

Original Research Article

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## Effect of Organic Manures on Antioxidant Activity and Essential Oil Composition of *Artemisia annua* cv. CIM Arogya

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### ABSTRACT

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Organic manure affect the essential oil composition of the flower of *Artemisia annua* cv. CIM Arogya, growing in Teari region of Uttarakhand, India, along with its antioxidant activity, is reported in the present study. More than sixty compounds were identified, representing 97.5% of the total oil. The major constituents of the oil were Eucalyptol (1-8 Cineol), Camphor and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol in all treatments except T3 (1, 5-Heptadien-4-one, 3,3,6-trimethyl-). Content (%) of bioactive compounds affect by organic manure. Isolated essential oil was tested for antioxidant activity with DPPH radical, exhibiting promising antioxidant profile.

### Introduction

*Artemisia annua* L. belonging to the family Asteraceae, is a fragrant annual herb. The genus *Artemisia* contains more than 400 species, and distributed mainly in the Northern Hemisphere, especially in Asia, Europe and North America (Pellicer *et al.*, 2006; Nibret & Wink 2010). This genus is represented by 32 species in India (Hajra *et al.*, 1995). Many species of the genus *Artemisia* are used in the folk and traditional medicine and also used as spices, and for

alcoholic drinks (Baser & Buchbauer 2010). Plant commonly named Sweet Annie, Sweet Wormwood Annie, Sweet Sagewort, Annual Wormwood or Qinghaosu. In Uttarakhand commonly known as “Paanti”. In Chinese traditional medicine use of this plant was recorded before 168 BC.

In 1972, artemisinin the active crystalline constituent was isolated from the aerial portions of the plant. Artemisinin is a sesquiterpene lactone which have antimalarial properties (Klayman, 1985). It is the only

commercial source of artemisinin (Ferreira *et al.*, 2013), a potent antimalarial drug (Woerdenbag *et al.*, 1993; Dhingra *et al.*, 2000; Bhakuni *et al.*, 2002; Caniato & Puricelli 2003). Since then *A. annua* has become one of the most extensively investigated plants in recent years. Besides its antimalarial activity (Bhakuni *et al.*, 2002) *A. annua* also shows several biological activities such as, anti-inflammatory, anti-oxidant, antipyretic (Huang *et al.*, 1993), anticancer (Zheng, 1994), antifungal (Liu *et al.*, 2001), antiulcerogenic (Foglio *et al.*, 2001), antiparasitic (Kim *et al.*, 2002), and cytotoxic (Nibret and Wink, 2010) activities.

The essential oil composition of *A. annua* plant has been studied thoroughly and hundreds of bioactive compounds have been identified up to date (Brown, 2010). Artemisia ketone, Camphor, and 1, 8-cineole and germacrene D, are usually found as the main bioactive compounds (Ahmad and Mishra, 1994; Tellez *et al.*, 1999; Malik *et al.*, 2009; Brown, 2010). This essential oil is used in the production of perfumes and cosmetics (Nedkov & Attanassovo 2004).

It is generally accepted that variability of chemical composition of essential oil of *A. annua* depends on plant development stage and geographical origin (Verma *et al.*, 2011; Lenardis *et al.*, 2011; Bhakuni *et al.*, 2002; Brown, 2010; Holm *et al.*, 1998). Thus, there is still a considerable research interest in the assay of biological properties and/or composition of essential oil of *A. annua* from different geographical origin. In the present work, we investigated that effect of treatment on essential oil content and composition of *A. annua* cv. CIM Arogya cultivated in Terai region of Uttarakhand. CIM Arogya is the cultivar variety of *A. annua* developed by Central institute of Aromatic and Medicinal Plants (CIMAP), Lucknow. In addition, the aim of this study was to determine the

antioxidant activity of the isolated essential oil.

## Materials and Methods

### Field trials

Field trials of the present study were carried out at the experimental field of G. B. PANT University, Uttarakhand, India, foot hills of Himalayas at 29° North latitude and 79.5° East latitude and the altitude of 243.83 meter above mean sea level in the year 2017–2018 and 2018–2019. The soil of the experimental field was coarse in texture having pH 7.1, electrical conductivity 0.11 ds m<sup>-1</sup>, nitrogen 281.35 kg ha<sup>-1</sup>, phosphorus 29.23 kg ha<sup>-1</sup> and potash 309.34 kg ha<sup>-1</sup>.

### Sowing and transplantation

Seeds of *A. annua* cv. CIM Arogya collected from the central institute of Medicinal and Aromatic Plant Lucknow, India were sown in 4m × 1 m nursery bed in the month of December. Three-month-old seedlings were then transplanted into the field. The plot size was 34.20 m<sup>2</sup> (5.70m × 6 m), row to row distance of 50 cm and plant to plant distance of 45 cm. The plants were irrigated in a week till the requirement.

**Treatments:** T1: Control (without manure), T2: 20 ton ha<sup>-1</sup> Farm yard manure, T3: 10 ton ha<sup>-1</sup> Vermicompost, T4: 10 ton ha<sup>-1</sup> Poultry manure, T5: 10 ton ha<sup>-1</sup> Farm yard manure + 5 ton ha<sup>-1</sup> Vermicompost + 5 ton ha<sup>-1</sup> Poultry manure, T6: Inorganic manure (NPK)

Urea, diammonium phosphate, murate of potash, farm yard manure, vermicompost and poultry were used as the source of nitrogen, phosphorus, potassium, and organic manure, respectively. Phosphorus and potassium fertilizers (50 kg ha<sup>-1</sup> each) were applied at the time of transplantation, while nitrogen

fertilizer 150 kg ha<sup>-1</sup> were applied in four equal splits, one at the time of transplantation and 2nd at bolting stage, respectively. Organic manure was also applied at the time of transplantation.

The experimental procedure was arranged in a completely randomized block design with three replicates of each treatment. The plants were harvested at full flowering stage.

### Essential oil extraction

Fresh flowers of *A. annua* cv. CIM Arogya was collected to extraction of essential oil via hydro-distillation method. 200 gm of flowers was subjected to Clevenger-type apparatus for 3 h in 500 ml water. The resultant data (%) were calculated as volume of essential oils per 200 gm of plant fresh weight basis. The oil was dried over anhydrous sodium sulphate and stored in refrigerator at 4°C till analysis.

### Gas chromatography- Mass spectrometry (GC-MS)

GC-MS analysis of essential oil of different treatments (Organic manures and combination of organic manure) was performed by using GC-MS QP2010 Plus (Gas Chromatograph Mass spectrometer, SHIMADZU, USA.) with Rxi ®-5Sil MS capillary GC column (5% phenyl 95% dimethyl polysiloxane) with dimension: 30 mts, ID: 0.25mm and df: 0.25µm. Flow rate of mobile phase (helium as a carrier gas) was set at 1.21 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 80°C raised to 250°C at 5°C/min and injected with 1µl volume.

### Antioxidant activity

DPPH Free radical scavenging activity was evaluated by measuring the scavenging activity of the Flower essential oil of *A. annua*

cv. CIM Arogya on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH). A 0.04 % solution of DPPH in methanol was prepared in methanol. Various concentrations (0.2-1.0 mg/mL) were added to 1.0 mL (0.04% DPPH) and final volume was made to 3.0 mL with methanol. The mixture was shaken thoroughly and incubate at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm on a UV-visible spectrophotometer. The decrease in the absorbance indicates an increase in DPPH-radical scavenging activity.

$$\text{DPPH radical scavenging (\%)} = [(A_t - A_0)] \times 100$$

Where  $A_t$  is the absorbance value of the tested sample and  $A_0$  is the absorbance value of blank sample, in particular time. L-Ascorbic acid served as positive control. The experiment was done in triplicate and mean values were recorded.

## Results and Discussion

### Essential oil content

The results presented in Table 1 showed that the content of essential oil was increased progressively irrespective of treatments over control. The essential oil content ranges from 0.72% to 1.2%. The maximum increase in essential oil content was recorded in T6 (1.2%) followed by T5 (1.08%), T4 (0.96%), T3 (0.88%), T2 (0.8%) over control.

### Impact of organic manures on Essential oil composition of *A. annua* cv CIM Arogya

The essential oil of *A. annua* cv CIM Arogya was subjected to detailed GC-MS analysis in order to determine the impact of the treatment on volatile constituents. Identification of the essential oil constituents was done on the basis of NIST library, WILEY and mass

spectrum. The relative percentages of the individual compound were calculated on GC peak area. GC-MS revealed the presence of more than 50 compounds. Major bioactive compounds listed in Table 2.

In T1 (control) 46 bioactive compounds were identified. Camphor (27.89%), Eucalyptol (25.39%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.97%) were recorded as the main components. In T2 50 compounds were identified of which the first three components are Eucalyptol (31.98%), Camphor (20.89%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.30%). In T3 54 compounds were identified. The most abundant components were Eucalyptol (23.84%), Camphor (19.68%) and 1, 5 Heptadiene-4-one, 3, 3, 6-trimethyl (8.90%).

In T4 51 compounds were identified of which Camphor (26.35%), Eucalyptol (25.39%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.30%). In T5 47 compounds were identified. The most abundant compound were Eucalyptol (33.20%), Camphor (24.89%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.30%). In T6 50 compounds were identified of which the first three compounds were Eucalyptol (29.80%), Camphor (26.00%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.60%).

Several bioactive compounds in *A. annua* essential oil have been identified. Significant

variations in the content percentage of different bioactive compounds in essential oils from *A. annua* have been recorded. Generally, camphor, 1,8-cineole, artemisia ketone, and germacrene D, artemisia alcohol, were the main bioactive compounds. But, the variation between chemical composition of the essential oil is generally influenced by the fertilizer, harvesting season and the pH of soils, the geographic location, the choice and stage of drying conditions, subspecies or chemotype, and choice of plant part or extraction method (Bilia *et al.*, 2014).

*A. annua* plants cultivated in India, camphor (10.5- 44.4%) was found to be the major bioactive compounds of essential oil. Bagchi *et al.*, (2003) revealed that the main components of the essential oil of *A. annua* were the artemisia ketone. Jain *et al.*, (2002) reported that artemisia ketone (52.9%), 1,8-cineole (8.4%) and camphor (6.0%) were the major components. According to Soylyu *et al.*, (2005) chemical composition of *A. annua* essential oil was rich in camphor (31.7%), 1,8-cineole (10.1%), caryophyllene oxide (7.1%),  $\alpha$ -copaene (3.4%) and camphene (3.3%). *A. annua* growing wild in Bulgaria, the main component of essential oil was found to be  $\alpha$ -caryophyllene (24.73%),  $\alpha$ -cuvabene (13.53%), artemisia ketone (8.45%),  $\alpha$ -selinene (8.21%),  $\alpha$ -copaene (7.24%), and camphor (3.61%) (Tzenkova *et al.*, 2010).

**Table.1** Impact of organic manure on essential oil content

S.No.	Treatment	Oil content (%)
1	T1	0.72
2	T2	0.8
3	T3	0.88
4	T4	0.96
5	T5	1.08
6	T6	1.2

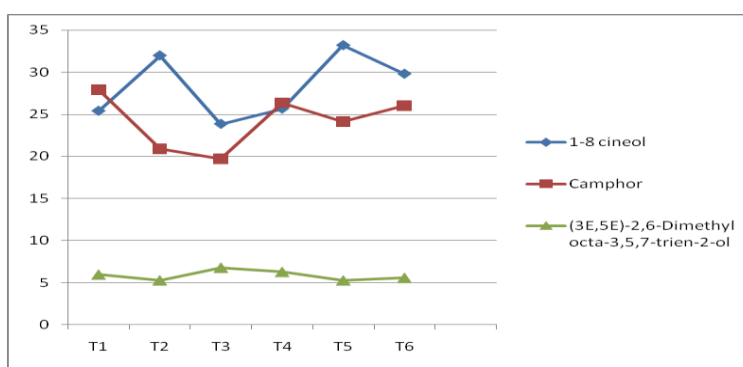
**Table.2** Major bio-active compounds found in Essential oil of *Artemisia annua* cv. CIM Aroogya

S.No.	Bioactive compound	Treatment					
		T1 (%)	T2(%)	T3(%)	T4(%)	T5(%)	T6(%)
1	Pinene <alpha->	2.16	1.99	1.53	2.53	2.11	2.06
2	Camphene	4.12	3.10	2.42	3.76	3.56	4.03
3	Sabinene	3.97	4.94	3.66	3.78	4.60	3.80
4	Pinene oxide <beta->	0.77	0.79	0.49	0.58	0.69	0.54
5	Myrcene	-	2.69	2.08	2.31	-	2.68
6	Eucalyptol	25.29	31.98	23.84	25.66	33.20	29.80
7	Terpinene <alpha->	0.58	0.75	0.47	0.72	0.76	0.73
8	Terpinene <gamma->	1.05	1.10	-	1.12	1.33	1.10
9	Sabinene hydrate <cis->	0.98	1.04	0.89	0.47	0.52	-
10	Butyrate <3-methylbutyl-, 2-methyl->	-	2.00	1.33	-	-	-
11	(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	5.97	5.30	6.78	6.28	5.29	5.60
12	Camphor	27.89	20.89	19.68	26.35	24.11	26.00
13	1,5,7-Octatrien-3-ol, 2,6-dimethyl-	4.32	4.07	4.57	4.41	3.72	4.01
14	Terpineol <delta>	1.6	-	-	1.25	-	-
15	Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylen	-	1.30	1.05	-	1.26	0.76
16	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLET	3.02	2.46	2.41	3.35	3.57	2.56
17	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)	1.86	1.90	2.33	-	2.11	-
18	Caryophyllene <(E)->	2.15	1.77	1.29	1.65	1.27	1.89
19	CINEOLE <DEHYDRO-1,8-> DB5-408	-	-	-	-	2.07	-
20	Farnesene <(E)-, beta->	1.83	1.68	1.77	1.54	1.69	2.29
21	1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-	2.52	2.48	1.79	2.17	1.91	2.52
22	Cymene <para>	-	-	5.06	-	-	-
23	Terpineol <delta >	1.61	-	-	1.25	-	-
24	1,5-Heptadien-4-one, 3,3,6-trimethyl-	-	-	8.90	-	-	-
25	BENZENE,METHYL (1-METHYLETHYL)-	-	-	-	3.14	-	-

(-)= absent

**Table.3** Impact of manure on DPPH radical scavenging activity (%)

Concentration (mg/ml)	Treatment						
	T1	T2	T3	T4	T5	T6	L-Ascorbic Acid
0.2	50.34±0.37	54.58±0.56	58.46±0.43	61.03±0.68	65.89±0.56	52.86±0.86	85.86±0.54
0.4	71.81±0.98	75.89±0.56	78.54±1.31	79.86±1.17	80.45±0.65	73.48±0.41	90.43±0.27
0.6	77.37±1.03	80.38±1.29	82.31±1.66	83.33±1.34	86.34±0.01	79.69±1.28	92.42±0.41
0.8	84.82±2.04	86.64±0.76	87.91±0.96	88.45±1.21	91.23±0.90	86.21±0.36	94.83±0.72
1.0	88.97±0.09	91.41±0.43	92.35±1.09	93.82±0.64	95.81±0.57	90.89±0.10	96.71±0.65

**Fig.1** Effect of treatment on variation in the major bioactive compounds of *A. annua* cv CIM Arogya

The percentage of bioactive compound in the essential oil of *A. annua* depends on time of harvesting of the plant material (Cavar *et al.*, 2012). Variation between chemical compositions of essential oils from *A.annua* plants harvested in different flowering stage. Yu *et al.*, (2011) stated that  $\beta$ -myrcene (37.71%), 1,8-cineole (16.11%), germacrene D (18.1%), camphor (15.84%) and (Z)- $\beta$ -farnesene (9.43%) were main compounds in the pre-flowering oil; camphor (16.62%), caryophyllene (16.27%),  $\beta$ -caryophyllene oxide (15.84%),  $\beta$ -farnesene (9.05%) and (-)-spathulenol (7.21%) were major components in the post flowering oil. Senkal *et al.*, (2014) reported that artemisia ketone (37.15%), camphor (23.80%) and 1,8-cineole (10.39%) were the main components in Full-flowering oil. Correspondingly, in our study, 1,8-cineole, camphor and (3E, 5E)-2, 6-Dimethylcota-3, 5, 7 trien-2-ol were the major bioactive compounds. The percentage and

chemical composition of essential oils are influenced according to the developmental stages of the plants and by the time of harvest. The vegetative stage of plants, biosynthesis of essential oil bioactive compounds is low. The necessary enzymes for the biosynthesis of some bioactive compounds are not active during the vegetative stage. Thus, the quality of essential oil mostly affected by plant harvest stage (Nurzyńska-Wierdak 2013; Rodrigues *et al.*, 2013).

### Antioxidant Activity

The antioxidant principle in their interaction depends on oxidative free radicals. The results presented in Table 2. revealed that the free radical scavenging activities of oil were maximum found to be the order of T5>T3>T4>T2>T6 and T1. Statistically significant effect (P<0.05) of different treatment.

In general, the antioxidative effectiveness of essential oil depends on the content of phenolic compounds (C'var et al 2012; Hasimi et al 2014). Lopes-Lutz *et al.*, (2008) reported that essential oils obtained from plants belonging to the *Artemisia* genus are markedly rich in non-phenolic compounds. According to Juteau *et al.*, (2002) the essential oil of *A. annua* aerial parts, consisting of camphor (44%), germacrene D (16%), trans-pinocarveol (11%),  $\beta$ -selinene (9%),  $\beta$ -caryophyllene (9%) and artemisia ketone (3%), have been weak antioxidant activity. Essential oils of several *Artemisia* species such as *A. absinthum*, *A. cana*, *A. biennis*, *A. longifolia*, *A. dracunculus*, *A. ludoviciana*, *A. frigida*, and *A. herba-alba*, shows similar results, which are dominant by non-phenolic components (LopesLutz *et al.*, 2008; Mighri et al 2010).

In conclusions our results indicated that the different treatment (organic manures) also affect the percentage, chemical composition and antioxidant activity of essential oil obtained from *A. annua* cv CIM Arogya. The essential oil content was low in T1 (0.72%), but rapidly increasing in the other treatment (0.8%, 0.88%, 0.96, 1.08% and 1.2 % for 50% T2, T3, T4, T5 and T6 respectively).

Major bioactive compounds (1.8-cineole (Eucalyptol), camphor and (3E, 5E)-2, 6-Dimethylcota-3, 5, 7 trien-2-ol) amounts were varied in different treatments. The essential oils exhibit marked radical-scavenging activity. The highest DPPH activity was recorded in essential oil of plant harvested in T5.

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