CHARACTERIZATION OF ANTIOXIDANT ACTIVITY OF EXTRACT FROM *ARTEMISIA VULGARIS*

ABEER TEMRAZ AND WALID H EL-TANTAWY*

Pharmacognosy Department, Faculty of Pharmacy for Girls, Al-Azhar University, Nasr City, Cairo, Egypt
**Drug Bioavailability Center, National Organization for Drug Control & Research, P.O. 29 Dokki, Cairo, Egypt*

ABSTRACT

Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. In this respect flavonoids and other polyphenolic compounds have gained the greatest attention. The present study was undertaken to evaluate the *in vitro* and *in vivo* antioxidant activities of aqueous extract of *Artemisia vulgaris*.

The plant extract was tested for DPPH (2, 2-diphenyl, 2-picryl hydrazyl) radical scavenging, nitric oxide radical scavenging, reducing power assays, total phenol, flavonoid and flavonol content. Determination of serum ascorbic acid level, blood glutathione level and superoxide dismutase activity in rats treated with 100 mg/Kg of *Artemisia vulgaris* extract.

The extract exhibited scavenging potential with IC_{50} value of 11.4 µg/ml for DPPH, the value were found to close to those of standard rutin (10 µg/ml).On the other hand *Artemisia vulgaris* extract exhibited nitric oxide scavenging activity with IC_{50} value 125 mg/ml. The reducing power of the extract depends on the amount of extract. The content of phenolic compounds (mg/g) in aqueous extract was found 19 ± 0.16 mg/g plant extract and expressed in gallic acid equivalents (GAE). The flavonoidal and flavonol contents were found to be 7.96 ± 0.76 and 3.4 ± 0.0 respectively mg/g plant extract in rutin equivalent. The treatment of rats with aqueous extract of *Artemisia vulgaris* resulted in a significant increase in blood glutathione level, superoxide dismutase activity and serum ascorbic acid level as compared to their corresponding controls.

The results obtained in the present study indicate that aqueous extract of *Artemisia vulgaris* is a potential source of natural antioxidants.

Keywords: *Artemisia vulgaris*; radical scavenging; DPPH; total phenolics; flavonoids; flavonols.

INTRODUCTION

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, ROS can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer (Ames,1998; Cox and Cohen 1996; Finkel and Holbrook 2000; Harman 1994).

Although many anti-oxidant defence systems consisting of enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic (ascorbic acid, glutathione and α-tocopherol) compounds can maintain the balance between ROS generation and protection from damage by ROS, these anti-oxidant systems do not provide complete protection from ROS attack under conditions of severe oxidative stress (Cesaratto, *et al.* 2004).

In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Deiana *et al.,* 1999; Lee and Shibamoto, 2000; Velioglu *et al.,* 1998, Wang and Jiao, 2000).

Artemisia vulgaris (Mugwort or Common Wormwood Family; Compositae) is one of several species in the genus *Artemisia* with names containing mugwort. It is native to temperate Europe, Asia and northern Africa, but is also present in North America where it is an invasive weed (wikipedia.org/wiki/*Artemisia_vulgaris*).

In traditional herbal medicine, aerial parts of *Artemisia vulgaris* are being used as antihelminth, antiseptic, antispasmodic, a tonic for vital organs and in various disorders including hepatosis (Duke *et al.* 2002).

The aim of this study is to investigate antioxidant activity of aqueous extract of *Artemisia vulgaris*.

MATERIALS AND METHODS

Preparation of the extract

Samples of *Artemisia vulgaris* were purchased from the Herbarium of the Faculty of Pharmacy, Cairo University, Egypt. The freshly cut plants were dried in the drying room with active ventilation at ambient temperature and packed in paper bags.

**Corresponding author:* Tel: 0020237496077, Fax: 0020233379445, e-mail: wldhmdy@yahoo.com

Table 1: Mean blood glutathione content, superoxide dismutase activity serum ascorbic acid level in rats treated with aqueous extract of *Artemisia vulgaris* in a dose of 100 mg/kg.

Tested parameter	Group (1)	Group (2)	Group (3)
Blood glutathione (mg/gm Hb)	1.96 ± 0.129	4.00 ± 0.26*	3.1 ± 0.526*
Blood superoxide dismutase (U/ml)	85.4 ± 3.81	174 ± 4.5*	167 ± 12.2*
Serum ascorbic acid (µg/ml)	3.58 ± 0.38	6.30 ± 0.96*	9.9 ± 1.74*

Group (1): normal control rats maintained on unrestricted standard diet and water ad libitum.
Group (2): treated orally with aqueous extract of *Artemisia vulgaris* in a dose of 100 mg/kg.
Group (3): treated orally with the reference drug (Silymarin) in a dose of 100 mg/kg.
Results are expressed in mean ± SE, Number of animals in each group equals 5.
P (comparison with control) < 0.05 is significant. *Significantly different from control value.

Dried plants were milled with sample mill (300 Waufn S2, Germany) and approximately (500 g of powder) in turn were extracted with water. The aqueous extract was evaporated to dryness under vacuum using a rotary. The process of maceration and evaporation was repeated till exhaustion of the plants powder, and then the residues were combined and weighed.

Identification of active constituents

Phytochemical analysis of *Artemisia vulgaris* showed that the major chemical constituents were eudesmanolides (sesquiterpene lactones), essential oils and flavonoids.

Determination of antioxidant activity of aqueous extract of the Artemisia vulgaris in vitro

DPPH• radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH• (2,2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH•_reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 520 nm on a UV/visible light spectrophotometer.

Radical scavenging activity of extracts was measured by slightly modified method of Brand-Williams *et al.*1995), as described below. Extract solutions were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. The solution of DPPH• in methanol ($6x10^{-5}$ M) was prepared daily, before UV measurements. Three ml of this solution were mixed with 77 (38 or 19 in additional assays) µl extract solution in 1 cm path length disposable microcuvettes (final mass ratio of extracts with DPPH• was approximately 3:1, 1.5:1, 0.75:1). Similar concentrations of rutin were used as reference standard. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH• solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

% inhibition = $[(A_B \_ A_A)/A_B] \times 100$

Where: A_B —absorption of blank sample (t=0 min);
A_A —absorption of tested extract solution (t=15 min).

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat,1964). In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Artemisia vulgaris* extract (25 to 125 mg/ml) or standard solution (rutin, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin was used as a standard.

Assay of reducing power

The reductive capability of the extract was quantified by the method of Oyaizu, 1986). One ml of (Extract) 100, 200 and 300 µg/ml in distilled water was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K3 Fe (CN) 6]. Similar concentrations of standard rutin were used as standard.

The mixture was incubated at 50°C for 20 min. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of

0.1%FeCl3. Blank reagent is prepared as above without adding extract. The absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Increased absorbance of the reaction mixture indicated greater reducing power.

Determination of the total amount of phenolic compounds

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu method 1927). For the preparation of calibration curve 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin–Ciocaleu reagent (diluted ten-fold) and 4 ml (75 g/l) sodium carbonate. The absorption was read after 30 min at 20^{o} C at 765 nm and the calibration curve was drawn. One ml aqueous plant extract (10 g/l) was mixed with the same reagents as described above, and after 1 h the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$C = c. V/m$

where: C—total content of phenolic compounds, mg/g plant extract, in GAE;
c—the concentration of gallic acid established from the calibration curve, mg/ml;
V—the volume of extract, ml;
m—the weight of pure plant methanolic extract, g.

Determination of flavonoid content

The content of flavonoids was determined by a pharmacopeia method (State Pharmacopeia of USSR, 1989) using rutin as a reference compound. One ml of plant extract in methanol (10 g/l) was mixed with 1 ml aluminium trichloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$X = (A.m_0.10)/ (A_0.m)$

where: X—flavonoid content, mg/g plant extract in RE; A—the absorption of plant extract solution; A_0—the absorption of standard rutin solution; m—the weight of plant extract, g; m_0—the weight of rutin in the solution, g.

Determination of flavonol content

The content of flavonols was determined by Yermakov, *et al*. 1987). The rutin calibration curve was prepared by mixing 2 ml of 0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml rutin ethanolic solutions with 2 ml (20 g/l) aluminum trichloride and 6 ml (50 g/l) sodium acetate. The absorption at 440 nm was read after 2.5 h at 20°C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of rutin solution. All determinations were carried out in duplicates. The content of flavonols, in rutin equivalents (RE) was calculated by the following formula:

$X = C.V/m$

where: X—flavonol content, mg/g plant extract in RE; C—the concentration of rutin solution, established from the calibration curve, mg/ml; V, m—the volume and the weight of plant extract, ml, g.

Animals and experimental protocol

Fifteen healthy male Wistar rats weighing between 150–200 g each were used for the investigation. The animals were housed at a temperature, humidity controlled room and a 12-h light-dark cycle (lights on at 0600 h). Rats were supplied with a standard pellet diet and tap water was freely available. The rats were randomly divided into 3 groups of 5.

Group (1): 5 Normal control rats maintained on unrestricted standard diet and water ad libitum and treated with distilled water (2 ml/kg p.o.) only.

Group (2): 5 treated orally with aqueous extract of *Artemisia vulgaris* in a dose of 100 mg/kg.

Group (3): 5 treated orally with the reference drug (Silymarin) in a dose of 100 mg/kg.

The animals were administered orally by orogastric catheter once daily for 42 days. At the end of treatment period, blood samples were collected from the tail veins of the rats at the same time of the day.

Antioxidant assays

Blood glutathione content was determined according to the method described by Beutler *et al.* (1963), blood superoxide dismutase activity, the method was carried out according to the pyrogallol method of Marklund and Marklund (1974) and serum ascorbic acid was estimated by the method of Jagota and Dani (1982).

RESULTS

DPPH radical scavenging activity

The aqueous extract of *Artemisia vulgaris* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 11.4 µg/ml. The IC_{50} value of the extract was found to be close to that of the standard; rutin (IC_{50} 10 µg/ml). Compared to rutin the extract exhibited a similar curve of antioxidant activity. This result demonstrated that *Artemisia vulgaris* extract has inhibitory activity against the DPPH radical, fig. 1.

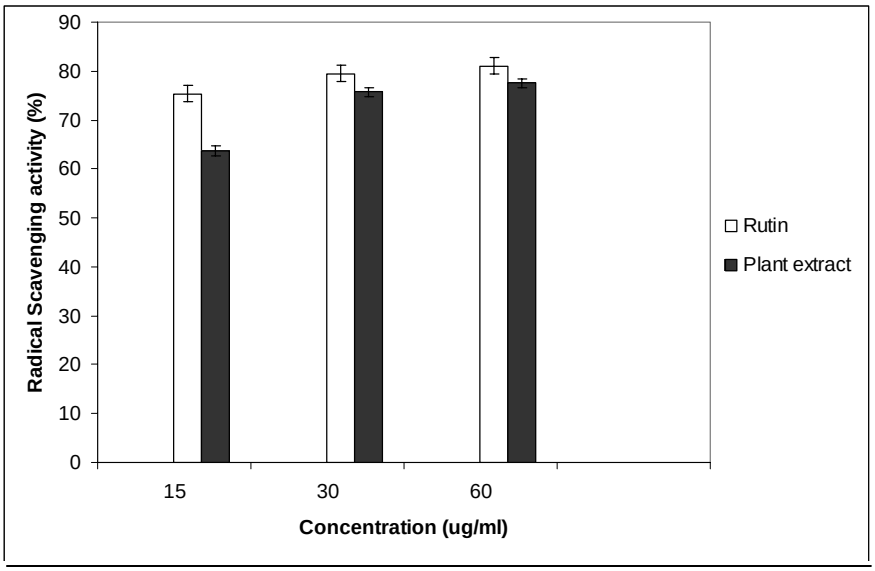

Fig. 1: **DPPH** radical scavenging activity of *Artemisia vulgaris* extract added to a methanolic solution of **DPPH** and radical scavenging activity was measured at 520 nm as compared to rutin.

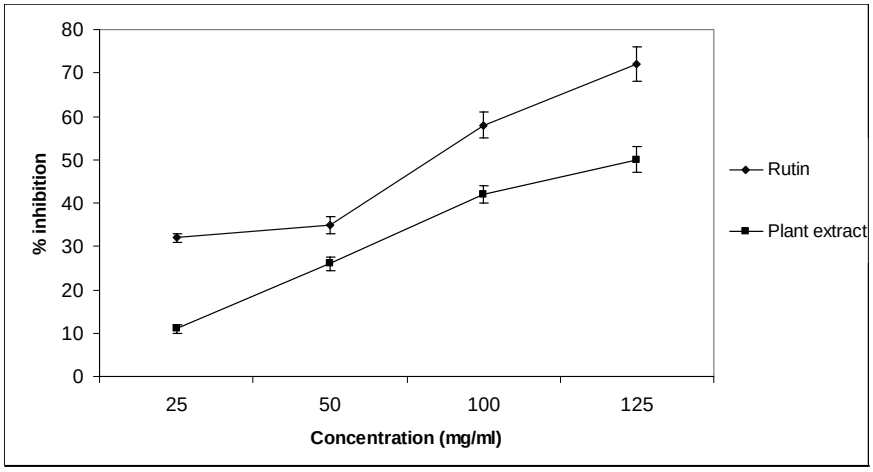

Fig. 2: Scavenging effect of *Artemisia vulgaris* extract and standard rutin on Nitric oxide radical.

Nitric oxide radical inhibition

The scavenging of nitric oxide by plant extract was increased in a dose dependent manner as illustrated in fig. 2. At concentration of 125 mg/ml of extract 50% of nitric oxide generated by incubation was scavenged, while IC_{50} value of standard rutin was found 86 mg/ml.

Reducing power

Fig. 3 shows the reductive capabilities of the plant extract compared to rutin. The reducing power of extract of *Artemisia vulgaris* was very potent and the reducing power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of rutin.

The content of phenolic, flavonoid and flavonol compounds

The content of phenolic compounds (mg/g) in aqueous extract was found 19 ± 0.16 mg/g plant extract and expressed in gallic acid equivalents (GAE). The flavonoidal and flavonol contents content were found to be 7.96 ± 0.76 and 3.4 ± 0.0 respectively mg/g plant extract in rutin equivalent.

Blood glutathione, superoxide dismutase and serum ascorbic acid

The treatment of rats with aqueous extract of *Artemisia vulgaris* resulted in a significant increase in blood glutathione level, superoxide dismutase activity and serum ascorbic acid level as compared to their corresponding controls ($P<0.05$), table 1.

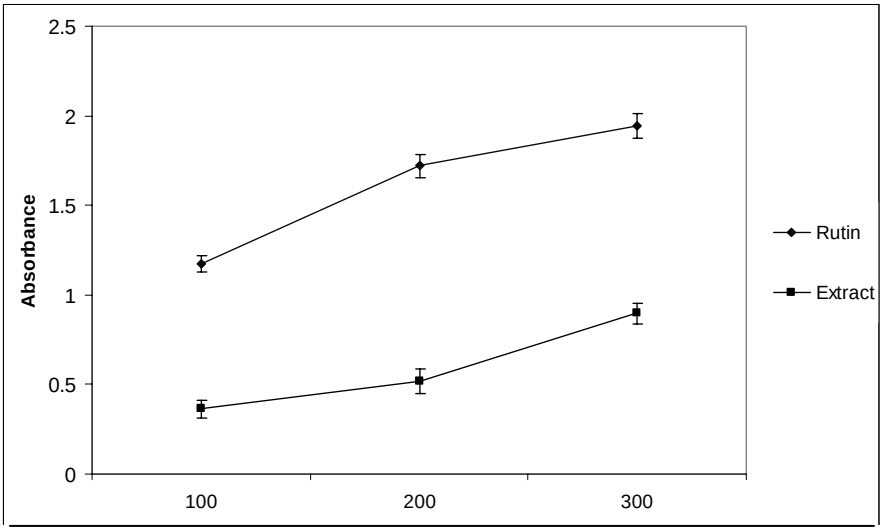

Fig. 3: The reductive ability of *Artemisia vulgaris* extract and rutin.

DISCUSSION

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants.

The present study was conducted to investigate the antioxidant potential of *Artemisia vulgaris*. Antioxidant activity of *Artemisia vulgaris* extract has been revealed in vitro by free radical scavenging, nitric oxide scavenging and reducing power assays and in vivo by determination of serum ascorbic acid, blood glutathione and superoxide dismutase assays in rats treated with *Artemisia vulgaris extract*. In addition, phenolic and flavonoid contents of the plant extract have been estimated. This study shows that *Artemisia vulgaris* extract clearly has antioxidant effects.

The model DPPH provides a method to evaluate antioxidant activity in a relatively short time compared to the other methods. In our present study, *Artemisia vulgaris* extract showed high scavenging activity of DPPH radical, which may be attributable to its hydrogen donating ability.

Nitric oxide radical inhibition assay proved that aerial part of the extract is a potent scavenger of nitric oxide. This nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci *et al.,* 1994).

Glutathione (GSH) acts as an antioxidant both intracellularly and extracellulary in conjunction with various enzymatic processes that reduced hydrogen peroxide and hydroperoxide by oxidizing GSH to GSSG and other mixed disulfides. In addition, the GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species (Arivazhagan *et al.,* 2000). The endogenous antioxidant enzymes are responsible for the detoxification of deleterious oxygen radicals. Superoxide dismutase (SOD) constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 (Hassan, 1988). Vitamin C is considered to be the most important antioxidant in extracellular fluids and has many cellular activities of an antioxidant nature as well. Vitamin C is likely to be involved in the detoxification of free radicals. In studies with human plasma lipids it was shown that vitamin C was far more effective in inhibiting lipid peroxidation (Estuo *et al.,* 1995). In the present study, the increase of these antioxidant systems in the rats treated with the extract of *Artemisia vulgaris* lead to the suggestion that the extract possess antioxidant activity.

The antioxidant activity of *Artemisia vulgaris* could be attributed to its flavonoidal content. Flavonoids act as scavengers of various oxidizing species i.e. super oxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen (Das and Ratty 1986). Numerous plant constituents have proven to show free radical scavenging or antioxidants activity (Aruoma and Cuppett, 1997).

Phenols are very important plant constituents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups (Vinson *et al.,* 1998). It was also reported that Phenolic compounds are

effective hydrogen donors, making them very good antioxidants (Yen *et al.,* 1993).

CONCLUSION

This study suggested that the *Artemisia vulgaris* extract possesses antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress- related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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