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Research Article

IN VITRO EVALUATION FOR FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC LEAF EXTRACT OF *ENTADA PURSAETHA*

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ABSTRACT

The objective of this study was to evaluate the antioxidant activity of methanolic leaf extract of *Entada pursaetha* by using Superoxide radical, Hydroxyl radical and DPPH radical scavenging methods. *Entada pursaetha* is a gigantic woody liana among legumes. The methanolic leaf extract of *Entada pursaetha* produced a dose dependent inhibition of *in vitro* free radical generation of superoxide anion (IC_{50} value **488.50** µg/ml), hydroxyl radical (IC_{50} value **509.00** µg/ml) and DPPH radical (IC_{50} value **390.00**µg/ml). The results clearly indicate the free radical scavenging activity of methanolic leaf extract of *Entada pursaetha* and this activity comparable with that of the standard drug ascorbic acid.

Keywords: *Entada pursaetha*, Antioxidant activity, Ascorbic acid.

INTRODUCTION

Entada pursaetha is a gigantic woody liana among legumes, which produces 90-150 cm long woody giant pods with 5 to 30 seeds. All parts of this species contain saponins and are thus used in the soap industry. This species is reported as tribal pulse[1]. Its semi ripe seeds are also used as a substitute for coffee. The plant material is used by the tribal's as a broad spectrum compound. This species can be used as a narcotic or as a tonic, etc, or used in curing liver troubles, allaying body pains, in warding off cold, curing eye diseases, arthritis, and paralysis[2]. This species is reported as endangered[3, 4, 5]. However, literature survey indicated no published reports on the in vitro antioxidant activities of this plant. Hence, a detailed study was carried out on the methanolic extract of *Entada pursaetha* for scavenging activity of superoxide radical, Hydroxyl radical and DPPH radical.

MATERIAL AND METHODS

Chemicals

All the chemicals and reagents used were of analytical grade. 1, 1-diphenyl-2-picrylhydrazyl was purchased from Sigma Chemical Company, St. Louis, USA), Riboflavin from Loba Chemie Pvt Ltd., Bombay, Deoxyribose and Nitroblue tetrozolium were purchased from Sisco Research Laboratories Pvt Ltd., Mumbai.

Plant Material

The plant material was collected in November 2009 from Katika village, Ananthagiri mandal, Andhra Pradesh, India and authenticated by Dr. P. Prayaga Murthy, Taxonomist. The Voucher specimens (BG/PMK/CG-11-09) were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University.

Methods

Preparation of Extract

The freshly collected aerial parts of the plant were shade dried and powdered. The powdered material was then subjected to triple maceration with methanol: water (70:30). The extract thus obtained was concentrated under vacuum at temperature of 43°C by using rotary evaporator (Buchi), dried completely, weighed and stored in a desiccator.

Preliminary Phytochemical Screening

The methanolic aerial parts extract on preliminary phytochemical screening showed the presence of sterols, triterpenes, saponins and flavonoids.

In-vitro Antioxidant Activity

The methanolic aerial parts extract of *Entada pursaetha* was screened for following free radical scavenging activity against Superoxide radical, Hydroxyl and DPPH radicals. The Percentage Inhibition and 50% Inhibition Concentration's (IC_{50}) were calculated. All experiments were performed thrice and the results were averaged.

Calculation of Percentage Inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula:

Formula-1

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

Calculation of 50% Inhibition Concentration

The optical density obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of the leaf extract was measured according to McCord and Fridovich method[6], 1969. It depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560nm (table-1, graphically plotted in Fig-1). The percentage inhibition was calculated from the above Formula-1.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao[7]1990, by studying the competition between deoxyribose and test extract for hydroxyl radicals generated by Fenton's reaction. The damage imposed on deoxyribose due to the free radicals was determined calorimetrically by measuring the thiobarbituric acid reactive substances (TBARS) at 532 nm. Percentage of inhibition was calculated using Formula-1. The results were showed in table-2 and graphically plotted in Fig-2.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was measured according to the method of Braca *et al* [8], 2003., S. D. Sanja et *al*[9], 2009., An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37° for 30 min. and absorbance of the test mixture was read at 517nm.The

percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using Formula-1. The results were placed in table-3 and graphically plotted in Fig-3.

Calculation of 50% Inhibition Concentration (IC_{50})

The optical density obtained with each concentration of test sample plotted taking concentration on X-axis and percentage inhibition on Y-axis, the graph was extrapolated to find the 50% inhibition concentration of test sample. The 50% Inhibition Concentration (IC_{50}) values of Superoxide, Hydroxyl and DPPH radicals were given in the table-4 and graphically represented in Fig-4.

Statistical Analysis

Values were expressed as mean ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA and linear regression analysis was used to calculate IC_{50} values. All determinations were done at least in triplicate and all were averaged (n=3).

RESULTS AND DISCUSSIONS

The methanolic extract of *Entada pursaetha* produced a dose dependent inhibition (Table 1, Table 2 & Table 3) of free radical generation of superoxide (IC_{50} value **488.50** µg/ml), hydroxyl radical (IC_{50} value **509.00** µg/ml) and DPPH radical (IC_{50} value **390.00** µg/ml) in *in vitro* (Table 4).

Table 1: Percent Inhibition of Superoxide Radical by Methanolic Aerial part Extract of *Entada pursaetha*

Extracts/ Compound	Percentage inhibition of Superoxide radical						
	Quantity of extracts/ ascorbic acid in micrograms (µg)						
	50	100	250	500	750	1000	2000
Alc. Ext of *E.pursaetha*	15.30±0.5	26.69±1.2	36.65±2.1	51.07±2.2	61.92±2.4	64.95±1.5	67.62±2.4
Ascorbic acid	43.17±0.7	52.41±0.2	61.10±0.2	75.31±1.3	81.52±1.6	86.48±0.5	87.17±1.4

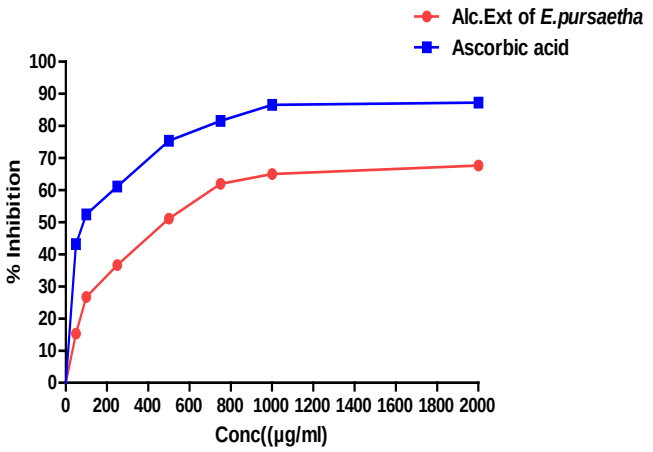

Fig. 1: Percent Inhibition of Superoxide Radical by Methanolic Aerial part Extract of *E.pursaetha*

Table 2: Percent Inhibition of Hydroxyl Radical by Methanolic Aerial part Extract of *Entada pursaetha*

Extracts/ Compound	Percentage inhibition of Hydroxyl radical						
	Quantity of extracts/ ascorbic acid in micrograms (µg)						
	50	100	250	500	750	1000	2000
Alc. Ext of *E.pursaetha*	13.97±0.6	24.94±1.3	31.67±1.4	49.88±2.2	59.10±1.5	64.59±2.2	68.83±2.5
Ascorbic acid	31.67±1.2	40.30±1.2	55.61±1.1	72.27±2.1	81.52±1.6	84.70±1.6	84.85±3.2

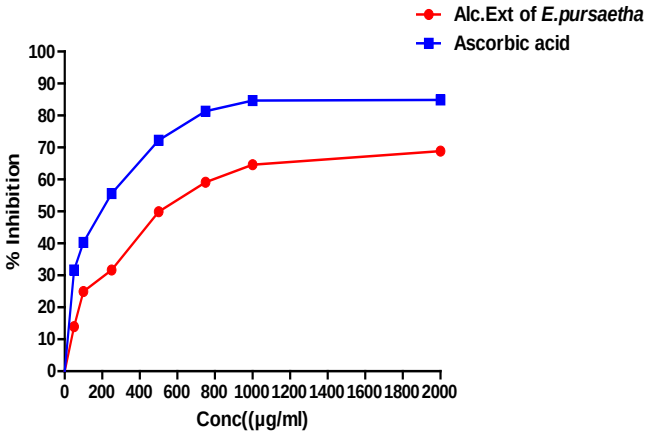

Fig. 2: Percent Inhibition of Hydroxyl Radical by Methanolic Aerial part Extract of *E.pursaetha*

Table 3: Percent Inhibition of DPPH Radical by Methanolic aerial Part Extract of *Entada pursaetha*

Extracts/ Compound	Percentage inhibition of DPPH radical						
	Quantity of extracts/ ascorbic acid in micrograms (µg)						
	50	100	250	500	750	1000	2000
Alc.Ext of *E.pursaetha*	18.60±2.2	27.53±1.5	43.90±1.2	55.21±2.5	63.54±2.3	68.15±1.2	70.09±2.4
Ascorbic acid	45.30±2.1	75.61±2.1	81.82±2.4	86.52±2.2	88.18±2.1	90.15±2.2	90.45±3.4

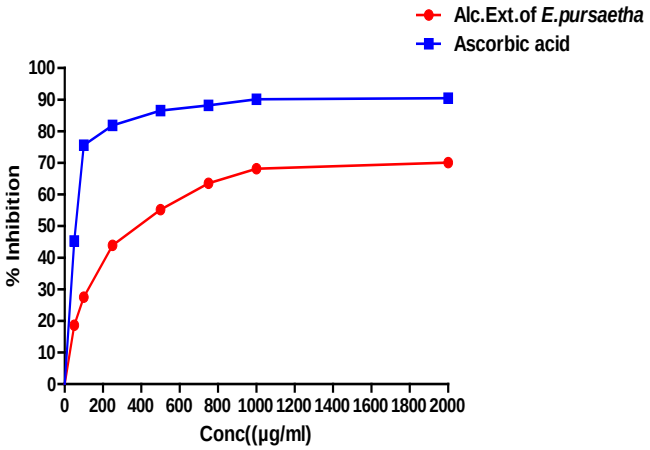

Fig. 3: Percent Inhibition of DPPH Radical by Methanolic aerial Part Extract of *E.pursaetha*

Table 4: Inhibitory activity (IC_{50}) of Methanolic aerial part Extract of *Entada pursaetha*

Extracts/ Compounds	Quantity of various extracts in micrograms		
	Free radicals, reactive oxygen species		
	Superoxide	Hydroxyl radical	DPPH
Alc. Ext of *E.pursaeta*	488.50±3.2	509.00±2.8	390.00±2.5
Ascorbic acid	80.2±2.1	190.20±2.5	60.24±1.3

*IC_{50} values are the mean ± standard error mean (SEM) of three assays.

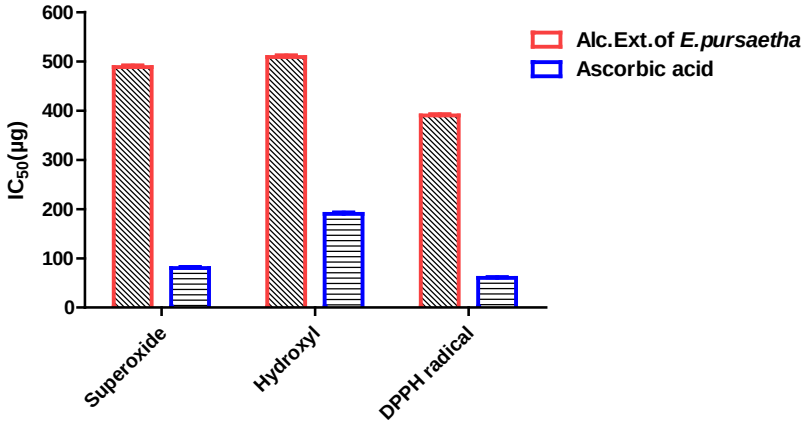

Fig. 4: Inhibitory activity (IC_{50}) of Methanolic aerial part Extract of *Entada pursaetha*

The results of *in-vitro* antioxidant activity of methanolic leaf extract of *Entada pursaetha* clearly indicate the presence of free radical scavenging activity and it produced dose dependent inhibition of free radical generation of superoxide anion, hydroxyl radical and DPPH radicals and they are compared to standard anti-oxidant drug, Ascorbic acid. Graphs were plotted from the observed values to find the percentage inhibition (Fig 1, Fig 2 & Fig 3) and the 50% inhibition concentration IC_{50} (Fig 4) of the methanolic extract of *Entada pursaetha*.

Preliminary Phytochemical screening of the methanolic aerial parts extract of *Entada pursaetha* showed the presence of sterols, triterpenes, saponins and flavonoids. Natural antioxidants such as plant-phenols, flavonoids and tannins possess potent antioxidant activity[10, 11]. Sterols like β-sitosterol have been reported for antioxidant activity[12]. Terpenoids are also reported to possess antioxidant activity[13]. The results of free radical scavenging activity of the methanolic extract of *Entada pursaetha* showed dose dependent *in vitro* anti-oxidant activity[14, 15]. These active constituents alone or in combination may be responsible for the observed antioxidant activity.

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REFERENCES

1. Siddhuraju, P., Vijayakumari, K. and Janardhanan, K. Genetic resources in tribal pulses. Plant Genetic Resources. 1993, 96: 47-49.
2. Johnson, T. Ethno botany Desk Reference 1999, pp. 302, CRC Press, Boca Raton, London, New York.
3. Jadhav, S. N., Ved, D. K., Reddy, K. N. and Reddy, Ch. S. Proceedings of the workshop on conservation assessment & management planning for medicinal plant of Andhra Pradesh 2001, pp. 4, FRLHT, Bangalore.
4. Janardhanan, K., Vadivelu, V. and Pugalenthi, E. Biotechnology in Indian tribal/under exploited pulses 2001, pp. 18-21, Kluwer Academic Publishers. Netherlands.
5. Varak, V. D. and Suryanarayana, M. C. Journal of Economic & Taxonomic Botany 1995, 19: 555-569.
6. Mc Cord JM, Fridovich I, Superoxide and Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein) J Biol Chem, 244(22), 1969, 6049-6055.
7. Elizabeth K, Rao M N A, Oxygen radical scavenging activity of curcumin, Int J Pharm, 58, 1990, 237-240.
8. Braca A, Tommasi ND,Bari LD,Pizza C, Politi M, Morelli I, Antioxidant principles from *Bauhinia terapotensis*, J Nat Prod 2001; 64:892-895.
9. Sanja S.D., Sheth N.R, Patel N.K, Dhaval Patel, Biraju Patel, Characterization and evaluation of Antioxidant activity of Portulaca oleracea, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 1, July-Sep. 2009, 74-84
10. Sanchez-Moreno C, Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems, Food Sci Technol Int 2002; 8:121-137.
11. Maryam zahin, Farrukh Aqil, Iqbal Ahmad, the in vitro Antioxidant activity and total phenolic content of four indian medicinal plants, , International Journal of Pharmacy and Pharmaceutical Sciences, 1(1) , Nov.-Dec. 2009, 88-95
12. Cai YZ, Luo Q, Sun M, Corke H, Antioxidant activity and phenolic compounds of 112 Traditional Chinese medicinal plants associated with anticancer, Life Sci. 74, 2004, 2157-2184.
13. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R: Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr, 133, 2003, 1286-1290.
14. Anita Murali, Purnima Ashok, Madhavan V. In vitro antioxidant activity and HPTLC Studies on the roots and rhizomes of *Smilax zeylanica* L. (smilacaceae). Int J Pharm Pharm Sci. 3(1), 2011,192-195.
15. Ganga Rao et *al*. Investigation on regional variation in Total Phenolic Content, Alkaloid Content and *In-Vitro* Antioxidant Activity of *Cleome chelidonii L*. Int J Pharm Pharm Sci. 3(4), 2011, 416-418.