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GCMS Analysis and Anti-microbial Activity of Essential Oil of *Artemisia minor* Jacq. ex Bess. from Lahaul & Spiti (Cold Desert) Region of North India

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Abstract

The essential oil analysis of *Artemisia minor* Jacq. ex Bess. has been done for the first time from Trilokinath (3020m) of Lahaul & Spiti (Cold Desert) region of North Indian higher altitude Himalayas in the month of July, 2007. Essential oil was isolated by hydro distillation from the aerial parts of the plants collected from the wild sources. The extraction yield for the essential oil of *A. minor* Jacq. ex Bess. was 0.40%. The oil was analyzed by GCMS; the components of the essential oil were identified by comparing their retention indices and mass spectra fragmentation patterns with those stored on the MS-computer library and also from the published literatures. The essential oil analysis led to the identification of 18 out of 22 constituents representing 65.37% of the composition of oil. The major constituents of the oil were: 1, 8-cineole (22.30%), camphor (12.64%), davanone (12.33%), ascaridole (11.11%) and α-phellandrene (5.23%). The presence of artedouglasia oxide-C in *A. minor* has not been reported earlier in the same species and therefore acts as a new chemotype from this study area. The present study describes the phytochemical profile and anti-microbial activity of essential oil of *A. minor*. Furthermore, anti-microbial activity of oil was evaluated using agar well diffusion method. The anti-microbial test results showed that the oil had a potential anti-microbial activity against all seven Gram+ve and Gram-ve bacterial strains such as: *Pseudomonas fluorescence, Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Staphylococcus epidermis* and *Acenetobactor calcoaceticus*. Essential oil showed maximum zone of inhibition and minimal inhibition concentration against *Bacillus subtilis* (MTCC-2451) and *Pseudomonas fluorescence* (MTCC-664) bacterial strains. These results permitted the conclusion to be made that, it is the first report of the GCMS analysis and anti-microbial activity on a new chemotype of *A. minor* Jacq. ex Bess., a naturally growing species from Trilokinath (3020m) of Lahaul & Spiti- a cold desert region of North India.

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1. Introduction

The family Asteraceae comprises of many aromatic and medicinal plants. The genus *Artemisia* known as "wormwood" belongs to the tribe Anthemideae and is one of the largest genera of the Asteraceae family. This family includes more than 800 species widely distributed throughout the world, especially, in South-West of Asia and Central Europe [1]. Several *Artemisia* species have been found to grow above 2600m. Out of 34 species of the genus *Artemisia* known from India, 15 species have been documented in the flora of Lahaul & Spiti [2]. The genus has always been of great interest to botanical, pharmaceutical and food industries [3]. *Artemisia* species are reported to possess anti-diabetic effect and have been used in many countries of middle east and Iran as a herbal medicine for treatment of diabetes, high blood pressure, anti-migraine, anti-fungal activity, useful as tonic, stomachic, anti-bacterial and anti-septic [4-5]. The essential oils from *Artemisia* genus are also used for various purposes such as flavoring, fragrances, rodents, mite repellents and for anti-spasmodic, anti-pyretic, anti-inflammatory and abortifacient activities. The *Artemisia* species have been known as a folk medicine resource, which is used for the treatment of epidemic hepatitis [6]. Because of application of *Artemisia* in traditional medicine, many species of this genus have been surveyed by phytochemists and pharmacologists [7-8]. Sesquiterpene lactones and acetylenes have been reported from some species of *Artemisia* such as *A. assoana, A. lantana* and *A. pedemontana* [9] and artemisia ketone, 1, 8-cineole, davanone, camphor, thujone, myrcene and germacrene-D have also been reported in essential oil of *A. absinthium, A. scoparis* and *A. vulgaris* [10-12]. The essential oil from various species of the genus are used in soaps, detergents, cosmetics, perfumes, as aromatherapy claims [13-14]. Chemical analysis of oils from different *Artemisia* species such as: *A. afra*, *A. annua, A. arborescene*, *A. elegantissima, A. maritima*,

A. myriantha, A. nilagirica, A. scoparia, A. vulgaris, etc. have already been done by many workers from India and abroad [15-19]. In spite of many studies on the genus *Artemisia,* there are still many problems in systematic interpretations. The quality and quantity of the essential oil varies a lot with the genetic makeup of the taxa and the prevalent environmental factors. As there is no report on the chemical analysis of essential oil and anti-microbial activity in *A. minor* Jacq. ex Bess. from this study area, the aim of this work is to provide the first report on the GCMS analysis of essential oil along with anti-microbial activity in the population of *A. minor* Jacq. ex Bess., a naturally growing species, from Trilokinath (3020m) - a cold desert region of India.

2. Materials and Methods

2.1. Plant materials

Aerial parts of *A. minor* at full flowering and fruiting stage were collected from Trilokinath, altitude range of (3020m) in the month of July, 2007 (Table 1). Plants specimens were identified by the Botanical Survey of India (BSI, Northern Circle), Dehradoon and specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (Punjab), India.

2.2. Volatile oil distillation

One hundred grams fresh aerial parts of leaves and fine stems were separated and then immersed in water in a round bottom flask and hydrodistilled for 3h in a full glass Clevenger-type apparatus, giving transparent light yellow oil. The oil was decanted to be used as essential oil. To improve the recovery and analysis, the essential oil was taken in *n*-pentane, dried over anhydrous sodium sulphate until the last traces of water were removed and then stored in a dark glass bottle at 4^{o}C prior to GC-MS analysis [20].

2.3. Gas chromatography-mass-spectrometry

GCMS (70ev) data were measured on GCMS (QP 2010 series Shimadzu, Tokyo, Japan) equipped with AOC 20i autosampler and BP20 Capillary column (SGC International

Ringwood, Australia) of 30m length, 0.25mm i.d. and 0.25µm film thickness. Temperature was programmed from 70-220°C at a rate of 4°C/min, held isothermally at 70°C and 220°C for 4 and 5 min, respectively. Mass spectrometer source temperature, 200°C; interface temperature, 220°C; injector temperature, 220°C. Sample injection volume 2µL (diluted 5µL oil in 2mL dichloromethane, HPLC grade); split ratio, 1:50 and mass scan, 50-600 amu. Helium was used as a carrier gas with 1.1mL/min flow rate.

2.4. Identification of components

The retention index was calculated for all volatile constituents using a homolog4us series of *n*-alkanes. The components of oil were identified by matching their mass-spectra with those stored in the computer library such as Wiley, New York mass spectral (MS) library, National Institute of Standards and Technology (NIST) [21] and their retention indices (RI) either with authentic compounds or with published data in the literature [22-25] based on retention indices of components on same phases of polar columns such as: BP-20, CW-20M, HP-20M and Supelcowax-10.

2.5. Microbial strains for anti-microbial activity

The microorganism strains used in the agar disc diffusion method were supplied by the Institute of Microbial Technology, Chandigarh, India. Gram-positive bacteria: *Bacillus subtilis* (MTCC-2451), *Staphylococcus aureus* (MTCC-740), *Staphylococcus epidermis* (MTCC-435), Gram-negative bacteria: *Escherichia coli* (MTCC-443), *Salmonella typhimurium* (MTCC-1251), *Pseudomonas fluorescence* (MTCC-664) and *Acenetobactor calcoaceticus* (MTCC-127).

2.6. Anti-microbial screening

In vitro anti-bacterial activity of the *A. minor* essential oil was studied against seven bacterial strains by the agar well diffusion method as described by Perez and

co-workers [25] with certain modifications. Nutrient agar (Hi Media, India) was used as the bacteriological medium. The anti-bacterial activity of essential oils was taken at different concentrations (5, 10, 20, 40 and 80µL/well). The nutrient agar was melted and cooled to 48-50°C and a standardized inoculum of 1 × 10^6 CFU/mL, (0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound was introduced in the well (8.5 mm). The plates were incubated overnight at 37°C. The anti-microbial spectrum of the oils was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, 20µL each of amoxicillin and ciprofloxacin (5mg/mL of autoclaved distilled water). These are commonly used anti-biotics to treat infections caused by several Gram-positive and Gram-negative bacteria. For each bacterial strain positive controls were maintained. The experiment was performed three times to minimize the error and the mean values are presented.

2.7. Minimal inhibition concentration

The essential oils that exhibited considerable activity were diluted with nutrient broth (1:1) in a series of seven sets of three test tubes for different microorganisms [26]. An aliquot of 1mL of the bacterial suspension (1x10^6) was inoculated into each tube. The control tubes were inoculated with same quantity of sterile distilled water and 75% ethanol. All tubes were incubated at 37°C for 24hrs. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration. The contents of all tubes that showed no visible growth were cultured on nutrient agar, incubated at 37°C for 24hrs. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony.

3. Results and Discussion

The essential oil obtained by hydrodistillation from the aerial parts of wild growing *A. minor,* with a yield of 0.40%, was pale yellowish and possessed a strong odour. By gas chromatography mass spectroscopy, 18 out of 22 components were identified, representing 65.37% of the composition of essential oil. The major constituents recorded from essential oil of *A. minor* were: 1, 8-cineole (22.30%), camphor (12.64%), davanone (12.33%), ascaridole (11.11%) and α-phellandrene (5.23%). The different constituents of essential oil of *A. minor* are well represented with their relative area percentage (Table 2). In the previous published reports on other species of the genus *Artemisia*, cineol, thujone and monoterpenes were reported to be the major constituents in *A. maritima* [27] and α-thujone (63.25%); sabinene (7.83%); 1, 8-cineole (6.54%) and germacrene-D (2.22%) were also considered as major components in *A. annua*. In one recent report on populations of *A. maritima* from Lahaul & Spiti, 1, 8–cineole, chrysanthenone, germacrene-D and borneol were recorded as major components, with small percentages of rare artedouglasia oxides was also recorded in one population [28].

In spite of many studies on the genus *Artemisia,* there is no report on essential oil composition of *A. minor* from the study area. Therefore, it is interesting to find such a high percentage of major constituents, 1, 8-cineole (22.30%), camphor (12.64%), davanone (12.33%), ascaridole (11.11%), α-phellandrene (5.23%) and one minor but rare constituent i.e. artedouglasia oxide-C (0.72%) first time in essential oil of the species in the population of *A. minor* from Trilokinath of Lahaul & Spiti (Cold Desert) region of North Indian higher altitude Himalayas. This observation about the presence of artedouglasia oxide-C in *A. minor* has not been reported earlier in the same species, but reported only in the oil of *A. maritima* [28] and *A. laciniata* [29], from which it is concluded that, *A. minor* oil from Trilokinath (3020m) is a new chemotype.

Anti-microbial activity showed that, the inhibition zones were found increased considerably when the concentration rate increased. Therefore it can be said that quantity of the oil was important for inhibition effect. Among all Gram-positive bacterial growths, the maximum zone of inhibition was recorded against *Bacillus subtilis* (MTCC-2451) i.e. 33mm, followed by *Staphylococcus epidermis* (MTCC-435) i.e. 27mm and 25mm zone of inhibition against *Staphylococcus aureus* (MTCC-740). On the other hand four different Gram-negative bacterial strains were tested and among these microorganisms *Pseudomonas fluorescence* (MTCC-664) showed maximum zone of inhibition i.e. 32mm, followed by *Acenetobactor calcoaceticus* (MTCC- 127) i.e. 23mm. The minimum zone of inhibition was recorded against the *Escherichia coli* (MTCC-443) strain i.e. 4mm (Table 3). The minimal inhibition concentration (MIC) was 1µL recorded in Gram-negative strain *Pseudomonas fluorescence* (MTCC-664) followed by a Gram-positive strains *Bacillus subtilis* (MTCC-2451) and *Staphylococcus aureus* (MTCC-740), both showed 5µL of minimal inhibition concentration (MIC) (Table 4).

From these it is concluded that the essential oil showed maximum zone of inhibition and minimal inhibition concentration against *Bacillus subtilis* (MTCC-2451) and *Pseudomonas fluorescence* (MTCC-664) bacterial strains, which indicate that *A. minor* Jacq. ex Bess. essential oil has capacity to inhibit the growth of both Gram-positive and Gram-negative bacterial strains when used in a higher amount. Further it can be concluded on the basis of previous studies on *Artemisia* genus and present results that *A. minor* Jacq. ex Bess. a higher altitude medicinal and aromatic plant act as an important anti-microbial agent against many Gram-positive and Gram-negative bacterial strains and a new chemotype with higher percentage of some most important chemical constituents, which was previously undescribed from this study area of "Cold Desert."

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Table 1. Collection details and essential oil yield of *A. minor* Jacq. ex Bess. from study area of North Indian higher altitude of Himachal Pradesh, Himalayas.

Species name	Place of collection	Herbarium Number	Altitude of study area (m)	Month & year of collection	Oil yield (%)
Artemisia minor Jacq. ex Bess.	Trilokinath (Lahaul & Spiti) North India	52168 (PUN)*	3020	July, 2007	0.40

*PUN: Abbreviation for Herbarium, Department of Botany, Punjabi University, Patiala, as indicated in Index Herbariorum.

Table 2. Essential oil composition of *Artemisia minor* Jacq. ex Bess. from Trilokinath (3020m).

S. No	Constituents	RI[a]	RA[b]%
1.	α-Phellandrene	1160	5.23
2.	*d1*-Limonene	1199	0.39
3.	1, 8-cineole	1208	22.30
4.	Dehydro 1,8-cineole	1228	0.55
5.	*cis*-Epoxy-ocimene	1228	2.29
6.	*trans*-Ocimene	1233	0.44
7.	γ-Terpinene	1241	0.91
8.	Artemisia ketone	1349	0.86
9.	Camphor	1509	12.64
10.	*l*-Linalool	1538	0.33
11.	*l*- Bornyl acetate	1573	0.31
12.	Caryophyllene	1594	0.67
13.	Terpene-4-ol	1604	2.66
14.	6,6-Dimethyl-bicyclo[3.1.1]hept-2-ene-2-ethanol	---	2.88
15.	Ascaridole	1715	11.11
16.	α-Curcumene	1763	1.44
17.	Artedouglasia oxide-C	---	0.39
18.	Methyl cinnamate	2051	1.13
19.	Spathulanol	2113	1.71
20.	Artedouglasia oxide-C	---	0.72
21.	Davanone	---	12.33
22.	Khusinol	2309	0.40

RI[a] : Actual retention indices of components on same phases of columns (BP-20).
RA[b] : Percentage of components.
--- : RI could not be calculated.

Table 3. Anti-microbial activity of essential oil of *A. minor* Jacq. ex Bess. against Gram-positive and Gram-negative bacteria strains.

S. No.	Nature of Bacterial Strains	Microorganisms (Bacterial Source Number)	Diameter of inhibition zone (mm) of essential oil concentration used for anti-microbial analysis (µL/well) (*n*=3)					Control +ve (*n*=3)	
								Amoxicillin 5mg/mL	Ciprofloxacin 5mg/ mL
			5 µL	10 µL	20 µL	40 µL	80 µL	20 µL	20 µL
1.	Gram +ve	*Bacillus subtilis* (MTCC-2451)	0.9	5.6	9	18	33	32	40
2.		*Staphylococcus aureus* (MTCC-740)	0.9	2.3	4	11	25	29	31
3.		*Staphylococcus epidermis* (MTCC-435)	N.A	2.9	6.2	14	27	25	42
4.		*Escherichia coli* (MTCC-443)	N.A	N.A	N.A	2.5	4	36	41
5.	Gram –ve	*Salmonella typhimurium* (MTCC-1251)	N.A	1.5	4	10	21	16	29
6.		*Pseudomonas fluorescence* (MTCC-664)	2.0	5	8	17	32	30	48
7.		*Acenetobactor calcoaceticus* (MTCC- 127)	1.3	2.7	6.1	11	23	29	36

All values are mean of triplicates (*n*=3); Gram +ve: Gram-positive; Gram –ve: Gram negative; N. A: No Activity.

Table 4. Minimal inhibition concentration (MIC) (µL) of essential oil of *A. minor* Jacq. ex Bess.

S. No.	Nature of Bacteria	Microorganisms (Bacterial Source Number)	MIC (µL)
1.	Gram +ve	*Bacillus subtilis* (MTCC-2451)	5
2.		*Staphylococcus aureus* (MTCC-740)	5
3.		*Staphylococcus epidermis* (MTCC-435)	6
4.	Gram-ve	*Escherichia coli* (MTCC-443)	30
5.		*Salmonella typhimurium* (MTCC-1251)	8
6.		*Pseudomonas fluorescence* (MTCC-664)	1
7.		*Acenetobactor calcoaceticus* (MTCC-127)	2.5

Gram +ve: Gram-positive; Gram –ve: Gram-negative; MIC: Minimal Inhibition Concentration.

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