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RESEARCH ARTICLE

INVITRO FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF THE LEAVES OF *BLEPHARIS MADERASPATENSIS (L) HEYNE EX ROTH*

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ABSTRACT

In this study, antioxidant potential of the aqueous extract of the leaves of *Blepharis maderaspatensis (L) Hyne Ex Roth* was evaluated by means of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, metal reducing power and total antioxidant capacity. The aqueous extract showed considerable actions in every antioxidant assays compared to the citation antioxidant ascorbic acid in a dose dependent manner. In DPPH scavenging assay the IC50 value of the extract was found to be 41.34µg/ml while the IC50 value of the reference standard ascorbic acid was 69.82 µg/ml. There is an increased total antioxidant activity was also found in a dose dependent manner. Besides, *Blepharis maderaspatensis* leaf extract showed strong metal reducing power. This result implies that *Blepharis maderaspatensis* leaf extract may act as a chemo preventive agent, and the antioxidant properties acts as efficient defense from free radicals.

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INTRODUCTION

The important research in medical field and also in the food industry is antioxidants. Recent research showed much interest on bioactive compounds responsible for antioxidant activities. Scientific research has been looking for novel bioactive compounds with antioxidants, which can be used in pharmaceuticals, it has been established that oxidative stress is amongst the chief contributory factors in initiation of many chronic and degenerative diseases together with atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). Thus, antioxidants with free radical scavenging activities may have immense consequence in the anticipation and therapeutics of diseases in which oxidants or free radicals are implicated. It is commonly accepted that reactive oxygen species, such as superoxide (O2˙-), hydroxyl (OH˙-), and peroxy (˙OOH, ROO˙) radicals, are produced under oxidative stress. Reactive oxygen species play important roles in degenerative or pathological processes, such as aging (Burns *et al*., 2001), cancer, coronary heart disease, Alzheimer's disease (Diaz *et al*., 1997), neurodegenerative disorders, atherosclerosis, diabetes, and inflammation (Chen *et al*., 2006). It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell, 1996). In vitro experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species (Ali *et al*., 2008). The study prepared on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. The free radical scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work. *Blepharis maderaspatensis (L) hyne ex Roth* is commonly known as creeping blepharis. It belongs to the family Acanthaceae. It is commonly named as creeping blepharis and seen commonly on slopes, among rocks, poor gravelly soil. *Blepharis* (Acanthaceae) is an Afro-asiatic genus comprising 129 species which occur in arid and semi-arid habitats. *Blepharis Maderaspatensis* is used for headache. Seeds are used as dysuria, diseases of nervous system, diuretic, aphrodisiac. It is used to cure cuts and wounds, juice extracted from leaf is heated with gingelly oil and applied on affected places to heal wound. Dry seeds of this plant contain steroids and the plant is

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used for brain disorders like Parkinson's disease. Leaves are economically important and are good medicine for throat troubles and asthma. *Blepharis maderaspatensis* is reported to contain alkaloids, phenols, flavonoids, steroids, saponins and tannins. It is used to study embryological studies, anthropogenic, brain disorder, bone fracture and Parkinson's disease and it is also used as anti- bacterial, anti- larvicidal, anti- inflammatory, anti-nociceptive and anti- cancer. This scrambling perennial herb is used traditionally for treatment of snakebites, wounds, oedema and gout. Phytochemical reviews shows that the bioactive substance such as terpenoids, phlobatannins, reducing sugar, flavonoids, phenols, glycosides, starch, proteins, peptide, amino acids, tannins, anthroquinones, sterols, steroids, coumarins, quinines, saponins and alkaloids were screened in the leaf extract of Blepharis maderaspatensis. In this paper we reported the study of the antioxidant activity of the leaves of *Blepharis maderaspatensis (L) hyne ex Roth*. The assay was performed in vitro by DPPH and metal reducing power

MATERIALS AND METHODS

Collection of plant material

The leaves of the plant part are taken for the research. The plant was collected from the area of chinnamanur Theni DT Tamil nadu India. The leaves were shade dried and after it completely dried they are powdered using pestle and mortar. Then the powdered plant was still size reduced with the help of a sieve. The fine powder was then packed in airtight container for further use to avoid the effect of humidity and then stored at room temperature.

Preparation of extract

The shade-dried leaves were extracted with aqueous by a Soxhlet apparatus at 35^{o}C. The solvent was completely removed by rotary evaporator and obtained crude exudates. This crude extract was used for further investigation for potential antioxidant properties.

Antioxidant activity assay

DPPH radical scavenging activity assay

The free radical scavenging capacity of the extracts was determined using DPPH. The reagent solution was prepared by adding 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. 300µL of the sample was added with 3mL of the reagent solution, mixed well and was incubated at 95°C for 90mins. After cooling the reaction mixture, the absorbance was measured at 695nm using UV spectrophotometer. Ascorbic acid was used as a standard. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95 % aqueous was served as blank. % scavenging of the DPPH free radical was measured by using the following equation:

$$\%\text{Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted and represented as % of mean inhibition ± standard deviation. IC50 values were obtained by probit analysis.

Metal reducing power

This assay was performed to find the metal reducing ability of the aqueous extract. 100µL of the extract was added with 400µL of distilled water. To this mixture, 1250µL of 0.2M phosphate buffer, 1250µL of 1% potassium ferricyanide was added and mixed well. Then this mixture was incubated in hot air oven at 50°C for 30mins. After cooling the reaction mixture, 1250µL of 10% trichloro acetic acid was added and mixed well. Then it was centrifuged at 3000rpm for 10mins. After centrifugation, the upper layer of 1250µL was transferred to a test tube and equal volume of distilled water was added and mixed well. Then to this, 500µL of 1% freshly prepared ferric chloride was added and mixed well. After mixing, the absorbance value was measured at 700nm using UV spectrophotometer. Ascorbic acid was used as a standard.

RESULTS AND DISCUSSION

Scavenging activity for free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. IC50 value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. In search of novel sources of antioxidants in the last years, medicinal plants have been broadly studied for their antioxidant activity. From ancient times, herbs have been used in many areas, including nutrition, medicine, flavoring, beverages, cosmetics, etc. The ingestion of fresh fruit, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases. Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials. Many naturally occurring triterpinoids exhibited a good anti-inflammatory activity have been isolated from various plants.

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *Blepharis maderaspatensis (L) hyne ex Roth* was given in figure 1. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 695 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC50 value of the extract was 43.26µg/ml, as opposed to that of ascorbic acid (IC50 55.89 µg/mL), which is a well known antioxidant.

Table 1. Spectrophotometric analysis of the silver nanoparticles of *Blepharis maderaspatensis (L) Heyne ex Roth* for *in vitro* anti oxidant activity (DPPH)

Aqueous extract:

Sample	OD value at 695nm	Mean value ± SD
100	1.047	
200	1.142	1.074±0.058
400	1.034	

Table 2. Spectrophotometric analysis of the aqueous extract of *Blepharis maderaspatensis (L) Heyne ex Roth* for *in vitro* antioxidant activity (Metal reducing)

Aqueous extract:

Sample	OD value at 700nm	Mean value ± SD
100	0.887	
200	0.865	0.857±0.033
400	0.821	

Fig. 1. Qualitative analysis of the aqueous extract of *Blepharis maderaspatensis (L) Heyne ex Roth* for *in vitro* antioxidant activity

Plant extracts made with water are nutritionally more relevant moreover herbs are traditionally ingested as hot-water infusions. On the other hand, acetone is preferred for more exhaustive extraction of polyphenols compounds. Results from this study will lead to a improved characterization of the antioxidant properties of the medicinal plants investigated and will divulge which of them are the best sources of dietary antioxidants. Several studies have investigated the relationship between the antioxidant activity and the content of polyphenols compounds in herbs. It can be concluded that the extracting solvent affects significantly the polyphenolic compound content and the antioxidant activity measured. From the above results and discussion it can be concluded that the aqueous extract of *Blepharis maderaspatensis* possesses the effective antioxidant substances which may be liable for its mechanism as well as justify the basis of using this plant's extract as folkloric remedies.

REFERENCES

Abimbola Sowemimo, Monsurat Onakoya, Muyiwa S. Fageyinbo, Titilayo Fadoju, 2013. Studies on the anti-inflammatory and anti-nociceptive properties of *Blepharis maderaspatensis* leaves Revista Brasileira De Farmacognosia. *Brazilian Journal of Pharmacognosy*, 3(5): 830-835

Aiyalu Rajasekaran, Vellaichamy Sivakumar and Sabarimuthu Darlinquine. 2012. Role of *Blepharis maderaspatensis* and *Ammannia baccifera* plant extracts on in vitro oxygen radical scavenging, secretion of gastric fluid and gastroprotection on ulcer induced rats. *Pharmaceutical Biology*, 50(9): 1085-1095

Alam MA, Nyeem MAB, Awal MA, Mostofa M, Alam MS, Subhan N and Rahman MM. 2008b. Antioxidant and hepatoprotective action of the crude methanolic extract of the flowering top of *Rosa damascena*. *Oriental Pharmacy and Experimental Medicine*, 8: 164-170.

Ayyanar M, Sankarasivaraman K, Ignacimuthu S. 2008. Traditional Healing Potential of Paliyars in Southern India. *Ethno Leaflets*, 12: 311-317.

Baskar, A.A., Numair, K.S.A., Alsaif, M.A., Ignacimuthu, S., 2012. In vitro antioxidant and antiproliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. *Redox Rep*., 17, 145-156.

Benzie IF & Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. *Analytical Biochemistry*, 239: 70–76

Braca A, Sortino C, Politi M 2002. Antioxidant activity of flavonoids from Licania licaniaeflora. *J. Ethnopharmacol*., 79: 379-381.

Fernandez MA, De las Heras B, Garcia MD, Saenz MT and Villar A. 2001. New insights into the mechanism of action of the anti inflammatory Triterpene lupeole. *Journal of Pharmacy and Pharmacology*, 53: 1533-39.

Gordon MH. 1990. The mechanism of antioxidant action in vitro. *In*: BJF Hudson (Ed.), Food antioxidants Elsevier Applied Science, London, pp. 1–18.

Graf E 1992. Antioxidant potential of ferulic acid. *Free RadicBiol Med*., 13:435–448. Doi: 10.1016/0891-5849(92) 90184-I.

Halliwell B. 1996. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research*, 25: 1–32.

Liao KL and Yin M C. 2000. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. *Journal of Agricultural and Food Chemistry*, 48: 2266-2270.

Mohan V.R, Amish Abragam D, Kalidass C and Maruthupandian A. 2010. Pharmacognostical and phytochemical investigation of whole plant of *Blepharis maderaspatensis* (L.) Heyne ex Roth. *Pharmacognosy Journal*, 2(14): 1-5.

Neelambika H S and Leelavathi S. 2015. Comparative Antioxidant Activity of Whole Plant of *Blepharis Maderaspatensis* (L.) Heyne Ex Roth. And *Blepharis Molluginifolia* Pers. of Mysore District by Dpph Method. *Indo American Journal of Pharmaceutical Research*, 5(3): 1191-1196

Prieto P, Pineda M and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337-341.

Saha MR, Alam MA, Akter R and Jahangir R. 2008. In-vitro free radical scavenging activity of *Ixora coccinea L, Bangladesh Journal of Pharmacology*, 3(2): 90-96.

Saha MR, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA & Mazumder MEH. 2008. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. *Bangladesh Journal of Veterinary Medicine*, 6(2): 197–202.

Suriyavathana Muthukrishnan, Indupriya Santhanam and Subha Palanisamy. 2014. Preliminary Phytochemical Screening and Antioxidant Potential of *Blepharis Maderaspatensis* (L.) B.Heyne Ex Roth Leaf. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(5): 658-668.

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