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Research Article

ANTIOXIDANT ANALYSIS OF ESSENTIAL OILS AND METHANOLIC EXTRACTS OF *Artemisia vulgaris*

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Abstract- Recent researches have focused on the isolation and identification of novel compounds which has strong biological activities. Anti-oxidant analysis is one of the several activities which has gained interest of many researchers and many plant species have been used for the analysis of this property. The present study focuses on the comparative analysis of Anti-oxidant properties of Methanolic Extracts and Essential oils of *Artemisia vulgaris*, a plant belonging to Asteraceae family. GC-MS study of the essential oils of this plant showed that the major phyto-constituents were (10.7%) followed by ß endesmol (8.95%). DPPH radical scavenging assay was used for the analysis of Antioxidant activity and it was found that the Methanolic extract had the maximum activity of 72.65% while the Essential oil had 88.65% of the activity. The TPC content of the Methanolic extracts was found to be 347.69 mg GAE/g and the TFC content was found to be 101.17 mg GAE/g respectively.

Keywords- Essential Oils, Anti-Oxidant, DPPH, TFC, TPC.

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Introduction

Artemisia vulgaris is an aromatic plant of Asteraceae family and is commonly found throughout the hills of India [7]. Location wise it is commonly found in High Himalayan Altitudes *i.e*., Western Himalayas, Kashmir and Ladakh and also at some parts of Western Ghats [15]. It is known to possess several medicinal properties like wound healing, anti-ulcerative, anti-oxidant, anti-diabetic, anti-microbial, anti-tuborculos, pesticidal and nematicidal [3, 7, 9, 11, 12, 16]. Essential oils of this plants have diverse chemical constituents like Sabinene, ß pinene, 1-8 Cineole, Artemisia Ketone, Cis and Trans Thujone, Germacrene D and other chemo-constituents [5, 8, 10, 17]. The secondary metabolites of essential oil of this plant have reported to have strong antimicrobial and antioxidant activities [5, 17] and hence they are commonly used in flavor and fragrance industries [1, 2]. The volatile compounds found in essential oil of this plant is also reported to have profound effect of nervous system and hence they are implemented in Neurotrophic medication too [1]. The DPPH mediated antioxidant activity of essential oil of this plant is found to be equivalent to that of BHT, which is a common chemical preservative used in food industry, and hence can replace it as a natural food preservative [2]. The current work focuses on the comparative analysis of DPPH mediated Antioxidant activity of *A. vulgaris* Methanolic extract and essential oils.

Materials and Methods

Collection of Plant Materials:

The plant materials were collected from Pauri region of Uttarakhand and were used further for extract preparation and essential oil extraction.

Preparation of the extracts

The aerial parts of the plants were dried under shade and powdered. Crude materials (50 g) were extracted successively with 500 ml of methanol, using a Soxhlet extractor for 6 h. The solvent was evaporated to dryness under reduced pressure on a rotary evaporator. Dried extracts were stored at 4 °C until studied.

Isolation of essential oils

The air-dried samples were subjected to hydro-distillation using a Clevenger apparatus for 6 h to obtain the essential oils. They were dried over anhydrous sodium sulfate, filtered, and stored at 4°C in darkness.

Gas chromatography (GC) and Gas Chromatography-Mass spectrometry (GC-MS)

GC analysis was performed by using HP 6890gas chromatograph equipped with FID detector and a HP-5 fused silica column (30m x 0.32 mmx 0.25 µm film thickness). Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210°Cand 230°C, respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in rate of 3°C/min. GC-MS analysis was carried out on a PerkinElmer mass spectrometer (Model Claurus 500) coupled to a Perkin Elmer Claurus 500 gas chromatograph with a 60 m x 0.32 mm x 0.25 µm film thickness column of rested make (Rtx-5). Helium was used as the carrier gas with a flow rate 1 ml/min. The mass range was scanned from40-600 Daltons. The oven temperature program ranged from 60°C to 220°C with an increase rate of 3°C/min. Other conditions were the same as described under GC. The identity of the constituents of the oils was established on the basis of GC retention indices, by comparing their 70 eV mass spectra with those reported in literature 23 and by computer matching with NIST and WILEY libraries, as well as, where possible, co-injection with authentic compounds available in our laboratory.

Determination of Antioxidant activity

The radical scavenging activity was measured using the stable radical DPPH according to the method described previously [14] with some modifications. The concentrations of 1mg/ml were made with extracts and essential oil. 10-100 µl of samples were added to different tubes and the volumes were made to 1 ml by addition of methanol and 2 ml of DPPH in each tubes. In case of Essential oils, they were first mixed in Acetone and rest all methodology was same as applied in case of plant extracts. Various concentrations of the extracts were added to 4 ml of 0.004% methanol solution of DPPH. The mixture was shaken and left for 30 min at room temperature in the dark, and the absorbance was then measured with a spectrophotometer at 517 nm on spectrometer using Methanol as Blank (Perkin-ELMER Lambda35 UV-Vis model) and converted into the percentage antioxidant activity using the following equation:

$$\text{DPPH anti-radical scavenging capacity (\%)} = \frac{AC-AT}{AC} X\ 100$$

where AC is the Absorbance of Control and AT is the Absorbance test sample, comprising of plant extract / Essential oil with DPPH reagent. An IC50 value of the Anti-oxidant activity was analyzed by using the non-linear sigmoid regression curves.

Determination of total phenols

Total phenols were determined by the Folin-Ciocalteau reagent using Gallic acid as a standard and the total phenols were expressed as mg Gallic acid equivalents (GAE) per g dry extract. 1 ml of the plant extracts (1 mg/ml) was mixed with the Folin-Ciocalteau reagent (1 ml) and an aqueous Na_2CO_3 solution (0.8 ml, 7.5%). The mixtures were allowed to stand at room temperature for30 minutes, and the absorbance of the reaction mixture was measured at 765 nm [16].

Determination of total flavonoids

The flavonoids content in extracts was determined spectrophotometrically using an aluminum chloride colorimetric method, based on the formation of a complex flavonoid-aluminum as described by Karabegović et al. (2011) with slight modification [11]. 1ml of the plant extract (2 mg/ml) was taken and mixed with 3 ml of methanol followed by the addition of 0.2 ml of 10% aluminum chloride ($AlCl_3$), 0.2 ml of 1 M potassium acetate (CH_3COOK) and 5.6 ml of distilled water. The reaction mixture was incubated at room temperature for 30 minutes, the absorbance of the reaction mixture and the absorbance was measured at 420 nm against the blank, which consisted of all the reagents except for the plant extract.

Results and Discussion

DPPH radicle scavenging method was used for determining the antioxidant activity of *A.vulgaris* Methanolic Extracts and Essential oils. The activity of Methanolic extract mediated antioxidant at 1 mg/ml, the activity was 72.65%. Depending on the concentration of extract used for the antioxidant activity, the Methanolic extract was found to scavenge 92.33% of DPPH [6]. In the case of Essential oil at the same concentration, the activity was found to be around 88.65% which is more as compared to the study done by previous researchers [2, 4] while the activity of the standard; Ascorbic acid at 1mg/ml was 96.565%. The antioxidant assay results and IC50 value of extracts and essential oils of the *A. vulgaris* are given in [Table-2] and [Fig-1] and [Fig-2]. Higher Phenolic content leads to the strong activity and has a good impact on the health factor. These Phenolic compounds have various components like caffeic acid, neochlorogenic acid, and ferulic acid which is known to be fund in higher concentration in *Artemisia vulgaris*. In the present study this value was found to be 347.69 mg GAE/g extract which is more as compared to the previous results. The Total Phenolic Content was evaluated from the regression equation of the calibration curve (R^2= 0.99275, y = 0.0231x± 0.3832), expressed in GAE as milligrams per gram of extract (mg GAE/g extract). Previous studies have reported that *Artemisia vulgaris* possess higher Phenolic content, thus imparting a high antioxidant potential. Some researchers have found the total phenolic content to be around 304 mg GAE/g extract [20] 321 mg GAE/g extract [6]. Flavonoids play a crucial role in providing antioxidant and biological properties to the plant systems. The flavonoid content was measured from the regression equation of the calibration curve (R^2= 0.99652, y = 0.0029x- 0.0138), expressed in GAE as milligrams per gram of extract (mg QE/g extract) and was found to be 101.17 mg QE/g extract which approximately as same as that of previous researches [11, 6, 20]. Total 24 compounds were identified from the essential oil analysis by GC. The average essential oil yield of *A. vulgaris* varied from 0.40-0.50 % (v/w) the percentage composition of the constituents in the oil is enlisted in [Table-1]. Camphor was found to be a major constituent (10.7%) followed by ß endesmol (8.95%), Trans-Caryophyllene (6.525%), Borneol (6.461%) Bornyl acetate (6.293%), cis Cadina-1,4- diene (4.323%), 1.8-Cineole (4.079%), Acoradiene (3.619%) followed by other constituents which were found in traces which are enlisted in [Table-1]. The quantity of phyto-constituents in the essential of this plant varied from the previous studies conducted on this plant's essential oil [2, 4, 13]. In conclusion, it can be deduced that the antioxidant mediated activities of *Artemisia vulgaris* plant is profound in essential oils as compared to the Methanolic extracts. Although the Results Are Vulnerable And May Increase With The Increase In Concentration. Secondly, essential oils of the aromatic plants are known to possess high concentration of secondary metabolites along with chemo-phyto-constituents which leads to an increased antioxidant activity as compared to extracts which possesses maximum quantity of chemo-phyto-constituents and less of secondary metabolites.

Table-1 *Phyto-constituents of Artemisia vulgaris Essential oils*

S. No.	Components Identified	%
1.	α pinene	0.90
2.	Camphene	2.54
3.	Sabinene	0.67
4.	Cymene	1.14
5.	Limonene	0.46
6.	1,8 Cineole	4.07
7.	γ terpinene	0.54
8.	Artemisia ketone	2.89
9.	Cis-Sabinene hydrate	1.08
10.	Trans Sabinene hydrate	0.55
11.	α thujone	3.18
12.	β thujone	1.19
13.	Camphor	10.75
14.	Borneol	6.46
15.	4- terpineol	1.44
16.	Bornyl acetate	6.29
17.	Trans-Caryophyllene	6.52
18.	Acoradiene	3.61
19.	AR curcumin	1.19
20.	Germacrene D	3.28
21.	Cadina-1,4-diene (cis)	4.32
22.	Caryophyllene Oxide	1.52
23.	β eudesmol	8.95
24.	Trans Pinocarveol	0.77
25.	Carvone	0.38
26.	Geraniol	0.84
27.	Thymol	0.39
28.	α Yalgene	0.21
29.	α Humulene	0.78
30.	γ Curcumene	1.55
31.	Geranyl Acetate	3.76
32.	β Selinene	0.45
33.	Carophyllene Oxide	0.78
34.	Davanone	0.15
35.	Trans Caryophyllene	6.94
36.	Trans β farnesene	0.88
Total % of Compounds Identified		91.42%

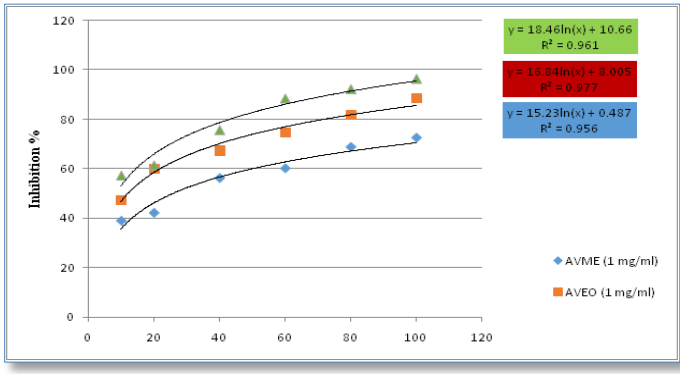

Fig-1- Antioxidant activity of *Artemisia vulgaris* Methanolic Extract and Essential Oil (%inhibition vs. concentration)

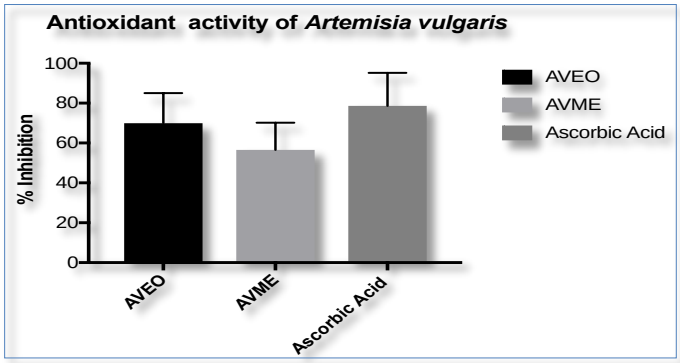

Fig-2 Antioxidant activity of *Artemisia vulgaris* Methanolic Extract and Essential Oil

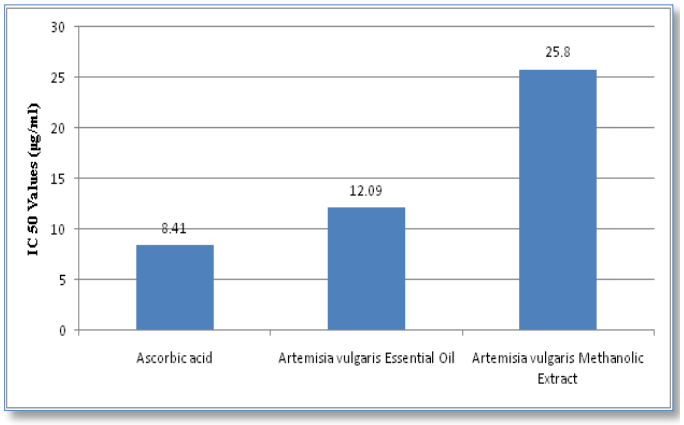

Fig-3 IC50 values of *Artemisia vulgaris* Methanolic Extract, Essential Oil and Ascorbic Acid

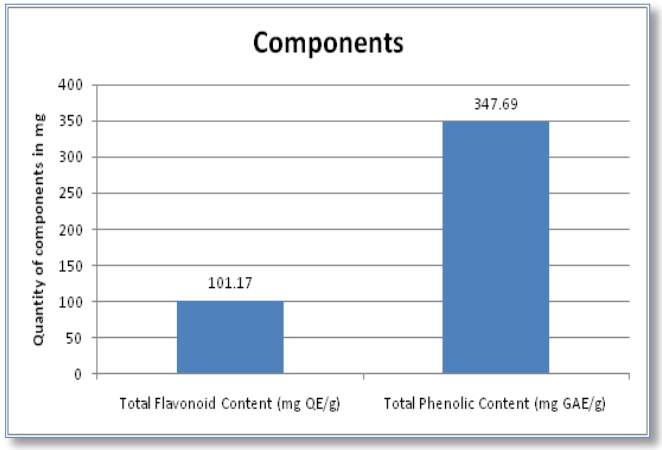

Fig-4 Total Phenolic and Flavonoid Content of *Artemisia vulgaris* Methanolic Extracts

Table-2 *Inhibition % of Artemisia vulgaris Methanolic extract and Essential oil at various concentrations.*

Antioxidant Activity of *Artemisia vulgaris*(Inhibition %)			
Sample quantity (µl)	AVME (1mg/ml)	AVEO (1 mg/ml)	Ascorbic Acid (1 mg/ml)
10	38.93	47.38	57.12
20	42.14	59.99	61.32
40	56.27	67.36	75.64
60	60.22	74.65	88.54
80	68.87	81.84	92.33
100	72.65	88.65	96.56

Table-3 *IC50 value of Artemisia vulgaris Methanolic extract and Essential oil at various concentrations*

IC50 value of *Artemisia vulgaris*		
S.No.	Sample	IC50 value (mcg/ml)
1.	Ascorbic acid	8.41
2.	*Artemisia vulgaris* Essential Oil	12.09
3.	*Artemisia vulgaris* Methanolic Extract	25.80

Conclusion

The study report proves that the anti-oxidant activity of essential oil of *Artemisia vulgaris* is more than its Methanolic extract. Secondly, the phenolic content of the Methanolic extracts of this plant (*Artemisia vulgaris*) is more as compared to the previous studies and may be responsible for the high antioxidant activity

Application of research: Artemisia vulgaris has been used in the form of tea in certain traditional medicine for curing of digestive and menstrual disorders. This study reveals the high phenolic content of the plant and a good anti-oxidant property, which can be also used in the form of an ethnomedicine with diverse applications.

Research Category: Phytochemicals and Antioxidants.

Abbreviations:

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