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Phytochemical screening, phenolic content and antioxidant activity of wild date palm (*Phoenix sylvestris* Roxb.) fruit extracted with different solvents

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Abstract

Fruits are known to have different levels of antioxidant capacity due to the presence of multiple types of phenolic compounds in varying amounts. In this study phytochemical constituents, phenolic content, flavonoid content, and antioxidant activity of *Phoenix sylvestris* fruit were investigated. Three solvents (methanol, ethanol, and acetone) with different concentrations (50%, 70% and 100%) and water were used for extraction. Phytochemical analyses showed the presence of tanins, flavonoids and saponins. Fruits extracted with 70% acetone showed highest total phenolic content (1554.85 mg GAE/100 g FW) and total flavonoid content (2121.77 mg CEQ/100 g FW) whereas lowest total phenolic content (212.94 mg GAE/100 g FW) and lowest total flavonoid content (130.41 mg CEQ/100 g FW) were found in H_2O extract. Antioxidant activities of different solvent extracts were evaluated using ferric reducing antioxidant power (FRAP) assay, total antioxidant capacity assay, reducing power assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assays. With all the assay methods, 70% acetone extract showed the highest antioxidant capacity. It was also observed that the phenolic content were strongly correlated with the antioxidant activity.

Introduction

Human body is characterized by continuous production of free radicals and other reactive oxygen species due to aerobic metabolism. At the same time antioxidants and antioxidant enzymes exert synergistic action in removing the free radicals (Uttara *et al*., 2009). Under physiological conditions oxidising agents and antioxidant defenses are in balance. However, if the production of free radicals exceeds the antioxidant capacity of a living system, it leads to several physiological and pathological abnormalities such as inflammation, gastric ulcer, cardiovascular disease, cancer, neuro-degenerative ailments, diabetes mellitus, AIDS and aging (Jacob and Burri, 1996; Stangeland *et al*., 2009; Ismail *et al.,* 2010).

According to the polyphenol content, different fruits show different antioxidant capacities (Saura-calixto and Goni, 2006) and recent research has confirmed that vegetables and fruits play an important role in the prevention and treatment of different diseases caused by oxidative damage (He *et al*., 2006; Galeone *et al*., 2007; Hung *et al*., 2015). Flavonoids are the largest group of phenols found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine and play a role against oxidative stress, inflammation, allergy, viral infection, and cancer (Middleton, 1998).

The phenolic composition, content and distribution of fruits varies with ripeness, cultivar specificities, cultural practices, geographic origin, growing season and postharvest storage conditions (Gul *et al*., 1998; Miletic *et al*., 2012). Different methods are used for the extraction of phenolic compounds from different parts of plant. These methods differ in solvent system and condition used. Solvents such as water and aqueous mixtures of methanol, ethanol, acetone and ethyl acetate have been used for the extraction of polyphenols from plant materials (Wizekoon *et al.*, 2011; Chavan and Amarowicz, 2013). The extraction method also plays a crucial role in estimating accurate antioxidant content and capacity. So it is very difficult to compare data from the literature reports (Asami *et al*., 2003; Alothman *et al.*, 2009; Do *et al.*, 2014).

Phoenix sylvestris Roxb., together with 13 other species, forms the genus Phoenix. In a phylogenetic study combining morphological, anatomical and genetic data, it appears close to the date palm (*Phoenix dactylifera* L.) and to *Phoenix theophrasti. P. sylvestris* is widely distributed in South Asia, from Pakistan to Myanmar, across India, Nepal, Bhutan and Bangladesh (Barrow, 1995). This palm

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produces edible fruits but it is generally called, "wild date palm" to distinguish it from the closely related *P. dactylifera* which is known as "date palm" and is cultivated agriculturally as the commercial source of dates (Bharti, 2015). The fruit of *P. sylvestris* Roxb., locally known as Khejur and ripened in the month of June-July, is underutilized in Bangladesh. It is cooling, oleaginous, cadiotonic, fattening, constipative, good in heart complaints, abdominal complaints, fevers, vomiting and loss of consciousness (Parmar and Kaushal, 1982). The fruit is also used to treat back pain, stomachache, toothache, headache, arthritis, pain of buttocks, piles, nervous debility, and as nervine tonic, restorative, sedative in ethnomedicine. Methanol extract of this fruit possesses strong central and peripheral antinociceptive activity as well as CNS depressant, sedative and anxiolytic activity (Shajib *et al.*, 2015). There are few studies on its antioxidant and pharmacological properties. Previously our research group (Aklima *et al*., 2014) showed highest phenolic content and antioxidative activities of this fruit among five Bangladeshi fruits by DPPH and ferric reducing methods using 70% ethanol as solvent whereas Prakash *et al*. (2013) determined the phenolic content and antioxidative activities of *P. sylvestris* fruit and leaf by same methods using 50% methanol. However effects of solvent systems on the extraction of phenols and antioxidant activities of the fruit have not been reported.

The main objectives of this study were to determine the phenolic contents and detail antioxidant activities of the *P. sylvestris* fruit grown in Bangladesh and to examine the efficiency of different solvent systems for the extraction of phenolic compounds. The phenolic constituents were extracted using three solvents, namely, methanol, ethanol and acetone with different proportions of water.

Materials and Methods

Plant materials

Fresh fruit sample without any physical and microbial damage were collected from Chittagong region (Bangladesh) at maturation stage and the species was identified consulting Siddiqui *et al.* (2007). The sample was stored at -20°C prior to analysis.

Chemicals and reagents

Folin–Ciocalteu's (FC) phenol reagent, sulphuric acid, hydrochloric acid, methanol, acetone and ethanol were from Merck (Darmstadt, Germany). Sodium nitrite, sodium acetate, aluminium chloride, ammonium phosphate, ammonium molybdate, ferric chloride and sodium carbonate were purchased from R and M Chemicals (Essex, UK). Ascorbic acid, potassium ferricyanide [K3Fe(CN)6], 2,4,6-Tris (1-pyridyl)-5-triazine (TPTZ), DPPH, Gallic acid and catechin were supplied by Sigma–Aldrich (St. Louis, MO, USA). All chemicals and reagents used in the study were of analytical grade.

Extraction of phenolic compounds with different solvents

First, the fruit was cleaned with distilled water and wiped with paper towel and weighed whole after removing the seeds. The edible portions were cut into small pieces and stored at -40°C before lyophilization using a freeze dryer. After lyophilization the fruits were pulverized into powder form with a grinder and kept at -40°C before analysis. Then 2 g fruit samples were mixed separately with 20 ml of three different solvents-water extraction systems (methanol, ethanol and acetone) at three different concentrations (50, 70 and 100%), and distilled H_2O. Each mixture was stirred with magnetic stirrer at room temperature (25^0C) for 4 hours and filtered with Whatman No. 41 filter paper. The residues remained after filtration were again treated in the same way and the resulting two filtrates for each solvent were mixed and stored at 4°C. All the analyses were done within three days of extraction.

Phytochemical screening of the fruit extract

Different solvent extracts were used for phytochemical screening of compounds which include tannins, flavonoids, alkaloids and saponins according to the methods of Harborne (1998) and Trease and Evans (1989) with slight modifications.

Test for tannins

Exactly 1.0 ml of fruit extract for each solvent was dissolved in 10 ml of distilled water and filtered (using Whatman No. 1 filter paper). A blue coloration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract.

Test for alkaloids

0.2 ml of extract for each solvent was taken in a conical flask and ammonia solution (3 ml) was added. It was allowed to stand for few minutes to evaluate free alkaloids. Chloroform (10 ml) was added to the conical flask, shaken by hand, and then filtered. The chloroform was evaporated from the crude extract by water bath and Mayer's reagent (3 ml) was added. A cream color precipitation was obtained immediately that showed the presence of alkaloids.

Test for flavonoids

1.0 ml of each solvent extract was taken in a test tube and few drop of dilute NaOH solution was added. An intense yellow color was appeared in the test tube. It became colorless upon addition of a few drop of dilute acid that indicated the presence of flavonoids.

Test for saponins

1.0 ml of extract for each solvent was taken in a test tube and diluted with 20 ml of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

Determination of total phenolic content

The total concentration of phenol (TP) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1998). 20 µl of sample extract was added to 2.58 ml distilled water and 100 µl of Folin-Ciocalteu reagent was added. After 1 min interval 300 µl of 20% sodium carbonate solution was added. After 2 hour incubation at room temperature, resulting blue color was read at 765 nm. Samples were analyzed in triplicates. A calibration curve was prepared, using a standard solution of gallic acid (0.005 - 0.05 mg/ml, $r^2 = 0.997$). Results were expressed on fresh weight basis (FW) of edible part as mg gallic acid equivalents (GAE)/100 g of sample.

Determination of total flavonoids

Total flavonoid contents (TF) of the fruit extracts were determined according to the colorimetric assay developed by Zhishen *et al.* (1999). 200 µl of fruit extract were mixed with 4.8 ml of distilled water. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH was added and the volume was made up to 10 ml, immediately by the addition of 2.4 ml of distilled water. The mixture was shaken vigorously and the absorbance was read at 510 nm. A calibration curve was prepared using a standard solution of catechin (20 - 200 mg/l, $r^2 = 0.988$). The results were also expressed on a FW basis (edible part) as mg catechin equivalents (CEQ)/100 g of sample.

DPPH free radical scavenging activity

DPPH free radical scavenging activity of the fruit sample was measured according to the method of Hossain *et al.* (2008) with slight modification. The reaction mixture (total volume, 3 ml) was consisted of 8 µl sample, 0.5 ml of a 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 1.492 ml of a 50% (v/v) ethanol aqueous solution. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured later, at 517 nm, against a blank of ethanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

% inhibition of DPPH= (Abs control -Abs sample)/ Abs control X 100

where Abs control is the absorbance of DPPH solution without extracts.

Total antioxidant capacity assay

The assay was based on the reduction of molybdenum (VI) to molybdenum (V) by the sample and subsequent formation of a green phosphate/ molybdenum (V) complex at acidic pH (Prieto *et al*., 1999). 400 µl of sample solution was combined with 4.0 ml of reagent solution (600 mM sulphuric acid, 28 mM ammonium phosphate, and 4 mM ammonium molybdate), incubated at 95°C for 90 min, and cooled to room temperature. The absorbance was measured at 695 nm against a blank. A calibration curve was prepared using a standard solution of ascorbic acid (0.005 - 0.16 mg/ml, $r^2 = 0.985$). The antioxidant capacity of extracts was expressed as mg equivalent of ascorbic acid/100g of FW (mg EAA/100 g).

Reducing power activity

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Phosphate buffers (2.5 ml, 0.2 M, pH 6.6) containing different concentrations of the extract were prepared and added to 2.5 mL of 1% (w/w) potassium ferricyanide, and mixed. After incubation at 50°C for 20 minutes, the mixtures were mixed with 2.5 mL of 10% (w/w) trichloro¬acetic acid followed by centrifugation at 650 g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absor¬bance was measured at 700 nm. A calibration curve was prepared using a standard solution of ascorbic acid (0.02 - 0.16 mg/ml, $r^2 = 0.989$). The antioxidant capacity of extracts was expressed as mg equivalent of ascorbic acid/100g of FW (mg EAA/100 g).

Ferric reducing/antioxidant power assay (FRAP assay)

FRAP assay was performed according to a modified method described by Benzie and Strain

Table 1. Phytochemical screening of different extract of *P. sylvestris* fruit

Solvents	Phytochemical compounds			
	Alkaloids	Tannins	Flavonoids	Saponins
100% H_2O	-	-	+	-
50 % Methanol	-	+	+	++
70% Methanol	-	-	+	++
100% Methanol	-	+	+	-
50% Ethanol	-	+	+	++
70% Ethanol	-	++	+	++
100% Ethanol	-	++	+	+
50 % Acetone	-	++	+	-
70% Acetone	-	+++	+	+
100% Acetone	-	+	+	-

+++ = appreciable amount (positive within 5 mins.); ++ = moderate amount (positive after 5 mins but within 10 mins); + = trace amount (positive after 10 mins but within 15 mins); - = completely absent.*Reducing sugar depending on intensity of color.

(1999). Briefly, a 40 µl fruit extract was mixed with 3 ml of FRAP reagent and the reaction mixture was incubated at 37ºC for 4 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 h. FRAP reagent was pre-warmed at 37° C and freshly prepared by mixing 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl with 2.5 ml of 20 mM FeCl3. $6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6. A calibration curve was prepared, using an aqueous solution of ferrous sulphate, FeSO4. 7H2O (0.2 - 1.6 mM, $r^2 = 0.997$). Ascorbic acid was used as positive control. Results are expressed as mM Fe^{2+}/ mg sample.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate measurements. Data were statistically analyzed using SPSS version. One-way analysis of variance (ANOVA) and Pearson correlation coefficients were determined and the significant difference was set at $p < 0.05$.

Results and Discussion

Phytochemical analysis

The phytochemical analysis of fruit *P. sylvestris* showed the presence of tannins, flavonoids and saponins (Table 1). Tannins play an important role in the prevention of cancer and also used for the treatment of inflamed and ulcerated tissue (Mota *et al*., 1985; Aiyegoro and Okoh, 2010). The major active nutraceuticals ingredients in plants are flavonoids. They can act as potent antioxidants and metal chelators. They also have long been recognized to possess antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (Tapas *et al.*, 2008). Saponins are also known to have many pharmacological properties including antiinflammatory (Hasan *et al*., 2012), anticholesterolemic, adjuvant hemolytic and anticancer (Tiwari *et al*., 2014). Saponins are also used as a major constituent of traditional Chinese medicine (Liu and Henkel, 2002). So, wild date palm fruit could be used as a traditional medicine for many health beneficial effects.

Alkaloid was not found in this fruit. A number of alkaloids are used as drugs. But many alkaloids block the action of neurotransmitter (Robert and Wink, 1998) and absence of alkaloids in this fruit has no risk to block the neurotransmitter. Moreover, it was found that the extraction of phytochemicals was solvent dependent e.g. although extraction of tannin was highest with 70% acetone but saponins level was higher with 50% and 70% methanol and ethanol compare to 70% acetone.

Total polyphenolic content

Table 2 represents the total phenolic content (TPC) of fruit extracts estimated using Folin-Ciocalteu's colorimetric method and expressed as gallic acid equivalents (GAE). The TPC of the fruit extracts ranged from 212.94 – 1554.85 mg of gallic acid/100 g FW of edible part of the fruit. Fruit extract prepared with 70% acetone showed highest TPC when compared to other solvents extracts. Previous study showed different phenolic contents in different variety of date fruits grown in different geographical locations. Among the six varieties of Tunisian dates Bejo showed highest phenolic content (576.48 mg GAE/100 g of FW) in 70% acetone (Kchaou *et al*., 2013). Al-Turki *et al.* (2010) used 80% acetone as solvent and showed that the range of total phenolic content in different cultivars of *Phoenix dactylifera* fruits was 225.0 to 507.0 mg GAE/100 g of FW. In another study Mohamed *et al*. (2014) reported that the phenolic content in 80% methanolic extract of the six varieties of Sudanese date fruits ranged from 35.82 to 199.34 mg GAE/100 g of dry weight. Thus it is evident that *P. sylvestris* fruit has higher phenolic content than the different variety of *P. dactylifera* fruits in spite of variation from other studies in the

Table 2. Total phenolic*content and total flavonoid*content of fruits extracts obtained from different solvent extraction systems

Solvents	Total Phenolics (mg GAE/100 g fresh weight)	Total flavonoids (mg CEQ/100 g fresh weight)
100% H_2O	212.94 ± 2.16^{a}	130.41 ±9.47^{k}
50 % Methanol	621.29 ± 20.69^{b}	633.27 ±46.43^{l}
70% Methanol	413.36 ± 19.88^{c}	171.80 ±13.21^{m}
100% Methanol	697.70 ± 12.08^{d}	690.95 ± 12.09^{n}
50% Ethanol	787.88 ± 19.28^{e}	272.12 ± 30.40^{o}
70% Ethanol	972.01 ±53.72^{f}	895.36 ±48.90^{p}
100% Ethanol	909.38 ±81.56^{gef}	823.88 ± 66.56^{q}
50 % Acetone	1430.47 ±25.02^{h}	1670.33 ±15.05^{r}
70% Acetone	1554.85 ±18.02^{i}	2121.77 ±30.10^{s}
100% Acetone	414.61 ±8.68^{jc}	678.41± 7.83^{tni}

*Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically significant at the 5% level

extraction method and sample preparation. High phenolic content (128.6±2.7 mg/g of dry weight) was also found in the *P. sylvestris* fruit grown in India (Prakash *et al.*, 2013).

The total flavonoid content (TFC) of this fruit extract was also measured. 70% acetone also showed the highest TFC comparing with the other solvent extracts (Table2). Correlation analysis was also performed between the TPC and TFC of the fruit extracts. The correlation between the TPC and TFC assay was 0.913 which was highly significant at the 0.01 level.

Effect of solvent system

Previously Methanol, ethanol, acetone, propanol, ethyl acetate and dimethylformamide have been commonly used as solvents at different concentrations in water for extraction of phenolic content from plant materials (Antolovich *et al*., 2000; Luthria and Mukhopadhyay, 2006).The recovery of phenolic content from fruit is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, the polarity of solvent plays a crucial role in increasing the phenolic compounds' solubility (Naczk and Shahidi, 2006). Therefore, it is difficult to develop an excellent extraction procedure suitable for extraction of all plant phenols.

From the results shown in Table 2, it is apparent that the recovery of phenolic compounds was dependent on the solvent used and its polarity. Among all the solvent extracts, 70% acetone was found as the most efficient for extracting phenolic compounds compared to all other solvent systems used, the level was 1554.85 mg GAE/100 g fresh weight, whereas H2O gave the lowest recovery of total phenolics (212.94 mg GAE/100 g fresh weight). Results also showed that 50% acetone was an efficient solvent after 70% acetone whereas 70% methanolic extract was an inefficient solvent for extracting phenolic compounds after H2O with significant differences between them ($p < 0.05$). On the other hand, for comparison among 50% ethanol, 70% ethanol and pure ethanol, there is no significant difference among the three solvent systems.

Various studies have shown the effect of different solvents on total phenolic compounds and antioxidant activities. Several studies showed 70% acetone as efficient solvent for extracting phenolic compounds from different fruits or vegetables (Alothman *et al*., 2009; Boulekbache-Makhlouf *et al.*, 2013; Kchaou *et al*., 2013; Boeing *et al.*, 2014). Again, of the three different aqueous solvents (50% acetone, 70% methanol and 70% ethanol), 50% acetone extracted the highest amount of phenolic compounds from two varieties of whole wheat and bran samples (Abozed *et al.*, 2014). Similarly, Rebey *et al*. (2012) indicated that the highest total phenolic contents were present in the 80% acetone extract of two provenances of mature green cumin seeds. However, the lowest total phenolic compounds were derived from water extract. Our result is similar to the above findings and suggests that acetone/water mixtures are good solvents for the extraction of phenolic compounds present in *P. sylvestris* fruit.

Antioxidant activity

Large varieties of different antioxidants present in fruits. So, it is very difficult to measure the all the antioxidants of plant extract through a single method. Different methods have been established to measure the antioxidant capacity of different plant materials (Guo *et al.*, 2003). Table 3 shows the antioxidative activity of *P. sylvestris* fruit extract in different solvents measured by different methods.

DPPH free radical-scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable organic nitrogen radical. DPPH react with phenol through two different processes: Direct separation of H atom from phenol and by electron transfer. Both of these pathways depend on either the nature of

Table 3. Antioxidant capacity* of fruit extracts obtained from different solvent extraction system

Solvents	(%) DPPH inhibition Concentration	Total antioxidant capacity (mg AAE/g fresh weight)	Reducing power activity(mg AAE/gfresh weight)	FRAP(Mm Fe(II)/g fresh weight
100% H_2O	18.86 ±3.18^{a}	10.32 ± 0.77^{k}	9.09 ±0.43^{A}	103.73±3.86^{K}
50% Methanol	36.77±2.62^{b}	21.05 ±1.27^{l}	77.68±3.41^{B}	616.38±4.35^{L}
70% Methanol	25.28±0.99^{c}	16.95 ±1.44^{m}	26.55±3.21^{C}	312.51±26.11^{M}
100% Methanol	39.42±3.86^{db}	21.56 ±0.56^{nl}	59.98±8.64^{D}	746.60±7.24^{N}
50% Ethanol	27.19±1.28^{ec}	14.36 ±0.76^{o}	23.60±4.60^{EC}	272.22±15.13^{OM}
70% Ethanol	58.82±2.48^{f}	24.62 ±0.15^{p}	85.55±2.95^{F}	793.88±3.39^{P}
100% Ethanol	52.68±1.04^{g}	22.92 ±0.59^{ql}	80.64±3.07^{G}	763.97±2.43^{Q}
50% Acetone	91.57±0.32^{h}	32.82 ±1.41^{r}	188.07±10.43^{H}	1445.80±28.82^{R}
70% Acetone	92.95±0.24^{i}	36.32 ±0.91^{s}	251.99±20.31^{I}	1964.57±11.29^{S}
100% Acetone	45.42±2.71^{j}	8.98 ±0.52^{t}	75.23±2.95^{JB}	655.93±10.13^{T}

* Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically significant at the 5% level

solvents or the redox potential of the involved species. Antioxidants react with DPPH and neutralize the free radical. The color of the reaction mixture changes from purple to yellow. The intensity of discoloration measures the potentiality of antioxidant scavenging activity (Vladimir-Knežević *et al*., 2011).

Table 3 shows that 70% and 50% acetone solvent extracts had strong DPPH free radical scavenging activities (92.95% and 91.57%, respectively) and H_2O had shown the lowest scavenging activity (18.86%). Furthermore, the TPC and DPPH scavenging activity showed strong correlation (0.912) between them. All solvent extracts showed dose dependency in scavenging DPPH free radical. From the dose dependency curve, it was again observed that 70% acetone exhibited strong inhibitory ability of free radicals (Figure 1).

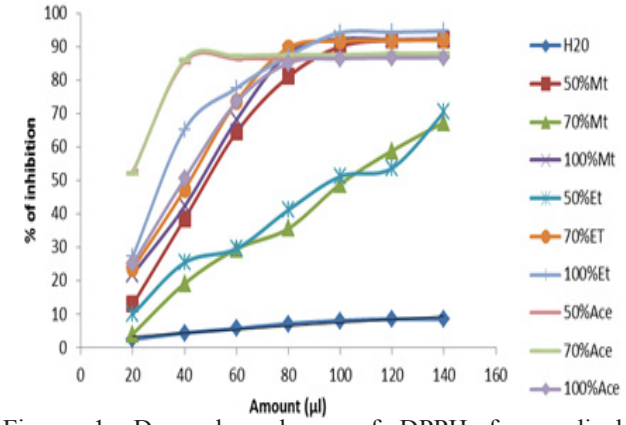

Figure 1. Dose dependency of DPPH free radical scavenging of *P. sylvestris* at different amount of fruit extracts.

Total antioxidant capacity

Total antioxidant capacities of different solvent extracts of *P. sylvestris* was measured by phosphomolybdenum method. From the Table 3 and Figure 2 it was found that 70% acetone had the highest total antioxidant activity (36.32 mg AAE/g of fresh weight). When compared, total antioxidant activity and phenolic content showed well correlation between them except for water. While H_2O had less phenolic content than 100% acetone but it showed higher total antioxidant activity. The potency of different solvent extracts was decreased in the following order: 70% acetone > 50% acetone > 70% ethanol > 100% ethanol> 100% methanol > 50% methanol > 70% methanol > 50% ethanol > H2O > 100% acetone. The correlation between TP and total antioxidant capacity was 0.917.

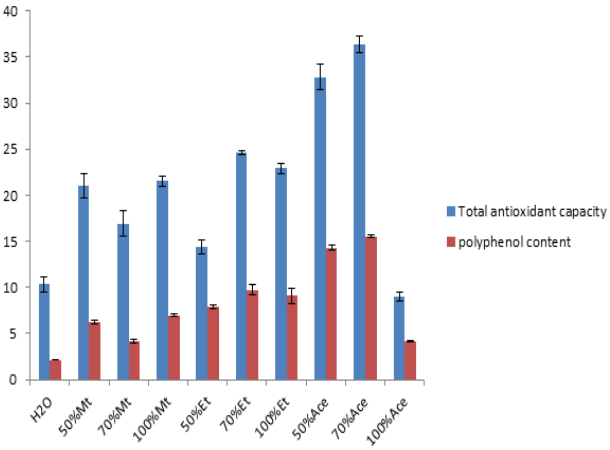

Figure 2. Total antioxidant capacities of different solvent extracts of *P. sylvestris* fruit in comparison with polyphenolic compounds.

Reducing power assay

Many studies have mentioned that the reducing power activity of bioactive compounds depends on the electron donating capacity which is associated with antioxidant activity. Reducing power properties are generally associated with the presence of reductones which exhibit antioxidant action by breaking the free radical chain. Furthermore reductones can diminish the oxidized intermediates of lipid peroxidation. The reducing power assay measures the electron donating ability of antioxidants using the potassium

ferricyanide reduction method. Antioxidants reduce the Fe^{3+}/ferricyanide complex and convert it to the ferrous form and activity is measured in the absorbance at 700 nm (Vladimir-Knežević *et al*., 2011).

The reducing power of different solvent extracts is presented in Table 3. Result showed that best reducing power was obtained from 70% acetone (251.98 mg AAE/g of fresh weight). Kchaou *et al.* (2013) also found highest reducing power of a different variety of Tunisian date extracts with 70% acetone. It was also observed that the phenolic contents of different solvents extract correlated with the reducing power activity, 70% acetone showed the highest reducing power activity in which highest phenolic compound was present. Similarly H_2O expressed lowest reducing power activity and H_2O contained lowest phenolic compound. And the correlation between TPC and reducing power was 0.885.

Ferric reducing/antioxidant power assay (FRAP assay)

The result of FRAP assay of different solvent extracts is expressed in Table 3. The highest reducing power was found in 70 % acetone (1964.57 mM Fe(II)/g fresh weight) when compared to the reducing power of ascorbic acid (4978.57 mM Fe(II)/g). The antioxidant activity of FRAP assay was correlated with the amount of total phenolic content. According to phenolic content 70% acetone showed the highest reducing activity and H_{2O} showed least reducing activity and they were significantly different. The correlation coefficient was 0.884.

Conclusion

This study confirmed the in vitro antioxidant activity of *P. sylvestris* fruit. The recovery of phenolic compounds was solvent dependent and 70% acetone was the most potent whereas H_2O was the least potent solvent for phenol extraction. A strong correlation between the total phenolic contents and antioxidants activities proved that phenolic compounds contributed to the antioxidant activity. Thus the fruit can be considered as a source of natural antioxidant for food and nutraceutical product.

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