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SHORT COMMUNICATION

CHEMICAL COMPOSITION, ANTIMICROBIAL AND TOPICAL ANTI-INFLAMMATORY ACTIVITY OF ESSENTIAL OIL OF *AMOMUM SUBULATUM* FRUITS

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Amomum is a genus of terrestrial, rhizomatous herb, distributed chiefly in Africa and tropical Asia, found in the eastern Himalayas and cultivated in Nepal, northern West Bengal, Sikkim and Assam hills (1). In folk medicine, the seeds are credited with stimulant, stomachic, alexipharmic and astringent properties, and are prescribed for the treatment of indigestion, vomiting, biliousness, abdominal pains and rectal diseases. They promote elimination of bile and are useful in congestion of liver; they are also used in gonorrhea. The pericarp is useful in headache and heals stomatitis. The aromatic oil extracted from the seeds is applied to the eyes to allay inflammation (1).

The seeds contained the glycosides petunidin 3,5-diglucoside and leucocynidin-3-O-β-D-glucopyranoside, and a new aurone glycoside, subulin. The presence of chalcone, cardamonin and a flavanone, alpinetin was also reported (1). The seeds on steam distillation yield a dark brown, mobile essential oil [2.5%] having a characteristic odor of cineol (1). Essential oil of *A. subulatum* has been studied extensively for its composition and its antimicrobial potential (2–6). Nigam and Purohit (7) obtained 2.5% oil from the seeds and fractionated the oil into different cineole-rich fractions. Lawrence (8) separated the components of the oil by preparative gas chromatography and identified them by their IR spectra and retention data and found the major component 1,8-cineole in 74%. Patra et al. (9) studied the oil by packed column GC and reported that it contains sabinene (9.1%), γ-terpinene (16.2%), 1,8-cineole (63.3%) as major components. In a latter study, Gupta et al. (10) have analyzed oils derived from different strains of *A. subulatum* growing wild in Sikkim and found 1,8-cineole content to vary from 77 to 89%. The oil and volatile concentrate produced by liquid carbon dioxide extraction of *A. subulatum* have been compared (11). More recently Gurudutt et al. (12) analyzed the oil using GC/MS and identified 25 components of which 16.3% were monoterpene hydrocarbons and 75.3% were of oxygenated monoterpenes with 1,8-cineole (61.3%), being the major component.

In the current study, chemical composition of essential oil isolated from fruits of *A. subulatum* obtained from Himachal Pradesh, India was determined and its potential as antimicrobial and anti-inflammatory agent has been studied.

EXPERIMENTAL

Amomum subulatum (dried fruits) was procured in October 2009 from the local market of Sunder Nagar, Distt. Mandi, Himachal Pradesh, India. The crude drug was identified and authenticated at National Bureau of Plant and Genomic Research (NBPGR), New Delhi. A specimen of the crude drug was submitted at NBPGR (Voucher number: EP 532).

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The air dried plant material (500 g) was hydrodistilled in an all-glass apparatus according to the method recommended by the British Pharmacopoeia, 2007. The pale yellow oil was dried over anhydrous sodium sulfate and stored at 4°C in the dark. The yield was 0.8% based on the dry weight of sample.

Analytical GC with FID detector was carried out on a Varian 3300 gas chromatograph fitted with Silicone DB-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was helium at a flow rate of 1.5 mL/min and with split mode, temperature programmed from 80 to 225°C at a rate of 4°C/min. The injector and detector temperature were 280°C and 300°C, respectively. The injection volume was 0.1 µL for all the samples.

Analytical GC-MS was carried out on a Shimadzu QP-2000 instrument at 70 eV and 250°C. GC column: Ulbon HR-1, fused silica capillary (50 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was helium at a flow rate of 2 mL/min. The initial temperature was 100°C for 6 min, rising at a rate of 10°C/min to 250°C.

Most constituents were identified by GC comparing their retention indices with those of authentic standards available or with retention indices in close agreement with reference (13). Further identification was achieved by GC-MS. The fragmentation

Table 1. Chemical composition of essential oil of *Amomum subulatum* fruits.

No.	Component	RI	Area (%)
1	n-Hexane	601	6.5
2	2-Methylheptane	662	9.1
3	n-Heptane	698	6.2
4	n-Non-2-ene	732	6.5
5	2-Methylnonane	901	7.2
6	n-Octanol	960	4.8
7	n-Nonanol	1015	3.9
8	n-Decanol	1260	3.8
9	n-Dodecanol	1473	3.8
10	n-Tridecanol	1480	4.0
11	n-Tetradecanol	1510	3.8
12	n-Hexadecane	1597	3.6
13	n-Heptadecanol	1701	3.3
14	n-Octadecane	1796	3.3
15	n-Nonadecane	1885	2.7
16	Hexadecanoic acid	1972	2.5
17	n-Eicosane	1993	3.1
18	Arachidic acid	1999	1.9
19	n-Docosane	2005	1.8
20	n-Tricosane	2290	1.0
21	n-Tetracosane	2325	0.8
22	n-Tetracosanoic acid	2350	8.0
23	Caryophylladienol I	2365	0.6
24	9-Epi-caryophyll-1(12),8(15) diene-14-ol	2385	4.7
25	Caryophylladienol II	2390	2.4
26	Caryophyllenol acetate	2425	0.7
	Total		100.0

Table 2. Antimicrobial study of essential oil of *Amomum subulatum* fruits.

No.	Microorganism	Zone of inhibition (mm)	
		STD	ASO
1	*Bacillus pumilus*	17	20
2	*Bacillus subtilis*	18	17
3	*Staphylococcus aureus*	15	15
4	*Micrococcus luteus*	17	14
5	*Staphylococcus epidermidis*	20	20
6	*Escherichia coli*	20	18
7	*Pseudomonas aeruginosa*	16	16
8	*Candida albicans*	19	15
9	*Aspergillus niger*	20	17
10	*Saccharomyces cerevisiae*	16	16

ASO = *Amomum subulatum* essential oil, STD = standard: for bacteria – ciprofloxacin, for fungi – fluconazole.

Table 3. Topical anti-inflammatory study of essential oil of *Amomum subulatum* fruits.

Treatment	T = 0 (μm)	T = 30 (μm)	Δ T	%I
Control	168.33 ± 0.25	373.33 ± 0.26	205	–
AS oil	170 ± 0.34	205 ± 0.3	35*	82.93
Diclofenac	158.33 ± 0.35	185 ± 0.29	26.67*	87.5

All the results were expressed as the mean ± standard error (SEM). Data were analyzed using one way ANOVA followed by Dunnett's multiple comparison test, *$p < 0.01$. – indicates no activity.

patterns of mass spectra were compared with those stored in the spectrometer database, using the NBS 54 KL and Wiley, L-built libraries and with those published in the literature (13–19). Other constituents were tentatively identified by means of GC-MS. The relative amounts of individual components are based on peak areas obtained without FID response factor correction. The retention indices were obtained from gas chromatograms by logarithmic interpolation between bracketing n-alkanes. The homologous series of n-alkanes (C_8 to C_{22}; Polyscience Inc.; Niles; USA) were used as standards.

The antimicrobial activity was carried out by disc diffusion technique (20). The filter paper discs of 6 mm diameter were prepared using Whatman filter paper no. 1. The discs were sterilized by autoclaving for 20 min at 15 lbs pressure. Then, the discs were soaked in essential oil (1 mg/disc) in methanol. The Petri dishes, antibiotic assay medium 1 (for bacteria) and potato dextrose agar medium (for fungi) were sterilized by autoclaving. Microorganisms like: *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus epidermidis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungal strains *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae* were used for the study. One day old bacterial culture (1 mL) was added to the sterilized medium. The cultures were inoculated, stirred well and this medium was added in Petri dishes. The filter paper discs impregnated with essential oil were placed aseptically and carefully on the solidified medium. The plates were kept in refrigerator for proper diffusion of essential oil. Then, the plates were incubated at 37°C for 24 h. and 28°C for 72 h. for bacteria and fungi, respectively. Afterwards, the plates were observed for clear zone of inhibition. The results obtained were compared with the control plates (i.e., plates having discs impregnated with methanol only) and standard drugs used (i.e., ciprofloxacin 10 μg/plate and fluconazole 20 μg/plate for bacteria and fungus, respectively). The experiment was repeated thrice

and a mean of the results obtained have been presented.

Topical anti-inflammatory activity was done by using xylene induced ear edema model (21). Edema was induced on the right ear of mice of either sex (18–24 g body weight) by topical application of xylene (20 µL) dissolved in 20 µL acetone (10 µL per ear) on both surfaces. The essential oil, dissolved in 20 µL acetone, was applied on the same ear 30 min before and simultaneously with the inflammatory agent, xylene. The thickness of the ears were measured using a digital vernier calliper, prior to the experiment (t = 0) and 30 min after (t = 30) induction of inflammation. Edema was expressed as the increase in ear thickness due to the inflammatory challenge and edema inhibition was expressed as the percent thickness reduction referred to the control group. The experiment was repeated thrice and a mean of the results obtained have been presented.

RESULTS AND DISCUSSION

The hydrodistillation of fruits of *A. subulatum* gave golden yellow colored oil, having strong odor with a yield of 0.8 mL per 100 g. The compounds identified and their relative proportions are listed in Table 1 according to their order of elution on silicon DB column. About 26 compounds were detected in the volatile oil.

Among 26 volatile oil components, there were 12 aliphatic hydrocarbons (51.8%), seven aliphatic alcohols (27.4%), three fatty acids (12.4%) and four sesquiterpenoids (8.4%).

The major aliphatic hydrocarbons were 2-methylheptane (9.1%), 2-methylnonane (7.2%), n-hexane (6.5%), n-non-2-ene (6.5%) and n-heptane (6.2%). n-Octanol (4.8%), n-nonanol (3.9%), n-decanol (3.8%), n-dodecanol (3.8%), n-tridecanol (4.0%) and n-tetradecanol (3.6%) occurred as the main aliphatic alcohols. n-Tetracosanoic acid (8.0%) was the predominant fatty acid followed by hexadecanoic acid (2.5%) and arachidic acid (1.9%). The predominant sesquiterpenoids were 9-epi-caryophyll-1(12),8(15)-dien-14-ol (4.7%) and caryophylladienol II (2.4%).

Table 2 shows results for antimicrobial activity. No zone of inhibition was seen in control plates suggesting a lack of antimicrobial activity of methanol itself. The essential oil isolated from fruits of *A. Subulatum* was found more active than standard drug used in case of *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae*.

Topical application of essential oil to the mouse ear resulted in potent suppression of acute edema induced by xylene (Table 3). Xylene causes instant irritation of the mouse ear, which leads to fluid accumulation and edema characteristic of the acute inflammatory response. Suppression of this response is likely indication of antiphlogistic effect. The topical anti-inflammatory effect suggests that constituents of the essential oil may relieve rheumatism and offer the additional advantage of suppressing the inflammatory response initiated by tissue injury (22). In case of essential oil isolated from *A. subulatum*, the topical anti-inflammatory activity was found slight less but comparable with standard drug used (diclofenac).

CONCLUSION

In conclusion, the volatile oil of *A. subulatum* was composed mainly of twelve aliphatic hydrocarbons, seven aliphatic alcohols, three fatty acids and four sesquiterpenoids. The major oil components were 2-methylheptane (9.1%), n-tetracosanoic acid (8.0%) and 2-methylnonane (7.2%). The results of the present study provide evidence for antimicrobial and topical anti-inflammatory activity of essential oil isolated from fruits of *A. subulatum*.

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