Asian Journal of Innovative Research 1(2) 39-43 (2016)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH
Available online at http://www.asianjir.com

Received 20 April 2016;
Accepted 12 June 2016
Online June 2016

Research Article **Siddha Medicine**

ANTIOXIDANT ACTIVITY OF *Evolvulus alsinoides* Linn

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ABSTRACT

Reactive oxygen species scavenging activity of methanolic extract of *Evolvulus alsinoides* Linn were carried out for proving its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. The methanolic extract was screened for in vitro antioxidant activity by radical scavenging such as DPPH scavenging, total anti-oxidant assay, superoxide anion radical scavenging, reducing power activity and metal chelation at different concentrations. Throughout the studies plant extract showed marked antioxidant activity. The antioxidant activity of the plant extract may be due to the phytochemicals present in it. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioactive compounds in the plants of *Evolvulus alsinoides* Linn. The results of the present study concluded that the plant extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases.

Keywords: *Evolvulus alsinoides* , Antioxidant, Free radicals

***Citation*:** Gomathi Rajashyamala L and Elango V. (2016) Antioxidant activity of *Evolvulus alsinoides* Linn. Asian Journal of Innovative Research 1(2): 39-43.

INTRODUCTION

The role of free radical reactions in disease pathology is well established, suggesting that these reactions are necessary for normal metabolism, but that they can be detrimental to aerobic life as well. Free radical reactions are involved in arteriosclerosis, ischemic heart disease, ageing, inflammation, diabetes, immunosuppression, neurodegenerative diseases, and many other diseases (Maxwell.,1995). During metabolism, oxygen consumption results in the constant generation of free radicals and reactive oxygen species. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like arteriosclerosis, stroke, diabetes, Alzheimer's disease, and cancer, have been developed during the last 3 decades (Kuo *et al*., 2002). The majority of active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds, which are found in natural foods, vitamins C and E, β-carotene, and α- tocopherol are known to possess antioxidant potential (Prior, 2003; Cai *et al*.,2004; . Kaur *et al*., 2002; . Aqil *et al*., 2006). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are

many phytochemicals fruits and herbs and each works differently (Arora *et al*., 1998). The plant kingdom is a rich source of potential drugs. In India, medicinal plants are widely used by all sections of the population, either directly in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicine. Research on natural resources has been encouraged by the World Health Organization since 1978).

The phenolic compounds in plants act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Velavan *et al*, 2007; Javanraedi *et al*., 2003). With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Evolvulus alsinoides* Linn. Hence, the anti-oxidant potential of *Evolvulus alsinoides* Linn was not evaluated.

These Plants have been used in traditional medicine for treatment of bronchitis, asthma (Chopra *et al*., 1986) and brain disorders like insanity, epilepsy, nervous disability, and scrofula. (Chatterjee, 1990; Nadkarni *et al*.,1982) *Evolvulus alsinoides* is well known for its memory enhancing property in traditional Indian system of medicine and extensively commercialized as nervin tonic in Asian countries. Therefore, the present study were to investigate the free radical scavenging activity of *Evolvulus alsinoides* Linn through the free radical scavenging such as DPPH (1,1-diphenyl-2- picryl hydrazyl) scavenging, total antioxidant assay ,superoxide anion radical scavenging, metal chelation and reducing power activity.

MATERIALS AND METHODS

Chemicals

Nitroblue tetrazolium (NBT), ethylenediamine - -tetra acetic acid (EDTA), Sodium nitroprusside (SNP), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Potassium hexa cyano ferrate [K3Fe(CN)6], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant materials

The fully mature *Evolvulus alsinoides* Linn leaf were collected from Marungulam, Thanjavur District, Tamil Nadu, India from a single herb.

Preparation of alcoholic extract

The collected *Evolvulus alsinoides* Linn were washed several times with distilled water to remove the traces of impurities from the flowers. The flowers were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Evolvulus* alsinoides Linn extract was stored in Desiccator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for in vitro antioxidant activity.

In vitro antioxidant activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al*., (1992). The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., (1999). The superoxide anion radicals scavenging activity was measured by the method of Liu *et al*., (1997). The chelating activity of the extracts for ferrous ions Fe2+ was measured according to the method of Dinis *et al*., (1994). The Fe3+ reducing power of the extract was determined by the method of Oyaizu (1986).

Statistical analysis:

Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC50, was graphically estimated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

In recent years there has been growing interest in the role of free radicals in cancer, arteriosclerosis, and ageing, and their prevention using antioxidants (Finkel T *et al*., 2000). The antioxidant activity of *Evolvulus alsinoides* Linn are presented in Table 1.

DPPH Assay

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude plant extracts (Navarro MC *et al*.,1992). With the DPPH test the ability of a compound to act as a donor for hydrogen atoms or electrons is measured spectrophotometrically. Hydroxyl radicals are the major active species that cause lipid oxidation and significant biological damage (Aruoma, *et al*., 1991). The ability of the tested extracts to quench hydroxyl radicals seems to be directly related to inhibiting the process of lipid peroxidation, and the methanalic extracts of *Evolvulus alsinoides* seemed to be good scavengers of reactive oxygen species. The percentage of hydroxyl radical scavenging increased as the concentration of the extracts increased. DPPH radical scavenging activity of plant extract and standard as ascorbic acid are presented in Table 1.

The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al*., 2003). Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterized as a stable free radical by

virtue of the delocalization of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Total antioxidant activity

The yield of the methanol extract of the plant extract and its total antioxidant capacity are given in Table 1. Total antioxidant capacity of *Evolvulus alsinoides* plant extract is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al*., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract.

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the extract from *Evolvulus alsinoides* assayed by the PMS-NADH system were shown in Table 1. The superoxide scavenging activity of *Evolvulus alsinoides* was increased markedly with the increase of concentrations. These results suggested that *Evolvulus alsinoides* had notably superior superoxide radical scavenging effects.

Table 1: Anti-oxidant activity of *Evolvulus alsinoides* Linn

Concentrations (µg/ml)	DPPH	Standard (Ascorbic acid)	Total Antioxidant Assay	Standard (Ascorbic acid)	Superoxide anion radical scavenging	Standard (Ascorbic acid)
20	14.54 ± 1.32	25.6±2.04	16.25 ±1.42	22.35± 1.80	13.57 ±1.02	31.25 ± 2.50
40	39.09 ±2.35	61.26±4.90	29.37 ±1.98	51.23± 4.09	27.14 ±1.65	64.23 ± 5.13
60	53.63 ±3.12	88.98±7.11	62.5 ±2.54	72.54± 5.80	64.28 ±2.65	89.54 ± 7.16
80	78.18 ±4.23	99.34±7.94	75.62 ±3.54	86.35± 6.91	85.00 ±5.32	98.51 ± 7.88

Values are expressed as Mean ±SD for triplicates

Reducing power activity

The measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Evolvulus* alsinoides Linn. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al*, 2000). Table 2 depicts the reductive effect of *Evolvulus alsinoides*. Similar to the antioxidant activity, the reducing power of *Evolvulus* alsinoides increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Evolvulus alsinoides* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine–Fe2+ complex is interrupted in the presence of methanolic extract of *Evolvulus alsinoides* (Table 2). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell, 1999). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Evolvulus* alsinoides demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Table 2: Anti-oxidant activity of *Evolvulus alsinoides* Linn

Concentrations (µg/ml)	Reducing power	Standard (Ascorbic acid)	Fe^{2+}chelating	Standard (Ascorbic acid)
20	0.57 ±0.23	0.41± 0.03	13.84 ±1.65	35.23 ± 2.81
40	0.60 ±0.35	0.71 ± 0.05	28.69 ±2.03	65.21 ± 5.28
60	0.63 ±0.42	0.89 0.07±	51.53 ±3.56	78.51± 6.28
80	0.79 ±0.56	0.98 ± 0.08	73.84 ±5.23	98.65 ± 7.89

Values are expressed as Mean ±SD for triplicates

CONCLUSION

The results of the present study showed that the extract of *Evolvulus alsinoides* Linn exhibits the greatest antioxidant activity through the scavenging of free radicals. Phytochemicals of *Evolvulus alsinoides* Linn are exhibited the greatest antioxidant activity DPPH, superoxide anion scavenging and metal chelator (iron chelator and iron reducing power) which participate in various pathophysiology of diseases including ageing. Overall, the plant extract is a source of natural antioxidants that can be important in disease prevention, health preservation and promotion of longevity promoter.

Acknowledgements

We would like to acknowledge Director, Harman Institute of Science Education and Research, Thanjavur, Tamil Nadu for providing facilities to carry out this work.

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Source of support: Nil;
Conflict of interest: None declared