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Compositional Characters and Antimicrobial Potential of *Artemisia stricta* Edgew. f. *stricta* Pamp. Essential Oil

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Abstract: Chemical and biological investigations were carried out to evaluate the composition and anti-microbial potential of a rare *Artemisia* species viz. *Artemisia stricta* Edgew. f. *stricta* Pamp. essential oil for the first time. GC and GC/MS analysis resulted in the identification of 27 compounds, which constituted 93.2% volatile constituents of the oil. The major constituents were capillene (41.6%), spathulenol (14.6%) and β-caryophyllene (13.4%). The oil was also assayed to determine its antimicrobial potential against eight bacterial and six fungal strains. The oil exhibited both antifungal and antibacterial activities. Among bacteria, the oil was most effective against S*taphylococcus epidermidis* (MIC 0.625 mg/mL) followed by S*taphylococcu. Aureus* (MIC 1.25 mg/mL). While among fungi, the oil was most effective against *Aspergillus flavus* followed by *Aspergillus niger* and *Sporothrix schenckii* with MIC as low as 0.625 mg/mL.

Keywords: *Artemisia stricta*; Asteraceae; Essential oil; Capillene; Antimicrobial activity.

1. Introduction

Plants of family Asteraceae possess both aromatic and medicinal properties. *Artemisia* is one of the largest and most widely distributed temperate aromatic genus of this family and about 37 species have been reported from India [1]. Several plants of this genus are important source of essential oil and commercially valued for a number of aroma-chemicals. Oils from these plants also exhibit diverse biological properties including anti-microbial and anti-inflammatory activities and are used in the preparation of different creams, soaps and toothpastes [2]. A number of *Artemisia* species are also used in folk [3] and modern systems of medicines [4]. The oil constituents of several *Artemisia* species have been studied in different parts of the world, both from wild (*in-situ*) and domesticated (*ex-situ*) plants [5-10]. In addition, studies have also been undertaken on the analysis of the biological activities of different species [11-13].

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However, so far no such studies have been undertaken on *A.stricta* Edgew. f. *stricta* Pamp, a rare species, which grows as small erect herbs usually upto 30 cm tall and possesses greenish white capitula. These plants are found in Western Himalayas from Ladakh to Uttarakhand between the altitudes of 3000-5000 m [1]. The present paper reports the composition of *A. stricta* essential oil and its antimicrobial activity.

2. Materials and Methods

2.1. Plant material

The aerial parts of *Artemisia stricta* Edgew. f. *stricta* Pamp. were collected from the Auli, Garhwal region of Himalayas in between 30º33′N, 79°37′E, 3052 m (India) in August 2010. The authenticity of the plant was confirmed in Department of Botany and Pharmacognosy, CIMAP, Lucknow and a voucher specimen (Acc no. 9427) was submitted in the herbarium of the department.

2.2. Extraction procedure

The dried plant material (400g) was subjected to hydro distillation in a Clevenger-type apparatus for two hours. The oil was then dried over anhydrous Na_2SO_4 and stored at 4°C prior to analysis.

2.3. Capillary GC and GC/MS

For capillary GC analysis, a PerkinElmer Auto System XL was used fitted with an Equity-5 column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven column temperature ranged from 70–250 °C, programmed at 3 °C/min, with initial and final hold time of 2 min, using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:30, an injection size of 0.03 µL neat, and injector and detector (FID) temperatures were 250°C and 280°C, respectively. GC/MS utilized a Perkin Elmer Auto System XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven temperature program was the same as described in capillary GC; injector, transfer line and source temperatures were 250°C; injection size 0.03 µL neat; split ratio 1:30; carrier gas He at 10 psi constant pressure; ionization energy 70 eV; mass scan range 40-450 amu. Characterization was achieved on the basis of retention time, Kovats Index, relative retention index using a homologous series of *n*-alkanes (C8-C25 hydrocarbons, Polyscience Corp. Niles IL), coinjection with standards in GC-FID capillary column (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data [14]. The relative amounts of individual components were calculated based on GC-FID peak area without using correction factors.

2.4. Microorganisms and medium

The antimicrobial activity of the essential oil was analyzed using strains procured as microbial type culture collections (MTCC) from the Institute of Microbial Technology, Chandigarh and All India Institute of Medical Sciences (AIIMS), New Delhi, India. The bacterial strains used were *Staphylococcus aureus*(MTCC96), *Enterococcus faecalis* (MTCC439), *Klebsiellapneumoniae* (MTCC109), *Staphylococcus epidermidis* (MTCC435), *Streptococcus mutans* (MTCC890), *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC723) and *Bacillus subtilis* (MTCC121), while the fungal strains used in the assays were *Cryptococcus neoformans* (AIIMS), *Candida albicans* (MTCC), *Aspergillusflavus* (AIIMS), *Sporothrixschenckii* (AIIMS), *Aspergillusniger* (AIIMS) and *Trichophytonrubrum* (AIIMS). Streptomycin for bacteria and Amphotericin for fungi were used as positive controls while DMSO was used as a negative control.

Table 1. Relative percentage composition of constituents in *Artemisia stricta* Edgew. f. *stricta* Pamp. essential oil.

Constituents	RI *	Percentage (SD)	MOI
α- pinene	927	0.9 ± 0.06	a,b,c
camphene	950	0.1 ±0.06	a,b,c
sabinene	974	2.1 ± 0.15	a,b,c
β- myrcene	989	6.3 ± 0.20	a,b
ρ-cymene	1026	3.2 ± 0.10	a,b,c
1,8-cineole	1034	0.7 ± 0.10	a,b
γ- terpenene	1061	0.4 ± 0.06	a,b,c
linalool	1102	0.6 ± 0.01	a,b,c
terpinene-4-ol	1180	*t*	a,b,c
β- citronellol	1231	0.2 ± 0.12	a,b,c
geranyl acetate	1387	0.4 ± 0.10	a,b
methyl eugenol	1410	0.1 ± 0.03	a,b,c
β- caryophyllene	1428	13.4 ± 0.10	a,b,c
α- humulene	1463	0.3 ± 0.06	a,b
germacrene-D	1490	0.2 ± 0.06	a,b
capillene	1499	41.6 ± 0.09	a,b
γ- cadinene	1521	0.2 ± 0.07	a,b
(*E*)-nerolidol	1562	2.8 ± 0.10	a,b
spathulenol	1592	14.6 ± 0.15	a,b
globulol	1577	0.3 ± 0.03	a,b
caryophyllene oxide	1599	2.1 ± 0.05	a,b,c
humelene epoxide	1606	0.8 ± 0.06	a,b
epi-*α*- muurolol	1654	0.5 ± 0.10	a,b
α- cadinol	1652	0.4 ± 0.07	a,b
bisabolol	1683	0.2 ± 0.08	a,b
farnesol	1722	0.1 ± 0.03	a,b,c
(*E*)- phytol	1949	0.7 ± 0.12	a,b
Monoterpene hydrocarbon		13.0%	
Oxygenated monoterpene		2.0%	
Sesquiterpene hydrocarbon		14.1%	

Oxygenated sesquiterpene	21.8%
Oxygenated diterpene	0.7%
Non-terpenoid	41.6%
Total	**93.2%**

*Retention Index (RI) on Equity-5 capillary columns using a homologous series of n-alkanes (C8-C25 hydrocarbons, Polyscience Corp. Niles IL), t:trace <0.1%, a:retention time, b:MS (GC-MS), c:co injection with sigma Standard, retention Index and elution order were used to confirm the identity of each constituent. SD Standard deviation (±). MOI: Mode of identification.

2.5. Disc diffusion assay

Antifungal and antibacterial disc diffusion assays were carried out following the method as described by Bauer et al. [15]. All strains were sub cultured from -80ºC stock culture into 5 mL Mueller-Hinton broth for bacteria, and Sabouraud dextrose broth (SDB) for fungi (Hi-Media) respectively, and incubated for 24 h at the desired temperatures. The turbidity was adjusted equivalent to 0.5 McFarland standards (approximately 1.5 x 108 cfu/mL), as described in NCCLS [16] protocols. Aliquots (100 µL) of inoculums were spread over the surface of nutrient agar plate with a sterile glass spreader. Five µL of oil was put on the paper disc (5 mm diameter, Whatman filter paper no.3); air-dried and then placed on the pre-made fungal and bacterial growths. The plates were incubated at 37ºC for 24 h for bacteria and at 28ºC for 48 h for fungi. The activity was measured in terms of zone of microbial growth inhibition. First, the diameter of the total zone was measured (including disc). Then the diameter of the disc was subtracted from the total zone to obtain the net zone of growth inhibition. The tests were performed in triplicate to confirm the findings. The net zone of growth inhibition above 10 mm was considered as highly active, 4-10 mm moderately active and less than 4 mm either weakly active or inactive.

2.6. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) was determined by two-fold serial dilution broth assay as described by Petersdorf and Sherris [17], and National Committee for Clinical Laboratory Standards and as described in our earlier publications [18,19], in a 96 well microplate. The oil was diluted into a final concentration of 10 to 0.625 mg/mL. The micro titre plates were inoculated with 10 µL of diluted 24 h grown culture of test organism with a titre equivalent to 0.5 McFarland standards. The inoculated microtitre plates were then incubated at 37°C for 24 hours for bacteria, and 28ºC for 48 h for fungi. The growth was recorded spectrophotometrically at 600 nm using a Spectramax 190-microplate reader (Molecular Devices, CA, and USA). The MIC values were taken as the lowest concentration of the test oil in the wells of the microtiter plate that showed no turbidity after 24 h of incubation at 37ºC for bacteria, and 28ºC for 48 h for fungi. The MFC and MBC values were detected from the turbidimetric data as the lowest concentration of oil where 99% of killing was observed. The MIC, MBC and MFC values reported are a mean of three experiments in replicate.

2.7. Statistical analysis

Data was subjected to analysis of variance (ANOVA). The statistical analysis was done using GenStat® Release 7.21. The least significant difference (LSD) at 5% level was used to compare the means of different test parameters. *p-values* < 0.05 were considered significant.

Table 2. Minimum inhibitory concentration (MIC) of *Artemisia stricta* essential oil against pathogenic bacterial strains.

Bacterial strains	***A. stricta* essential oil (mg/mL)**		**Streptomycin (µg/mL)**	
	ZOI ± SD	**MIC (MBC)**	**ZOI ± SD**	**MIC (MBC)**
SA 96	07 ± 0.25	1.25 (3.5)	09 ± 0.10	12.5 (25)
EF 439	3.5 ± 0.06	5 (5)	10 ± 0.15	25 (50)
KP 109	05 ± 0.10	2.5 (2.5)	09 ± 0.15	12.5 (12.5)
SE 435	11 ± 0.06	0.625 (1.25)	09 ± 0.06	25 (50)
SM 890	05 ± 0.25	1.25 (2.5)	13 ± 0.12	12.5 (25)
PA 741	03 ± 0.17	5 (10)	07 ± 0.09	25 (25)
EC 723	2.5 ± 0.15	>10	09 ± 0.10	12.5 (25)
BS 121	ND	>10	18 ± 0.06	1.6 (3.125)

ZOI: Zone of Inhibition (mm), MIC: Minimum Inhibitory Concentration, *SA* 96: *Staphylococcus aureus, EF 439: Enterococcus faecalis, KP 109: Klebsiella pneumonia, SE435: Staphylococcus epidermidis, SM890: Streptococcus mutans,PA* 741: *Pseudomonas aeruginosa, EC 723: Escherichia coli, BS121: Bacillus subtilis.*

Table 3. Minimum inhibitory concentration (MIC) of *Artemisia stricta* essential oil against fungal strains.

Bacterial strains	***A. stricta* essential oil (mg/mL)**		**Streptomycin (µg/mL)**	
	ZOI ± SD	**MIC (MFC)**	**ZOI ± SD**	**MIC (MFC)**
CN AIIMS	06 ± 0.06	5.0 (10)	11 ± 0.06	1.56 (3.125)
CA MTCC	06 ± 0.21	5.0 (5)	06 ± 0.16	1.56 (3.125)
AF AIIMS	18 ± 0.06	0.625 (1.25)	04 ± 0.12	3.12 (6.25)
SS AIIMS	12 ± 0.15	0.625 (1.25)	04 ± 0.12	3.12 (6.25)
AN AIIMS	13 ± 0.10	0.625 (0.625)	06 ± 0.21	1.56 (3.125)
TR AIIMS	10 ± 0.06	5.0 (5)	03 ± 0.15	1.56 (3.125)
CN AIIMS	06 ± 0.06	5.0 (10)	11 ± 0.06	3.12 (6.25)
CA MTCC	06 ± 0.21	5.0 (5)	06 ± 0.16	3.12 (6.25)

ZOI: Zone of Inhibition (mm), MIC: Minimum Inhibitory Concentration, *CN* AIIMS: *Cryptococcus neoformans, CA* MTCC: *Candida albicans, AF* AIIMS: *Aspergillus flavus, SS* AIIMS: *Sporothrix schenckii, AN* AIIMS: *Aspergillus niger, TR* AIIMS: *Trichophyton rubrum.*

3. Results and Discussion

The oil yield of *Artemisia stricta* f. *stricta* was 0.46% (v/w) on dry weight basis. The results of the qualitative and quantitative oil analyses listed in order of elution in Equity-5 fused silica capillary column are shown in Table 1. In total, 27 compounds were identified, accounting for 93.2% of its volatile constituents. It was observed that non-terpenoid constituent comprised the major portion (41.6%) of oil followed by oxygenated sesquiterpene (21.8%), sesquiterpene hydrocarbon (14.1%) and monoterpene hydrocarbon (13.0%). While, total oxygenated monoterpenes, and oxygenated diterpene comprised only minor fraction (2.7%) of the oil.

The major constituent of the oil was capillene which constituted 41.6% of the total aroma components, followed by spathulenol (14.6%), *β*-caryophyllene (13.4%) and *β*-myrcene (6.3%).Other important oil constituents were *p*-cymene (3.2%), caryophyllene oxide (2.1%) and sabinene (2.1%). The major constituent of this oil, capillene, has the potential to be used as a starting material for the synthesis of several bioactive molecules [6]. It also reported to possess anti-feedant activity against certain insect larvae [13] and has the potential to be used as fumigants.

When tested for antimicrobial activity, the oil was observed to possess effective antifungal and antibacterial properties. Out of eight bacterial strains, the oil exhibited activity against seven strains where it was found to be very effective against *Staphylococcus epidermidis* and moderately against by *Staphylococcus aureus* followed by *Streptococcus mutans*. The mean readings derived from three independent experiments are given in Table 2. On the other hand, oil of this plant showed antifungal activity against all the six test fungi but activity against four fungi viz. *Aspergillus flavus*, *Aspergillus niger*, *Sporothrix schenckii* and *Trichophyton rubrum* was very high (Table 3). It strongly inhibited the growth of *Aspergillus flavus* and *Sporothrix schenckii* giving values of MIC, as less as 0.625mg/mL.

Capillene is the characteristic oil constituents of *Artemisia stricta* f. *stricta*. Besides this plant, it has also been reported from the oils of other members of sect. Dracanculus eg. *Artemisia capillaries* (syn *Artemisia scoparia*) [5], *Artemisia dracanculus* [6]**,** *Artemisia glauca* [7] and *Artemisia campastris* var. g*lutinosa* [8]. Therefore, this constituent of the oil has chemotaxonomic value for the identification and characterization of these plants. In the present global scenario, disease causing microbes are acquiring resistance against most of the antimicrobials used for treating antifungal and antibacterial infections [20].Therefore, it is imperative to search the structurally different antimicrobial agent(s) that can kill the drug-resistant mutants with fewer side effects. Additionally, in view of growing interest in research concerning alternative pesticides and antimicrobial active compounds including plant extracts and essential oils that are known for their relatively less damaging effect to the mammalian health and environment, essential oil *Artemisia stricta* f. *stricta*, which posses very high antimicrobial activity can be used as a source of potential antimicrobial agent.

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