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Antioxidant Potential of Leaves of *Plectranthus amboinicus* (Lour) Spreng

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ABSTRACT

The plant Plectranthus amboinicus (Lour.) Spreng belongs to family Lamiaceae, known as country borage in English. The anti-oxidant potential of aqueous and ethanolic extract of leaves of Plectranthus amboinicus (Lour) Spreng was investigated by DPPH assay, reducing power assay and nitric acid screening methods. The preliminary phytochemical analysis showed presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids. The total phenolic content is also evaluated as they contribute in anti-oxidant property. The anti-oxidant potential and reducing power of both extracts increased with increasing concentration of extract (50 g – 250 g). Phenolic contents of ethanolic and aqueous extracts were found 11.6 µg and 9.4 µg gallic acid equivalent per mg of extracts respectively. This study is to verify the anti-oxidant potential and to evaluate total phenolic contents in Plectranthus amboinicus (Lour.) Spreng leaves.

Keywords: Anti-oxidant, reducing power, phenolic contents, *Plectranthus amboinicus*.

INTRODUCTION

Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. When the balance between ROS production and antioxidant defences is lost, 'Oxidative stress' results which through a series of events deregulates the cellular functions and leads to various pathological conditions including aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurodegenerative diseases [1]. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA [2].

There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage and mutagenesis. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals [3]. Therefore an attempt has been made to evaluate anti-oxidant potential of *Plectranthus amboinicus* (Lour.) Spreng as natural source. The plant *Plectranthus amboinicus* (Lour.) Spreng belongs to family Lamiaceae, known as country borage in English [4]. It is large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose [5]. It is found or cultivated throughout India, Ceylon and Moluccas [6]. Upon literature review it was found that the plant contains butylanisode, β-caryophyllene, quercetin, ursolic acids, triterpenic acids, α-pinene, β-pinene, thymol, eugenol, carvacrol, 1,8-cineole, β-phellandrene, p-cymene, salvigenin, crisimaritin and chrysoeriol [7-12]. Many pharmacological properties have reported including urolithiasis [13-14], antiepileptic [15], antitumour and antimutagenic [16], neuropharmacoligical [17], radioprotective effect [18], antioxidant [19], anti-microbial [20-21], anti-bacterial, anti-fungal [22-23], diuretic [24], antipyretic and analgesic properties [25].

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Plectranthus amboinicus* (Lour) Spreng were collected from the fields of Kanchipuram, Tamil Nadu. It was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen no. PARC/2007/89 has been deposited in the institute.

Preparation of extracts

The air-dried leaves were made into coarse powder. The powdered material was successively extracted with petroleum ether, chloroform, ethanol and water by cold maceration in increasing order of their polarity [26]. In addition the fresh powder was defatted with pet. ether and extracted with 95% ethanol (72 hours) and water (24 hours) separately. The extracts were filtered with muslin cloth and solvent was distilled off. Final traces of solvent were removed under vacuum.

Phytochemical investigation

The pet. ether (PEPA), chloroform (CEPA), ethanolic (EEPA) and aqueous (AEPA) extracts of *P. amboinicus* were subjected to preliminary, qualitative phytochemical investigation [27]. The percentage yield of PEPA, CEPA, EEPA, AEPA were found to be 2.47, 3.69, 12.20 and 18.10 % respectively.

DPPH Assay

Antioxidant potential of the extracts was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Plant extracts were mixed with 95% methanol to prepare the stock solution (5 mg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and extracts were added followed by serial dilutions (50 µg to 250 µg) to every test tube so that the final volume was 3 ml and after 10 min, the absorbance was read at 515 nm using a spectrophotometer. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was served as blank. % scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ Scavenging Activity} = (Ac-As)/Ac)\times 100$$

Where, Ac is the absorbance of the control reaction
As is the absorbance in the presence of the sample of the extracts.

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation [28].

Nitric oxide screening method

Both the extracts of *Plectranthus amboinicus* were screened for nitric oxide (NO) radical scavenging activity 8.1 ml sodium nitropruside (10 mM) in 0.5 M phosphate buffer (pH 7.4) was mixed with 3.0 ml of the different concentrations (25 – 100 µg/ml) of the sample dissolved in methanol and incubated at 25^0C for 15 min. Above samples were reacted with Greiss reagent (1% sulphanilamide in 5% H3PO4 and 0.1% N-(1-napthyl) ethylenediamine dihydrochloride in water). The absorbance of the chromophore formed during the diazotization of nitrate with sulphanilamide and subsequent coupling with N- (1-napthyl) ethylenediamine was read at 546 nm. The same reaction mixture without extract of plant but with equivalent amount of 0.5 M phosphate buffer served as control [29].

Reducing Power Assay

The different concentrations of the both extracts (50-250 µg/ml) in 1 ml distilled water were mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 3000g (rpm) for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700 nm [28].

Determination of total phenolic content [30]

1 ml of extract solution (1000 µg of the extract) in a volumetric flask diluted with distilled water (46 ml). Folin-Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 minutes, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in aspectrophotometer. The amount of total phenolic content in the both extracts was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.00816 \text{ X Total phenols [Gallic acid equivalents (µg)]} - 0.0135.$$

Statistical analysis

SPSS (version 13.0) statistical program was used to carry out a one-way analysis of variance was used to carry out a one-way analysis of variance (ANOVA) on data followed by Dunnett's t test. Values are expressed as mean ± SEM of three parallel measurements. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The phytochemical tests revealed that the PEPA extract contained carbohydrates, tannins and terpenoids; CEPA extract had alkaloids; and EEPA and AEPA had alkaloids, carbohydrates,

glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids.

DPPH Assay

In DPPH assay the ability of compound to act as donor for hydrogen atom or electron was measured spectrophotometrically. Reduction capacity of DPPH radical is obtained by decrease in its absorbance at 515 nm. The percentage inhibition of DPPH radicals were increased as concentration of extracts was increased [Table 1].

Table 1: DPPH free radical scavenging activity of ethanolic and aqueous extracts of *Plectranthus amboinicus*

Concentration (µg/ml)	% inhibition	
	Ethanolic extract	Aqueous Extract
50	16.29	13.25
100	40.43	35.32
150	48.19	43.89
200	59.45	54.21
250	71.70	67.23

Nitric Oxide screening method

In nitric oxide scavenging activity, the sodium nitropruside solution spontaneously generates nitric oxide which reacts with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric ions [29]. Results showed that the nitric oxide scavenging activity of extracts was increased with concentration [Table 2].

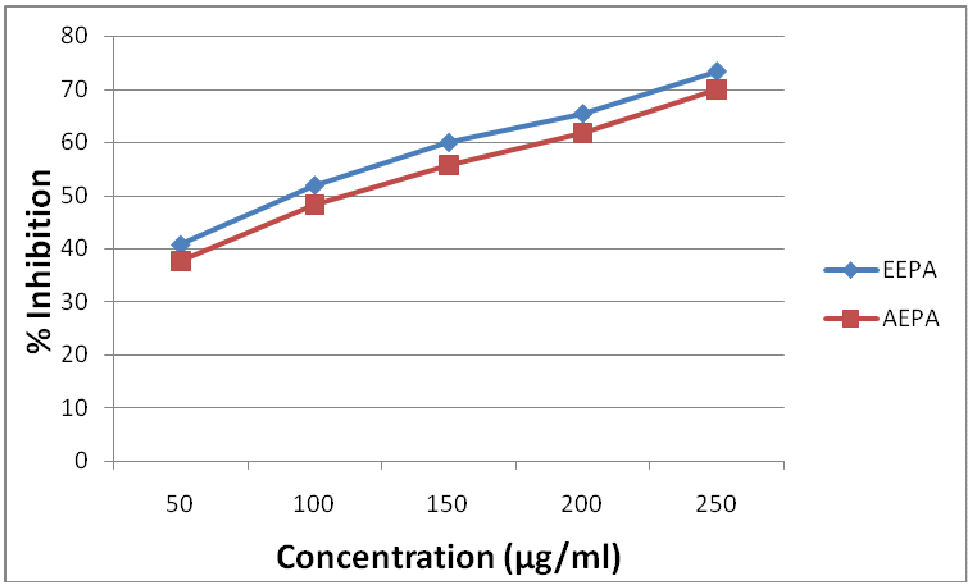
Table 2: Nitric oxide radical scavenging activity of ethanolic and aqueous extracts of *Plectranthus amboinicus*

Concentration (µg/ml)	% inhibition	
	Ethanolic extract	Aqueous Extract
50	31.56	29.56
100	44.83	40.23
150	59.09	56.19
200	63.25	61.81
250	73.56	71.21

Reducing Power Assay

Reducing power of extracts is measured by investigating the transformation of Fe^{3+} - Fe^{2+}. Graph 1 showing that reducing power is increased with increasing of concentration of extracts.

Graph 1: Antioxidant Potential of ethanolic and aqueous extract of *Plectranthus amboinicus* in Reducing Power Method



Total Phenolic contents

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups [1]. Phenolic contents of EAPA and AEPA were found 11.6 µg and 9.4 µg gallic acid equivalent per mg of extracts respectively.

CONCLUSION

The free radicals are one of the common causes of several diseases. The results showed that ethanolic and aqueous extracts of *Plectranthus amboinicus* had significant antioxidant activity. Ethanolic extract was more potent as compare to aqueous extract. The preliminary phytochemical investigation indicates the presence of flavonoids and tannins in the extract. So the antioxidant potential may be due to these polyphenols. Hence the further studies are needed to evaluate the in-vivo antioxidant activity of the plant in various animal models.

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