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Research Article

Identification and quantification of fruit phenolic compounds of *Malus communis*

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ABSTRACT

Malus communis (apple) is fruits belong to family *Rosaceae*. Apple is important source of phytochemicals substance, which has good effect in human health and antioxidant activity. The aim of this work is to identify the polyphenols compounds and the antioxidant activity of *Malus communis*. The analyses phytochemicals of polyphenols is estimated by UPLC method. The flavonoids content were determinate by the Aluminum chloride method. The antioxidant activity was evaluated by the DPPH, ABTS and reducing power. The results suggested that *Malus communis* have high content of sugars, tannin (795 ± 0.05 mg D- glucose equivalents per gram dry weight, 31.38 ± 0.006 mg tannic acid equivalents per gram dry weight and important amount of flavonoids 5.08 ± 0.001 mg quercetin equivalents per gram of dry weight) respectively. The chromatogram of apple demonstrated that this fruits contain various substances such as Gallic acid and Chlorogenic acid. The extract exerted good effect in antioxidant activity. Apple can scavenge free radicals ABTS and DPPH with values of IC_{50} (0.64 ± 0.02 , 0.60 ± 0.03 mg/ml respectively). Finally the consumption dietary of fruits can reduce the risk the chronic disease.

Keywords: *Malus communis*, UPLC, tannin, flavonoids, antioxidant activity.

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INTRODUCTION

Free radicals are normal molecules product by cell body. These compounds are very reactive and have important role in natural processes [1]. The high level of free radical induced stress oxidative which associated with various diseases such as cancers, atherosclerosis, arthritis, stroke, asthma [2]. Fruits and vegetables may play an important role to decrease of the mortality rate of cardiovascular and reduce blood pressure and others diseases [3]. This positive influence is attributed to some natural antioxidant phytonutrients [4]. The most thoroughly investigated dietary compounds in fruits acting as antioxidant are fiber, polyphenols, flavonoids and vitamins [5]. The amount of polyphenols is very important than the ascorbic acid in fruits and have good antioxidant activity [6]. Polyphenols act as anticancer or cardio-protective agents by a variety of mechanisms [7]. In this research, the apple used and is growing in Algeria. It has high nutritional value with reasonable amounts of sugars, amino acids and minerals like sodium, potassium, calcium, magnesium and iron [8]. It has also higher dietary fiber level than most common fruits and vegetables, giving excellent results in the treatment of constipation and intestine inflammation [9]. Apple also contain other nutritional and bioactive components as polyphenols [10]. The aim of this study is the determination,

analysis of polyphenols compounds and evaluates the antioxidant activity in vitro of Apple fruit extract. The continuous eating of fruits reduces the incidence of serious diseases.

MATERIALS AND METHODS

Fruits materials

The fruits of *Malus communis* were purchased from commercial market in Amouche from Setif (Algeria). In this study, the fruit used freshly.

Preparation of extract

The extract fruit was obtained according the method of Markham [11]. 100g of fruits after homogenized mixed with 1L methanol- water (85:15 v/v, 50:50 v/v). After 5 day the mixture was filtered. The supernatant was evaporated using vacuum rotary evaporator at 40 C°.

Identification of polyphenols

A ultra -performance liquid chromatographic method equipped with diode-array detection was used to identified the major phenolic compounds (gallic acid, naringenin, chlorogenic, catechin, syringic acid) in apple. Chromatographic separation was performed on a Hypersil column ($1.9\mu\text{m} \times 3\text{nm} \times 50\text{mm}$). The flow rate was kept

constant throughout the analysis at 0.6 ml/min and the injection volume was 20 μ l. The operating condition were as follows: mobile phase water (A) and acetonitrile (B): gradient 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 10 min, 5% B from 10 to 13 min. The column was maintained at 30° and UV detection was recorded in the range 165 nm- 365 nm. The peak identification in samples was also based on comparisons of the retention times (TR) of the isolated phytochemical standards.

Total flavonoids contents

The total flavonoids content was determined by the using the Aluminum chloride colorimetric method of Bahromun [12]. The standard curve was prepared using 0-40 μ g/ml solution of Quercetin. The results are expressed in milligram of quercetin equivalent per gram dried extract.

Tannin content

The determination of tannin content is based to the capacity of these compounds for precipitate the hemoglobin. The amount of tannin was estimated according the method of Gharzouli *et al* [13] and they was expressed as mg equivalent tannic acid /g dry extract.

Total soluble sugars content in the fruit extract

The total sugars content was determined using the method of Dubois *et al* [14]. The amount of sugar was expressed in mg/g dried weight.

In vitro antioxidant activity

DPPH radical scavenging activity

The ability of fruit extract to scavenge DPPH radicals was estimated by the procedure of Yardpiroom [15]. A 0.5 ml of extract with different concentration was mixed with 1 ml

DPPH solution (0.1Mm). After 30 min of incubation, the absorbance was recorded at 517 nm. The percentage inhibition was calculated using the following formula.

Percentage inhibition = $(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}} * 100$. The concentration having 50% radical inhibition activity (IC_{50}) expressed as mg extract/ml.

ABTS radical scavenging activity

The method used was described by Re *et al* [16]. This assay is based the ability of extract to inhibit the ABTS radical compared the reference standard vitamin C. The stock solution of ABTS was prepared by the mixed of ABTS (7mM) and 2.45 mM potassium persulfate. After 16 h of incubation the mixture was diluted by the methanol to give 0.7± 0.02 absorbance at 734 nm. 50 μ l of extract was added to 1 ml of ABTS solution. After 30 min of incubation in the dark at temperature room, the absorbance was measured at 734 nm. The percentage inhibition was calculated by the same equation of DPPH assay.

Statistical analysis

The result was expressed in the Mean ± SD. All analyses were determined by the Graph pad (version 5).

RESULT AND DISCUSSION

Identification of polyphenols

The UPLC chromatogram of *Malus communis* fruit extract belong to Rosaceae family revealed the presence of various phenolic acid and flavonoids such as gallic acid, protocachic acid, Syringic acid, naringenin. The phenolic compounds were identified according to their retention times, molecular masses, fragmentation patterns, characteristic spectra, and bibliographical sources. The compound major in this family is Gallic and protocachic acid.

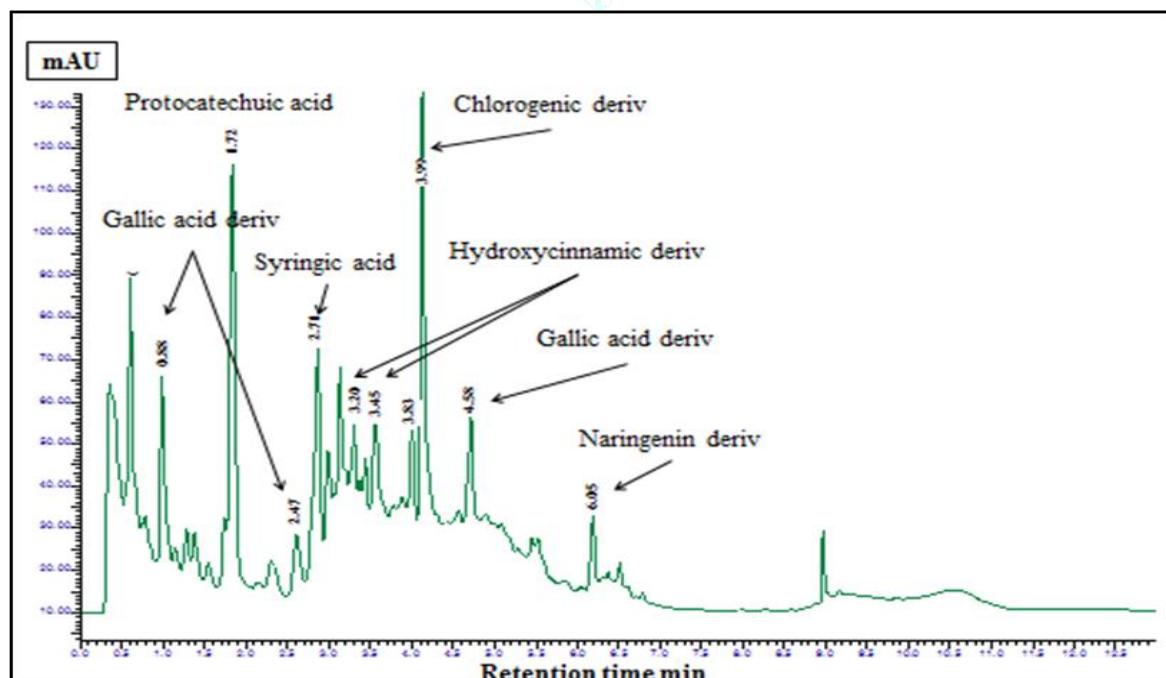


Figure 1: UPLC chromatogram of *Malus communis* fruit methanol extract.

Total flavonoids, tannin content and total sugars in the fruit extract.

Apple is good source of phytochemicals such as phenolic compounds. Which have good health benefits [17]. Those health benefits of polyphenol consumption originate from their antioxidant properties [18]. Flavonoids are the most and important group of phenolic compounds, which are characterized by a benzo-pyrone structure [19]. The total flavonoid content was expressed as mg quercetin equivalents (QE)/ g sample. Tannins are naturally occurring phenolic compounds which precipitate protein. Which are

found in legumes and fruits and important source of dietary antioxidant [20].

The total flavonoids, tannin and total sugars were calculated from the regression equation of calibration curve ($y = 0.035x - 0.046$, $R^2 = 0.996$; $y = -0.0021x + 1.4181$, $R^2 = 0.988$; $y = 0.0107x + 0.157$, $R^2 = 0.994$) respectively. Table 1 represented the amount of flavonoids, tannin and total sugars in *Malus communis*. The result showed that the apple extract contained high level of sugars 795 ± 0.05 flowed by tannin and flavonoids with values $(31.38 \pm 0.006$ mg TAE/g 5.08 ± 0.001 mg QE/g) respectively.

Table 1: flavonoids and tannins contents and total sugars in fruits extracts.

Extract	Total sugar (mg d-glucose E/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)
<i>Malus communis</i>	795 ± 0.05	5.08 ± 0.001	31.38 ± 0.006

QE : Quercetin Equivalent . TAE : Tannic Acid Equivalent. Results expressed as means \pm SD.

Secondary plant metabolites, such as flavonoids or tannins, can be also involved in complex system of antioxidant defense. The basic mechanisms of antioxidant activity of tannins are free radical scavenging activity, chelation of transition metals and inhibition of prooxidative enzymes. Tannins show various health benefit activities, especially antioxidant, antitumor, cardioprotective, anti-inflammatory and antimicrobial activity [21]. There is some research showed that sugars having antioxidant activity and the mechanisms of sugars for exerted the antioxidant effect is not clearly [22, 23].

In vitro antioxidant activity

DPPH radical scavenging activity

DPPH is commercially stable free radical with purple colour. Generally used for evaluated the scavenging activity. This method based the reduction of DPPH solution by the hydrogen and electron donating antioxidant present in the extract, and the formation of new molecule (biphenyl hydrazine), which have yellow colour. The change of colour is result of scavenging of DPPH by the antioxidant. DPPH molecule have high absorbance at 517 nm which decreased when DPPH accept electron or hydrogen [24, 25]. Figure 1. Present the percentage inhibition of DPPH by fruit extract and standard. The results show that fruit extract and BHT can reduce the signal intensity of DPPH and their radical-scavenging activities increased with increasing concentration.

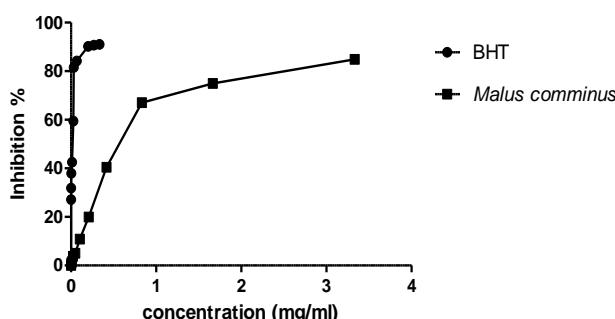


Figure 2: DPPH free radical scavenging activity of fruit extract and standard.

ABTS radical scavenging activity

In this assay, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical, which has a peak absorbance at 734 nm, should be performed by mixing ABTS and potassium persulfate ($K_2S_2O_8$). When antioxidants were added, the ABTS radical, which has a blue-green colour, is reduced to ABTS (no colour) [26]. The ABTS activity was quantified in terms of percentage inhibition of the ABTS radical cation by antioxidant in sample. Figure 2. Shows the ABTS scavenging abilities of apple and reference standards. At 0.01 mg/ml apple extract reduced ABTS only 10% compared Vit C reduced 92 %.

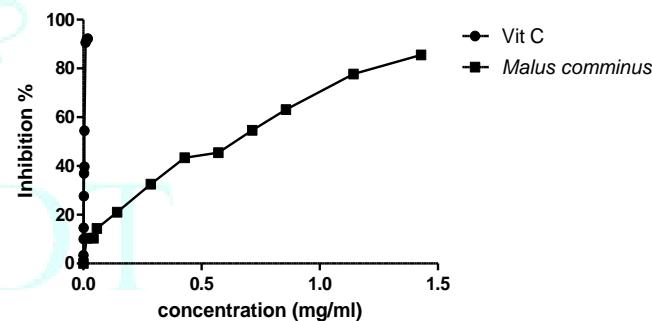


Figure 3: ABTS free radical scavenging activity of fruit extract and standard.

CONCLUSION

In conclusion, fruit extract used in the present study were grown in Algeria. This fruit is widely consumed by the Algerian population. This fruit contain appreciable amounts of total flavonoids and tannins which are responsible for the in vitro properties of these fruits and their consumption can reduce cardiovascular diseases and other pathologies associated with free radicals.

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