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ANTIDANDRUFF ACTIVITY AND CHEMICAL CONSTITUENTS OF THE ROOTS OF *SAUSSUREA COSTUS* (FALC.) LIPSCH

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ABSTRACT
Saussurea costus (Falc.) Lipsch. (Asteraceae) grows in the Himalayan region and its roots are used to treat abdominal, blood, kidney and liver disorders, respiratory and skin diseases, cholera, convulsion, general debility, erysipelas, fever, headache, helminthiasis, malaria, irregular menses, paralysis, rheumatism and ulcers. The air-dried root powder was extracted exhaustively with methanol in a Soxhlet apparatus. The concentrated methanolic extract was dissolved in 95% aqueous methanol and fractionated by partitioning the solution with petroleum ether, chloroform, ethyl acetate and *n*-butanol. The chloroform fraction exhibited marked antidandruff activity against the growth of human skin fungus *Malassezia furfur*. It was adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was loaded over silica gel columns. The column was eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate nonadecan-1-ol (**1**), stearic acid (**2**), henetriacontan-1-ol (**3**), pentatriacontan-1-ol (**4**), *n*- pentatriaconta-1,19β-diol (**5**), *n*-nonadecanyl stearate (**6**) and hexatetracontan-1-ol (**7**). Their structures were established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Saussurea costus*, roots, antidandruff activity, phytoconstituents, isolation, characterization.

INTRODUCTION
Dandruff is a skin disorder that mainly affects the scalp resulting in flakes, mild itchiness, skin inflammation and dry skin. It is characterized by excessive shedding of skin cells from the scalp. A fungus *Malassezia furfur*, syn. *Pytirosporum ovale* is the causative organism for dandruff.[1] It transforms the sebum lipid into fatty acids and triglycerides, which accelerate hyperproliferation of keratinocytes.[2] The ointments, lotions and shampoos are used to treat dandruff which are composed of zinc pyrithione, salicylic acid, imidazole and tar derivatives, selenium sulphide and ketocanazole. These ingredients are unable to prevent reoccurrence of dandruff and have side effects. An in vitro hair strand test was a reliable test model for evaluation of the antifungal activity of antidandruff preparations. Climbazole proved to be an effective antidandruff agent.[3] There are some medicinal plants and essential oils which possess antidandruff properties.[4-7] A formulation composed of neem and lemongrass oils, *Aloe vera* gel, hena oil, glycerin and EDTA was effective for treating dandruff without any eye irritation.[8] The plant extracts having antifungal activity could be safely used for treating dandruff.[9]

Saussurea costus (Falc.) Lipsch., syn., *Aplotaxis lappa* Decne., *Saussurea lappa* (Decne.) Sch.Bip. (Asteraceae), known as costus and kut root, grows in the Himalayan region and in Kashmir and Himachal Pradesh at an altitude of 2,600-3,600 m. It is a perennial, up to 3 m tall herb, stem is upright, stout and fibrous, root is long stout, with a characteristic odour; leaves lobate, stalked, membranous, irregularly dentate; upper leaves are small, basal leaves are large with long lobately winged stalks; flowers are stalk less, dark purple to black, arranged in terminal and axillary heads (Fig. 1).

The roots are acrid, alternative, analgesic, anodyne, anthelmintic, antiseptic, antispasmodic, aphrodisiac, aromatic, bitter, carminative, deodorant, depurative, diaphoretic, digestive, diuretic, disinfectant, emmenagogue, expectorant, febrifuge, galactagogue, stimulant, stomachic, sweet, thermogenic, tonic and vermifuge. The roots are used to treat abdominal distension, asthma, blood, liver and kidney disorders, bronchitis, bruises, cancer of the intestine, cardiac disorder, chest pain, cholera, colds, constipation, convulsion, cuts, cough, general debility, diarrhoea, dysentery, oedema, epilepsy, erysipelas, fever, flatulence, headache, helminthiasis, hiccough, hysteria,

gout, inflammation of the lungs, itching, jaundice, leprosy, leucoderma, malaria, irregular menses, paralysis, pulmonary disorders, ringworm, scabies, skin diseases, tuberculosis and ulcers. The root essential oil is used in perfumery, incenses and hair tonic, effective to cure leprosy, rheumatism, swelling and fullness of the stomach.[10-12]

(a) Roots **(b) Twig**

Fig. 1: *Saussurea costus* (a) root and (b) twig.

The roots contained costunolide derivatives, lappadilactone, mokkolactone, betulinic acid, betulinic acid methyl ester, cynaropicrin, reynosin, santamarine, saussureamines, α-cyclocostunolide, alantolactone derivatives, β-cyclocostunolide, 1β-hydroxyarbusculin A, soulangianolide A, syringaresinol, scopoletin, aloeemodin- and rhein-8-O-β-D- glucopyranosides, chrysophanol, rutin, luteolin- and apigenin-7-O-β-D-glucosides, acylated flavonoids, phytosterols, α-amyrin, saussurine, chlorogenic acid and 3β-acetoxy-9(11)-baccharene.[13-25] The root essential oil was mainly consisted of dehydrocostus lactone, costunolide, 8-cedren-13-ol, α-curcumene, β-costol, germacrenes, δ-elemene, α-selinene, β-selinene, α-costol, 7,10,13-hexadecaterinal, dehydrocostus lactone, elemol and valerenol.[26- 32] The chloroform and alcoholic extracts of the roots exhibited antibacterial activity against tested Gram positive and Gram negative bacterial strains.[33-35] Keeping in view the high reputation and wide application of *S. costus* for the treatment of many body disorders in indigenous systems it has been aimed to carry out antidandruff activity, isolation and characterization of chemical constituents from the roots of this plant.

MATERIALS AND METHODS

General procedure

Melting points were determined on a Perfit melting point apparatus and were uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on Shimadzu FTIR-8400 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were scanned on Bruker DRX 300 MHz instrument using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/*z values of the more intense peaks are mentioned and the figures in bracket attached to each *m/*z values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours, UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant material

The roots of *Saussurea costus* were procured from a local market, Khari Bawli, Delhi, India. The plant was taxonomically authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources (Ref. no. NISCAIR/RHMD/Consult/-2010-11/1593/191). A voucher specimen (MSIP/ 2010/ 08/ 13) was deposited in the herbarium section of the Pharmacognosy Division, Maharaja Surajmal Institute of Pharmacy, Janakpuri, New Delhi.

Extraction

The dried powdered roots (2.0 kg) of *S. costus* were extracted with methanol exhaustively in a Soxhlet apparatus for 40 hrs. The methanolic extract was concentrated on a steam bath and dried under reduced pressure to get a dark brown viscous mass (262 g). The crude methanolic extract (200 g) was dissolved in 95% aqueous methanol and fractionated into various fractions by partitioning the solution successively with petroleum ether (60-80ºC), chloroform, ethyl acetate and *n*-butanol to obtain petroleum ether soluble fraction (54 g), chloroform fraction (81 g), ethyl acetate fraction (31 g) and *n*-butanol fraction (28 g).[36]

Total aqueous extracts of the roots of *S. costus* was prepared by extracting the root powder (100 g) with hot water at 80-83°C for 8 hrs. The extract was cooled down at room temperature, filtered through Whatman No. 1 filter paper and the filtrate dried under reduced pressure using rotary evaporator to get dark brown viscous mass (9.1 g).

Antidandruff activity

The cup plate method was used to determine antidandruff activity through a solidified agar layer in a Petri dish or plate. The growth of the added micro-organism was prevented entirely in a zone around the cylindrical hole containing a solution of the antifungal.

Micro-organism

A standard isolate of *Malassezia furfur* was procured from the Institute of Microbial Technology, Chandigarh, India (strain No. MTCC 1374) for the *in vitro* study. The petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions of the methanolic extract of the roots of *S. costus* and total leaf aqueous extract were evaluated, for

most potential extract responsible for antidandruff activity (Bioassay guided). The isolate was maintained in Sabouraud's dextrose agar (SDA) supplemented with corn oil.

Media

Sabouarauds dextrose agar medium was prepared by taking dextrose (20 g), peptone (10 g), agar (20 g) and sterilized corn oil (5 ml) at pH 5.6 and temperature 25°C. The material was suspended in 1 It of distilled water. It was heated to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Preparation of test microorganism

For antidandruff activity evaluation *Malassezia furfur* (MTCC 1374) was maintained on Sabouraud-dextrose agar media and incubated at 32ºC for 96 hours. After completion of incubation, the growth on the slant was washed with 10 ml sterile normal saline with vortexing. Culture concentration was taken at 560 nm by spectrophotometer. A sterile swab is dipped into the slant containing normal saline and excess inoculums were removed by pressing the swab against the inner wall of the test tube. Inoculums were uniformly spread over the plate and left at room temperature for 20 minutes to dry. Wells were punched in each plate with the help of 6 mm steel agar borer and filled with 10 mg/ml extract solution. Plates were incubated at 32ºC for 96 hours. Results were noted at the end of incubation period.[37-38]

Zone of inhibition study

Diffusion dependent activities of various extracts were studied by zone of inhibition. The broth culture was uniformly swabbed onto the surface of the Sabouraud's medium. All the extracts were dissolved in their respective solvents at 10 mg/ml concentration. The plates were incubated at 32°C for 96 hours and the zone of inhibition was measured as follows:

(i) A 24 hrs broth culture was swabbed over the surface of Sabouraud's agar and all the extracts were dissolved in their respective solvents at 10 mg/ml concentration.
(ii) A well of 6 mm diameter was cut at the centre of the agar and above prepared different test extracts were loaded on the well.
(iii) The zone of inhibition of respective solvents were taken as blank and ketoconazole was taken as standard.
(iv) The plates were incubated at 32°C for 96 hrs.
(v) After incubation the zone of inhibition was measured and recorded.

Table 1: Zone of inhibition (mm) of *Malassezia furfur* with different test extracts of *S.costus* and standard ketoconazole.

Extract	Concentration	Zone of inhibition (mm) mean± SD
Petroleum ether	10 mg/ ml	10.07 ± 0.32
Chloroform	10 mg/ ml	32.03 ± 1.2
Ethyl acetate	10 mg/ ml	6.25 ± 0.23
***n*-Butanol**	10 mg/ ml	9.67 ± 0.25
Total aqueous	10 mg/ ml	2.03 ± 1.8
Ketoconazole (standard)	10 µg/ ml	25 ± 0.0

The above mentioned readings are inclusive of disc diameter. Values are expressed as mean ± standard deviation, where n = 3.

Determination of Minimal Inhibitory Concentration (MIC)

Dilution susceptibility testing method was used to determine the minimal concentration of plant extracts to inhibit growth of *Malassezia furfur*. This was achieved by dilution of the extracts to inhibit the micro-organism and was achieved by dilution of extract in either agar or broth media (PDA). Procedure for performing the minimum inhibitory concentration test inoculums preparation was performed as discussed earlier in well diffusion method.

Procedure

i. Different concentration of plant extract in (10 µl, 20 µl….up to 100µl) to the tube to respective tubes were added.
ii. From the inoculums 10 µl of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106 cells/ml. This procedure was performed for all the positive extracts antifungal activity which were obtained by primary screening.
iii. Then all sets of tubes of dilution broth were incubated at 37°C for 24 hours in an incubator.

All sets of tubes were observed for determination of MIC to the susceptible fungus tested and note down the results.

Among all the extracts and fractions the chloroform fraction of the methanolic extract of *S. costus* exhibited marked antidandruff activity, i.e., the chloroform fraction was the most active displaying the largest zone of inhibition (32.03 mm). The aqueous extract was least active against the growth of *Malassezia furfur*.

Isolation of phytoconstituents

The dried chloroform extract (70 g) was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The slurry was dried in air and chromatographed over silica gel

columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether (b. p. 60 - 80°C). The column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*) and chloroform successively. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

1-Nonadecanol (1)

Elution of column with petroleum ether yielded pale yellow powder of compound **1**, yield 51 mg (0.0102%), R_f 0.66 (chloroform-acetone, 9:1), m. p. 62 – 64°C, IR *V*max (KBr): 3422, 2922, 2851, 1626, 1445, 1260, 1069, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.80 (2H, d, J = 10.4 Hz, H_2-1), 2.11 (2H, m, CH_2), 1.50 (2H, m, CH_2), 1.46 (2 H, m, CH_2) 1.38 (2H, m, CH_2), 1.24 (26H, brs, 13 x CH_2), 0.87 (3H, t, *J*= 6.5 Hz, Me-19); ^{13}C NMR ($CDCl_3$): 60.41 (C-1), 27.23 (15 x CH_2), 25.31 (CH_2), 22.67 (CH_2), 14.15 (C-19); TOF MS *m/z* (rel. int.): 284 $[M]^+$ ($C_{19}H_{40}O$) (38.3).

Stearic acid (2)

Elution of column with petroleum ether- chloroform (1: 1) yielded colourless amorphous mass of compound **2**, yield 72 mg (0.0144%), R_f 0.79 (chloroform-acetone, 1:1), m. p. 71- 73°C; IR *V*max (KBr): 3412, 2926, 2855, 1702, 1626, 1415, 1382, 1252, 1210, 1179, 1071, 800 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.50 (2H, m, H_2-2), 2.25 (2H, m, CH_2), 2.07 (2H, m, CH_2), 1.51 (2H, m, CH_2), 1.21 (24H, brs, 12 x CH_2), 0.83 (3H, t, J = 6.5 Hz, Me-18); TOF MS *m/z* (rel. int.): 284 $[M]^+$ ($C_{18}H_{36}O_2$) (95.3).

1-Henetricontanol (3)

Further elution of the column with petroleum ether - chloroform (1:1) afforded colourless amorphous powder of **3**, yield 47 mg, m. p. 86 - 87°C; IR (KBr): *V*max 3423, 2917, 2852, 1417, 1261, 1163, 1062, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.54 (2H, t, J = 6.8 Hz, H_2-1), 1.65 (2H, m, H_2-2), 1.56 (2H, m, CH_2), 1.31 (2H, m, CH_2), 1.29 (8H, brs, 4 × CH_2), 1.25 (44H, brs, 22 × CH_2), 0.88 (3H, t, J = 6.5 Hz, Me-31); ^{13}C NMR ($CDCl_3$): δ 63.15 (C-1), 32.81 (C-2), 31.95 (CH_2), 29.73 (19 × CH_2), 29.69 (CH_2), 29.65 (CH_2), 29.56 (CH_2), 29.52 (CH_2), 29.43 (CH_2), 29.37 (CH_2), 25.78 (CH_2), 22.69 (CH_2), 14.16 (Me-31); FAB MS (+ve ion) *m/z* (rel. int.): 452 $[M]^+$ ($C_{31}H_{64}O$) (32.4).

***n*- Pentatricontanol (4)**

Elution of column with acetone-methanol (4:1) furnished colourless amorphous powder of compound **4**, yield 63 mg, R_f 0.68 (acetone), m. p. 83-85°C; IR *V*max (KBr): 3398, 2933, 2851, 1642, 1408, 1262, 1203, 1079, 780 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.63 (2H, m, H_2-1), 2.53 (2H, m, CH_2), 1.98 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.24 (60H, brs, 30 x CH_2), 0.86 (3H, t, J= 6.3 Hz, Me- 35); TOF MS *m/z* (rel. int.): 508 $[M]^+$ ($C_{35}H_{72}O$) (8.1).

***n*- Pentatriconta-1,19β-diol (5)**

Elution of column with chloroform- methanol (49: 1) produced pale yellow powder of **5**, yield 52 mg, R_f 0.36 (acetone-methanol, 9:1), m. p. 108-109°C; IR *V*max (KBr): 3399, 2931, 2851, 1633, 1407, 1262, 1204, 1078, 1041, 781 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.74 (1H, brm, $w_{1/2}$= 16.0 Hz, H-19α), 3.35 (2H, m, H_2 - 1), 1.95 (2H, m, CH_2), 1.55 (2H, m, CH_2), 1.24 (60H, brs, 30 x CH_2) 0.87 (3H, t, J= 6.5 Hz, Me-35); TOF MS *m/z* (rel. int.): 524 $[M]^+$ ($C_{35}H_{72}O_2$) (9.1), 255 (10.3).

***n*- Nonadecanyl stearate (6)**

Elution of column with chloroform - methanol (19: 1) gave a colourless amorphous mass of compound **6**, 112 mg, R_f 0.48 (acetone-methanol, 1:1), m. p. 128 -130°C; IR *V*max (KBr): 2921, 2851, 1721, 1630, 1439, 1201, 1083, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.72 (2H, t, J = 5.6 Hz, H_2 – 1′), 2.27 (2H, t, J = 7.2 Hz, H_2 - 2), 2.02 (2H, m, CH_2), 1.87 (2H, m, CH_2), 1.55 (4H, m, 2 x CH_2), 1.38 (4H, m, 2 x CH_2), 1.31 (6H, m, 3 x CH_2), 1.28 (6H, m, 3 x CH_2), 1.24 (40H, brs, 20 x CH_2), 0.88 (3H, t, J= 6.5 Hz, Me-18), 0.85 (3H, t, J= 6.3 Hz, Me-19); TOF MS *m/z* (rel. int.): 536 $[M]^+$ ($C_{36}H_{72}O_2$) (81.6), 267 (21.3), 283 (17.4).

1-Hexatetracontanol (7)

Further elution of the column with chloroform - methanol (19: 1) yielded colourless crystals of **7**, yield 76 mg, m. p. 171 - 173°C; IR *V*max (KBr): 3398, 2932, 2847, 1634, 1408, 1261, 1203, 1079, 924, 872, 735 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.79 (2H, t, J = 6.9 Hz, H_2-1), 2.26 (2H, m, CH_2), 1.94 (2H, m, CH_2), 1.74 (2H, m, CH_2), 1.55 (4H, m, 2 x CH_2), 1.39 (4H, m, 2 x CH_2), 1.32 (6H, m, 3 x CH_2), 1.28 (8H, brs, 4 x CH_2), 1.24 (60H, brs, 30 × CH_2), 0.85 (3H, t, J = 6.5 Hz, Me-46); ^{13}C NMR ($CDCl_3$): δ 62.91 (C-1), 32.84 (C-2), 31.93 (CH_2), 29.71 (8 × CH_2), 29.67 (25 x CH_2), 29.55 (CH_2), 29.53 (CH_2), 29.48 (CH_2), 29.43 (CH_2), 29.40 (CH_2), 29.38 (CH_2), 29.36 (CH_2), 25.72 (CH_2), 22.69 (CH_2), 14.17 (Me-46); ESI MS *m/z* (rel. int.): 662 $[M]^+$ ($C_{46}H_{94}O$) (21.3).

RESULTS AND DISCUSSIONS

Compound **1** was an aliphatic alcohol identified as nonadecan-1-ol (CAS No. 1454-84-8).[39-40] Compound **2** was a fatty acid characterized as stearic acid (octadecanoic acid).[41-42] Compounds **3** and **4** were the higher aliphatic alcohols and their structures were established as henetriacontan-1-ol[43-44] and 1-pentatriacontanol[45- 46], respectively (Fig 2).

The IR spectrum of **5** showed IR absorption bands for hydroxyl groups (3399 cm^{-1}) and long aliphatic chain (781 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 524 corresponding to an aliphatic diol, $C_{35}H_{72}O_2$. An ion fragment arising at *m/z* 255 $[CH_3(CH_2)_{15}CHOH]^+$ indicated the presence of one of the hydroxyl group at C-19. The ^1H NMR spectrum of **5** displayed a one-proton broad multiplet at δ 3.74 ($w_{1/2}$ = 16.0 Hz) and a two- proton multiplet at δ 3.35 assigned to α-oriented secondary H-19 carbinol and primary

hydroxylmethylene H_2-1 protons, respectively. Two two-proton multiplets at δ 1.95 and 1.55 and a broad singlet at δ 1.24 (60 Hz) were ascribed to the methylene protons. A three- proton triplet at δ 0.87 (J = 6.5 Hz) was accounted to terminal C-35 primary methyl protons. On the basis of this discussion, the structure of **5** has been formulated as *n*- pentatriaconta-1,19β-diol, a new aliphatic diol (Fig 2).

The compound **6** exhibited IR absorption bands for ester group (1721 cm^{-1}) and long aliphatic chain (720 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 536 consistent with a molecular formula of a fatty acid ester, $C_{36}H_{73}O_2$. The ion peaks arising at *m/z* 267 $[CH_3(CH_2)_{16}CO]^+$ and 283 $(CH_3(CH_2)COO)^+$ indicated that stearic acid was esterified with a C_{19} alcohol. The ^1H NMR spectrum of **6** showed a two - proton triplet at δ 3.72 (J = 5.6 Hz) assigned to oxygenated methylene H_2 - 1′ protons, a two - proton triplet at δ 2.27 (J = 7.2 Hz) ascribed to methylene H_2-2 adjacent to the ester group, other methylene protons as multiplets at δ 2.02 (2H), 1.87 (2H), 1.55 (4H), 1.38 (4H), 1.31 (6H) and 1.28 (6H) and as a broad singlet at 1.24 (40 H) and two three-proton triplets at δ 0.88 (J = 6.5 Hz) and 0.85 (J = 6.3 Hz) accounted to terminal C- 18 and C- 19′ primary methyl protons, respectively. The absence of any signal beyond δ 3.72 supported saturated nature of the molecule. On the basis of foregoing account the structure of **6** has been elucidated as *n*- nonadecanyl stearate (Fig 2).

Compound **7** was a known aliphatic alcohol characterized as hexatetracontan-1-ol.[47-48]

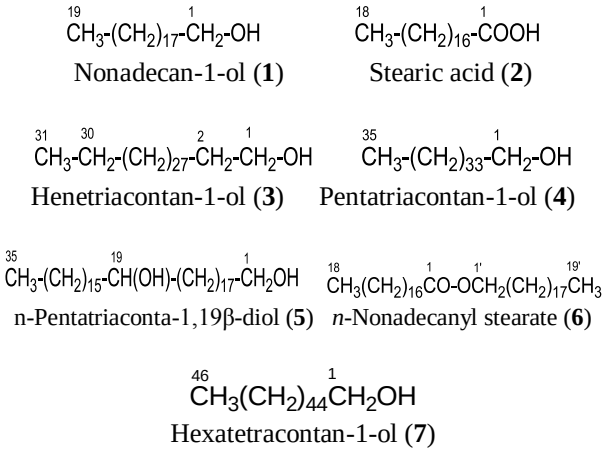

Fig. 2: Chemical constituents 1 - 7 isolated from the roots of *Saussurea costus*.

CONCLUSION

A chloroform fraction of the methanolic extract of the roots of *Saussurea costus* exhibited marked antidandruff activity against the growth of *Malassezia furfur*. Phytochemical investigation of the chloroform fraction resulted in the isolation of four long chain aliphatic alcohols, viz., nonadecan-1-ol (**1**), henetriacontan-1-ol (**3**), pentatriacontan-1-ol (**4**), and hexatetracontan-1-ol (**7**), a dihydroxy alcohol pentatriaconta-1,19β-diol (**5**) and one each of fatty acid stearic acid (**2**) and fatty ester *n*- nonadecanyl stearate (**6**). This work has enhanced understanding about the phytoconstituents of the plant roots. These compounds may be used as chromatographic markers for standardization of the plant roots.

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REFERENCES

1. Arora P, Nanda A, Karan M. Plants used in management of Dandruff. The Indian Pharmacist, 2011; March: 28-31.
2. Singla C, Drabu S, Ali M. Potential of herbals as antidandruff agents. International Research Journal of Pharmacy (IRJP), 2011; 2(3): 16-18.
3. Mayser P, Argembeaux H, Rippke F. The hair strand test- A new method for testing antifungal effects of antidandruff preparations. J Cosmet. Sci, 2003; 54(3): 263-270.
4. Vijayakumar R, Muthukumar C, Kumar T, Saravanamuthu R. Characterization of *Malassezia furfur* and its control by using plant extracts. Indian J Dematol, 2006; 51(2): 145-148.
5. Lee, Hyun J, Lee J-S. Chemical composition and antifungal activity of plant essential oils against *Malassezia furfur*. Kor. J Microbiol. Biotechnol, 2010; 38(3): 315-321.
6. Arora P, Nanda A, Karan M. Screening of plant essential oils for antifungal activity against *Malassezia furfur*. Int. J Pharm Pharm Sci, 2013; 5(2): 37-39.
7. Potluri A, Shaheda ASK, Rallapally N, Durraivel S, Harish G. A review on herbs used in anti-dandruff shampoo and its evaluation parameters. Indo American Journal of Pharm Research, 2013a; 3(4): 3266 – 3278.
8. Potluri A, Harish G, Kumar BP, Durraivel S. Formulation and evaluation of herbal anti-dandruff shampoo. Indian Journal of Research in Pharmacy and Biotechnology, 2013b; 1(6): 835- 839.
9. Pingili M, Vanga S, Raparla R. Antifungal activity of plant extracts against dandruff causing organism *Malassezia furfur*. International Journal of Bioassays, 2016; 5(11): 5047-5049.
10. Anonymous. The Wealth of India, Raw Materials, New Delhi; Council of Scientific and Industrial Research, 2000; 9: 240-242.
11. Kirtikar KR, Basu BD. Indian Medicinal Plants with Illustrations, Delhi; Oriental Enterprises, 2006; 5: 1421-1423.
12. Zahara K, Tabassum S, Sabir S, Arshad M, Qureshi R, Amjad MS, Chaudhari SK. A review of therapeutic potential of *Saussurea lappa* - an endangered plant from Himalaya. Asian Pac J Trop Med, 2014; 7(Suppl 1): S60-S69.

13. Yoshikawa M, Hatakeyama S, Inoue Y, Yamahara J. Saussureamines A, B, C, D, and E, new antiulcer principles from Chinese Saussureae Radix. Chem Pharm Bull (Tokyo), 1993; 41(1): 214-216.
14. Yang H, Xie JL, Sun HD. Study on chemical constituents of *Saussurea lappa* II. Acta Botanica Yunnanica, 1997; 19: 92-96.
15. Jung IH, Kim Y, Lee CO, Kang SS, Park JH, Im KS. Cytotoxic constituents of *Saussurea lappa*. Arch Pharm Res, 1998; 21: 153-156.
16. Chhabra BR, Gupta S, Jain M, Kalsi PS. Sesquiterpene lactones from *Saussurea lappa*. Phytochemistry, 1998; 49: 3801-3804.
17. Matsuda H, Kageura T, Inoue Y, Morikawa T, Yoshikawa M. Absolute stereo structures and syntheses of saussureamines A, B, C, D and E, amino acid-sesquiterpene conjugates with gastroprotective effect from the roots of *Saussurea lappa*. Tetrahedron, 2000; 56: 7763-7777.
18. Sun CM, Syu WJ, Don MJ, Lu JJ, Lee GH. Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa*. J Nat Prod, 2003; 66(9): 1175-1180.
19. Yin HQ, Fu HW, Hua HM, Qi XL, Li W, Sha Y et al. Two new sesquiterpene lactones with the sulfonic acid group from *Saussurea lappa*. Chemical and Pharmaceutical Bulletin, 2005; 53: 841-842.
20. Rao KS, Babu GV, Ramnareddy YV. Acylated favone glycosides from the roots of *Saussurea lappa* and their antifungal activity. Molecules, 2007; 12(3): 328-344.
21. Rao RN, Raju SS, Babu KS, Vadaparthi PRR. HPLC determination of costunolide as a marker of *Saussurea lappa* and its herbal formulations. Int J Biochem, 2013; 3(1): 99-107.
22. Robinson A, Kumar TV, Sreedhar E, Naidu VG, Krishna SR, Babu KS, Srinivas PV, Rao JM. A new sesquiterpene lactone from the roots of *Saussurea lappa*: structure-anticancer activity study. Bioorg Med Chem Lett, 2008; 18(14): 4015-4017.
23. Robinson A, Yashvanth S, Katragadda SB, Roa JM, Madhavendra SS. Isolation of α-amyrin eicosanoate, a triterpenoid from the roots of *Saussurea lappa* Clarke - Differential solubility as an aid. Journal of Pharmaceutical Science and Technology, 2010; 2(4): 207- 212.
24. Choi JY, Na M, Hyun Hwang I, Ho Lee S, Young Bae E, Yeon Kim B, Seog Ahn J. Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenoids with protein tyrosine phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C. B. Clarke. Molecules, 2009; 14(1): 266-272.
25. Julianti T, Hata Y, Zimmermann S, Kaiser M, Hamburger M, Adams M. Antitrypanosomal sesquiterpene lactones from *Saussurea costus*. Fitoterapia, 2011; 82(7): 955-959.
26. Maurer B, Grieder A. Sesquiterpenoids from Costus root oil (*Saussurea lappa* Clarke). Helv Chim Acta, 1997; 60(7): 2177-2190.
27. Yang H, Xie JL, Sun HD. Resarch progress on the medicinal plant *Saussurea lappa*. Nat Prod Res Dev, 1998; 10(2): 90-98.
28. De Kraker JW, Franssen MC, de Groot A, Shibata T, Bouwmeester HJ. Germacrene from fresh costus roots. Phytochemistry, 2001; 58(3): 481–7.
29. Pandey MM, Rastogi S, Rawat AK. *Saussurea costus*: Botanical, chemical and pharmacological review of an Ayurvedic medicinal plant. J Ethnopharmacol, 2007; 110(3): 379-390.
30. Chang KM, Kim GH. Comparison of volatile aroma components from *Saussurea lappa* C.B. Clarke root oils. Journal of Food Sciences and Nutrition, 2008; 13: 128-133.
31. Gwari G, Bhandari U, Andola HC, Lohani H, Chauhan N. Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India. Pharmacognosy Res., 2013; 5(3): 179–182.
32. Benedetto C, D Auria M, Mecca M, Prasad P, Singh P, Singh S, Sinisgalli C, Milella L. Chemical and biological evaluation of essential oil from *Saussurea costus* (Falc.) Lipsch. from Garhwal Himalaya collected at different harvesting periods. Natural Product Research, 2018; 19: 1-4. DOI: 10.1080/14786419. 2018. 1440219.
33. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research, 2007; 10: 175-181.
34. Hasson SSA, Al-Balushi MS, Alharthy K, Al-Busaidi JZ, Aldaihani MS, Othman MS et al. Evaluation of antiresistant activity of Auklandia (*Saussurea lappa*) root against some human pathogens. Asian Pacific Journal of Tropical Biomedicine, 2013; 3(7): 557-562.
35. Alaagib RMO, Ayoub SMH. On the chemical composition and antibacterial activity of *Saussurea lappa* (Asteraceae). The Pharma Innovation Journal, 2015; 4(2): 73-76.
36. Sarker SD, Latif Z, Gray AI. Methods in Biotechnology-Natural products isolation; Purification by solvent extraction using partition coefficient;(Hideaki Otsuka), 2nd ed., NJ; Humana Press, Totowa, 2006; 20: 269-274.
37. Tiwari AK, Mishra RK, Kumar A, Srivastava S, Dikshit A, Pandey A, Bajaj K. A comparative novel method of antifungal susceptibility for *Malassezia furfur* and modification of culture medium by adding lipid supplement. Journal of Phytology, 2011; 3(3): 44-52.
38. Prabhamanju, M, Shankar SG, Babu K, Ranjith MS. Herbal vs. chemical substances as antidandruff ingredients: which are more effective in the management of dandruff? - An overview. Egyptian Dermatology Online Journal, 2009; 5(2): 1- 8.
39. Guleria S, Saini R, Jaitak V, Kaul VK, Lal B, Rahi P, Gulati A, Singh B. Composition and antimicrobial activity of the essential oil of *Heracleum thomsonii* (Clarke) from the cold desert of the western

Himalayas, 2011; 25(13): 1250-60. doi: 10.1080/14786419.2011.557375.
40. Alemu MA, Mekonnen HG, Milkyas Endale Annisa. Phytochemical analysis and antibacterial activity on seed and pod extracts of *Crotalaria incana*. Journal of Pharmacy & Pharmacognosy Research, 2015; 3(4): 100-108.
41. Chung IM, Upadhyaya K, Ahmad A. Isolation of fatty acids and other constituents from *Callicarpa macrophylla* fruits. Asian J. Chem., 2006; 18(3): 1751 – 1758.
42. Soliman HM, Basuny AM, Arafat SM. Utilization of stearic acid extracted from olive pomace for production of triazoles, thiadiazoles and thiadiazines derivatives of potential biological activities. J Oleo Sci., 2015; 64(9): 1019-32. doi: 10.5650/jos.ess14261.
43. Singh S, Reddu SK, Sharma SK, Sanjrani MA. New Unsaturated Fatty Acid from Roots of *Bougainvillea spectabilis* Willd. Asian J Chem, 2009; 21(6): 4744-4748.
44. Shiradkar M, Pawankumar G, Shah K. Pharmacological evaluation of *Cassia auriculata* bark extract. International Journal of Pharma and Bio Sci, 2011; 2(1): 758-766.
45. Parveen M, Ghalib RM, Khanam Z, Mehdi SH, Ali M. A novel antimicrobial agent from the leaves of *Peltophorum vogelianum* (Benth.). Nat Prod Res., 2010; 24(13): 1268-73. doi: 10.1080/14786410903387688.
46. Li XJ, Liu ZZ, Kim K-W, Wang X , Li Z , Kim YC, Yook CS, Liu XQ. Chemical Constituents from Leaves of *Pileostegia viburnoides* Hook.f.et Thoms. Natural Product Sciences, 2016; 22(3): 154-161.
47. Ndom JC, Kouam, Vardamides JC, Wansi JD, Kamdem AW, Mbafor JT, Fomum ZT. Constituents of *Erythrina sigmoidea*. Bulletin of Chemical Society of Ethiopia, 2001; 15(2): 151-156.
48. Sultana S, Ali M, Mir SR. Chemical constituents from the leaves of *Adenanthera pavonina* and Erythrina variegata and roots of *Heliotropium eichwaldii*. International Journal of Advances in Pharmacy Medicine and Bioallied Sciences, 2017; 5(4): 217-224.