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Evolution of Free Radical Scavenging Potential of *Embelia Basal*

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ABSTRACT

Reactive Oxygen Species (ROS) is a metabolic side product of oxidative stress process which causes several diseases like atherosclerosis, cancer etc. In defense of ROS, antioxidants play a key role in combating them. Plant products, phenolics and flavonoids serve a best source for controlling these activities by its own metabolic pathway. Thus in this aspect the spectrophotometric determination of phenolics and flavonoids content from acetone, ethanol and methanol extracts of fruits of Embelia basal using Folin –Ciocalteau reagent and Quercetin as standard were carried out. The results show high contents of phenolics and flavonoids which are responsible for the antioxidant activity of the plant. Considering the above facts plant extracts further screened in-vitro, for their possible radical scavenging antioxidant activity by employing 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and total reducing power with ascorbic acid as a standard. The results of the present study revealed that the fruit extracts of E. basal can be a potential source of natural antioxidant, which can be applied as new source of Antioxidant Activity Index (AAI).

Key words: *Embelia basal*, Phenolics, Flavonoids, DPPH, Antioxidant activity.

INTRODUCTION

The Reactive Oxygen Species (ROS) is highly reactive side product of metabolic process in living organisms. These ROS plays key role in generation of the diseases like atherosclerosis, cancer, DNA and protein damage, lipid peroxidation, ageing, inflammatory activities etc.

Phenols and flavonoids the aromatic compounds with hydroxyl groups are plant secondary metabolites widely distributed in plant kingdom occurring in all parts of the plants [1]. They offer resistance to diseases and reduce the risk of tumors like cancer. Higer the phenolics content stronger is the antioxidant activity. In Ayurveda, there are number of plants reported to possess antioxidant activity, which could be the best external source. Thus in this aspect the present work is subjected to determine the antioxidant potential of fruits of *E. basal.*

The genus Embelia has been investigated for a variety of purposes in Ayurveda [2]. One of the species, *E. ribes* is used in dental caries [3]. It is also used in the process of formulating anti-AIDS Ayurvedic pharmaceutical compositions [4]. This species shows an antispermatogenic [5] effect and also acts as a contraceptive [6]. The antibacterial activity of embelin, isolated from berries of *E. ribes* and *E. robusta* has been reported [7] but apparently no work has been reported on isolation or identification of phytoconstituents from the fruits of *E. basal*. It is a shrub from family Myrsinaceae, an Indian variety, is widely distributed throughout India and commonly known as Vidanga. The larger elliptical leaves of the plants are used in combination with ginger, as a gargle for sore throats. The dried bark of the root is used as a remedy for toothache and the finely powered berries are formulated as an ointment for treating pleurities [8]. *E. basal* is highly esteemed in Ayurvedic medicine as a powerful anthelmintic [9] and also an important constituent of number of formulations [10-11]. In addition decoction is widely used in the treatment of insanity and heart diseases [11]. Preliminary phytochemical analysis of the plant revealed the presence of major constituents of phenolics, terpenoids, alkaloids, flavonoids and steroids.

The present work is carried out in order to evaluate the efficacy of the plant in view of phenolic and flavonoid contents. Quantitative determination of phenol and flavonoid of fruit extracts were performed using spectrophotometric method. Total flavonoid content was determined as quercetin equivalent and phenolic content was determined as pyrrocatechol equivalent using Folin Ciocalteau reagent. Fruits were screened for their antioxidant activity by employing radical scavenging assay; DPPH (2, 2-Diphenyl -1- picrylhydrazyl). Ascorbic acid was used as a standard. From the standard curves, their concentrations in the test samples were calculated.

EXPERIMENTAL SECTION

Plant Material

The fruits of *E. basal* (R. & S.) A. DC was parches from market, Pune, Maharashtra, India. The taxonomic identification is accomplished with the help of flora of Bombay Presidency [12] and Flora of Maharashtra [13] for identification. The fruits are authenticated at Agharkar Research Institute Pune, India. Its authentication number is AHMA F- 084.

Preparation of Extract

Air shade dried and powdered fruit material (10 gm) was extracted with the Acetone, Ethanol and Methanol by keeping for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extracts. These extracts were further used for experiments. Folin-Ciocalteau reagent, Catechol, Quercetin, Ascorbic acid and al other chemicals used were

from Merck. The UV spectrophotometer (UV-VisS1700 Pharma Spectrometer Schimadzu) was employ for the measurement of absorbance at various concentrations of the extracts under study.

Total Phenolic Content

The total phenolic contents of prepared fruit extracts were determined according to the method developed by Malik and Singh [14]. The Folin Ciocalteau reagent and sodium carbonate were added in alkaline solution of test sample. A blue coloured complex was developed due to phosphomolybdic acid, which is present in Folin-Ciocalteu reagent. Calibration plot was expressed as pyrrocatechol (2 -10 µg/ml) equivalent of phenol per gram of sample. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

Total Flavonoid Content

Aluminum chloride colorimetric method was used for flavonoids determination [15]. Each extract of the plant material (0.5 ml of 1:10 g ml^{-1}) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam using UV -VIsS1700 Pharma spectrophotometer Schimadzu. The calibration plot was generated by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

In Vitro DPPH Scavenging Activity

DPPH (2, 2-Diphenyl -1- picrylhydrazyl, 4.3mg) was dissolved in methanol (6.6 ml); it was protected from light by covering the test tubes with aluminum foil. DPPH solution (150 µl) was added to 3ml methanol and absorbance was noticed immediately at 516nm for control reading. A different volume of test samples that is 50 µl, 100 µl, 150 µl, 200 µl, 250 µl 300 µl and 350 µl was taken. Each of the sample was diluted with methanol up to 3ml and to it 150 µl DPPH was added. Absorbance was observed after 15 minutes at 516 nm using methanol as blank. IC_{50} values for the samples were calculated and compared with Ascorbic acid as a positive control [16-17]. The % reduction and IC_{50} values were calculated as follows. The free radical scavenging activity (% antiradical activity) was calculated using the equation:

$$\% \text{ Antiradical Activity} = \frac{\text{Control Abs. - Sample Abs.}}{\text{Control Absorbance}} \times 100$$

Each experiment was carried out in triplicates and results were recorded as mean % antiradical activity ± SD.

RESULT AND DISCUSSION

Plant extracts with a high phenolic content also enclosed high flavonoid content. The amount of total phenolics, flavonoids and the graph plots for the test samples are summarized **(Table 1 and 2, Graph 1 and 2)**. DPPH Radical Scavenging Activity for the test samples and the graph plots are recorded **(Table 3, Graph 3, 4 and 5)**.

Table 1: Total Phenolics Content of Extracts

Total Phenolics Contents mg/g ± SEM		
Acetone	Ethanol	Methanol
19.26±0.1	65.6±0.42	27.62±0.034

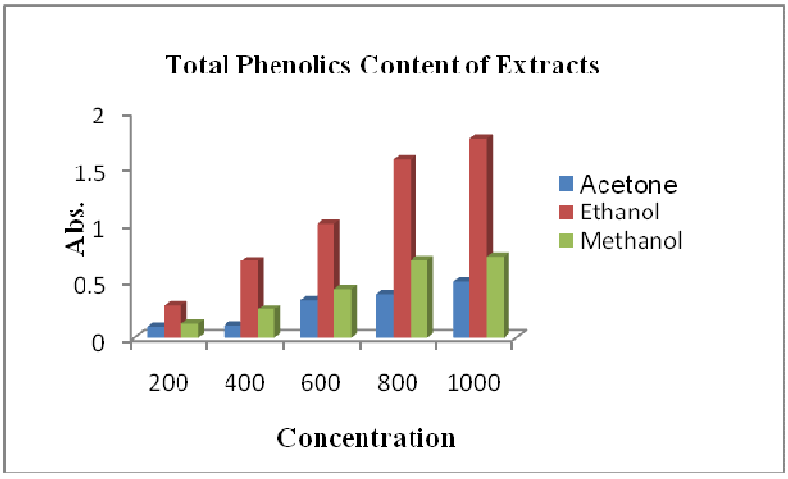
Table 2: Total Flavonoids Content of Extracts

Total Flavonoids Contents mg/g ± SEM		
Acetone	Ethanol	Methanol
6.85±0.5	7.7±0.36	12.56±0.55

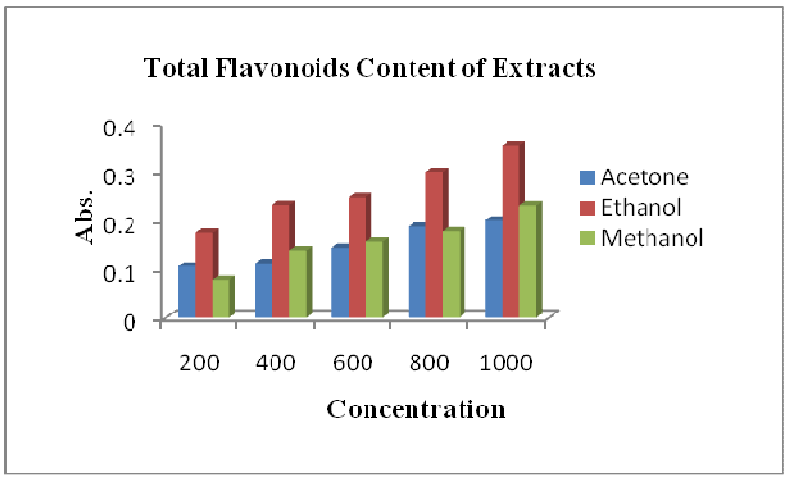
Table 3: DPPH Scavenging Activity of Extracts

IC_{50} Value µg/ml ± SD			
Ascorbic Acid	Acetone	Ethanol	Methanol
3.028±0.05	42.62	9.87	33.35

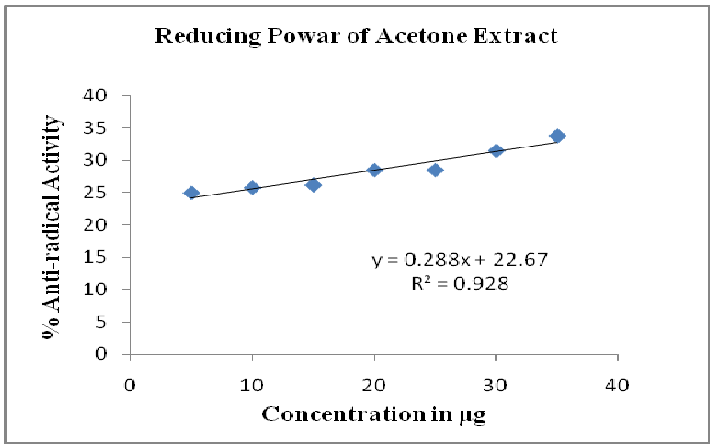
Graph 1: Total Phenolics Content of Extracts



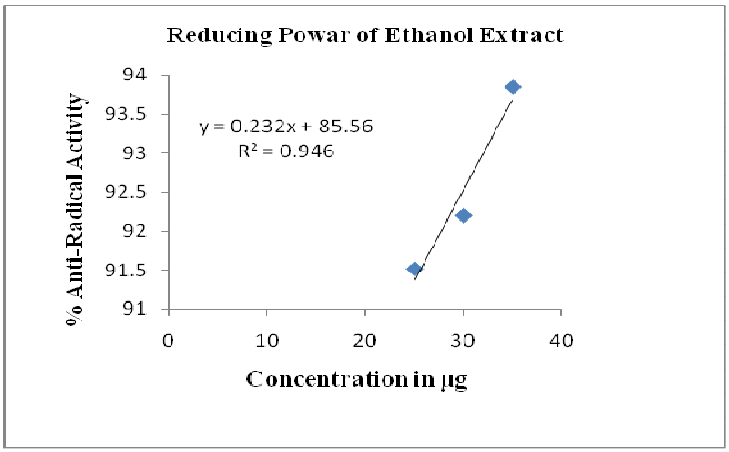
Graph 2: Total Flavonoids Content of Extracts



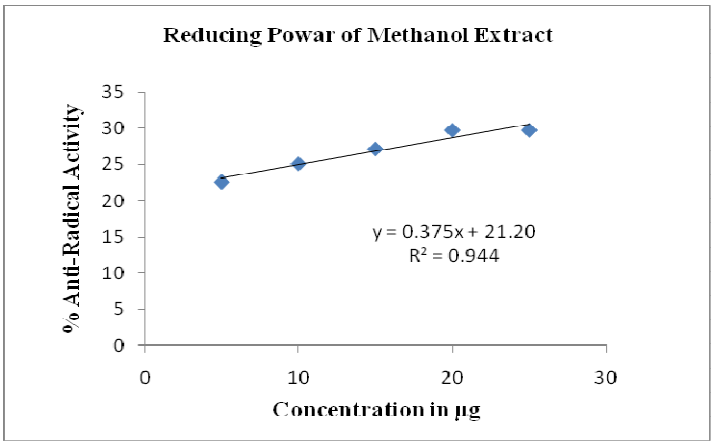
Graph 3: DPPH Radical Scavenging Activity of Acetone Ext.



Graph 4: DPPH Radical Scavenging Activity of Ethanol Ext.



Graph 5: DPPH Radical Scavenging Activity of Methanol Ext.



Statistical Analysis
Results are expressed as the standard error mean of three independent experiments. Student's *t*-test was used for statistical analysis; P values > 0.05 were considered to be significant.

CONCLUSION

This study indicates that the ethanol extract obtained from fruits of the medicinally important plant- *E. basal* contain high amount of phenolics and flavonoids compounds. It also exhibited the significant antioxidant activity. The high scavenging activity is due to hydroxyl groups existing in the phenolic compounds and chemical structure that can provide the necessary component as a radical scavenger.
In the longer term, the constituents of fruits of *E. basal* identified as having high antioxidant activity may be of the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

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