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Phenolic compounds of *Pyrus communis* fruit methanol extract and evaluation of antioxidant activity

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ABSTRACT

Pear (*Pyrus communis*.) fruit is popular among consumers due to its high nutritive value, good taste and low caloric level. This study examined polyphenolic content, antioxidant activity and phenolic profile of methanol extract of *Pyrus communis* fruit. Extract were screened for possible antioxidant activities using free radical scavenging activity of DPPH, hydroxyl scavenging activity and reducing power assays. This extract contain high polyphenolic and flavonoids contents (172.5 ± 0.005 mg GAE/g dry extract and 5.34 ± 0.003mg QE/g dry extract) respectively. Similarly, this extract showed high antioxidant activity using DPPH, hydroxyl radical scavenging and reducing power with an IC_{50} value of 0.682 ± 0.022 mg/ml, 0.47 ± 0.06 mg/ml and 1.17 ± 0.029 mg/ml respectively. The phenolic profile in the methanolic extracts was investigated using Ultra high liquid chromatography (UPLC). Four different phenolic acids gallic acid, chlorogenic acid, hydroxybenzoic acid and protocatechuic acid have been identified. Some flavonoids have also been identified such as rutin and kaempherol derivative. In conclusion, the present study showed that *Pyrus communis* exhibited good antioxidant activity which is probably related to phenolics and flavonoids present in the extract.

Keywords: *Pyrus communis,* phenolic compounds, flavonoids, antioxidant activity.

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INTRODUCTION

Pyrus communis is a usual fruit crop of temperate climates such as North Africa. It is the fifth most widely produced fruit in the world, being produced mainly in China, Europe, and the United States. *Pyrus communis* belongs to *Rosaceae* family. Pears are typically eaten fresh [1]. The quality of pear fruit is influenced by both external (size, shape, and color) and internal appearances (nutritional and taste qualities) [2]. *Pyrus* has high nutritional value with reasonable amounts of sugars, amino acids and minerals Contents of free sugars and organic acids play an important role in the nutritive value and quality of the fruit, due to the formation of sour taste and regulation of chemical reactions in the body [3, 4]. It has also higher dietary fiber level than most common fruits and vegetables [1]. Pears contain also other nutritional and bioactive components such as polyphenols. Which are important bioactive compounds known for their health benefits. Those health benefits of polyphenol consumption derive from their antioxidant and anti-inflammatory properties [5]. Other prohealth properties that are attributed to pears are related to the content of triterpenoids, due to their antioxidative, anti-inflammatory and anticancer properties [6]. Phenolic compounds contribute also to the sensory quality of fruit (color, astringency, bitterness and flavor). Phenolic compounds are generally more concentrated in the peel than in the fruit flesh [7].

Therefore, goal of the present study is to evaluate antioxidant activity and the determination of bioactive compounds (phenolic compounds, flavonoids and tannin) in pear pulp and peel.

MATERIALS AND METHODS

Fruit materials

Fresh *Pyrus communis* fruit was purchased from commercial market in Amoucha. Sétif (Algeria).

Extraction procedure

Phenolic cmopounds were extracted from the fruit according the method of Markham [8] with slight modification. 100 g of the consumed parts of the fruits were washed with water mixed with 1 liter of methanol- water solution (85:15 v/v, 50:50 v/v) and kept at room temperature for 5 days. The filtrate was evaporated using a vacuum rotary evaporator at 40 C° to obtain crude methanol extract and the crude extract was dreid.

Identification of phenolics compounds

Phenolic compounds were identified using UPLC coupled with diode array detector. The analytes were determined at temperature of 30° on an analytical column (1.9µm* 3nm* 50mm) .The mobile phase consisted of the solvent (A) water and (B) and acetonitrile using a gradient elution of 5% B from 0 to 1 min, 5%-21% B from 1 to 5 min, 21%-50% B from 5 to 7 min, 50%-100% B from 7 to 10min, 5% B from 10 to 13 min. Phenolic compounds were identified by the comparison between the retention time and spectrograms of extract with that of standards.

Estimation of polyphenols content

Total soluble phenolics in the methanol extract of *Pyrus communis* were determined using Folin– Ciocalteu reagent according to Li [9] with slight modifications. Briefly, 200 µl of extract mixed with 1000 µl of Folin–Ciocalteu reagent after 5 min 800 µL of sodium carbonate (7.5%) was added to the mixture. This was incubated at room temperature in the dark for 90 min. The absorbance was measured against a blank at 760 nm. The standard curve was made using gallic acid. Phenolic contents were expressed as mg equivalent gallic acid /g dry extract (EGA).

Estimation of flavonoids content

Determination of total flavonoid was based on a colorimetric test using aluminum trichloride, described by Bahorun, [10]. In brief, 1 ml extract mixed with 1ml of $AlCl_3$ solution. After 10 min the absorbance was measured at 430 nm. The result was expressed as mg equivalent quercetin /g dry extract (EQ).

DPPH radicals scavenging activity of the extract

The bleaching rate of a stable free radical DPPH was monitored at a characteristic wavelength in the presence of the pear sample. The radical form of DPPH absorbs at 517 nm upon reduction by an antioxidant. This activity was measured according the previously described method of Yardpiroon [11], briefly 0.5 ml of DPPH solution (0.1 mM) was added to 0.250 mL of pear extract. After 30 min, the absorbance was measured at 517 nm.

DPPH scavenging effect (%)= $[(A_{Control}-A_{Sample}/A_{Control})\times 100]$,

Where $A_{Control}$ is the absorbance of the DPPH reaction and A_{Sample} is the absorbance in the presence of pear extract. Rutin was used as a standard.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by the ability of the fruit extract to scavenge the hydroxyl radicals according to Smirnoff and Cumbes [12] with slight modifications. 100 µL of varying concentration of samples or Vit C standard antioxidants mixed with 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (6 mM), 0.3 ml of sodium salicylate (20 mM). This mixture was incubated at 37 C° for 1 h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as flows: Scavenging rate = [Acontrol – Asample] / Acontrol× 100.

Where $A_{control}$ was the absorbance of the control (without sample) and A_{sample} was the absorbance in the presence of the sample

Reducing power of extract

The assay was based on the reducing power of a compound (antioxidant) as previously described by Ebrahimzadeh [13]. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}), the latter forms a blue complex, which increases the absorption at 700 nm. Briefly, 100 µl of the extract was mixed with an equal volume of 0.2 M phosphate buffer (pH= 6.6) and 1% of potassium ferricyanide [$K_3Fe(CN_6)$]. The reaction was incubated at 50°C in a water bath for 20 min. Then 250 µl of 10% trichloroacetic acid was added to stop the reaction followed by centrifugation for 10 min at 3000 rpm. 250 µl of the upper layer of solution was mixed with 250 µl of distilled water and 500 µl of $FeCl_3$ and the absorbance was measured at 700 nm against a blank. BHT was used as a positive standard.

Statistical analysis

The experiments were performed in triplicate. The results were expressed as mean ± SD.

RESULTS AND DISCUSSION

Identification of phenolics compounds and flavonoids in the extract

The UPLC chromatogram of *Pyrus* fruit extract (Fig 1) showed the presence of phenolic compounds such as gallic acid, chlorogenic acid, Kaempferol and protocatchuic acid. The major constituent is rutin and it derivatives.

Many studies on polyphenol composition conclude that hydroxycinnamic acids and arbutin are the main phenolic compounds in pear [4, 14,15].

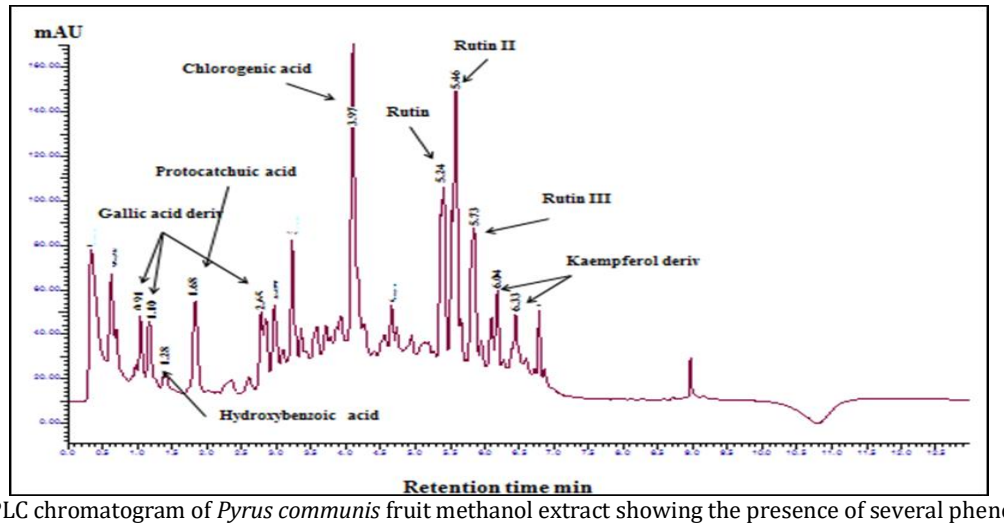

Figure 1: UPLC chromatogram of *Pyrus communis* fruit methanol extract showing the presence of several phenolic acid and flavonoids.

Estimation of polyphenols and flavonoids content

Phenolic compounds represent an extremely chemically diverse subclass of secondary metabolites that occur ubiquitously in plants and therefore constitute an integral part of the human diet. Total phenolic and flavonoids compounds are determined by the Folin–Ciocalteu reagent and $AlCl_3$ method respectively. In this study, the amount of polyphenols and flavonoids in peel and pulp pear fruit are important. This amount is very high compared to other studies. Kolniak-Ostek, [16] reported that peel of pear contains 9.176 mg/1 g DW and pulp contains only 2.342 mg of phenolic compounds in 1 g of dry pears. In this study pear contain 172.5 mg GAE/ g DW. The content of phenolic compounds in pears is related to the anatomical part. Variability of phenolics in plant tissues depends on many factors, such as environmental conditions, including temperature, UV light, and nutrition [17].

Table 1: Total phenolics and flavonoids contents in pears fruit methanol extract.

Extract	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg QE/ g DW)
Pyrus communis	172.5 ± 0.005	5.34 ± 0.003

Results are expressed as means ± SD (n=3).

DPPH radicals scavenging activity of the extract

Free radical scavenging potentials of pear extract at different concentrations were tested by the DPPH method, the results are shown in Fig 2. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α, α-diphenyl-*β*-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract**.** In this study, free radical was scavenged by fruit extract with an inhibition percentage of 75.23% and an IC_{50} of 0.61 ± 0.03 mg/ml, this result is low compared to the IC_{50} values obtained for rutin (0.15 ± 0.005 mg/ml). Similar result was reported by Manzoor *et al* [18].

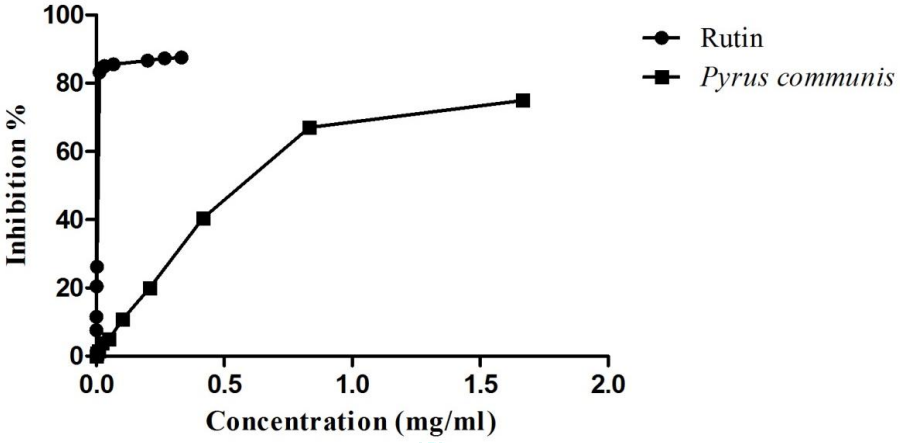

Figure 2: DPPH free radical scavenging activity of fruit extract and standard

Hydroxyl radicals scavenging activity of the extract

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [18]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity [19]. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids [20]. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using H_2O_2- salicylate acid. The hydroxyl radical scavenging activity of extract is shown in Figure 3. In the present investigation, the methanol extract of pear exhibits hydroxyl radical scavenging activity 66 % at a concentration of 1mg/ml**.**

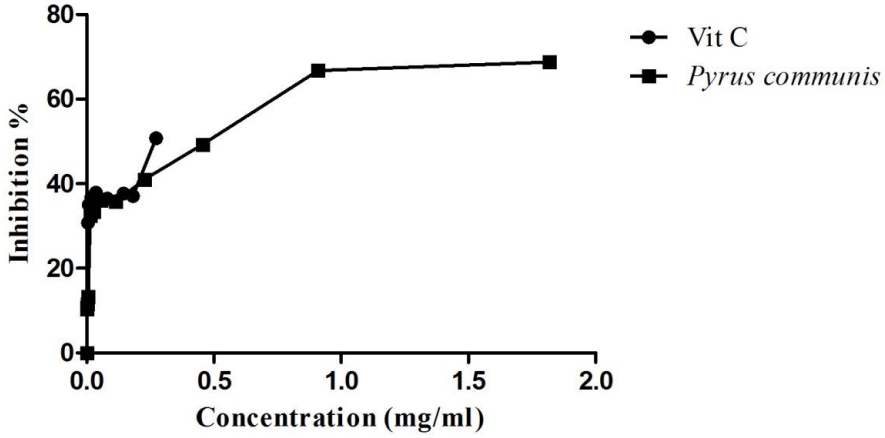

Figure 3: Hydroxyl radical scavenging activity of fruit extract and standard.

Reducing power of the extract

The reducing power method reflects the ability of an antioxidant present in the extract to donate electron to convert Fe^{3+} into Fe^{2+}. The amount of the Fe^{2+} complex was followed by measuring the formation of Perls' Prussian blue color at the absorbance of 700 nm [21]. The reducing power of fruit extract present in the figure 4. Our finding showed that *Pyrus* has an important ability to reduce ferric. At 0.1 mg/ml reducing power of extract and standard compound (BHT) exhibited the following order: BHT (0.769± 0.001)> extract (0.064 ± 0.002). Similar study established that extract of pears (pulp and peel) had a weaker activity with 0.06 at 7.5 mg/ml [22].

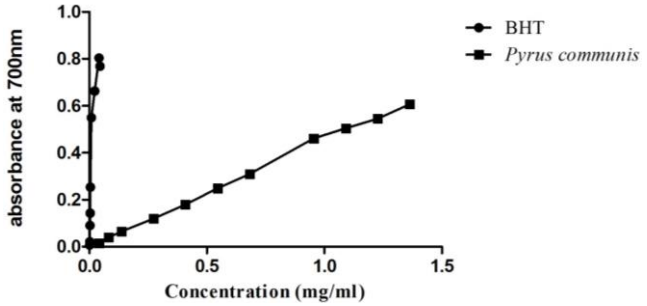

Figure 4: reducing power activity of fruit extract and standard

Fruits contain a large family of phytochemicals like vit C, carotenoids, organic acid and phenolic acid. Several study related the potential of antioxidant activity with the presence of phenolic compounds in the extract. In this study, pear contains several compounds and the major compound present is rutin and their derivatives. Yang and their colleagues [23] demonstrate that rutin has a noticeable effect on scavenging free radicals. Kolniak-Ostek [24] obtained an important relationship between the DPPH radical and reducing power and antioxidant potential of Kaempferol (r= 0.70 for DPPH and r= 0.86 for reducing capacity). For biologically active substances found in fruits and vegetables, among others polyphenol compounds can be included. The strong antioxidant properties of these compounds are releated to the number of hydroxyl groups present in the molecule. Depending on the location and amount of hydroxy, methoxy, and the rest of the glycosides, the biological activity of polyphenolic compounds and their physical and chemical properties differ [25].

CONCLUSION

In this study, polyphenols contents and identification and antioxidant capacity of pear fruit was evaluated. Consumed parts of the fruit contain considerable amounts of phenolic compounds. This research suggests that the use of pear can be a better and cheaper source of phytochemicals. The consumption of pear to us full in the prevention of diseases associated with free radicals.

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