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Extraction and standardization of oil yield from pomegranate fruit

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Abstract
The pomegranate fruit, scientifically known as *Punica granatum*, is a fascinating and delicious fruit with a rich history and a wide range of health benefits including reduced inflammation, improved heart health and potential anticancer effects. Additionally, consuming pomegranate helps to lower blood pressure, enhances digestion and supports overall immune function. Pomegranate fruits are extracted to obtain valuable antioxidants and other beneficial compounds for various culinary, medicinal and cosmetic applications. In the present paper, the soxhlet extraction of pomegranate fruit with ethanol (99.9%) and methanol (99.8%) as solvent were studied. The oil extraction process was influenced by nature and amount of solvent used, the quantity of sample, temperature and number of hours. The extraction with ethanol as solvent at 65 and 75 °C, was carried out for 1, 3, 9 and 12 hours. Similarly with methanol as solvent at 60 and 65 °C for 12 and 24 hours. The highest yield of 82.66% (w/w) was obtained with 220 ml ethanol as solvent, under the conditions of 75 °C, when extracted for 12 hours. Whereas an oil yield of 80.58% (w/w) was obtained using 250 ml solvent extracted for 24 hours at 65 °C.

Keywords: Pomegranate, solvent extraction, soxhlet apparatus, ethanol, methanol

Introduction
The Pomegranate, (*Punica granatum*) belongs to the family *Punicaceae*. It is a fruit that has been revered for centuries because of its distinct flavour, eye-catching colour, and potential health advantages (Wu and Tian, 2017) [11]. Pomegranate is indigenous to parts of Iran and the Mediterranean, and has gained popularity all around the world. Today, it is cultivated in Asia, North Africa and tropical Africa, North and South America and even in Europe for its fruit as well as decorative tree and shrub. India is the world's top producer of pomegranate followed by Iran and Turkey. Egypt, Afghanistan and the United States are also major producers (Saroj and Kumar, 2019) [5]. Pomegranate fruit is a rounded berry with a thick reddish skin covering approximately 200-1400 white to deep red or purple seeds (Sharma *et al*., 2017) [6].
Pomegranate contains abundant nutrients when compared to many fruits. They include 80 calories per 100 g serving, no fat, no sugar, and 5 g of fibre and 15% of the daily recommended dose of vitamin C. Pomegranate supplies not only vitamin C, but also vitamin B5, B9 and K, it also contains vital minerals potassium and zinc (Sharma *et al*., 2018) [7]. Pomegranate production and consumption have dramatically expanded as people become more aware of the fruit's exceptional therapeutic benefits like lower blood pressure, improved heart health, potential anticancer effect and strong antioxidant activity (Jose *et al*., 2017) [2].
Pomegranate has significant amounts of anthocyanins, which have antioxidant characteristics and are responsible for many of its health advantages. According to Amri *et al*., (2017) [1] various pomegranate parts have different levels of phytochemicals, fatty acids and potential antioxidant effect. The peel has higher quantities of phenolic compounds, the seeds are abundant in bioactive substances such as ellagitannins, anthocyanins and flavonoids. In addition, the pomegranate juice and arils also possess strong antioxidant activity. Punicalagins, which are present in both the juice and peel of the pomegranate, are exceptionally strong antioxidants (Lu *et al*., 2008) [3]. Pomegranate juice has been discovered to have eight times the amount of antioxidant activity as grape, grapefruit and orange juices, respectively and has three times the antioxidant activity of red wine and green tea. The extract and powder of pomegranates are primarily prepared from the peel due to its high punicalagin and antioxidant content (Suman and Bhatnagar 2019) [8].
The objective of the present study was to extract oil from pomegranate fruit with selected solvents such as ethanol and methanol using soxhlet extraction process.

The effects of parameters such as temperature, time and amount of solvent was studied to maximize oil yield.

Materials and Methods

Well ripened pomegranate fruits was obtained from a farm in Vengoor in Ernakulam district. The study was conducted in the Horticulture lab, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore.

Sample Preparation

The fruits were washed thoroughly under running tap water to remove any dirt and entire fruits (including peel and arils along with seeds) were cut into small pieces and dried under room temperature for one week. The cut pieces were pulverized into powder using a heavy-duty mixer to increase the surface area and facilitate the extraction process.

Solvent Extraction and Rotary Evaporation

According to Negi *et al*., (2003) [4] the solvent extraction was carried out in the soxhlet apparatus (Fig.1). A thimble was homogenously packed with powder of whole fruit in filter paper with 460 ml of ethanol and 450 ml of methanol separately to obtain the fruit extract. This process was carried out at boiling temperature of 60 and 65 °C separately using for 99.9% ethanol for 1, 3 and 9 hours. Similarly, the process carried out at boiling temperature of 60 and 65 °C separately using for 99.8% methanol for 12 and 24 hours. The boiling temperature varied according to the solvents and weight of fruit sample taken. The extract was collected in a round bottom flask and evaporated using a rotary evaporator. After that the extract was collected in a petriplate and was left open for some time until the entire solvent had evaporated. Then the extract was preserved in refrigerator at 4 °C until further process (Tunchaiyaphum *et al*., 2013) [9].

Fig 1: Soxhlet Apparatus

Oil Yield Estimation

The yield of oil from the pomegranate fruit extract was estimated using the formula as follows,

Oil Yield (%) = W1/W2×100

Were,
W1 is the weight of the crude extract and W2 is the weight of the pomegranate fruit powder.

Result and Discussion

The extracted oil from the whole pomegranate fruit was evaluated to standardize the quantity of solvent, temperature and time (in hours). The quantity of the extract varied according to the change in the temperature, time and the weight of the sample taken (Bereket *et al*., 2017) [12].

Table 1: Result of Soxhlet extraction using ethanol and methanol

Solvent used for extraction	Hours of extraction	Amount of Solvent (ml)	Temperature (ºC)	Sample weight (g)	Crude extract (g)	Oil Yield%
Ethanol	1	130	65	3	1.79	59.66%
	3	150	65	6	4.32	72%
	9	180	75	9	6.89	76.55%
	12	220	75	12	9.92	82.66%
Methanol	12	200	60	10	7.76	77.6%
	24	250	65	12	9.67	80.58%

The highest oil yield was obtained using ethanol as solvent, under the conditions of 220 ml solvent extracted for 12 hours at 75 °C, is 82.66% (w/w). While for methanol, 80.58% (w/w) under the condition of 250 ml solvent extracted for 24 hours at 65 °C. For ethanol it is shown that increase in time, and higher amount of solvent produces more oil yield. The temperature was set according to the boiling point of the solvents. On the other hand, methanol exhibited oil yield while using more amount of solvent and more time than ethanol. Ethanol has a strong solubilizing capability and high electro negativity.

Table 2: The extraction process was repeated as second time, which is displayed

Solvent used for extraction	Hours of extraction	Amount of Solvent (ml)	Temperature (ºC)	Sample weight (g)	Crude extract (g)	Oil Yield%
Ethanol	9	180	75	9	8.27	91.88%
	12	220	75	12	11.45	95.41%
Methanol	12	200	60	10	9.36	93.6%
	24	250	65	12	11.32	94.33%

It is clearly evident that the highest oil yield obtained from ethanol with 95.41% (w/w) while 94.33% (w/w) for methanol, when repeated for a second time. According to Bereket *et al*., (2017) [12] the oil yield increases according to the different parameters. In this experiment, the oil yield was higher for ethanol using 12 g of sample and temperature set to

the boiling point of the solvent as compared to methanol. Similarly, the yield of crude extract was also higher using 220 ml of ethanol when compared to 250 ml of ethanol. This confirms that ethanol as a solvent is more reliable than methanol for the oil yield estimation from pomegranate fruit.

Conclusion and Scope for Future Research

To sum up, this research work carried out to evaluate and standardize the parameters of oil yield obtained from the pomegranate fruit include the extraction time, temperature and the amount of solvent and samples. It was further observed that with ethanol as solvent, led to an increased oil yield even while using less amount of solvent than methanol, suggesting that ethanol is more reliable than methanol for the solvent extraction of pomegranate fruits. Pomegranate fruits have the potential to be one of the best natural antioxidants that could eventually replace some of the highly toxic synthetic antioxidants. Furthermore, this extraction makes it possible to extract phenolic compounds from pomegranate fruits at small and commercial scale for use in the food, pharmaceutical and nutraceutical industries.

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