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Estimation of Total Phenolic, Total Flavonoid Content and Assessment of in vitro Antioxidant Activity of Extracts of plant Ehretia Laevis bark

Umesh Prabhakar Joshi *, Rajendra Dayaram Wagh
DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra, India, 424006
Corresponding Author: Umesh Prabhakar Joshi

Abstract: *Barks of Ehretia laevis are very popular and well known for its astringent, anthelmintic, diuretic, demulcent, expectorant and veneral diseases. In this study the Total phenolic, Total flavonoid content, Antioxidant effect methanolic, aqueous and hydroalcoholic extract of dried dried Barks of Ehretia laevis Roxb was evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods and compared. Result indicated that methanolic extract of the dried barks exhibited potent antioxidant activity.*

Keywords: *Ehretia laevis, Antioxidant, Total phenolic, Total flavonoid, DPPH scavenging activity, Nitric oxide scavenging activity, Reducing power, Hydrogen peroxide etc.*

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I. Introduction

Free radicals are naturally occurring by-products of our own metabolism. Free radicals are electrically charged molecules that attack various cells, tearing through impermeable cellular membranes to react with the nucleic acids, proteins and enzymes present in the body. Free radicals can also be cause the lipid peroxidation in foods which leads to their deterioration[2]. Oxidation is known as to be the major cause of foods and materials degradation[3]. Oxidation is a chemical process that allow transfer of electron from a substance to an oxidizing agent. Oxidation reaction can be produce various free radicals which are act as start chain reactions that damage cells[1]. The free radicals are species which having very short half life, high reactivity and damaging activity for macromolecules like as proteins, DNA and lipids. Free radicals may defined as the molecular sharks which damage the molecules in cell membranes, mitochondria, DNA and they are very unstable, tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses. The most commonly reactive oxygen species are includes superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are like as nitric oxide (NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N_2O_3) [4].

Reactive oxygen species (ROS) like as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or sometimes from exogenous factors[5]. *In vivo*, some of these Reactive oxygen species (ROS) play an important role in cell metabolism activities like as energy production, phagocytosis and intercellular signaling[6]. The reactive oxygen species are circulating in the body which tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions like as carcinogenesis, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis, coronary heart disease, diabetes mellitus, liver disorders, Parkinson‟s disease, autoimmune disease, Alzheimer's and AIDS.[7]

The most effective way is to carry out elimination and diminish the action of various free radicals which can cause the oxidative stress is called as antioxidative defense mechanisms. The Antioxidants are the substances which have possess free radical chain reaction breaking properties. Recently there is find an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re- antioxidants in reducing oxidative stress-induced tissue injury[8]. Among the various naturally occurring antioxidants like as Vitamin C (ascorbic acid), carotenoids (Carotenes) and phenolic compounds which are more effective [9]. These substances are use as to inhibit lipid peroxidation (by making inactivation of enzyme lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions[10].

Antioxidants are substances which are provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking[11]. There are many substances from natural sources have been shown to contain antioxidant and they are under the study. Antioxidant compounds like as Phenolic acids, polyphenolic substances and flavonoids which scavenge

the free radicals like as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[12]. Ethnomedical literature has reveals that the large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There are many plants that have been found to possess strong antioxidant activity[13].

Antioxidants are compounds which have ability to bring either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are substances used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals[14].

Ehretia laevis is fast-growing small tree belonging to family Ehretiaceae. The plant is native to India, Pakistan, Laos, Myanmar, Vietnam, China, Bhutan. The plant *Ehretia laevis* is located at hilly forests, in ravine and on hill slopes. The plant is known as Dant-Rang, Vadhvarni, Chamror[15]. The inner bark of *E. laevis* is used as food. Leaves are applied to ulcers, skin diseases and in headache. Fruit is used as urinary passage, lung and spleen diseases, astringent, anthelmintic, diuretic, demulcent, expectorant. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are anthelmintic. Barks are used in throat infection. Root for veneral diseases. The plant contains chemical constituents like s fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides, and benzoquinones [16,17].

This paper reports total phenolic, total flavonoid and antioxidant potential of methanolic, hydroalcoholic and aqueous extracts of Barks of *Ehretia laevis* evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods.

II. Materials and Methods

2.1 Plant Collection:

The fresh barks of plant *Ehretia laevis* were collected from haripura and manudevi region of Taluka Yawal, District Jalgaon, India. The selected plants were authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule, Maharashtra . Barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

2.2 Preparation of Plant extract:

The bark of plant were thoroughly washed with tap water, dried at room temperature and transformed to coarse powder. The bark powder were extracted with three solvents i.e methanol, water and water-ethanol separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract.

2.3 Chemicals:

2-2 diphenyl-1 picryl hydrazyl (DPPH), Methanol, Sodium nitroprusside, Sulphanilamide, Potassium ferricyanide, Trichloroacetic acid, Ascorbic Acid, Ferric chloride, N-(1- naphthyl) ethylenediamine dihydrochloride), Hydrogen Peroxide solution, Phosphate Buffer and all other reagents were of analytical grade.

2.4 Instrument: Shimadzu UV – visible spectrophotometer

2.5 Determination of Total phenolics content[18]

The Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent according to the method reported by Singletion *et al.,* (1999) using gallic acid as a standard phenolic compound. 1 About 500µl (20mg/ml) of plant sample was added to 25ml of distilled water and 1ml of Folin-Ciocalteu reagent (1:10). Then this mixture was kept at room temperature for 3 minutes, after then 1.5ml of 2% sodium bicarbonate was added, soon after vortexting the reaction mixture for 1 hour at room temperature, the absorbance was measured at 760nm. All the tests were performed in triplicates and the results were averaged. The concentration of total phenolic compounds in methanolic leaf and root extracts was determined as microgram of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph (10- 100 µg/ml).

2.6 Determination of Total flavonoids content[19]

The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Zhishen *et al.* (1999). 100µl (20mg/ml) of the extract was mixed with 2.5 ml of distilled water and 300µl of 5% sodium nitrate. Then, it was incubated at room temperature for 5 minutes and 300µl of 10% aluminium chloride, 2ml of 1M sodium hydroxide and 1ml of distilled water were added. Then, absorbance of the reaction mixture was measured at 512nm, along with the standard, quercetin and blank. The total flavonoids content was

determined as microgram, quercetin equivalent by using the standard, quercetin graph, obtained by comparing the calibration curve prepared from a reference solution containing quercetin (10- 100µg/ml).

2.7 DPPH radical scavenging assay[20]

The antioxidant activity of the methanolic, aqueous and hydroalcoholic extracts of dried bark of the plant *Ehretia laevis* was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux. About 1 ml of 100 µM DPPH solution in methanol, equal volume of the extract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30 min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using a spectrophotometer at 517 nm. The different concentrations of ascorbic acid were used as reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition:-} \quad \frac{\text{Absorbance Control - Absorbance test}}{\text{Absorbance Control}} \times 100$$

2.8 Nitric oxide radical scavenging assay[21]

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan *et al*. When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions. About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the methanolic, aqueous and Hydroalcoholic extract (25 - 200 µg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding *Ehretia laevis* extract. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition:-} \quad \frac{\text{Absorbance Control - Absorbance test}}{\text{Absorbance Control}} \times 100$$

2.9 Reducing power assay[22]

The reducing power was determined according to the method of Berker *et al*. The methanolic, aqueous and hydroalcoholic extracts of dried stembark of the plant *Ehretia laevis* (25-200 µg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After the addition of 2.5 ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

2.10 Hydrogen peroxide radical scavenging Assay

Hydrogen peroxide radical scavenging activity was determine by the method of Ruch et al. (1984)[23]. Hydrogen peroxide (H_2O_2) is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems[24, 25]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of the methanolic, aqueous and hydroalcoholic extracts of dried stembark of the plant *Ehretia laevis* (25-200 µg/ml) in phosphate buffer were added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of *Ehretia laevis* and Ascorbic acid as standard compound was calculated as

H_2O_2radical scavenging activity (%) = [{Ao – A1/Ao}] ×100.

Where, Ao is the absorbance of the H_2O_2,

A1 is the absorbance of the presence of the extract in H_2O_2 solution [26, 27].

III. Result

3.1 Total phenolics content

The Folin-Ciocalteu method is sensitive to reducing compounds, polyphenols there by producing blue colored complex. The quantative phenolics estimation was performed at 760 nm by change in intensity of Folin-phenolic compounds complex. In methanolic, hydroalcoholic and aqueous extracts of stem bark of plant *Ehretia laevis*, the total phenolic content was found to be 89.55µg/ml, 71.33µg/ml and 53.55µg/ml respectively in terms of gallic acid equivalent (Table No. 1 and Figure No. 1). In addition it has been determined that the highest extraction yield was found in stembark extract.

3.2 Total flavonoids content

The aluminium chloride forms acid stable complexes with the C-4 keto group and either with C-3 or C-5 hydroxyl group of flavones and flavonols. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. In methanolic, hydroalcoholic and aqueous extracts of stem bark of plant *Ehretia laevis*, the total flavonid content was found to be 78.75µg/ml, 60.33µg/ml and 52.66µg/ml respectively in turms of quercetin equivalent (Table No. 2 and Figure No. 2)

3.3 DPPH radical scavenging activity

The DPPH assay is purely based on the assumption that an antioxidant serve as a hydrogen donor and thus reduces the DPPH free radicals (the color turns from purple to yellow). This assay is known as a basic and quick tool to carry out evaluation of antioxidant activity of plant extracts. The antioxidant potency of a compound is relative to loss of DPPH free radicals that can be quantified through a decrease in the maximum absorption of DPPH at 517 nm. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 3 and Figure No. 3)The DPPH-derived IC_{50} values of plant extracts are also illustrated in Table No.3 The methanolic, hydroalcoholic and aqueous extract of plant inhibited DPPH upto 87.13%, 68.55% and 72.35% at concentration 200ug/ml. Amongst the plant extracts of *Ehretia laevis*, methanolic extract *was* found to be the most potent DPPH scavengers, as they could inhibit DPPH free radicals up to 87.13% at 200ug/ml concentration compared to the rest aqueous and hydroalcoholic extract where as standard Ascorbic acid were shows 94.29% of DPPH scavenging activity.

3.4 Nitric oxide radical scavenging assay

Nitric oxide is an unstable free radical which involved in many biological processes and associated with several diseases. It react with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be act as toxic and inhibition of over production is an important goal. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 4 and Figure No. 4). The Nitric oxide-derived IC_{50} values of plant extracts are also illustrated in Table 4. The methanolic, hydroalcoholic and aqueous extract of plant inhibited Nitric oxide upto 85.08%, 74.74% and 69.38%, at concentration 200ug/ml.. Amongst the plant extracts of *Ehretia laevis*, methanolic extract was found to be the most potent Nitric oxide scavengers, as they could inhibit Nitric oxide free radicals up to 85.08% at 200 ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 93.13% of Nitric oxide scavenging activity.

3.5 Hydrogen peroxide scavenging activity

Scavenging of H_2O_2 by extracts may be attributed to their polyphenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of plant extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch, where they are compared with that of tocopherol as standard (Ruch et al., 1984). The plant extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner (Table No. 5 and Figure No. 5). Although hydrogen peroxide itself is not very reactive, but sometimes it can cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removal of H_2O_2 is very important living systems. The Hydrogen peroxide -derived IC_{50} values of plant extracts are also illustrated in Table 5. The methanolic, hydroalcoholic and aqueous extract of plant inhibited Nitric oxide upto 87.55%, 76.39% and 71.77% at concentration 200ug/ml.. Amongst the plant extracts of *Ehretia laevis*, methanolic extract *was* found to be the most potent Hydrogen peroxide scavengers, as they could inhibit Hydrogen peroxide free radicals up to 87.55% at 200ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 93.84% of Hydrogen peroxide scavenging activity.

3.6 Reducing Power Assay

Reducing capability of an antioxidant substance can be assessed using its ability to convert Fe^{3+} to Fe^{2+}. Intensity of Perl's Prussian blue color caused by this reduction is measured at 700 nm. Higher absorbance indicates higher reducing power. The reducing power of the compound can be contributed to its antioxidant

potency. The reducing power assay of the plant extracts of plant *Ehretia laevis* were tested in this study illustrated in Table No. 6 and Figure No. 6. The findings revealed that the values of reducing power of the plant extracts of *Ehretia laevis* were functions of their concentrations. In this study, results showed that all plant extracts had significant levels of Reducing Power activity in a dose dependent manner. At concentration 200ug/ml, methanolic, aqueous and hydroalcoholic extract had reducing power values of 0.115, 0.103, 0.105 as compare to standard Ascorbic acid 0.176. At this concentration, methanolic extract showed a remarkable reducing power that was significantly greater than those of the hydroalcoholic and aqueous extract as compared to standard ascorbic acid.

IV. Figures And Tables

Table 1 Total phenolic content present in extracts of bark of plant *Ehretia laevis*

Plant Extracts	**Absorbance mean ± SEM (n=3)**	**Total Phenolic content (mg gallic acid equivalent/g of dried extract)**
Methanolic	0.268±0.002	89.55±0.67
Hydroalcoholic	0.214±0.001	71.33±0.51
Aqueous	0.160±0.001	53.55±0.29

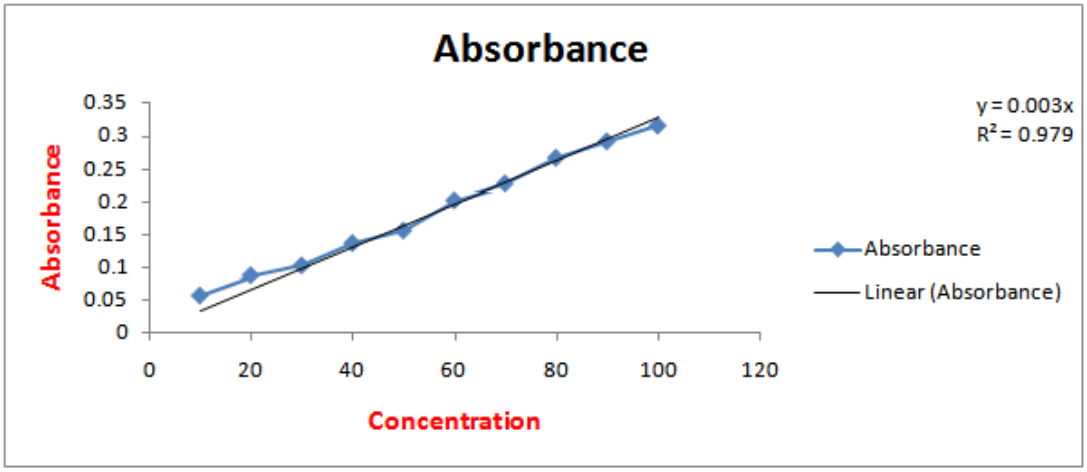

Fig.1 Calibration curve of Gallic acid

Table 2 Total flavonoid content present in extracts of bark of plant *Ehretia laevis*

Plant Extracts	**Absorbance mean ± SEM (n=3)**	**Total flavonoid content (mg querecetin equivalent/g of dried extract)**
Methanolic	0.315±0.001	78.75±0.43
Hydroalcoholic	0.241±0.002	60.33±0.50
Aqueous	0.210±0.003	52.66±0.79

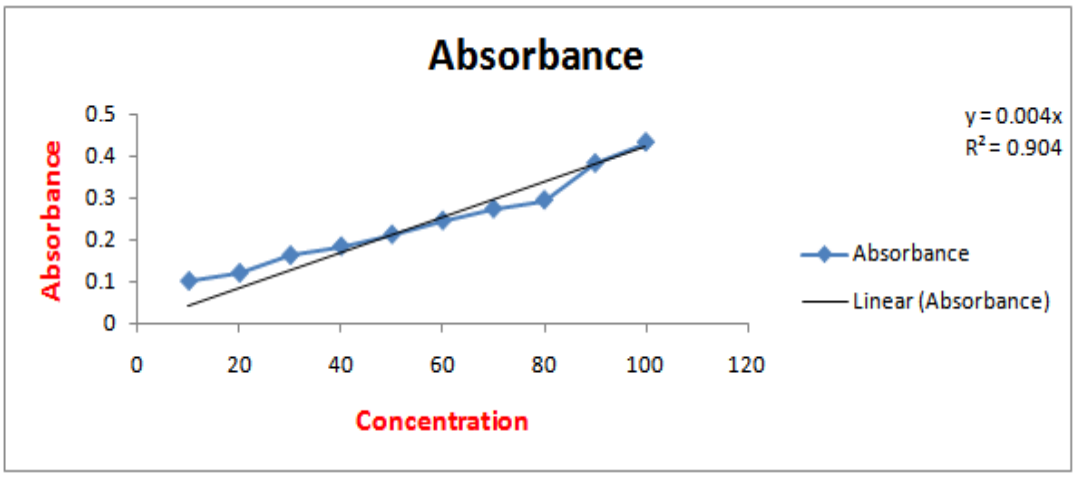

Fig. 2 Calibration curve of Querecetin

Table 3 DPPH free radical scavenging activity of extracts of bark of plant *Ehretia laevis*

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	15.68±0.09	10.04±0.19	6.11±0.18	8.82±0.18
50	28.4±0.18	17.54±0.12	11.8±0.10	13.2±0.10
75	41.36±0.03	32.27±0.07	21.2±0.04	24.17±0.14
100	53.98±0.16	45.09±0.15	37.6±0.30	41.96±0.26
125	64.74±0.15	56.79±0.22	44±0.38	49.76±0.06
150	72.54±0.33	63.55±0.26	48.88±0.14	55.52±0.14
175	84.97±0.03	74.41±0.13	60.01±0.10	61.41±0.10
200	94.29±0.51	87.13±0.09	68.55±0.18	72.35±0.08
IC50	101.21	115.74	136.98	147.52

Where, ASC-Ascorbic acid, MET-Methanolic extract, HAL-Hydroalcoholic extract, AQE-Aqueous extract

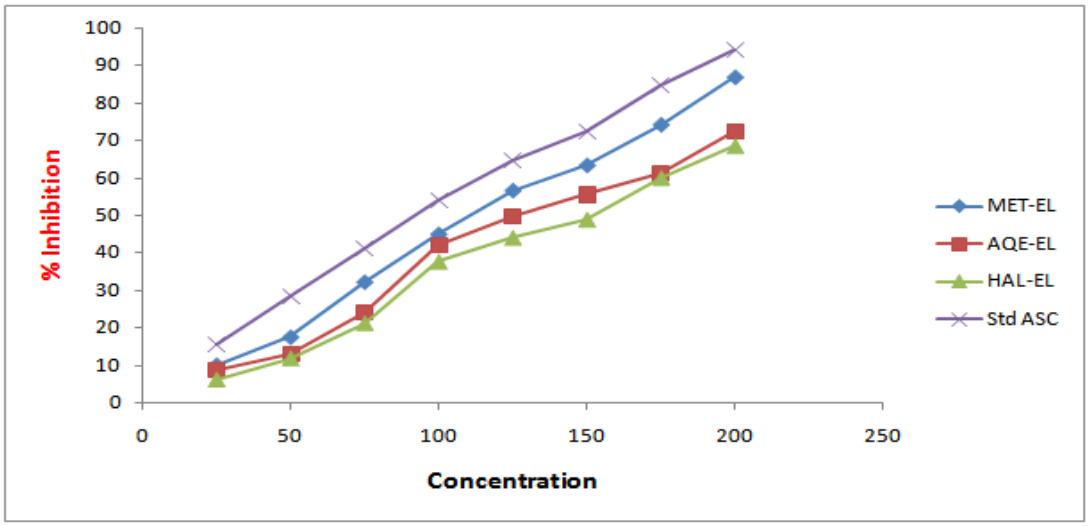

Fig. 3 DPPH free radical scavenging activity of extracts of bark of plant *Ehretia laevis*

Table 4 Nitric oxide free radical scavenging activity of extracts of bark of plant *Ehretia laevis*

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	18.84±0.10	12.92±0.19	7.9±0.16	5.09±0.21
50	30.81±0.22	22.13±0.16	12.29±0.15	10.84±0.24
75	44.98±0.10	38.52±0.16	32.93±0.15	19.36±0.11
100	57.8±0.10	49.02±0.16	42.21±0.17	33.28±0.10
125	66.02±0.10	59.22±0.16	51.18±0.15	46.12±0.04
150	77.08±0.10	68.19±0.19	57.66±0.18	54.17±0.19
175	86.48±0.20	78.35±0.12	67.35±0.15	63.55±0.16
200	93.13±0.10	85.08±0.17	74.74±0.22	69.38±0.10
IC50	98.61	110.86	129.53	144.09

Where, ASC-Ascorbic acid, MET-Methanolic extract, HAL-Hydroalcoholic extract, AQE-Aqueous extract

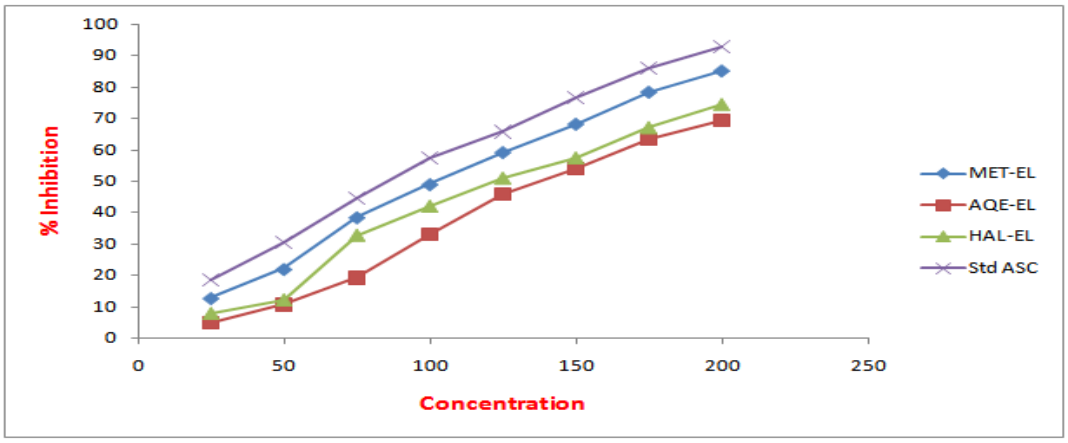

Fig 4 Nitric oxide free radical scavenging activity of extracts of bark of plant *Ehretia laevis*

Table 5 Hydrogen peroxide scavenging activity of extracts of bark of plant *Ehretia laevis*

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	19.22±0.13	14.03±0.189	7.35±0.14	4.69±0.22
50	32.03±0.1	21.34±0.18	12±0.15	10.42±0.18
75	45.83±0.1	34.26±0.22	24.92±0.39	18.36±0.18
100	60.73±0.09	49.03±0.19	39.21±0.21	29.59±0.18
125	72.62±0.13	53.89±0.17	50.83±0.30	45.09±0.14
150	81.38±0.1	71.71±0.1	65.1±0.15	56.76±0.3
175	89.36±0.13	78.48±0.21	72.58±0.18	65.16±0.10
200	93.84±0.16	87.55±0.18	76.39±0.15	71.77±0.18
IC50	94.87	110.61	125.94	142.04

Where, ASC-Ascorbic acid, MET-Methanolic extract, HAL-Hydroalcoholic extract, AQE-Aqueous extract

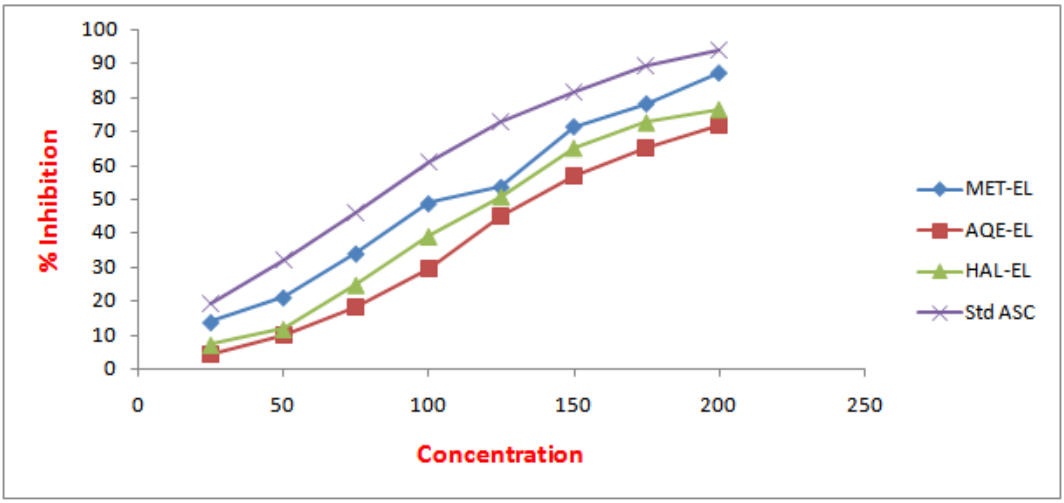

Fig. 5 Hydrogen peroxide free radical scavenging activity of extracts of bark of plant *Ehretia laevis*

Table 6 Reducing Power Assay activity of extracts of extracts of bark of plant *Ehretia laevis*

Concentration (ug/ml)	Absorbance (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	0.039±0.0008	0.03±0.0011	0.016±0.0018	0.023±0.0014
50	0.053±0.0008	0.047±0.0008	0.029±0.0012	0.04±0.0012
75	0.08±0.0012	0.058±0.0003	0.038±0.002	0.047±0.0012
100	0.086±0.0008	0.072±0.0008	0.046±0.0017	0.055±0.0015
125	0.1±0.0004	0.077±0.0011	0.06±0.0008	0.066±0.0012
150	0.113±0.0023	0.099±0.0011	0.075±0.0015	0.078±0.0008
175	0.138±0.002	0.104±0.0011	0.086±0.0012	0.091±0.0011
200	0.176±0.002	0.115±0.0011	0.103±0.0014	0.105±0.0012

Where, ASC-Ascorbic acid, MET-Methanolic extract, HAL-Hydroalcoholic extract, AQE-Aqueous extract

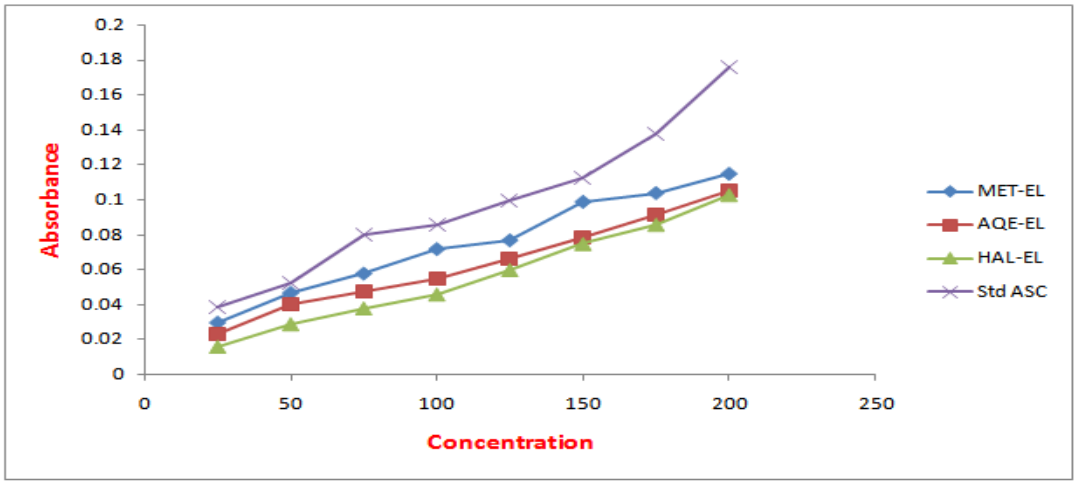

Fig 6 Reducing Power Assay activity of extracts of bark of plant *Ehretia laevis*

V. Discussion

Polyphenolic compounds are known as powerful chain breaking antioxidant[28] and they are very important constituents in plant because of their scavenging ability, which is due to their hydroxyl groups[29].

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antioxidant, anti-inflammatory, hepatoprotective, antiulcer, antiallergic, antiviral and anticancer activities[30]. Flavonoids are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and so they are potent antioxidants[31].

In the present study several biochemical constituents and free radical scavenging activities of extracts of medicinal plants were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, inflammation, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the control and management of these diseases. DPPH is a stable free radical which is a sensitive way to determine the antioxidant property of plant extracts [32, 33].

The DPPH method as antioxidant activity was evidently introduced nearly 50 years ago by Blois and is used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant potency. The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) [34].

Free Radical scavenging activities are important due to the deleterious role of free radicals in foods and in biological systems. It is known that free radicals can cause auto-oxidation of unsaturated lipids in food[35].

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and other cells, involved in the regulation of various physiological processes. Excess concentration of NO is always associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of NO via a five electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components[36].

The degree of inhibition of the NO free radicals was found to be increased in increasing concentration of the *Ehretia laevis* extracts, this indicates that the extract may contain compounds capable of inhibiting the generation of nitric oxide and offers scientific evidence for the use of *Ehretia laevis* in the treatment of various diseases. The antioxidant principles present in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that *Ehretia laevis* greater inhibition comparative to other plant extracts but less than ascorbic acid which has shown good inhibition of NO.

Hydrogen peroxide is a weak oxidizing agent and because of its ability to penetrate biological membranes, once inside the cell it can probably react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects[37].

All the extracts of *Ehretia laevis* when added to the reaction mixture scavenge hydroxyl radicals in a concentration dependent manner. The scavenging activity of the hydroxyl radicals may be due to the presence of polyphenolic compounds in the extracts which can donate electrons to H_2O_2, thus neutralizing it to water.

The reducing capacity of a extract may act as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, degradation of peroxides, reducing capacity and radical scavenging[38].

VI. Conclusion

Nature is always a source of medicinal agents for thousands of years and an impressive number of modern drugs are isolated from natural sources, many based on their use in traditional medicine. Higher plants are act as a sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Present study shows that polyphenolic content in the methanolic bark extract of *Ehretia laevis* is high and these extracts exhibit strong antioxidant activities compared to that of the hydroalcoholic, aqueous and with reference to standard compound. The results would help to determine the potency of the crude methanolic, hydroalcoholic and aqueous extract from plant *Ehretia laevis* as a potential source of natural antioxidants. Presence of adequate amount of polyphenol and flavonoid compounds may account for this fact. So these findings of present study suggest that this plant *Ehretia laevis* have a potential source of natural antioxidant. Further studies are suggested for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

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References

[1]. Vaibhav D Aher, Arunkumar wahi, Abhijit M. pawdey, Antioxidants as Immunomodulator: an expanding research avenue, *International journal of current pharmaceutical research*; 3(1), 2011, 8-10.
[2]. S.S. Naphade, S.S. Khadabadi, S.L. Deore, N.S. Jagtap, and S.P. Hadke, Antioxidant Activity of different extracts of plant *Tricholepsi Glaberrima* DC (Asteraceae), *International Journal of Pharm Tech Research. 1(3),* 2009, 502-505.
[3]. Mohammad hassanzadeh-khayyat, Mehdi mojarab and Seyyed ahmad emami, Antioxidant activity of methanolic extracts of different species of Artemisia from Iran, *Pharmacology online, 2,* 2009, 797-807.
[4]. Rathore K.S, Sisodia S.S. and panchawat S. A review on herbal antioxidant *International Journal Of Pharma Tech Research, 2(1),* 2010, 232-239.
[5]. Kikuzaki H and Nakatani N. Antioxidant effects of some ginger constituents. *Journal of Food Science, 58(6),* 1993, 1407-1410.
[6]. Ottolenghi A., Interaction of ascorbic acid and mitochondria lipids, *Arch. Biochem. Biophy. 79,* 1959, 355-363.
[7]. Halliwel B, Gutteridge JMC. *Free Radical in Biology and Medicine* New York, Oxford University Press, 3rd ed, 1998, 100-105.
[8]. Pourmorad F., Hosseinimehr S.J. and Shahabimajd N., Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. *S. Afr. J. Biotechnol. 5*, 2006, 1142-1145.
[9]. Duh P.D., Tu Y.Y. and Yen GC, Antioxidants activity of aqueous extract of Harnjyur (*Chrysanthemum morifolium* Ramat). *Lebensmwiss Technol. 32*, 1999, 269-277.
[10]. Raja Sudarajan N, Ahamad H. and Kumar V., *Cytisus scoparius* Link-A natural antioxidant. *BMC Complement Altern Med , 6(8),* 2006, 1-7
[11]. Srinivasan R., Chandrasekar M.J.N, Nanjan M.J, Suresh B., Antioxidant Activity of *Caesalpinia Digyna* Root. *Journal of Ethnopharmacology, 113,* 2007, 284-291.
[12]. Kumaraswamy M. V. and Satish S., Antioxidant and Anti-Lipoxygenase activity of *Thespesia lampas* Dalz & Gibs, *Advan. Biol. Res., 2(3-4),* 2008, 56-59.
[13]. Badami S., Gupta M.K. and Suresh B., Antioxidant activity of ethanolic extract of *Striga orobanchioides*, *J. Ethnopharmacol., 85,* 2003, 227-230.
[14]. Aurelia Magdalena Pisoschi1 and Gheorghe Petre Negulescu, Methods for Total Antioxidant Activity Determination: A Review, *Biochemistry & Analytical Biochemistry, 1(1),* 2011, 1-10
[15]. Joshi SG. Medicinal Plants, Oxford And IBH Publishing Co. Pvt. Ltd. New Delhi, 102 (2000).
[16]. Abha Shukla and Amanpreet Kaur. A Systematic Review Of Traditional Uses Bioactive Phytoconstituents Of Genus *Ehretia*. *Asian Journal Of Pcal And Clinical Research, 11(6),* 2018, 88-100.
[17]. Mukherjee P. K., *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals* New Delhi, Business Horizons, 5th reprint, 2012, 405-407.
[18]. Singletion VL., Orthofer R. and Lamuela-Raventos RM., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent Methods, Enzymol., *299*, 1999, 152-178.
[19]. Zhishen J., Mengcheng T. and Jainming W., Research on antioxidant activity of flavonoids from natural materials. *Food Chem. 64*, 1999, 555-559.
[20]. Molyneux P., The Use of Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity, *J Sci Technol, 26,* 2004, 211-219.
[21]. R Govindharajan, S Rastogi, M Vijayakumar, AKS Rawat, A Shirwaikar, S Mehrotra, P Pushpangadan, Studies on the antioxidant activities of Desmodium gangeticum, *Biol Pharm Bull, 26*, 2003, 1424-27.
[22]. K Berker, K Guclu, I Tor, B Demirata, R Apak, Total antioxidant capacity assay using optimized ferricyanide/Prussian blue method, *Food Anal Methods, 3*, 2010, 154-68.
[23]. Ranju Pal, Kundlik Girhepunje, Ashutosh Upadhayay And Thirumoorthy N., Antioxidant and free radical scavenging activity of ethanolic extract of the root of *Morinda citrifolia*, *African journal of pharmacy and pharmacology, 6(5),* 2012, 278-282.
[24]. Priscilla M. Clarkson and Heather S. Thompson, Antioxidant: what role do they play in physical activity and health, *The American journal of clinical nutrition, 72(2),* 2000, 637S-646S.
[25]. Fereidoon Shahidi, *Antioxidant: Science, technology, and Applications*, Bailey"s industrial oil and fat products, 6th edition; 6, 431-489.
[26]. Nitai Chand Chaulya, Pallab Kanti Haldar and Arup Mukherjee, In Vitro Free Radical Scavenging Activity Of Methanolic Extract Of Rhizome Of *Cyperus Tegetum* Roxb.(Cyperaceae*), International Journal of Current Pharmaceutical Research*; *2(3),* 2010, 39-43.
[27]. Buricova L., Andjelkovic M., cermakova A., Reblova Z., Jurcek O., Kolehmainen E., Verhe R., and Kvasnicka F. Antioxidant capacity and antioxidants of strawberry, blackberry and raspberry leaves, *Czech J. food sci., 29(2),* 2011, 181-189.
[28]. Shahidi F. and Wanasundara PKJPD. Phenolic antioxidants, *Food Sci. Nut. 32(1),* 1992, 67-103.
[29]. Hatano T., Edamatsu R. and Mori A., Effect of interaction of tannins and related poyphenols on superoxide anion radical and on DPPH radical, *Chem Pharm Bull. 37*, 1989, 2016-2021.
[30]. Umamaheswari and Chatterjee TK. In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *Afr. J. Traditional, complementary and Alternative Medicines. 5(1),* 2008, 61-73.
[31]. Cao G., Sofic E. and Prior RL. Antioxidant and pro-oxidative behavior of flavonoids: Structure activity relationships. *Free Radical.Biol. Med. 22*, 1997, 749-760.
[32]. Koleva II, Van Beek T.A., Linseen J.P.H., de Groot A., and Evstatieva L.N., Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal. 13*, 2002, 8-17.
[33]. Suresh P.K., Sucheta S., Sudarshana V.D., Selvamani P., and Latha S., Antioxidant activity in some selected Indian medicinal plants. *Afr. J. Biotechnol. 7*, 2008, 1826-1828.
[34]. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1958, 1199–1200.
[35]. Ak T. and Gülçin I. Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact. 174(1),* 2008, 27–37.
[36]. Rozina Parul, Sukalayan Kumar Kundu and Pijush Saha, In Vitro Nitric Oxide Scavenging Activity Of Methanol Extracts Of Three Bangladeshi Medicinal Plants, *The Pharma Innovation - Journal, 1(12),* 2013 83-88.

[37]. Halliwell, B. and Gutteridge, J.M.C. *Free radicals in biology and medicine* 2007, 4th Edition, Oxford University Press, New York, 10-15.
[38]. Yildirim,A., Mavi,A., Oktay,M., Kara,A.A., Algur,O.F. and Bilaloglu,V., Comparision of antioxidant and antimicrobial activities of Tilia (*Tilia argentea*Desf Ex DC), Sage (*Savia trilob*aL.) and Black tea (*Camellia sinensis*) extracts, *J Agric Food Chem.,48(10),* 2000, 5030-5034.

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