Research article

ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF *Artemisia vulgaris* COLLECTED FROM SUB-TROPICAL REGION OF BAGMATI PROVINCE, NEPAL

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ABSTRACT

Artemisia vulgaris is one of the highly used plant species as traditional medicine because of its antioxidant activity, anti-allergic effect and other several health benefits. This study was carried out with the objectives to explore the phytochemical constituents and assess the antioxidant property of essential oils of *A. vulgaris* found in sub- tropical regions of Bagmati province. Hydro distillation method was used for the extraction of essential oil and gas chromatography–mass spectrometry (GC-MS) analysis was performed to identify various phytochemicals present in essential oil. Evaluation of antioxidant activity by *in vitro* method was carried out by observing hydrogen peroxide scavenging effects of essential oils at different concentrations. GC-MS analysis showed 41 different phytochemicals including mono- and sesquiterpenes. Among them, highly expressed phytochemicals were Cadinene<gamma-> (14.95%), Caryophyllene(E) (9.32%), Camphor (8.74%), Thujone<alpha-> (6.57%), Eucalyptol (6.25%). Essential oil also showed scavenging effect against hydrogen peroxide with EC_{50} value of 48.49. This preliminary study shows that essential oil of *A. vulgaris* can be used as a source of natural antioxidants, but *in vivo* and other clinical trials must be done for oral use.

Keywords: *Artemisia vulgaris*, essential oil, antioxidants, phytochemicals

INTRODUCTION

A. vulgaris is widely distributed in mountain regions of Nepal. It is commonly known as mugwort, a perennial weed growing wild and abundantly in temperate and cold-temperature zones. *A. vulgaris* species, have been the most used species since long ago traditionally till now, as they are the rich source of flavonoids and steroids compounds (Xie et al., 2014), but little is known about the flavonoids contents. *A. vulgaris* has received increasing interest in the scientific community for their medicinal applications, especially with regards to the flavonoids. Flavonoids have been known for their high antioxidant activities and associated with several health benefits (Iqbal et al., 2012; Tang et al., 2000).

Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of" the plant material from which they were extracted. They are complex mixtures of volatile compounds such as terpenes (mostly monoterpenes and sesquiterpenes), phenolics and alcohols (Lucchesi et al., 2004). Essential oils are known to possess potential as natural agents for food preservation. Many of them recently have been qualified as natural antioxidants and proposed as potential substitutes for synthetic antioxidants in specific sectors of food preservation where their use is not in contrast with their aroma (Ruberto & Baratta, 2000).

Free radicals are molecules that contain an unpaired electron in an atomic orbital and are capable of independent existence that gives them the property of being highly reactive and unstable species. They attack the important macromolecules like the lipids, nucleic acids and proteins leading to cell death and homeostatic disruption.

Antioxidants are compounds that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radicals by one (or more) of several mechanisms such as scavenging free radicals, chelating metal ions, preventing formation of peroxides, breaking the autoxidative chain reactions and reducing localized O2 concentrations (Brewer, 2011). Current use of plant based natural antioxidants

in the form of phenolic compounds such as flavonoids, phenolic acids and tocopherols in food as well as in medicinal world are alluring much recognition because of their comparatively safe status and their putative protective effects against the deleterious oxidationinduced injuries (Ormancey et al., 2001; Cieslik et al., 2006). Not much studies on *A. vulgaris* have been done in Nepal to explore its chemical components. So, this investigation was carried out to explore the phytochemical constituents and assess the antioxidant property of essential oils of *A. vulgaris.*

MATERIALS AND METHODS

Plant Materials

Plant samples of *Artemisia* species were collected from subtropical regions (1200m-1400m height) of Bagmati Province of Nepal. The plant samples were stored in plastic containers in a refrigerator until essential oils were extracted.

Extraction of Essential Oil

Essential oils were extracted by using hydro distillation method. To extract the essential oils, leaves of *A. vulgaris* were air dried at room temperature for 24 hours. These were then sliced into small pieces and subjected to extraction with water by hydro-distillation method for 3 hours using a clevenger-type apparatus. The essential oils thus obtained were dried over anhydrous sodium sulphate and stored at 4 ºC until use.

GCMS Analysis

The component of volatile oil samples from the leaves of plant samples were identified using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The analysis of essential oils was performed using GC-2010 plus gas chromatograph coupled to a GCMS-QP2010 SE mass spectrometry detector and equipped with an AOC20i auto-injector. A capillary Rtx-5MS column was used for separation. Helium (at a flow rate of 1.0 mL/min) was used as carrier gas. Temperature was kept at 60ºC for 5 min and programmed to reach 240ºC at the rate of 3ºC per min. The samples were injected at the injected temperature of 250ºC. The injection volume was 1.0 µL in 1:30 split ratio. The mass spectra were obtained with electron impact ionization (70 eV) at full scan mode (40 to 500 m/z), using an ion source at 200 ºC. The compounds were identified by comparing retention indices (RI) and mass spectra with data from the FFNSC 4.O, FFNSC 1.3 library and the literature.

Evaluation of antioxidant activity by *in vitro* methodHydrogen peroxide scavenging effects

A total of 4 ml of (1.2510 µl/ml) essential oil/ascorbic acid was added to 0.6 ml of hydrogen peroxide solution (4 mM) in phosphate buffer (0.1 M and pH7.4). After incubating for 10 min at 37°C, the absorbance at 230 nm was measured. Corresponding blanks were taken. The experiment was performed in triplicate. The absorbance of phosphate buffer as control was measured at 230 nm. Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals is seen by the decrease in absorbance at 230 nm with increasing concentration of the test sample. The scavenging effect (%) was measured using the following equation:

Percent scavenging = $[(A_0-A_1)/A_0]$ x 100%,
where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the sample.

Data Analysis

Microsoft Office Excel 2010 was used to compute mean along with the standard deviation (±SD). Statistical analysis was done using one-way ANOVA and considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Essential Oil

The essential oils extracted were clear, yellowish in color. The maximum percentage yield was 0.95% v/w. The phytochemical constituents of the essential oil extracted showed 41 different compounds. The 20 compounds with their peak area percentage are shown in Table 1 and Figure 1. Among these compounds

Cadinene <gamma-> (14.95%), Caryophyllene(E) (9.32%), Camphor (8.74%), Thujone <alpha-> (6.57%) and Eucalyptol (6.25%) were the major five compounds (In which basis these five compounds were major compounds?). The experimental results were different from those previously reported. Pandey, et al., (2017) reported that the major components were sabinene (11.29%), beta-thujone (19.99%), chrysanthenone (4.48%), camphor (11.89%), borneol (4.44%) and germacrene D (8.42%). However, although in lesser amount, the presence of sabiene (2.58%), beta-thujone (4.15%), chrysanthenone (4.61%) and borneol (1.53%) were seen in these essential oils (in your experiment or what?). The variation in chemical compositions of essential oil may be due to harvesting seasons, extraction methods, geographical sources and adaptive metabolism of plants, etc. (Al-Reza et al., 2010; Kamazeri et al., 2012).

The essential oil of *A. vulgaris* contained thujone which was reported to have gamma-amino butyric acid receptor antagonist (Karin et al., 2000). Similarly, compound borneol is used as a natural insect repellent and presence of thujone and borneol makes the strong insect repellent properties.(Maria et al., 2012)

Table 1. GC–MS chemical composition of essential oil from *A.vulgaris*

Name of Compounds	Contain %	S.N.	Name of compounds	Contain %
Cadinene <gamma->	14.95	11	Humulene <alpha->	2.66
Caryophyllene (E)->	9.32	12	Sabinene	2.58
Camphor	8.74	13	Cymene <para->	1.97
Thujone <alpha->	6.57	14	Pinene <a1pha->	1.75
Eucalyptol	6.52	15	Muurolene<gamma->	1.70
Cadinene <de1ta->	4.91	16	Borneol	1.53
Chrysanthenone	4.61	17	Camphene	1.44
Thujone <beta->	4.15	18	Caryophyllene oxide	1.39
Gurjunene<alpha>	3.85	19	Amorphene <gamma->	1.37
Bicyclogermacrene	3.25	20	Copaene <a1pha->	1. 36

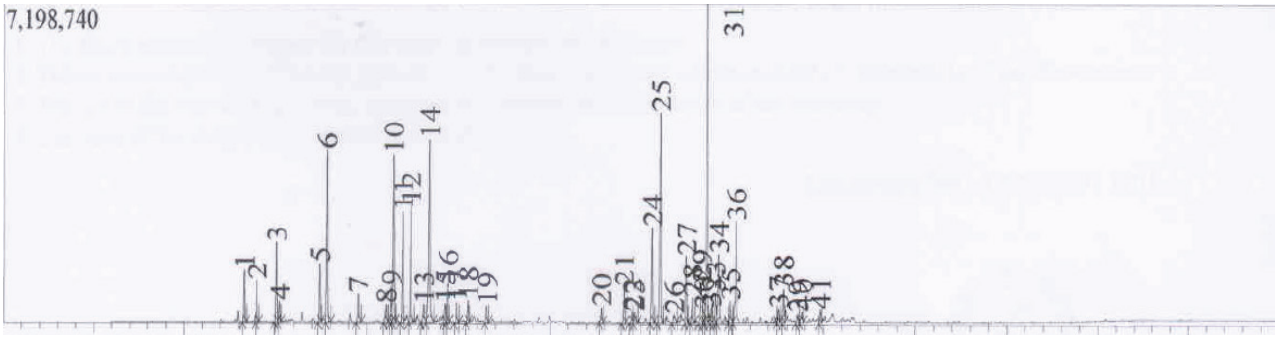


Figure 1. **GC–MS spectrum of essential oil from *A.vulgaris*.**

Antioxidant Activity

Hydrogen peroxide scavenging effects

It was seen that hydrogen peroxide scavenging effect increased with the increase in concentration of essential oil of *A. vulgaris*. However, the essential oil showed less scavenging activity when compared with ascorbic acid. H_2O_2 was scavenged by 68.75 % at 60 ug/ml concentration of essential oil where same amount of scavenging effect was shown by L-ascorbic acid at 15ug/ml as shown in Table 2. The greater scavenging capacity means the higher antioxidant activity. The concentration required for 50% inhibition (EC_{50}) value of ascorbic acid was found to be 8.86 while that of essential oil of *A. vulgaris* was 48.49. This shows that essential oil of *A. vulgaris* exhibits antioxidant property but less than L-ascorbic acid.

Table 2. Hydrogen Peroxide assay for antioxidant activity of essential oil & L-Ascorbic acid

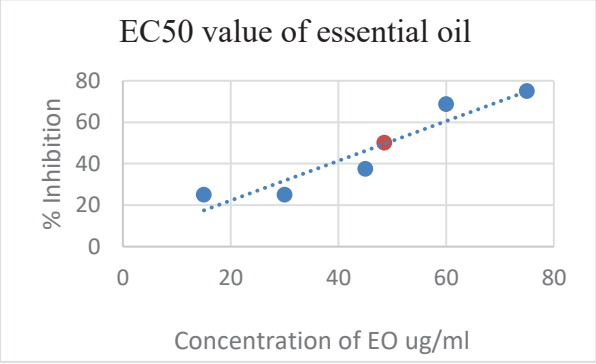
Concentration of essential oil	% Scavenging
15 ug/ml	25.00
30ug/ml	25.00
45ug/ml	37.50
60ug/ml	68.75
75ug/ml	75.00

Concentration of L-Ascorbic acid	% Scavenging
5ug/ml	37.50
7.5ug/ml	43.75
10ug/ml	56.25
12.5ug/ml	62.50
15ug/ml	68.75

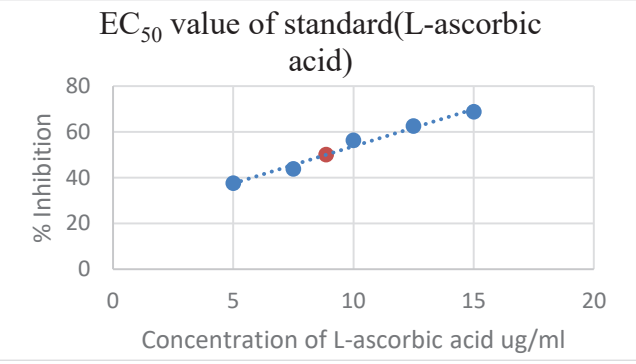
Table 3. E50 value of essential oil of *A. vulgaris* and L-ascorbic acid

Sample	EC50 value
Essential oil	48.49
L-ascorbic acid	8.86

Artimisia vulgaris essential oil contained mono- and sesquiterpenes compounds such as alpha and beta-thujone, sabinene, chrysanthenone, camphor, borneol, germacrene D etc. These compounds make the essential oil to possess the antioxidant activity. Beside this, oxygenated monoterpenes like cineole, terpineol are also responsible for the antioxidant property of essential oil (Szabolcs et al., 2000). The presence of strongly activated methylene groups in these compounds is probably the main reason for antioxidant behavior. However, the effectiveness of essential oils for scavenging free radicals may be due to synergistic effect of their own chemical compositions existing in oils (Andrade et al., 2013).



Graph 1. EC_{50} value of *A. vulgaris* essential oil



Graph 2. EC_{50} value of L-ascorbic acid

CONCLUSION

Essential oil extracted from *A. vulgaris* contained 41 different phytochemicals. Some of these compounds were oxygenated monoterpenes whereas some were sesquiterpenes. Due to the presence of these various compounds, the essential of *A. vulgaris* showed strong scavenging activity against hydrogen peroxide. Thus, from this preliminary study we can conclude that essential oil of *A. vulgaris* can be used as a source of natural antioxidants. Furthermore, the oil can be used in pharmaceutical application, natural therapy, and food supplements. But *in vivo* studies and other clinical trials are needed to justify and evaluate the potential use of the oil as antioxidant agent in topical or oral applications.

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