IN-VITRO ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT FROM WHOLE PLANT OF *Teramnus labialis (Linn.)*

G.Alagumani Vasagam*,A.Kottai Muthu , R.Manavalan.

Department of Pharmacy, Annamalai University, Annamalai nagar-608 002. Tamil Nadu, India.

***Corresponding author:**

G.Alagumanivasagam,
Assistant professor,
Department of Pharmacy,
Annamalai Universlty,
Annamalai nagar, 608002,
Tamil Nadu, India.
Contact No: +9194872 37131

E-mail : manivasagam78@rediffmail.com

Summary

In vitro antioxidant activity of whole plant of *Teramnus labialis (Linn.)extracts obtained by methonolic solvent was evaluated by three different in vitro methods.*. DPPH (2, 2-diphenyl -1- picryl hydrazyl) method, superoxide anion scavenging and iron chelating activity. The DPPH radical scavenging activity of Methanolic extract (IC_{50} =210 µg/ml) was better than that of standard Rutin (IC_{50}=480 µg/ml), The superoxide anion scavenging activity of methanolic extract (IC_{50}=70µg/ml) was better when compared the standard Quercetin(IC_{50}=60µg/ml), and iron chelating activity of methanolic extract (IC_{50}=160µg/ml) was superior to standard EDTA (IC_{50}=65µg/ml).The above result of possess good production against oxidative damage an antioxidant activity when compare to the above all standard.

Keywords: Antioxidant, *Teramnus labialis (Linn.)*, DPPH method, Superoxide anion scavenging activity, Iron chelating activity.

Introduction

Plant derived natural chemicals, known as secondary metabolites, are effective in their roles of protection, adaptation and pollination. Secondary metabolites are mainly used in food, pharmaceutical, chemical, cosmetic industries and agriculture [**1-2].**

Oxidative stress induced ROS and free radicals are believed to be major cause of physiological disorders like alzhemers, Parkinsons, arthritis ,atherosclerosis,coronary heart diseases,, Emphysema, gastric ulcer, diabetics mellitus, cirrhosis, aging and cancer [**3-4]. e**pidemiological and in vitro studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological system[**5-7**].

Teramnus labialis(L) spreng (Family; Fabaceae) is a herb, commonly known as mashaparni and a well known medicinal plant in the Ayurvedic system of medicine. It has been reported to be useful in treating rheumatism, tuberculosis, nerve disoders, paralysis and catarrhs [**8-10],** and chemical analysis and nutritional assessment [**11].** the plant used as antihyperglycemic activity [**12],** anti-inflammatory activities [**13], a** noval bioactive flavonol glycoside from teramnus labialis **[14].**

Therefore, the present investigation was to evaluate the free radical scavenging activity of methanolic extract of whole plant of teramnus labialis with three invitro antioxidant methods..

Material and Methods

Collection and Identification of Plant materials

The Whole plant of *Teramnus labialis*, were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai,Tamilnadu. The whole plant of *teramnus labialis* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus [**15**] for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques:

DPPH photometric assay [16]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[16.]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity [17]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[17]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity [18]

The method of Benzie and strain (1996) [18] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Result and Discussion

1.Dpph Method

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule**[19].** The methanolic extract of *Teramnus labialis* exhibited a maximum DPPH scavenging activity of 67.50% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the methanol extract of *Teramnus labialis was found to be* 210 µg/ml and Rutin(standard) was found to be 480µg/ml . The percentage of DPPH radical scavenging activity of methanolic extract of *Teramnus labialis* presented in Table 1 .

Table 1: Effect of Methanolic extract of whole plant of *Teramnus labialis* on DPPH assay

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	43.51±0.054	18.85 ± 0.076
2	250	55.42± 0.031	22.08 ± 0.054
3	500	59.23±0.013	52.21 ± 0.022
4	1000	67.50±0.031	69.83 ± 0.014
		IC_{50} = 210µg/ml	**IC_{50} = 480µg/ml**

All values are expressed as mean ± SEM for three determinations

II. SUPER OXIDE METHOD

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes.Superoxide dismutase enzymes present in aerobic

and anaerobic organisms catalyses the breakdown of superoxide radical**[20]**. The methanolic extract of *Teramnus labialis* exhibited a maximum Superoxide anion scavenging activity of 85.71% at 1000 µg/ml whereas for Quercetin (standard) was found to be 98.01% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Teramnus labialis* was found to be70µg/ml and Quercetin (standard) was found to be 60µg/ml. The percentage of Superoxide anion scavenging activity of methanolic extract of *Teramnus labialis* presented in Table 2.

Table 2: Effect of Methanolic extract whole plant of *Teramnus labialis* (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	65.05±0.022	73.81 ± 0.006
2	250	67.25±0.035	91.31 ± 0.011
3	500	80.55±0.048	92.99 ± 0.024
4	1000	85.71±0.021	98.01 ± 0.012
		IC_{50} = 70µg/ml	**IC_{50} = 60µg/ml**

*All values are expressed as mean ± SEM for three determinations

III. IRON CHELATING METHOD

Iron is essential for life because it is required for oxygen transfort, respiration and activity of many enzyme.However, iron is an extremely reactive metal and catalyzes oxidative chanches in lipids, proteins and other cellular components**[21]**. The methanolic extract of *Teramnus labialis* exhibited a maximum iron chelating activity of 94..23% at 1000 µg/ml whereas for EDTA (standard) was found to be 97.90% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Teramnus labialis* was found to be 160µg/ml and EDTA(standard) was found to be 65µg/ml. The percentage of iron chelating activity of methanolic extract of *Teramnus labialis* presented in Table 3.

Table 3: Effect of Methanolic extract whole plant of *Teramnus labialis* on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	32.53±0.03	58.68 ± 0.007
2	250	53.22±0.06	65.87 ± 0.018
3	500	76.23±0.04	83.83 ± 0.012
4	1000	94.23±0.06	97.90 ± 0.019
		IC_{50} = 160µg/ml	**IC_{50} = 65µg/ml**

*All values are expressed as mean ± SEM for three determinations

The results of the above investigation indicated that the methanolic extract of whole plant of *Teramnus labialis* showed significant antioxidant activity was found in DPPH method, superoxide anion scavenging activity and Iron chelating method when compared to the reference standard Rutin, Quercetin and EDTA respectively.

Conclusion

The results of the present study was clearly indicated that the methanolic extract of whole plant of *Teramnus labialis* showed significant natural antioxidant activity and used as food supplement in pharmaceutical industry. Phytochemical screening of extract showed presence of Triterpenoids ,flavonoids, glycosides, tannins and alkaloids . Hence it can be concluded that these components might be helpful in preventing the progress of various oxidative stress..Therefore, it is suggested that further work should be performed on the isolation and identification of the antioxidant compounds in *Teramnus labialis*

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