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Original Article

Exhaustive Extraction of Compounds from Pomegranate Peel and Flesh Using Solvents of Varying Polarity

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Abstract

Pomegranate fruit is the most studied part of *punica granatum* shrub. The fruit contains enormous amount of polyphenol compounds in the peel and arils (flesh) which are responsible for its antioxidant activity. The polyphenols present are of varying degree of lipophilicity and thus would require solvents of varying polarity to extract them. In this study, the effects of solvent type and homogenisation on extraction yield were considered. The fruit was first separated into peel and flesh and subsequently, one half of each of the peel and flesh were separately homogenised. Ethanol, ethylacetate and hexane were used to extract the polyphenol content of each of the four samples; non-homogenised peel (NP), non-homogenised flesh (NF), homogenised peel (HP) and homogenised flesh (HF) in decreasing order of polarity using maceration method. The extraction was carried out successively using the residue recovered from previous extraction. Ethanol was used for a second time to complete the extraction process. The total extractive yield from the four samples were 27.19, 26.04, 25.03 and 15.61 for HP, NP, HF and NF respectively. The experiment has demonstrated that maceration process can be used to extract compounds from pomegranate to give a yield similar to more sophisticated method and ethanol is a suitable solvent for extracting hydrophilic compounds from the fruit.

Keywords: extraction, pomegranate, polyphenol, solvents, maceration
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Introduction

Antioxidants have aroused scientific interest in the last few decades. Safety issues like carcinogenicity and toxicity of artificial antioxidants have shifted attention to natural sources of these substances[1, 2]. The major antioxidant compounds in plants are phenolic acids, flavonoids, ascorbic acid, tocopherols and the carotenoids. The latter three are essential vitamins while the former two are collectively called polyphenols[3, 4].

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms[5, 6]. They have found tremendous application in the food and cosmetic industry as well as medicine.

Phenolic compounds basically contain at least one phenolic ring with one or more hydroxyl groups attached to it [7]. They can be grouped into phenolic acids, flavonoids, tannins and the less common stilbenes and lignans [5].

A lot of extensive studies have been carried out to determine the most efficient method to extract antioxidant compounds from plant matrices to enable laboratory investigations and commercialisation. The physicochemical properties of the antioxidant to be extracted, the nature of plant matrix, and the polarity of solvent are some factors to consider when choosing any method of extraction. Other parameters like sample-solvent ratio, temperature, size of sample particle and duration of extraction process also affect extraction yield [8, 9].

For food and pharmaceutical application of extracts, ethanol is the most suitable for extracting polyphenols from plants[10]. It has minimal toxicity and it is relatively cheap. Water is also a good extracting solvent but it is limited by its inability to extract non-polar polyphenols and its susceptibility to contamination. Solubility of polyphenols is affected by the number of hydroxyl groups they possess, the length of hydrocarbon and the molecular mass [11].

Pomegranate fruits like other berries have been extensively studied for its antioxidant property. This property has been attributed to the large amount of polyphenols it contains. There are a lot of studies on the pharmacological activity of the fruit. It has found traditional application in the treatment of intestinal worms, diarrhoea, respiratory tract infections and many more.[12-14] The fresh fruit, jams, jellies, and wines are commercially available. Although the economic impact of pomegranates on the food and beverage industry is huge, there is great potential for the use of pomegranate extracts as ingredients in functional foods, cosmeceuticals, nutraceuticals, phytoceuticals, and botanical dietary supplements (BDS).[15] Recently, the fruit extract including the peel is been formulated into dosage form for large scale pharmaceutical production. The non-edible peel is usually discarded as agricultural waste after processing the fruit for its commercial products. However, the peel has been found to be superior in antioxidant activity and polyphenol content compared to the fruit pulp[16].

A lot of extraction techniques have been adopted to maximise polyphenol yield. Polar solvents like water, methanol[6] and ethanol are the most frequently used solvents to extract antioxidant compounds from pomegranate fruit.

In this study, solvents of varied polarity would be employed to extract the polar and non-polar antioxidant components of the peel and flesh (arils) of pomegranate fruit. To also minimise the loss of antioxidant during drying process, the fresh fruit will be used as the extracting matrix.

Materials and Methods

Sample collection and preparation

Fresh pomegranate fruits were purchased from available sources in Terengganu, Malaysia and stored at 4°C until required for extraction. The fruits were then peeled and separated into peel and flesh (aril with intact seeds).

The carpellary membrane was removed and discarded. The flesh was then divided into two; the first half was homogenised (HF) while the second half was left intact (NF). The same was also done for the peel, homogenising half of it and labelled HP and the non-homogenised half labelled NP.

Extraction of sample

100g of each of HF, NF, HP and NP were weighed, separately placed in 1000ml conical flask and extracted with 500ml of 95% ethanol for 72hrs in a dark cupboard with vigorous shaking at intervals. The mixture was then filtered using whatmann No 4 filter paper and the filtrate was concentrated using rotatory evaporator. The marc (residue) for each sample were successively re-extracted using ethyl acetate, hexane and 95% ethanol for the second time using the same procedure as above. The concentrated extracts were then placed in the oven at 50°C to allow for complete evaporation.

Extraction yield

The extraction yield was calculated according to the method of Zhang (2007) using the formula;

W2/W1 * 100% (W1= original weight of sample= 100g, W2= weight of dried extract)

Results and Discussion

The percentage yield of extract from 100g of each of the four samples are shown in the table 1 below.

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Table 1. Percentage yields of extracts

sample	Extract yield (%)				
	ethanol	Ethyl acetate	hexane	Second ethanol	Total yield
HP	18.73	6.46	0.72	1.28	27.19
NP	17.93	8.11	0.54	0.36	26.04
HF	10.86	0.41	1.10	12.66	25.03
NF	10.69	3.65	0.55	0.72	15.61

Methanol, ethanol and water are the most frequently used solvents for the extraction of polyphenols from plant matrix. Due to toxicity associated with methanol and the susceptibility of water to contamination, ethanol has been picked as the polar solvent of choice for this study. Although a study has shown that methanol and water give higher yield than ethanol [17]. This study aimed to exhaust the extractable content of the flesh and peel of pomegranate fruit by considering the effect of homogenisation and using solvents of varying polarity to successively extract sample residue.

From the table above, it is observed that a large percentage of the extract was removed by the first ethanol extraction except for HF where the first ethanol yield was 10.86g while the second ethanol extraction gave 12.66g yield. This could be due to the presence of high amount of fat contained in the seeds of HF hindering extraction. But the removal of fat by hexane before the second ethanol extraction made it easy for the polyphenols to be extracted. Nonetheless, the 1:5 sample to solvent ratio is still justified as reported by Chidambara-Murthy who used a 1:6 ratio with a yield of 10.38% lower than that obtained in this study (27.19**).** The result obtained was similar to that obtained from the result of Yasoubi, who got a yield of about 28% with a sample/solvent ratio of 1:16.[18] However, the extraction methods adopted were different. The exposure of the fat content of the seeds by homogenising HF also gave the highest hexane yield. Also comparing the hexane yield of the two peel samples, it would be observed that there was higher yield in HP than NP which could be due to the exposure of more fatty content from the matrix by homogenisation.

Comparing the homogenised versus non-homogenised samples, the homogenised samples gave higher extract yield (HP, 27.19 and HF, 25.03) as compared to the non-homogenised samples (NP, 26.04 and NF, 15.61). This is due to the increase in surface area of the marc.

The ethyl acetate yield of the peel samples were higher than those of the flesh samples. This could be attributed to the presence of highly polar anthocyanins and flavonoids present in the flesh as compared to less polar ellagitannins present in the peels [EA yield at 0.75%][19].

Homogenisation did not have much impact on yield from the peel samples.

Sample-solvent ratio of the extraction process was 1:5 and proved to be quiet effective considering the low amount of ethanolic extract recovered after a second extraction, except for HF where there is a possibility of interference of exposed fat from the seeds. This high amount of total extract from HF as compared to NF also suggests that homogenisation can increase the extractable compounds from a plant material.

Conclusion

The study has demonstrated that ethanol is one of the most suitable solvents for the extraction of compounds from pomegranate fruit. The high yield obtained using simple maceration method makes it suitable for easy commercialisation knowing that researches have affirmed its enormous antioxidant property as well as other pharmacological effects on physiological organs. The yield obtained from this study is similar or superior to those obtained from other studies using the same or other polar solvents. However, cultivar variation also affects the polyphenolic content and composition of the fruit.

Homogenisation of the samples did not affect the yield of the peel but significantly affected that of the flesh. Thus, maceration which is an age long extraction procedure should still be considered for pomegranate fruit extraction. It is simple to execute and requires little expertise to extract target compounds. This study has also demonstrated that homemade extraction of pomegranate compounds can be achieved with minimal resources since a sample/solvent ratio of 1:5 adopted gave similar value to researches that adopted higher ratios.

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