ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 9:4 (2015) 546-552

records of natural products

Nothoapiole and *α*-Asarone Rich Essential Oils from Himalayan *Pleurospermum angelicoides* Benth

Chandra S. Mathela[1*], Rakesh K. Joshi[1], Balam S. Bisht[1] and Subhash C. Joshi[2]

[1]*Department of Chemistry, Kumaun University, Nainital-263002, Uttarakhand, India*

[2]*Jubilant Life Sciences Ltd, R&D, C-26, Sector 59, Noida, UP, India*

(Received February 18, 2012; Revised January 11, 2015; Accepted February 05, 2015)

Abstract: *Pleurospermum angelicoides* Benth., a herb used in folk medicine, was collected from near Milam glacier of Uttarakhand. Different plant parts were investigated for their terpenoid compositions, antifungal, antibacterial and antioxidant potential. Nothoapiole (5-allyl-4,6,7-trimethoxy-benzo[1,3] dioxide **1**) was observed as a single major constituent (87.3%) in the root essential oil while *α*-asarone was the major constituent of leaf and flower essential oils (23.2%, 20.7% respectively). Monoterpenes *viz.* limonene, α-pinene, γ-terpinene and perilla aldehyde were other constituents of leaf and flower oils. All the essential oils exhibited broad range of antioxidant potential. The leaf essential oil showed significant antifungal activity against *Candida albicans* and *Candida glabrata*.

Keywords: *Pleurospermum angelicoides*; Apiaceae; nothoapiole; 5-allyl-4,6,7-trimethoxy-benzo[1,3] dioxide; α-asarone; antimicrobial; antioxidant.

1. Introduction

Pleurospermum angelicoides Benth. (Apiaceae) is a herb native to India, Yunnan province, China and Nepal. The roots are used in folk medicine as antipyretic and diaphoretic agent [1]. It is locally known as "Chippi" in the Himalayan region and used for the treatment of typhoid and dysentery [2]. Previous reports have shown angelicoidenol, isocumarins and 1-propenyl 2,3,4-trimethoxybenzene as chemical constituents in *Pleurospermum angelicoides* along with antifungal activity of its essential oil against animal pathogens [3-7].

Considering the significant variation in the essential oil constituents and its commercial potential, the detailed terpenoid composition, antioxidant, antifungal and antibacterial activities of the leaf, flower and root essential oils of *Pleurospermum angelicoides* growing in sub-alpine Himalayan region has been undertaken.

2. Materials and methods

2.1. Plant material

Roots, leaves and flowers of *Pleurospermum angelicoides* were collected from adjoining areas of Milam glacier (3600 m) in August-September. Plant was identified from Botanical survey of India (BSI), Dehradun and voucher specimen *viz*. KU/ Chem/ PA/10/003 have been preserved in the Phytochemistry Lab, Chemistry Department, Kumaun University, Nainital.

* Corresponding author: E-Mail: mathelacs@gmail.com; Phone: +91 9412351488

2.2. Extraction of essential oils

Fresh plant materials (roots, leaves and flowers; 1 kg each) were subjectd to steam distillation (2 h). The distillates (saturated with NaCl) was extracted with *n*-hexane and methylene chloride. Both organic layers were separately, mixed, demoisturised over anhydrous sodium sulphate and the solvents were removed in a rotary vacuum evaporator at 30°C to get essential oils. Essential oil yields were calculated on the basis of dry weight of fresh plant materials.

2.3. GC and GC/MS analysis

The essential oils were analyzed using Nucon 5765 gas chromatograph fitted with FID and Rtx-5 column (30 m length × 0.32 mm diameter, 0.25 μm film coating) with a split ratio of 1: 48. Nitrogen was used as a carrier gas at a flow of 4 kg/cm^2. The GC/MS analysis was done on Thermo Quest Trace GC 2000 interfaced with Finnigan MAT PolarisQ Ion Trap Mass spectrometer with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 μm film coating). The column temperature was programmed 60-210°C at 3°C/min using helium as carrier gas at a flow of 1.0 mL/min. The injector temperature was maintained at 210°C, injection volume 0.1μL (1% solution in hexane) and split ratio 1:40. Mass spectra were recorded at 70 eV with a mass range of 40-450 Da.

2.4. Isolation of major constituents

The essential oils were fractionated on 600 × 25 mm silica gel (230-400 mesh) column packed in hexane. Elution was done with hexane followed by gradient with diethyl ether (1-20%). Isolation was monitored on pre-coated silica gel TLC plates. Fractions having similar pattern on TLC were mixed and concentrated.

2.5. Spectral analysis and identification

The IR spectral analysis of the pure compounds was done in Perkin Elmer FT-IR (Spectrum bx II). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-Avance 400 MHz NMR spectrometer. Multiplicities of ^{13}C NMR spectra were assigned by DEPT 135 and DEPT 90 experiments. The identification was done on the basis of Linear Retention Index determined with reference to homologous series of *n*-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL under identical experimental conditions), co-injection with standard (Sigma), MS Library search (NIST and WILEY), by comparison of the MS literature data [8] and IR and NMR of major isolates. The relative contents of components were determined by GC peak areas (FID response) without using correction factor.

2.6. Antimicrobial activity

The *in vitro* antibacterial activity of the root, leaf and flower essential oils was performed against *Salmonella typhi* (clinically isolated), *Klebsiella pneumoniae*, (MTCC-109), *E. coli* (MTCC-1610), *Staphylococcus aureus* (MTCC-96), *Streptococcus mutans* (MTCC-890) and *Bacillus subtilis* (MTCC-121). The antifungal activity of the oils was performed against *Candida albicans* (MTCC-1637) and *Candida glabrata* (MTCC-3019). The bacterial and fungal cultures were maintained on appropriate agar slants at 4°C throughout to use as stock cultures. The antibacterial and antifungal activities of the essential oils were investigated by the disc diffusion method using 24-48 h grown strains reseeded on nutrient Broth (bacterial strains) and Potato Dextrose Agar (fungal strains) [9]. The mean values of diameter of zone of inhibition (ZOI) were measured in millimeter. All experiments were performed in triplicate.

2.7. Antioxidant activity

2.7.1. Free radical scavenging activity

The free radical scavenging activity of essential oils was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical [10]. 1.0 mL of 0.1 mM DPPH solution (in ethanol) was added to 3.0 mL of essential oil solution in water at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher DPPH free radical scavenging activity. The DPPH radical concentration was calculated by the equation:

$$\text{DPPH scavenging activity (\%)} = (Ac\text{-}As)/Ac \times 100$$

where, Ac = control and As = absorbance of sample.

2.7.2. Reducing power activity

The reductive potential was measured by taking different concentrations (5, 10, 15, 20 mg/mL) of the essential oils in 1 mL of distilled water mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%) [11]. The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture which was then centrifuged for 10 min at 1000 rpm (MSE Mistral 2000, UK). The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the mixture indicated greater reductive potential.

2.7.3. Chelating activity

The chelating of ferrous ions by the essential oils and standards was estimated by the method of Dinis *et al* [12]. Essential oils were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm. The experiments were run in triplicate and averaged. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was given as:

$$\text{\% Inhibition} = (A_0\text{-}A_1)/A_1 \times 100$$

Where A_0 = the absorbance of the control and A_1 = the absorbance in the presence of the of sample/ standard.

3. Results and discussion

3.1 Terpenoid compositions

The yield (% v/w) of leaf, root and flower essential oils of *P. angelicoides* was found 1.10, 1.12 and 0.90 respectively. A total of 40 compounds were identified in GC and GC/MS analysis of the leaf, root and flower essential oils which represented a range of 97.8% to 99.2% of the total composition. The identified constituents are listed in Table 1 in order of their elution pattern in Rtx-5 column.

The root essential oil was found to be rich in phenylpropanoids (90.8%) dominated by nothoapiole (87.3%; Figure 1) as the single major constituent. Nothoapiole **1** was isolated and characterized followed by comparison with previously reported spectral data [13-15]. This highly oxygenated phenylpropanoid, previously reported in a few *Carum* species is structurally and biogenetically related to myristicin, apiole and dill-apiole [13,16]. The latter are widespread in Apiaceae herbs, but nothoapiole is rare. The presence of nothoapiole in the root essential oil of *P. angelicoides* is not surprising which could have biosynthetized by successive enzymatic methoxylations of other related phenylpropanoids [16]. The major constituents in the leaf and flower oils were limonene (48.4%, 14.8%) and α-asarone (23.2%, 20.7%) along with minor presence of β-asarone (4.8%, 1.7%) followed by α-pinene and perilla aldehyde. Occurrence of α-asarone as major constituent in the leaf and flower parts of *P. angelicoides* is significant.

3.2. Antifungal/ antibacterial activity

The leaf, root and flower essential oils of *P. angelicoides* showed a wide range of antifungal and antibacterial potential (Table 2-3).

The leaf essential oil showed significant antifungal activity against *Candida albicans* (zone of inhibition, 16 mm) and *Candida glabrata* (zone of inhibition, 11 mm) comparable with amphotericin B under identical conditions. The root essential oil exhibited comparable antifungal activity against *Candida albicans* (12.0 mm) followed by flower essential oil (zone of inhibition, 10.0 mm). The flower essential oil showed minimum activity against *Candida glabrata* while root essential oil was found to be ineffective.

The leaf, root and flower essential oils exhibited moderate antibacterial activities against one or more among the tested bacteria. All the essential oils were found to be effective against *S. mutans* (6.0-8.0).

Table 1. Essential oil composition of *Pleurospermum angelicoides* Benth.

Compound	LRI	Leaf (%)	Flower (%)	Root (%)
α-pinene	941	0.2	**22.3**	t
camphene	946	0.3	0.2	-
sabinene	978	1.2	1.0	-
β-pinene	982	0.7	3.0	-
myrcene	994	-	0.3	-
p-cymene	1029	0.1	0.4	0.1
limonene	1034	**48.4**	**14.8**	0.1
1,8-cineole	1038	t	0.7	t
(*E*)-β-ocimene	1050	2.6	t	-
γ-terpinene	1064	**11.0**	0.3	
terpinolene	1086	t	0.1	-
(*E*)-sabinene hydrate	1098	0.1	t	-
linalool	1101	t	0.1	-
(*Z*)-*p*-menth-2-en-1-ol	1121	t	0.2	-
borneol	1167	t	0.1	-
terpinen-4-ol	1180	t	0.2	-
(*Z*)-anethole	1249	t	0.1	t
p-anisaldehyde	1250	t	4.5	t
perilla aldehyde	1269	3.3	**16.8**	0.3
bornyl acetate	1285	0.2	7.5	-
(*E*)-anethole	1287	0.2	1.8	-
δ-elemene	1335	-	0.1	-
α-copaene	1374	-	0.2	-
β-elemene	1389	0.2	0.1	-
β-caryophyllene	1418	0.2	0.2	-
β-gurjunene	1428	-	0.1	-
(*E*)-α-bergamotene	1432	t	0.1	-
(*Z*)-β-farnesene	1440	-	0.2	-
germacrene D	1481	t	0.1	1.5
α-muurolene	1500	0.2	0.1	4.8
cubebol	1514	0.8	0.1	t
δ-cadinene	1522	t	t	0.2
elemicin	1555	0.7	t	t
(*E*)-isoelemicin	1568	t	t	0.5
(*Z*)-isoelemicin	1569	t	t	1.7
caryophyllene oxide	1581	0.4	t	-
β-asarone	1616	4.8	1.7	1.2
10-*epi*-γ-eudesmol	1622	t	0.2	-
α-asarone	1675	**23.2**	**20.7**	0.1
nothoapiole	1740	0.4	t	**87.3** (**1**)
Monoterpene hydrocarbons		64.5	42.4	0.2
Oxygenated monoterpenes		3.6	30.1	0.3
Sesquiterpene hydrocarbons		0.6	1.2	6.5
Oxygenated sesquiterpenes		1.2	0.3	-
Phenylpropanoids		29.3	24.3	90.8
Total identified		**99.2**	**98.3**	**97.8**

LRI= Linear retention indices, t = trace

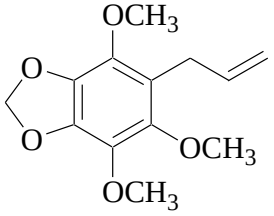

Figure 1. Nothoapiole **(1)**

Table 2. Antifungal activity of *P. angelicoides* essential oils.

Essential oil/ standard	*Candida albicans*	*Candida glabrata*
Flower oil	10.0 ± 0.18	8.0 ± 0.08
Leaf oil	16.0 ± 0.22	11.0 ± 0.22
Root oil	12.0 ± 0.12	na
Amphotericin B (20µg)	16	11

(40µg of oil/well), values are mean of three replicates (± SD)

Table 3. Antibacterial activity of *P. angelicoides* essential oils.

Essential oil/ standard	*S. typhi*	*K. pneumoniae*	*E. coli*	*S. aureus*	*S. mutans*	*B. subtilis*
Flower oil	8.0 ± 0.20	7.0 ± 0.22	na	na	6.0 ± 0.14	na
Leaf oil	na	9.0 ± 0.20	na	8.0 ± 0.18	8.0 ± 0.21	8.0 ± 0.16
Root oil	7.0 ± 0.18	na	9.0 ± 0.11	na	7.0 ± 0.13	na
Chloramphenicol (10µg/disc)	25	25	21	22	30	24

(10 µg of oil/ disc; ZOI in mm), na = not active, values are mean of three replicates (± SD)

3.3. Antioxidant activity

The leaf, root and flower essential oils of *P. angelicoides* showed antioxidant potential as evidenced by DPPH scavenging, reducing power and chelating power (Table 4-6). The flower essential oil was found to possess the highest DPPH free radical scavenging power (61.66%) at the lowest concentration followed by the leaf and root essential oils (51.34 and 7.266%). The highest reducing power was shown by the root essential oil (0.525 mg/mL) as compared to other tested samples against the standard linoleic acid. The highest chelating power was shown by the leaf essential oil (91.75%) which is quite comparable to the standard EDTA (89.32%) at its lowest concentration.

Table 4. DPPH free radical scavenging activity of *P. angelicoides* essential oils.

Essential oil/ standard	5 mg	10 mg	15 mg	20 mg
Flower oil	61.66 ± 2.54	63.52 ± 2.40	68.30 ± 2.92	68.58 ± 3.45
Leaf oil	51.34 ± 3.20	66.57 ± 3.12	69.20 ± 3.08	86.43 ± 2.68
Root oil	7.266 ± 1.86	92.38 ± 1.70	51.55 ± 2.55	64.70 ± 1.98
BHT	93.60	94.50	97.30	97.70
Catechin	91.90	92.46	94.10	94.70
Gallic acid	84.48	85.65	85.93	85.95

values are mean of three replicates (± SD)

Table 5. Reducing power activity of *P. angelicoides* essential oils.

Essential oil/ standard	5 mg	10 mg	15 mg	20 mg
Flower oil	2.077 ± 0.22	2.291 ± 0.30	2.633 ± 0.58	2.912± 0.90
Leaf oil	2.077 ± 0.16	2.730 ± 0.28	3.031 ± 0.62	3.515± 0.88
Root oil	0.525 ± 0.18	0.678 ± 0.26	0.749 ± 0.56	0.830± 0.78
BHT	2.303	2.413	2.456	2.497
Gallic acid	4.000	4.000	4.000	4.000
Linoleic acid	0.373	0.379	0.395	0.411
Catechin	4.000	4.000	4.000	4.000

values are mean of three replicates (± SD)

Table 6. Chelating power activity of *P. angelicoides* essential oils.

Essential oil/ standard	5 mg	10 mg	15 mg	20 mg
Flower oil	81.24 ± 2.48	81.77 ± 2.28	82.87 ± 3.60	84.74 ± 3.32
Leaf oil	91.75 ± 2.22	95.55 ± 1.88	99.22 ± 3.90	99.77 ± 2.96
Root oil	81.84 ± 3.10	80.32 ± 3.20	81.49 ± 3.44	81.94 ± 2.58
EDTA	89.32	87.63	88.04	88.11
Citric acid	86.18	87.04	89.36	89.56

values are mean of three replicates (± SD)

4. Conclusion

The essential oil composition of different plant parts of *P. angelicoides* revealed it as a rich source of phenylpropanoids *viz*. nothoapiole and α-asarone. The leaf essential oil showed significant antifungal activity against *C. albicans* and *C. glabrata* comparable with the standard. The oils also showed a broad range of antioxidant activities.

Acknowledgements

The authors are grateful to Council of Scientific and Industrial Research, New Delhi (E.S. Scheme) for financial support and BSI Dehradun for plant identification. Authors are also grateful to Prof. A.K. Pant and Dr. Om Prakash, Chemistry Department, CBSH, GBPUA & T, Pantnagar, for bioactivity support.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] J. D. Hooker (1889). Flora of British India Vol. II, L. Reeves, Kent, pp.103.
[2] R. Shan (1979). Flora Reipublicae Popularis Sinicae, Science Press, China, 55, pp. 152-153.
[3] S.K. Uniyal, A. Awasthi and G.S. Rawat (2002). Current status and distribution of commercially exploited medicinal and aromatic plants in upper Gori valley, Kumaon Himalaya, Uttaranchal, *Curr. Sci.* **82**, 1246-1252.
[4] U. Mahmood, S.B. Singh and R.S. Thakur (1983). Angelicoidenol, a bicyclic monoterpene from the seeds of *Pleurospermum angelicoides*, *Phytochemistry* **22**, 774-776.
[5] M. Shibano, H. Naito, M. Taniguchi, N. Wang and K. Baba (2006). Two isocoumarins from *Pleurospermum angelicoides*, *Chem. Pharm. Bull.* **54**, 717-718.
[6] A. Dikshit and A. Husain (1984). Antifungal action of some essential oils against animal pathogens, *Fitoterapia*, **55**, 171-176.
[7] U. Mahmood and R.S.Thakur (1981). Chemical constituents of *Pleurospermum angelicoids*, *Ind. Jour. Pharm. Sci*. **43**, 157-158.
[8] R.P. Adams (1995). Identification of Essential Oil Components by Gas Chromatography /Mass Spectroscopy, Allured publishing Co. Carol Stream, Illinois.
[9] J.L. Rios, M.C. Recio and A. Vilar (1988). Screening methods for natural products with antimicrobial activity: A review of the literature, *J. Ethnopharmacol.* **23,** 127-149.
[10] K. Shimada, K. Fujikawa, K. Yahara and T. Nakamura (1992). Antioxidative properties of xnthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J. Agri. Food Chem.* **40**, 945-948.
[11] M. Oyaizu (1986). Studies on product of browning reaction prepared from glucose amine, *Jap. J. Nutr.* **44**, 307-315.
[12] T. Dinis, V. Madeira and T. Almeida (1994). Action of phenolic derivative (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, *Arch. Biochem. Biophy*. **315**, 161-169.
[13] H. Laouer, E.K. Meriem, S. Prado and N. Baldovini (2009). An antibacterial and antifungal phenylpropanoid from *Carum montanum* (Coss. et Dur.) Benth. et Hook. *Phytother. Res.* **23**, 1726-1730.

[14] A. Rahman, M.I. Choudhary, S. Hayat, A.M. Khan, A. Ahmed and S. Malik (1999). Spatozoate and varninasterol from the brown alga *Spatoglossum variabile*, *Phytochemistry* **52**, 495-499.
[15] V. Formacek and K. H. Kubeczka (1982) "Essential Oils Analysis by Capilary Gas Chromatography and Carbon'13 NMR Spectroscopy, Willey Heyden, U K., pp.316.
[16] M. Ito, M. Toyoda, A. Yuba and G. Honda (1999). Genetic analysis of nothoapiole formation in *Perilla frutescens*, *Biol. Pharm. Bull.* **22**, 598-601.