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Antidandruff activity and chemical constituents of the leaves of *Betula cylindrostachya* Lindl. ex Wall

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Abstract
Dandruff is a skin disorder caused by a fungus *Malassezia furfur*. There are some medicinal plants and essential oils which possess antidandruff properties. The stem bark and young shoots of *Betula cylindrostachys* Lindley (Betulaceae) are used as an anti-inflammatory, analgesic, astringent, rubefacient, tonic and to treat bladder infections, neuralgia, rheumatism skin diseases. The air-dried leaf 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 *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 1-heptadecanol (1), behenyl alcohol (1-docosanol, 2), 1-tricosanol (3), lignoceric acid (*n*-tetracosanoic acid, 4) and 1-nonacosanol (5). Their structures were established on the basis of spectral data analysis and chemical reactions.

Keywords: *Betula cylindrostachya*, leaves, antidandruff activity, phytoconstituents, isolation, characterization

1. Introduction
Dandruff is a skin disorder that mainly affects the scalp resulting flaking, mild itchiness, skin inflammation and dry skin. It is characterized by excessive shedding of skin cells from the scalp. A fungus *Malassezia furfur*, syn. *Pityrosporum 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].
Betula cylindrostachya Lindl. ex Wall., syn. *B. rhombibracteata* P. C. Li (Betulaceae), known as pareng , saur, sangli-kung and flame of the forest, is distributed in southern China, north-eastern India, Nepal, Pakistan and Myanmar. It is deciduous, much branched, up to 35 m tall tree; shoots rough with small warts, hairless; leaves small, simple, acute, dentate, triangular, base broad base, tip pointed, ovate-acuminate, elliptic, irregularly serrate, the underside nerves covered with brownish hairs; bark smooth, shining, reddish white, with horizontal smooth lenticels, outer bark consists of numerous thin papery layers, exfoliating in broad horizontal rolls; flowers orange – pink, in pendulous spikes [10, 11]. The stem bark and young shoots are used as an anti-inflammatory, analgesic, astringent, rubefacient and tonic, used to treat bladder infections, neuralgia and rheumatism. An essential oil from the bark and branches is mainly added for flavouring medicines [12]. In Darjeeling Himalayas, the bark is used to treat skin diseases [13]. In Sikkim, the fruits are eaten; a young shoot decoction is drunk as a tea [14].

Fig 1: *Betula cylindrostachya* plant

This paper described screening of antidandruff activity of various fractions of a methanolic extract of leaves of *B. cylindrostachya* against *Malassezia furfur* and to isolate chemical constituents from the active chloroform fraction.

Material and methods

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were scanned on a 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 and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant Material

The leaves of *B. cylindrostachya* were collected from botanical garden of the Forest Research Institute (FRI), Dehradun, India and identified by Dr. S. Biswas, Botany Division, FRI, Dehradun. A voucher specimen (MSIP/ 2010/ 08/ 12) was deposited in the herbarium section of the Pharmacognosy Division, Maharaja Surajmal Institute of Pharmacy, Janakpuri, New Delhi.

Extraction

The air-dried powdered leaves (2kg) of *B. cylindrostachya* 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 dark brown viscous mass (162 g, 8.1% yield). The dried methanolic extract (150 g) was dissolved in 95% aqueous methanol and fractionated successively with petroleum ether (60-80 ºC), chloroform, ethyl acetate and *n*-butanol to obtain their respective fractions. The fractions were filtered and the filtrates were evaporated under reduced pressure to get correspondingly 23.15g, 76.1g, 28.45g and 22.3g of the dried extracts.

Preparation of aqueous extract

Total aqueous extract of *B. cylindrostachya* leaves was prepared by extracting the leaf powder (250g) with hot water (500 ml) at 80-83 °C for 8 hrs. The extract was cooled at room temperature, filtered and the filtrate dried under reduced pressure to get a dark brown semisolid mass (24.7g).

Antidandruff activity

The cup plate method was used to determined 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 leaves of *B. cylindrostachys* 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 (20g), peptone (10g), agar (20g) and sterilized corn oil (5ml) at pH 5.6 and temperature 25 °C. The material was suspended in 1lt 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 32 ºC for 96 hours. Results were noted at the end of incubation period [4, 15, 16].

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:

1. 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.
2. 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.
3. The zone of inhibition of respective solvents were taken as blank and ketoconazole was taken as standard.
4. The plates were incubated at 32 °C for 96 hrs.
5. After incubation the zone of inhibition was measured and recorded.

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

Extract	Concentration	Zone of inhibition (mm) mean± SD
Petroleum ether	10 mg/ml	9.1 ± 0.36
Chloroform	10 mg/ml	22.47 ± 1.14
Ethyl acetate	10 mg/ml	9.37 ± 0.38
n-Butanol	10 mg/ml	10.86 ± 0.26
Total methanol	10 mg/ml	12.14 ± 0.32
Total aqueous	10 mg/ml	11.43 ± 0.42
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 Minimum Inhibitory Concentration (MIC)

Dilution susceptibility testing method was used to determine the minimal concentration of leaf 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 discuss earlier in well diffusion method.

Procedure

1. Different concentrations of plant extract in (10 µl, 20 µl….up to 100µl) to the tube to respective tubes were added.
2. From the inoculums 10 µl of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106cells/ml. This procedure was performed for all the positive extracts antifungal activity which were obtained by primary screening.
3. 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 *B. cylindrostachys* leaves exhibited marked antidandruff activity against *M. furfur*, i.e., the chloroform fraction was more active with larger zone of inhibition (22.47 mm). The petroleum ether extract was least active against the growth of *M. furfur*.

Isolation of phytoconstituents

The dried chloroform extract (70 g) was dissolved in minimum amount of methanol and a silica gel for column chromatography was then mixed slowly with continuous mixing until the whole chloroform solution of the extract adsorbed on silica gel particles. It was dried in the air; the large lumps were broken by rubbing and finally passed through a sieve (No. 8) to get uniform particle size. The dried slurry was chromatographed over silica gel columns loaded 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-Heptadecanol (1)

Elution of column with petroleum ether- chloroform (9: 1) gave creamy white amorphous mass of compound 1, yield 38 mg (0.0076 %), R_f 0.37 (petroleum ether - chloroform, 3:1), m. p. 51 - 52 °C; IR *V*max (KBr): 3337, 2928, 2842, 1639, 1442, 1277, 1082, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.26 (2H, t, J = 7.5 Hz, H_2-1), 1.95 (2H, m, CH_2), 1.92 (2H, m, CH_2), 1.58 (2H, m, CH_2) 1.25 (24H, brs, 12 x CH_2), 0.85 (3H, t, J = 6.5 Hz, Me-17); ESI MS *m/*z (rel. int.): 256 $[M]^+$ ($C_{17}H_{36}O$) (12.8).

Behenyl alcohol (2)

Elution of the column with petroleum ether - chloroform (4:1) furnished colourless amorphous powder of compound **2**, yield 45 mg (0.009%), R_f 0.55 (chloroform-acetone, 9:1), m. p. 68 - 71 °C; IR *V*max (KBr): 3436, 2924, 2853, 1637, 1417, 1096, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.25 (2H, t, J = 6.8 Hz, H_2-1), 1.98 (2H, m, CH_2), 1.51 (2H, m, CH_2), 1.51 (2H, `m, CH_2), 1.38 (2H, m, CH_2), 1.32 (4H, m, 2 x CH_2), 1.28 (32H, brs, 16 x CH_2), 0.85 (3H, t, J = 6.3 Hz, Me-21); ESI MS *m/*z (rel. int.): 326 $[M]^+$ ($C_{22}H_{46}O$) (21.5).

1-Tricosanol (3)

Elution of the column with petroleum ether - chloroform (3 : 1) afforded colourless amorphous mass of compound 3, yield 64 mg (0.0128%), R_f 0.48 (acetone), m. p. 74 - 76 °C; IR *V*max (KBr): 3435, 2927, 2852, 1638, 1425, 1216, 1096, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.32 (2H, t, J = 6.3 Hz, H_2-1), 1.98 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.29 (8H, brs, 4 × CH_2), 1.23 (30H, brs, 15 × CH_2), 0.87 (3H, t, J = 6.6 Hz, Me-23); ^{13}C NMR ($CDCl_3$): δ 62.91 (C-1), 33.21 (CH_2), 31.68 (CH_2), 29.87 (16 × CH_2), 27.61 (CH_2), 25.39 (CH_2), 22.69 (CH_2), 14.23 (C-23); ESI MS *m/*z (rel. int.): 340 $[M]^+$ ($C_{23}H_{48}O$) (32.8).

Lignoceric acid (4)

Elution of column with chloroform gave colourless amorphous powder of compound 4, yield 29 mg (0.0058%), R_f 0.67 (chloroform-acetone, 1:1), m. p. 83 - 84 °C; UV λmax (MeOH): 273, 325 nm; IR *V*max (KBr): 3433, 2924, 2854, 1704, 1636, 1460, 1260, 1158, 1025, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.53 (2H, t, J = 7.8 Hz, H_2-2), 2.11 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.38 (2H, m, CH_2), 1.24 (36H, brs, 18 x CH_2), 0.86 (3H, t, J= 6.3 Hz, Me-24); ESI MS *m/*z (rel. int.): 368 $[M]^+$ ($C_{24}H_{48}O_2$) (34.2).

1-Nonacosanol (5)

Further elution of the column with chloroform yielded pale yellow powder of compound 5, yield 35 mg (0.007%), R_f 0.76 (acetone-methanol, 9:1), m. p. 69 – 70 °C; IR *V*max (KBr): 3428, 2926, 2854, 1639, 1403, 1077, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.31 (2H, t, J = 7.1 Hz, H_2-1), 1.98 (2H, m, CH_2), 1.55 (2H, m, CH_2), 1.35 (50H, brs, 25 x CH_2), 0.87 (3H, t, J = 6.7 Hz, Me-29); ESI MS *m/*z (rel. int.): 424 $[M]^+$ ($C_{29}H_{60}O$) (29.3).

Discussions

Compound 1: $[M]^+$ at *m/*z 256 ($C_{17}H_{36}O$), showed IR absorption bands for a hydroxyl group (3337 cm^{-1}) and long aliphatic chain (720 cm^{-1}). Its ^1H NMR spectrum displayed a two-proton triplet at δ 3.26 (J = 7.5 Hz) assigned to hydroxymethylene H_2-1 protons, methylene protons as two proton multiplets at δ 1.95, 1.92 and 1.58 and as a broad singlet at δ 1.25 (24H) and a three-proton triplet at δ 0.85 (J = 6.5 Hz) ascribed to terminal C-17 primary methyl protons. On the basis of these informations the structure of 1 has been identified as 1-heptadecanol (Fig 2) [17, 18].

Compound 2: $[M]^+$ at *m/*z 326 ($C_{22}H_{46}O$), exhibited IR absorption bands for a hydroxyl group (3436 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its ^1H NMR spectrum displayed a two - proton triplet at δ 3.25 (J = 6.8 Hz) ascribed to hydroxymethylene H_2-1 protons, methylene protons as multiplets between δ 1.98 - 1.32, as a broad singlet at δ 1.28 (32 Hz) and a three-proton triplet at δ 0.85 (J = 6.3 Hz) accounted to terminal C-21 primary methyl proton. These data led to characterized the structure of **2** as behenyl alcohol (1-docosanol) (Fig 2) [19, 20].

Compound 3: $[M]^+$ at *m/*z 340 ($C_{23}H_{48}O$), exhibited IR spectrum absorption bands for hydroxyl group (3435 cm^{-1}) and long aliphatic chain (721 cm^{-1}). The ^1H NMR spectrum of 3 displayed a two- proton triplet at δ 3.32 (J = 6.3 Hz) assigned to hydroxymethylene H_2-1 protons and methylene protons from δ 1.98 to 1.23. A three- proton triplet at δ 0.87 (J = 6.6 Hz) was accounted to terminal C-23 primary methyl protons. The ^{13}C NMR spectrum of **3** showed signals for hydroxymethylene carbon at δ 62.91 (C-1), other methylene carbon in the range of δ 33.21- 22.69 and methyl carbon at δ 14.23 (C-23). On the basis of above discussion, the structure of **3** has been established as 1-tricosanol (Fig 2) [21, 22].

Compound 4: $[M]^+$ at *m/*z 368 ($C_{24}H_{48}O_2$), produced effervescences with sodium bicarbonate solution and showed IR spectrum absorption bands for carboxyl function (3433, 1704 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its ^1H NMR spectrum of displayed a two-proton triplet at δ 2.53 (J = 7.8 Hz) assigned to methylene H_2-2 protons adjacent to the carboxylic group, other methylene proton signals between δ 2.11 - 1.24 and a three proton triplet at δ 0.86 (J = 6.3 Hz) accounted to terminal C-24 primary methyl protons. On the basis of above discussion, the structure of 4 has been elucidated as lignoceric acid (*n*- tetracosanoic acid) (Fig 1) [23, 24].

Compound 5: $[M]^+$ at *m/*z 424 ($C_{29}H_{60}O$), showed IR absorption bands for hydroxyl group (3428 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The ^1H NMR spectrum of B- 5 exhibited a two- proton triplet at δ 3.31 (J = 7.1 Hz) assigned to hydroxyl methylene H_2 -1 protons, other methylene protons from δ 1.98 to 1.35 (50 H) and a three-proton triplet at δ 0.87 (J = 6.7 Hz) attributed to terminal C- 29 primary methyl protons. On the basis of these evidences, the structure of 5 has been elucidated as 1-nonacosanol (Fig 2) [25, 26].

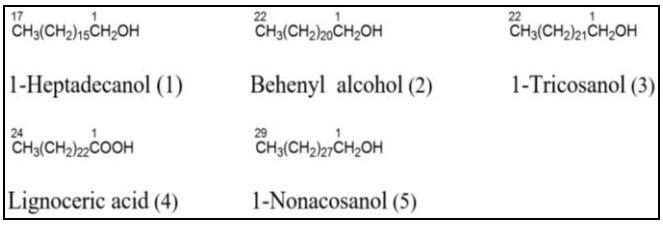

Fig 2: Structural formulae of compounds 1 - 5 isolated from the leaves of *Betula cylindrostachya*

Conclusion

Phytochemical investigation of a chloroform fraction of the methanolic extract of the leaves of *B. cylindrostachya* resulted in the isolation of four long chain aliphatic alcohols, viz., 1-heptadecanol (1), behenyl alcohol (2), 1-tricosanol (3) and 1-nonacosanol (5) and a fatty acid identified as lignoceric acid (4). This work has enhanced understanding about the phytoconstituents of the plant leaves. These compounds may be used as chromatographic markers for standardization of the plant leaves.

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References

1. Arora P, Nanda A, Karan M. Plants used in management of Dandruff. The Indian Pharmacist. 2011, 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 JS. 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. Fredskild B. The genus Betula in Greenland - Holocene history, present distribution, and synecology. Nord. J Bot. 1991; 11(4):393-412.
11. Chettri N, Sharma E. Introgression and taxonomy of the mountain birch in SW Greenland compared with related results from Iceland and Finnish Lapland. Meddel. Grfnlan, Bioscience. 2000; 3(3):21-29.
12. Devi TI, Devi KU, Singh EJ. Wild medicinal plants in the hill of Manipur, India: a traditional therapeutic potential. International Journal of Scientific and Research Publications (IJSRP). 2015; 5(6):1-9.
13. Sharma BC. Ethnomedicinal plants used against skin diseases by indigenous population of Darjeeling Himalayas, India. Indian Journal of Fundamental and Applied Life Sciences. 2013; 3(3): 299-303.
14. Lokendra SS, Vats P, Praveen S. Studies on wild edible plants of ethnic people in east Sikkim. Asian J. of Bio Sci. 2006; 1(2):117-125.
15. 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.

16. Tiwari AK, Mishra RK, Kumar A, Srivastava S, Dikshit A, Pandey A et al. 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.
17. El-Youssef HM, Murphy BT, Amer ME, Abdel-Kader MS, Kingston DJI. Phytochemical and biological study of the aerial parts of *Lotus lalambensis* growing in Saudi Arabia. Saudi Pharmaceutical Journal. 2008; 16(2):122-134.
18. Zhen HS, Zhu XY, Lu RM, Lianq J, Qiu Q, Meng QM. Research on chemical constituents from stem of *Gymnema sylvestre*. Zhong Yao Cai. 2008; 31(8):1154-1156.
19. Pope LE, Marcelletti JF, Katz LR, Katz DH. Anti-herpes simplex virus activity of n-docosanol correlates with intracellular metabolic conversion of the drug. Journal of Lipid Research. 1996; 37(10):2167-2178.
20. Vergara M, Olivares A, Altamirano C. Antiproliferative evaluation of tall-oil docosanol and tetracosanol over CHO-K1 and human melanoma cells. Electronic Journal of Biotechnology. 2015; 18(4):291-294.
21. Khan KM, Perveen S, Ayattollahi SA, Saba N, Rashid A, Firdous S et al. Isolation and structure elucidation of three glycosides and a long chain alcohol from *Polianthes tuberosa* Linn. Nat. Prod Lett. 2002; 16(4):283-290.
22. 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.
23. Hansen RP, Shorland FB, Cooke NJ. Isolation and identification of the high molecular weight saturated fatty acids of butterfat. J Dairy Res. 1959; 26 (2):190-195.
24. Habe H, Morita T, Sakaki K, Kitamoto D. Isolation and characterization of thermotolerant fungi producing lignoceric acid from glycerol. J Oleo Sci. 2008; 57(4):251-255.
25. Mendez E, Blanco M, Laguna A, García E. Isolation and characterization of a mixture of higher primary aliphatic alcohols of high molecular weight from henequen (*Agave furcroydes* L.) wax. Revista CENIC Ciencias Quimicas. 2003; 34(1):35-38.
26. Singh V, Ali M, Pahuja C. A new steroidal ester from Tinospora cordifolia Miers. stems. Der Pharmacia Lettre. 2016; 8(20):47-51.