

## EVALUATION OF THE ANTIOXIDANT ACTIVITIES OF *PRUNUS DOMESTICA* WHOLE FRUIT: AN *IN VITRO* STUDY

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### ABSTRACT

**Objective:** *Prunus domestica* is known for its nutritional values and therapeutic properties. It contains rich amount of natural phenolic phytochemicals such as phenolic acids, flavonoids and anthocyanin. Previous reports suggest that prune extract and juice inhibit peroxidation process. As no detailed antioxidant property, particularly on the whole fruit was studied, in this investigation, we not only evaluated the detailed antioxidative activities of different fractions of *P. domestica* fruit but also tried to correlate the same with total phenol and flavonoids contents.

**Methods:** Simple warring blender method for extraction, folin- ciocalteu method for total phenolic content, aluminium chloride colorimetric method for total flavonoid content were employed. Different antioxidant activities such as total antioxidant, iron chelating, hydrogen peroxide scavenging of superoxide radical and reducing power method were performed.

**Result:** Simple warring blender method, used in the present study for the preparation of whole fruit extract exhibited better yield potential, total phenolic and flavonoid contents. A comparative study of different fraction types indicated that ethyl acetate fraction had very high phenolic and flavonoid contents that also exhibited maximum antioxidant activity. Butanol fraction also showed somewhat similar results.

**Conclusion:** Ethyl acetate and n-butanol fractions showed maximum antioxidative potential. Further studies will be helpful to explore its therapeutic potential in treating different chronic diseases.

**Keywords:** *Prunus domestica*, Total phenol, Total flavonoid, Antioxidant activities.

### INTRODUCTION

Antioxidant compounds are known to scavenge free radicals and can prevent or delay oxidative damage of lipids, proteins and nucleic acids. Scavenging of the free radicals is extremely important in inhibiting oxidative mechanisms that leads to degenerative diseases [1]. A previous review indicated that intake of fruits rich in antioxidants increases the antioxidant capacity of plasma and reduces the risk of chronic health ailments including cancer and cardiovascular disease [2]. The major part of the fruit comprises antioxidants such as phenolics and flavonoids which have reducing power, free radical scavenging and metal chelating properties [3]. Recently interest has been focused on natural antioxidants, because, on one hand synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ), although commonly used in foods, found to be toxic and carcinogenic [4]. On the other hand polyphenols which are widely present in fruits are believed to possess antioxidant properties. In fact, novel natural antioxidants from different fruits and vegetables have been extensively studied for their antioxidant and/or free radical scavenging activity [5-7]. *Prunus domestica*, which belonging to family rosacea is known for its nutritional values and therapeutic properties. It contains rich amount of natural phenolic phytochemicals such as phenolic acids, flavonoids and anthocyanin which constitute valuable components of our diet both in terms of dietary and medicinal values [8-9]. A previous report suggests that prune extract and juice inhibit oxidation of isolated human low-density lipoprotein [10]. In vitro studies also reveal the antioxidant activity of *P. domestica* fruit peel and flesh with high content of total phenolics and flavonoids, suggesting its radical scavenging abilities [11, 12]. However, no trial was made so far with its whole fruit for its detailed antioxidant property except its DPPH scavenging activity, reported very recently [13].

In fact, no attempt was made so far in *P. domestica* to evaluate its detailed antioxidative activities (total antioxidant, iron chelating,

hydrogen peroxide, scavenging of superoxide radical and reducing power) and to correlate the same with its total phenol and flavonoid contents. Therefore, main objectives of this study were (I) to reveal the diverse antioxidant capacity of different fractions of *P. domestica* fruit and then (II) to correlate the total phenolic and flavonoid contents with its antioxidative activities.

### MATERIALS AND METHODS

#### Chemicals used

Aluminium chloride, ammonium molybdate, ascorbic acid, butylated hydroxytoluene (BHT), ferric chloride (FeCl<sub>3</sub>), ferrozine, Folin-Ciocalteu's reagent, gallic acid, nitroblue tetrazolium (NBT), potassium ferricyanide, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), quercetin, sodium hydroxide (NaOH), sodium nitrate (NaNO<sub>2</sub>), sodium phosphate, trichloroacetic acid and methionine were procured from Sigma Chemicals Co., St. Louis, USA. Ferrous sulphate (FeSO<sub>4</sub>), H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> and all solvents were purchased from Merck, Bangalore, India. Riboflavin was purchased from Himedia, Mumbai, India. All chemicals used were of analytical grade.

#### Plant materials

Fruits of *P. domestica* were collected from Kullu, Himachal Pradesh, India; authenticated by the local taxonomist and a voucher specimen (PD-10/02) of this collection was deposited in the School of Life Sciences, Devi Ahilya University, Indore, India.

#### Extraction

Seeds were removed from *P. domestica* fruits and were extracted thrice with acetone by warring blender method. Briefly, 5 kg of seedless fruit (peel + flesh) were extracted with 80% acetone (w/v) in the ratio of 1: 10 in the warring blender for 5 minutes and then homogenized by polytron homogenizer for 3 minutes. The residue was processed with the same procedure for three times. The combined extracts were filtered through buchner funnel and

evaporated under reduced pressure at 50 °C up to 90 %. The remaining liquid was successively partitioned with hexane, ethyl acetate and n-butanol. The hexane, ethyl acetate and n-butanol extracts were separately pooled and evaporated to dryness under reduced pressure, while the aqueous layer was lyophilised to dryness. The fractions were designated as hexane fraction (HF), ethyl acetate fraction (EF), butanol fraction (BF) and aqueous fraction (AqF) respectively [13].

#### Total phenolic content

Total polyphenols were determined by Folin-Ciocalteu method with some modifications [14]. Briefly, 500 µl of sample was dissolved in 2.5 ml Folin-Ciocalteu's reagent and kept for 2 minutes. Then 2 ml of Na<sub>2</sub>CO<sub>3</sub> was added and adjusted the volume up to 10 ml with distilled water. The solution was well mixed and heated at 45 °C for 15 min and the absorbance was measured after cooling the solution against the blank at 765 nm. Comparisons were made with the standard prepared similarly with known gallic acid concentrations. Results were expressed as gallic acid equivalent per gram of material (mg GAE/g).

#### Total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method [15]. Briefly, 500 µl of the sample extract was diluted with 2 ml of distilled water. To it 0.15 mL of a NaNO<sub>2</sub> (5%) solution was added, followed by 0.15 ml of aluminium chloride hexahydrate (10%) solution after 6 min, and the mixture was allowed to stand for another 6 min. To it 2 ml of 4% NaOH was added, and the total volume was made up to 5 ml with distilled water. The solution was well mixed and was allowed to stand for 15 min. The absorbance was measured immediately against the blank at 510 nm, while the standard was prepared similarly with known quercetin concentrations. Results were expressed in quercetin equivalent (mg quercetin/g).

#### Total antioxidant assay

Total antioxidant was determined using phosphomolybdate reagent with little modification [16]. This method is based on the principle of the reduction of Mo (VI) to Mo (V) by the different antioxidants present in the extracts and ultimately the formation of green colour phosphate/Mo (V) complex is observed [17]. Briefly, 0.1 ml of extract/standard was mixed with 1 ml of phosphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and was incubated at 95 °C for 90 min. After cooling absorbance was taken against the blank at 695 nm. The antioxidant activity is expressed as ascorbic acid equivalent (µg AAE/ml).

#### Determination of antioxidant activity (AA) by different methods

##### Iron chelating assay

Iron is the chief peroxide and is able to generate lipid peroxidation through the Fenton reaction or by accelerating the dissociation of lipid hydroperoxides to their respective peroxy and alkoxy radicals [18]. The ferrous ion-chelating ability was determined according to the method of Dinis et al., (1994) with minor modifications [19]. One ml of sample (with different dilution) was mixed with 2.16 ml of distilled water and 1 ml of 0.125 mM FeSO<sub>4</sub> in a test tube. The reaction was initiated by the addition of 1 ml of 0.3125 mM ferrozine. The solutions were well mixed and allowed to stand for 10 min at room temperature. BHT was used as standard. After incubation, the ability of the sample to chelate ferrous ion was calculated relative to the control at 562 nm using the following formula

$$\text{Chelating effect (\%)} = \frac{((A_0 - A_1))}{A_0} * 100$$

where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance of the samples or standard.

##### Hydrogen peroxide assay

The hydrogen peroxide assay is relevant as phenols contribute electron to hydrogen peroxide and reduce it to water [20]. This assay

was performed according to the method of Ruch et al. (1989) with minor modifications [21]. A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (50 mmol/L, pH 7.4) and its concentration was determined by absorption at 230 nm using a spectrophotometer. Test extract in phosphate buffer was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. BHT was used for comparison. The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\% \text{ Scavenging (H}_2\text{O}_2) = \frac{(A_0 - A_1)}{A_0} * 100$$

where A<sub>0</sub> is the absorbance of control and A<sub>1</sub> is the absorbance of test/standard.

##### Scavenging of superoxide radical

The superoxide radical is a lethal free radical. The hydroxyl radical produced through Haber-Weiss reaction produces toxic effects by interacting with DNA, membrane lipid and proteins. NBT causes the photochemical reaction in the presence of riboflavin and the plant extracts are believed to reduce it. NBT is reduced into tetrazolyl radical in the presence of O<sub>2</sub>, finally getting transformed to the formazan. In the presence of an antioxidant, it donates an electron to NBT and the purple colour of the formazan diminishes [17]. The effect of the extract on superoxide anion radicals was estimated according to the method described previously [22-23]. The reaction mixture contained 1 mL each of riboflavin (3.3×10 mol L<sup>-1</sup>), methionine (0.01 mol L<sup>-1</sup>) and NBT (4.6×10 mol L<sup>-1</sup>). After adding 1 mL of sample of different concentrations, the reaction mixture was illuminated at 4000 lx and 25 °C for 30 min. BHT was used as standard. The absorbance of the reaction mixture was measured at 560 nm and the scavenging percentage was calculated using the following formula:

$$\% \text{ Scavenging (superoxide radical)} = \frac{(A_0 - A_1)}{A_0} * 100$$

where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the sample/standard.

##### Reducing power assay

The basic principle of the assay is the conversion of Fe<sup>3+</sup> ferricyanide complex used in this procedure to the ferrous form, as the colour change occurs with the presence or absence of ferrous ions in solution. An estimation of the extracts reducing power was determined by the procedure described by Oyaizu [24]. Briefly, 2.5 ml of each concentration of the extract (2.5 mL) was mixed with phosphate buffer (2.5 mL; 0.2 M; pH 6.6) and potassium ferricyanide solution (2.5 mL; 1% w/v) and incubated at 50 °C on a water bath for 20 min. After cooling, trichloroacetic acid (2.5 mL, 10% w/v) was added. An aliquot of 2.5 mL was withdrawn and 0.5 mL of ferric chloride solution (0.1% w/v) was added. The absorbance of the resulting prussian blue solution was measured at 700 nm after 2 min against a reagent blank. The EC<sub>50</sub> values were calculated both for the extract as well as for the standard, BHT.

##### Statistical analysis

Readings for all scavenging assays were taken in triplicate. Graph Pad Prism 5 software was used to calculate the IC<sub>50</sub> values. Pearson correlation coefficient for phenolic and flavonoids was also employed.

## RESULTS AND DISCUSSION

### Total phenolic, flavonoid content and extraction yield (Table 1)

Phenolic compounds are very important constituents of plants and their radical scavenging activity is due to their hydroxyl group present in it. They undergo complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent. Following the extraction by different solvent systems different yields were obtained for these compounds. Maximum percentage extraction yield was obtained for aqueous fraction (21.88 ± 2.56 %) followed by n-butanol, ethyl acetate and hexane fraction respectively. Ethyl acetate fraction of *P. domestica* had highest

amount of polyphenol ( $33.88 \pm 0.18$  mg GAE/g), while the lowest was observed in hexane fraction ( $6.28 \pm 0.12$  mg GAE/g) (Table 1). Our results are somewhat different from the previous reported work. According to one report, methanolic extract contains 4.42 mg GAE/g dry weight [25]; while another report indicated 0.27 to 0.54 mg/ g fresh weight [26]. However, Mariaa et al., reported that, phenolics content ranged from 0.42 to 1.09 mg/ g fresh weight in five plum cultivars, while, Kim et al., found a range from 1.74 to 3.75 mg/ g fresh weight in six plum cultivars [27, 28]. As flavonoids are well known antioxidants, it is believed that the plants which are rich in flavonoids have potent antioxidant activity. This may also be true in our study, in which a good amount of total flavonoid content ( $47.96 \pm 0.36$  mg quercetin/g) was found to be present in ethyl acetate fraction of *P. domestica*; while minimum concentration was present in hexane fraction ( $2.06 \pm 0.3$  mg quercetin/g). Result of an earlier study reported 1.03 mg rutin /g fresh weight of flavonoids in *P. domestica* pulp [29]. Thus our process appears to provide a better yield in ethyl acetate fraction.

**Table 1: Total phenolic and flavonoid contents and extraction yield of *P. domestica* fractions**

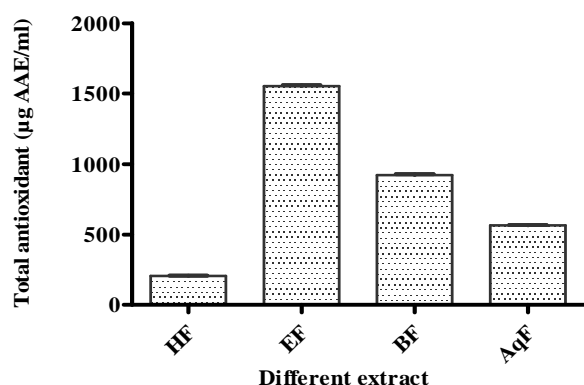
Plant extracts	Total phenolic (mg GAE/g)	Total flavonoid (mg quercetin/g)	Percentage extraction yield
HF	$6.28 \pm 0.12^b$	$2.06 \pm 0.3^b$	$1.02 \pm 0.3^b$
EF	$33.88 \pm 0.18^c$	$47.96 \pm 0.36^d$	$8.97 \pm 0.53^c$
BF	$32.65 \pm 0.27^c$	$37.2 \pm 0.21^c$	$11.77 \pm 2.12^d$
AqF	$15.25 \pm 0.19^a$	$32.2 \pm 0.43^a$	$21.88 \pm 2.56^a$

Each value in the table is represented as mean  $\pm$  SD (n = 3). Means not sharing the same letter (a-d) are significantly different at  $P < 0.01$  in each column.

### Antioxidant activities

#### Total antioxidant assay

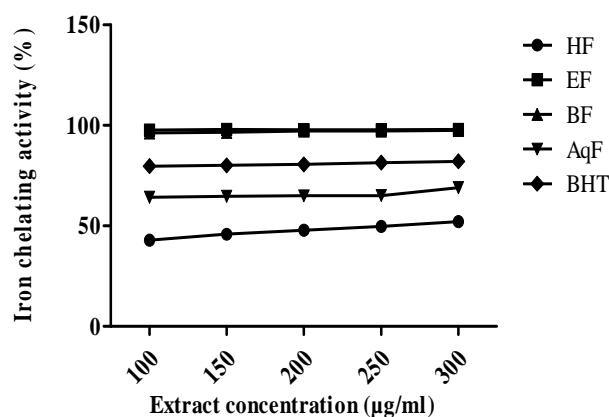
Figure 1 shows the total antioxidant activity of all fractions. All the test samples exhibited noticeable total antioxidant activity, which for the different fractions were determined in the range from  $205.82 \pm 2.52$  to  $1554.15 \pm 5.73$   $\mu$ g AAE/ml and the total antioxidant were found in the order of EF>BF>AqF>HF (Table 2). However, the highest phosphomolybdenum reduction was observed in ethyl acetate fraction ( $1554.15 \pm 5.73$   $\mu$ g AAE /ml), which was probably because of higher phenol and flavonoids content. Contrast to our finding, in another study total antioxidant capacity of three plum cultivars was found to be much less [30].



**Fig. 1: Total antioxidant capacity of different fractions of *P. domestica* at different concentrations. (HF, hexane fraction; EF, ethyl acetate fraction; BF, butanol fraction; AqF, aqueous fraction).**

### Iron chelating assay

Iron generates LPO by accelerating the dissociation of lipid hydroperoxides to their respective peroxy and alkoxy radicals [18]. In our study iron chelating percentage was higher for ethyl acetate and n-butanol fraction and it increased with increase in concentration. Ethyl acetate and n-butanol fraction showed highest metal chelating activity (97.92 % and 97.49% respectively, (Fig. 2). The former fraction had the highest  $Fe^{2+}$  chelating effect with  $IC_{50}$  value of  $0.116 \pm 0.000$ , followed by n-butanol, aqueous and hexane with  $IC_{50}$  value of  $0.116 \pm 0.000$ ,  $0.170 \pm 0.001$  and  $0.230 \pm 0.001$  mg/ml respectively (Table 2). Other species of *Prunus* showed somewhat similar results exhibiting ethyl acetate fraction with highest iron chelating effect, but with high  $IC_{50}$  value of  $480 \pm 0.47$  mg/ml for pulp and  $450 \pm 0.22$  mg/ml for peel [31].



**Fig. 2: Iron chelating activities of different fractions of *P. domestica* at different concentrations. (HF, hexane fraction; EF, ethyl acetate fraction; BF, butanol fraction; AqF, aqueous fraction)**

### Scavenging of hydrogen peroxide

Different fractions of *P. domestica* scavenged hydrogen peroxide in a concentration dependent manner (Fig. 3). When a comparison for  $IC_{50}$  value was made, the hydrogen peroxide scavenging activity of ethyl acetate and n-butanol with value of  $0.116 \pm 0.000$  mg/ml and  $0.120 \pm 0.000$  mg/ml respectively were more effective than that of aqueous and hexane extracts and BHT with value of  $0.131 \pm 0.000$ ,  $0.227 \pm 0.002$  and  $0.136 \pm 0.001$  respectively (Table 2). The potential of all fractions to scavenge hydrogen peroxide as shown by  $IC_{50}$  values were significantly different from  $IC_{50}$  value obtained for standard BHT. The scavenging abilities on hydrogen peroxide were in order of EF > BF > AqF > BHT > HF. Interestingly, in comparison to a previous report [32] in which *P. domestica* showed 30-79 % of scavenging activity, in the present study, ethyl acetate and n-butanol fraction exhibited a better activity of about 99 % scavenging activity for hydrogen peroxide.

### Scavenging of superoxide radical

While the maximum scavenging ability of 64-75 % was observed for ethyl acetate fraction, BHT showed 52-59 % at different concentrations (Fig. 4). Probably the higher scavenging activity of ethyl acetate is due to its high content of total phenol and flavonoids with hydroxyl group (O-H) that is easily liberated for stabilization of superoxide anion. The highest superoxide scavenging activity of ethyl acetate fraction is also supported by its  $IC_{50}$  value of  $0.167 \pm 0.000$  mg/ml, whereas n-butanol had  $IC_{50}$  value of  $0.168 \pm 0.000$  mg/ml as compared to BHT having  $IC_{50}$  value of  $0.195 \pm 0.000$  mg/ml. Lowest superoxide scavenging activity was reported for aqueous and hexane fractions with  $IC_{50}$  value of  $0.196 \pm 0.000$  mg/ml and  $0.318 \pm 0.001$  mg/ml respectively (Table 2).

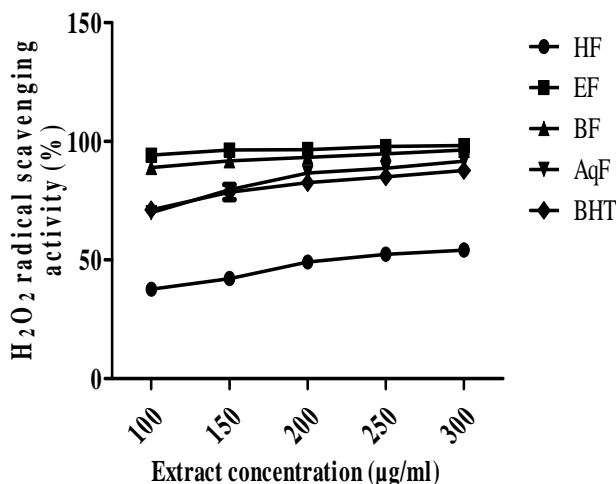


Fig. 3: Hydrogen peroxide scavenging activities of different fractions of *P. domestica* at different concentrations. (HF, hexane fraction; EF, ethyl acetate fraction; BF, butanol fraction; AqF, aqueous fraction)

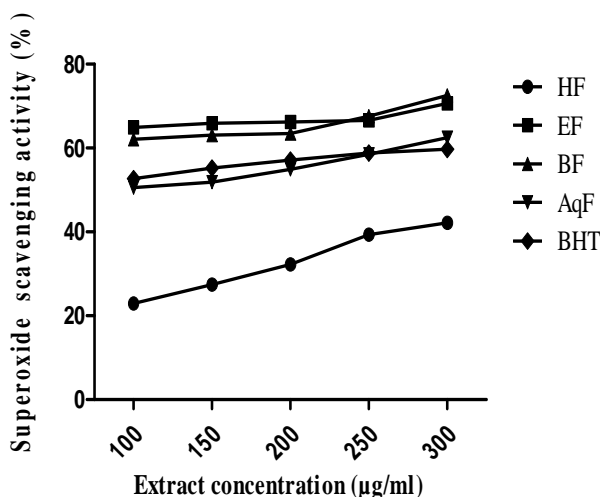


Fig. 4: Superoxide scavenging activities of different fractions of *P. domestica* at different concentrations. (HF, hexane fraction; EF, ethyl acetate fraction; BF, butanol fraction; AqF, aqueous fraction)

#### Reducing power

The increase in absorbance with increased concentrations of various fractions of *P. domestica* indicated that the plant contains plenty of electron donors that can reduce the oxidized intermediates of lipid peroxidation processes [30]. Reducing power of all fractions increased with increase in concentration (Fig. 5). However, ethyl acetate showed maximum reducing power (99%), followed by n-butanol (97-98%) and aqueous fraction (92-97%); whereas least reducing power was observed for hexane fraction (40-60%). The ethyl acetate fraction exhibited  $EC_{50}$  value of  $0.113 \pm 0.000$  mg/ml and proved to have higher reducing power than the n-butanol ( $0.114 \pm 0.000$  mg/ml), aqueous fraction ( $0.117 \pm 0.000$  mg/ml) and hexane fraction ( $0.204 \pm 0.001$  mg/ml). By comparing these findings with that reported by Paul et al. [33], we observed that the reducing power of ethyl acetate fraction of *Prunus sp.* obtained from whole

fruit were higher than the pulp and peel fractions, which with the same solvent system showed a reducing power of 18-20% [33].

#### CORRELATION OF PHENOLS AND FLAVONOIDS CONTENT WITH ANTIOXIDANT ACTIVITIES

All the antioxidant activities showed significant correlations with both total phenolics and flavonoids. Superoxide radical scavenging property showed high correlation with total phenol ( $R^2=0.99$ ) and total flavonoids ( $R^2=0.82$ ).  $IC_{50}$  of hydrogen peroxide scavenging showed high significant correlation with both total flavonoids ( $R^2=0.97$ ) and total phenols ( $R^2=0.81$ ). Similarly, iron chelating activity showed highly significant value for total flavonoids ( $R^2=0.94$ ) and moderate value for total phenol ( $R^2=0.68$ ). Nearly similar results were obtained for reducing power for total flavonoid ( $R^2=0.90$ ) and total phenols ( $R^2=0.63$ ). Total antioxidant also showed significant correlation with both total phenols ( $R^2=0.83$ ) and total flavonoid ( $R^2=0.80$ ). Thus, a positive correlation was established with the active components of the test material.

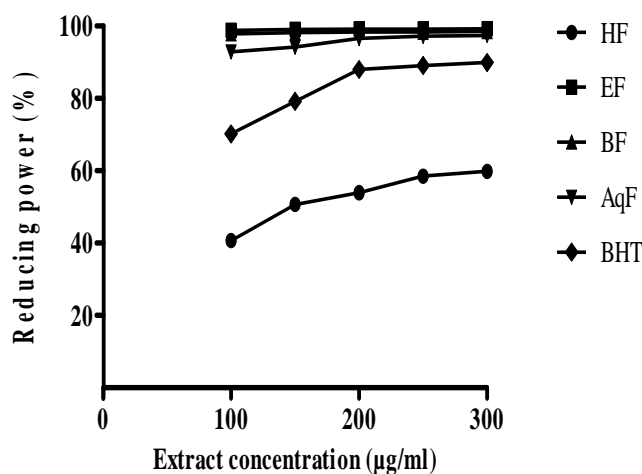


Fig. 5: Reducing power of different fractions of *P. domestica* at different concentrations. (HF, hexane fraction; EF, ethyl acetate fraction; BF, butanol fraction; AqF, aqueous fraction)

#### CONCLUSION

For the first time, in the present investigation using four different solvent systems (hexane, ethyl acetate, n-butanol and water), phenolic and flavonoid contents as well as different antioxidant activities were evaluated in whole fruit of *P. domestica*. We suggest that simple warring blender method, used in the present study for the preparation of whole fruit extract, may have better yield potential and more total phenolic and flavonoid contents. A comparative evaluation of different fractions of *P. domestica* whole fruit showed that ethyl acetate fraction contains highest antioxidative activities. Similar results were observed in n-butanol fraction. In the light of these results, we conclude that highest antioxidant activity in ethyl acetate fraction was possibly due to its high phenolic and flavonoid contents. Therefore, we suggest that the ethyl acetate and n-butanol fractions of *P. domestica* may be further studied to explore the therapeutic potential in treating different chronic diseases.

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#### CONFLICT OF INTEREST

The authors have no conflict of interest.

**Table 2: Antioxidant effect, IC<sub>50</sub> values of DPPH; iron chelating, hydrogen peroxide, superoxide scavenging activities and EC<sub>50</sub> of reducing power with total antioxidant of *P. domestica* fruit**

Plant extracts/ chemicals	IC <sub>50</sub> /EC <sub>50</sub> mg/ml				
	Iron chelating	H <sub>2</sub> O <sub>2</sub>	Superoxide radical	Reducing power*	Total antioxidant (µg AAE/ml)
HF	0.230 ± 0.001 <sup>b</sup>	0.227 ± 0.002 <sup>b</sup>	0.318 ± 0.001 <sup>b</sup>	0.204 ± 0.001 <sup>b</sup>	205.82 ± 2.52 <sup>b</sup>
EF	0.115 ± 0.000 <sup>a</sup>	0.116 ± 0.000 <sup>a</sup>	0.167 ± 0.000 <sup>a</sup>	0.113 ± 0.000 <sup>a</sup>	1554.15 ± 5.73 <sup>d</sup>
BF	0.116 ± 0.000 <sup>a</sup>	0.120 ± 0.000 <sup>a</sup>	0.168 ± 0.000 <sup>a</sup>	0.114 ± 0.000 <sup>a</sup>	920.48 ± 7.07 <sup>c</sup>
AqF	0.170 ± 0.001 <sup>c</sup>	0.131 ± 0.000 <sup>c</sup>	0.196 ± 0.000 <sup>c</sup>	0.117 ± 0.000 <sup>c</sup>	564.26 ± 3.72 <sup>a</sup>
BHT	0.138 ± 0.001 <sup>a</sup>	0.136 ± 0.001 <sup>a</sup>	0.195 ± 0.000 <sup>a</sup>	0.131 ± 0.000 <sup>a</sup>	-

Each value in the table is represented as mean ± SD (n = 3). Means not sharing the same letter (a-d) are significantly different at P < 0.01 and probability level in each column. \*Value is expressed in EC<sub>50</sub>

**Table 3: Correlation between *P. domestica* fractions antioxidant activities and total phenolic and flavonoid contents**

Assays	Correlation R <sup>2</sup>	
	Phenols	Flavonoids
IC <sub>50</sub> of superoxide radical scavenging	0.9972	0.8222
IC <sub>50</sub> of hydrogen peroxide radical scavenging	0.8182	0.9732
IC <sub>50</sub> of Iron chelating assay	0.6894	0.9402
Reducing power assay	0.6333	0.9078
Total antioxidant	0.8328	0.8072

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