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IN VITRO ANTIOXIDANT POTENTIAL OF ETHANOLIC BARK EXTRACT OF SYMPLOCOS RACEMOSA ROXB

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Abstract

Aim of study: This study examines the *in vitro* antioxidant activity of the ethanol extract of *Symplocos racemosa* (EESR) Roxb bark.

Material and methods:

The coarse powdered materials of *Symplocos racemosa* was extracted with ethanol (95% v/v) by using soxhlet apparatus. The ethanol extract was concentrated *in vacuo* and kept in a vacuum desiccator for complete removal of solvent. The crude extract was screened for Free radical scavenging assays such as DPPH (2, 2-diphenyl-1-picrylhydrazyl), Nitric oxide, Hydroxyl radical and ABTS [2, 2'-azinobis-(3-ethyl-enzothiazoline-6-sulfonic acid)] assay method.

Results and Discussion:

The extract exhibits antioxidant activity with an IC₅₀ value of 131.76 ± 6.58 µg/ml in the DPPH radical scavenging method, 574.8 ± 8.23 µg/ml in the Nitric oxide assay, 131.7 ± 7.2 µg/ml in the Hydroxyl radical method, and 41.45 ± 0.51 µg/ml in the ABTS assay method which was compared with the standard drugs Ascorbic acid and Rutin.

Conclusion:

The results of the study indicated that the Ethanolic extract of *Symplocos racemosa* (EESR) showed potent antioxidant activity against ABTS assay method.

Key words: *Symplocos racemosa*, DPPH, Nitric Oxide, Hydroxyl radical, ABTS assay.

1. INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes (1-3). Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias (4-6).

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components (4). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. Antioxidant based

drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis; stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (7, 8).

This has attracted a great deal of research interest in natural antioxidants. The present study was taken up on the medicinal plant namely *Symplocos racemosa* also called as Lodhra belongs to the family Symplocaceae (9). The plant is small evergreen tree with stems upto 6m height and 15 cm diameter. Bark dark grey, rough. Blaze 7.5-13 mm, shortly fibrous, pale yellow, finely mottled with pale brown. It is distributed throughout India. Literature review reveals that the bark contains carbohydrates, glycosides, alkaloids, triterpenoids, sterols, saponnins, phenolic compounds and hydrolysable tannins, etc., (11, 12). Traditionally it is used for treatment of diarrhoea, dysentery, liver complaints and dropsy. It is also used as antioxytotic, amoebicide, anticancer, conjunctivitis and ophthalmia, decoction of bark is used to treat bleeding gums, menorrhagia and other uterine disorders (13, 14). Here the bark is screened for *In vitro* antioxidant activity study using DPPH, Nitric oxide, Hydroxyl radical and ABTS assay methods. Ascorbic acid and Rutin used as a standard.

2. MATERIALS AND METHODS

2.1 Collection of plant Material and Extraction

The plant *Symplocos racemosa* (Family: Symplocaceae) was collected from Kolli Hills at Namakkal District, Tamilnadu, India. Further it was authenticated by the taxonomist Dr.V.Sathyanathan, Epoch Pharma and Research Labs Pvt. Ltd. Chennai, and its voucher specimen was deposited in the Herbarium for further reference. After due authentication the barks were dried in shade and powdered to obtain coarse powder. The coarse powder material (250g) was extracted with ethanol (95%v/v) by using soxhlet apparatus. The ethanol extract was concentrated *in vacuo* and kept in a vacuum desiccator for complete removal of solvent. The yield was 9.3% w/w with respect to dried powder (15).

2.2 Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) [Sigma Chemicals Co], Methanol, DMSO (Dimethyl Sulphoxide), Sodium nitroprusside, Naphthyl ethylene diamine dihydrochloride, Sulphanilic acid, Potassium persulphate, Ferric chloride, Hydrogen peroxide, Thiobarbituric acid, Trichloro acetic acid, Ascorbic acid and Rutin were purchased from Merck India Ltd.

3. PHARMACOLOGICAL SCREENING

3.1 Antioxidant Assay

The antioxidant activity of plant extract was determined by different *in vitro* methods such as DPPH free radical scavenging assay, Nitric oxide, Hydroxyl radical and ABTS assay method. All the assays were carried out in triplicate and average values were considered.

3.2 DPPH Assay

The free radical scavenging capacity of Ethanolic extract of *Symplocos racemosa* (EESR) was determined using DPPH method. DPPH solution (0.004% w/v) was prepared in 95% ethanol. EESR was mixed with 95% ethanol to prepare the stock solution (10mg/100ml). The concentration of this solution was 10 mg /100 ml or 100µg/ml. From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing EESR (20 µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer (HACH 4000 DU UV– Visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10 mg/100ml or 100µg/ml) of EESR. Control sample was prepared which contains the same volume without any extract and reference ascorbic acid. 95% ethanol was used as blank. % scavenging of the DPPH free radical was measured using the following equation (16-18).

Absorbance of control - Absorbance of test Sample

$$\% \text{ DPPH radical-scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test Sample}}{\text{Absorbance of control}} \times 100$$

3.3 Nitric oxide scavenging activity

Nitric oxide scavenging was carried out using the alkaline Dimethyl Sulfoxide (DMSO) method (18). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200 μ l) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 μ M), EDTA (10 μ M) and potassium phosphate buffer (10 μ M, pH 7.4). Test solutions at different concentrations (5-100 μ g/ml) were added and absorbances were recorded at 540 nm against the control.

3.4 Hydroxyl radical scavenging activity

The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of ferric chloride, 0.1 ml of hydrogen peroxide, 0.36 ml of deoxyribose, 1.0 ml of test solutions (5-100 μ g/ml) in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 $^{\circ}$ C for 1 hr and 1.0 ml portion of the incubated mixture was mixed with 10 % TCA and 1.0 ml of 0.5 % TBA to develop the pink chromogen and measured at 532 nm (19).

3.5 ABTS radical cation assay

The assay measures ABTS.+ radical cation formation induced by metmyoglobin and hydrogen peroxide. Trolox [6-Hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid], a water soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner. The antioxidant activity in biological fluids, cells, tissues, and natural extracts can be normalized to equivalent Trolox units to quantify the composite antioxidant activity present. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•1) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the

dark at room temperature for 12-16 h before use. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of phenolic compounds and food extracts, the ABTS•1 solution was diluted with ethanol and for plasma antioxidants with PBS, pH 7.4, to an absorbance of 0.70 (60.02) at 734 nm and equilibrated at 30°C. Stock solutions of phenolics in ethanol, carotenoids in dichloromethane and plasma antioxidants in water were diluted such that, after introduction of a 10ml aliquot of each dilution into the assay, they produced between 20%–80% inhibition of the blank absorbance. After addition of 1.0 ml of diluted ABTS•1 solution to 10 ml of antioxidant compounds or Trolox standards (final concentration 0-15 mM) in ethanol or PBS the absorbance reading was taken at 30°C exactly 1 min after initial mixing and up to 6 min. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times, and in triplicate, on each occasion and at each separate concentration of the standard and samples (20).

4. RESULTS AND DISCUSSION

Crude ethanolic extract may contain thousands of phytochemical constituents. Concentration of sample at which the inhibition percentage reaches 50% is the IC₅₀ value. IC₅₀ value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower the IC₅₀ value, the higher is the antioxidant activity of the tested sample. The IC₅₀ values of EESR for DPPH radical, Nitric Oxide radical, hydroxyl radical, ABTS radical Scavenging activities are summarized in Table 1.

Table 1 Free radical scavenging activity of EESR

Extract /Standards	IC ₅₀ values ± SEM µg/ml* by methods			
	DPPH	Nitric oxide	ABTS	H ₂ O ₂
Ascorbic acid	2.69 ±0.05	-----	11.25 ±0.49	187.33 ±1.93
Rutin	-----	65.44 ±2.56	0.51 ± 0.01	36.66 ± 0.22
EESR	131.76 ±6.58	574.8 ±8.23	41.45 ±0.51	131.7 ±7.20

*Average of triplicate determinations. Values were mean ± S.E.M

EESR exhibited potent ABTS radical scavenging activity with IC₅₀ value 41.45±0.51µg/ml which is significantly lower than those of the standard Ascorbic acid and Rutin. The IC₅₀ value of extract for DPPH, Nitric Oxide radical, Hydroxyl radical was 131.76±6.58, 574±8.23 and 131.7±7.20µg/ml respectively, which were significantly higher than those of the standard Ascorbic acid and Rutin. According to the results in table 1, the EESR possessed moderate antioxidant activity.

5. CONCLUSION

The results of the study indicated that the Ethanolic extract of *Symplocos racemosa* (EESR) showed potent antioxidant activity in ABTS assay method than other methods and can be used as accessible source of natural antioxidants and a possible food supplement.

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