Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 6 pp.1183–1187, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

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

ONKAR SINGH, MOHAMMED ALI* and SHAHNAZ S. HUSAIN

Phytochemical Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi – 110 062, India

Keywords: *Centratherum anthelminticum*, Asteraceae, seeds, naphthalene derivatives, antifungal activity

Centratherum anthelminticum O. Kuntze, syn. *Vernonia anthelmintica* Willd. (Asteraceae), is known as kalajiri or purple fleebane. It is an erect, branched, leafy annual herb, 50 to 90 cm in height, distributed throughout India up to 1650 m altitude in the Himalayas, Afghanistan and Malaysia. The seeds are acrid, astringent, anthelmintic, diuretic, stomachic, tonic and used to treat fever, skin diseases, leucoderma, ulcers, asthma, kidney troubles, hiccough, intestinal colics, inflammation, sores and itching eyes (1). The seeds contained carbohydrates (2), steroids (3–5), acetylated triterpenoid saponins (6, 7), fatty acids (8, 9), aliphatic constituents (5), chalcone, flavone and butin (10, 11) and elemanolide lactone (12). The seeds exhibited analgesic and antipyretic (13), larvicidal (14), antimicrobial (15), antifilarial (16), antiviral (17), spermicidal (18) and anthelmintic (19) activities. The present manuscript describes the isolation and characterization of two new naphthalene derivatives and antifungal activity of the seeds of *C. anthelminticum*.

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. ^{1}H and ^{13}C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Rheinstetten, 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 (Qualigens, Mumbai, India) 60–120 mesh. TLC was run on silica gel G (Qualigens). 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).

* Corresponding author: e-mail: maliphyto@gmail.com

Various fractions were collected separately and checked by TLC for homogeneity. Similar fractions (having the same R_f 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 acetone-methanol (1:1, v/v), 125 mg (0.0062% yield).R_f: 0.70 (petroleum ether). UV λ_{max} (MeOH): 277 nm (log e 5.3). IR ν_{max} (KBr): 3500, 3350, 2950, 2845, 1725, 1650 cm^{-1}. +ve FAB MS *m*/*z* (rel. int.): 620 $[M]^+$ $C_{39}H_{72}O_5$ (2.1).

Glyceryl diricin (2)

Elution of column with petroleum ether-chloroform (1:1, v/v) gave cream-colored semisolid mass of **2**, purified by TLC using acetone-methanol (4:1, v/v), 350 mg (0.017% yield). R_f: 0.80 (toluene : ethyl acetate : acetic acid; 5:4.5:0.5, v/v).UV λ_{max} (MeOH): 246 nm (log e 4.3). IR ν_{max} (KBr): 3450, 2926, 2855, 1725, 1640 cm^{-1}. +ve FAB MS *m*/*z* (rel. int.): 652 $[M]^+$ $C_{39}H_{72}O_7$ (2.5).

Glyceryl ricinolpalmitein (3)

Elution of column with petroleum ether-chloroform (2:3, v/v) furnished colorless semisolid mass of **3**, recrystallized from chloroform-methanol (1:1, v/v), 475 mg (0.02375% yield). R_f: 0.60 (toluene : ethyl acetate : acetic acid; 5:4.5:0.5, v/v).UV λ_{max} (MeOH): 247 nm (log e 4.6). IR ν_{max} (KBr): 3455, 2928, 2856, 1743 cm^{-1}. +ve FAB MS *m*/*z* (rel. int.): 606 $[M]^+$ $C_{37}H_{66}O_6$ (5.1).

Centratherumnaphthyl pentol (4)

Elution of column with chloroform-methanol (97:3, v/v) yielded pale yellow amorphous mass of **4**, recrystallized from chloroform-methanol (1:1, v/v), 220 mg (0.011% yield). R_f: 0.60 ($CHCl_3$). UV λ_{max} (MeOH): 243 nm (log e 5.2). IR ν_{max} (KBr): 3410, 3350, 2955, 2845, 1640, 1560, 1175, 1162, 1042, 950, 860 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ, ppm): 7.16 (1H, brs, H-1), 7.03 (1H, brs, H-4), 6.57 (1H, d, *J* = 9.5 Hz, H-6), 6.35 (1H, d, *J* = 9.5 Hz, H-7), 4.76 (1H, dd, *J* = 5.6, 11.8 Hz, H-14α), 4.36 (1H, dd, *J* = 11.8, 5.9 Hz, H-15α), 4.01 (1H, d, *J* = 10.2 Hz, H_2-11a), 3.95 (1H, d, *J* = 10.5 Hz, H_2-11b), 3.79 (1H, brm, *w*1/2 = 14.7 Hz, H-20α), 2.81 (1H dd, *J* = 11.8, 10.2 Hz, H-13), 2.56 (1H, m, H-12), 2.50 (1H, m, H-16), 1.89 (1H, m, H-17), 1.59 (2H, m, H_2-19), 1.23 (2H, m, H_2-18). ^{13}C NMR (DMSO-d_6, δ, 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 $[M]^+$ $C_{20}H_{22}O_6$ (10.3).

Centratherumnaphthyl 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). R_f: 0.80 ($CHCl_3$); UV λ_{max} (MeOH): 242 nm (log e 4.9); IR ν_{max} (KBr): 3410, 3355, 2960, 2855, 1650, 1587, 1481, 1046, 795 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ, ppm): 7.21 (1H, brs, H-1), 7.06 (1H, brs, H-4), 6.60 (1H, d, *J* = 9.3 Hz, H-6), 6.39 (1H, d, *J* = 9.3 Hz, H-7), 4.78 (1H, dd, *J* = 11.4, 5.5 Hz, H-14α), 4.33 (1H, dd, *J* = 11.4, 5.7 Hz, H-15α), 4.03 (1H, d, *J* = 10.5 Hz, H_2-11a), 3.97 (1H, d, *J* = 10.5 Hz, H_2-11b), 3.81 (1H, dd, *J* = 5.7, 8.9 Hz, H-20α), 3.67 (1H, brm, *w*″= 15.6 Hz, H-19α), 2.81 (1H, dd, *J* = 11.4, 10.5 Hz, H-13), 2.56 (1H, m, H-12), 2.50 (1H, m, H-16), 1.90 (1H, m, H-17), 1.59 (1H, m, H_z-18a), 1.51 (1H, m, H_2-18b). ^{13}H NMR (DMSO-d_6, δ, 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 $[M]^+$ $C_{20}H_{22}O_7$ (9.8).

Antifungal activity

The antifungal activity was performed on *Aspergillus 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^{o}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

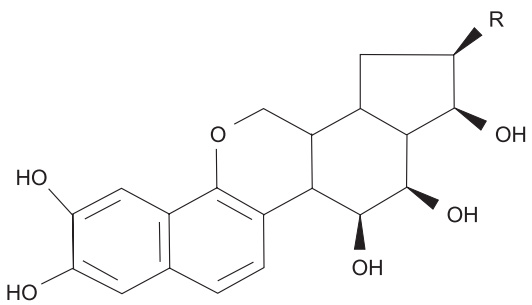
Compounds **1**, **2** and **3** were the fatty acid glycerides characterized as glyceryl diolein, glyceryl diricin and glyceryl ricinolpalmitein, respectively, on the basis of spectral data analyses.

Compound **4**, designated as centratherumnaphthyl pentol, was obtained as a pale yellow amorphous mass from chloroform-methanol (97:3) eluents. It gave positive tests for phenols and showed characteristic IR absorption bands for hydroxyl groups (3410, 3350 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 ^{13}C NMR and FAB mass spectra the molecular weight of **4** was determined at *m*/*z* 358 corresponding to a molecular formula of a naphthalene derivative $C_{20}H_{22}O_6$. The ^1H NMR spectrum of **4** exhibited 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 H_2-11a and H_2-11b, respectively. The remaining methylene and methine protons resonated between δ 2.81–1.23 suggesting their saturated nature. The ^{13}C 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; H_2-11 with H-12; and H-20 with H-16 and H_2-19. The HMBC spectrum of **4** showed interactions of C-2 with H-1 and H-4; C-9 with H-1 and H_2-11; C-15 with H-13, H-14 and H-16; and C-20 with H-16, H_2-19 and H-17. On the basis of foregoing discussions the structure of **4** has been elucidated as 2,3-dihydroxynaphthyl-[c,d]-14β,15β,20β-trihydroxy[16, 17]-cyclopentanocyclohexyl tetrahydropyran. This is a new naphthalene derivative reported from *Centratherum* or other species.

Compound **5,** designated as centratherumnaphthyl hexol, was obtained as a colorless amorphous mass from chloroform-methanol (97:3) eluents. It responded positively to $FeCl_3$ 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 ^{13}C 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 $C_{20}H_{22}O_7$. The ^1H 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 H_2-11 methylene protons. The remaining methylene and methine protons resonated between δ 2.81–1.51 ppm. The ^{13}C 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 carbinol 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 ^1H-^1H COSY spectrum of 5 showed correlations of H-6 with H-4 and H-7; H_2-11 with H-12; H-14 with H-13 and H-15; and H-19 with H_2-18 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 H_2-11; and C-19 with H_2-18 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

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

Tested material	Concentration (mg/mL)	Mean inhibition zone (mm)		
		Aspergillus flavus	*Candida albicans*	*Penicillium citrinium*
Metanolic extract	1	15	14	12
	5	17	17	15
	10	18	17	16
	20	13	18	17
Compound **4**	25	12	11	10
	40	12	12	11
	100	12	18	11
	200	13	19	12
Compound **5**	25	–	–	–
	40	11	–	11
	100	12	11	11
	200	13	12	12
Fluconazole	30	19	18	18



4. R = H, **5.** R = OH

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

derivative isolated from a natural of synthetic source.

The methanolic extract of the seeds of *C. anthelminticum* exhibited antifungal activity against *Aspergillus flavus*, *Candida albicans* and *Penicillium citrinium* 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 fluconazol 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 antifungal activity against all the fungal strains at 200 mg/mL (Table 1).

REFERENCES

1. Mhaskar K.S., Blatter E., Caius J.F.: Kirtikar and Basu' Illustrated Indian Medicinal plants, p. 1832, Sri Satguru Publications, Delhi 2000.
2. Yadava R.N., Barsainya D.: Asian J. Chem. 8, 813 (1996).
3. Akihisa T., Hayashi Y., Patterson G.W., Shimizo N., Tamura T.: Phytochemistry. 31, 1759 (1992).
4. Mehta B.K., Mehta D., Verma M.: Nat. Prod. Res. 19, 435 (2005)
5. Verma M., Deshiraju S, Jafri M., Mehta B.K.: Indian J. Chem. 43 B, 442 (2004).
6. Mehta B.K., Mehta, D., Itoriya A.: Carbohydr. Res. 339, 2871 (2004).
7. Mehta B.K., Mehta D., Itoriya A.: Nat. Prod. Res. 24, 120 (2010).
8. Singh C., Kaul B.L.: J. Med. Arom. Plant Sci. 21, 308 (1999).
9. Rajput N., Lakhani A.: J. Tropical Med. Plants 5, 2 (2005).
10. Tian G., Zhang U., Zhang T., Yang F., Ito Y.: J. Chromatogr. A 1049, 219 (2004).
11. Yadava R.N., Barsainya D.: J. Indian Chem. Soc. 74, 822 (1997).

12. Asaka Y., Kubota T., Kulkarni A.B.: Phytochemistry 36, 1838 (1997).
13. Purnima A., Koti B.C., Tikare V.P., Viswanathaswamy A.H.M., Thippeswamy A.H.M., Dabadi P.: Indian J. Pharm. Sci. 71, 461 (2009).
14. Srivastava A., Bartarya R., Tonk S., Srivastava S.S., Kumari K.M.: J. Environ. Biol. 29, 669 (2008).
15. Sharma S., Mehta B.K.: J. Hyg. Epidemiol. Microbiol. Immunol. 35, 157 (1991).
16. Singhal K.C., Sharma S., Mehta B.K.: Indian J. Exp. Biol. 30, 546 (1992).
17. Bhakuni D.S., Dhar M.L., Dhar M.M., Dhawan B.N., Mehrotra B.N.: Indian J. Exp. Biol. 11, 250 (1969).
18. Setty B.S., Kamboj V.P., Khanna N.M.: J. Exp. Biol. 7, 213 (1977).
19. Singh R.S., Sen S.P., Pandey S.N.: J. Res. Indian Med. 14, 133 (1979).

Received: 30. 12. 2010