In vitro antioxidant activity of *Entada pursaetha, Toddalia aculeata,* and *Ziziphus mauritiana.*

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Abstract

The antioxidant activity of the crude methanolic extract of the seeds of *Entada pursaetha*, the stem of *Toddalia aculaeta*, and the fruit of *Ziziphus mauritiana* was investigated. The total phenolic composition of methanolic extract was calculated to be 5.5mg catechol equivalents/g of sample. Antioxidant activity of the extract was evaluated on the basis of ability of scavenging free radical and hydroxyl radical with the Ic 50 values 2.12mg/ml and 1.034mg/ml respectively. Total antioxidant capacity of crude plant extract was found to be 1.43mg ascorbic acid equivalents at 250µg/ml extract concentration. The reducing power of the extract increased dose dependently and the extract reduced the most Fe^{3+} ions to the extent less than the standard ascorbic acid.

Keywords: Antioxidants, Entada *pursaetha, Toddalia aculeata, Ziziphus mauritiana*, DPPH, Hydroxyl radical, Reducing power

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INTRODUCTION

Reactive oxygen species(ROS) are highly reactive molecules derived from the metabolism of oxygen causing damage to DNA, proteins, carbohydrates and lipids.ROS include super oxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen(1). All these radicals exert oxidative stress towards the cells of human body and this leads to a number of physiological disorders. Antioxidants are of great importance in terms of reducing oxidative stress that is thought to cause damage to biological molecules(2).Phenolic compounds have been reported to play key antioxidant roles using the mechanism of delocalization of the single electron of the radical(3).

The commonly used synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) are suspected to have some toxic effects and possible carcinogens (4). Therefore development and utilization of more effective antioxidants of natural origins are desired. A great number of natural medicinal plants have been tested for their antioxidant activities and results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants in vitro than BHT or vitamin E(5).

The plants used for the present study were *Entada pursaetha, Toddalia aculeata, and Ziziphus mauritiana. Entada pursaetha* is a gigantic creeper with giant pods among legumes and is an endangered species belonging to the family Fabaceae. It can be used as a narcotic or as a tonic or used in curing liver troubles, allaying body pains, in warding of cold, curing eye diseases, arthritis and paralysis(6). *Toddalia aculeata* is a thorny large shrub belongs to the family Rutaceae. It has been used by traditional health practitioners in East Africa for management of diseases (7). *Ziziphus mauritiana* is a tropical fruit tree species belonging to the family Rhamnaceae. The fruits are sweet, cooling, anodyne purgative, mucilaginous, pectoral. Styptic, aphrodisiac, invigorative, depurative, appetizer and tonic. They are useful in encephalopathy, opthalmopathy, dipsia, cough, asthma. Wound vitiated conditions of pitta burning sensation, diarrhea, vomiting, leucorrhoea, general debility and insomnia (8).

MATERIALS AND METHOD

Extraction of plant materials

The seeds of *Entada pursaetha*, the stems of *Toddalia aculeata* and the fruits of *Ziziphus mauritiana* were collected from Kolli hills, Namakkal, Salem District, Tamilnadu, India. The samples were air dried in shade at room temperature and then ground to a fine powder in a mechanic grinder.10 g of the mixed powdered plant material was extracted

with 400ml of methanol in a soxhlet extractor for 24hrs. The resultant crude methanolic extract was evaporated to dryness and then stored in the freezer until ready for use.

Quantification of total phenolic compound.

Total phenolics were quantified and expressed as catechol equivalents according to a method proposed by Singleton et al 1999 (9).To 0.1ml of methanolic extract added 3.9ml of distilled water and 0.5ml of Folin –Ciocalteau reagent.

The tubes were incubated at room temperature for 30 min. To this added 2ml of 20% sodium carbonate and kept at boiling water bath for 3 min.The blue colour formed was read at 650 nm with spectrophotometer. The standard graph was prepared with a plot of various concentrations against corresponding absorbance values for catechol .Total phenol values are expressed as catechol equivalents (mg/g dry mass).

Determination of Total antioxidant activity of plant extracts.

Antioxidant activity of plant extracts was determined by the method of Prieto et al 1999 (10). The tubes containing 0.2 ml of plant extract, 1.8 ml of distilled water and 2 ml of phosphomolybdenum reagent solution (0.6M sulphuric acid,28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 minutes. The mixture was cooled to room temperature and the absorbance was measured at 695 nm using an UV/VIS spectrophotometer. The antioxidant activity is expressed as number of equivalents of ascorbic acid.

Measurement of free radical scavenging activity.

The ability of the extract to scavenge free radical was assayed with use of a synthetic free radical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The method of Brand Williams et al 1995 (11). has been adapted. 200 µl of the extracts of concentration between 50–250 µg were mixed with 2 ml of DPPH reagent (0.1 mM DPPH in methanol). The disappearance of pink color of DPPH was read spectrophotometrically at 517 nm after 30 mins of incubation at room temperature in the dark. The same solvent was used as a control. The same procedure was repeated with methanolic solutions of synthetic antioxidant Quercetin as positive control. Free radical scavenging capacity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation;

% inhibition = 100 × (1 – Absorbance of sample/ Absorbance of control).

Measurement of hydroxyl radical scavenging activity.

The hydroxyl radical scavenging activity was measured by a modified deoxyribose method determining thiobarbituric acid reactive substances (TBARS) proposed by Res at Apak et al 2006 (12). To a test tube added 3 ml of phosphate buffer (pH 7.0) , 1 ml of 10 mM 2-deoxy-D-ribose, 0.5 ml of 20 mM Na_2 EDTA , 0.5 ml of 20mM $Fecl_3$ solution , 3.8 ml distilled water , 0.2 ml of plant extract (200-1000 µg) and 1 ml of 10mM of H_2O_2 in the given order and the mixture of incubated for 4 hours at 37°C in a water bath . At the end of the period the reaction was arrested by adding 5 ml of 2.8% TCA. To this added 5 ml of 1% TBA and the reaction mixture was kept in a boiling water bath for 10 minutes. The mixture was cooled and the absorbance was measured at 520 nm. Hydroxyl radical scavenging capacity was expressed as percentage inhibition of Hydroxyl radical and was calculated by the following equation;

% inhibition = 100 × (1 – Absorbance of sample/ Absorbance of control).

Determination of reducing power of plant extracts.

The reducing power of the methanolic plant extract was determined according to the method of Oyaizu 1986 (13). To 1 ml of plant extract 2.5 ml of phosphate buffer of pH 6.6 and 2.5 ml of 1% potassium fericyanide were added. The mixture was incubated at 50°C for 20 min 2.5 ml of 10% Trichloroacetic acid was added to the mixture and the mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml 0.1% $Fecl_3$. Absorbance was measured at 700 nm using spectrophotometer.

RESULTS

10 g of powdered sample yielded 1.6g of extract after concentration and drying with rotary evaporator. Percentage yield was calculated to be 16 %.

Total Phenolic content of methanolic plant extract was estimated by Folin-Ciocalteu method as 5.5 mg catechol equivalent /gm of sample. Fig –1 shows the antioxidant activity exhibited by the plant extract of various concentrations (50–250 µg). The study reveals that antioxidant activity of the plant extract was increased in a dose dependent manner. Total antioxidant capacity of plant extract is expressed as the mg equivalents of ascorbic acid. Total antioxidant capacity of plant extract was found to be 1.430 mg ascorbic acid equivalents at 250 µg/ ml of extract concentration. The DPPH free

radical scavenging activity of the plant extract is showed in fig–2. The extract exhibited a notable dose dependent inhibition of the DPPH activity with a 50% inhibition (Ic50) at a concentration of 2.12 mg/ml while the Ic50 value of the positive control quercetin was found to be 0.75 mg/ml.Hydroxyl radical scavenging activity of crude plant extract is given in Fig–3.The ability of the extract to scavenge these radicals was evaluated by the Fenton mediated 2-deoxy ribose assay. The Ic 50 value of the crude extract was found to be 1.034mg/ml while the Ic

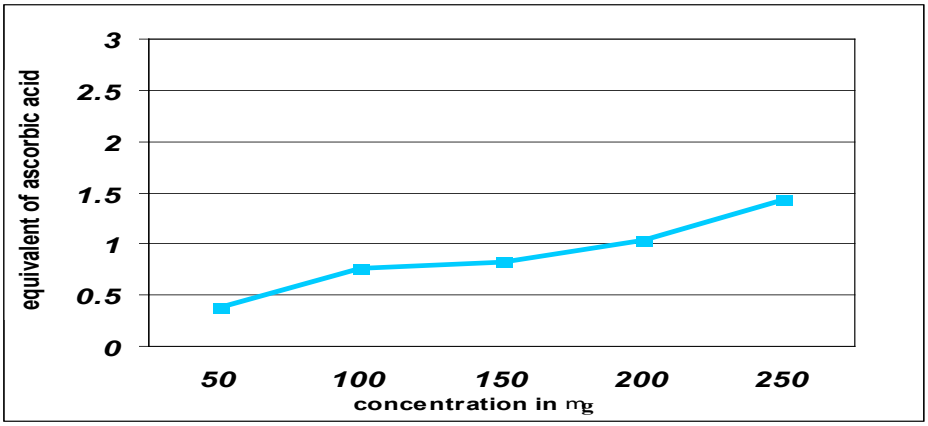

Figure 1 TOTAL ANTIOXIDANT ACTIVITY.

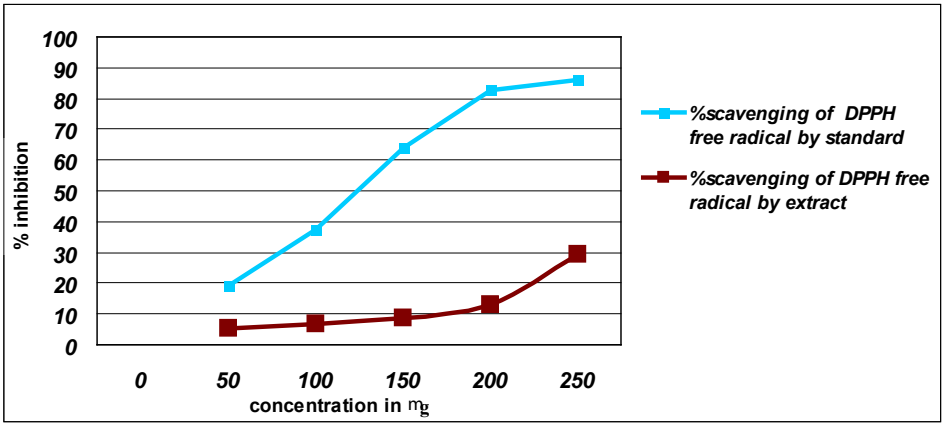

Figure 2 SCAVENGING POTENTIAL AGAINST DPPH FREE RADICAL.

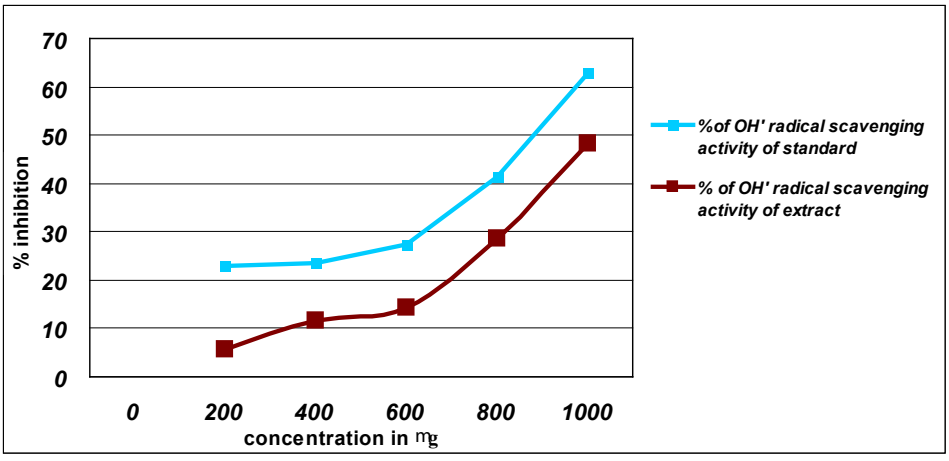

Figure 3 HYDROXYL RADICAL SCAVENGING ACTIVITY.

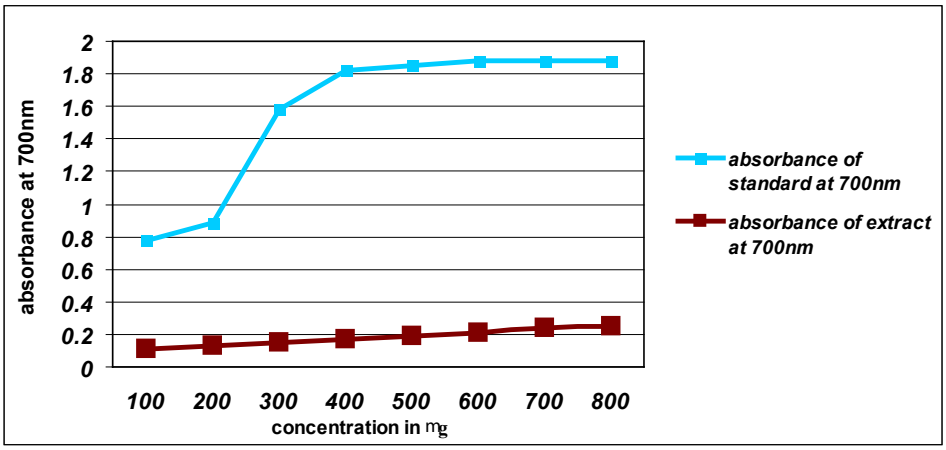

Figure 4 REDUCING POWER.

50 value of standard quercetin was found to be 0.793mg/ml.The reducing power of the crude plant extract is given in fig–4. The reducing power of the extract was increased with concentration of the sample. The plant extract could reduce the most Fe^{3+} ions which had a lesser reductive activity than the standard ascorbic acid.

DISCUSSION

Total phenolic content of plant extract was estimated to be 5.5 mg catechol equivalent /gm of sample. Phenolic compounds present in the molecular structure of natural antioxidants help in enhancing their antioxidant activity (14–15). Phenolic acids have implicated as natural antioxidants in fruits, vegetables and other plants. For ex- Caffeic acid, Ferulic acid and vanillic acid are widely distributed in plant kingdom (16). Phosphomolybtenum assay used to determine the total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate /Mo (V) complex at acid pH (17). Total antioxidant capacity of crude plant extract was found to be 1.430 mg ascorbic acid equivalent at 250 µg/ml extract concentration. The study reveals that the antioxidant activity of the extract exhibits increasing trend with increasing concentration of the plant extract. The DPPH antioxidant assay is based on the ability of 1, 1 diphenyl 1-2 picryl – hydrazyl, a stable free radical to be decolorized in the presence of antioxidants (18). The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and for deep purple colour.When DPPH accepts an electron donated by an antioxidant compound the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The extract exhibited a notable dose dependent inhibition with Ic50 value of 2.12mg /ml while the Ic50 value of standard quercentin was found to be 0.75 mg/ml. The higher Ic50 value of extract found in the experiment was because the sample used was a crude extract with the compound (s) react as antioxidant. Oxidative DNA damage has been implicated to be involved in various degenerative diseases including Alzheimer's disease, Parkinson's disease, and Hodgkin's disease (19). In view to make ascertain hydroxyl radical scavenging activity of crude extract, Fenten reaction generated hydroxyl radical damaging assay to deoxy ribose was studied. In the present study the Ic50 value of the crude plant extract was found to be 1.034mg/ml. The Ic50 value of Euphorbia hirta for hydroxyl radical scavenging activity was found to be 0.162mg/ml(20). In comparison to this earlier work our plant extract showed good hydroxyl radical scavenging activity. The reducing ability of a compound depends on the presence of reductants which exhibit antioxidant activity by breaking the free radical change through donation of a hydrogen atom (21). The reducing power of the extract increased dose dependently. However the extract reduced most Fe^{3+} ions to the extent less than ascorbic acid.

CONCLUSION

This study suggested that the crude plant extract of Entada *pursaetha, Toddalia aculeata and, Ziziphus mauritiana* possess antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress related diseases. Further works may be performed for the isolation and characterization of antioxidant component(s) in the plants.

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