

**REACTIVE OXYGEN AND NITROGEN SPECIES SCAVENGING
ACTIVITY OF *APLOTAXIS AURICULATA* RHIZOMES****R. Shalini¹ and S. Velavan^{2*}**

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ABSTRACT

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention. Natural antioxidants occur in all parts of plants. In the present study to investigate the antioxidant potential of *Aplotaxis auriculata* rhizome extract. The *Aplotaxis auriculata* extract was screened for in vitro antioxidant activity by nitric oxide radical scavenging, oxygen radical scavenging such as DPPH scavenging, superoxide anion radical scavenging, total antioxidant, hydroxyl radical, nitric oxide scavenging and metal chelation activity at different

concentrations. Throughout the studies extract showed marked antioxidant activity. The antioxidant activity of the 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 bioflavonoids content in the of *Aplotaxis auriculata*. Overall, the plant extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging.

KEYWORDS - Antioxidant activity, *Aplotaxis auriculata*, Radical scavenging, Reactive Oxygen and nitrogen species.

INTRODUCTION

Antioxidants are believed to play a very important role in the body defense system against Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) (Boxin *et al.* 2002; Vivek and Surendra, 2006). In another term antioxidant is “any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Halliwell and Gutteridge, 1995). Halliwell (2007) reported that an antioxidant is “any substance that delays, prevents or removes oxidative damage to a target molecule. Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc (Peter, 2007).

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Regular consumption of anti-oxidative vegetables and fruits has been recognized as reducing the risk of chronic diseases (Dembinska-Kiec *et al.*, 2008). The human body is protected from cardiovascular, neurological and carcinogenic diseases, delaying chronic health problems like cataracts by the use of antioxidants. The recommendations based on epidemiological studies are such that fruits and vegetables ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract. Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention (Diwani *et al.*, 2009). Natural antioxidants occur in all parts of plants. In the present study to investigate the antioxidant potential of *Aplotaxis auriculata* rhizomes.

MATERIALS METHODS

Plant materials

The *Aplotaxis auriculata* rhizomes were collected in January 2015 from Kolli hills, Nammakal District, Tamil Nadu, India from a single herb. The rhizomes were identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu, India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The collected *Aplotaxis auriculata* rhizomes were washed several times with distilled water to remove the traces of impurities from the rhizomes. Then examined carefully old, infected and fungus damaged portion of the rhizomes were removed. Healthy rhizomes were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. 25gms of the powder of rhizomes was transferred into different conical flask (250ml). The conical flask containing 100ml of Alcoholic solvents. The conical flask containing rhizomes powder and solvent was shaken it well for 2 hours by free hand. After 1 day, the extracts were filtered using Whatmann filter paper No.1. and was transferred into china dish. The supernatant was completely removed by keeping the china dish over water bath at 45°C. The obtained extracts were stored at 4°C in air tight bottle until further use.

In vitro antioxidant studies

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 scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al.*, (2004). Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC₅₀, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically / mean± standard deviation.

RESULTS

ANTIOXIDANT ACTIVITY OF *Aplotaxis auriculata*

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals

maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides. Traditional herbal medicines, dietary foods were the main source of antioxidant for ancient peoples that protected them from the damage caused by free radicals. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.

DPPH radical scavenging activity

DPPH radical scavenging activity of plant extract of SRLE and standard as ascorbic acid are presented in Fig 1. The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* extract and ascorbic acid were $48.51\mu\text{g/ml}^{-1}$ and $41.81\mu\text{g/ml}^{-1}$ respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity (Table. 1). 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.

Table 1: DPPH radical scavenging activity of *Aplotaxis auriculata* extract.

Samples	Concentrations				IC_{50} $\mu\text{g/ml}$
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	
<i>Aplotaxis auriculata</i>	20.46 ± 1.43	36.82 ± 2.57	62.28 ± 4.35	87.28 ± 6.10	48.51
Ascorbic acid (Std.)	26.54 ± 0.95	38.6 ± 2.04	79.26 ± 4.90	96.98 ± 7.11	41.81

Values are expressed as Mean \pm SD for triplicates.

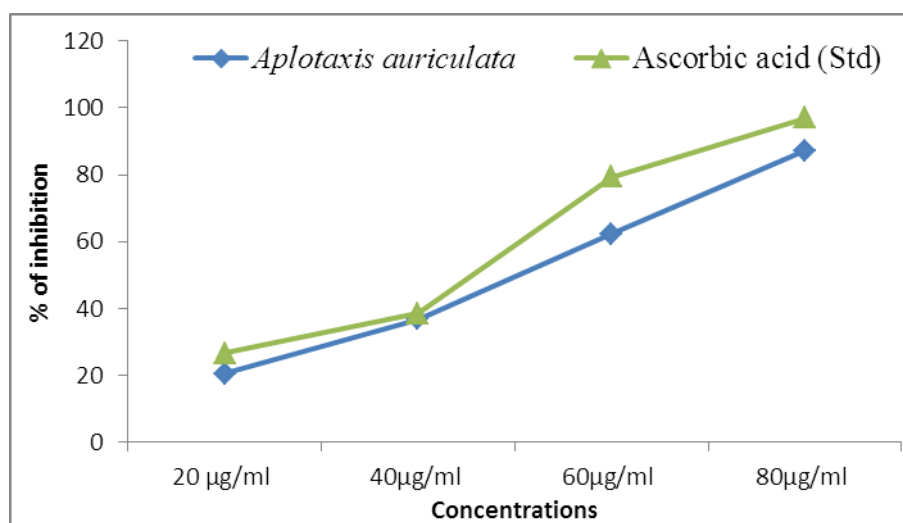


Fig.1: DPPH radical scavenging activity of *Aplotaxis auriculata* extract.

Total antioxidant activity

The yield of the ethanol extract of the plant and its total antioxidant capacity are given in Fig. 2. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract (Table. 2). The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were $50.80\mu\text{g/ml}^{-1}$ and $45.68\mu\text{g/ml}^{-1}$ respectively.

Table 2: Total antioxidant activity of *Aplotaxis auriculata* extract.

Samples	Concentrations				IC_{50} $\mu\text{g/ml}$
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	
<i>Aplotaxis auriculata</i>	19.37 ± 1.35	38.75 ± 2.71	58.12 ± 4.06	80.62 ± 5.64	50.80
Ascorbic acid (Std.)	22.35 ± 1.80	43.67 ± 2.61	51.23 ± 4.09	72.54 ± 5.80	45.68

Values are expressed as Mean \pm SD for triplicates

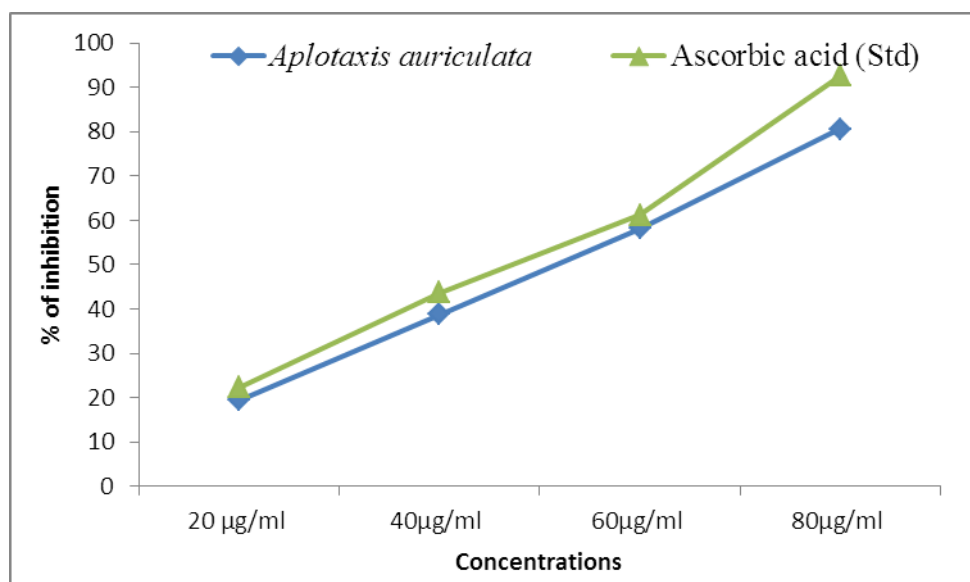


Fig 2: Total antioxidant assay of *Aplotaxis auriculata* rhizomes.

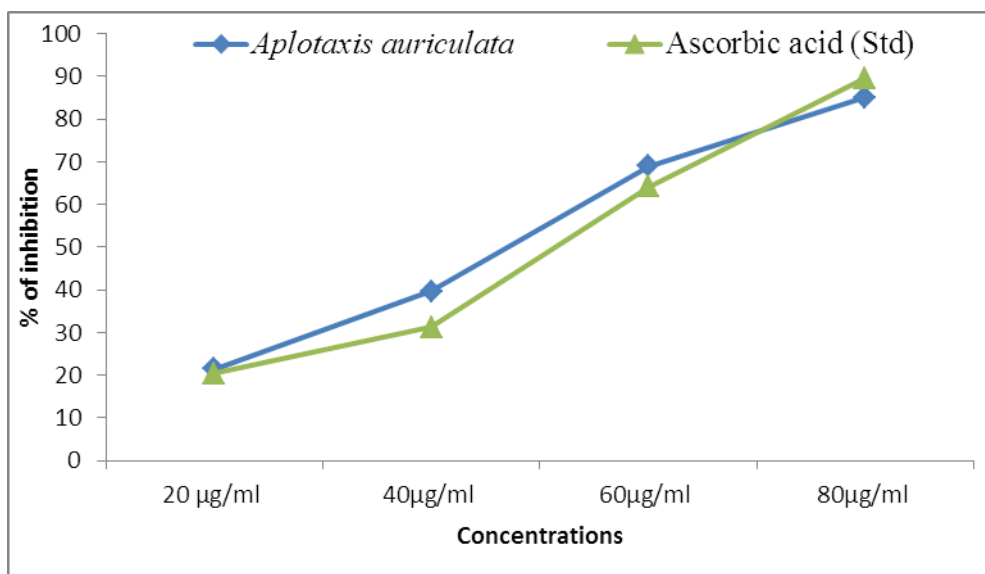
Superoxide scavenging activity

The superoxide anion radical scavenging activity of the extract from *Aplotaxis auriculata* assayed by the PMS-NADH system was shown in Fig. 3. The superoxide scavenging activity of *Aplotaxis auriculata* was increased markedly with the increase of concentrations (Table. 3). The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* was $48.89\mu\text{g/ml}^{-1}$ and ascorbic acid were $46.60\mu\text{g/ml}^{-1}$ respectively.

Table.3: Superoxide radical scavenging activity of *Aplotaxis auriculata*

Samples	Concentrations				IC ₅₀ μg/ml
	20 μg/ml	40μg/ml	60μg/ml	80μg/ml	
<i>Aplotaxis auriculata</i>	21.42± 0.01	39.64±2.77	68.92± 4.82	85.00± 5.95	48.89
Ascorbic acid (Std.)	20.37 ±0.97	31.25±2.50	64.23±5.13	89.54±7.16	46.60

Values are expressed as Mean± SD for triplicates.

**Fig 3: Super oxide scavenging activity of *Aplotaxis auriculata* rhizomes.**

The ferrous ion chelating activity of rhizome extract of *Aplotaxis auriculata*

The formation of the ferrozine– Fe²⁺ complex is interrupted in the presence of aqueous extract of *Aplotaxis auriculata*, indicating that have chelating activity with an IC₅₀ of 51.36 μg/ml and ascorbic acid was 45.91 μg/ml respectively (Fig. 4 and Table. 4).

Table 4: Ferrous ion chelating activity of *Aplotaxis auriculata*.

Samples	Concentrations				IC ₅₀ μg/ml
	20 μg/ml	40μg/ml	60μg/ml	80μg/ml	
<i>Aplotaxis auriculata</i>	17.69± 1.23	35.00±2.45	53.46± 3.74	87.69± 6.13	51.36
Ascorbic acid (Std.)	19.17±0.93	42.23±2.81	65.21±5.28	93.51±6.28	45.91

Values are expressed as Mean± SD for triplicates.

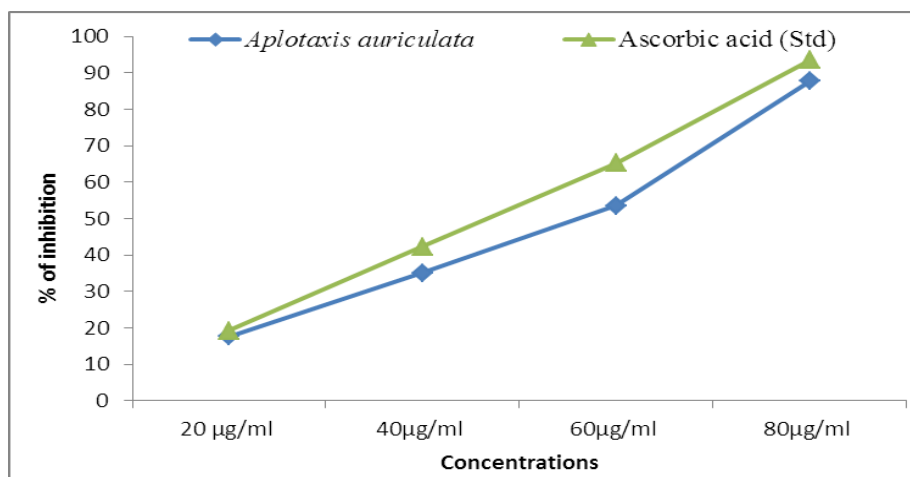


Fig 4: Ferrous iron chelating activity of *Aplotaxis auriculata* rhizomes.

Hydroxyl radical scavenging activity

Fig. 5 depicts the hydroxyl radical scavenging activity of *Aplotaxis auriculata*. The hydroxyl radical scavenging activity of *Aplotaxis auriculata* increased with increasing concentration (Table. 5). The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* was $46.10 \mu\text{g/ml}^{-1}$, and ascorbic acid were $35.26 \mu\text{g/ml}^{-1}$ respectively. *Aplotaxis auriculata* has potential hydroxyl radical scavenging activity and near to standard.

Table 5: Hydroxyl radical scavenging activity of *Aplotaxis auriculata*.

Samples	Concentrations				IC_{50} $\mu\text{g/ml}$
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	
<i>Aplotaxis auriculata</i>	23.33± 1.63	39.16±2.74	67.91± 4.75	88.33± 6.18	46.10
Ascorbic acid (Std.)	32.21±2.51	56.45±4.40	78.65±6.13	92.75±7.2	35.26

Values are expressed as Mean± SD for triplicates.

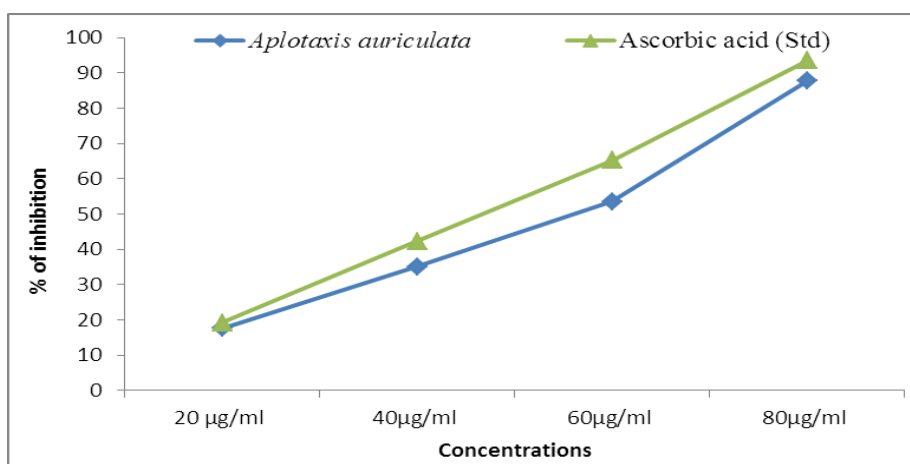


Fig 5: Hydroxyl radical scavenging activity of *Aplotaxis auriculata* rhizomes.

Nitric oxide scavenging activity

Fig. 6 depicts nitric oxide scavenging activity of *Aplotaxis auriculata* rhizome extract. The nitric oxide scavenging activity of *Aplotaxis auriculata* increased with increasing dosage (Table. 6). The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* was $46.73 \mu\text{g/ml}^{-1}$ and ascorbic acid were $35.88 \mu\text{g/ml}^{-1}$ respectively.

Table 6: Nitric oxide scavenging activity of *Aplotaxis auriculata*.

Samples	Concentrations				IC_{50} $\mu\text{g/ml}$
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	
<i>Aplotaxis auriculata</i>	23.80 \pm 1.66	36.19 \pm 2.53	66.19 \pm 4.63	88.57 \pm 6.19	46.73
Ascorbic acid (Std.)	26.21 \pm 2.04	59.62 \pm 4.65	84.23 \pm 6.56	96.45 \pm 7.52	35.88

Values are expressed as Mean \pm SD for triplicates.

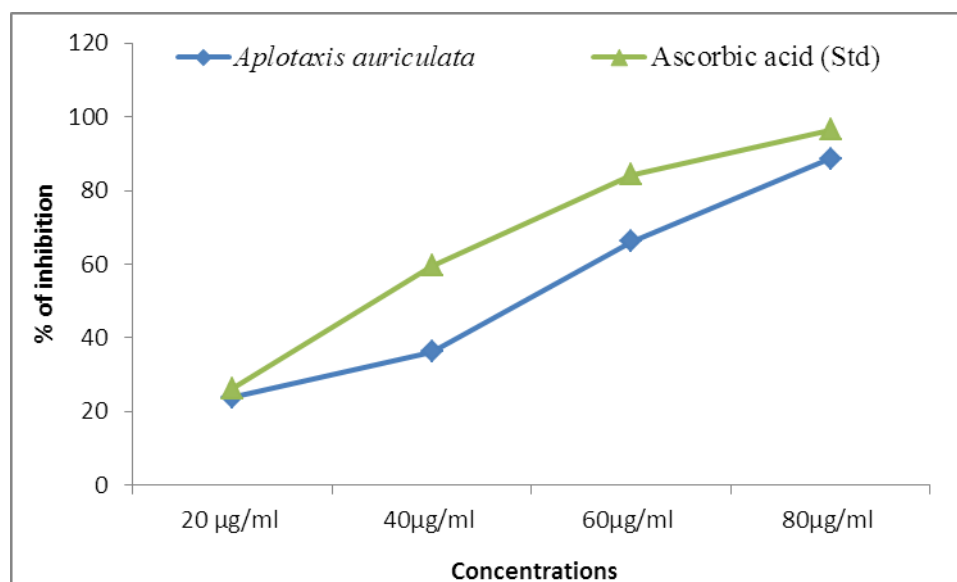


Fig 6: Nitric oxide scavenging activity of *Aplotaxis auriculata* rhizomes.

DISCUSSION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They may protect cells from damage caused by unstable molecules known as free radicals. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism. Majority of the diseases are mainly linked to oxidative stress due to free radicals (Velavan *et al.*, 2007).

IN VITRO ANTIOXIDANT ACTIVITY OF *Aplotaxis auriculata*

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants (Badarinath *et al.*, 2010). Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Velavan, 2011; Smith *et al.*, 2000). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term (Blokhina *et al.*, 2003).

Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.) (Nur Alam *et al.*, 2013). In the present study was to investigate the antioxidant activity of *Aplotaxis auriculata*.

DPPH assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. DPPH is gained its stability as free radical molecules due to the delocalization of odd electron throughout the molecules. This more stabilized DPPH produce intense violet colour in ethanol solution. The antioxidant present in the extracts reacts with DPPH free radical solution and converts them into reduced form either by donating hydrogen atom or transferring electron followed by proton. This oxidation reaction is accompanied with loss of violet colour which can be measured quantitatively at 517 nm (Nuutila *et al.*, 2003). The half inhibition concentration (IC₅₀) of plant extract and ascorbic acid were 48.51 µg/ml⁻¹ and 41.81 µg/ml⁻¹ respectively. The *Aplotaxis auriculata* 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 of plant extract is near to standard as ascorbic acid.

Total antioxidant activity

Total antioxidant capacity of *Aplotaxis auriculata* 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. The half inhibition concentration (IC₅₀) of *Aplotaxis auriculata* extract and ascorbic acid were 50.80 µg/ml⁻¹ and 45.68 µg/ml⁻¹ respectively.

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 and Richardson, 1978). The superoxide anion radical scavenging activities of the extract from *Aplotaxis auriculata* assayed by the PMS-NADH system. The superoxide scavenging activity of *Aplotaxis auriculata* was increased markedly with the increase of concentrations. The half inhibition concentrations (IC₅₀) of *Aplotaxis auriculata* extract was 48.89 µg/ml⁻¹ and ascorbic acid were 46.60 µg/ml⁻¹ respectively. These results suggested that *Aplotaxis auriculata* had notably superior superoxide radical scavenging effects.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red coloured) formation is interrupted and as a result, the red colour of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of colour reduction. The formation of the ferrozine Fe²⁺ complex is interrupted in the presence of aqueous extract of *Cissus vitifolia*, indicating that have chelating activity with an IC₅₀ of 51.36 µg/ml and ascorbic acid was 45.91 µg/ml⁻¹. Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals (Halliwell, 1991; Fridovich, 1995). Metal chelating activity can contribute in reducing the concentration of the

catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form's 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). This study demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of ethanolic extract was measured by 1, 10 phenanthroline- Fe^{2+} complex oxidation method. Fe^{2+} was formed when ferrous sulphate added to hydrogen peroxide. This formed ferrous ion reacts with 1,10 phenanthroline and forms 1,10 phenanthroline- Fe^{2+} complex which is acts as indicator in oxidation reduction reaction. Simultaneously the hydroxyl radical formed from the H_2O_2 - Fe^{2+} reaction mixture oxidize Phenanthroline- Fe^{2+} into Phenanthroline- Fe^{3+} complex. Presence of free radical scavenger in the extract reduces the oxidation reaction accompanied with reduction in the absorbance which can be measured quantitatively at 560 nm (Olabinri et al., 2010). Hydroxyl radical scavenging activity of *Aplotaxis auriculata* increased with increasing dosage. The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* extract and ascorbic acid were $46.10 \mu\text{g/ml}^{-1}$ and $35.26 \mu\text{g/ml}^{-1}$ respectively.

Nitric oxide scavenging activity

Nitric oxide ($\text{NO}\bullet$) released from sodium nitroprusside (SNP) has a strong NO^+ character which can alter the structure and function of many cellular components. The extract of *Aplotaxis auriculata* exhibited good $\text{NO}\bullet$ scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The $\text{NO}\bullet$ scavenging capacity was concentration dependent with $80 \mu\text{g/ml}$ scavenging most efficiently. The *Aplotaxis auriculata* extract in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of $\text{NO}\bullet$ liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of $\text{NO}\bullet$ increases when it reacts with superoxide to form the peroxynitrite anion ($\bullet\text{ONOO}^-$), which is a potential strong oxidant that can decompose to produce $\bullet\text{OH}$ and NO_2 (Pacher et al., 2007). The present study shows that *Aplotaxis auriculata* rhizome extract has a potent nitric oxide scavenging activity. The nitric oxide scavenging activity of *Aplotaxis auriculata* increased with increasing concentrations. The half inhibition concentration (IC_{50}) of *Aplotaxis auriculata* extract and ascorbic acid were $46.73 \mu\text{g/ml}^{-1}$ and were $35.88 \mu\text{g/ml}^{-1}$ respectively.

CONCLUSION

The results of the present study showed that the extract of *Aplotaxis auriculata* extract which contains of flavonoids and polyphenols. These phytochemicals are exhibited the greatest antioxidant activity DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation, hydroxyl radical and nitric oxide scavenging activity which participate in various pathophysiology of diseases including cancer, diabetic, ageing etc. This work has gathered experimental evidence on the *Aplotaxis auriculata* extract as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be an effective against oxidative stress. In addition, the *Aplotaxis auriculata* extract found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Thus, it can be concluded that *Aplotaxis auriculata* extract can be used as an accessible source of natural antioxidants with consequent health benefits.

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