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Evaluation of Antioxidant Activities of Various Solvent Extracts of Fruits and Leaves of *Ehretia serrata*

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Ehretia serrata belongs to the genus Ehretia which is well known for its many important biological properties. Different species of Ehretia are used by traditional practitioners for curing several diseases. The present study was aimed to evaluate the antioxidant properties and reducing power of various solvent fractions of fruits and leaves of *Ehretia serrata*. Ethyl acetate fraction of leaves contain the highest flavonoid (752.753 ± 1.5 µg/mL of rutin equivalent) as well as phenolic content (942.33 ± 2.3 µg/mL of gallic acid equivalent). It is also found to be most active against free radicals and reducing agents when investigated by various assays, *i.e.*, ABTS (TEAC 1.76 ± 0.004 µmol), DPPH (EC_{50} 120.499 µg/mL), FRAP (270.44 ± 1.00 µmol of ascorbic acid equivalent, AAE), Phosphomolybdate (156.92 ± 4.63 µg/mL of AAE) and reducing power assays. However, the ethyl acetate fraction of fruits showed the highest lipid peroxidation value followed by the leaves fraction. The 1-butanolic and chloroform fractions of leaves and ethyl acetate fraction of fruits showed appreciable results in all the assays. The non-polar hexane fractions were less effective against free radicals. The significant correlation coefficient calculated for TEAC, $1/EC_{50}$, FRAP and total phenolic content assay indicates the presence of highly active phenolic compounds. An insignificant correlation was found between the total flavonoid and phenolic content. Thus, the findings of this research indicate ethyl acetate fractions of fruits and leaves, to be a possible lead in the discovery of natural antioxidants.

Key Words: *Ehretia serrata*, Free radical scavenging, Antioxidants.

INTRODUCTION

The use of plants as folk medicine for curing diseases has been the part of all the cultures[1] and are still the largest source of medication. According to World Health Organization approximately 75-80 % of world's population uses plant medicines either in part or completely[2]. In Pakistan, use of plant based medicines is also a common practice. In 2006, Shinwari *et al.*[3] had published a pictorial guide which has enlisted more than 500 species of flowering plants of Pakistan which have served medicinal purposes. Plants are a rich source of natural products and nutraceuticals necessary for the maintenance of good health and combat diseases. One of the main causes of diseases such as aging, cardiovascular diseases, neural disorders, arteriosclerosis, skin irritations, inflammations and cancers are the free radicals produced in human body[4]. They are formed in the living body when cells utilize oxygen molecules in order to generate energy. They include superoxides ($O^{\cdot -}$), hydroxyl ($HO^{\cdot}$), hydroperoxyl ($HOO^{\cdot}$), peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals[5], collectively known as reactive oxygen species (ROS). The damage to the cell targets *i.e.*, DNA, lipids and proteins occurs if there is a disproportion between the free radicals and the natural antioxidants in the body[6]. Antioxidants scavenge the odd electron of free radicals either by donating electron, hydrogen atom or by chelating metals, thus inhibiting the radical chain reaction[7]. Naturally occurring antioxidants are not only safer than their synthetic counter parts, also have antimutagenic, antitumor and hepatoprotective activities[8]. Thus there is a growing need to explore newer and safer natural antioxidants. Plants constitute a promising avenue for such substances. A number of antioxidant capacity assays are used to detect the potential of a compound or herbal extract to scavenge a free radical which include Folin-Ciocalteu reagent assay, ferric reducing antioxidant power (FRAP) assay, 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) scavenging assay, N,N-dimethyl-*p*-phenylenediamine (DMPD) assay and Trolox equivalent antioxidant capacity (TEAC) assay[9]. The mechanism of the assay is governed by the structure of an antioxidant and pH of the reaction[10].

The plant *Ehretia serrata* (syn. *Ehretia acuminatavar. serrata*) belongs to the genus Ehretia which comprises about 50 species, distributed mainly in the tropical Asia and Africa[11]. Different species of Ehretia have been explored to isolate alkaloids[12,13], phenolic acids, flavonoids, benzoquinones,

cyanogenetic glycosides and fatty acids[14] which are associated with many biological activities including antiinflammatory[15], antitrypanosomal[16], antisnake venom[17] and antitumor[18]. The roots, bark, leaves, fruit, heartwood of the plants of genus Ehretiaare used as traditional medicines against inflammation, cough, itches, swellings, diarrhea, dysentery, fever, cachexia and syphilis[18,19]. In Zimbabwe, different parts of *Ehretia obtusifoliaare* used for treating sore throat, teething pains in infants, menstrual pain, abdominal pains and infertility in women[20]. In China, the species *Ehretia thyrsiflora* has been used to make kudingcha, a bitter tea[21]. In India, *Ehretia laevis* is used to treat headache and ulcers, it also possess potent anthelmintic, diuretic, demulcent, expectorant and astringent properties. The inner bark of *E. laevis* is used as food[22]. *Ehretia serrata*, a plant native to Pakistan[23] is locally known as Puna[24]. The wood of this plant is used for fuel purposes and leaves for fodder. The tree is also used for erosion control in farm forestry and for a gunstock purpose. The unripe fruit is used as pickle in food[24,25]. Literature survey has revealed no research work to evaluate the antioxidant activity of fruits and leaves of *E. serrata*. In view of that, the present exhaustive *in vitro* anti-oxidant study has been carried out on fractions of fruits and leaves of *E. serrata* in solvents of different polarity.

EXPERIMENTAL

Ascorbic acid, ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH [1,1-diphenyl-2-picrylhydrazyl] radical were purchased from MP biomedicals, Inc. (France). Butylatedhydroxyanisole (BHA) and Tween-20 were obtained from Merck (Darmstadt, Germany). Trolox, Rutin, Linoleic acid and Folin-Ciocalteu reagent were purchased from Sigma Aldrich. Gallic acid was obtained from Scharlau-Switzerland and 2,4,6-(2-tripyridyl)-*s*-triazine (TPTZ) was obtained from Alfa Aesar, (Germany). All the other solvents and chemicals used were of analytical grade or purer.

Collection and preparation of samples: Fresh fruits and leaves of *Ehretia serrata* were collected from the campus of Forman Christian College, Lahore, Pakistan in June 2010 and identified by the taxonomist of the college. The fruits and leaves were air dried in the shade for 7 days and ground to a fine powder. The powdered fruits and leaves 200 g each were extracted in 80 % aqueous methanol (1 L × 15 days × 2 days) and filtered. The filtrate was concentrated under reduced pressure on rotary evaporator. The crude methanolic extract of fruits and leaves (6 g each) were suspended in double distilled water and extracted with hexane, chloroform, ethyl acetate and 1-butanol, respectively. Thus five fractions of each extract were prepared:hexane, chloroform, ethyl acetate, 1-butanolic and aqueous after partition.

Total flavonoid content assay: The total flavonoid content (TFC) assay was performed following the protocol set by Park *et al.*[26]. Briefly, 3 mg of fraction was dissolved in 10 mL of methanol. An aliquot (300 µL) was dissolved in 30 % aqueous methanol (3.4 mL) and 150 µL each of 0.5 M $NaNO_2$ and 0.3 M $AlCl_3 \cdot 6H_2O$ were added. After the interval of 5 min, 1 M NaOH (1 mL) was added. The absorbance of the samples was read immediately at 506 nm on UV/visible spectrophotometer against a blank. The standard calibration curve of rutin was made through the same procedure. The total flavonoid content was determined using the following equation,

$$\text{Rutin equivalent (RE)} = \left[\frac{(\text{Absorbance (506 nm)} - 0.008141)}{0.0002428}\right]$$

R^2 0.9987

Total phenolic content assay: The total phenolic content (TPC) was determined by the method reported by Singleton and Rossi[27]. For each test, 3 mg of an extract was dissolved in 10 mL of methanol. 40 µL aliquot waspoured ina glass vial. To it, distilled water (3.16 mL) was added. The solution was then mixed with Folin-Ciocalteu reagent (200 µL). After an interval of 8 min, 20 % sodium carbonate solution (600 µL) was added. The mixture was incubated at 40 ºC for 0.5 h. The absorbance was measured at 765 nm. The standard curve for total phenolics was made with standard solution (50-500 mg/L) of gallic acid following the same procedure.The gallic acid equivalent (GAE) was determined from the following equations obtained from the standard curve of gallic acid.

$$\text{GAE} = \left[\frac{(\text{Absorbance (765 nm)} + 0.02091)}{0.0009567}\right]$$

R^2 0.9995 (for fruits samples)

$$\text{GAE} = \left[\frac{(\text{Absorbance (765 nm)} + 0.02035)}{0.0008921}\right];$$

R^2 0.9997 (for leaves samples)

$ABTS^{\cdot+}$ decolourization assay: The $ABTS^{\cdot+}$ decolourization assay was carried out following the method developed by Re *et al.*[28]. The stock solution was prepared by making a solution of ABTS (0.038 g) in deionised water (10 mL) and then potassium persulfate (0.27032 g) was added. The stock solution was mixed well and placed in dark for 18 h. The working solution was prepared by dilutingthe stock solution with phosphate buffer saline (PBS) till the absorbance of 0.700 (+ 0.02) was reached at 734 nm and equilibrated at 30 ºC. Then 10 µL of the sample (1 mg/mL) was dissolved in diluted ABTS solution (2.99 mL) and absorbance was measured at 734 nm after every 0.5 min for 8 min. The per cent inhibition in the absorbance was determined using the following formula[29]:

$$\text{Inhibition (\%)} = \left[\frac{(\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}}\right] \times 100$$

The same protocol was carried out with Trolox, a standard antioxidant, used to generate a calibration curve. Trolox equivalent antioxidant capacity (TEAC) values (mM) of the samples were determined from the following equation:

$$\text{TEAC Value} = \left[\frac{(\text{Absorbance (734 nm)} - 3.684)}{0.04393}\right];$$

$R^2 = 0.9793$

DPPH radical scavenging assay: The DPPH radical scavenging assay was done according to the method of Brand-Williams *et al.*[30]. The stock solution of DPPH was prepared by dissolving DPPH (24 mg) in methanol (100 mL) and stored at 20 ºC until needed. The working solution was obtained by diluting DPPH solution with methanol till absorbance reaches

0.980 ± 0.02 at 517 nm. Theworking solution (3 mL) was taken in a glass vial and mixed with the sample (100 µL) of concentration (1 mg/mL). The vials were incubated in the dark for 0.5 h. Absorbance was measured at 517 nm. The scavenging activity was calculated by using the formula:

Inhibition (%)

$$= \left[\frac{(\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \right] \times 100$$

Using the same protocol, EC_{50} values *i.e*., effective concentration that causes the inhibition of 50 % of DPPH radicals[31] was also calculated.

Ferric reducing antioxidant power (FRAP) assay: The ferric reducing power assay (FRAP) was done according to the method proposed by Benzie and Strain[32]. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6, 25 mL), 10 mM TPTZ solution (2.5 mL) in 40 mM HCl solution (20 mL) and 20 mM $FeCl_3 \cdot 6H_2O$ solution (2.5 mL), the reagent was kept at 37 ºC throughout the experiment. After incubation of 10 min, its absorbance was measured at 593 nm. Then FRAP reagent (3 mL) was added to 100 µL of sample (250 µg/mL). Absorbance of the solution was measured at 593 nm after 5 min. The blank was prepared by dissolving FRAP reagent (3 mL) with methanol (100 µL). Same protocol was followed with various concentrations of ascorbic acid, a standard antioxidant, in order to generate a calibration curve. The results were expressed as µmol of ascorbic acid equivalent (AAE) per 250 µg of the dried extract, as determined from the following equation of straight line.

Ascorbic acid equivalent (AAE)

$$= \left[\frac{(\text{Absorbance at 593 nm} - 0.004}{0.002} \right]; R^2 = 1$$

Phosphomolybdate assay: The phosphomolybdate assay was done according to the protocol given by Umamaheswari and Chatterjee[33]. The fraction (250 µg) was dissolved in methanol (1 mL) an aliquot (0.1 mL) was poured in a test tube along with 1 mL of reagent solution, which was prepared by mixing equal quantities of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The test tubes were capped with silver foil and incubated in water bath at 95 ºC for 90 min. After samples were cooled to room temperature, the absorbance were measured at 765 nm against a blank. Ascorbic acid was used as a standard antioxidant. Various concentrations of the standard (50-500 mg/L) were prepared and tested using the same procedure in order toplot a standard curve. The µg of ascorbic acid equivalent (AAE) per 250 µg of the driedweight of the fraction was determined from the following equation obtained from the standard curve.

$$\text{AAE} = \left[\frac{(\text{Absorbance (765 nm)} + 0.0593)}{0.0025} \right]; R^2 = 0.997$$

Reducing power assay: The reducing power of fruits and leaves was determined by the method reported by Oyaizu[34]. The sample was prepared by dissolving dried extract (10 mg) in methanol (2 mL). It was then mixed with 2 mL each of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide. The reaction mixture was incubated at 50 ºC for 20 min. Then, trichloroacetic acid (2 mL) was added, mixed and centrifuged at 650 rpm for 10 min. After centrifugation, the upper layer (2 mL) of the solution was mixed with deionised water (2 mL) and 0.1 % (w/v) ferric chloride (0.4 mL). The absorbance was recorded at 700 nm. Increased absorbance showed a high reducing power. Gallic acid was used as a standard.

Lipid peroxidation value in linoleic acid emulsion system: This assay was done according to the method reported by Mitsuda *et al*.[35]. Linoleic acid emulsion was prepared by mixing Tween-20 (175 µg) and linoleic acid (155 µL) and adding to it, the potassium phosphate buffer (pH 7, 50 mL) to form an emulsion. The dried fraction (5 mg) was dissolved in methanol (1 mL). An aliquot (100 µL) was dissolved in potassium phosphate buffer (0.04 M, pH 7, 2.4 mL) and linoleic acid emulsion (2.5 mL). The mixture was incubated at 37 ºC. The aliquot (100 µL) from the incubated solution was regularly taken at 24 h intervals and allowed to react with 20 mM $FeCl_2$ (100 µL) and 30 % ammonium thiocyanate (100 µL). The absorbance was measured at 500 nm after every 24 h for 7 days. A 5 mL solution consisting of equal quantities of linoleic acid emulsion and potassium phosphate buffer was used as a blank. Butylated hydroxy anisole (BHA), a synthetic antioxidant, was used as a standard and same protocol was followed.

Statistical analysis: Triplicate determinations were made for each sample and results were expressed as mean ± SD (n = 3) unless mentioned otherwise. Different statistical methods *i.e*., Pearson correlation coefficient, linear regression analysis and one way ANOVA was used to compare and analyze the results obtained from different assays. The p value < 0.05 indicated the significant difference.

RESULTS AND DISCUSSION

Total flavonoid content assay: Flavonoids are the important class of plants secondary metabolites ubiquitous in nature and famous for their role against many ailments. Results showed the presence of highest flavonoid content (752.753 µg/mL of rutin equivalent, RE) in ethyl acetate fraction of leaves followed by 1-butanolic, chloroform and methanolic extracts, respectively. However in fruits fractions, the highest flavonoid content (748.637 µg/mL of RE) was exhibited by hexane fraction followed by 1-butanolic and chloroform fractions, respectively. The total flavonoid content in all the fractions ranged from 752.753-62.815 µg/mL of RE. The total flavonoid content (TFC) has shown an insignificant correlation with the antioxidant assays (Table-5), which is also reported by Heinonen[36] and other researchers[37,38]. This is due to the fact that only those flavonoids have the potential to act as antioxidants which have right positioning of hydroxyl group substitution[39,40]. For instance, the substitution of electron donating group at *para*-position increase the antioxidant potential of the aryloxyl radical, similarly the electron withdrawing group substitution at *para*-position will have the negative effect on the antioxidant activity of the compound[41].

Total phenolic content assay: The plants having phenolic content may show antioxidant activity[42]. The total phenolic content was determined by Folin-Ciocalteu method, in which, the reagent comprising oxides of tungsten and molybdenum undergoes chemical reduction[43]. The results of the total

phenolic content expressed as GAE are given in (Table-1). The ethyl acetate fraction of leaves showed the highest phenolic content, 942.33 ± 2.3 µg/mL of gallic acid equivalent (GAE). Among the fruits fractions, the highest phenolic contentwas 125.68 ± 8.89 µg/mL, showed by the ethyl acetate fraction. The fruits extracts showed lower amount of phenolics as compared to the leaves (Table-1). The high flavonoid and phenolic contents of the ethyl acetate fraction of leaves of *Ehretia serrata*is in accord with the values reported for the ethyl acetate fraction of leaves of *Ehretia thyrsiflora*, a closely related species native to China[44]. An insignificant correlation was observed between the results of total flavonoid and phenolic content assays (Fig. 1), a fact observed by other researchers as well[45]. The significant correlation was observed between the total phenolic content and antioxidant assays (Table-6). The ethyl acetate fraction of leaves of *Ehretia serrata*, which possessed the highest phenolic content had also been found to be highly active against free radicals and showed significant correlation with the antioxidant assays based on the aqueous medium, a trend also reported by Baderschneider *et al.*[46].

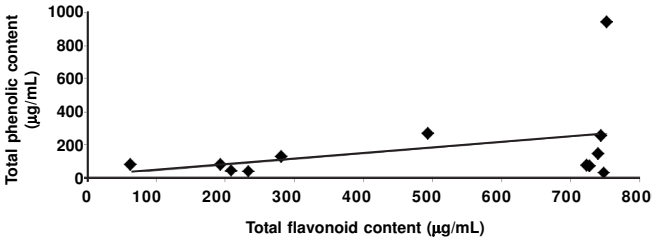

Fig. 1. An insignificant correlation between the total phenolic content and total flavonoid content was observed, $R^2 = 0.133$

ABTS$^{\cdot+}$ decolourization assay: The ABTS$^{\cdot+}$ [2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] radical undergoes reduction in the presence of an antioxidant. Trolox, a water soluble analog of vitamin E was used to plot a standard curve. The results are expressed as TEAC values *i.e.*, Trolox equivalent of antioxidant capacity[28]. The results listed in (Table-2) showed the presence of highest antioxidant activity in the ethyl acetate fraction of leaves followed by the fruits fraction. The TEAC values of fruits and leaves range from 0.20 ± 0.005 to 1.76 ± 0.004 mM. The lowest TEAC value was given by the hexane fraction of fruits. The decrease in absorbance of fruits and leaves extracts over a period of time is shown in Fig. 2. The correlation between the total flavonoid content and TEAC was insignificant (Table-5) while between total phenolic content and TEAC value was significant (Table-6). Thus, the antiradical activity of the fractions was possibly present due to the phenolic content.

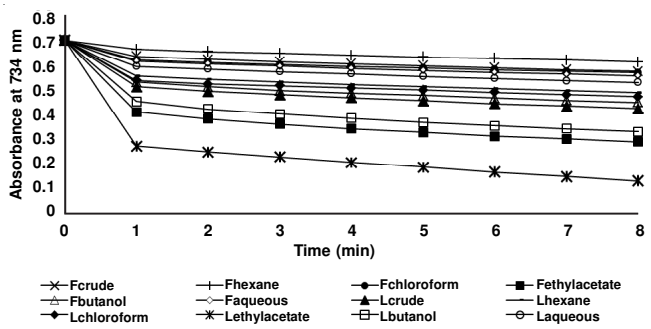

Fig. 2. Decrease in absorbance of various fractions of fruits and leaves against ABTS$^{\cdot+}$ radical over a period of time

DPPH radical scavenging assay: DPPH (2,2-diphenyl-1-picrylhydrazyl) is an organic nitrogen centered free radical which is stable at room temperature. The principle of DPPH decolourization assay is based on the ability of this radical to accept an electron or a hydrogen atom from any reducing agent

TABLE-1
TOTAL FLAVONOID (RE) AND TOTAL PHENOLIC CONTENT (GAE)
OF FRUITS AND LEAVES OF *Ehretia serrata*

Fraction/extract	Rutin equivalent (%) µg/mL		Gallic acid equivalent (GAE) µg/mL	
	Fruits	Leaves	Fruits	Leaves
Methanolic	209.366 ± 4.5	493.409 ± 1.6	38.58 ± 2.8	264.18 ± 1.7
Hexane	748.637 ± 8.0	62.815 ± 2.1	27.78 ± 1.2	79.60 ± 2.6
Chloroform	728.054 ± 6.6	740.404 ± 4.9	69.59 ± 0.6	141.62 ± 1.1
Ethyl acetate	281.817 ± 2.3	752.753 ± 1.5	125.68 ± 8.9	942.33 ± 2.3
1-Butanolic	723.937 ± 3.9	744.520 ± 2.8	73.07 ± 2.1	250.73 ± 3.2
Aqueous	234.065 ± 1.8	192.899 ± 2.1	34.05 ± 2.2	77.73 ± 6.2

*Concentration of fruits and leaves was 300 µg/mL. **Each value listed in the table is represented as mean ± SD (n = 3).

TABLE-2
TEAC(ABTS ASSAY), PER CENT INHIBITION**AND EC_{50} VALUES (DPPH ASSAY)
OF DIFFERENT FRACTIONS OF FRUITS AND LEAVES OF *Ehretia serrata*

Fraction/extract	TEAC(mM)		Percent inhibition (%)		EC_{50}(µg/mL)	
	Fruits	Leaves	Fruits leaves	Leaves	Fruits	Leaves
Methanolic	0.32 ± 0.002	0.77 ± 0.007	12.05±1.081	93.91±0.869	>2500	290.491
Hexane	0.20 ± 0.005	0.59 ± 0.012	04.50±0.500	64.36±0.650	Nill	850.263
Chloroform	0.40 ± 0.003	0.67 ± 0.011	39.51±1.194	72.75±2.203	1400.226	589.818
Ethyl acetate	1.22 ± 0.006	1.76 ± 0.004	84.33±1.041	96.38±0.544	450.213	120.499
1-Butanolic	0.72 ± 0.003	1.16 ± 0.011	29.90±0.661	81.92±2.696	2000.415	269.999
Aqueous	0.34 ± 0.004	0.42 ± 0.004	06.28±0.625	44.71±4.990	>2500	1600

*The values are the mean of triplicate determinations. **The concentration of fruitsand leaves for TEAC values(ABTS assay) and %inhibition by DPPH was 1mg/mL.

to form a stable non-radical diamagnetic molecule[47]. EC_{50} (Effective concentration) is the concentration of a sample which can scavenge 50 % of the free radicals. The ethyl acetate fraction of leaves had given the lowest EC_{50} value *i.e.*, 120.499 µg/mL indicating its highest radical scavenging potential. The EC_{50} value of ethyl acetate fraction of fruits was 450.213 µg/mL.The EC_{50} values of methanolic and aqueous fractions of fruits were greater than 2500 µg/mL showing their low antioxidant potential. The EC_{50} ofascorbic acid, a standard antioxidant is listed in (Table-3). The Pearson correlation between the TEAC and $1/EC_{50}$ was also very significant with r 0.9072 and $p < 0.0001$ (Table-6), which showed that the antioxidant potential determined by the DPPH and TEAC assays was significantly correlated.

TABLE-3
DPPH ASSAY: EC_{50} VALUE OF ASCORBIC ACID, A STANDARD ANTIOXIDANT

Standard antioxidant	EC_{50}(µg/mL)
Ascorbic acid	99.9176

*The values are the mean of triplicate determinations.

Ferric reducing antioxidant power (FRAP) assay: The ferric reducing antioxidant power is used to compare the total quantity of antioxidants present in a sample. Since FRAP assay involves electron transfer, it cannot detect the compounds which stabilize radicals through hydrogen transfer. Moreover, since FRAP activity is observed within 5 min of the reaction, there is a chance that slow reacting polyphenols are not detected[48]. The FRAP reaction is carried out at low pH, *i.e.*, 3.6, in order to keep the iron solubility constant during the reaction. During the reaction, ferric tripyridyltriazine complex changes into its ferrous form having intense blue colour and gives maximum absorbance at 593 nm[32]. In our study on *Ehretia serrata*, the ethyl acetate fraction of leaves showed the highest FRAP value of 270.44 ± 1.00 µM of ascorbic acid equivalent (AAE) while the hexane fraction of fruits and 1-butanolic fraction of leaves showed intermediate powers (151.04 ± 0.60 and 108.60 ± 1.56 µM of ascorbic acid equivalent). The methanolic extract of fruits gave the poorest result (3.39 ± 0.60 µM of ascorbic acid equivalent). The chloroform and 1-butanolic fraction of leaves showed 10.03 ± 0.60 and 108.60 ± 1.56 µM of ascorbic acid equivalent (Table-4). The change in trend of antioxidant capacity of some of the fractions of fruits and leaves observed in FRAP assay is mainly because FRAP assay does not give good correlation with other antioxidant assays[10]. However, FRAP assay showed significant correlations with the total phenolic content and($1/EC_{50}$) DPPH assays (Table-6).

Phosphomolybdate assay: In the presence of a reducing agent, the Mo(VI) reduces to Mo(V) and forms a green coloured phosphomolybdenum(V) complex, which gives maximum absorbance at 700 nm[49]. The results of this assay were expressed as µg/mL of ascorbic acid equivalent (AAE). The antioxidant activity of leaves fractions were in the range of 66.95 ± 1.91 µg/mL of AAE for aqueous to 156.92 ± 4.63 µg/mL of ascorbic acid equivalent for ethyl acetate fraction which was also the highest amongst all the fractions of fruits and leaves. Whereas, in case of fruits extracts the values range from 33.47 ± 0.78 µg/mL of ascorbic acid equivalent for aqueous to 101.08 ± 3.67 µg/mL of ascorbic acid equivalent for ethyl acetate fractions. The comparison among the same solvent fractions of fruits and leaves indicated that all the fractions of leaves possessed higherantioxidant potential than the same polarity fractions of fruits (Table-4). This trend showed that the leaves possess more potent chemical constituents which are effective against free radicals.

Reducing power assay: The reducing power of an extract is often due to the presence of reductones, have the ability to break the radical chain reaction by providing a hydrogen atom[50]. The reduction of Fe^{3+}-Fe^{2+} is determined by the change in colour to greenish blue which absorbs at 700 nm. Gallic acid was used as a standard. The order of reducing capacity of extracts of fruits was;ethyl acetate > 1-butanolic > chloroform > hexane > methanolic > aqueous. Whereas, for leaves fractions, the order was;chloroform > hexane > ethyl acetate ≈ 1-butanolic > aqueous > methanolic. All the fractions of leaves were relatively more active from gallic acid. However, the ethyl acetate fraction of fruits showed a reducing capacity comparable to gallic acid.

Lipid peroxidation in linoleic acid emulsion system: Lipids having unsaturation are prone to peroxidation which results in their rancidity. To prevent their deterioration, antioxidants are added. Since the synthetic antioxidants are not free from side effects, it is desirable to explore natural antioxidants and free radical scavengers for a substitute. In the present research, the lipid peroxidation value of extracts of fruits and leaves of *E. serrata* was determined by linoleic acid emulsion method[35]. The assay is based on the fact that the oxidation of linoleic acid generatesperoxyl radicals, which are scavenged in the presence of an antioxidant. The radicals remained are then allowed to oxidize the Fe^{2+}-Fe^{3+} which forms a coloured complex with a thiocyanate solution. The absorbance of the complex was determined at 500 nm. The low absorbance indicates the presence of high antioxidant activity, *i.e.*, increase in lipid peroxidation value indicates low antioxidant potential

TABLE-4
THE ASCORBIC ACID EQUIVALENT (AAE) OF DIFFERENT FRACTIONS OF FRUITS AND LEAVES* AS DETERMINED BY FRAP (µM) AND PHOSPHOMOLYBDATE ASSAY (µg/mL)

Fraction/extract	FRAP Assay		Phosphomolybdate assay	
	Fruits	Leaves	Fruits	Leaves
Methanolic	3.39 ± 0.60	48.04 ± 2.80	48.21 ± 6.82	114.01 ± 1.36
Hexane	151.04 ± 0.60	36.20 ± 0.80	53.38 ± 6.23	86.34 ± 1.18
Chloroform	49.61 ± 0.40	10.03 ± 0.60	76.10 ± 5.79	93.07 ± 13.28
Ethyl acetate	5.47 ± 0.80	270.44 ± 1.00	101.08 ± 3.67	156.92 ± 4.63
1-Butanolic	Negligible	108.60 ± 1.56	57.12 ± 2.42	117.50 ± 5.00
Aqueous	Negligible	7.81 ± 0.40	33.47 ± 0.78	66.95 ± 1.91

*Concentration of fruits and leaves was 250 µg/mL. ** Each value listed in the table is represented as mean ± SD (n = 3).

TABLE 5
THEPEARSON CORRELATION COEFFICIENT 'r' FOR TOTAL FLAVONOID CONTENT ASSAYWITH TEAC, FRAP AND $1/EC_{50}$ (DPPH) *i.e.*; p VALUE > 0.05**

Pearson correlation	TEAC			FRAP			$1/EC_{50}$		
	r	P	R^2	r	P	R^2	R	P	R^2
TFC	0.2671	0.3483	0.08827	0.5246	0.0799	0.2752	0.3429	0.2752	0.1176

*R^2 is linear regression coefficient. ** $p > 0.05$ indicates insignificant correlation.

of the sample and *vice versa*. The fractions of fruits and leaves of *Ehretia serrata* were analyzed for 7 days for their lipid peroxidation value. Butylated hydroxyanisole was used as standard. Results (Fig. 3) showed that the ethyl acetate fractions of fruits and leaves and aqueous and 1-butanolic fraction of leaves were the most active against lipid peroxidation. This trend also correlated with their high phenolic content (Table-1). Overall the fruits extracts exhibited better antioxidant potential against lipid peroxidation which was comparable with the standard. The hexane fraction of fruits was the least active amongst all, which is in agreement with its low value of antioxidant potential when investigated with other antioxidant assays. The chloroform fraction of leaves showed high lipid peroxidation value indicating the low antioxidant activity.

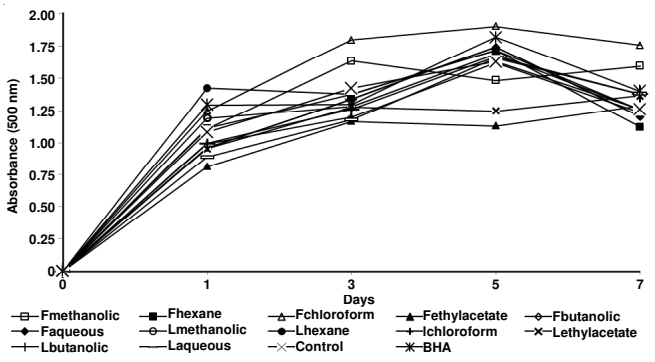

Fig. 3. Increase and decrease in absorbance of various fractions of fruits and leaves along with Butylatedhydroxyanisole (BHA), a standard, over a period of time indicating their lipid peroxidation value. *Each value is the mean of triplicates. **The capital F and L before the fraction name refers to fruits and leaves, respectively

Statistical analysis: ANOVA test of one way analysis was used to compare the means of all the assays. The results indicated the significant difference of $p < 0.05$. Pearson correlation between various assays is listed in Tables 5 and 6. The R^2 values obtained by linear regression analysis are shown in (Fig. 1 and Table-5).

TABLE-6
PEARSON CORRELATION* COEFFICIENT 'r' STUDIED FOR THE TOTAL PHENOLIC CONTENT (TPC), TEAC, $1/EC_{50}$ (DPPH) AND FRAP VALUES ($p < 0.05$)

	TPC	$1/EC_{50}$	TEAC
TEAC	0.8394	0.9072	–
$1/EC_{50}$	0.9697	–	–
FRAP	0.8114	0.7523	0.0548**

**Ehretia serrata* fruits and leaves fractions were used in correlation.
**Insignificant value of P more than 0.05 was observed.

Conclusion

The *in vitro* antioxidant and radical scavenging assays carried out on fruits and leaves of *Ehretia serrata* have indicated that the ethyl acetate fraction of leaves extract was the most active amongst all the fruits and leaves fractions. The high activity of this fraction is credited to its high phenolic content. Ethyl acetate fraction of fruits also showed promising results. The chloroform and 1-butanolic fractions of leaves also exhibited appreciable free radical scavenging potential. On the other hand, hexane fractions of both, fruits and leaves were least effective. Based on this study, it is proposed that the phytochemical investigation into the ethyl acetate fractions may result in the isolation of chemical constituents which can be used as natural antioxidants.

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