ejpmr, 2020,7(8), 564-570

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SECONDARY METABOLITES PRODUCED DURING THE GERMINATION OF *STREPTOMYCES COELICOLOR* AND FROM THE LEAVES OF *OXALIS DEBILIS*

Asif Ali[1,2], Mohammed Ali[1]*, Showkat Rassol Mir[1], Md. Kamaruz Zaman[1,3] and Shahnaz Sultana[1,4]

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

[2]National Institute of Science Communication and Information Resources (NISCAIR), CSIR, 14 Satsang Marg, Institution Area, New Delhi – 110067, India.

[3]Pharmacognosy Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh - 786004 (Assam), India.

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

***Corresponding Author: Prof. Mohammed Ali**
Phytopharmaceuticals Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, P.O. Hamdard Nagar, New Delhi – 110062, India.

Article Received on 09/06/2020 **Article Revised on 29/06/2020** **Article Accepted on 19/07/2020**

ABSTRACT

Streptomyces coelicolor produces various type of compounds by means of specialized biosynthetic pathways. This paper reports the isolation and identification of chemical constituents found in a fermentation broth of *S. coelicolor*. A methanol extract of the cells of *S. coelicolor* was chromatographed over a silica gel column. The column was eluted with various solvent mixtures of increasing polarity to isolate the chemical constituents. Elution of the column with petroleum ether - chloroform (1:1) yielded a new 2,3-dihydro-1-benzopyran-4-one identified as 2α-methyl-8-hydroxy-4-chromanone (**1**). Elution of the column with chloroform gave yellow crystals of a new alkylated β-naphthol carboxylic acid characterized as 1-(2'-pentanyl-1'-aldehyde)-2-hydroxy-8-naphthoic acid (**2**). Lawsonyl-1,3-dimethyl-2α-(10,14-dimethyl nonan-7β-olyl)cyclohexan-1-oate (**3**), a new diterpenic ester with lawsone, was isolated when the column was eluted with chloroform-methanol (19:1) mixture. Elution of the column with chloroform-methanol (9:1) furnished red crystals of 3-[1-(2'-hexan-1'-al-4'-one)-2-hydroxy-8-naphthoic acid]-3"-[1"-(2'"-hexan-1'"-al)-2"-hydroxy-8"-naphthoic acid]-hydroxy methine (**4**), a new dinaphthol-1, 1"-dihexanal-8, 8"-dioic acid carbinol derivative. The leaves of *Oxalis debilis* Kunth (Oxalidaceae) possess analgesic, anodyne, antiscorbutic, astringent, diuretic, emmenagogue, expectorant and stomachic properties and are used to treat earache, fevers and to quench the thirst. Phytochemical investigation of the leaves of *O. debilis* furnished a known phytosterol, stigmasterol (**5**) and a higher aliphatic alcohols identified as 1-heptatriacontanol (**6**). The structures of these natural products were elucidated on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Streptomyces coelicolor*, spore germination, *Oxalis debilis* leaves, chemical constituents, isolation, identification.

INTRODUCTION

Streptomycetes are Gram-positive, filamentous, soil-dwelling saprophytic bacteria which are responsible for more than 50% of the known microbial metabolites including many antibiotics used in human and veterinary medicines. *Streptomyces coelicolor* and other species are known to produce polyketides (actinorhodins, kalafungin, phenocyclinone, 5-hydroxyaloesaponarin),[1-4] ribose trisaccharide, 2-O- and 5-O-(α-mannosyl)-myo-inositols, trehalose and D-ribulose,[5] γ-butyrolactones, furans, fatty acids, oligopyrroles, deoxysugars, butenolides, siderophores, alkylprodiginines, cyclic depsipeptides, aminocoumarins, carbohydrates, α- and γ-pyrone derivatives, nalidixic acid, actinoperylones, albaflavenone, germicidin, chalcone and terpenic compounds by means of specialized biosynthetic pathways.[6-19] These compounds may provide defence, competition, signalling, or interspecies interactions depending on the environmental cues, thus increasing the likelihood of survival in an inhospitable environment.[20,21]

Oxalis debilis Kunth, syn. *Acetosella debilis* (Kunth) Kuntze (Oxalidaceae), known as the large-flowered pink-sorrel, pink wood sorrel and South American wood sorrel, is a native to South America, and distributed in the tropical countries including Australia, Hawaii, Fiji, New Caledonia, and China, Taiwan, Hong Kong and in India mainly in the Brahmaputra valley region.[22,23] It is a perennial herb, 10-35 cm high, arising from a dense cluster of sessile bulblets, bulb scales 3-nerved; leaves all basal, leaflets 3, rounded-obcordate, lobes apically

convex, hirsute, oxalate deposits in tiny crystals around leaf margins; flowers in irregular cymes, petals violet to lavender or rose-purple; capsules absent. Its flowers, leaves and roots are edible. The whole plant is anthelmintic, antidiabetic, astringent, catalytic, diuretic, febrifuge, refrigerant and stomachic, used to treat asthma, diarrhoea, diabetes, edema, fevers, liver and digestive disorders, mouth sores, piles, scurvy, skin caners, swollen gums, urinary problems, wounds and as an antidote for toxicity. The plant juice is laxative, and applied to cure boils, cuts and injuries and to treat labour pain and swellings.[24-26] The leaves are analgesic, anodyne, antiscorbutic, astringent, diuretic, emmenagogue, expectorant, febrifuge, irritant and stomachic. A leaf decoction is taken to relieve fevers, to quench the thirst and to calm down earache.[26] *Oxalis debilis* is richer in nutraceutical components.[27] The leaves contained oxalic acid, ascorbic acid, mucilage, luteolin and apigenin derivatives.[26] The presence of flavonoids, alkaloids and glycosides were detected in the plant.[28]

This paper reports the isolation, identification and structure elucidation of chemical constituents found in a fermentation broth of *Streptomyces coelicolor* and leaves of *Oxalis debilis.*

MATERIALS AND METHODS

General procedures

UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were scanned on Bruker DRX Spectrometer (Rheinstetten, Germany) using $CDCl_3$ and DMSO-d_6 as solvents and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) instrument with a +ve and –ve ESI techniques. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size and petroleum ether, chloroform, methanol and other solvents used were purchased from Merck Specialties (E. Merck, Pvt. Ltd., New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapours or under UV radiations and spraying with ceric sulphate solution.

Bacterial cultivation

Submerged cultivations of *Streptomyces coelicolor* M145 were carried out in 1.0 L flat bottom flasks containing 100 mL of the medium (g/ L) yeast extract 2 (Difco); ammonium sulphate 2; calcium carbonate 5; sodium chloride 5; dipotassium phosphate 0.5; magnesium sulfate 0.1; and glycerol 30.0 for inoculum, 70.0 for the production medium. After 30 h of cultivation, the seed culture was used as an inoculum (15%, v/v) for the production medium. Cultivations were performed on an orbital shaker (162 rpm) at 28 ^{o}C for 7 days until complete utilization of the carbon source. During the cultivation, the pH of the culture was checked at 12 h intervals and 2 M NaOH was added to maintain a mild alkaline value (pH 9).[5] Glucose, glycerol or mannitol was used as a carbon source. For boosting synchronicity of the population, spores were incubated for 10 min at 50°C, followed by 6-h germination at 37°C before screening for produced secondary metabolites.[29]

Isolation of chemical constituents from the bacterial cultivation of *Streptomyces coelicolor*

The cells from two cultivation flasks were separated by centrifugation and supernatant (200 mL) was lyophilized. Petroleum ether was added to the dried matter to remove pigments and other nonpolar substances. The dark brown viscous mass (25 g) was dissolved in a small portion of methanol and adsorb on silica gel (60-120 mesh) to prepare a slurry. It was dried in air and chromatographed over silica gel columns (80 cm x 16 mm x 2 mm) packed in petroleum ether. Various solvent mixtures of increasing polarity, viz., petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and chloroform - methanol (19.9: 0.1; 99: 1; 97: 3; 19: 1; 93: 7; 9: 1, v/v) were used to eluted the column. The 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 the following pure compounds:

2*α*-Methyl-8-hydroxy-4-chromanone (1)

Elution of the column with petroleum ether - chloroform (1:1) yielded pale yellow crystals of **1**, yield 38 mg, recrystallized from chloroform – methanol (1:1), UV λmax (MeOH) 233, 270 nm (log ε 4.8, 2.1); IR υ_{max} (KBr): 3345, 3024, 1698, 1606, 1521, 1367, 1234, 1153, 1072, 889, 812, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.38 (1H, dd, J = 2.8, 8.4 Hz, H-7) 6.53 (1H, m, H-6), 6.42 (1H, dd, J = 2.8, 8.4 Hz, H-5), 4.58 (1H, brm, $w_{1/2}$ = 10.8 Hz, H-2β), 2.67 (2H, d, J = 5.6 Hz, H_2-3), 1.27 (3H, d, J = 10.1 Hz, Me-11); ^{13}C NMR ($CDCl_3$): δ 73.81 (C-2), 43.84 (C-3), 198.50 (C-4), 107.30 (C-5), 109.18 (C-6), 138.17 (C-7), 162.12 (C-8), 161.70 (C-9), 108.05 (C-10), 20.84 (C-11); ESI MS *m/z* (rel. int): 178 $[M]^+$ ($C_{10}H_{10}O_3$) (100).

1-(2'-Pentanyl-1'-aldehyde)-2-hydroxy-8-naphthoic acid (2)

Elution of the column with chloroform gave yellow crystals of **2**, yield 53 mg, recrystallized from chloroform – methanol (1:1), UV λmax (MeOH): 226, 283 nm (log ε 5.1, 2.7); IR υ_{max} (KBr): 3409, 3211, 2968, 2847, 1701, 1679, 1524, 1473, 1351, 1297, 1231, 1106, 782 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.53 (1H, d, J = 8.4 Hz, H-3), 7.23 (1H, dd, J = 8.4, 2.6 Hz, H-5), 6.89 (1H, dd, J = 8.0, 1.1 Hz, H-7), 6.78 (1H, m, H-6), 6.42 (1H, d, J = 8.4 Hz, H-4), 9.93 (1H, d, J = 8.5 Hz, H-1'), 3.13 (1H, m, H-2'), 2.40 (2H, m, H_2-3'), 1.73 (2H, m, H_2-4'), 1.01 (3H, t, J = 7.2

Hz, H_3-5'); ^{13}C NMR ($CDCl_3$): δ 135.32 (C-1), 168.02 (C-2), 135.67 (C-3), 110.38 (C-4), 110.14 (C-5), 109.03 (C-6), 108.36 (C-7), 160.67 (C-8), 156.81 (C-9), 111.34 (C-10), 183.72 (C-11), 207.86 (C-1'), 28.07 (C-2'), 20.71 (C-3'), 17.83 (C-4'), 13.92 (C-5'); ESI MS *m/z* (rel. int.): 272 $[M]^+$ ($C_{16}H_{16}O_4$) (12.6).

Lawsonyl-1,3-dimethyl-2α-(10,14-dimethyl non-7β-olyl) cyclohexan-1-oate (3)

Elution of the column with chloroform-methanol (19:1) afforded red crystals of **3**, yield 112 mg, recrystallized from chloroform – methanol (1:1), UV λmax (MeOH): 212, 255, 293, 325, 450 nm (log ε 4.9, 5.7, 5.6, 1.8, 1.2); IR υ_{max} (KBr): 3368, 2990, 2905, 1721, 1667, 1541, 1458, 1421, 1338, 1094, 1055, 886 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.18 (1H, ddd, J = 2.6, 7.3, 2.5 Hz, H-7α), 3.11 (1H, dd, J = 2.6, 5.9 Hz, H-2β), 2.99 (1H, m, H-3α), 2.82 (1H, m, H_2-4α), 2.75 (1H, m, H_2-4β), 2.53 (2H, m, H_2-5), 2.34 (1H, m, H-10 α), 2.15 (1H, m, H-14α), 2.11-1.75 (10H, m, 5 x CH_2), 1.10 (3H, s, Me-16), 0.98 (3H, d, J = 7.4 Hz, Me-15), 0.90 (3H, d, J = 6.5 Hz, Me-20), 0.87 (3H, d, J = 6.5 Hz, Me-18), 0.83 (3H, d, J = 6.8 Hz, Me-19), 7.38 (1H, m, H-8'), 7.27 (1H, m, H-7'), 7.16 (1H, s, H-3'), 6.97 (1H, dd, J = 8.3, 2.5 Hz, H-9'), 6.82 (1H, dd, J = 8.3, 2.5 Hz, H-6'); ^{13}C NMR ($CDCl_3$): δ 59.15 (C-1), 56.25 (C-2), 56.31 (C-3), 45.48 (C-4), 36.01 (C-5), 45.42 (C-6), 60.46 (C-7), 36.84 (C-8), 28.35 (C-9), 53.48 (C-10), 28.12 (C-11), 22.74 (C-12), 22.51 (C-13), 37.83 (C-14), 21.28 (C-15), 22.47 (C-16), 166.31 (C-17), 22.36 (C-18), 19.18 (C-19), 16.08 (C-20), 170.36 (C-1'), 155.91 (C-2'), 116.16 (C-3'), 169.63 (C-4'), 128.77 (C-5'), 130.35 (C-6'), 126.88 (C-7'), 129.77 (C-8'), 129.24 (C-9'), 135.91 (C-10'); ESI MS *m/z* (rel. int.): 482 $[M]^+$ ($C_{30}H_{42}O_5$) (9.8), 309 (14.1), 173 (33.7).

Dinaphthol-1, 1''-dihexanal-8, 8''-dioic acid carbinol (4)

Elution of the column with chloroform-methanol (9:1) furnished red crystals of **4**, yield 89 mg, recrystallized from acetone, UV λmax (MeOH): 221, 287, 323 nm (log ε 4.7, 5.3, 1.3); IR υ_{max} (KBr): 3411, 3227, 3195, 2963, 2841, 1706, 1701, 1692, 1541, 1529, 1452, 1335, 1098, 1049, 879 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.51 (1H, s, COOH-11), 7.22 (1H, dd, J = 2.7, 8.0 Hz, H-7), 6.88 (1H, dd, J = 2.5, 8.0 Hz, H-5), 6.39 (1H, m, H-6), 6.12 (1H, s, H-4), 10.02 (1H, s, H-1'), 3.11 (1H, m, H-2'), 2.39 (4H, m, H_2-3', H_2-5'), 1.01 (3H, t, J = 7.2 Hz, Me-6'), 3.84 (1H, s, CHOH), 12.49 (1H, s, COOH-11''), 7.52 (1H, dd, J = 2.1, 8.4 Hz, H-7''), 7.09 (1H, dd, J = 2.9, 8.8 Hz, H-5''), 6.41 (1H, m, H-6''), 6.09 (1H, s, H-4''), 9.98 (1H, s, H-1'''), 2.77 (1H, m, H-2'''), 1.69 (2H, m, H_2-3'''), 1.57 (2H, m, H_2-4'''), 1.27 (2H, m, H_2-5'''), 0.98 (3H, t, J = 7.1 Hz, Me-6'''); ^{13}C NMR (DMSO-d_6): δ 150.71 (C-1), 161.92 (C-2), 155.18 (C-3), 111.28 (C-4), 110.31 (C-5), 135.25 (C-6), 135.65 (C-7), 160.67 (C-8), 136.05 (C-9), 108.22 (C-10), 183.64 (C-11), 205.33 (C-1'), 57.27 (C-2'), 31.93 (C-3'), 198.53 (C-4'), 17.78 (C-5'), 14.13 (C-6'), 74.49 (CHOH), 150.74 (C-1''), 160.97 (C-2''), 156.78 (C-3''), 110.37 (C-4''), 122.54 (C-5''), 136.05 (C-6''), 135.24 (C-7''), 161.43 (C-8''), 140.46 (C-9''), 108.43 (C-10''), 183.59 (C-11''), 207.85 (C-1'''), 46.69 (C-2'''), 43.85 (C-3'''), 33.52 (C-4'''), 29.70 (C-5'''), 20.69 (C-6'''); ESI MS *m/z* (rel. int.): 614 $[M]^+$ ($C_{35}H_{34}O_{10}$) (6.1), 315 (21.4), 301 (18.3).

Collection of the leaves of *Oxalis debilis*

The leaves of *O. debilis* were collected from wild fields of Dibrugarh district, Assam, India. The plant material was identified and authenticated by Botanical Survey of India, Eastern Regional Centre, Shillong, India, and a voucher specimen (BSI/ERC/2014/Plant identification/360) was deposited in the herbal centre for future reference.

Extraction and isolation: The air dried leaf powder of *O. debilis* (1 kg) was extracted with methanol in a Soxhlet apparatus. The methanolic extract was concentrated under reduced pressure to yield a dark brown viscous mass (214 g). A slurry of silica gel (60-120 mesh) was prepared by adsorbing the dried extract (200 g) in a small amount of methanol. It was dried in air and chromatographed over silica gel columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether. Various solvent mixtures of increasing polarity, viz., petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and chloroform - methanol (49 : 1; 97: 3; 19: 1; 93: 7; 9: 1, v/v) were used to eluted the column. The 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.

Isolation of phytoconstituents from the leaves of *Oxalis debilis*

Stigmasterol (5)

Elution of the column with chloroform yielded a colourless amorphous powder of **5**, yield 189 mg; m. p. 165-167 °C; R_f 0.43 (petroleum ether - chloroform - methanol, 7:1:2, v/v/v); UV λmax (MeOH): 211 nm (log ε 5.8); IR υ_{max} (KBr): 3425, 2920, 2852, 1641, 1463, 1373, 1225, 1173, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (1H, m, H-6), 5.12 (1H, m, H-22), 5.02 (1H, m, H-23), 3.63 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3α), 2.28 to 1.25 (25 H, m, 9 x CH_2, 7 x CH), 1.01 (3H, brs, Me-19), 0.93 (3H, d, J = 6.3 Hz, Me-21), 0.86 (3H, d, J = 6.6 Hz, Me-26), 0.82 (3H, d, J = 6.0 Hz, Me-27), 0.80 (3H, d, J = 6.6 Hz, Me-29), 0.69 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 36.58 (C-1), 31.97 (C-2), 71.88 (C-3), 42.88 (C-4), 140.76 (C-5), 121.71 (C-6), 31.71 (C-7), 31.97 (C-8), 51.34 (C-9), 37.32 (C-10), 21.17 (C11), 39.84 (C-12), 42.35 (C-13), 56.83 (C-14), 24.13 (C-15), 29.01 (C-16), 556.02 (C-17), 12.09 (C-18), 19.48 (C19), 36.58 (C-20), 18.87 (C-21), 138.40 (C-22), 129.33 (C-23), 45.90 (C-24), 27.29 (C-25), 19.82 (C-26), 18.94 (C-27), 23.14 (C-28), 12.05 (C-29); ESI MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (30.1), 411 (12.1), 396 (43.5), 394 (100), 381 (19.8), 271 (31.8), 255 (63.4), 240 (23.7), 213 (32.6).

1-Heptatriacontanol (6)

Further elution of the column with chloroform gave colourless amorphous powder of **6**, yield 143 mg, m. p. 92 - 94 °C; IR υ_{max} (KBr) : 3419, 2954, 2849, 1471, 1264, 1123, 1024, 729 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.63 (2H, t, J = 6.4 Hz, H_2-1), 2.42 (2H, m, H_2-2), 2.23 (2H, m, H_2-3), 2.16 (2H, m, H_2-4), 2.12 (2H, m, H_2-5), 1.58 (2H, m, H_2-6), 1.36 (2H, m, H_2-7), 1.31 (2H, m, H_2-8), 1.29 (4H, m, H_2 -9, H_2-10), 1.25 (52H, br s, 26 × CH_2), 0.88 (3H, t, J = 6.8 Hz, Me-37); ^{13}C NMR ($CDCl_3$): δ 63.13 (C-1), 32.89 (C-2), 31.96 (C-3), 29.72 (C-4), 29.65 (25 x CH_2), 29.62 (C-30), 29.57 (C-31), 9.52 (C-32), 29.47 (C-33), 29.37 (C-34), 25.79 (C-35), 22.71 (C-36), 14.10 (C-37); ESI MS *m/z* (rel. int.): 536 $[M]^+$ ($C_{37}H_{74}O$) (100).

RESULTS AND DISCUSSION

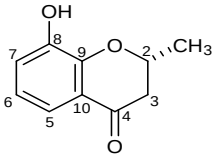
Compound **1** gave positive tests of phenols, showed UV absorption maximum at 270 nm for aromatic compounds and IR absorption bands for a hydroxyl group (3345 cm^{-1}), carbonyl function (1698 cm^{-1}) and aromatic ring (1606, 1521, 1072 cm^{-1}). Its molecular ion peak was established at *m/z* 178 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a 2,3-dihydro-1-benzopyran-4-one derivative, $C_{10}H_{10}O_3$. The ^1H NMR spectrum of **1** exhibited two one-proton deshielded double doublets at δ 7.38 (J = 2.4, 8.4 Hz) and 6.42 (J = 2.8, 8.4 Hz) assigned to meta, ortho-coupled aromatic H-7 and H-5 protons, respectively, a one-proton multiplet at δ 6.53 due to H-6 proton, a one-proton multiplet at δ 4.58 with half-width of 10.8 Hz ascribed to oxymethine H-2β proton, a two-proton doublet at δ 2.67 (J = 5.6 Hz) due to methylene H_2-3 protons and a three-proton doublet at δ 1.27 (J = 10.1 Hz) accounted to secondary C-11 methyl protons. The ^{13}C NMR spectrum of **1** exhibited signals for aromatic carbons between δ 162.12 - 107.30, oxymethine carbon at δ 73.81 (C-2), carbonyl carbon at δ 198.50 (C-4), methylene carbon at δ 43.84 (C-3) and methyl carbon at δ 20.84 (C-11). The HMBC spectrum of **1** showed correlations of H-2 with C-11; H-2, H_2-3 and H-5 with C-4; and H-6 and H-7 with C-8. On the basis of these evidences, the structure of **1** was established as 2α-methyl-8-hydroxy-4-chromanone, a new 2,3-dihydro-1-benzopyran-4-one (Fig. 1).

Compound **2** produced effervescence with sodium bicarbonate solution, had UV absorption maximum at 283 nm for aromatic compounds and IR absorption bands for a hydroxyl group (3409 cm^{-1}), carboxyl function (3211, 1679 cm^{-1}), carbonyl group (1701 cm^{-1}), aromatic ring (1524, 1106 cm^{-1}) and aliphatic chain (782 cm^{-1}). Its molecular ion peak was determined at *m/z* 272 on the basis of mass and ^{13}C NMR spectra corresponding to molecular formula of an alkylated naphthol moiety, $C_{16}H_{16}O_4$. The ^1H NMR spectrum of **2** exhibited two one-proton deshielded doublets at δ 7.53 (J = 8.4 Hz) and 6.42 (J = 8.4 Hz) assigned to ortho-coupled aromatic H-3 and H-4 protons, respectively, two one-proton double doublets at δ 7.23 (J = 8.4, 2.6 Hz) and 6.89 (J = 8.0, 1.1 Hz) ascribed to ortho-, meta-coupled H-5 and H-7, respectively, and a one-proton multiplet at δ 6.78 due to H-6 proton, a one-proton doublet at δ 9.93 (J = 8.5 Hz) accounted to aldehydic H-1' proton linked to a tertiary carbon C-2', a one-proton multiplet at δ 3.13 attributed to methine H-2' proton, two methylene protons as multiplets at δ 2.40 (H_2-3') and 1.73 (H_2-4') and a three-proton triplet at δ 1.01 (J = 7.2 Hz) associated with the primary methyl H_3-5' protons. The ^{13}C NMR spectrum of **2** displayed signals for carboxylic carbon at δ 183.72 (C-11), aromatic carbons between δ 168.02 - 108.36, aldehydic carbon at δ 207.86 (C-1'), methine carbon at δ 28.07 (C-2'), methylene carbons at δ 20.71 (C-3') and 17.83 (C-4') and methyl carbon at δ 13.92 (C-5'). The HMBC spectrum of **2** showed interactions of H-3 and H-4 with C-2; H-4 and H-5 with C-10; H-6 and H-7 with C-8; and H-2' and H_2-3′ with C-1'. On the basis of these evidences, the structure of **2** was elucidated as 1-(2'-pentanyl-1'-aldehyde)-2-hydroxy-8-naphthoic acid, a new alkylated β-naphthol carboxylic acid (Fig. 1).

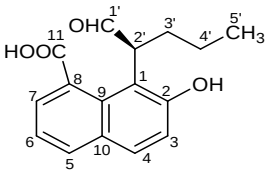
Compound **3**, $[M]^+$ at *m/z* 482 ($C_{30}H_{42}O_5$), had UV absorption maxima at 293, 325, 450 nm for aromatic compounds and exhibited IR absorption bands for a hydroxyl group (3368 cm^{-1}), ester function (1721 cm^{-1}), naphthoquinone group (1667 cm^{-1}) and aromatic ring (1541, 1094 cm^{-1}). Its molecular ion peak was determined at *m/z* 482 on the basis of mass and ^{13}C NMR spectra relating to a molecular formula of an esterified naphthol moiety, $C_{30}H_{42}O_5$. The mass ion fragments generated at *m/z* 173 [C_{17} – O fission, $C_{10}H_5O_3]^+$ and 309 [M – 173, $C_{20}H_{37}O_3]^+$ suggested that lawsone was esterified with a monocyclic diterpenic acid unit. The ^1H NMR spectrum of **3** displayed two one-proton deshielded multiplets at δ 7.38 and 7.27, a one-proton singlet at δ 7.16 and two one-proton double doublets at δ 6.97 (J = 8.3, 2.5 Hz) and 6.82 (J = 8.3, 2.5 Hz) assigned to naphthoquinone protons. A one-proton triple doublet at δ 4.18 (J = 2.6, 7.3, 2.5 Hz) was accounted to α-oriented carbinol H-7 proton. A three-proton singlet at δ 1.10 and four three-proton doublets at δ 0.98 (J = 7.4 Hz), 0.90 (J = 6.5 Hz), 0.87 (J = 6.5 Hz) and 0.83 (J = 6.8 Hz) were attributed to tertiary C-18 and secondary C-15, C-20, C-18 and C-19 methyl protons, respectively. The remaining methine and methylene protons resonated as a one-proton double doublet at δ 3.11 (J = 2.6, 5.9 Hz) due to methine H-2β and as multiplets between δ 2.99 – 1.75. The ^{13}C NMR spectrum of **3** showed signals for ester carbon at δ 166.31 (C-17), naphthoquinone carbons at δ 170.36 (C-1') and 169.63 (C-4'), aromatic carbons in the range of δ 155.91 – 116.16, carbinol carbon at δ 60.46 (C-7) and methyl carbons between δ 22.47 – 16.08. The DEPT spectrum of **3** exhibited the presence of ten methine, eight methylene and five methyl carbons. The ^1H- ^1H COSY spectrum of **3** showed correlations of H-6', H-7' and H-9' with H-8'; H-2 and H_2-6 with Me-16; H-2, H-3 and H_2-4 with Me-18; H-2, H-3, H_2-8 and H_2-9 with H-7; H_2-9, H_2-11 and H-10 with Me-19; and H_2-12, H_2-13, Me-15 and Me-20 with H-14. The HMBC spectrum of **3** displayed interactions of H-3' and H-9' with C-1'; H-3' and H-6' with C-4'; H-3', H-6' and H-7' with C-5'; H-2,

H_2-6 and Me-16 with C-17; H-2, H_2-8 and H_2-9 with C-7; H_2-8, H_2-9, H_2-11 and Me-19 with C-10; and H_2-12, H_2-13, Me-15 and Me-20 with C-14. On the basis of these evidences, the structure of **3** was formulated as lawsonyl-1,3-dimethyl-2α-(10,14-dimethyl nonan-7β-olyl)cyclohexan-1-oate, a new diterpenic ester with lawsone (Fig. 1).

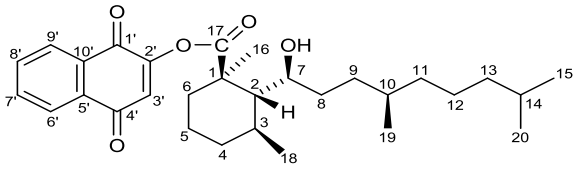
Compound **4**, named dinaphthol-1, 1"-dihexanal-8, 8"-dioic acid carbinol, $[M]^+$ at *m/z* 614 ($C_{35}H_{34}O_{10}$), showed UV absorption maxima at 287, 323 nm for aromatic compounds and IR absorption bands for hydroxyl groups (3411, 3227 cm^{-1}), carboxylic functions (3195, 1692 cm^{-1}), carbonyl groups (1706, 1701 cm^{-1}) and aromatic ring (1541, 1529, 1098 cm^{-1}). The mass ion fragments generated at *m/z* 299 [C_3 – CHOH fission, $C_{17}H_{15}O_5]^+$ and 315 [M – 301, $C_{18}H_{19}O_5]^+$ suggested that two units of hexyl naphthoic acid were linked to a hydroxymethine carbon. The ^{1}H NMR spectrum of **4** displayed two one-proton deshielded singlets at δ 12.51 and 12.49 due to C-11 and C-11" carboxylic protons, two one-proton singlets δ 10.02 and 9.98 assigned to aldehydic H-1' and H-1'" protons, respectively, eight aromatic protons as one-proton double doublets at δ 7.22 (J = 2.7, 8.0 Hz, H-7), 6.88 (J = 2.5, 8.0 Hz, H-5), 7.52 (J = 2.1, 8.4 Hz, H-7") and 7.09 (J = 2.9, 8.8 Hz, H-5"), as one-proton multiplets at δ 6.39 (H-6) and 6.39 (H-6") and as one-proton singlets at δ 6.12 (H-4) and 6.09 (H-4"), two one-proton multiplets at δ 3.11 (H-2') and 2.77 (H-2'") attributed to methine protons, four multiplets at δ 2.39 (4H), 1.69 (2H), 1.57 (2H) and 1.27 (2H) associated with the methylene protons, two three-proton triplets at δ 1.01 (J = 7.2 Hz) and 0.98 (J = 7.1 Hz) ascribed correspondingly to primary C-6' and C-6'" methyl protons and a one-proton singlet at δ 3.84 due to hydroxymethine proton. The ^{13}C NMR spectrum of **4** showed signals for aldehydic carbons at δ 205.33 (C-1') and 207.85 (C-1'"), keto carbon at δ 198.53 (C-4'), carboxylic carbons at δ 183.64 (C-11) and 183.59 (C-11"), aromatic carbons between δ 161.92 – 108.22, methyl carbons at δ 14.13 (C-6') and 20.69 (C-6'") and hydroxymethine carbon at δ 74.49 (CHOH). The HMBC spectrum of **4** displayed interactions of H-2' and H_2-3' with C-1'; H_2-3' and H_2-5' with C-4'; H-6 and H-7 with C-8; H-4 and H-4" with CHOH; H-6" and H-7" with C-8"; and H-2'" and H_2-3'" with C-1'". These evidences led to established the structure of **4** as 3-[1-(2'-hexan-1'-al-4'-one)-2-hydroxy-8-naphthoic acid]- 3"- [1"- (2"'-hexan -1'"-al) -2"-hydroxy- 8"-naphthoic acid]-hydroxy methine, a new dinaphthoic acid hydroxymethine derivative (Fig. 1).



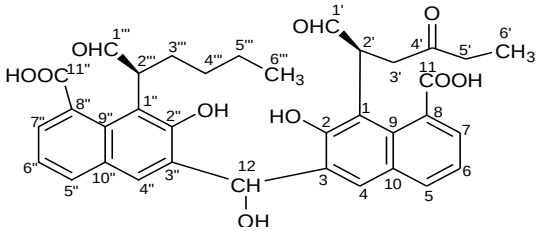
2α-Methyl-8-hydroxy-4-chromanone (**1**)



1-(2'-Pentanyl-1'-aldehyde)-2-hydroxy naphthoic acid (**2**)



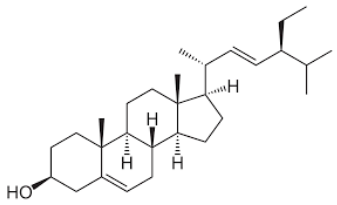
Lawsonyl-1,3-dimethyl-2α-(10, 14-dimethyl non-7β-olyl) cyclohexane-1-oate (**3**)



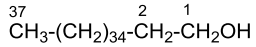
Dinaphthol-1,1"-dihexanal-8,8"-dioic acid carbinol (**4**)

Fig. 1: Structural formulae of the chemical constituents 1 – 4 isolated from the bacterial cultivations of *Streptomyces coelicolor*.

Compounds **5** was a known phytosterol and its structure was elucidated as stigmasterol.[30-32] Compounds **6** was a long chain aliphatic alcohols identified as 1-heptatriacontanol[33,34] (Fig. 2).



Stigmasterol (**5**)



1-Heptatriacontanol (**6**)

Fig 2: Chemical constituents 5 and 6 isolated from the leaves of *Oxalis debilis*.

CONCLUSION

The fermentation broth of *Streptomyces coelicolor* was extracted with methanol. The extract was subjected to column chromatography to afford 2α-methyl-8-hydroxy-4-chromanone (**1**), 1-(2'-pentanyl-1'-aldehyde)-2-hydroxy-8-naphthoic acid (**2**), lawsonyl-1,3-dimethyl-2α-(10,14-dimethyl nonan-7β-olyl)cyclohexan-1-oate (**3**), a diterpenic ester with lawsone and a dinaphthol-1, 1"-dihexanal-8, 8"-dioic acid carbinol derivative (**4**). Two known phytoconstituents, stigmasterol (**5**) and 1-heptatriacontanol (**6**), were isolated from the leaves of *Oxalis debilis*. This work has enhanced understanding

about the phytoconstituents of the undertaken natural sources.

ACKNOWLEDGEMENTS

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

REFERENCES

1. Zhang HL, He XG, Adefarati A, Gallucci J, Cole SP, Beale JM, Keller PJ, Chang CJ, Floss HG. Mutactin, a novel polyketide from *Streptomyces coelicolor*. Structure and biosynthetic relationship to actinorhodin. Journal of Organic Chemistry, 1990; 55(5): 1682-1684.
2. Bystrykh LV, Fernandez-Moreno MA, Herrema JK, Malpartida F, Hopwood DA, Dijkhuizen L. Production of actinorhodin-related "blue pigments" by *Streptomyces coelicolor* A3(2). Journal of Bacteriology, 1996; 178: 2238–2244; doi: 10.1128/jb.178.8.2238-2244.1996.
3. Bystrykh LV, Herrema JK, Kruizinga W, Kellogg RM. 5-Hydroxyaloesaponarin II, a inor blue pigment in an actinorhodin-negative mutant of *Streptomyces coelicolor* A3(2). Biotechnology and Applied Biochemistry, 1997; 26: 195-201; https://doi.org/10.1111/j.1470-8744.1997.tb01331.x
4. Zhang H, Zhan J, Su K, Zhang Y. Kind of potential food additive produced by *Streptomyces coelicolor*: Characteristics of blue pigment and identification of a novel compound, λ-actinorhodin. Food Chemistry, 2006; 95(2): 186-192; https://doi.org/10.1016/j.foodchem.2004.12.028
5. Pospíšil S, Sedmera P, Halada P, Petříček M. Extracellular carbohydrate metabolites from *Streptomyces coelicolor* A3(2), Journal of Natural Products, 2007; 70(5): 768-771; https://doi.org/10.1021/np0606188.
6. Challis GL, Ravel J. Coelichelin, a new peptide siderophore encoded by the *Streptomyces coelicolor* genome: structure prediction from the sequence of its non-ribosomal peptide synthetase. FEMS Microbiology Letters, 2000; 187(2): 111–114; https://doi.org/10.1111/j.1574-6968.2000.tb09145.x
7. Poralla K, Muth G, Härtner T. Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiology Letters, 2000; 189(1): 93–95; https://doi.org/10.1111/j.1574-6968.2000.tb09212.x
8. Takano E, Nihira T, Hara Y, Jones JJ, Gershater CJL, Yamada Y, Bibb M. Purification and Structural Determination of SCB1, a γ-Butyrolactone That Elicits Antibiotic Production in *Streptomyces coelicolor* A3(2), The Journal of Biological Chemistry, 2000; 275: 11010-11016; doi: 10.1074/jbc.275.15.11010.
9. Hojati Z, Milne C, Harvey B, Gordon L, Borg M, Flett F, Wilkinson B, Sidebottom PJ, Rudd BAM, Hayes MA. Structure, biosynthetic origin, and engineered biosynthesis of calcium-dependent antibiotics from *Streptomyces coelicolor*. Chemistry & Biology, 2002; 9(11): 1175–1187.
10. Barona-Gómez F, Wong U, Giannakopulos AE, Derrick PJ, Challis GL. Identification of a cluster of genes that directs desferrioxamine biosynthesis in *Streptomyces coelicolor* M145. Journal of American Chemical Society, 2004; 126(50): 16282-16283.
11. Eustáquio AS, Gust B, Li SM, Pelzer S, Wohlleben W, Chater KF, Heide L. Production of 8'-halogenated and 8'-unsubstituted novobiocin derivatives in genetically engineered *Streptomyces coelicolor* strains. Chemistry & Biology, 2004 Nov; 11(11): 1561-1572; DOI: 10.1016/j.chembiol.2004.09.009.
12. Lautru S, Deeth RJ, Bailey LM, Challis GL. Discovery of a new peptide natural product by *Streptomyces coelicolor* genome mining. Nature Chemical Biology, 2005; 1(5): 265-269; Epub 2005 Sep 11.
13. Mo, S. J., Kim, B. S. and Reynolds, K. A.: Production of novel alkylprodiginines in *Streptomyces coelicolor* by replacement of the 3-ketoacyl ACP synthase III initiation enzyme (RedP), Chemistry & Biology, 2005; 12: 191-200.
14. Yang Y-H, Joo H-S, Lee K, Liou K-K, Lee H-C, Sohng J-K, Kim B-G. Novel method for detection of butanolides in *Streptomyces coelicolor* culture broth, using a His-Tagged Receptor (ScbR) and mass spectrometry, Applied and Environmental Microbiology, 2005; 71: 5050–5055; DOI: 10.1128/AEM.71.9.5050-5055.2005.
15. Jiang J, He X, Cane DE. Geosmin biosynthesis. *Streptomyces coelicolor* germacradienol/ germacrene D synthase converts farnesyl diphosphate to geosmin. Journal of American Chemical Society, 2006; 128(25): 8128-8129; https://doi.org/10.1021/ja062669x.
16. Lin X, Hopson R, Cane DE. Genome mining in *Streptomyces coelicolor*: Molecular cloning and characterization of a new sesquiterpene synthase. Journal of American Chemical Society, 2006; 128(18): 6022-6023; https://doi.org/10.1021/ja061292s.
17. Song L, Barona-Gomez F, Corre C, Xiang L, Udwary DW, Austin MB, Noel JP, Moore BS, Challis Gy L. (2006) Type III polyketide synthase beta-ketoacyl-ACP starter unit and ethylmalonyl-CoA extender unit selectivity discovered by *Streptomyces coelicolor* genome mining. Journal of the American Chemical Society, 2006; 128(46): 14754-14755; doi:10.1021/ja065247w
18. Čihák M, Kameník Z, Šmídová K, Bergman N, Benada O, Kofroňová O, Petříčková K, Bobek J. Secondary metabolites produced during the Germination of *Streptomyces coelicolor*. Frontiers in

Microbiology, 2017; 8: 2495; doi:10.3389/fmicb.2017.02495.
19. Arora N, Kumar S, Satti NK, Ali A, Gupta P, Katoch M. A strain of *Streptomyces* sp. isolated from rhizospheric soil of *Crataegus oxycantha* producing nalidixic acid, a synthetic antibiotic. Journal of Applied Microbiology, 2018; 124(6); DOI:10.1111/jam.13736.
20. Brachmann, A. O., Brameyer, S., Kresovic, D., Hitkova, I., Kopp, Y., Manske, C., Schubert K, Bode HB, Heermann. Pyrones as bacterial signaling molecules. Nature Chemical Biology, 2013; 9: 573–578; doi: https://doi.org/10.1038/nchembio.1295
21. Martinez G, Regente M, Jacobi S, Del Rio M, Pinedo M, de la Canal L. Chlorogenic acid is a fungicide active against phytopathogenic fungi. Pesticide Biochemistry and Physiology, 2017; 140: 30–35; https://doi.org/10.1016/j.pestbp.2017.05.012.
22. Luo S, Zhang D, Renner SS. *Oxalis debilis* in China: Distribution of flower morphs, sterile pollen and polyploidy, Annals of Botany, 2006; 98(2): 459–464; doi: 10.1093/aob/mcl121.
23. Nesom GL. Taxonomic notes on *Acaulescent oxalis* (Oxalidaceae) in the United States. Phytologia, 2009; 91(3): 501 – 526.
24. Singh A, Dubey NK. An ethnobotanical study of medicinal plants in Sonebhadra district of Uttar, Pradesh, India with reference to their infection by foliar fungi. Journal of Medicinal Plants Research, 2012; 6: 2727-2746.
25. Panda E, Pradhan C, Das AB. Variations in phytoconstituents and antimicrobial activities in ecotypes of *Oxalis corniculata* L. and *Oxalis debilis* Kunth, International Journal of Pharmacy and Pharmaceutical Sciences, 2016; 8(10): 270-275.
26. Qasem JRS. The Coloured Atlas of Medicinal and Aromatic Plants of Jordan and Their Uses. Cambridge Scholars Publishing, Newcastle, UK. Cambridge Scholars Publishing, 2020; 3: 619.
27. Sarma S, Sarmah P, Dolai DK, Pandu, Medhi, P. *Oxalis debilis* var. *corymbosa* (DC.) Lourteig and *Oxalis corniculata* L: A comparative study of nutraceutical properties. International Journal of Current Research, 2015; 7(01): 11307-11310.
28. Junejo JA, Gogoi G, Zaman K, Islam J, Hazarika G. Phytochemical profiling and toxicological studies of *Oxalis debilis* Kunth leaves, International Journal of Green Pharmacy, 2016; 10(3): 165 – 171.
29. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. Practical Streptomyces Genetics, 2nd Edn., John Innes Foundation, Norwich, England, 2000.
30. Ali M, Shahnaz Sultana S & Mir SR. A triterpenic constituent from the aerial parts of *Ardisia thyrsiflora* D. Don. Advanced & Applied Pharmaceutical Science Journal, 2016; 1(1): 16-19.
31. Ali M, Alam P, Singh V, Jameel M & Sultana S (2017). Phytochemical investigations of the leaves and flowers of *Hibiscus rosa-sinensis* L. Indian Drugs, 2017; 54(10): 30-37.
32. Sultana S, Ali M, Jameel M. Aliphatic constituents from the leaves of *Dillenia indica* L., *Halothamus bottae* Jaub. and *Xylosma longifolium* Clos. Chemistry Research Journal, 2018; 3(3): 109-117.
33. Gohar AA. Heptatriacontanol and phenolic compounds from *Halochris hispida*. Journal of Biological Sciences, 2001; 1(9): 843–845; DOI: 10.3923/jbs.2001.843.845.
34. Nyemb JN, Magnibou LM, Talla E, Tchinda AT, Tchuenguem RT, Henoumont C, Laurent S, Mbafor JT. Lipids constituents from *Gardenia aqualla* Stapf et Hutch. Open Chemistry, 2018; 16(1): 371–376; DOI: https://doi.org/10.1515/chem-2018-0035.