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RESEARCH ARTICLE

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IN-VITRO ANTIOXIDATIVE ACTIVITY OF PHENOLIC AND FLAVONOID CONTENTS OF LEAVES OF MEDICINAL PLANT – *EHRETIA LAEVIS*

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ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoid content and to evaluate in vitro antioxidant activity of methanolic extract of *Ehretia laevis*. The methanol extract **(A)** of leaves of *E.laevis* was analysed for the total phenolic and flavonoid contents and antioxidant activity. The content of total phenolics in the extract was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as catechol equivalent. The content of total flavonoids in the extract was determined and calculated as quercetin equivalent. The possible radical scavenging antioxidant activity of the methanol extract was investigated in vitro methodology using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and total reducing power was compared with that of ascorbic acid as a standard compound. The obtained results were substantial. So, study was extended for gummy mass of crude methanol extract **(B)** and hexane fraction of gummy mass of crude methanol extract **(C)**.

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KEY WORDS

Ehretia laevis, Phenols, Flavonoids, Antioxidant activity, DPPH.

INTRODUCTION

Plant derived natural chemicals, known as secondary metabolites, are effective in their roles of protection, adaptation and pollination. Secondary metabolites are mainly used in food, pharmaceutical, chemical, cosmetic industries and agriculture[1-2]**.** Plant derived natural products are abundant in nature. Many of them exhibit numerous biological activities and some can be employed as food additives. Synthetic antioxidants have been used in the food industry since the 1940s, but trends in many health related industries tend to shift preferences to natural sources. Therefore, investigation of natural antioxidants has been a major research interest for the past two decades as many research groups and institutions have been screening plant materials for possible antioxidant properties[2]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxy radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's).

Ehretia laevis is a small tree. It is generally found in Asia and Australian tropics[3]. Literature survey revealed wide biological activity of family Boraginaceae. The inner bark of *E. laevis* is used as food[3]. Leaves are applied to ulcers and in headache[4]. Fruit is astringent, anthelmintic, diuretic, demulcent, expectorant and used in affections of urinary passages, diseases of lungs and spleen[4]. Powdered kernel mixed with oil is a remedy in ringworm[4]. Seeds are anthelmintic[4].

The present study was undertaken to evaluate phenolic, flavonoid content of methanol extract of leaves. Quantitative determination of phenol and flavonoid of the sample was performed using spectrophotometric method. Total flavonoid content was determined as quercetin equivalent and phenolic content was determined as pyrrocatechol equivalent using Folin Ciocalteau reagent. Methanol extract of leaves was 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 of *E. laevis* was collected from Pune; Maharashtra, India during the month of July. The taxonomic identification is accomplished with the help of flora of Bombay Presidency[5] and Flora of Maharashtra[6] 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 (60.0 g) of leaves of *E. laevis* was used. It was extracted successfully with methanol (360 ml, 1:6 w/v) by keeping it for 72 hours at room temperature. It was filtered using Whatman no. 1 filter paper. The solvent was evaporated to dryness in vaccum using a rotary evaporator to yield crude methanol extract (5.8 %). This methanol extract **(A)** was used for total content of phenols, flavonoids and for the assessment of antioxidant capacity.

Fractionation of methanol extract

The methanol extract **(A,** 15g**)** was stirred with water (300ml) for 6 hours at room temperature. It was filtered to detach gummy mass **(B,** 48.88 %**)** from the aqueous layer. The above gummy mass **(B**, 8 g**)** stirred with hexane (200ml) for 6 hours at room temperature and filtered. The hexane filtrate was evaporated to dryness in vaccum using a rotary evaporator to acquire gummy mass of hexane **(C,** 42.16 %**)**.

Estimation of total phenolic content [7]

The total phenolic contents of methanol extract **(A)** and its fractions, Gummy mass extract **(B)** and Gummy mass hexane extract **(C)** were determined according to the method developed by Malik and Singh[7]. 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 (2-10µg/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[8]. 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 solution 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 [9-10]. The % reduction and IC_{50} values were calculated as follows. The free radical scavenging activity (% antiradical activity) was calculated using the equation:

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

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

RESULT AND DISSCUSSION

The amount of total phenolic for the test samples are summarized **(Table 1)** and the amount of total flavonoids for the test samples are summarized **(Table 2)**. IC_{50} values for the test samples are recorded **(Table 3)** and the graphs are plotted **(Graph 1-4)**.

Anti-oxidant activity of extract (**A)** and fraction **(C)** is mainly depending on the concentration of total phenolic contents. As total phenolic contents increases, anti-oxidant activity also increases. In case of fraction (**B)**, anti-oxidant activity is mainly depend on the concentration of total flavonoid contents. No significant

correlation is noticed between the total phenol, flavonoid content and antioxidant activity.

Table 1
Total Phenolic Content of Extracts

Total Phenolic Contents mg/g ± SEM		
A	B	C
49.43±4.91	41.5635±0.544	260.25±0.314

Each value represents mean± SEM (n=3)

Table 2
Total Flavonoid Content of Extracts

Total Flavonoid Contents mg/g ± SEM		
A	B	C
16.46±2.61	62.63±4.4	11.56±1.32

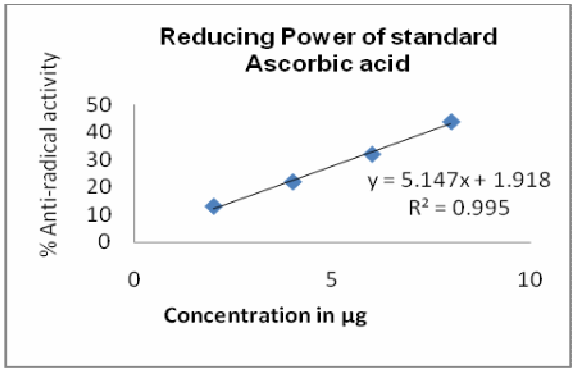
Each value represents mean± SEM (n=3)

Table 3
Antioxidant Activity of Extracts

	IC_{50} Value µg/ml		
Ascorbic Acid	A	B	C
3.028	13.52	46.43	25.67

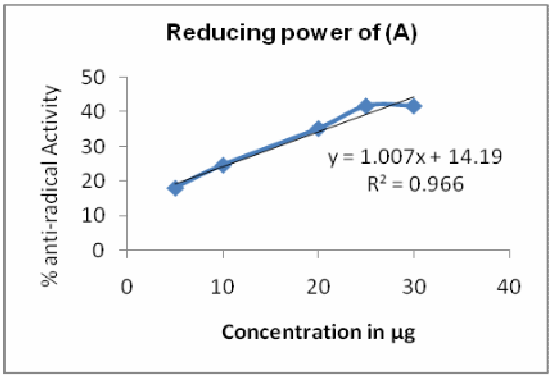
n=3

Graph 1
DPPH radical scavenging activity of Ascorbic Acid



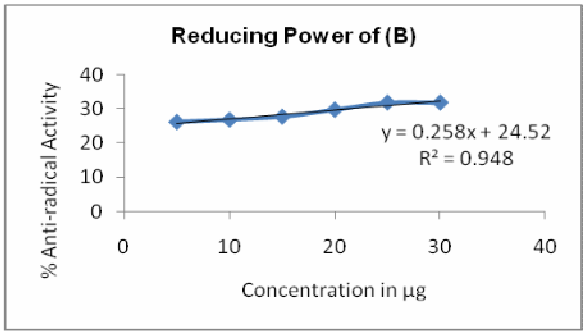
IC_{50}=3.028µg/ml

Graph 2
DPPH radical scavenging activity of (A)



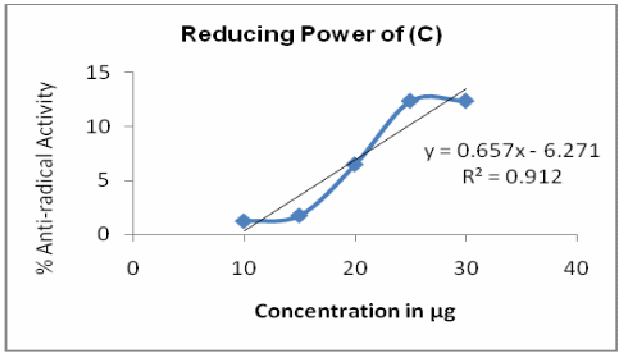
IC_{50}=13.52 µg/ml

Graph 3
DPPH radical scavenging activity of (B)



IC_{50} = 46.43µg/ml

Graph 4
DPPH radical scavenging activity of (C)



IC_{50} = 25.67 µg/ml

CONCLUSION

It is realized that the total phenolic and flavonoid content does not give a full picture of the quantity of the total phenolic and flavonoid constituents in the extracts. In addition, there may be some interference rising from other chemical components, present in the extracts[11]. As observed from the present data, antioxidant activity does not necessarily correlate with high amounts of phenolic and flavonoid, concurrently. Yet the results gained by these methods provide simple data that make it promising to classify extracts in respect to their antioxidant potential.

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