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REACTIVE OXYGEN AND NITRIC OXIDE SCAVENGING ACTIVITY OF *APLOTAXIS AURICULATA* RHIZOMES

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ABSTRACT

The Present investigation was to screen the antioxidant activity in order to find novel antioxidants in future pharmaceutical formulations. The methanolic extract of *Aplotaxis auriculata* rhizome extract (AARE) was screened for *in-vitro* antioxidant activity by DPPH radical scavenging assay, total antioxidant assay, superoxide anion scavenging activity, nitric oxide scavenging activity, Fe^{2+} chelating activity and reducing power assay at different concentrations. From the results, the AARE contains active biocompounds which participate in various pathophysiology of diseases including stress, cardiovascular diseases, inflammation, ageing, diabetes etc. Thus, this study performs experimental evidence on AARE as natural antioxidant due to its capacity to scavenge ROS & RNS and protect cells or organism from oxidative damage and thus could be effective against oxidative stress.

KEYWORDS: Antioxidants, *Aplotaxis auriculata*, oxidative stress and Phytochemicals.

INTRODUCTION

In recent years, the requirement of medicinal plant products has been increased in a tremendous way. (Shelly Rana *et al*., 2016). The unlimited traditional knowledge of mega biodiversity nations like India and China mainly accounts on medicinal plants and healthcare has been provide the basis for research and development to produce novel drugs for major ailments(Polterait,1990). Now a day's more attention has been given to natural oxidants due to their potentiality in health benefits (Finkel *et al*., 2000). Antioxidants are the compounds

produced from the plant extract which plays significant role of protection against reactive oxygen species (ROS) and free radicals (Valko *et al*., 2007).

ROS and free radicals act as alarming substances in the body may cause disruption of membrane fluidity, lipid peroxidation and protein denaturation etc, (Anderson *et al*., 1996; Velavan *et al*., 2007). Oxidative process organizes the main causative routes for generating free radicals in foods, drugs and even in living systems (Halliwell *et al*., 1994) because oxygen is the most essential factor indispensable to our life (Geetha *et al*., 2016).

Free radicals, also known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are occur due to exogenous sources of ionizing radiation, cigarette smoke, sunlight, toxic chemicals, atmospheric pollutants, over nutrition, changing food habits etc. and/or endogenous sources are pro inflammatory cytokines of TNF - α, IL - 8, IL-1B, mitochondrial electron transport chain, β –oxidation of fat etc., (Devasagayam *et al*., 2009). The most common reactive oxygen species includes superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH).The nitrogen derived free radicals are nitric oxide (NO), peroxynitrite anion (ONOO), nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) (Kokate and Panchawat *et al*., 2004).

By naturally free radicals either as ROS and RNS carrying beneficial effects only in a regulated manner eg, in maintaining homeostasis at the cellular level, work as signaling molecules, ATP generation in mitochondria, apoptosis, phagocytosis, xenobiotics detoxification are of in our body system.(Yoshikawa etal.,2000).When the level of free radical is more than its demand it may cause chronic and degenerative diseases such as cancer, auto immune disorders, cardio vascular diseases, arthritis, neuro disorders and the most notorious pathological effects are Alzheimer's disease and ageing (Lian, 2008).

Antioxidants are the chemical substances that inhibit free radical activities by donating the missing electrons without joining the chain of reactions (Sahu *et al*.). They are produced by the natural mechanisms in our body or externally supplied through foods and/or supplements when the inner source is in inadequate level (Valko, Rhodes *et al*., 2006). These compounds acquired significant role in slowing the harmful action of free radicals by scavenging them and detoxify the organism (Anagnostopoulouetal, 2006). They are also known as radical scavengers, metal chelators, reducing agents and singlet quenchers (Proestos *et al*., 2006). Our body naturally safeguarded by endogenous antioxidants and classified into enzymatic

and non-enzymatic. The major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and reductase (GPx, GRx) are involved in neutralization of ROS and RNS (Bahrain etal,) the metabolic and nutrient antioxidants belongs to non-enzymatic. The antioxidants which cannot be produced in the body must provided through foods and supplements such as vitamin E, C, carotenoids, trace metals (Se, Cu, Mn, Zn), omega-3 & omega-6 fatty acids (Willox *et al*., 2004).

Recent studies revealed that number of plant products including polyphenols, terpenes, alkaloids, flavanoids and various plants extract exerts antioxidant action. Several studies have been conducted inorder to correlate the amount of phenolic compounds in plants and antioxidant activity (Maja kazaic *et al.*, 2016) based on these properties, the present study was designed to evaluate the antioxidant activity of *Aplotaxis auriculata* rhizomes in methanolic extract.

MATERIAL AND METHODS

The rhizome of *Aplotaxis articulate* (family: Astreaceae) were collected in the month of Janauary 2016 from the Koli hills, Tamil Nadu, India. The rhizome of the plant was identified and authenticated by Dr. S. Alagumanian M.Sc., Ph.D. Assistant Professor in Botany, H.H.The Rajah's College, Pudukkottai, Tamil Nadu, India.

Preparation of extract

Aplotaxis auriculata rhizome was washed several times with distilled water to remove the traces of impurities from the rhizome. The rhizomes were dried at room temperature and finely 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 different concentrations (20, 40, 60 and 80µg/ml) of extract were used for in vitro activity.

In vitro antioxidant activity of *Aplotaxis auriculata* extract

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 Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986).

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 scavenging activity of sample was expressed as 50% effective concentration (EC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, 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

DPPH Assay

DPPH radical scavenging activity of plant extract of PMLE and standard as ascorbic acid are presented in Fig 1. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 54.73µg ml^{-1} and 34.91µg 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: % of DPPH Radical scavenging activity of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Aplotaxis auriculata extract	15.30 ±1.2	36.21±1.72	69.54±3.2	86.09±4.41	54.73
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	34.91

Values were expressed as Mean ± SD for triplicates

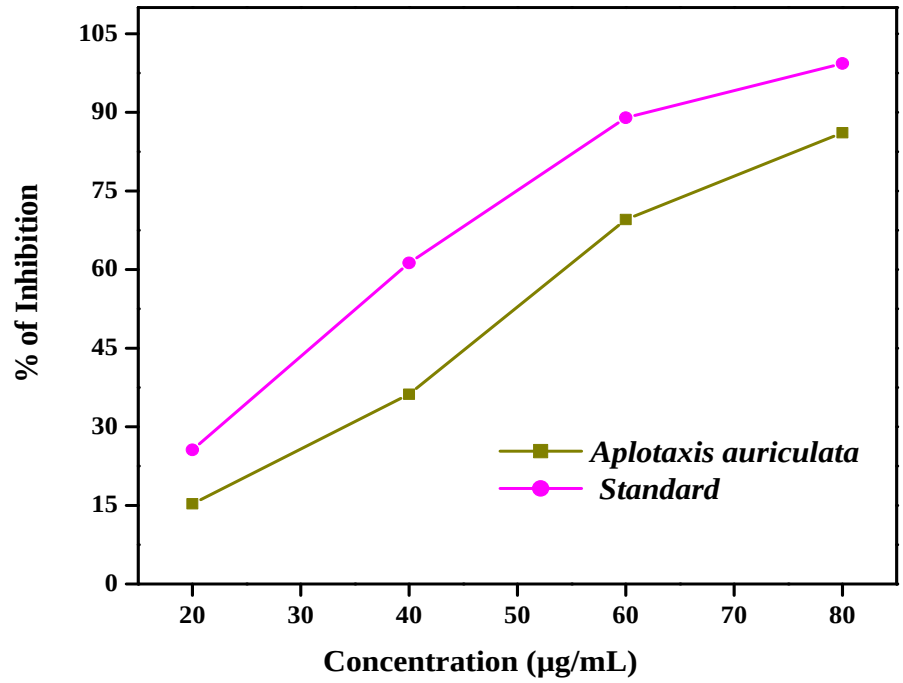

Fig 1: DPPH radical scavenging activity of *Aplotaxis auriculata.*

Total antioxidant activity

The yield of the ethanol extract of the plant extract 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 54.55µg ml^{-1} and 42.41 µg ml^{-1} respectively.

Table 2: % of Total antioxidant activity of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Aplotaxis auriculata extract	15.81±0.4	43.88±0.35	57.75±0.34	81.62±0.4	54.55
Standard (Ascorbic acid)	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	42.41

Values were expressed as Mean ± SD for triplicates.

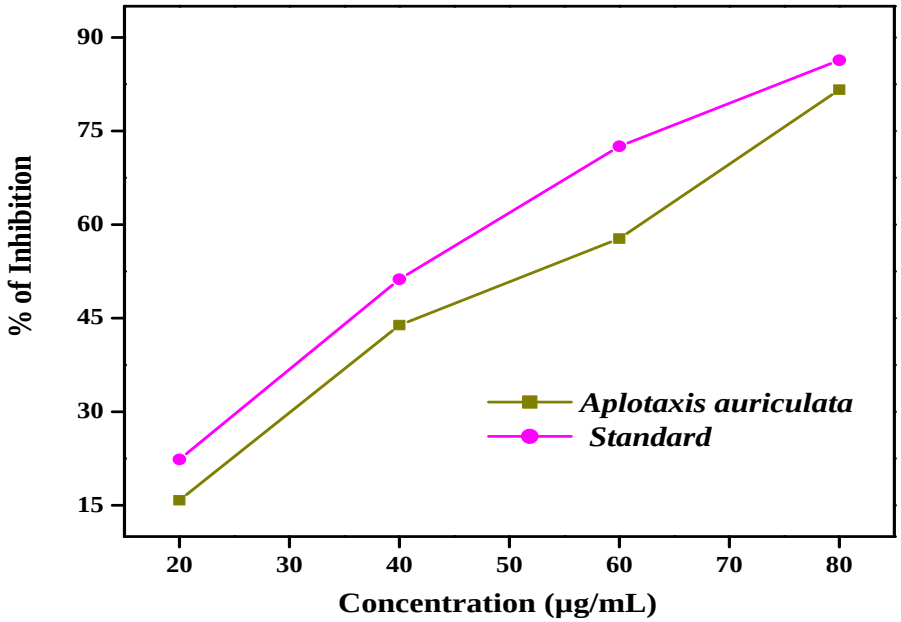

Fig 2: Total antioxidant assay of *Aplotaxis auriculata.*

Superoxide scavenging activity

The superoxide anion radical scavenging activities of the extract from *Azima tetracantha* assayed by the PMS-NADH system was shown in Fig 3. The superoxide scavenging activity of *Azima tetracantha* was increased markedly with the increase of concentrations (Table.3) The half inhibition concentration (IC_{50}) of *Azima tetracantha* was 55.93 µg ml^{-1} and ascorbic acid were 31.62µg ml^{-1} respectively.

Table 3: of Superoxide Radical scavenging activity of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Aplotaxis auriculata extract	11.93±0.86	22.01±0.4	48.59±0.85	79.56±1.15	55.93
Standard (Ascorbic acid)	31.25 ± 2.50	64.23± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.62

Values were expressed as Mean ± SD for triplicates

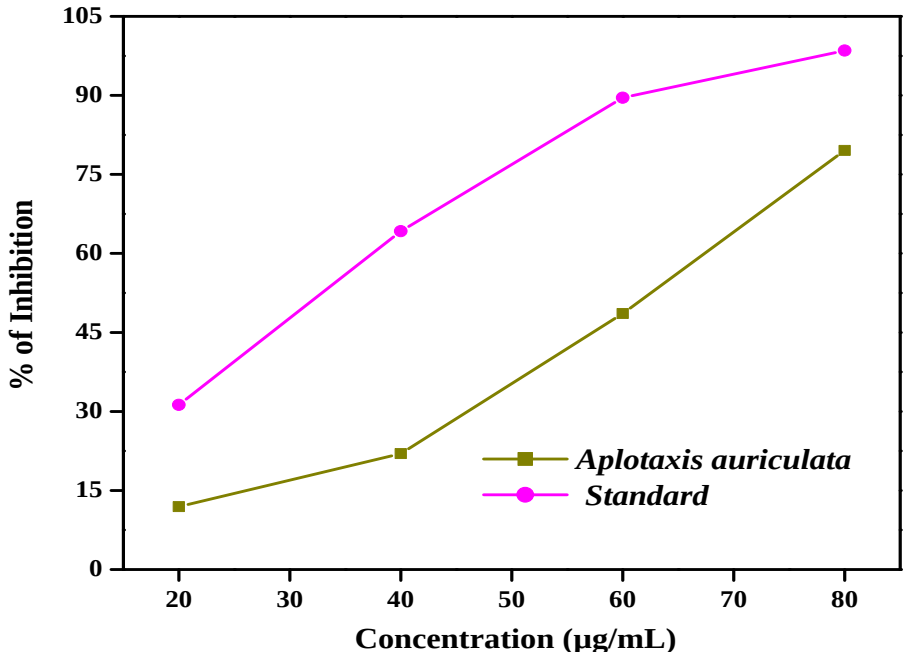

Fig 3: Superoxide scavenging activity of *Aplotaxis auriculata.*

The ferrous ion chelating activity

The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of aqueous extract of *Physalis minima L*, indicating that have che Tlating activity with an IC_{50} of 50.83 µg ml^{-1} and ascorbic acid was 30.96µg ml^{-1} respectively (Fig. 4, Table 4).

Table 4: % of Ferrous iron chelating activity of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Aplotaxis auriculata extract	11.44±7.3	38.09±4.76	52.38±4.76	73.02±7.27	50.83
Standard (Ascorbic acid)	35.23 ± 2.81	65.21± 5.28	78.51± 6.28	98.65 ± 7.89	30.96

Values were expressed as Mean ± SD for triplicates.

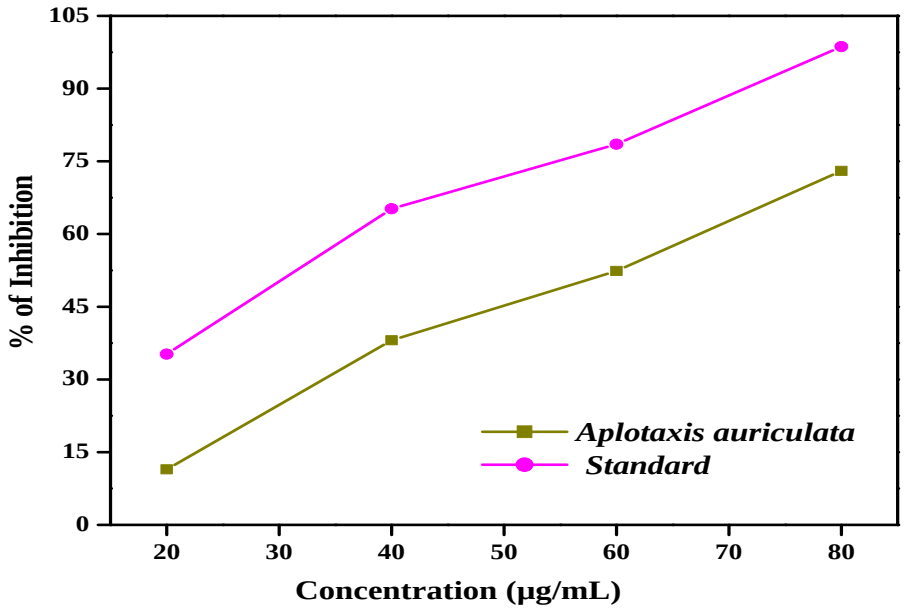

Fig 4: Ferrous iron chelating activity of *Aplotaxis auriculata.*

Reducing power activity

Fig. 5 depicts the reductive effect of *Aplotaxis auriculata.* The reducing power of *Aplotaxis auriculata* increased with increasing dosage (Table 5). All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Aplotaxis auriculata* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table 5: Reducing power assay of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**
Aplotaxis auriculata extract	0.163±0.047	0.33±0.031	0.56±0.016	0.75±0.03
Standard (Ascorbic acid)	0.41± 0.03	0.71 ± 0.05	0.89± 0.07	0.98 ± 0.08

Values were expressed as Mean ± SD (Optical density) for triplicates.

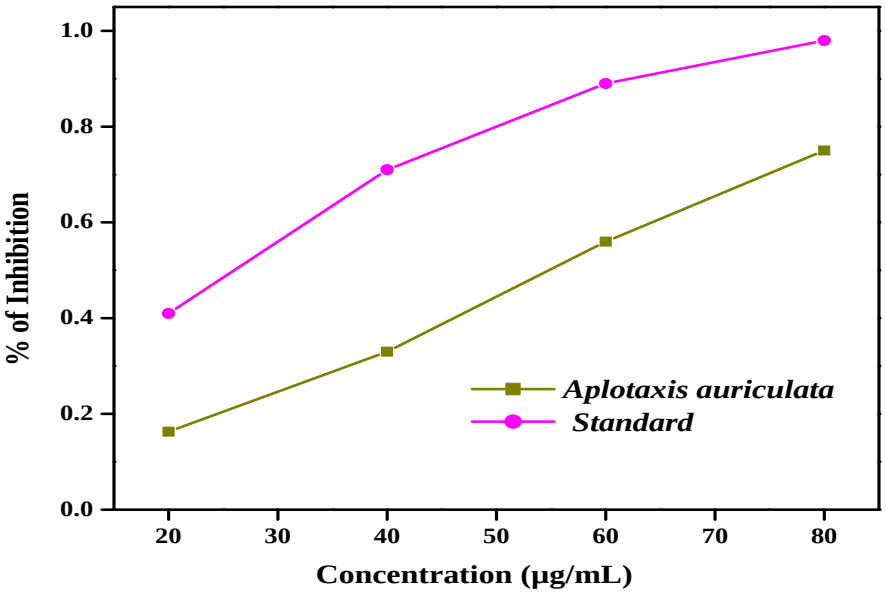

Fig. 5: Reducing power assay of *Aplotaxis auriculata.*

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Nitric oxide scavenging activity

Fig. 6 depicts the Nitric oxide scavenging activity of *Aplotaxis auriculata*. Similar to the antioxidant activity, the Nitric oxide scavenging activity of *Aplotaxis auriculata* increased with increasing dosage (Table 6). All the doses showed significantly higher activities than the control exhibited greater Nitric oxide scavenging activity, indicating that *Aplotaxis auriculata* consist of hydrophilic polyphenolic compounds that cause the greater Nitric oxide scavenging activity.

Table 6: Nitric oxide scavenging activity of *Aplotaxis auriculata* extract at different concentrations.

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Aplotaxis auriculata extract	14.21±7.9	44.44±11.11	70.36±6.41	85.17±6.41	52.31
Standard (Ascorbic acid)	26.21 ± 2.04	59.62± 4.65	84.23 ± 6.56	96.45 ± 7.52	46.63

Values were expressed as Mean ± SD (Optical density) for triplicates

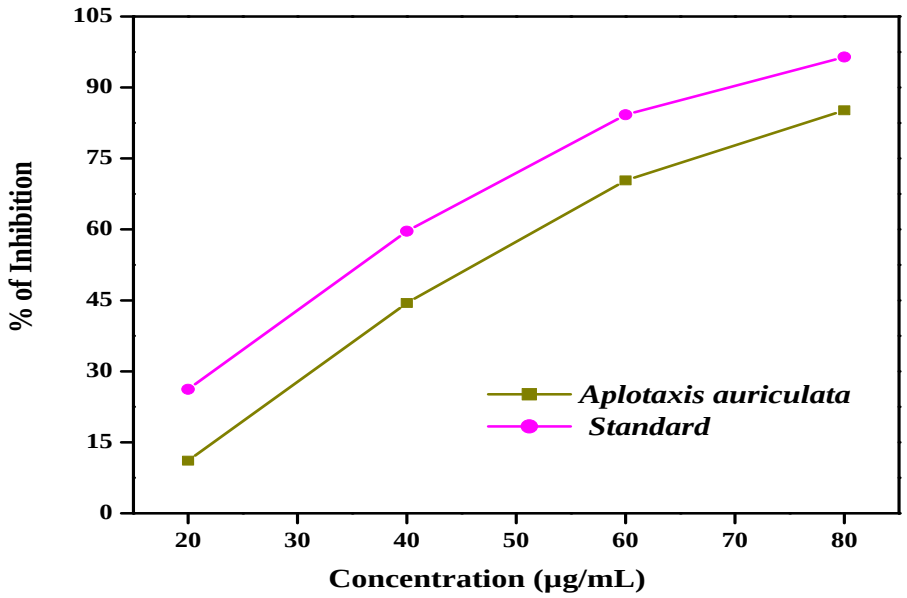

Fig 6: Nitric oxide scavenging activity of *Aplotaxis auriculata*.

DISCUSSION

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 extracts of *Aplotaxis auriculata* seemed to be good scavengers of reactive oxygen species. The percentage of hydroxyl radical scavenging increased as the concentration of the extracts increased. 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

Total antioxidant capacity of *Aplotaxis auriculata* 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

The superoxide anion radical scavenging activities of the extract from *Aplotaxis auriculata* assayed by the PMS-NADH system. 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 scavenging activity of *Aplotaxis auriculata* was increased markedly with the increase of concentrations. These results suggested that *Aplotaxis auriculata* had notably superior superoxide radical scavenging effects.

Reducing power activity

The measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Aplotaxis auriculata*. 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). The reducing power of *Aplotaxis auriculata* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Aplotaxis auriculata* 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 extract of *Aplotaxis auriculata*. 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, *Aplotaxis auriculata* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Nitric oxide scavenging activity

Nitric oxide (NO•) 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 NO• scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The NO• scavenging capacity was concentration dependent with 80 µg/ml scavenging most efficiently. The *Aplotaxis auriculata* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO• liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO• increases when it reacts with superoxide to form the peroxynitrite anion (•ONOO-), which is a potential strong oxidant that can decompose to produce •OH and NO_2 (Pacher *et al*., 2007). The present study shows that a *Aplotaxis auriculata* has a potent nitric oxide scavenging activity.

CONCLUSION

The results of the present study showed that the extract of *Aplotaxis auriculata* Linn exhibits the greatest antioxidant activity through the scavenging of free radicals. Phytochemicals of *Aplotaxis auriculata* are exhibited the greatest antioxidant activity DPPH, superoxide anion scavenging, nitric oxide 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.

REFERENCES

1. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). *Food Chem*, 2006; 94: 19-25.
2. Anderson D, Antioxidant defences against reactive oxygen species causing genetic and other damage, *Mutat Research Journal*, 1996; 350: 103-108.
3. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol, 2006; 30: 177-83.
4. Aruoma OI, Kaur H, Halliwell H. Oxygen free radicals and human diseases. J Royal Soc Health, 1991; 111: 172-77.
5. Cai Y, Luo Q, Sun M. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci, 2004; 74: 2157-84.
6. Chatterjee A. "Treaties of Indian Medicinal Plants," Council for Scientific and Industrial Research, New Delhi, India, 1990; 327.
7. Chopra R. N., Nayer S. L., Chopra I. C. "Glossary of Medicinal Plants," Publication and Information Directorate, CSIR, New Delhi, India, 1986; 116.
8. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidant in human health: current status and future prospects. *JAPI,* 2004; 52: 794–804.
9. Dinis TCP, Madeira VMC, Almeidam LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radicals scavengers. Achieves of Biochemistry and Biophysics, 1994; 315: 161-169.
10. Diplock AT. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? Free Radical Research, 1997; 27: 511-532.
11. Finkel, T., Holbrook, N.J., Oxidants, oxidative stress and biology of ageing, *Nature,* 2000; 408: 239–2479.
12. Garrat D.C. *The quantitative analysis of drugs.* Japan: Chapman and Hall, 1964; 3: 456-458.

13. Geetha T.S and Geetha.N, Antioxidant Profiles of Cymbopogan Citratus (DC) Stapf. Leaves, *International Journal of Pharmaceutical Sciences and Business Management*, January- 2016; 4(1): 1-12.
14. Gordon MH. The mechanism of the antioxidant action in vitro. In B. J. F. Hudson, Food Antioxidants, 1990; 1-18., London: Elsevier.
15. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine, 1999; 419-422. Oxford: Clarendon.
16. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet, 1994; 344: 721-724
17. Kokate C. K and Purohit A.P, "*Text book of pharmacognosy*", 2004; 29,317-18,336-37.
18. Korycka-Dahl M, Richardson M. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and aminoacids. Journal of Dairy Science, 1978; 61: 400-407.
19. Lian Ai Pham-Huy, Hua He, Chuong Pham- Huy. Free radicals, antioxidants in disease andhealth. *International Journal of Biomedical Science*, 2008; 4(2): 89-96.
20. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci, 1997; 60: 763-771.
21. Maja Kazazic, Maida Djapo and Emina Ademovic, Antioxidant activity of water extracts of some medicinal plants from Herzegovina region, *Int. J. Pure App. Biosci*, 2016; 4(2): 85-90.
22. Navarro MC, Montilla MP, Martin A *et al*. Free radicals and antihepatotoxic activity of Rosmarinus tomentosus. Planta Med, 1992; 59: 312-14.
23. Nuutila, A. M., Pimia, R. P., Aarni, M., & Caldenty, K. M. O. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry, 2003; 81: 485–493.
24. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 1986; 44: 307-315.
25. Panchawat S., Rathore K.S., Sisodia S.S., A review on herbal antioxidants, *Int J. Pharma Tech Research*, Jan-March 2010; 2(1): 232-239.
26. Potterat O. Antioxidants and free radical scavengers of natural origin. Current Organic Chemistry, 1997; 1: 415-440.

27. Prieto, P., Pineda, M., & Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Speci. c application to the determination of vitamin E. Analytical Biochemistry, 1999; 269: 337–341.
28. Proestos C, Boziaris, I. S., Nychas, GJ E and Komaitis M. Analysis of Flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and Food Chem, 2006; 95: 664-67.
29. Sahu, K.G., Khadabadi, S.S. &Bhide, S.S. Evaluation of in vitro antioxidant activity of Amorphophalluscampanulatus (Roxb.) Ex Blumedecne. *International Journal of Chemical Sciences,* 2009; 7(3): 1553-1562.
30. Shelly Rana, Ved Prakash and Anand Sagar, Medicinal and Antioxidant properties of some Medicinal Plants, *Journal of Drug Delivery & Therapeutics,* 2016; 6(3): 1–6.
31. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 1992; 40: 945–948.
32. Sindhu M, Abraham TE. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food and Chemical Toxicology, 2006; 44: 198–206.
33. Valko M, Rhodes CJ, Moncol J, Izakovic M *et al*. Free radicals, metabolism and antioxidants in oxidative stress- induced cancer. *Chem Biol Interact,* 2006; 160: 38-40.
34. Valko, M., Leibfritz, D., Moncol, J., Cronin M.T.D., Mazur, M., Telser, J., Free radicals and antioxidants in normal physiological functions and human disease, *The International Journal of Biochemistry & Cell Biology*, 2007; 39(1): 44–84.
35. Velavan S, Nagulendran K, Mahesh R. *In vitro* antioxidant activity of *Asparagus racemosuss* root. Pharmacog. Magaz, 2007; 26-33.
36. Willcox JK, Ash SL, Catignan GL. Antioxidants and prevention of chronic disease. *Crit Rev Food Sci Nutri,* 2004; 44: 275-95.
37. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf Ex DC), Sage (Salvia triloba L.) and Black Tea (Camellia sinensis) extracts. Journal of Agricultural and Food Chemistry, 2000; 48: 5030-5034.
38. Yoshikawa T, Toyokuni S, Yamamoto Y and Naito Y, (eds) Free Radicals in Chemistry Biology and Medicine, *OICAInternational, London,* 2000.
39. Young I, Woodside J. Antioxidants in health and disease *J. Clinc Path*, 2001; 54: 176-86.