GC-MS ANALYSIS OF ESSENTIAL OIL OF *ARTEMISIA ANNUA* AND BIOLOGICAL ACTIVITIES OF ITS EXTRACTS

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Medicinal plants form the basis of healthcare in many societies. Approximately 80 per cent of traditional medicines used in primary healthcare are of indigenous origin. Uttarakhand Himalayas are considered to be a rich source of important medicinal and aromatic plants. Traditionally, medicinal plants are used by the local people to obtain health benefits. *Artemisia annua* is a wild growing aromatic plant known for its anti-malarial activity and an array of secondary metabolites. The present study was performed to investigate the chemical composition, antioxidant and antimicrobial activity of essential oil and different extracts of *A. annua.* The essential oil was analysed by GC-MS (Gas chromatography–mass spectrometry) and has shown thirty-seven components. The result found that this plant can be a useful raw material for development of nutraceutical agent due to the presence of various secondary metabolites. The extracts possessed significant antioxidant and antimicrobial activity and thus, can be utilised as a nutraceutical agent.

Keywords: Medicinal plant, *Artemisia annua*, antioxidant activity, antimicrobial activity and essential oil.

Introduction

Plants have played a key role in day-to-day life support system of human beings from time immemorial. Medicinal plants are the base of many societies, for their primary healthcare system. In primary healthcare, about 80 per cent of traditional medicines used are derived from plants. Traditional medical knowledge of plants is not only useful for conservation of biodiversity but also useful to healthcare and the development of new drug. Himalaya is a global biodiversity hotspot with much diversified geographical, ecological and evolutionary factors for species diversity which support 18,440 species of plants of which 25.3 per cent is of endemic (Phondani, P.C., *et al.* 2010). The Indian state Uttarakhand located in Himalayan hotspot, has a huge wild diversity, which occupies 17.3 per cent of India's total land area including 92.57 per cent area under hills and 7.43 per cent under plains. Geographically, it is located between 28°43'–31°27'N latitudes and 77°34'–81°02'E longitudes (Samant, 1993). The state is considered rich source of important medicinal and aromatic plants. Local people of Uttarakhand heavily use traditionally easily available medicinal plants for health benefits and are without side effects. However, at present, the traditional knowledge on the use of plant resources is dwindling due to several reasons, including shift in attitude towards a more western lifestyle and declining interest of younger generations to carry forward the

tradition. There are areas in the remote hills where people still practise traditional way of life and hence use nearby plant species for curing diseases and other purposes. Ton's watershed being tugged deep into the hills the inhabitants of this area are relatively less influenced by the modern forces (Kala, 2015).

Artemisia annua belonging to the family Asteraceae, is a short-day, annual, cross-pollinated medicinal plant, grows in wild in Asia, Europe and North America. Over a period of time, it has been naturalised and grows wild in many other regions of the world. It produces an array of secondary metabolites including sesquiterpenoids, triterpenoids, monoterpenoids, phenolics, coumarins, steroids, lipids, flavonoids and aliphatic compounds (Bhakuni, 2001). Artemisinin is a naturally occurring endoperoxide with anti-malarial properties so has been used clinically as an anti-malaria drug (Mueller, 2004). In 1967, a national research project against malaria was initiated in China. Around 380 herbal extracts were evaluated by a Chinese scholar for their anti-malarial activities and *A. annua* was found to be the most active in them (Tu, 2011). Beside anti-malarial effects, *A. annua* has biological activities, such as antibacterial, anti-inflammatory, angiotensin converting enzyme inhibitory and antitumor effects (Efferth, 2017). In the present study, we investigated the chemical composition of *A. annua* growing wild in Uttarakhand Himalayan region. In addition, the aim of the study is to determine the antioxidant activity and antimicrobial activity of the *A. annua.*

Material and Methods

Plant Material

Aerial part of plant, *Artemisia annua* were collected from Dhanulti region (Tehri District) of Uttarakhand and were identified by Department of Botany, H.N.B.G.U. Srinagar Uttarakhand. The fresh leaves were hydro distilled for 8 hours to get the green coloured essential oil. Dried leaves were successively extracted using different solvents (petroleum ether, chloroform and methanol). The essential oil was stored in an air tight bottle for further analysis.

Gas Chromatography and Mass Spectroscopy

The essential oil of *A. annua* was analysed by GC-MS using Perkin Elmer Clarus 680 Gas Chromatograph with SQ 8 mass Spectrometer.

GC Parameters

GC Column: PE Elite-5 (30 m x 0.25mm x 0.25um) Oven programming: 60 °C–240 °C at the rate of 3°c/min and final hold of 2 minutes.

Injector temp: 290 °C, Split 1:100, Carrier: Helium at 1 ml/min flow.

MS Parameters

Ionisation: Electron Ionisation, Mass range: 40 to 500 amu (atomic mass unit), Scan time: 0.8 seconds Inter scan delay: 0.01 seconds

GC Inlet and Source Temp: 220 °C Identification of constituents were done on the basis of Retention Index, MS library search (NIST and WILEY) and by comparison with MS literature data (Adams, 2001).

Phytochemical Analysis of Extracts

The phytochemical screening of solvent extracts viz. petroleum ether, chloroform and methanol was performed for identification of the chemical constituents, such as

alkaloids, glycosides, carbohydrates, proteins, phenolics, flavonoids, saponins, amino acids, steroids and triterpenoids, according to the standard and conventional methods (Harborne, 1998).

Antioxidant Activity

The antioxidant activity of crude extracts of different solvents viz. petroleum ether, hexane, chloroform, methanol, hydro-alcoholic (50% v/v) and water were determined using DPPH assay (Mishra *et al.* 2018). Decline in absorbance at 517 nm reflected the decrement of DPPH radical. Ascorbic acid was considered as reference. Scavenging capacity was estimated as DPPH scavenging activity (%) =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Ascorbic acid (0.5 mm) was used as the positive control. The antioxidant activity was determined on the basis of IC_{50} (inhibitory concentration). IC_{50} is the amount of concentrated solution of sample needed to inhibit 50 per cent of DPPH free radicals.

Antimicrobial activity

Pathogenic Microbes Used in the Study

The pure bacterial cultures of test organisms (*Bacillus subtilis* NCS/10034, *Staphylococcus aureus* NCS/954 and *Escherichia coli* NCS/126 were procured from Microbiology Department of NCS Group, Nagpur, Maharashtra, India. These cultures were further sub cultured and glycerol stocks were maintained and kept at 4°C till further use.

Inoculum in Liquid Broth

Each bacterial culture was inoculated separately into sterilised Soyabean casein digest broth and incubated at 37°C for 18 hours. The suspension of each bacterial culture growth was checked to provide yield approximately, 105 colonies (CFU/ml).

Determination of Diameter of Zone of Inhibition by Well Diffusion Method

The agar well diffusion method (Saklani *et al.* 2012) was modified. Soyabean casein digest agar medium (SCDM) was used for bacterial cultures. Bacteria suspended in digest broth were inoculated into the culture media, respectively. Punched a total of 8 mm diameter wells into the agar and filled with plant extracts (100 µg/ml) and solvent blanks. Different solvents used in the study, viz. petroleum ether, hexane, chloroform, methanol, hydro-alcoholic (50% v/v) and water were utilised as negative controls. The standard antibiotic (Azithromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were further incubated at 37 °C for 18 h. The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition observed. The procedure for assaying antibacterial activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organisms.

Results and Discussion

GC-MS of Essential Oil

The identification of the components was based on comparison of their mass spectral fragmentations pattern with those of the data reported in Wiley and NIST Libraries and those described by Adams (2001). The result of composition of essential oil are shown in Fig. 1 and Table 1.

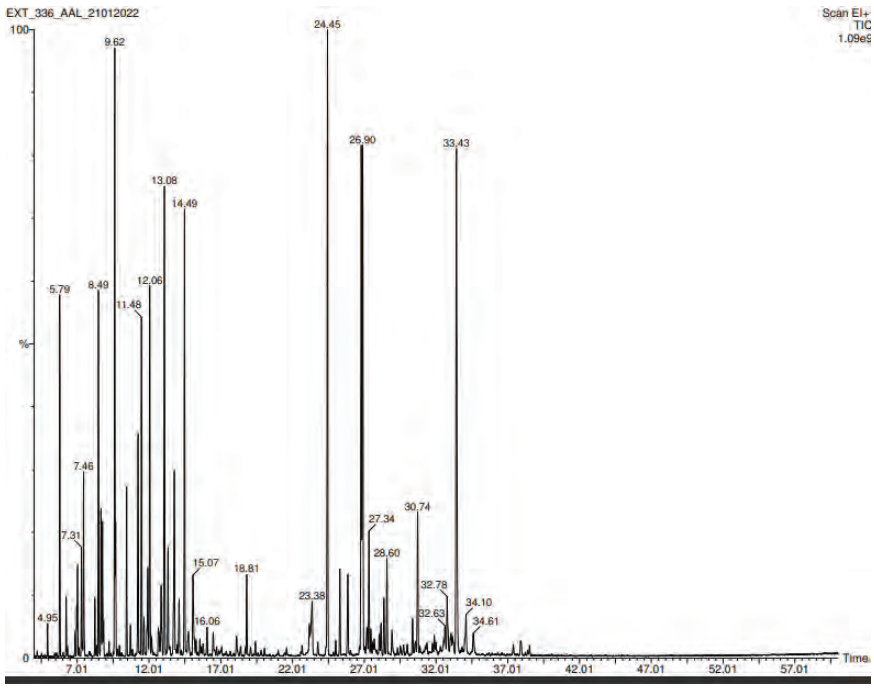

Fig. 1. GC-MS Chromatogram of the Composition of Essential Oil of *Artemisia annua*

Table 1. Chemical Composition of Essential Oil of *Artemisia Annua* Leaves

S.No.	Compound	Molecular Formula	Molecular Weight	Rf
1.	α-Bisabolol	$C_{10}H_{16}$	222	34.61
2.	β-Eudesmol	$C_{10}H_{16}$	222	33.43
3.	Cis-Caryophyllene	$C_{10}H_{16}$	204	32.78
4.	Gamma Cis-sesquicyclogeranial	$C_{10}H_{16}$	222	30.73
5.	Trans-gamma-bisabolene	$C_{10}H_{16}$	204	30.37
6.	β-funebrene	$C_{10}H_{16}$	204	28.59
7.	Cadinene	$C_{10}H_{16}$	204	28.37
8.	α-Cubebene	$C_{15}H_{24}$	204	28.17
9.	trans-α–Bergamotene	$C_{15}H_{24}$	204	27.33

10.	Aromadendrene	$C_{15}H_{24}$	204	27.20
11.	Curcumene	$C_{15}H_{24}$	202	26.90
12.	Gamma-Curcumene	$C_{15}H_{24}$	204	26.79
13.	A-Humulene	$C_{15}H_{24}$	204	25.87
14.	β-sesquiphellandrene	$C_{15}H_{24}$	204	25.36
15.	Cis-Caryophyllene	$C_{15}H_{24}$	204	24.94
16.	α-Farnesene	$C_{15}H_{24}$	204	23.18
17.	Carneal	$C_{10}H_{16}$	152	16.06
18.	α-terpineol	$C_{10}H_{18}O$	154	1.07
19.	Terpineol	$C_{10}H_{18}O$	154	14.68
20.	3-Carerne	$C_{10}H_{16}$	136	5.78
21.	Camphene	$C_{10}H_{16}$	136	6.23
22.	Octen-3-ol	$C_{10}H_{16}O$	128	6.96
23.	Geraniol	$C_{10}H_{16}O$	182	7.03
24.	α-Pinene	$C_{10}H_{16}$	136	7.30
25.	α-Terpinene	$C_{10}H_{16}$	136	8.24
26.	Cymene	$C_{10}H_{14}$	134	8.48
27.	D-Limonene	$C_{10}H_{16}$	136	8.66
28.	Eucalyptol	$C_{10}H_{18}O$	154	8.77
29.	Filifone	$C_{10}H_{14}O$	150	11.25
30.	Thujone	$C_{10}H_{16}O$	152	11.48
31.	β-Thujone	$C_{10}H_{16}O$	152	11.92
32.	Trans-pinocarveol	$C_{10}H_{16}O$	152	12.86
33.	Camphor	$C_{10}H_{16}O$	152	13.07
34.	Santolina triene	$C_{10}H_{16}$	136	13.33
35.	Lavandulol	$C_{10}H_{18}O$	154	13.77
36.	Borneol	$C_{10}H_{18}O$	154	14.10

Phytochemical Screening

The solvent extracts viz. petroleum ether, chloroform and methanol were used for phytochemical screening. The results showed the presence of all phytochemicals viz. carbohydrates, alkaloids, glycosides and steroid in the petroleum ether extracts of the *A. annua* except flavonoids. Chloroform extracts were found to have carbohydrates, alkaloids, saponins, flavonoids, steroids while phenol and glycosides were found to be absent. Methanolic extracts were found to have all phytochemicals except steroid. The results are shown in Table 2.

Table 2: Phytochemical Screening of Crude Extracts of *A. annua*

Phytoconstituents	Petroleum Ether Extract	Chloroform	Methanol
Carbohydrate	+	+	+
Alkaloid	+	+	+
Phenol	-	-	+
Glycoside	+	-	+
Flavonoids	-	+	+
Saponins	+	+	+
Steroids	+	+	-

Antioxidant Activity

The results of the antioxidant activity suggest that, methanolic extracts of *A. annua* had significant antioxidant potential in comparison to petroleum ether and chloroform extracts. The result of antioxidant activity is shown in Table 3.

Table 3: Antioxidant Activity of *A. annua*

Concentration	Petroleum Ether	Chloroform	Methanol
1.	75.23	76.88	89.75
2.	70.98	72.68	85.21
3.	67.23	65.09	83.23
4.	75.88	64.12	82.07
5.	61.91	52.10	83.13

Antimicrobial Activity

The antimicrobial activities of polar and non-polar solvent extracts of the leaves of *A. annua* were determined against *E. coli*, *B. subtilis* and *S. aureus* via well diffusion method. The significant highest zone of inhibition was recorded of polar extracts against all the bacterial strains studied in comparison to non-polar extracts. The aqueous extract showed highest antimicrobial activity but methanol extract has showed the maximum zone of inhibition against all the microbes. The results are shown in Table 4 and Fig. 2.

Table 4: Antimicrobial Activity of Solvent Extracts of Leaves of *A. annua*

Bacterial Pathogen	Diameter of Zone of Inhibition (mm)			
	Methanol (100 µg/ml)	Aqueous (100 µg/ml)	Chloroform (100 µg/ml)	Petroleum ether (100 µg/ml)
Bacillus subtilis (MTCC 441)	18.0	15.2	14.2	12.0
Staphylococcus aureus (MTCC 441)	15.0	12.0	9.7	5.0
Pseudomonas aeriginosa (MTCC 441)	-	10.0	8.8	6.0
Proteus vulgaris (MTCC 441)	12.0	9.2	7.0	5.0
Escherichia coli (MTCC 441)	11.5	9.0	8.0	7.0
Klebsiella pneumniae (MTCC 441)	13.0	10.5	11.4	9.0

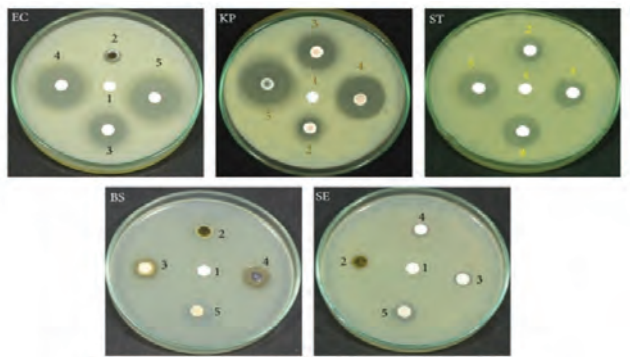

Fig. 2. Antimicrobial Activity of Different Extracts of *A. annua*

Conclusion

The result of this study concluded that essential oil of *Artemisia annua* has various components. The plant has also shown effective antioxidant and antimicrobial property and hence, can be further used in the formulation of effective nutraceutical.

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