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Extraction of phenolic composition and quantification of the total phenolic of pomegranate pomace

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ABSTRACT

In this study total phenolics of pomegranate pomace were determined using the Folin-Ciocalteu method. Total phenolics of the pomegranate pomace were extracted with aqueous ethanol (80 % v/v) using a pomace-to-solvent ratio of 1:4 (w/v).Total phenolic content of pomegranate pomace was 9.58 mg tannic acid equivalent/g. pomegranate pomace extract was rich in total phenolic content showed its potential for antioxidant activity and could be further evaluated as dietary supplements.

Key words: Extraction, pomegranate pomace, total phenolic.

INTRODUCTION

Pomegranate (*Punica granatumL.*) is one of the oldest edible fruits and has been used extensively in the folk medicine of many cultures. Popularity of pomegranate has increased tremendously especially in the last decade because of anti-microbial, anti-viral, anti-cancer, potent anti-oxidant, and anti mutagenic effects of the fruit. The antioxidants contained in fruits and vegetables are including ascorbic acid, carotenoids, flavonids, and hydrolysable tannins [16]. However, pomegranates also have some anti-nutritional factors such as tannins and other secondary compounds.Tannins are polyphenolic substances with various molecular weight and a variable complexity [8]. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups.They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans [6]. Tannin effects on ruminant nutrition have been studied for several years, and are often seen only in terms of their negative impacts on intake and production: decreased nutrient utilization, particularly protein [17]; decreased palatability and consequently the amount of food ingested; decreased digestibility [15,14,13]; volatile fatty acids production reduction, and decreased digestibility of organic matter and fiber [2]; damage of kidney and liver, tissue damage in the rumen, intestine ulceration and morphological changes at the microvilli level [9]. However, besides these anti-nutritional and toxic effects, there is an increasingly awareness of tannin's beneficial roles on animal nutrition and health [5], namely influences on the cell signaling pathways [1], anti-oxidative effects [10], and anti-helmintic and anti-microbial activities [11]. In ruminants a particularly important positive effect of tannins is dietary protein protection from ruminal microflora attack [7]. Due to the binding of tannins to dietary protein, and also to a reduction in the activity of a large proportion of microflora, there is an increased rate of amino acid absorption in the intestine, which improves the utilization of nitrogen by ruminants [12]. As well as binding to protein, tannins can also bind to carbohydrates, leading also to a reduction in ruminal gas production [15]. Due to a combination of these activities tannins can be associated with improvements in animal growth and productivity and consequentially minimization of effects to the environment. The objectives of this study were therefore to determine the levels of phenolic compounds in extracts prepared from pomegranate pomace for further evaluation of their potential in animal nutrition.

MATERIALS AND METHODS

Reagents and chemicals

Folin-Ciocalteu phenol reagent (Sigma Chemical Co), Sodium carbonate, ethanol, tannic acid was obtained from Merck.

Sample collection

Pomegranate pomace (PP) sample were collected from Spoota factories in Urmia West Azerbaijan province, Iran. Different parts of the fruits were used in the study (rind and seeds). whole fruit extracted with ethanol (80 % v/v) using a pomace-to-solvent ratio of 1:4 (w/v).

Tannin extract preparation

A fresh sample of PP (500 g DM/kg fresh weight) was dried and ground to pass a 1 mm sieve. Fifty grams (oven dry matter, Gallenkamp, UK) of PP was treated with 120 ml of an aqueous solution of ethanol (80%). The mixture was heated for 5 minutes. After heating, the pomace was cooled, washed and filtered through filter paper. Then 100 ml of the ethanol was again added to pomace and heated for 10 minutes and filtered through filter paper. The washed liquid was evaporated to a moderate concentration (rotary evaporator, temperature: 50 ◦C) for 30 minutes.

Determination of total phenolics (TP) content of pomegranate pomace

Aliquots (1 ml) of each of the extracts were mixed with 5 ml Folin-Ciocalteu reagent in 100 ml volumetric flasks that contained 70 ml of deionised water. Sodium carbonate solution (15 ml of 20 % m/v anhydrous sodium carbonate in deionised water) was added after 1 min but before 10 min. The volumetric flasks were then made up to volume with deionised water. After standing for 2 h at room temperature, the absorbance was read at a wavelength of 640 nm in the visible range of the spectrum using a UV/Visible spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Cambridge, England). The estimation of total phenols in the extracts was carried out in triplicate. Tannic acid was used as a standard on various concentrations and the results obtained were expressed as mg tannic acid equivalent/g of sample, on a dry weight basis. The TP content was determined according to the Folin–Ciocalteu method reported by reference [4].

Statistical analysis

The least signification different (LSD) was used to compare the data and all tests were considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Standard curve was obtained from plotting of light absorption against different concentration of tannic acid and are shown in figure 1 and the Equation of light absorption against different concentration of tannic acid are shown in Table1. The contents of total phenolics of pomegranate pomace extract are given in Table 2.

Figure 1: Standard curve was obtained from plotting of light absorption against different concentration of tannic acid

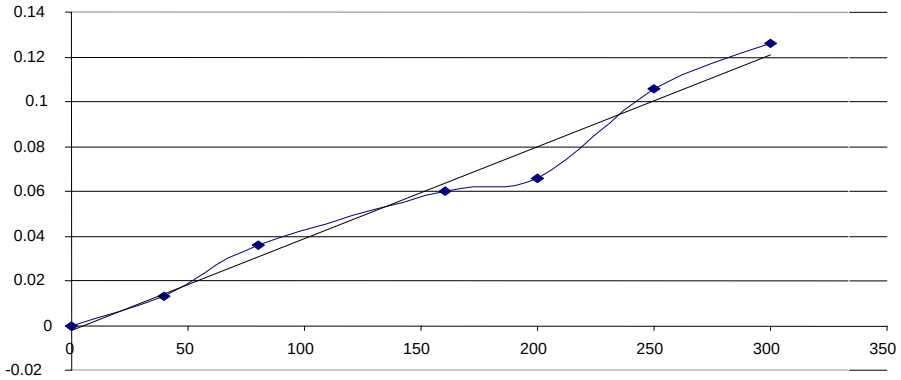

Table 1: Equation of light absorption against different concentration of tannic acid

Standard	curve	Equation	R^2
Tannic ac	Tannic acid standard curve	Y = 0.0004x - 0.0023	0.977

x concentration (in milligrams/milliliters), Y absorbance at 640 nm

Table 2: Total phenolics content (expressed as mg tannic acid equivalent/g) of pomegranate pomace extract on a dry matter basis

Sample	Total phenols
Pomeg Pomegranate pomace	9.58 ±0.32

Values are the means ± standard deviation (n = 3)

Reference [19] reported that in pomegranate peel extracts the amount of phenolic compounds in the acetone extracts in either solvent or ultrasound-assisted solvent extraction methods were the highest (40.0 and 35.0 % for sonication and solvent extraction, respectively (P< 0.05)), followed by methanol (34.5 and 31.0 %), ethanol (25.3 and 23.0 %), and water (10.0 and 12.0 %), and ethyl acetate extracts (0.2 and 0.2 %). ethyl acetate extraction gave the highest content of the total phenolics (20.24%), proanthocyanidins (2.65%) and flavonoids (3.92%) in the extracts. Reference [18] reported that the highest content of the total phenolics (20.24%), proanthocyanidins (2.65%) and flavonoids (3.92%) in the extract.The phenolic composition of pomegranate depends on multiple actors, including climate and variety. Pomegranate pomace extract was rich in total phenolic content showed its potential for antioxidant activity and could be further evaluated as dietary supplements.

CONCLUSION

Pomegranate pomace is a natural product rich in dietary polyphenols. The results obtained from this work show that pomegranate pomace may be exploitable as a potential source of total phenolic such as tannins for possible use as antimicrobial agents and to be use in animal nutrition.

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