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Research Article

ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *PHASEOLUS TRILOBUS* ROOT POWDER

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ABSTRACT

The antioxidant activity of the methanolic extract of powder of roots of *Phaseolus trilobus* was evaluated. Methanolic extract was evaluated for its reducing power, hydrogen peroxide scavenging activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. High content of flavonoids in the roots may account for the antioxidant activity of the species. Study shows the potential of the methanolic extract of *P. trilobus* as a natural antioxidant.

Keywords: Antioxidant, DPPH, Reducing power, Scavenging activity

INTRODUCTION

Free radicals are chemical species that have an unpaired electron and play very important role in human health [1-2]. Free radicals are known to be associated with normal natural metabolism of aerobic cells. Oxygen consumption in cell growth leads to the formation of oxygen free radicals that further react with molecules of lipidic nature to form new radicals such as hydroperoxides and different peroxides. These radicals thus formed interact with biological systems in a cytotoxic manner, more is the amount of free radicals formed more will be the damage to cells and tissues leading to several diseases [3-4]. They can cause oxidative damage to lipids, protein and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans. Thus there is a need of antioxidants of natural origin that can protect the human body from the diseases caused by free radicals [5].

Antioxidants are important in preventing human diseases. Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce oxidative damage associated with diseases like, cancer, diabetes, arthritis, cardiovascular diseases, cataracts and ageing [6-8]. Antioxidant compounds may function as free radical scavengers, reducing agents and quenchers of singlet oxygen species [9]. Synthetic antioxidants like, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are carcinogenic. Therefore, need of natural antioxidants has increased in recent years.

*Phaseolus trilobus,*Ait. syn *Vigna trilobata* (L) Verdc. Synonyms:African gram or jungle mat bean. In hindi, it is known as ranmoong and mugani. It belongs to family Fabaceae (alt. Leguminosae). *Phaseolus* is a genus of annual or perennial herbs or twiners found throughout the tropics [10] and warm temperate regions of the world. About five species occur in India, three of them are cultivated throughout the world mainly for their edible pods [11]. *Phaseolus trilobus* [12] is native to Asia. It is found throughout India, in forests at low altitudes and in open rich soil and on Himalayas up to 7000 ft. It also grown in Africa, Australia, Madagascar, Mauritius and South America. Plant has been reported to contain dalbergioidin, kievitone, phaseollidin and flavonoid glycosides viz. Quercitin, kaempferol, vitexin, isovitexin [13] have been reported. The fruit of this plant is found to contain proteins, minerals and vitamin K [14], vitamin C [15]. The plant also contains friedelin, epifriedelin, stigmasterol and tannins.

Plant is extensively used by tribal people of Nandurbar district of Maharashtra, India in the treatment of jaundice and other liver disorders [14]. Plant is used as febrifuge in Bihar [16]. Leaves are considered as sedative, coolant, antibilious [16, 17]; tonic and they are used in cataplasms for week eyes [18]. Leaves are also applied in the form of paste to the eyes to improve the sight, and also in ophthalmia and in hemorrhoids [19]. Fruit is aphrodisiac, astringent, styptic, anthelmintic and good for eyes. Its consumption cures inflammation, fever, burning sensation, thirst, piles, dysentery, cough, gout and biliousness [20]. In Ayurveda, the juice of the plant is prescribed in rat- bite fever. Roots are used for curing fever, cough, diarrhea, hemorrhoids, ophthalmology and dyspepsia [21].

Here, this present work is aimed to investigate antioxidant activity of the methanolic extract of *P. trilobus* root powder by various *in vitro* models.

MATERIAL AND METHODS

Instrument and chemicals

Shimadzu UV-1800 spectrophotometer was used for the measurement of absorbance of solution mixtures. Ascorbic acid (Loba Chemie, Mumbai), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was obtained from Sigma Germany while hydrogen peroxide was obtained from Merck, India.

Plant material and extract preparation

The roots of Phaseolus trilobus was collected from Garhwal region of Uttrakhand. The collected plant material was authenticated from Head, Botany Division, Forest Research Institute, Dehradun. Specimen of the collected material is kept in the Chemistry Division for future reference. The collected plant material was cleaned properly and then dried in shade. Shade dried plant material was chopped and ground to coarse powder. Powder was extracted by methanol using maceration process. The extract was filtered and concentrated in vacuum for complete removal of solvent. The dried extract (MEPT) thus obtained was used for the assessment of antioxidant activity by in vitro assays.

Phytochemical screening of the plant extracts

Small portion of the dried extract was used for the phytochemical tests for components like tannins, flavonoids, alkaloids, saponins and steroids. To the 1ml methanolic extract of the plant few drops of ferric chloride solution was added. Formation of blue to greenish violet colouration indicated the presence of flavonoids [22]. 2g of plant powder

was vigorously shaked with 10 ml of water. Formation of soapy lather indicated the presence of saponin. The soapy liquid thus obtained failed to reduce Fehling's solution but on hydrolysis with 2N HCl it reduced Fehling's solution confirming it to be saponin [23]. About 0.5 g of the extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was carefully added to the filtrate to form lower layer. A reddish brown colour at the interface was taken as positive for steroid ring. 5 ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish- brown colouration at the interface was formed to show positive results for the presence of terpenoids [24,25].

Determination of Total Flavonoids

10 g of root powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 m). The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight [26]. The amount of flavonoid was determined as mg/g.

Scavenging of hydrogen peroxide

The ability of MEPT to scavenge hydrogen peroxide was determined according to the method of Ruch et al [27]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffered saline (PBS; pH7.4).

Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Different concentrations of MEPT (10, 20, 40, 80, 160, 320 µg/ml) in distilled water was added to hydrogen peroxide (0.6 ml, 40 mM) and absorbance at 230 nm was determined after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide. L-Ascorbic acid is used as standard for hydrogen peroxide scavenging activity. The percentage of hydrogen peroxide scavenging activity of both extract and standard was calculated as,

$$\% \text{ hydrogen peroxide scavenging} = [(A_{cont} - A_{test})/A_{cont}] \times 100$$

Where, A_{cont} is the absorbance of control and A_{test} is the absorbance in presence of the extract.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986) [28]. Various concentrations of MEPT (2.5 ml) were mixed with 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 1000 rpm for 8 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The assays were carried out in triplicate and the results expressed as mean values ± standard deviations. The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 700 nm against extract concentration. L- Ascorbic acid was used as standard.

DPPH radical scavenging activity

The free radical scavenging activity of the methanolic extract of *Phaseolus trilobus* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2,2-diphenyl-2-picryl hydrazyl). 0.1 mM of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (10-320 µg/ml). After 30 min the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0 \times 100$$

Where A_0 was the absorbance of control (blank without extract) and A_t was the absorbance in presence of extract. All the tests were performed in triplicate and graph was plotted with mean values [29].

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical analysis conducted on *P. trilobus* extract revealed the presence of flavonoids, steroids, terpenoids and saponins (Table 1). Total flavonoid content present in the root powder of the plant was found to be 92 mg/g. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study.

Table 1: Qualitative analysis of *Phaseolus trilobus* root extracts (Exh. & Seq.)

S. No.	Plant constituents	Methanol extract	
		Ex	Seq.
1.	Alkaloids	-	-
2.	Carbohydrates	-	-
3.	Glycosides	-	-
4.	Proteins and Amino- acids	-	-
5.	Saponins	+	+
6.	Phenolic compounds and tannins	-	-
7.	Fixed oils and fats	-	-
8.	Flavonoids	+	+
9.	Sterols	+	-
10.	Terpenoids	+	+
11.	Free amino acids	-	-

(+ indicates the presence of constituents and — indicates the absence of constituents)

Exh. = exhaustive and Seq. = sequential extract

Hydrogen peroxide scavenging activity

Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains [30]. Hydrogen peroxide can act directly or indirectly via its reduction product, OH^- that can act as a second messenger in the synthesis and activation of several inflammatory mediators [31]. When a scavenger is incubated with hydrogen peroxide then the loss of hydrogen peroxide can be measured. Fig. 1 shows the scavenging ability of MEPT and ascorbic acid on hydrogen peroxide at different concentrations and the scavenging ability of extract were found to be comparable with that of standard ascorbic acid. IC_{50} value of MEPT was 23.906 µg/ml while that of ascorbic acid was found to be 31.217 µg/ml. These findings show that MEPT is comparable scavenging activity with that of ascorbic acid (Table 2).

Table 2: Data of Hydrogen peroxide scavenging activity

Concentration (µg/ml)	% Scavenging activity	
	Ascorbic acid ± SD	Extract ± SD
10	27.46 ± 1.284	25.53 ± 1.942
20	48.66 ± 1.050	47.19 ± 3.496
40	55.52 ± 0.882	52.2 ± 1.094
80	61.57 ± 0.125	59.15 ± 1.314
160	79.68 ± 0.120	68.95 ± 0.420
320	81.77 ± 0.508	73.85 ± 0.110

Reducing power assay

Fig. 2 shows the reducing power of MEPT as a function of their concentration. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the compound. The presence of the reducers (antioxidants) causes the conversion of ferric ions/ ferricyanide complex used in the study to ferrous form. Therefore by measuring the absorbance of these colored solutions at 700 nm, we can determine the concentration of ferrous ions formed. Higher absorbance of the given solutions indicates higher reducing power.

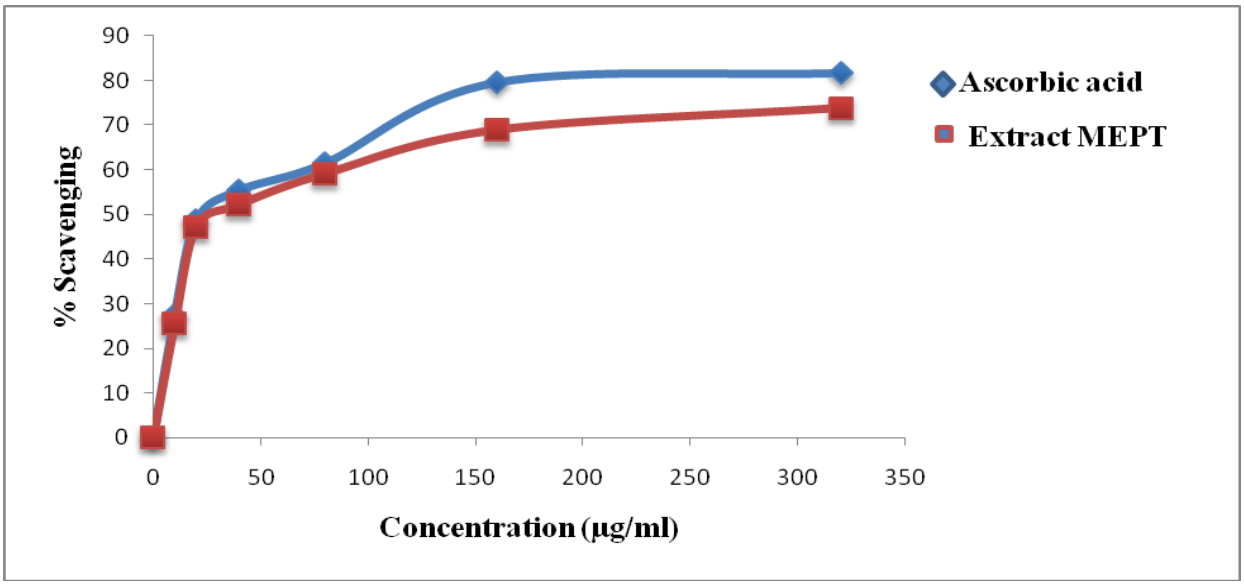

Fig. 1: Plots of ascorbic acid and extract for Hydrogen peroxide scavenging activity. Figure shows that IC $_{50}$ of Ascorbic acid is 23.906 µg/ml and IC $_{50}$ of Extract is 31.217 µg/ml

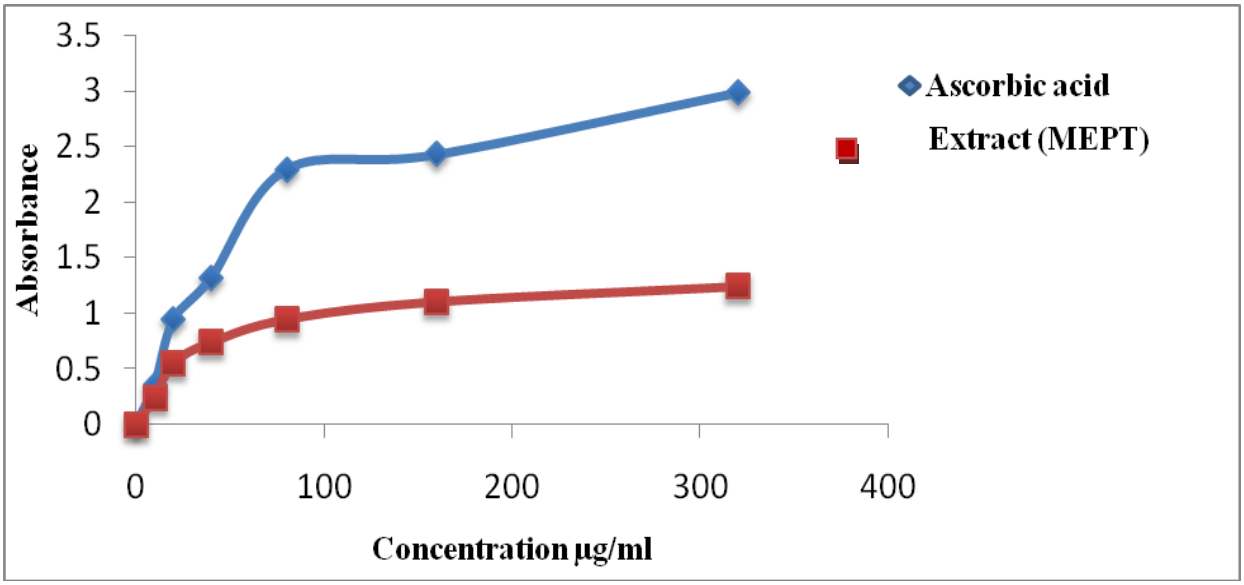

Fig. 2: Plots of ascorbic acid and extract for their respective Reducing power. Extract shows maximum reducing power at 320 µg/ml EC$_{50}$ of ascorbic acid was found to be 12.42 µg/ml and that of extract was 18.317 µg/ml

Table 3: Data of reducing power assay

Concentration (µg/ml)	Absorbance at 700 nm	
	Ascorbic acid ± SD	Extract ± SD
10	0.357 ± 0.019	0.243 ± 0.007
20	0.941 ± 0.0081	0.552 ± 0.0096
40	1.316 ± 0.0101	0.738 ± 0.008
80	2.294 ± 0.0095	0.946 ± 0.0192
160	2.428 ± 0.013	1.105 ± 0.0168
320	2.984 ± 0.0108	1.243 ± 0.0208

The reducing power of MEPT increased with concentration. At 320 µg/ml we obtained an excellent reducing power. Reducing power of ascorbic acid at 320 µg/ml was 2.984 (Table 3). It was reported that the reducing power depends on the presence of reductones, which

have been shown to exert antioxidant action by breaking free radical chain by donating a hydrogen atom.

DPPH scavenging activity

The free radical DPPH possesses a characteristic absorbance at 517 nm (purple in color) which decreases significantly on exposure to radical scavengers by providing hydrogen atoms or by electron donation. A lower absorbance at 517 nm indicates higher radical-scavenging activity of the extract. Free radical-scavenging is one of the mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity studies. This technique provides a means of rapid screening of radical-scavenging activity of a specific compound or extract.

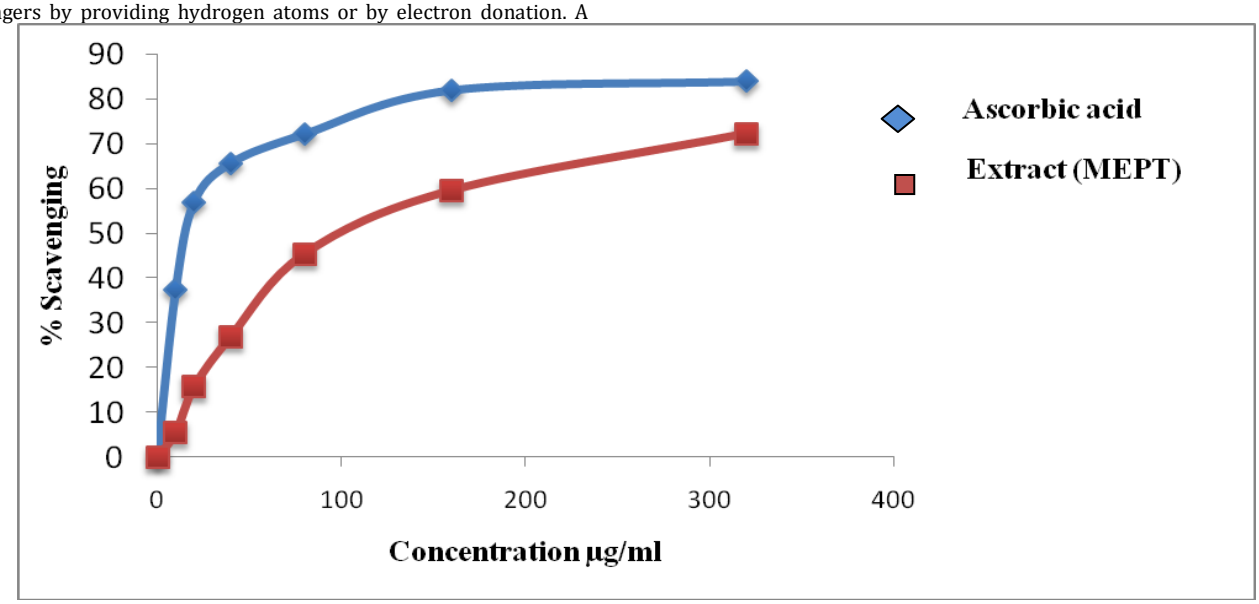

Fig. 3: Plots of ascorbic acid and extract for DPPH scavenging activity. Figure shows that IC $_{50}$ of Ascorbic acid is 16.468 µg/ml and IC $_{50}$ of Extract is 104.33 µg/ml

The values of DPPH scavenging activity of MEPT are shown in Fig. 3. Results are shown as the ratio of percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. From the analysis we can interpret that the effect of the extract o DPPH radicals increased with concentration (Table 4).

Table 4: Data of DPPH scavenging activity

Concentration (µg/ml)	% Scavenging activity	
	Ascorbic acid ± SD	Extract ± SD
10	37.326 ± 1.185	5.405 ± 1.351
20	56.92 ± 1.01	15.653 ± 1.436
40	65.646 ± 1.151	26.914 ± 1.354
80	72.123 ± 1.015	45.379 ± 1.456
160	81.923 ± 0.845	59.572 ± 1.124
320	83.893 ± 1.096	72.241 ± 1.312

CONCLUSION

From the study we can clearly demonstrate that the MEPT can effectively scavenge various reactive oxygen species or free radicals under in vitro conditions. This may be due to a number of stabilized oxidation products that it can form after oxidation or radical scavenging. Further studies are needed to clarify the in vivo potential of this plant in the management of human diseases resulting from oxidative stress and this is a subject of investigation in our group.

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