Research Article

An *In-Vitro* Free Radical Scavenging Activity of Methanolic Extracts of Whole Plant of *Teramnus labialis (Linn.)*

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

Department of Pharmacy, Annamalai University, Annamalai nagar, Tamil Nadu, India.

ABSTRACT

In vitro antioxidant activities of methanol extract of tuberous root of *Ipomoea digitata* (Linn.) was investigated. The free radical scavenging activity to evaluate by Hydroxyl radical scavenging activity**,** FRAP method and Estimation of total phenol, Nitric oxide method. cccc, Estimation of flavonoid method. Hydroxyl radical scavenging activity of methanolic extract and reference standard Ascorbate IC50 values was found to be 230 μg/ml and 410 μg/ml. FRAP method of methanolic extract and reference standard Ascorbate IC50 values was found to be 800 μg/ml and 50 μg/ml. The total phenol content of methanolic extract was found to be 7.51mg/g respectively. Nitric oxide method activity of methanolic extract and reference standard Ascorbate IC50 values was found to be 760 μg/ml and 410 μg/ml. Total antioxidant activity method activity of methanolic extract and reference standard Ascorbate IC50 values was found to be 180 μg/ml and 410 μg/ml. Total antioxidant activity method activity of methanolic extract and reference standard Ascorbate IC50 values was found to be 180 μg/ml and 410 μg/ml Estimation of flavanoid was found to be 2.502mg/g respectively.The above result possess significant antioxidant activity when compare to the above all standard.

Keywords: Antioxidant, Hydroxyl radical scavenging activity, FRAP method, Estimation of total phenol.

INTRODUCTION

Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), as well as non-free radicals species (H_2O_2) and the singlet oxygen ($^1O2$)[1]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[2]

Teramnus labialis L) spreng (Family; Fabaceae) is a herb, commonly known asmashaparni 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[3-5]**,** and chemical analysis and nutritional assessment[6]**.** The plant used as antihyperglycemic activity[7]**,** anti-inflammatory activities[8]**,** a noval bioactive flavonol glycoside from teramnus labialis[9]**.**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[10] 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:

1. Hydroxyl radical scavenging activity[11]

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves *in-vitro* generation of hydroxyl radicals using Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods the hydroxyl radicals formed by the oxidation is made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow color with Nash reagent (2M ammonium acetate with 0.05m acetic acid and 0.02m acetyl acetone in distilled water). The intensity of yellow color formed is measured at 412 nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging.

2. Frap method[12]

FRAP (Ferric Reducing Ability of Plasma) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6 – tri) (2-pridyl)–S-triazine) and $FeCl_3 6H_2O$. The absorbance is measured spectrophotometrically at 595nm. Antioxidant activity of plant extracts is reported by this method.

3. Estimation of total phenol[13]

The measurement of total phenol is based on Mallick and Singh (1980), To 0.25g of sample, added 2.5ml of ethanol and centrifuged at 2ºc for 10 mins. The supernatant was Preserved. Then, the sample was re-extracted with 2.5ml of 80./. ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3ml of water to the dried supernatant. To which added 0.5ml of folins phenol reagent and 2ml of sodium corbonate (20/.). The reaction mixture was kept in boilingwater bath for 1mins.The absorbence was measured at 650nm in a spectrophotometer.

4. Nitric oxide method[14]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964). The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

5. Total antioxidant activity[15] (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo(V) to form phosphomolybdenum complex (Prieto et al., 1999). An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and

4 mM ammonium molybdate). These vials were capped and incubated in a water bath at 95ºc for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

6. Estimation of flavonoid method[16]

0.2g of the plant material was ground with ethanol-water in two different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these two ratios were obtained. This was evaporated to dryness until most of the ethanol has removed. The resultant aquous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aquous layer was concentrate.0.5

RESULT AND DISCUSSION

1. Hydroxy radical method

Freeradical scavenging activity of the methanolic extract of *Teramnus labialis (Linn.)* studies was determined by hydroxyl radical method.The free radical scavenging potential shown maximum activity is 65.71 at 1000µg/ml for as Standard (ascorbate) was found to be 54.00 at 1000 µg/ml. The IC_{50} of the methanol extract of *Teramnus labialis (Linn.)* and standard (Ascorbate) was found to be 230µg/ml and 410µg/ml better antioxidant is respectively.

Table 1: Antioxidant Effect of tuberous root of Methanolic extract of *Teramnus labialis (Linn.)* on Hydroxy radical method

S. No.	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	22.11±0.04	27.63±0.076
2	250	47.34±0.08	33.53 ±0.054
3	500	53.52±0.05	59.12±0.022
4	1000	65.71±0.04	54.00±0.014
		IC_{50}=420 µg/ml	IC_{50}=410µg/ml

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

2. Frap method

Freeradical scavencing activity of the methanolic extract of *Teramnus labialis (Linn.)* was determined by FRAP method. The antioxidant of scavenging activity of free radical potential shown maximum activity is 78.66 at 1000µg/ml for as Standard (Ascorbate) was found to be 98.07 at 1000 µg/ml. The IC_{50} of the methanol extract of *Teramnus labialis (Linn.)* and standard (Ascorbate) was found to be 180µg/ml and 50µg/ml in better antioxidant is respectively.

Table 2: Antioxidant Effect of tuberous root of Methanolic extract of *Teramnus labialis (Linn.)* on FRAP method

S. No.	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (Ascarbate)
1	125	32.47±0.04	72.04±0.014
2	250	45.11±0.02	82.05±0.034
3	500	61.84±0.05	86.04±0.026
4	1000	78.66±0.03	98.07±0.041
		IC_{50}= 180 µg/ml	IC_{50}=50µg/ml

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

3. Estimation of total Phenol

Free radical scavenging activity of the methanolic extract of *Teramnus labialis (Linn.)* was determined by estimation of total phenol. The maximum Estimation of total phenol scavenging activity of 8.176mg/g respectively.

Table 3: Antioxidant Effect of tuberous root of Methanolic extract of *Teramnus labialis (Linn.)* on Estimation of total phenol

S. No.	Extract	Total Phenolic compound (mg/g Catechol)	Total phenolic content ±SEM
1	Methanolic Extract *Teramnus labialis (Linn.)*	8.12 8.23 8.18	8.176±0.03

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

4. Nitric oxide method

Free radical scavenging activity of the methanolic extract of *Teramnus labialis (Linn.)* was determined by nitric oxide method. The free radical scavenging potential shown maximum activity is 57.23% at 1000µg/ml for as Standard (ascorbate) was found to be 62% at 1000 µg/ml. The IC_{50} of the methanol extract of *Teramnus labialis (Linn.)* and standard (ascorbate) was found to be 760µg/ml and 410µg/ml better antioxidant is respectively.

Table 4: Antioxidant activity of tuberous root of Methanolic extract of *Teramnus labialis (Linn.)* by nitric oxide free radical scavenging method

S. No.	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (ascorbate)
1	125	32.65±0.02	27.63±0.076
2	250	43.21±0.19	31.53 ±0.054
3	500	48.11±0.02	55.12±0.022
4	1000	57.23±0.06	62.00±0.014
		IC_{50}= 760 µg/ml	IC_{50}=410µg/ml

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

5. Total antioxidant method

Total antioxidant activity of the methanolic extract of *Teramnus labialis (Linn.)* was determined by phosphomolybdate method. The free radical scavenging potential shown maximum activity is 81.30% at 1000µg/ml for as Standard (ascorbate) was found to be66 % at 1000 µg/ml. The IC_{50} of the methanol extract of *Teramnus labialis (Linn.)* and standard (ascorbate) was found to be 180µg/ml and 410µg/ml better antioxidant is respectively.

Table 5: Antioxidant activity of tuberous root of Methanolic extract of *Teramnus labialis (Linn.)* by Phosphomolybdate method

S. No.	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (ascorbate)
1	125	49.92±0.02	27.63±0.076
2	250	68.49±0.02	31.53 ±0.054
3	500	74.85±0.03	60.12±0.022
4	1000	81.30±0.15	66.00±0.014
		IC_{50}=180 µg/ml	IC_{50}=410µg/ml

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

6. Estimation of flavonoid method

The flavonoid content of methanol extract of whole plant of *Teramnus labialis (Linn.)* was presented table 6. Based on the report of methanolic extract of whole plant of *Teramnus labialis (Linn.)* was found 2.502mg/g of flavonoid compound.

Table 6: Flavonoid content of Methanolic extract of *Teramnus labialis (Linn.)*

S. No.	Extract	flavonoid compound (mg/g)	Flavonoid compound ±SEM
1	Methanolic extract of *Teramnus labialis (Linn.)*	2.524 2.498 2.486	2.502± 0.05

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

CONCLUSION

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. In the present work, the high antioxidant capacity observed for methanolic extract of whole plant of *Teramnus labialis (Linn.)* suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

REFERENCES

1. Adedapol AA, Jimoh FO, Afolayan AJ and Masika PJ. Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis Africana*. Rec Nat Prod. 2009;3:23-31.
2. Halliwell B. Advances in pharmacology. 1997;38:3-17.
3. viswanathan MB, Thangadurai D, Tamil vendan K and Ramesh N. Chemical analysis and nutritional assessement of teramnes labialis, Plant Foods For Human Nutrition. 1999;54:345-352.
4. Pineda M and Aguilar M. Anal Biochem. 1999;269:337-341.
5. Fort DM, Rao K, Jolad SD, Luo J, Carlson TJ and King SR. Antihyperglycemic activity of Teramnus labialis. Phytomedicine. 2000;6(6):465-7.
6. Sridhar C, Krishnaraju AV and Subbaraju GV. Antiinflamamatory constituents of teramus labialis. Indian J Pharm.sci. 2006;68(1):111-114.
7. Yadava RN and Jain S. A noval bioactive flavonol glycoside from teramnus labialis. Nat Prod Res. 2004; 18(6):537-42.
8. Harborne JB. Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 1984:4-5.
9. Waynforth BH. Injection techniques. Experimental and surgical techniques in the rats, Academic Press, London, 1980:3.
10. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990;58:237-240.

11. Prieto P, Pineda M and Aguilar M. Anal Biochem. 1999;269:337-341
12. Waynforth BH. Injection techniques. Experimental and surgical techniques in the rats, Academic Press, London. 1980:3.
13. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990;58:237-240.
14. Prieto P, Pineda M and Aguilar M. Anal Biochem. 1999;269:337-341.
15. Mallick CP and Singh MB. Plant enzymology and Histoenzymology (eds), Kalyani publishers, New Delhi. 1980:286.
16. Garrat DC. The quantitative analysis of drugs, Champman and Hall, Japan. 1964;3:456-458.
17. Prieto P, Pineda M and Aguilar M. Anal Biochem. 1999;269:337-341.