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*Full Length Research Paper*

# Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage

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***Artemisia absinthium* (*Asteraceae*) is widely used in Iranian traditional medicine. Its effects may be correlated with the presence of antioxidant compounds. Methanolic extract of *A. absinthium* aerial part at flowering stage was screened for antioxidant activities by five complementary test systems. Also, its antidepressant activity was determined by forced swimming (FST) and tail suspension tests (TST). The extract showed good antioxidant activity. Also, the extract showed good reducing power activity between 50 and 800 µgml$^{-1}$. The extract exhibited a good activity in $H_2O_2$ scavenging ($IC_{50}$ = 243 ± 12.15 µg ml$^{-1}$). $IC_{50}$ for iron ion chelating activity was 419 ± 20.95 µg ml$^{-1}$. Quercetin, BHA, EDTA and ascorbic acid used as positive controls in parallel experiments. The extract showed high phenolic and flavonoid contents. Extract showed good antidepressant activity in FST. The extract shortened remarkably the immobility period during the FST and TST and exhibited a dose dependent activity. All test groups were significantly different form control group ($P < 0.001$). Extract at 500 mg kg$^{-1}$ showed similar activity as imipramine 10 mg kg$^{-1}$ ($p > 0.05$) in TST. $LD_{50}$ was 3700 mg/kg. These results introduced *A. absinthium* aerial parts as an easily accessible and edible source of natural antioxidants and antidepressant.**

**Key words:** Antidepressant, Antioxidant activity, *Artemisia absinthium*, DPPH, forced swimming test, tail suspension test.

## INTRODUCTION

Depression constitutes the second most common chronic condition in clinical practice (Whooley and Simon, 2000) and will become the second leading cause of premature death or disability worldwide by the year 2020 (WHO, 1999). Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing (Mora et al., 2006). Although there are many effective antidepressants available today (Hadizadeh et al., 2009), the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated. This necessitates the development of newer and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential has been assessed in a variety of animal models (Zhang, 2004). In other way, plants are rich sources of natural antioxidants.

Among the various medicinal and culinary plants, some endemic species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou et al., 2002). Reactive oxygen species (ROS) have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease (Halliwell, 1997). Thus, recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals (Hou et al., 2003; Nabavi et al., 2008a). Additionally, it has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavornoids, phenolic acids, tannins and phenolic diterpenes (Pietta, 2000). Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has

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considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis (Zhou et al., 2000).

*Artemisia absinthium* L. (wormwood) (*Asteraceae*) is an aromatic-bitter herb, used as traditionally in Iran. this species known to possess ethnomedical and biological properties related to anthelmintic activity (Tariq et al., 2009), antifungal (Kordali et al., 2005), antimicrobial activity (Lopes-Lutz et al., 2008) choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukaemia and sclerosis (Canadanovic-Brunet et al., 2004). Essential oil composition of this species was reported previously (Nin et al., 1995). Antioxidant activity have been reported for essential oil of *A. absinthium* L. (Kordali et al., 2005; Lopes-Lutz et al., 2008) and its methanol extract (by DPPH and hydroxyl radical scavenging methods) (Canadanovic-Brunet et al., 2004). To the best of the authors' knowledge, antioxidant activity of aerial part of *A. absinthium* L. at flowering stage has not been reported to date. Also, nothing was found about antidepressant activity of this plant. Therefore, the aim of the present work is to determine the antioxidant activity of aerial parts of *A. absinthium* L. at flowering stage examined employing five various *in vitro* assay systems, that is, DPPH and nitric oxide and hydrogen peroxide radical scavenging, reducing power and iron ion chelating and its antidepressant activity by forced swimming test (FST) and tail suspension test (TST) in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

## MATERIALS AND METHODS

### Chemicals

Ferrozine, $H_2O_2$, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Quercetin, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

### Animals

Swiss albino mice (20 ± 2 g) of either sex were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45 - 55% relative humidity, with a 12 h light:12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water *ad libitum*. Experiments were conducted between 8:00 and 14:00 h. The experiments were conducted according to the norms of committee for the purpose of control and supervision of experiments in animal. Swiss albino mice were divided into five different groups (n = 10 per group) and tested in FST and TST.

### Plant material and preparation of freeze-dried extract

Aerial parts of *A. absinthium* L. at flowering stage was collected from Golestanak protege area central Elburz, Iran, in summer 2007. After identification of the plant by Dr. Bahman Eslami a voucher (No. 345) has been deposited in the Faculty of Pharmacy herbarium. The aerial part was dried at room temperature and coarsely ground before extraction. Aerial part was extracted at room temperature by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum for complete solvent removal until a crude solid extract was obtained.

### Determination of total phenolic and flavonoid content

Total phenolic compound content was determined by the Folin-Ciocalteau method (Ebrahimzadeh et al., 2008b; Ghasemi et al., 2009). The extract sample (0.5 ml of different dilutions) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoid was estimated according to method of our recent papers (Ebrahimzadeh et al., 2009a, d). Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

### DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extract (Nabavi et al., 2008a, Ebrahimzadeh et al., 2008a; Ghasemi et al., 2009). Different concentrations of extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. $IC_{50}$ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

### Reducing power determination

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action (Nabavi et al., 2008a, Ebrahimzadeh et al., 2009b,c). The reducing power of *A. absinthium* was determined according to the method of our recent paper (Ebrahimzadeh et al., 2008b). 2.5 ml of extract (50-800 µg $ml^{-1}$) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

### Assay of nitric oxide-scavenging activity

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide

compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of extract dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without extract but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% $H_3PO_4$ and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Ebrahimzadeh et al., 2008d, 2009d; Nabavi et al., 2009b).

#### Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extract (0.1 - 1 mg $ml^{-1}$) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compounds was calculated as follows: % Scavenged $[H_2O_2] = [(A_o - A_1)/A_o] \times 100$ where $A_o$ was the absorbance of the control and $A_1$ was the absorbance in the presence of the sample of extract and standard (Nabavi et al., 2008b; Nabavi et al., 2009a,b).

#### Metal chelating activity

The chelating of ferrous ions by extract was estimated according to our recently published paper (Ebrahimzadeh et al., 2008c; Dehpour et al., 2009). Briefly, the extract (0.2 - 3.2 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- $Fe^{2+}$ complex formation was calculated as $[(A_0 - A_s)/A_s] \times 100$, where $A_0$ was the absorbance of the control and $A_s$ was the absorbance of the extract/standard. $Na_2EDTA$ was used as positive control.

#### Forced swimming test

The mouse was dropped into glass cylinder (20 cm in height) and 12 cm in diameter containing 8 cm deep water at 24 - 25°C and left there for 6 min. The duration of immobility during the final 4 min interval of the swimming test was measured (Luo et al., 2000; Morteza-Semnani et al., 2007). Control group was treated with solvent. The other groups of mice received an interperitoneal (i.p.) injec-tion of extracts (125, 250, 500 and 1000 mg $kg^{-1}$) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (5 and 10 mg $kg^{-1}$), 1 h before the experiment. Imipramine was utilized as positive control of the test.

#### Median lethal dose

$LD_{50}$ was assumed using 50% deaths within 72h after i.p. administration of the extract at different doses. Male Swiss mice weighing 20 - 25 g (10 per group) were used in this experiment (Morteza-Semnani et al., 2007).

#### Tail suspension test

This test is a variant of the behavioural despai r test in which immobility is induced by suspending a mouse by its tail. On day 3, 1 h after the extract treatment, mice were hung individually on a wire in an upside down posture so that its nostril just touches the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5 min observation period were noted (Steru et al., 1985; Chermat et al., 1986). This test is reliable and rapid screening method for anti-depressants including those involving the serotonergic system (Bhattacharya et al., 1999).

#### Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test. The $EC_{50}$ values were calculated from linear regression analysis.

## RESULTS AND DISCUSSION

### Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total phenolic content of *A. absinthium* L. was 194.9 ± 9.7 mg gallic acid equivalent/g of extract. The total flavonoid contents was 12.4 ± 0.6 mg quercetin equivalent/g of extract, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (Nabavi et al., 2008b).

### DPPH radical-scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2008b). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh et al., 2008d). It was found that the radical- scavenging activities of extract increased with increasing concentration. $IC_{50}$ for DPPH radical-scavenging activity was 612 ± 30.6 µg $ml^{-1}$. The $IC_{50}$ values for Ascorbic acid, Quercetin and BHA were 1.26 ± 0.11, 1.32 ± 0.07 and 13.49 ± 1.04 µg $ml^{-1}$, respectively.

### Reducing power of *A. absinthium* extract

In the reducing power assay, the presence of reductants

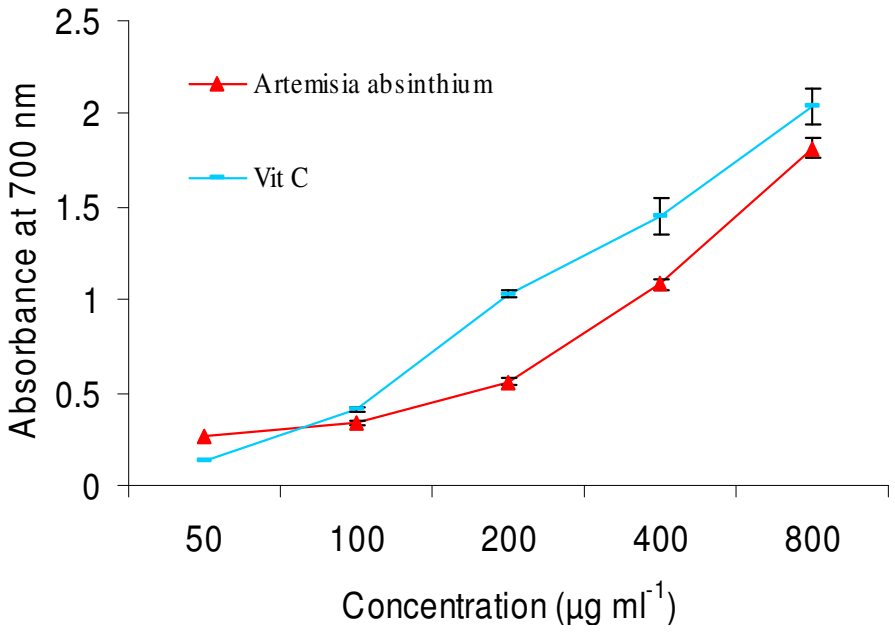


**Figure 1.** Reducing power of methanolic extract of *Artemisia absinthium* L. aerial part at flowering stage.

(antioxidants) in the samples would result in the reducing of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. Amount of $Fe^{2+}$ complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows the dose-response curves for the reducing powers of the extract from *A. absinthium* L. It was found that the reducing powers of extract also increased with the increase of its concentration. There were no significant differences among the extract and Vitamin C in reducing power ($p > 0.01$). Because the reductive ability of extract, it was evident, that *A. absinthium* did show reductive potential and could serve as electron donors, terminating the radical chain reaction.

### Assay of nitric oxide-scavenging activity

The extract showed very weak nitric oxide-scavenging activity between 0.4 and 3.2 mg $ml^{-1}$. $IC_{50}$ was 1.77 ± 0.08mg $ml^{-1}$. Quercetin showed very good activity. $IC_{50}$ for quercetin was 17.01 ± 0.03µg $ml^{-1}$. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Ebrahimzadeh et al., 2008d). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

### Scavenging $H_2O_2$

Scavenging of $H_2O_2$ by *A. absinthium* L. aerial part extract may be attributed to their phenolics, which can donate electrons to $H_2O_2$, thus neutralizing it to water (Nabavi et al., 2008b). $H_2O_2$ scavenging ability of extract may be attributed to the structural features of its active components, which determine its electron donating abilities (Nabavi et al., 2008b). The *A. absinthium* extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner. $IC_{50}$ for $H_2O_2$ scavenging activity was 243 ± 12.15 µg $ml^{-1}$. The $IC_{50}$ values for Ascorbic acid and BHA were 21.4 ± 1.07 and 52.0 ± 2.6 µg $ml^{-1}$, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing $H_2O_2$ is very important throughout food systems.

### $Fe^{2+}$ chelating ability

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (Ebrahimzadeh et al., 2008c). Because $Fe^{2+}$ causes the production of oxyradicals and lipid peroxidation, minimizing its concentration affords protection against oxidative damage. In the presence of other chelating agents, the ferrozine complex formation is disrupted with the result that the red color of the complexes decreases. Tested extract exhibited good $Fe^{2+}$ chelating ability ($IC_{50}$ = 419 ± 20.95 µg $ml^{-1}$). EDTA showed very strong activity ($IC_{50}$ =

**Table 1.** Effect of methanol extract of *Artemisia absinthium* L. on the duration of immobility during forced swimming test and tail suspension test.

| Group | Dose (mg/kg) | Duration of immobility (s), FST | Duration of immobility (s), TST |
|---|---|---|---|
| Control | - | 150.2 ± 8.9 | 159.6 ± 12 |
| *Artemisia* | 125 | 130.8 ± 8.2* | 93.4 ± 1.2* |
| *Artemisia* | 250 | 111.6 ± 5.1* | 83.2 ± 1.4* |
| *Artemisia* | 500 | 49.2 ± 2.3* | 71.2 ± 2.4* |
| *Artemisia* | 1000 | 21.0 ± 0.9* | --------- |
| Imipramine | 5 | 21.6 ± 0.7* | --------- |
| Imipramine | 10 | 14.4 ± 0.5* | 73.8 ± 4.2* |

*ANOVA followed by Newman–Keuls multiple comparisons test shows that all test groups are significantly different from control group (P<0.001). Values are mean ± SD (n = 10).

18 ± 0.05 µg $ml^{-1}$).

## Effect of *A. absinthium* on immobility in FST

The swimming test has been widely employed to evaluate the effect of various agents on the central nervous system (CNS), such as CNS depressants, anti-depressants, sedative-hypnotics, psychostimulants, euphorics, nootropics, adaptogens, etc. According to Porsolt et al. (1978), the immobility seen in rodent during swimming reflects behavioral despair as seen in human depression. The swimming test has also been used extensively to assess the anti stress activity of plants in mice and rats (Ozturk et al., 1995; Maity et al., 2000). The forced swimming test is a classic animal model for antidepressant drug screening (Daudt et al., 2000). The result of effect of methanol extract of *A. absinthium* on the duration of immobility during forced swimming test is shown in Table 1. The extract shortened remarkably the immobility period during the forced swimming test in comparison with negative control and exhibited a dose dependent antidepressant activity. ANOVA analysis followed by Newman-Keuls multiple comparisons test shows that all test groups were significantly different form control group ($P < 0.001$). The duration of immobility of 1000 mg $kg^{-1}$ extract-treated mice was similar to imipramine 5 mg/kg ($p > 0.05$). Median lethal dose ($LD_{50}$) was calculated as 3700 mg/kg.

## Effect of *A. absinthium* on immobility time in TST

*A. absinthium* (125, 250 and 500 mg $kg^{-1}$) significantly ($P < 0.001$) and dose dependently decreased the immobility time as compared to control mice (Table 1). The extract at the dose of 500 mg $kg^{-1}$ showed the same activity as imipramine ($P > 0.05$), in decreasing immobility period. Tail suspension test (Willner, 1984; Steru et al., 1985) represents the behavioural despair model, claimed to reproduce a condition similar to human depression. The test is based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (that is, behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli (Lucki, 1997). It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity (Thierry et al., 1986). Remarkably, TST detects the anti-immobility effects of a wide array of antidepressants, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), electro-convulsive shock (ECS) and even atypical antidepressants. Thus, the activity of *A. absinthium* could involve one of the mechanisms of the established agents as described above.

## Conclusion

The finding of the present investigation suggests the antidepressant activity of *A. absinthium* in FST and TST models of depression. *A. absinthium* significantly reduced the immobility period in both FST and TST. The extract also exhibited good but different levels of antioxidant activity in some models studied. However, further studies are necessary for complete understanding the antidepressant activity of *A. absinthium*. Such identified potential and natural constituents could be exploited as cost effective food additives for human and animal health.

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