Extraction and Physicochemical Analysis of *Punica granatum l.* (Pomegranate) Seed Oil

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Abstract: **Pomegranate (*Punicagranatum l*) is an edible fruit with high nutritional value. It has an outstanding medical history in many cultures around the world. This research is aimed at creating awareness on the nutritional content of the pomegranate seed and seed oil, so as to encourage local cultivation and consumption of the fruit and seed. The extraction of the pomegranate seed oil (PSO) was done using soxhlet apparatus using petroleum ether (60-80^{o}C) as solvent. PSO forms approximately 9.90% (percentage yield oil) of the seeds. This research shows the PSO has a refractive index of 1.4723 at 25^{0}C which shows that the oil is clear oil. Proximate analysis shows the moisture content (10.40 ± 0.35)% , ash content (1.38 ± 1.58)%, crude fiber (35.55 ± 0.1)% , crude protein (8.80 $\pm$ 0.42) %, crude fat (12.46 ± 1.5)% and total carbohydrates analyzed by difference (30.91 ± 0.35)%, this shows that the seeds are edible and nutritional. Physicochemical analysis of the seed oil reveals the saponification value (228.95 ± 0.7)mgKOH/g, acid value (10.39 ± 1.13)mgKOH/g, peroxide value 80.07 ± 0.43 mgO_2/g, iodine value (131.38 ± 0.98)mgI_2/100g and ester value ($2.18.55 \pm 0.42$)mgKOH/g. The Pomegranate seed oil is compared with the Nigeria industrial standards (NIS) and Standard Organization of Nigeria (SON) values for industrial oils used in cosmetology. The values are well fitted to be used in the production of soap, body creams and hair creams.**

Keywords: **Proximate analysis, Nutritional, Pomegranate seed oil, Extraction.**

I. INTRODUCTION

The pomegranate (*Punica granatum*) is a fruit bearing delicious shrub or small tree in the family of *Lythraceae* that grows between 5 and 10 m (15 and 33 ft.) tall. The pomegranate has multiple spiny branches and is extremely long lived, with some specimens in France surviving for 200 years. *P. granatum* leaves are opposite or sub opposite, glossy, narrow oblong, entire, 3-7 cm (1.2-2.8 in) long and 2cm (0.79 in) broad. The flowers are bright red and 3cm (1.2 in) in diameter, with 3-7 petals. Some fruitless varieties are grown for the flowers alone (Abbasi *et al.* 2008).

Botanically, the edible fruit is a berry with seeds and pulp produced from the ovary of a single flower. The fruit is intermediate in size between a lemon and a grapefruit, 5-12cm (2-5 in) in diameter with a round shape and thick, reddish husk (Mirzae, 2014).

In traditional medical practice, the ancient Egyptians used Pomegranates to eliminate intestinal worms. The imagery of the fruit was found on temple pillars, mosaics, on the robes of priests, and on coinage. Roman women fashioned headdresses out of Pomegranate plant twigs to indicate their marital status, and armies carried spears that replaced the spikes on their tips with Pomegranates, which were seen as a sign of strength (Mirzae, 2014).

The name Pomegranate is likely derived from the Medieval Latin words for "apple" – pomum – and for "seeded" – granatum. A second meaning of the word granatum is "of a dark red color," with reference to its seeds. It is believed that, during the first Moorish period, the Spanish city of Granada was named after the Pomegranate, and today the coat of arms for the province of Granada bears a Pomegranate (Abbasi *et al.* 2008).

Pomegranate seed oil can be obtained by different methods such as soxhlet extraction (solvent extraction), super-heated hexane extraction (SHHE), supper critical carbon dioxide extraction, normal stirring extraction, microwave assisted extraction and cold pressing method (Basiri *et al.,* 2013).

Mainly, 12-20% of total seed weight is made up by pomegranate seed oil. Conjugated octadecatrienoic fatty acids are responsible for approximately 80% of seed content and pomegranate seed oil is considered to be a rich source of those fatty acids; in particular, punicic acid (cis9, trans11,cis13 acid) which is the main fatty acid among them. Other isomers of conjugated linolenic acids are catalpic acid (C18:3-9trans, 11trans, 13cis acid) and α-eleostearic acid (C18:3-9cis, 11trans, 13trans) but the oil content of the seed and fatty acid composition are affected by cultivation sites, harvesting time, fruit genotypes and climatic conditions.Punicic acid is a conjugated α-linolenic acid molecule which is found in pomegranate seed oil and it contribute to a lot of health benefits associated with pomegranate (Celik *et al.,* 2009).

Punicic acid contains a third double bond, is known as cis9, Trans 11, cis 13, and is referred to as 18:3 fatty acids. It is an omega 5 long chain polyunsaturated fatty acid and a positional and geometric isomer of α-linolenic acid (Carvalho *et al.,* 2010).

Pomegranate seed oil contains up to 85% of punicic acid, an omega 5 conjugated fatty acid. It has been reported that conjugated linoleic acid, has favorable physiological effects, such as anti-atherosclerosis, anti-obesity, anti-tumor and anti-hypertension. The conjugated fatty acids give pomegranate seed oil anti-inflammatory properties, which helps to reduce swelling and ease muscular aches (Dadashi *et al.*, 2013).

Pomegranate seed oil inhibits two inflammatory enzymes: cyclooxygenase and lipoxygenase, which may help protect the skin against age-accelerating effect of inflammation. Pomegranate seed oil has some therapeutic effects on the human skin and hair. It is used in cosmetic products as an anti-aging agent, to revitalize dull or mature skin, reduce wrinkles and to soothe minor skin irritations (Lansky and Newman, 2007).

The aim of this research is to extract and analyze the physicochemical composition of pomegranate seed oil.

II. MATERIALS AND METHODS

Sample Treatment

- *Collection of sample*
  Pomegranate seeds sample was obtained from a farmer who has a plantation of pomegranate trees that grows pomegranate fruits at its season. The farm is located in Rantya Jos, Nigeria.
- *Air drying of sample*
  The seeds after collection are spread on a tray and dried in the room at normal room temperature of 25 ℃.
- *Pulverization*
  This is the process of grinding/pounding the dried seeds to fine powder using mortar and pestle in the laboratory.
- *Sieving*
  After obtaining the pomegranate seed powder, I sieved it using a mesh of about 0.01m in size to obtain a finer powder.

Extraction of Pomegranate Seed Oil Using Soxhlet Extractor (Solvent Extraction)

Using petroleum ether (boiling point 60-80) as solvent, 50g of sample (pomegranate seed powder) was put into the thimble. The solvent was heated to reflux; the solvent vapor then travels up through a distillatory arm and floods into the chamber housing the sample. The condenser ensures that any solvent vapor cools and drips back down into the chamber housing the sample. The chamber containing the sample material is called the thimble is slowly filled with warm solvent, when the soxhlet chamber is emptied by the siphon, the solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. The cycle maybe allowed repeating many times over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the solvent, just one batch of solvent is recycled (Liu et al., 2009).

III. PROXIMATE ANALYSIS

Proximate analysis is the partitioning of compounds in a given sample into different categories based on chemical properties of the compound. These categories are: moisture, crude protein, crude fat, crude fiber and ash content. The proximate composition of the sample is carried out based on the AOAC Methods for analyzing proximate composition in foods. (AOAC, 2015)

Digestible carbohydrate

The carbohydrate of the sample is contained in two fractions, the crude fiber (CF) and the nitrogen-free extractives (NFE). The former is determined by subjecting the residual sample from ether extraction to successive treatments with boiling acid and alkali of defined concentration; the organic residue is the crude fiber (AOAC, 2015).

Carbohydrate was calculated by difference after the analyses of all other items using the formula:

$$\text{NFE} = 100 - (\%\ \text{Moisture} + \%\text{Crude protein} + \%\text{Crude fat} + \%\text{Crude fibre} + \%\text{Ash})$$

Acid value

Acid value (or neutralization number or acid number or acidity) is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the number of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds (Firestone, 2013).

Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1g of the substance. The saponification number is a measure of the average molecular weight of the triacylglycerol in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerol present i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length) (Firestone, 2013).

Ester value

The ester value is defined as the mg of KOH required to react with glycerin (glycerol / or glycerin) after saponifying one gram of fat. It is calculated from the saponification Value *(SV)* and the acid Value (AV):

Ester Value (EV) = Saponification Value (SV) – Acid Value (AV)

$$\% \text{ glycerin} = \text{Ester Value} \times 0.054664$$

Iodine value

The iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of substance (Firestone, 2013).

Peroxide value

Peroxide value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulphate in the presence of KI. Starch is used as indicator.

Refractive index

The refractive index RI of the pomegranate seed oil was determined at 25 degrees Celsius by refractometer (ATAGO hand refractometer, N-3E, Japan) According to AOAC 921.08 Test method (AOAC, 2015).

IV. RESULT AND DISCUSSION

Results

Table 1: Proximate Analysis of the Pomegranate

S/N	Parameters	Values	Reported Values (Tuting *Et Al.*, 2013)
1	Moisture Content (%)	10.40 ± 0.35	9.80
2	Ash Content (%)	1.38±1.58	1.68
3	Protein (%)	8.80 ± 0.42	10.70
4	Crude Fat (%)	12.46±1.5	11.34
5	Crude Fiber (%)	35.55±0.1	42.40
6	Carbohydrate (%)	30.91±0.35	24.09

Table 1 is a result showing the proximate composition of the pomegranate seed compared with the results obtained by Tuting *et al.*, (2013) who worked on the pomegranate seed from another location. The moisture content of the seed shows that the seed has a short shelf-life. The ash content of 1.38±1.58% shows that it contains less noncombustible minerals which mean it cannot be used as a source of alkali for local soap production. The crude fiber content of 35.55±0.1% points that the seed is not an oil rich seed, though rich in fiber than carbohydrate which makes it very good for diabetic patients. Generally, the seeds are edible and highly nutritional.

Table 2: Physico-Chemical Analysis of the Pomegranate Seed Oil

S/N	Parameters/Units	Values	Son/Nis Standard (2013)
1	Percentage Yield (%)	9.90	-
2	Density At 20^0c (G/ML)	0.9202±0.0051	-
3	Viscosity	0.063±0.004	-
4	Texture At 37^0c	Liquid	Liquid
5	Colour	Yellow (Amber)	-
6	Saponification Value (Mgkoh/G)	228.95±0.7	195.00-205.00
7	Acid Value (Mgkoh/G)	10.39±1.13	7.00
8	Peroxide Value (Mgo_2/G)	80.07±0.43	10.00
9	Iodine Value (Mgi_2/100g)	131.38±0.98	110.00-140.00
10	Refractive Index At 25^0c	1.47±0.1	1.38-1.50
11	Ester Value (Mgkoh/G)	2.18.55±0.42	190.00-200.00

Table 2 is the results of the physicochemical composition of the pomegranate seed oil.The values are compared with the Standard Organization of Nigeria (SON) and Nigeria Industrial Standard (NIS) values for industrial oils. The percentage yield of the oil indicates that the pomegranate seed oil is not oil rich oil. The iodine value of 131.38±0.98 mgI_2/100g gives the degree of unsaturation of the oil, showing that the oil is a liquid at room temperature which will constitute high degree of unsaturated fatty acids when characterized with a gas chromatography mass spectrometer (GC-MS). The acid value of 131.38±0.98 mgKOH/100g is a pointer that the oil can undergo trans-esterification for perfectly for the production of bio diesel.

The refractive index shows that the oil is clear oil. The other parameters fall within the range for industrial based oils.

V. CONCLUSION

Pomegranate seeds should not be discarded as it has high nutritional benefits and the Pomegranate seed oil is good for industrial application as it has good to no bad physicochemical composition. In conclusion, Pomegranates should be widely grown in Nigeria as it has lot of economic importance.

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