**PHYTOCHEMICAL INVESTIGATION AND ANTIFUNGAL ACTIVITY OF THE SEEDS OF CENTRATHERUM ANTHELMINTICUM**

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**EXPERIMENTAL**

Melting points were determined on a Perfit melting point apparatus (Ambala, India) and are uncorrected. IR spectra were recorded using KBr discs with a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong). UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. ¹H and ¹³C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Rheinsteen, Germany), respectively, with TMS as an internal standard. FAB mass spectra were obtained using a JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column chromatography was performed on silica gel (Qualigen, Mumbai, India) 60–120 mesh. TLC was run on silica gel G (Qualigen). Spots were visualized by exposure to iodine vapor, UV radiation and by spraying reagents.

**Plant material**

The seeds of *C. anthelminticum* were procured from the Khari Baoli market of Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard. New Delhi. A voucher specimen No. PRL/JH/09/09 is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

**Extraction and isolation**

The dried seeds (2 kg) were coarsely powdered, defatted with petroleum ether and then exhaustively extracted with ethanol (95%) in a Soxhlet apparatus. The combined ethanol extracts were concentrated on a steam bath and dried under reduced pressure to obtain 85 g (4.25% yield) of dark viscous brown mass. It was dissolved in small quantity of methanol and adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, mixtures of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3, v/v), pure chloroform and finally mixtures of chloroform and methanol (99:1, 97:3, 95:5, 90:10, v/v).
Various fractions were collected separately and checked by TLC for homogeneity. Similar fractions (having the same Rf values) were combined and crystallized. The isolated compounds 4 and 5 were recrystallized to get pure compounds. The physiochemical and spectral data of the isolated compounds are reported below.

**Glyceryl diolein (1)**

Elution of column with petroleum ether-chloroform (4:1, v/v) afforded colorless sticky mass of 1, purified by TLC using acetonitrile-methanol (1:1, v/v), 125 mg (0.0062% yield). Rf: 0.70 (petroleum ether). UV $\lambda_{max}$ (MeOH): 277 nm (log e 5.3). IR $\nu_{max}$ (KBr): 3000, 3350, 2950, 2845, 1725, 1650 cm$^{-1}$. $^1$H NMR (DMSO-d$_6$, ppm): 1.18 (1H, brm, Hz-6), 1.36 (1H, dd, $J$ = 9.5 Hz, Hz-15), 2.30 (1H, m, Hz-18a), 1.58 (2H, m, Hz-19), 1.23 (1H, m, Hz-18b). 13C NMR (DMSO-d$_6$, ppm): 35.27 (C-18), 37.46 (C-19), 50.06 (C-17), 50.24 (C-16), 51.76 (C-13), 52.14 (C-12), 61.31 (C-11), 68.65 (C-20), 70.03 (C-15), 70.46 (C-14), 115.06 (C-7), 123.55 (C-6), 127.74 (C-10), 132.77 (C-4), 138.38 (C-5), 140.69 (C-1), 141.88 (C-8), 163.75 (C-3), 164.45 (C-2), 166.73 (C-9). $^{+ve}$ FAB MS $m/z$ (rel. int.): 358 [M]$^+$ C$_{30}$H$_{52}$O$_6$ (10.3).

**Centratherum napthyl hexol (5)**

Further elution of column with chloroform-methanol (97:3, v/v) produced colorless amorphous mass of 5, recrystallized from chloroform-methanol (1:1, v/v), 725 mg (0.036 % yield). Rf: 0.80 (CHCl$_3$). UV $\lambda_{max}$ (MeOH): 242 nm (log e 4.9); IR $\nu_{max}$ (KBr): 3410, 3355, 2960, 2855, 1650, 1587, 1481, 1046, 795 cm$^{-1}$. $^1$H NMR (DMSO-d$_6$, ppm): 7.21 (1H, brs, Hz-1), 7.06 (1H, brs, Hz-4), 6.60 (1H, d, $J$ = 9.3 Hz, Hz-6), 6.39 (1H, d, $J$ = 9.3 Hz, Hz-7), 4.78 (1H, dd, $J$ = 11.4, 5.5 Hz, Hz-14a), 4.33 (1H, dd, $J$ = 11.4, 5.7 Hz, Hz-15a), 4.03 (1H, dd, $J$ = 10.5 Hz, Hz-11a), 3.97 (1H, d, $J$ = 10.5 Hz, Hz-11b), 3.81 (1H, dd, $J$ = 5.7, 8.9 Hz, Hz-20a), 3.67 (1H, brm, $w_r$= 15.6 Hz, Hz-19a), 2.81 (1H, dd, $J$ = 11.4, 10.5 Hz, Hz-13), 2.56 (1H, m, Hz-12), 2.50 (1H, m, Hz-16), 1.90 (1H, m, Hz-17), 1.59 (1H, m, Hz-18a), 1.51 (1H, m, Hz-18b). $^1$H NMR (DMSO-d$_6$, ppm): 37.44 (C-18), 50.15 (C-17), 50.21 (C-16), 51.80 (C-13), 52.11 (C-12), 60.30 (C-11), 68.67 (C-19), 69.18 (C-20), 70.01 (C-15), 70.55 (C-14), 115.16 (C-7), 123.55 (C-6), 127.81 (C-10), 133.26 (C-4), 138.31 (C-5), 140.67 (C-1), 141.87 (C-8), 163.75 (C-3), 164.44 (C-2), 166.74 (C-9). $^{+ve}$ FAB MS $m/z$ (rel. int.): 374 [M]$^+$ C$_{30}$H$_{52}$O$_7$ (9.8).

**Antifungal activity**

The antifungal activity was performed on *Asperillus flavus* (MTCC-277), *Candida albicans* (MTCC-3958) and *Penicillium citrinum* (MTCC-3395). A fungal suspension in sterile normal saline was prepared. An aliquot of 1.5 mL was uniformly seeded on the malt extract media (15 mL, 4 cm thick) in Petri dishes, left aside for 15 min, excess was drained and discarded properly. Wells of 6 mm in diameter and 2 cm apart were punctured into culture media using a sterile cork borer (6 mm). Concentrations of 25, 50, 100, and 200 mg/mL of the test extract or compound were prepared in dimethyl sulfoxide (DMSO). The standard drug – fluconazole (30 mg tablet) was obtained from Cipla Laboratories (Mumbai, India). The plates were then incubated at 30°C for 48 h. After incubation, bioactivity was determined by measuring the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls included solvent with-
out test compounds, although no antifungal activity was noted in the solvent employed for the test.

RESULTS AND DISCUSSION

Compounds 1, 2 and 3 were the fatty acid glyc erides characterized as glyceryl diolein, glyceryl diricin and glyceryl ricinopalmitine, respectively, on the basis of spectral data analyses.

Compound 4, designated as centratherumnaph thyl pentol, was obtained as a pale yellow amor phous mass from chloroform-methanol (97:3) elu ents. It gave positive tests for phenols and showed characteristic IR absorption bands for hydroxyl groups (3410, 3355 cm -1) and aromatic nucleus (1640, 1560, 950 cm -1). This was supported by its UV absorption maximum at 243 nm. On the basis of 13C NMR data of 4 exhibiting two one-proton broad signals at δ 7.16 and 7.03 ppm correspondingly attributable to para-coupled H-1 and H-4 aromatic protons. Two ortho, ortho-coupled H-6 and H-7 aromatic protons appeared as two doublets, one-protons each, at δ 6.57 (J = 9.5 Hz) and 6.35 (J = 9.5 Hz), respectively. Three carbinol proton signals, one-proton each, resonated as double doublets at δ 4.76 (J = 5.6, 11.8 Hz) and 4.36 (J = 5.9, 11.8 Hz) and a broad multiplet at δ 3.79 ppm (w1/2 = 14.7 Hz) were ascribed, respectively, to α-oriented H-14, H-15 and H-20 protons. Two downfield one-proton doublets at δ 4.01 (J = 10.2 Hz) and 3.95 (J = 10.5 Hz) were attributed to oxygenated methylene protons H2-11a and H2-11b, respectively. The remaining methylene and methine protons resonated between δ 2.81–1.23 suggesting their saturated nature. The 1H NMR data of 4 exhibited signals for twenty carbons in the molecule. The oxygenated aromatic carbons C-2, C-3 and C-9 appeared at δ 164.45, 163.75 and 166.73 ppm, respectively. The signals between δ 140.69–127.74 ppm were due to the remaining aromatic carbons. The carbinol carbons resonated at δ 61.31 (C-11), 70.03 (C-15) and 68.65 (C-20) ppm. The DEPT spectrum of 4 showed the presence of three methylene, eleven methine and six quaternary carbons. The 1H–1H COSY spectrum of 4 exhibited correlations of H-6 with H-4 and H-7; H-14 with H-13 and H-15; H2-11 with H-12; and H-20 with H-16 and H2-19. The HMBC spectrum of 4 showed interactions of C-2 with H-1 and H-4; C-9 with H-1 and H2-11; C-15 with H-13, H-14 and H-16; and C-20 with H-16, H2-19 and H-17. On the basis of foregoing discussions the structure of 4 has been elucidated as 2,3-di hy droxy naphthyl-[c,d]-14β,15β,20β-trihydroxy[16, 17]-cyclopentanocyclohexyl tetrahydropyran. This is a new naphthalene derivative reported from Centrtherum or other species.

Compound 5, designated as centratherumnaph thyl hexol, was obtained as a colorless amorphous mass from chloroform-methanol (97:3) eluents. It responded positively to FeCl3 test for phenols. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3410, 3355 cm -1) and aromatic moiety (1650, 1587 cm -1). Its UV absorption maximum at 242 nm indicated a conjugated system in the molecule. On the basis of 13C NMR and FAB mass spectra the molecular weight of 5 was established at m/z 374 consistent with the molecular formula of a naphthalene derivative C20H22O7. The 'H NMR spectrum of 5 exhibited two one-proton broad signals at δ 7.21 and 7.06 ppm assigned correspondingly to para-coupled H-1 and H-4 aromatic protons. Two ortho, ortho-coupled aromatic protons H-6 and H-7 appeared as two doublets, one proton each, at δ 6.60 (J = 9.3 Hz) and 6.39 (J = 9.3 Hz) ppm, respectively. Three one-proton double doublets at δ 4.78 (J = 11.4, 5.5 Hz), 4.33 (J = 11.4, 5.7 Hz) and 3.81 (J = 5.7, 8.9 Hz) ppm were attributed to α-oriented H-14, H-15 and H-20 carbinol protons, respectively. A one-proton broad multiplet at δ 3.67 ppm with half-width of 15.6 Hz was ascribed to α-oriented H-19 carbinol protons. Two one-proton doublets at δ 4.03 (J = 10.5 Hz) and 3.97 (J = 10.5 Hz) were due to the oxygenated H2-11 methylene protons. The remaining methylene and methine protons resonated between δ 2.81–1.51 ppm. The 13C NMR of 5 exhibited signal for 20 carbon atoms in the molecule. The aromatic carbons resonated between δ 166.74–123.55 ppm. Signals at δ 70.55, 70.01, 68.67 and 69.18 ppm were due to the carbonyl C-14, C-15, C-19 and C-20 carbons, respectively. The oxygenated methylene C-11 carbon resonated at δ 60.30 ppm. The other methine and methylene carbons appeared between δ 37.44–52.11 ppm. The DEPT spectrum of 5 showed the presence of two methylene, twelve methine and six quaternary carbons. The 'H–'H COSY spectrum of 5 showed correlations of H-6 with H-4 and H-7; H2-11 with H-12; H-14 with H-13 and H-15; and H-19 with H2-18 and H-20. The HMBC spectrum of 5 exhibited interactions of C-2 with H-1 and H-4; C-9 with H-1 and H2-11; and C-14 with H2-18 and H-20. On the basis of these evidences the structure of 5 has been elucidated as 2,3- dihydroxynaphthyl-[c,d]-14β, 15β, 19β, 20β-tetrahydroxy[16, 17]-cyclopentanocyclohexyl tetrahydropyran. This is also a new naphthalene
The methanolic extract of the seeds of *C. anthelminticum* exhibited antifungal activity against *Aspergillus flavus*, *Candida albicans* and *Penicillium citrinum* in increment of the concentration. It showed the highest activity against *C. albicans* at 200 mg/mL and the activity was equivalent to that of control drug fluconazole at 30 mg/mL.

Compound 4 was active against all the fungal strains from 25 to 200 mg/mL. It exerted maximum inhibition of *C. albicans* at 100 and 200 mg/mL. Compound 5 was inactive against all fungal strains at 25 mg/mL. However, it showed significant anti-

derivative isolated from a natural of synthetic source.

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Table 1. Antifungal activity of methanolic extract of the seeds of *C. anthelminticum*, centratherumnaphthyl pentol (4) and centratherumnaphthyl hexol (5).

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Figure 1. Structure of new naphthalene derivatives from *Centratherum anthelminticum*

REFERENCES


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