Free Radical Scavenging Activity and Kinetic Behaviorof Essential Oil from *Artemisia vulgaris*

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The radical scavenging activity of *Artemisia vulgaris* essential oil was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay in this study. Essential oil exhibited a significant free radical scavenging activity, with the highest activity at 15 μL/mL concentration. The reaction rate was slow and concentration-dependent

Key words : *Artemisia vulgaris* essential oil, Free radical scavenging activity, Butylated hydroxyanisole, α-Tocopherol, Concentration-dependent manner

Introduction

Plant volatile oils areknown to have potential natural agents for food preservation as their effectiveness against a wide range of microorganisms has been well established. Essential oils have been reported to have a range of biological properties. The major properties amongst are their antibacterial, antifungal and antioxidant properties[1]. Recently many essential oils have been qualified as natural antioxidants[2-4] and are suggested as a potential substitute of synthetic antioxidants. Furthermore, essential oils and their components are gaining aninterest because of their relatively safe status, wide acceptance by consumers, and exploitation for potential multi-purpose functional use[5,6].

Artemisia vulgaris Linn (family: Compositae) is a common shrub found in mountains of Nepal (1,500-3,600 m). It is one of the most religious plants in Nepal and is offered in many ritual celebrations. It is also used extensively in spiritual treatment of patient. For example local healers use the foliage in chasing the evils away from the patient's body. Infusion of leaves and flowering tops are used in nervous and spasmodic affections, asthma and diseases of brain[7]. Many *Artemisia* species have a characteristic scent or taste, caused by monoterpenes and sesquiterpenes, which in many cases are the reason for their application in folk medicine[8]. So far a few species of Nepalese plants have been studied for their antioxidant activity[9-11] To our best knowledge, there have been no previous studies regarding the free radical scavenging activity of *Artemisia vulgaris* essential oil. Therefore, the present study was conducted to identify antioxidant properties of essential oil of this plant.

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Materials and Methods

1. Instrument and reagents

Octadecyl-functionalized silica gel, butylated hydroxy anisole, α-tocopherol and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. All reagents used, including solvents, were of analytical grade. Recordings were made in a UV-vis Diode Array Spectrophotometer, Hewlett Packard 8453.

2. Plant materials

Artemisia vulgaris essential oil (steam distillation of the leaves) was obtained from Gharelu Herb Processing Centre, Chapagaun, Lalitpur in Nepal in March 2004. The oil has a pale yellow colour with powerful, fresh-camphoraceous aroma.

3. DPPH assay

The ability of the *Artemisia vulgaris* essential oil to donate hydrogen atoms or electrons was measured from the bleaching of purple coloured methanol solution of DPPH[12]. Briefly, 0.5 mL of methanolic solution of 0.24 mM DPPH was mixed with 0.5 mL of *Artemisia* essential oil (in methanol) in different concentrations (5, 10, 15, 20, 25 and 30 μL/mL) and reference compounds. The mixture was shaken vigorously and left to

stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. Radical scavenging activity was expressed as the percentage of DPPH elimination after 30 minutes and calculated as follow.

$$\text{Scavenging ability (\%)} = [A_0 - A_1/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the test compound. BHA and α-tocopherolwere used as positive controls. Moreover, to understand its kinetic behavior, the decrease in absorbance of essential oil and reference standards was studied at 517 nm for every minute until 90 minutes.

4. Statistical analysis

The data are results of triplicate experiments. Microsoft Excel was used to compute means and standard deviation. Differences among all sample means were determined by analysis of variance (ANOVA) using Origin, version 5(Micro cal Software, Inc, 1991-1997) and were considered significant at $p<0.05$.

Results and Discussion

Radical scavenging activity and kinetic behavior of different concentrations of essential oil and that of standard antioxidants was studied. Essential oil exhibited a concentration dependent radical scavenging activity, with the highest activity at the concentration of 15 μL/mL(Fig. 1).

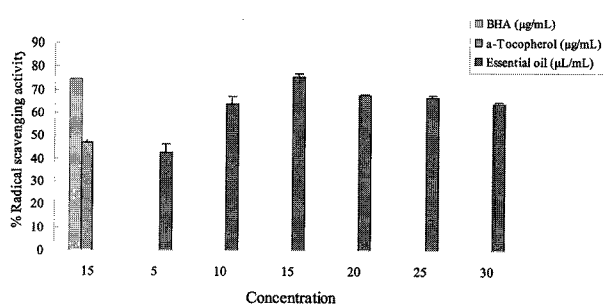

Fig. 1. Free radical scavenging activity (%) of *Artemisia vulgaris* essential oil and reference compounds at 30 minutes. The results are mean ± S.D. of triplicate experiments. Significantly different from the control values: $p<0.05$.

The reaction rate was slow and depended on concentration. However, there was a gradual decrease in scavenging activity with increasing concentration above 15 μL/mL (Fig. 2 & 3). Antioxidants are believed to intercept the free radical chain of oxidations and to contribute hydrogen from the phenolic hydroxyl groups themselves; thereby forming stable free radicals which do not initiate or propagate further oxidation of lipids[13].

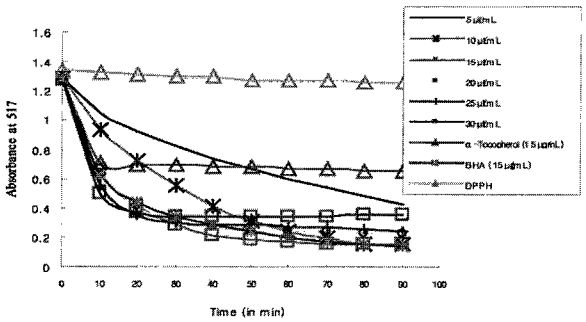

Fig. 2. Kinetics of *Artemisia vulgaris* essential oil and reference compounds against the DPPH method with various concentrations.

Furthermore, the presence of available hydrogen atoms in phenol and/or allylic groups represents a good barrier against the primary oxidative process[14]. The present study revealed that the *A. vulgaris* essential oil is an effective hydrogen donor and a primary antioxidant by reacting with the lipid radical.More than 40 components have been identified from the essential oil of *A. vulgaris*, among which the oxygenated monoterpenes as major components (1,8-cineole, camphor and [alpha]-terpineol) followed by sesquiterpenes and one acyclic non-terpenic compound[15]. Similarly, the leaf oil was found to be rich in 1,8-cineole (2.2-12.2 %), [alpha]-thujone (0-11.4 %), camphor (15.7-23.1 %) and isobomeol (9.3-20.9 %)[16]. Oxygenated monoterpenes are mainly responsible for the antioxidant property of the plant oils which contain them[17,18]. The presence of strongly activated methylene groups in these molecules is probably the reason for this behaviour[14].

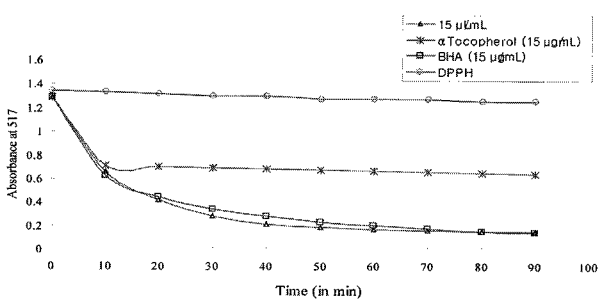

Fig. 3. Kinetics of *Artemisia vulgaris* essential oil (15 μL/mL) by DPPH method with compared to reference antioxidants.

The kinetic classification, according to the time at the steady state, has been reported as rapid <5 minutes, intermediate 5 - 30 minutes and slow >30 minutes[19]. In this study, the reaction rate of essential oil and BHA was slow while that of α-tocopherol as intermediatewhich agreed with previous studies[19,20] showing that the kinetic classification of BHA was slow and α-tocopherol was intermediate.

Finally, we standardized *Artemisia vulgaris* essential oil by nuclear magnetic resonance (NMR) spectrometry (Varian Unity

500, 500 MHz, Janpan). The essential oil of *Artemisia vulgaris* was analysed by ^{1}H-NMR spectroscopy of $CDCl_3$ solubles. It gave the main biological compound whose signals was clearly visible in the ^{1}H-NMR spectrum of the essential oil. Probably obvious were chemical shifts between weak 5.6 ppm and 5.7 ppm indicative of lactone of exocyclic-α-methylene group, and olefinic groups between 4.6 ppm and 5.4 ppm in germacranolide-type compounds.It showed IR absorptions at 3474 (OH), 3073 (olefinic C-H stretching), 1746 (αβ-unsaturated five-ring lactone) and 1657 cm^{-1} (non-unconjugated alkene group)(Fig. 4)[21-23].

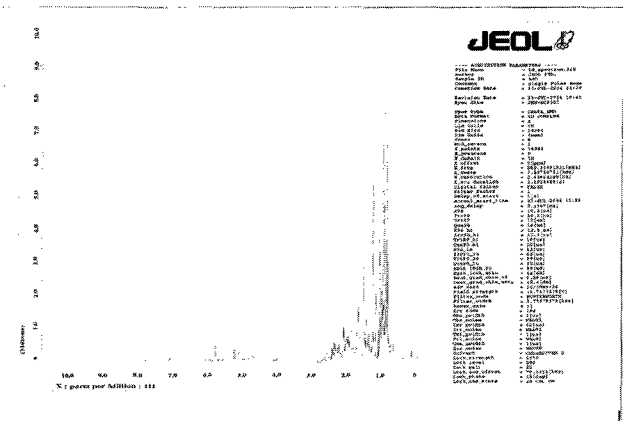

Fig. 4. ^{1}H-NMR Spectrum of *Artemisia vulgaris* essential oil

Table 1. Absorption frequencies of functional groups in the IR spectrum of *Artemisia vulgaris* essential oil.

Peak No	Band (cm-1)	Functional group	Remarks
2	3474 (m)	H-bonded-OH (liquid)	Often broad but may be sharp for some intramolecular single bridge hydrogen bonds: the lower frequency the stronger the hydrogen bond.
3	3073 (m)	>C=CH2	Olefinic C-H stretching.
4	2961-2850 (s)	>CH2, -CH3	Two or three stretching bands usually.
5	2728 (w)	-CHO	Aldehydic C-H stretching band
6	1746 (s)	αβ-Unsaturated ketone	αβ-Unsaturated five-ring lactone
7	1684 (m)	αβ-Unsaturated ketone	αβ-Unsaturated ketone
8	1657 (v)	>C=C<	Non-unconjugated alkene group
9	1603 (w)	αβ-Unsaturated carbony compound with olefinic group	αβ-Unsaturated carbony compound with olefinic band
10	1453 (s)	>CH2, -CH3	C-H Deformation
12	1372 (s)	-O-CO-CH	C-H Stretching band
25	880 (s)	>C=CH2	C-H Alkene stretching band

The present results revealed that the *A. vulgaris* essential oil possessed a remarkable radical scavenging activity, and might be effective against diseases caused by over production of free radicals. Since there is no report on the radical scavenging activity of A. vulgaris essential oil, our study may be considered as the first report. We hope that the results obtained will provide a starting point for the further investigations especially to evaluate its in vivo potential in animal models and different antioxidant mechanisms.

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