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Research Article

ANTIOXIDANT CAPACITY OF LEAVES AND STEM OF *EHRETIA LAEVIS*

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ABSTRACT

Phenols from plants are a class of antioxidant agents which act as free radical terminators.Flavonoids show antioxidant activity and their mechanism of action are through scavenging or chelating process. The aim of this work is to estimate the total phenolic and flavonoid content, and to evaluate in-vitro antioxidant activity of alcoholic extract of medicinally important plant *Ehretia laevis.* It belongs to family Boraginaceae. Total phenolic compounds in ethanol extract of leaves and stem is found to be 19.02 and 7.84 mg/g of extract calculated as Folin Ciocalteau equivalent (r^2= 0.991). Total flavonoids in ethanol extract of leaves and stem is found to be 18.19 and 1.29 mg/g calculated as Quercetin equivalent (r^2= 0.997). The greater amount of phenolic and flavonoid compounds are observed in leaves extract than that of stem extract. The antioxidant activity is compared with ascorbic acid as a standard. The leaves of *E. laevis* show the highest antioxidant activity for DPPH than the standard reference ascorbic acid.

Keywords: *Ehretia laevis*, Boraginaceae, Phenol, Flavonoid, Antioxidant activity

INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS[1-2]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induced abnormal proteins. Oxidation process is one of the most important route for producing free radicals in food, drugs and even living systems.

Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or prompt negative health effects. They are suspected of being responsible for liver damage and carcinogenesis in laboratory animals[3]. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[4].

Ehretia laevis is a small tree. It is generally found in Asia and Australian tropics[5]. Literature survey revealed wide biological activity of family Boraginaceae.The inner bark of *E. laevis* is used as food[5]. Leaves are applied to ulcers and in headache[6]. Fruit is astringent, anthelmintic, diuretic, demulcent, expectorant and used in affections of urinary passages, diseases of lungs and spleen[6]. Powdered kernel mixed with oil is a remedy in ringworm[6]. Seeds are anthelmintic[6].

The present study was undertaken to evaluate phenolic, flavonoid content of leaves and stem of the plant. Quantitative determination of phenol and flavonoid of both the samples 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. Leaves and stem 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.

MATERIAL AND METHODS

Phytochemical evaluation

Plant material

The leaves and stem of *E. laevis* were collected from Pune; Maharashtra, India during the month of July. The taxonomic identification is accomplished with the help of flora of Bombay Presidency[7] and Flora of Maharashtra[8] for identification. It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI / WC / Tech / 2006 /185.

Extraction procedure

Air shade dried and pulverized material (0.100 g) of leaves and stem of *E. laevis* was used. Each material was grind using ethanol (10 ml, 80%). It was centrifuge for 20 minutes and filtered. The extract was evaporated to dryness in vaccum using a rotary evaporator. This extract was used for total content of phenols, flavonoids and for the assessment of antioxidant capacity.

Estimation of total phenolic content [9]

The total phenolic contents of leaves and stem material were determined according to the method developed by Malik and Singh[9]. 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.

Estimation of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination[10]. 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.

Evaluation of in vitro antioxidant 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 and 300 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 [11-12]. The % reduction and IC_{50} values were calculated as follows. The free radical scavenging activity (% antiradical activity) was calculated using the equation:

% Antiradical Activity = Control Absorbance - Sample Absorbance/Control Absorbance × 100

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

RESULTS AND DISCUSSION

Plant extracts with high phenolic content also enclosed high flavonoid content[13]. The amount of total phenolic and flavonoids for the test samples are summarized (Table 1) and the graphs are plotted (Figure 1 and 2). IC_{50} values for the test samples are recorded (Table 2) and the graphs are plotted (Figure 3 -5).

Table 1: Total phenolic and flavonoid content (mg/g) of *Ehretia laevis* ethanol extract

Total phenolic content (mg/g ± SEM)		Total flavonoid content (mg/g ± SEM)	
Leaves	Stem	Leaves	Stem
19.02±1.57	7.84± 0.7636	18.19± 1.198	1.29± 0.07

Each value represents mean ± SEM (n=3)

Table 2: Antioxidant capacity (µg/ml) of ascorbic acid and *Ehretia laevis* ethanol extract by DPPH free radical method

IC_{50} Value (µg/ml ± SD)		
Ascorbic Acid	**Leaves**	**Stem**
3.028±0.05	2.44±0.09	29.88±0.1

Values are mean of three replicate determinations (n=3) ± Standard deviation

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.

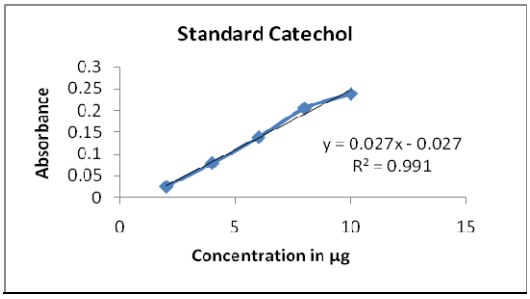

Fig. 1: Total phenolic content of standard catechol

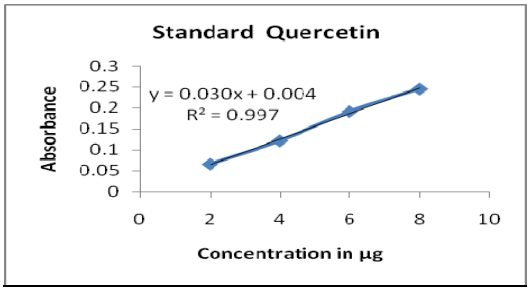

Fig. 2: Total flavonoid content of standard quercetin

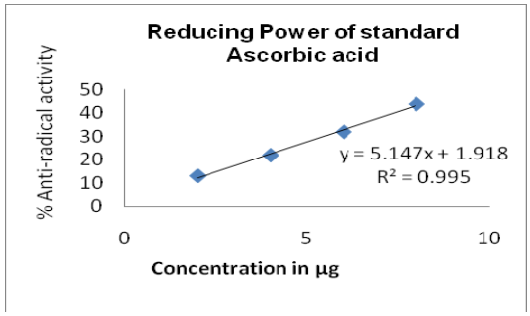

Fig. 3: DPPH radical scavenging activity of Ascorbic acid IC_{50} = 3.028±0.05 µg/ml

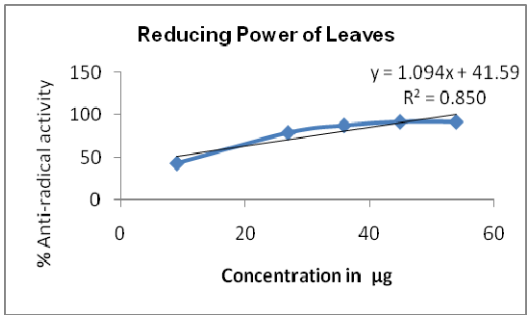

Fig 4: DPPH radical scavenging activity of ethanol extract of leaves of *E. laevis* IC_{50} = 2.44±0.09 µg/ml

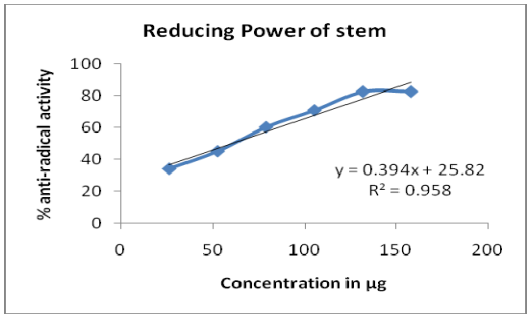

Fig 5: DPPH radical scavenging activity of stem ethanol extract of *E. laevis* IC_{50} = 29.88±0.1µg/ml

CONCLUSION

This study indicates that the ethanol extract obtained from the leaves and stem of the medicinally important plant- *E. laevis* contain high amount of phenolic and flavonoid compounds. It also exhibited the significant antioxidant activity. The high scavenging activity may be 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 leaves of *E. laevis* 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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