OMe), 2.32 (2H, t, J = 7.3 Hz, H-2'), 1.65 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.27 (12H, brs, 6 x CH_2), 0.85 (3H, t, J = 6.3 H_Z, Me-12'); ^{13}C NMR (DMSO-d_6): δ 153.67 (C-1), 148.08 (C-2), 163.25 (C-

3), 151.41 (C-4), 137.67 (C-5), 132.18 (C-6), 129.95 (C-7), 122.87 (C-8), 115.09 (C-9), 113.16 (C-10), 205.12 (C-11), 170.32 (C-1′) 54.75 – 22.67 (C-2' – C-11'), 14.16 (12'), 55.51 (OMe); ESI MS *m/z* (rel. int) : 386 $[M]^+$ (C_{24} H_{34} O_4) (2.7).

Myristyl picraldehyde (**2**)

Further elution of the column with chloroform produced colourless crystals of **2**, recrystallized from chloroform-methanol (1:1), 125 mg, m. p. 66 – 67 °C; IR ν_{max} (KBr): 1723, 1702, 1641, 1521, 769 cm^1; $^{\cdot 1}$H NMR ($CDCl_3$) : δ 9.87 (1H, d, J = 8.9 Hz, H-11), 7.67 (1H, d, J = 2.2 Hz, H-2), 6.83 (1H, d, J = 8.5, H-5), 6.54 (1H, d, J = 2.2, 8.5 Hz, H-6), 5.25 (1H, d, J = 4.7 Hz, H-7), 5.25 (1H, m, H-8), 4.95 (1H, m, H-9), 4.93 (1H, m, H-10), 3.78 (3H, brs, OMe) 2.33 (2 H, t, J = 7.2 Hz, H_2-2′), 2.07 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.35 (18 H, brs, 9 x CH_2), 0.86 (3H, t J = 6.6 Hz, Me-14'). ^{13}C NMR ($CDCl_3$): δ 145.06 (C-1), 143.31 (C-2), 153.18 (C-3), 148.06 (C-4), 138.87 (C-5), 132.11 (C-6), 129.08 (C-7), 128.35 (C-8), 123.56 (C-9), 119.13 (C-10), 201.11(C-11), 167.74 (C-1') 34.41 – 28.16 (C-2' to C-12'), 22.26 (C-13'), 14.09 (14'), 55.37 (OMe); ESI MS *m/z* (rel. int) : 414 $[M]^+$ (C_{26} H_{38} O_4) (9.1), 203 (10.8), 187 (17.6).

Capryl vanillic acid (**3**)

Elution of the column with chloroform – methanol (49 : 1) yielded pale yellow crystals of **3**, recrystallized from acetone, 254 mg, m. p. 113-115 °C; IR ν_{max} (KBr): 3310, 1721, 1695, 1635, 1515, 1015, 767 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.42 (1H, d, J = 2.3 Hz, H-2), 6.88 (1H, d, J = 8.9 Hz, H-5), 6.67 (1H, dd, J = 2.3, 8.9 Hz, H-6), 3.79 (3H, brs, OMe), 2.43 – 1.34 (8H, m, 4 x CH_2), 1.23 (8H, brs, 4 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me -10'); ^{13}C NMR (DMSO-d_6): δ 142.37 (C-1), 131.15 (C-2), 153.07 (C-3), 148.24 (C-4), 127.38 (C-5), 115.32 (C-6), 178.14 (C-7), 55.52 (OMe), 168.06 (C-1'), 34.26 – 22.67 (8 CH_2), 14.06 (C-10'); ESI MS *m/z* (rel. int) : 322 $[M]^+$ (C_{18} H_{26} O_5) (4.9).

α-Glucovanillin (4)

Elution of the column with chloroform: methanol (19:1) gave pale yellow crystals of **4**, recrystallized from methanol, 206 mg, m. p. 188 - 190 °C.; IR ν_{max} (KBr): 3395, 3263, 2928, 2847, 1702, 1641, 1518, 1427, 1284, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.35 (1H, s, H-7), 7.33 (1H, d, J = 2.2 Hz, H-2), 6.87 (1H, dd, J = 8.5, 2.2 Hz, H-6), 6.62 (1H, d, J = 8.5 Hz, H-5), 5.02 (1H, d, J = 6.1 Hz, H-1′), 4.63 (1H, m, H-5′), 4.21 (1H, dd, J = 6.1, 6.8 Hz, H-2′

), 3.76 (1H, m, H-3′), 3.33 (1H, m, H-4′), 3.08 (2H, d, J = 7.8 Hz, H_2-6′), 3.25 (3H, brs, OMe); ^{13}C NMR ($CDCl_3$): δ 142.37 (C-1), 133.67 (C-2), 153.12 (C-3), 148.23 (C-4), 128.29 (C-5), 116.03 (C-6), 207.49 (C-7), 55.51 (OMe), 103.27 (C-1'), 73.21 (C-2'), 68.16 (C-3'), 65.21 (C-4'), 76.29 (C-5'), 60.19 (C-6'); ESI MS *m/z* (rel. int): 314 $[M]^+$ (C_{14} H_{18} O_8) (3.8).

Picraldehyde α-D-diglucoside (5)

Elution of the column with chloroform : methanol (17:3) afforded yellow crystals of **5**, recrystallized from methanol, 208 mg, m. p. 117-119 °C; IR ν_{max} (KBr): 3458, 3387, 2928, 2847, 3268, 1705, 1643, 1519, 1457, 1371, 1281, 1156, 1075 cm^{-1}; ^{1}H NMR (DMSO-d_6) : δ 9.79 (1H, d, J = 5.1 Hz, H-11), 7.45 (1H, d, J = 2.5 Hz, H-2), 7.35 (1H, dd, J = 2.5, 8.6 Hz, H-6), 6.92 (1H, d, J = 8.6 Hz, H-5), 6.62 (1H, d, J = 8.5 Hz, H-5), 5.12 (1H, d, J = 9.6 Hz, H-7), 5.09 (1H,d d, J = 9.6, 8.9 Hz, H-8), 5.06 (1H, dd, J = 8.9, 11.2 Hz, H-9), 5.04 (1H, dd, J = 4.7, 11.2 Hz, H-10), 5.01 (1H, d, J = 5.9 Hz, H-1′), 4. 59 (1H, m, H-5′), 4.39 (1H, m, H-2′), 3.74 (1H, m, H-3′), 3.39 (1H, m, H-4′), 3.15 (2H, d, J = 9.6 Hz, H_2-6′), 4. 95 (1H, d, J = 6.0 Hz, H-1″), 4. 57 (1H, m, H-5″), 3.85 (1H, m, H-2″), 3.66 (1H, m, H-3″), 3.34 (1H, m, H-4″), 3.04 (2H, d, J = 8.7 Hz, H_2-6″), 3.69 (3H, s, OMe); ^{13}C NMR (DMSO-d_6): δ 152.96 (C-1), 148.57 (C-2), 159.81 (C-3), 154.41 (C-4), 145..07 (C-5), 143.91 (C-6), 129.04 (C-7), 128.39 (C-8), 116.27 (C-9), 115.55 (C-10), 199.86 (C-11), 102.66 (C-1'), 77.39 (C-2'), 73.41 (C-3'), 70.12 (C-4'), 81.79 (C-5'), 62.88 (C-6'), 101.97 (C-1″), 75.24 (C-2″), 72.39 (C-3″), 69.13 (C-4″), 76.71 (C-5″), 61.02 (C-6″), 58.41 (OMe); ESI MS *m/z* (rel. int) : 528 $[M]^+$ (C_{24} H_{32} O_{13}) (2.8), 325 (12.6), 179 (9.1).

Picrortetraglucoside (6)

Elution of the column with chloroform: methanol (4:1) yielded yellow crystals of **6**, recrystallized from methanol, 145 mg, m. p. 122-124 °C; IR ν_{max} (KBr): 3452, 3395, 3263, 2926, 2849, 1723, 1645, 1517, 1453, 1367, 1286, 1159, 1074 cm^{-1}; ^{1}H NMR (DMSO-d_6) : δ 7.55 (1H, d, J = 2.0 Hz, H-2), 7.51 (1H, dd, J = 2.0, 8.4 Hz, H-6), 6.93 (1H, d, J = 8.4 Hz, H-5), 3.36 (2H, t, J = 9.6 Hz, H_2-10), 2.52 (2H, t, J = 5.1 Hz, H_2-7), 1.24 (4H, m, H_2-8, H_2-9), 3.69 (3H, s, OMe), 7.48 (1H, d, J = 2.2 Hz, H-2′), 7.41 (1H, dd, J = 2.2, 8.6 Hz, H-6′), 6.87 (1H, d, J = 8.6 Hz, H-5′), 5.19 (1H, d, J = 9.7 Hz, H-7′), 5.11 (1H, dd, J = 9.5, 9.7 Hz, H-8′), 5.09 (1H, dd, J = 9.7, 9.6 Hz, H-9′), 5.07 (1H, d, J = 9.6 Hz, H-10′), 3.64 (3H, s, OMe), 5.04 (1H, d, J = 6.5 Hz, H-1a), 5.01 (1H, d, J = 6.3 Hz, H-1b), 4.98 (1H, d, J = 5.5 Hz, H-1c), 4.93 (1H, d, J = 6.3 Hz, H-1d), 4.78 – 4.63 (4H, m, H-5a, H-5b, H-5c, H-5d), 4.43 – 4.25

(4H, m, H-2a, H-2b, H-2c, H-2d), 3.81 – 3.76 (3H, m, H-4a, H-4b, H-4c), 4.21 (1H, m, H-4d), 3.58 – 3.43 (4H, m, H-3a, H-3b, H-3c, H-3d), 3.28 (2H, d, J = 6.6 Hz, H_2-6a), 3.23 (2H, d, J = 9.6 Hz, H_2-6b), 3.19 (2H, d, J = 11.2 Hz, H_2-6c), 3.09 (2H, d, J = 10.4 Hz, H_2-6d); ^{13}C NMR (DMSO-d_6): δ 144.62 (C-1), 133.85 (C-2), 165.61 (C-3), 151.81 (C-4), 128.97 (C-5), 123.78 (C-6), 41.79 (C-7), 37.39 (C-8), 35.16 (C-9), 63.05 (C-10), 55.64 (OMe), 141.06 (C-1′), 130.53 (C-2′), 160.26 (C-3′), 147.48 (C-4′), 128.37 (C-5′), 119.98 (C-6′), 117.87 (C-7′), 115.79 (C-8′), 115.23 (C-9′), 112.65 (C-10′), 170.05 (C-11′), 58.41 (OMe), 102.68 (C-1a), 74.87 (C-2a), 72..43 (C-3a), 73.82 (C-4a), 77.35 (C-5a), 62.89 (C-6a), 101.93 (C-1b), 74.74 (C-2b), 70.24 (C-3b), 73.35 (C-4b), 77.27 (C-5b), 61.32 (C-6b), 97.82 (C-1c), 74.28 (C-2c), 69.87 (C-3c), 79.68 (C-4c), 76.36 (C-5c), 61.11 (C-6c), 92.95 (C-1d), 74.17 (C-2d), 73.23 (C-3d), 79.67 (C-4d), 75.28 (C-5d), 60.39 (C-6d); ESI MS *m/z* (rel. int) : 942 $[M]^+$ (C_{47} H_{66} O_{26}) (2.3), 739 (11.9), 203 (43.1) .

RESULTS AND DISCUSSION

Compounds **1** -**3** were the known chemical constituents identified as 3- methoxy-4-dodecanoxy phenyl- *n*-pent-7,9-dien-11-al (lauryl picraldehyde), 3-methoxy-4-tetradecanoxy-phenyl *n*-pent- 7,9 –diene-11-al (myristyl picraldehyde) and 3-methoxy-4-decanoxy benzoic acid (capryl vanillic acid), respectively[14].

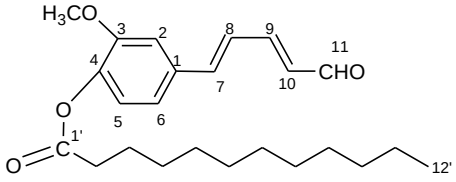
Compound **4**, named α-glucovanillin, gave positive tests for aldehydes and glycosides and exhibited distinct IR absorption bands for hydroxyl groups (3395, 3263 cm^{-1}), aldehydic function (1702 cm^{-1}) and aromaticity (1641, 1518, 1065 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of compound **4** was established at *m/z* 314 corresponding to an aromatic aldehydic glycoside, $C_{14}H_{18}O_8$. The ^{1}H NMR spectrum of compound **4** showed a one-proton singlet at δ 9.35, two one-proton doublets at δ 7.33 (J = 2.2 Hz) and 6.62 (J = 8.5 $H_{Z,}$) and a one-proton double doublet at δ 6.87 (J = 8.5, 2.2 Hz) assigned to aldehydic H-7 and aromatic H-2, H-5 and H-6 protons, respectively. A one-proton doublet at δ 5.02 (J= 6.1 Hz), three one-proton multiplets at δ 4.63, 3.76 and 3.33 , a one-proton double doublet at δ 4.21 (J= 6.1, 6.8 Hz) and a two-proton doublet at δ 3.08 (J = 7.8 Hz) were ascribed correspondingly to α-oriented anomeric H-1′ and other sugar protons. A three-proton broad singlet at δ 3.25 was attributed to the methoxy protons. The ^{13}C NMR spectrum of compound **4** displayed signals for aldehydic carbon at δ 207.49 (C-7), aromatic carbons from δ 153.12 to 116.03, methoxy carbon at δ 55.51, anomeric carbon at δ 103.27 (C-1′) and other sugar carbons between δ 76.29 to 60.19. Acid hydrolysis of **4** yielded

vanillin, m. p. 81 - 83° C, R_f 0.568 (toluene - 1, 4- dioxin - acetic acid 9 : 2.5 : 0.4)[15] and D-glucose, R_f 0.55 (*n*-butanol - acetic acid - water, 2 : 1 : 1). On the basis of these results, the structure of compound **4** has been characterized as vanillin-α-D-glucopyranoside.

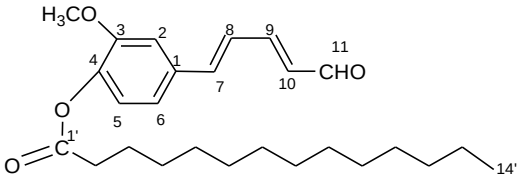
Compound **5**, designated as picraldehyde α-D-diglucoside, responded to aldehydic and glycosidic tests positively and showed IR absorption bands for hydroxyl groups (3458, 3387 cm^{-1}), aldehydic function (1705 cm^{-1}) and aromaticity (1643, 1519, 1075 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of compound **5** was determined at *m/z* 528 corresponding to an aromatic aldehydic diglycoside, $C_{24}H_{32}O_{13}$. The ion peaks arising at *m/z* 179 $[C_6 H_{11} O_6]^+$ and 325 $[C_{12} H_{21} O_{10}]^+$ indicated that two hexose units were linked to the aldehyde. The ^1H NMR spectrum of compound **5** showed four one-proton doublets at δ 9.79 (J = 5.1 Hz), 7.45 (J = 2.5 Hz), 6.92 (J = 8.6 Hz) and 6.62 (J = 8.5 H_Z) and a one-proton double doublet at δ 7.35 (J = 2.5, 8.6 Hz) assigned to aldehydic H-11 attached to a methine group and aromatic H-2, H-5 and H-6 protons, respectively. A one-proton doublet at δ 5.12 (J = 9.6 Hz) and three one-proton double doublets at δ 5.09 (J = 9.6, 8.9 Hz), 5.06 (J = 8.9, 10.2 Hz) and 5.04 (J = 4.7, 10.2 Hz) were ascribed correspondingly to *cis*-oriented vinylic H-7. H-8, H-9, and H-10 protons. Two one-proton doublets at δ 5.01 (J = 5.9 Hz) and 4. 95 (J = 6.0 Hz, H-1″) were accounted to α-oriented anomeric H-1′ and H-1″ protons, respectively. The other sugar protons appeared as one-proton multiplets from δ 4.59 to 3.34 and as two-proton doublets at δ 3.15 (J = 9.6 Hz, H_2-6′) and 3.04 (J = 8.7 Hz, H_2-6″). A three-proton broad singlet at δ 3.69 was due to the methoxy protons. The ^{13}C NMR spectrum of **5** displayed signals for aldehydic carbon at δ 199.86 (C-11), aromatic and vinylic carbons from δ 159.81 to 116.27, methoxy carbon at δ 58.41, anomeric carbons at δ 102.66 (C-1′) and 101.97 (C-1″) and other sugar carbons between δ 81.79 - 60.92. The presence of oxymethylene H_2-6′ proton signal in the deshielded region at δ 3.15 and its carbon signal at δ 62.88 (C-6') suggested (6′→1″) linkage between the sugar moieties. Acid hydrolysis of **5** yielded picraldehyde and D-glucose, R_f 0.55 (*n*-butanol - acetic acid - water, 2: 1: 1). On the basis of these results, the structure of **5** has been elucidated picraldehyde 4-O-α-D-glucopyranosyl-(6′→1″)- O-α-D-glucopyranoside.

Compound **6**, named picrortetralucoside, gave positive tests for phenolic compounds and glycosides. Its IR spectrum showed absorption bands for hydroxyl groups (3452, 3395, 3263 cm^{-1}), ester function (1723 cm^{-1}) and aromatic compounds (1645, 1517, 1074 cm^{-1}). Its molecular ion peak was determined on the basis of mass and ^{13}C NMR spectra at *m/z* 942

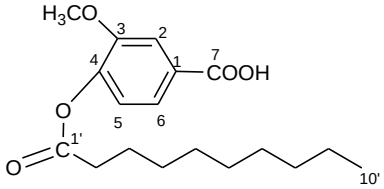
consistent with a molecular formula of alkylated diphenolic tetra glycoside, $C_{47}H_{66}O_{26}$. The ion peaks arising at *m/z* 195 $[MeO(OH)C_6H_3\text{-}(CH_2)_3\text{-}CH_2\text{-}O]^+$, 203 $[MeO(OH)C_6H_3\text{-}(CH{=}CH)_2\text{-}CO]^+$ and 739 $[M - 203]^+$ indicated the presence of alkyl phenyl ring at one end of the tetra glycoside and alkenylated phenyl ester at another side of the molecule. The ^1H NMR spectrum of compound **5** displayed aromatic protons as one-proton doublets at δ 7.55 (J = 2.0 Hz, H-2), 6.93 (J = 8.4 Hz, H-5), 7.48 (J = 2.2 Hz, H-2′), 6.87 (J = 8.6 Hz, H-5′) and as one-proton double doublets at δ 7.51 (J = 2.0, 8.4 Hz, H-6) and 7.41 (J = 2.2, 8.6 Hz, H-6′), *cis*-oriented vinylic H-7′ to H-10′ protons from δ 5.19 to 5.07 with coupling interactions between 9.5 – 9.7 Hz, oxymethylene H_2-10 protons as a two-proton triplet at δ 3.36 (J = 9.6 Hz), other methylene protons as a two-proton triplet at δ 2.52 (J = 5.1 Hz, H_2-7) and as a four-proton multiplet at δ 1.24 (H_2-8, H_2-9), four α-oriented anomeric protons as one – proton doublets at δ 5.04 (J = 6.5 Hz, H-1a), 5.01 (J = 6.3 Hz, H-1b), 4.98 (J = 5.5 Hz, H-1c) and 4.93 (J = 6.3 Hz, H-1d) and the remaining sugar protons from δ 4.78 to 3.09. Two three-proton singlets at δ 3.69 and 3.64 were due to methoxy protons. The ^{13}C NMR spectrum of **6** exhibited signals for ester carbon at δ 170.05 (C-11′), aromatic and vinylic carbons from δ 165.61 to 112.65, oxymethylene carbon at δ 63.05 (C-10), methoxy carbon at δ 55.64 and 58.41 (2 x OMe), three methylene carbons at δ 41.79 (C-7), 37.39 (C-8) and 35.16 (C-9), anomeric carbons at δ 102.68 (C-1a), 101.93 (C-1b), 97.82 (C-1c) and 92.95 (C-1d) and other sugar carbons from δ 77.35 to 60.39. The presence of oxymethylene proton signals in the deshielded region at δ 3.28 (H_2-6a), 3.23 (H_2-6b) and 3.19 (H_2-6c) and carbon signals at δ 62.89 (C-6a), 61.32 (C-6b) and 61.11 (C-6c) suggested linkages of the sugar anomeric carbons with the oxymethylene carbons. The appearance of H-4d ^1H signal in the downfield region at δ 4.21 and carbon signal at δ 79.68 (C-4c) indicated the presence of the ester linkage at C-4d. 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 compound **6** has been formulated 3-methoxy-4-hydroxyphenyl-*n*-butanyl-α-O-D-glucopyranosyl-(6a→1b)- α-O-D-glucopyranosyl-(6b→1c)- α-O-D-glucopyranosyl-(6c→1d)- α-O-D-glucopyranosyl-4d-3′-methoxy-4′-hydroxyphenyl-*n*-pent-7′,9′-dien-11′-oate, a new diphenyl tetraglucoside.



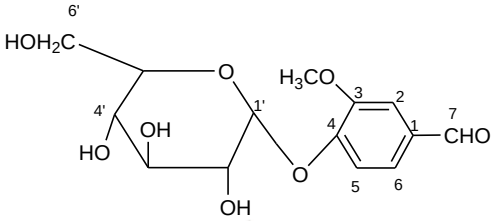
Lauryl picraldehyde **(1)**



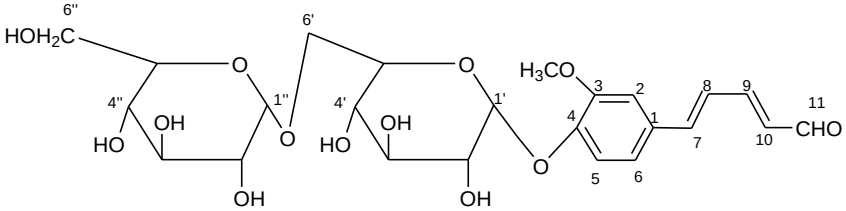
Myristyl picraldehyde **(2)**



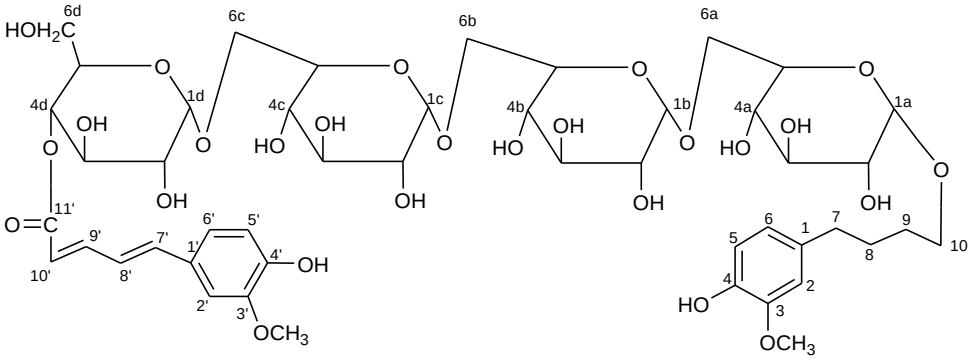
Capryl vanillic acid **(3)**



Vanillin-α-D-glucoside **(4)**



Picraldehyde diglucoside **(5)**



Picrortetraglucoside **(6)**

CONCLUSION

Phytochemical investigation of a methanolic extract of the rhizomes of *P. kurroa* led to the isolation of acyl picraldehydes, capryl vanillic acid, α-glucovanillin, picraldehyde α-D-diglucoside and picrortetraglucoside. 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.

ACKNOWLEDGEMENTS

The authors are thankful to the instrumentation centers, Central Drug Research Institute, Lucknow and Jawaharlal Nehru University, New Delhi for recording spectral data of the compounds.

REFERENCES

1. Kapahi BK, Srivastava TN, Sarin YK. Description of *Picrorhiza kurroa*, a source of the Ayurvedic drug kutaki. Pharmaceutical Biology. 2008, 31(3): 217-222. DOI: 10.3109/13880209309082945.
2. Kumar R, Bhandari P, Singh B, Ahuja PS. Evaluation of *Picrorhiza kurroa* accessions for growth and quality in northwestern Himalayas. J Med Plants Res. 2012, 6(13): 2660-2665.
3. Anonymous. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial products, Raw materials. New Delhi: National Institute of Science Communication and Information Resources, CSIR; 1992, 8, 49.
4. Arya D, Bhatt D, Kumar R, Tewari LM, Kishor K, Joshi GC. Studies on natural resources, trade and conservation of Kutki (*Picrorhiza kurroa* Royle ex Benth., Scrophulariaceae) from Kumaun Himalaya. Scientific Research and Essays. 2013, 8(14), 575-580.
5. Masood M, Arshad M, Qureshi R, Sabir S, Amjad MS, Qureshi H, Tahir Z. *Picrorhiza kurroa*: an ethnopharmacologically important plant species of the Himalayan region. Pure Appl. Biol., 201, 4(3): 407-417.
6. Thyagarajan P, Jayaram S, Gopalakrishnan V, Hari V, Jeyakumar P, Sripathi M. Herbal medicines for liver diseases in India. J Gastroenterol Hepatol. 2002, 17:370–376.
7. Weinges K, Kloss P, Henkels WD. Natural products from medicinal plants. XVII. picroside-II, a new 6-vanilloylcatapol from Picrorhiza kurroa Royle ex Benth. Justus Liebigs Annalen der Chemie. 1972, 759: 173-182.
8. Stuppner H, Wagner H. New cucurbitacin glycosides from *Picrorhiza kurroa*. Planta Medica. 1989, 55: 559-563.
9. Singh N, Gupta AP, Singh B, Kaul VK. Quantification of picroside I and picroside II in *Picrorhiza kurroa* by HPTLC. J Liq Chromatogr. 2005, 28: 1679–1691.
10. Sharma N, Pathania V, Singh B, Gupta RC Intraspecific variability of main phytochemical compounds in *Picrorhiza kurroa* Royle ex Benth. from North Indian higher altitude Himalayas using reversed-phase high-performance liquid chromatography. J Med Plants Res. 2012, 6(16): 3181-3187.
11. Sharma N, Pathania V, Singh B, Gupta RC Intraspecific variability of main phytochemical compounds in *Picrorhiza kurroa* Royle ex Benth. from North Indian higher altitude Himalayas using reversed-phase high-performance liquid chromatography. J Med Plants Res. 2012, 6(16): 3181-3187.
12. Thapliyal S, Mahadevan N, Nanjan MJ. Analysis of Picroside I and Kutkoside in *Picrorhiza kurroa* and its Formulation by HPTLC. Int J Res Pharm Biomed Sci. 2012, 3(1): 25-30.
13. Sultan P, Jan A, Pervaiz Q. Phytochemical studies for quantitative estimation of iridoid glycosides in *Picrorhiza kurroa* Royle. Bot Stud. 2016, 57:7. DOI 10.1186/s40529- 016- 0121-2.

14. Kumar N, Sharma SK. A new aromatic ester from *Picrorhiza kurroa* Royle ex Benth. J Pharmacog Phytochem. 2014, 3 (1): 96-98.
15 Hingse SS, Digole SB, Annapure US. Method development for simultaneous detection of ferulic acid and vanillin using high-performance thin-layer chromatography. J Analytical Sci Technol. 2014, 5:21, 1-9.