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ANTIOXIDANT ACTIVITY OF METHANOLIC SEED COAT EXTRACT OF ENTADA PURSAETHA DC

S. Valarmathi* and K. Raju

Department of Botany, Kandaswami Kandar's College, Velur – Namakkal- 638 182, Tamil Nadu, India.

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***Corresponding Author**
S. Valarmathi
Department of Botany,
Kandaswami Kandar's
College, Velur – Namakkal-
638 182, Tamil Nadu, India.

ABSTRACT

The antioxidant activity of the crude methanolic extract of the seed coat of *Entada pursaetha*, was investigated. The total phenolic composition of methanolic extract was calculated to be 5.6mg 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 2.15mg/ml and 1.005mg/ml respectively. Total antioxidant capacity of crude plant extract was found to be 1.525mg 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: Antioxidant, Methanolic Extract, seed coat, *Entada pursaetha,* and Kolli hills.

INTRODUCTION

Rosemary Stanton [1] stated that oxygen is essential to life, but as our bodies use oxygen, we generate by-products. The by-products are reactive oxygen species (ROS) or, more commonly, free radicals such as super oxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen.[2] They damage to biological molecules such as DNA, proteins, carbohydrates, lipids and also exert oxidative stress towards the cells of human body and this leads to a number of physiological disorders.[3]

Antioxidants neutralise the effects of free radicals, but activity may be limited to specific antioxidants such as Phenolic compounds, which have been reported to play key roles in using the mechanism of delocalization of the single electron of the radical.[4] Numbers of

medicinal plants have been tested for antioxidant activities and their results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants than the commonly used synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) which are suspected to have some toxic effects and possible carcinogens.[5-6] The plant used for the present study is *Entada pursaetha*, which is a gigantic creeper with giant pods among legumes and is an endangered species of Fabaceae. It can be used as a narcotic, as a tonic, used in curing liver troubles, allaying body pains, curing eye diseases, arthritis and paralysis.[7]

MATERIALS AND METHODS

Extraction of plant materials

The seed coat of *Entada pursaetha* was collected from Kolli hills, Namakkal, Salem District, Tamilnadu, India. The sample was air dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. About 10g of the leaves 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 refrigerator set at 2- 6 ºC for further use.[8]

Antioxidant screening methods

Different methods have been developed to ascertain the potential of natural products as antioxidant agents with *In vitro* condition.[9-10] These include the total phenolic content, total antioxidant capacity, DPPH free radical scavenging assay, hydroxyl radical scavenging activity and ferric reducing power assays and. Due to the complexity of phytochemicals and their various reaction mechanisms, it is essential to do more than one assay method to assess the antioxidant capacity of plant extracts.

1. Total phenolic content

Plant flavonoids, phenolic acids and tannins are chemicals; they make them potent radical scavengers.[11] Usually the total phenolic content is measured by a method using Folin-Ciocalteu reagent.[12] The method is based on the reduction of the Folin-Ciocalteu's reagent by phenolic compounds to form a mixture of blue oxides with UV absorption maxima in the 760 nm region. Tannic acid is used as a standard and the total phenolic content is expressed in mg/g tannic acid equivalent (TAE) (mg/g dry mass).

2. Total antioxidant activity capacity

Antioxidant activity of plant extracts was measured.[13] The tubes containing 0.4 ml of plant extract 3.6 ml of distilled water and 4 ml of phosphomolybdenum reagent solution 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.

3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The ability of the extract to scavenge free radical was assayed with use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH).[14] DPPH is a free stable radical ion that reacts with compounds that can donate a hydrogen atom. The DPPH radical scavenging assay is based on the reduction of methanol solution of DPPH by a hydrogen donating antioxidant to form the non-radical form DPPH-H ("Fig. 1").

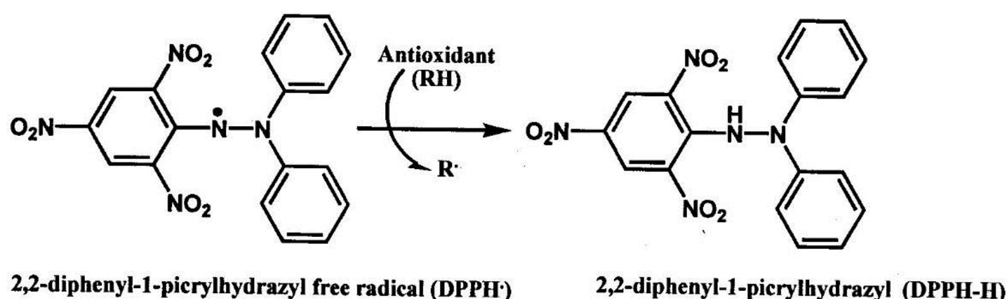


Fig. 1: DPPH scavenging by an antioxidant.

About 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;

$$\% \text{ inhibition} = 100 \times (1 - \text{Absorbance of sample}/\text{ Absorbance of control}).$$

4. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by a modified deoxyribose method determining thiobarbituric acid reactive substances (TBARS) proposed by Bektasoglu *et*

al.[22] 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;

$$\% \text{ inhibition} = 100 \times (1 - \text{Absorbance of sample/ Absorbance of control}).$$

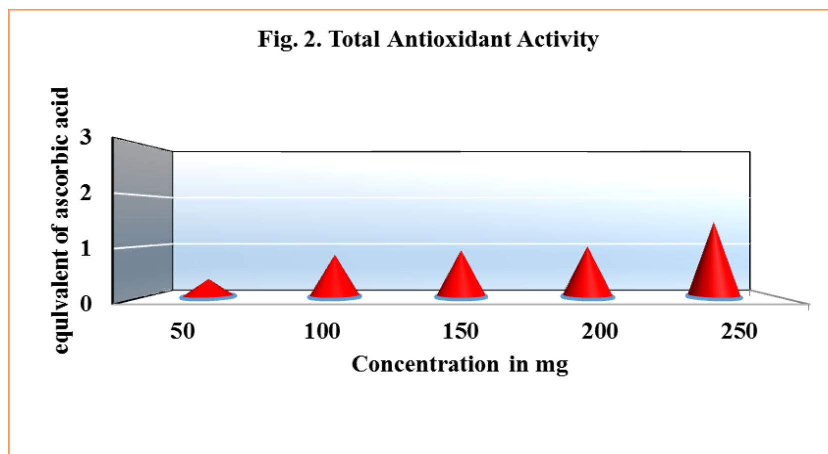
5. Ferric Reducing Power Assays

The reducing power of the methanolic plant extract was determined according to the method.[15] To 1 ml of plant extract 2.5 ml of phosphate buffer of pH 6.6 and 2.5 ml of 1% potassium ferricyanide 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.

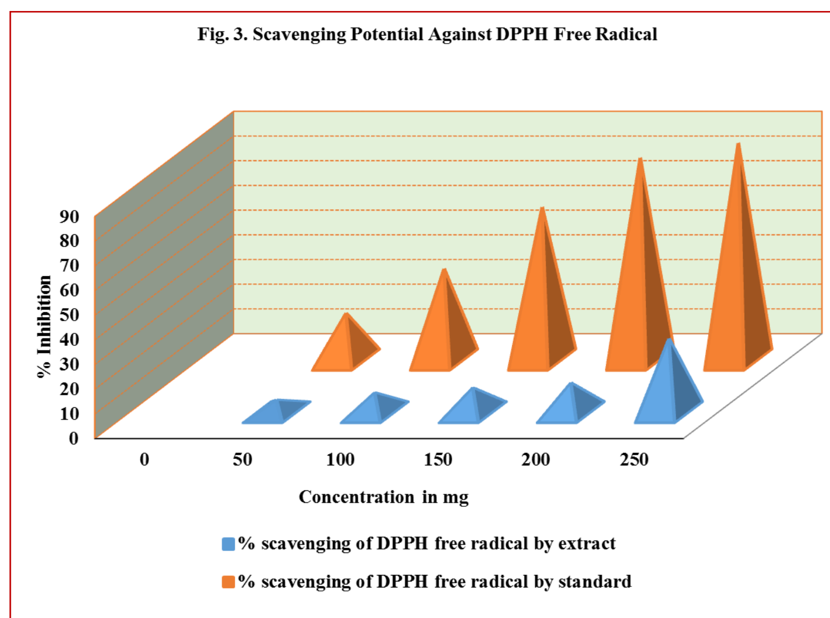
RESULTS AND DISCUSSION

Total phenolic content of methanolic seed coat extract of *Entada pursaetha* was estimated by Folin-Ciocalteu method as 5.6 mg catechol equivalent/gm of sample. The antioxidant activity exhibited by the seed coat extract of *Entada pursaetha* with various concentrations of 50–250 µg. The study showed that antioxidant activity of the seed coat extract of *Entada pursaetha* was increased in a dose dependent manner.

Total antioxidant capacity of *Entada pursaetha* seed coat extract is expressed as the mg equivalents of ascorbic acid. Total antioxidant capacity of the extract was found to be 1.525 mg ascorbic acid equivalents at 250µg/ml of extract concentration (Fig. 2). Phenolic compounds present in *Entada pursaetha* seed coat extract showed like as natural antioxidants in fruits, vegetables and other plants.[16-18] The study reveals that the antioxidant activity of the extract exhibits increasing trend with increasing concentration of the seed coat crude extract.

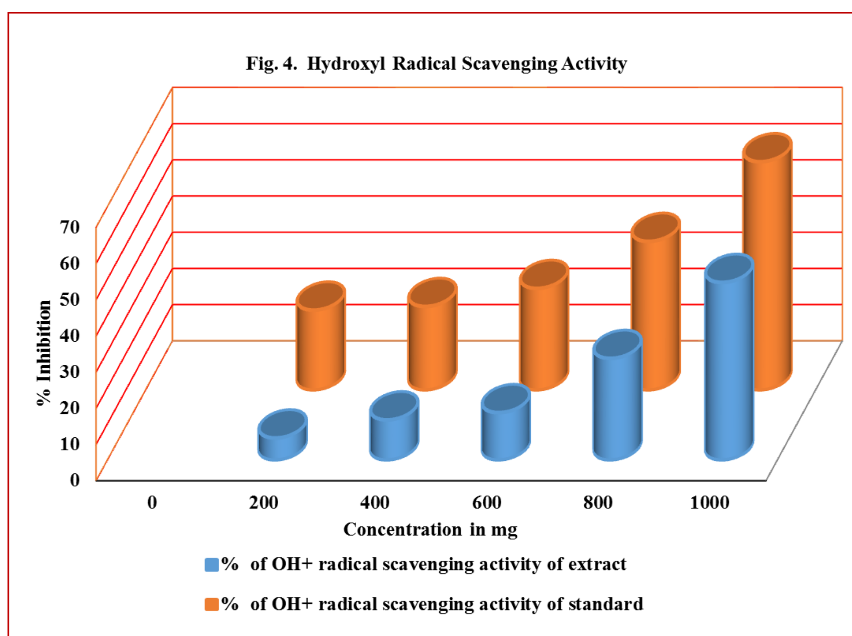


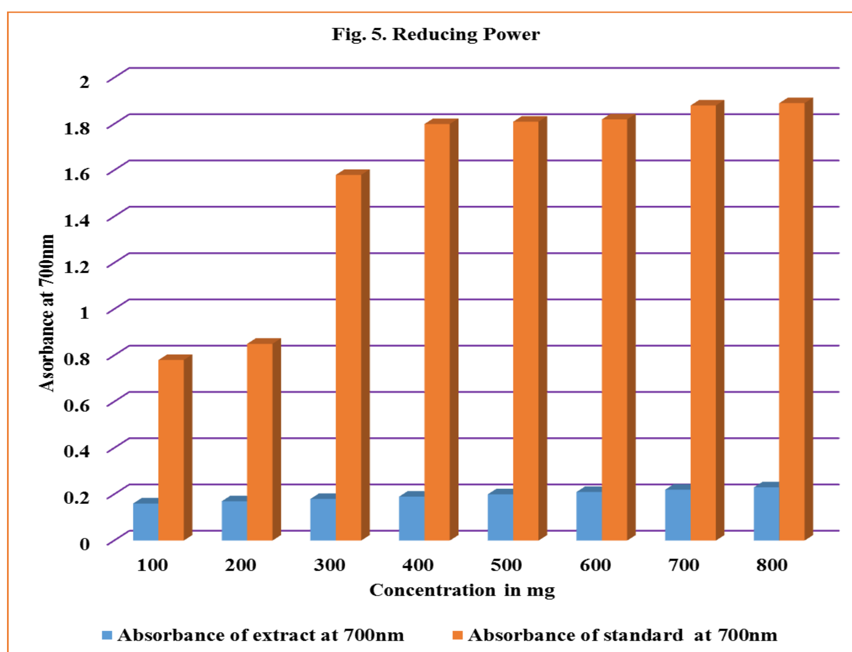
The DPPH free radical scavenging activity of the *Entada pursaetha* plant seed coat extract is showed in "Fig. 3". The extract exhibited a notable dose dependent inhibition of the DPPH activity with a 50% inhibition (IC_{50}) at a concentration of 2.15 mg/ml while the IC_{50} value of the positive control quercetin was found to be 0.75 mg/ml. The higher IC_{50} value of extract found in the experiment was because the sample used was a crude extract with the compound (s) react as antioxidant.[19]



Hydroxyl radical scavenging activity of *Entada pursaetha* plant seed coat crude extract is given in "Fig.4". 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 1.005mg/ml while the IC_{50} value of standard quercetin was found to be 0.790mg/ml. In comparison to this earlier work our plant extract showed good hydroxyl radical scavenging activity.[20]

The reducing power of the crude plant extract is given in "Fig. 5". 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. 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 seed coat crude plant extract of *Entada pursaetha* 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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