Extraction and Characterization of Oil from Pistachio Nutshell

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Abstract. The soxhlet extractor was applied to extract the oil from a pistachio nutshell. The proposed technique was accurate and straightforward for extraction, and the percentage yield was quite acceptable. Solubility and density of the extracted oil were also determined. The acid and saponification value indicated the amount of free fatty acid available and can be used to prepare soap in the industry. The saponification value was found to be 270.4 to 274.5 mg KOH/g of the extracted oil. The other vital parameters like % ash content, % crude fibre, peroxide value, and iodine value were determined and indicated the oil stability for consumption and oxidation. Phenolic compounds and alcohol present in the oil are confirmed by Fourier transform infrared spectroscopy. All results were reproducible and easily adapted to extract oil from different waste materials as routine extraction and valuable technique.
Keywords: Soxhlet extraction; pistachio nutshell; oil; saponification value; iodine value; peroxide value.

Resumen. Se utilizó un extractor soxhlet para extraer el aceite de las cáscaras de pistacho. La técnica propuesta es precisa y sencilla para la extracción, y el porcentaje de rendimiento fue bastante bueno. También se determinaron la solubilidad y la densidad del aceite extraído. El valor de acidez y saponificación muestra la cantidad de ácidos grasos libres disponible y se puede utilizar para preparar jabón en la industria. Se determinó que el valor de saponificación fue de 270.4 a 274.5 mg de KOH g^{-1} del aceite extraído. También se determinaron los parámetros vitales como los porcentajes de contenido de cenizas, de fibra cruda, índice de peróxido e índice de yodo e indicaron la estabilidad del aceite para el consumo y su oxidación. Los compuestos fenólicos y el alcohol presentes en el aceite se confirmaron mediante espectroscopía infrarroja por transformada de Fourier. Todos los resultados fueron reproducibles y se adaptaron fácilmente para extraer aceite de diferentes materiales de desecho como extracción de rutina y es una técnica valiosa.
Palabras clave: aceite; cáscara de pistacho; extracción Soxhlet; valor de saponificación; índice de yodo; valor de peróxido.

Introduction

The attention of extracting biologically active materials are getting broad interest due to its possible protection of human health against various diseases. The presence of phenolic and non-polar compounds such as vitamins, unsaturated fatty acid, etc., in the different parts of plants, helps achieve this goal. Studies will extract these materials from the agricultural wastes to replace the synthetic equivalents [1].

Pistachio nut is one of the most nutrient tree nuts widely used from the old year onwards. According to a survey, the United States produces 47 % of the pistachio, followed by Iran (38%) and turkey (9%) in the world market [2]. Currently, after the nuts' processing, the shells are removed, and it becomes waste material. It can be a feedstuff for livestock, fancy fabrics, enriching the soil texture, etc. Studies show that pistachio shells and nuts have a high protein content, minerals, antioxidants, and vitamins [3-5]. The oil extracted has suitable antimicrobial and antimutagenic activities.[6] Recently, several studies were performed to extract and characterise the essential oil extracted from nutshells, leaves, peels, seeds of different fruits, and nuts [7-12]. These methods include solvent extraction, ultrasound-assisted extraction, supercritical fluid extraction, and microwave-assisted extraction [13-15]. Goli et al. reported that the extracted oil with a concentration of 0.06% has a similar effect to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butyl-hydroxytoluene (BHT) [13]. Ozel et al. [17], in their studies using comprehensive gas chromatography coupled with time of flight mass spectrometry, indicated that α-pinene and (z) -α- terpineol were the significant components in the oil.

A soxhlet extraction method was developed for the extraction of oil from the pistachio nutshell. n-hexane and diethyl ether used as a solvent for the extraction process. Physical parameters were determined and characterised using FTIR and presented for the extracted oil.

Experimental

Chemicals and apparatus
Methanol, n-hexane, toluene, diethyl ether purchased from Sigma Aldrich, USA.

Sodium hydroxide, potassium hydroxide, was bought through local vendors belongs to PanReac AppliChem ITW Reagents, Germany.

Soxhlet apparatus purchased from Merck KGaA, Darmstadt, Germany.

The distilled water was prepared in the laboratory of Jubail Industrial College, Saudi Arabia.

All the chemicals were of analytical grade and used without any further purification.

The moisture content of the pistachio shell
The pistachio shells were ground and transferred into a beaker and tabulated its mass (W_1) g. The sample was kept in the oven at 105ºC for 24 hours. Then, it cooled the sample and again resulted in its weighed (W_2) g and calculated the moisture content using the below equation (Eq. 1).

$$\% \text{ Moisture} = ((W_1-W_2)/W_1) \times 100 \qquad \text{Eq. 1}$$

Extraction of oil
Pistachio shell was cleaned, dried, and ground into small particles. After that, 25 g (Sample A) pistachio particles were transferred into the soxhlet apparatus and added hexane into the round bottom flask. The heating mantle fixed at 70°C, and yellow colour was observed in the round bottom flask after extraction due to extracted oil mixed with the solvent. Then extracted oil with solvent was removed from the soxhlet and performed simple distillation. The hexane layer evaporated, and oil was remained in the RBF and collected. The procedure continued with different amounts of pistachio nutshell; Sample B (40 g), Sample C (50 g), Sample D (60 g), and Sample E (75 g) particles. The above process was repeated with diethyl ether, ethanol, acetone, chloroform, dichloromethane, and calculated % yield for all samples.

Determination of ash content
An empty crucible was placed at 600ºC for two hours in a muffle furnace; it cooled inside a desiccator and recorded the weight (W_0). The oil sample (W_1 g) was transferred into the crucible and kept for 3 hours at 600ºC. The sample converted into grey-white ash showed complete oxidation of all organic matter and cooled. Recorded the weighed (W_2 g) and % ash content[12] calculated using the below Eq. 2.

$$\% \text{ Ash content} = \frac{(W2-W0)g}{W1\,g} \times 100 \qquad \text{Eq. 2}$$

Determination of crude fibre

The oil sample (W_1 g) and 100 ml distilled water added to an Erlenmeyer flask. 20 ml of 20% sulfuric acid (H_2SO_4) was mixed with it and gently boiled for 30 minutes. After cooling, the mixture filtered through Whatman paper. The solid residue was collected and transferred to another Erlenmeyer flask, adding 100 ml distilled water and 20 ml NaOH (10%). The mixture was boiled for 30 minutes and filtered again to collect the residue. The residue washed in the following manner, i. hot water, ii. 10% HCl, iii. ethanol iv. petroleum ether. Then, the content was dried at 105 °C and tabulated the weighed (W_2 g). After ashed at 600 °C for 3 hours, colled the content and recorded the weight (W_3 g) [14].

$$\% \text{ Crude fibre} = \frac{(W2-W3)g}{W1\,g} \times 100 \qquad \text{Eq. 3}$$

% Where: W_1= weight of oil; W_2= weight,after drying; W_3= weight of ash sample.

Physicochemical properties of pistachio oil
Solubility

The extracted oil's solubility was determined with water, toluene, benzene, hexane, diethyl ether. 2 ml of each solvent transferred into the test tubes, and ten oil drops were added to it and shook well. The appearance of two layers means it is insoluble, and one layer means it is soluble.

Density and specific gravity

The empty graduated cylinder weighed out (W_0 g), added 5 ml of pistachio oil in it, and weighed (W_1 g) again to determine the oil mass. Later on, oil was substituted with water and recorded the weighed (W_2 g). The density and specific gravity of the sample were calculated using the below equations (Eq. 4-5).

$$\text{Density of oil} = \frac{\text{Mass of oil } (W1-W0)g}{\text{Volume of Oil (ml)}} \qquad \text{Eq. 4}$$

$$\text{The specific gravity of oil} = \frac{\text{Density of oil}}{\text{Density of water}} \qquad \text{Eq. 5}$$

Where, W_0=Mass of the empty cylinder, W_1-W_0=Mass of oil, W_2-W_0=Mass of water.

Saponification value of oil

Initially, the empty conical flask was weighed out, then added 1 ml of pistachio oil and weighed again to calculate the oil mass (W g). 25 ml of 0.5M KOH was transferred into the same flask and gently heated the flask for 30 minutes. After heating, immediately cooled the solution and titrated it with 0.5M HCl. The above procedure was applied for blank without the sample, repeated the process four more times for sample and reference, and recorded the average value for calculation. Similarly, the above procedure was applied with oil extracted from different amounts of pistachio shell particles, tabulated the results, and calculated the saponification value[18]. The following equation (Eq. 6) determines the saponification value

$$\text{Saponification value (SV)} = \frac{56.1 \times N \times V}{W} \qquad \text{Eq. 6}$$

Where W=weight of the sample used (g), N=normality of HCl solution, V=V_2-V_1=volume of HCl solution used with the sample, V_2=volume of HCl solution used for blank.

Acid Value

The definite amount of extracted oil was transferred into an Erlenmeyer flask, adding two drops of phenolphthalein indicator drops. Manual titration was continuous with 0.1M KOH. The procedure repeated four more times to get the average value and calculate the acid value. As well, a similar process was adopted for other samples[19]. The acidic value and free fatty acid present in the oil were computed using the following Eq. 7.

$$\text{Acid value (AV)} = \frac{56.1 \times N \times V}{m} \qquad \text{Eq. 7}$$

Where V=the average volume of 0.1N KOH, N=normality of KOH, m=mass of oil.

The free fatty acid of the oil was evaluated from the acid value using the following equation (Eq. 8).

$$\% \text{ free fatty acid} = \text{acid value}/2 \qquad \text{Eq. 8}$$

Determination of peroxide

The extracted oil sample (0.5 g) and potassium iodide (KI) (1 g) was transferred into an Erlenmeyer flask and added 10 ml chloroform and acetic acid mixture (volume ratio 1:2). Then boiled it for two minutes and poured the hot solution into another Erlenmeyer flask containing 10 ml 5% KI. Three drops of the starch solution were added with it as an indicator and titrated with 0.01N sodium thiosulfate ($Na_2S_2O_3$), and the peroxide value [20] determines the following equation. The volume recorded and calculated the peroxide value as per the below Eq. 9:

$$\text{Peroxide value (PV)} = \frac{S \times N \times 10^3}{w} \qquad \text{Eq. 9}$$

Where S=Volume of sodium thiosulfate (ml), N=normality of sodium thiosulfate, W=weight of the sample (g).

Iodine Value

The oil sample (W g) was treated with excess iodobromine (IBr) in the presence of glacial acetic acid. Then, added KI, converted to iodine (I_2) by reacted with unreacted IBr. After that, titrated with $Na_2S_2O_3$ (0.1 N), as well, continued without sample for a blank Eq. 10 [19,20].

$$\text{Iodine value (IV)} = \frac{(A-B) \times N \times 126.9}{W \times 10} \qquad \text{Eq. 10}$$

Where A=Quantity of sodium thiosulfate for blank, B= Quantity of sodium thiosulfate with the sample, N=normality of sodium thiosulfate, W=weight of the sample (g) the molecular weight of I2=126.9 g/mole.

Characterization
FTIR analysis

The pistachio nutshell and oil sample's FTIR analysis was performed with Thermo NICOLET 6700 FTIR spectrophotometer at a scan rate of 32 scans, resolution of 4 cm^{-1} from wavelength region 600–4000 cm^{-1}.

TGA analysis

Thermal stability of pistachio nutshell was executed applying the thermogravimetric analyser, Pyris-6 (Perkin Elmer). Typically, 5 mg of nutshell sample was heated from 30–800°C in an inert, nitrogen atmosphere at a scanning rate of 10°C/min.

Results and discussion

% Moisture content
The % yield of oil using the soxhlet extraction method for pistachio shell depends on the amount of particles utilised. It is directly related to the % moisture content present in a nutshell. The % moisture content for all samples is almost similar, and it is in the range of 0.71–0.74% (Table 1).

Table 1. Determination of moisture content of nutshell and density, specific gravity, and extracted pistachio oil solubility.

Sample	Color	% H$_2$O	Density (g/ml)	Specific gravity	Solubility
A	Pale yellow	0.72	0.851	0.861	Soluble in n-hexane, diethyl ether, slightly soluble ethanol, chloroform, and insoluble with water

Solubility and density
The extracted oil was soluble in all non-polar solvents like benzene, toluene, hexane, and diethyl ether but insoluble in polar solvent like water. The density of the extracted oil was in the range of 0.849–0.851 g/ml.

Yield of extracted oil
Before extraction, the pistachio nutshell particles were kept in the oven at 105ºC for 10 hours. The experimental findings testified that the increased heating time has no impact on the % yields. Therefore, the maximum heating duration was constant at 10 hours throughout the experiment to extract oil from the pistachio nutshell. The % yield of the oil extracted from the nutshell was in the range of 0.484–0.581% (Table 2). The % yield was maximum with n-hexane but almost similar to diethyl ether. It decreased with solvent polarity and found minimum yield with acetone due to the nature of oil polarity.

Table 2. Determination % yield of extracted oil with different solvents.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	0.484	0.491	0.008	0.256	0.225	0.003
B	0.503	0.488	0.009	0.257	0.223	0.005
C	0.528	0.535	0.101	0.259	0.228	0.006
D	0.555	0.569	0.100	0.261	0.229	0.007
E	0.581	0.578	0.101	0.277	0.231	0.008

Ash and fibre content
The ash content of the oil sample was higher with acetone and decreases using n-hexane and diethyl ether. The range between 2.15–3.78 % (Table 3) for all samples extracted with different solvents. As well, the % of the crude fibre found to be 4.5–8.4 % (Table 4), indicating the presence of a low level of undigested cellulose.

Table 3. Determination of ash content (%) of the extracted oil.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	3.35	2.65	2.98	3.23	3.23	3.78
B	3.45	3.23	3.11	3.18	3.18	3.01
C	2.15	3.18	3.24	2.88	2.89	2.65
D	3.01	2.15	2.89	3.45	3.43	3.23
E	2.85	3.01	3.32	2.66	2.99	3.18

Table 4. Determination of fibre content (%) of the extracted oil.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	5.7	6.7	5.2	5.5	6.4	5.9
B	7.4	7.1	8.2	6.4	7.1	5.9
C	8.2	8.1	7.6	8.1	6.6	6.4
D	5.9	6.2	6.7	6.1	4.5	5.7
E	6.4	6.4	6.4	8.4	5.9	8.1

Acid and saponification value of oil

The extracted oil's acid value was within the range of 1.5 to 1.9 mg NaOH/g (Table 5), indicating free fatty acid. As per AOAC,[18] the maximum acceptable level was 4mgKOH/g but resulted below it, representing a non-degraded state used for daily eating. The extracted oil saponification values were found to be within the range of 270.4 to 274.5 mg KOH/g (Table 6); therefore, the high proportion of fatty acid with low molecular weight could produce soap.

Table 5. Determination of acid value (mg NaOH/g) of the extracted oil.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	1.5	1.7	1.6	1.7	1.8	1.6
B	1.9	1.8	1.8	1.6	1.7	1.9
C	1.6	1.7	1.7	1.5	1.6	1.8
D	1.8	1.6	1.5	1.8	1.8	1.7
E	1.7	1.5	1.7	1.8	1.7	1.6

Table 6. Determination of saponification value (mg KOH/g) of the extracted oil.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	270.4	271.2	270.6	272.2	270.9	271.2
B	272.2	270.8	271.2	273.3	271.3	272.5
C	271.5	270.1	271.7	272.5	273.2	272.7
D	273.3	272.4	272.1	270.3	270.8	271.9
E	274.5	273.2	273.5	272.8	272.8	271.8

Peroxide and iodine value

The peroxide value indicated the stability of the oils during storage conditions. The values were within the range of 2–10 meq KOH/g[18] and presented in Table 7. The values resulting from the proposed method were 2.25–4.96 showed the excellent quality of the oil. The iodine value measured the degree of unsaturation of the extracted oil. The iodine value directed its stability to oxidation and the present double bond's reactivity in the oil. The iodine values have resulted from extracted oil in Table 8.

Table 7. Determination of peroxide value (meq/kg) of the extracted oil.

Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	4.33	4.34	3.88	2.58	4.86	4.37
B	4.21	4.85	4.55	3.05	4.23	4.07
C	3.99	4.22	4.32	3.69	3.35	4.51
D	3.75	3.32	4.01	4.21	4.12	4.89
E	2.25	3.55	4.66	4.96	2.93	4.65

Table 8. Determination of iodine value (g I_2/100 g) of the extracted oil.

| Sample	n-Hexane	Diethyl ether	Ethanol	Dichloromethane	Chloroform	Acetone
A	92.1	81.2	83.2	82.6	84.1	96.6
B	83.5	84.5	87.8	86.4	92.9	94.5
C	87.2	92.7	93.4	90.1	96.7	97.8
D	95.7	96.3	98.3	92.8	95.4	90.4
E	98.8	99.9	98.8	97.1	98.3	93.3

FTIR and TGA Analysis

Thermogravimetric (TGA) analysis was performed to investigate the thermal stability of the samples. Derivative thermogravimetric (DTG) and TGA curves of raw pistachio nutshell were presented in Fig. 1. The curve showed that the temperature increases; consequently, mass loss occurs for the pistachio nutshell. Initially, a slight weight loss occurred between 30-150˚C, which is due to the evaporation of moisture absorbed in a nutshell. Two degradation peak could be seen in the DTG curved in the region between 200-325˚C and 350-400˚C with peak maxima value of 287˚C and 356˚C. The first degradation peak was attributed to the degradation of hemicellulose, which has less thermal stability. The second degradation is due to cellulose's degradation, which presents in the pistachio nutshell, and a residue of 13.74% was observed at 800˚C.
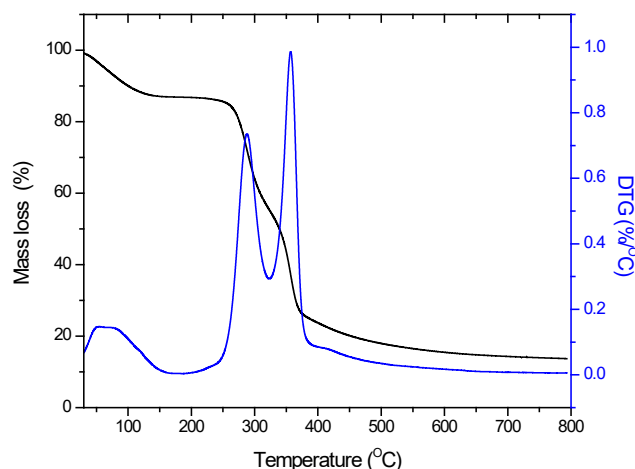
Fig. 1. TG and DTG curves of Pistachio nutshell.

The broadband O-H's stretching vibrations were observed due to the hydroxyl group present in the cellulose within the spectra region of 3600–3000 cm^{-1}. The peaks at 2900-2800 cm^{-1} were related to C-H

stretching, indicating cellulose in the pistachio shell. The band at 1735 cm^{-1} corresponds to the elongating of carbonyl groups (C=O) associated with the presence of acetyl ester and carbonyl aldehyde groups of hemicellulose and lignin. The absorption peak at 1648 cm^{-1} corresponds to O-H bending vibrations of absorbed water. The peaks at 1500 cm^{-1} and 1238 cm^{-1} were related to C-C bonds in aromatic rings in lignin and the C-O-C stretching. The peaks related to C–O stretching and C–H rock vibrations of cellulose at 1033 cm^{-1} and 895 cm^{-1} (Fig. 2).
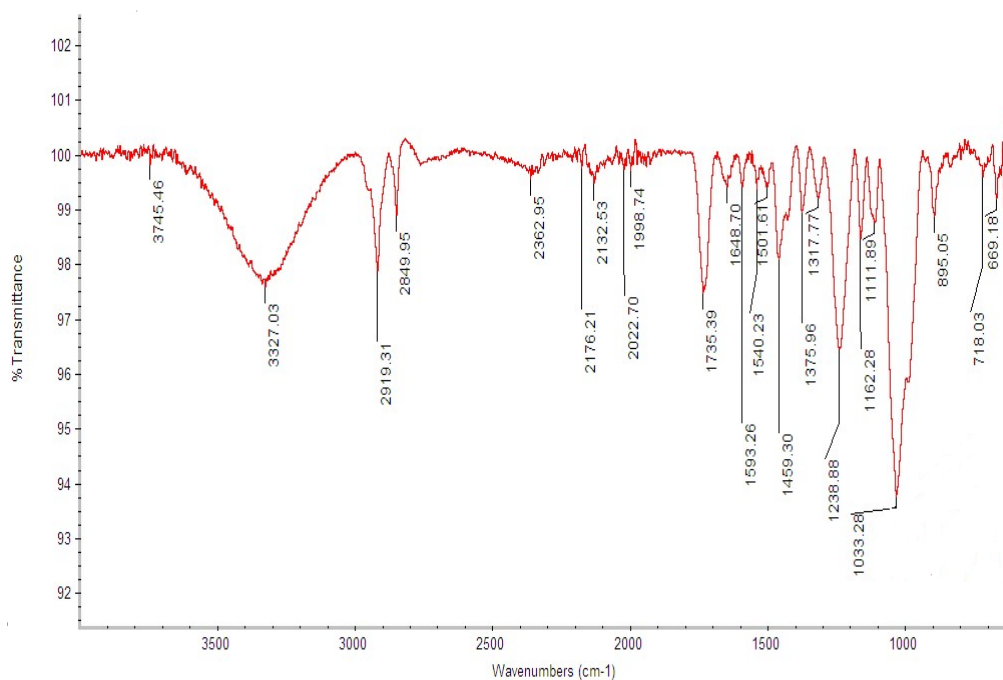

Fig. 2. FTIR of Pistachio nutshell.

Fig. 3 shows the Fourier transform-infrared (FT-IR) spectra of the oil extracted from the pistachio shell. Broadband starting from 4000-3400 cm^{-1} indicates–OH group from phenolic compounds and alcohol. The C-H stretching causes a peak in the region of 3200-2900 cm^{-1}. The C-H deformation peak was available between 1350 and 1475 cm^{-1}. A sharp peak at 1740 cm^{-1} was showed the presence of the ketone or aldehyde group. The peaks between 1525 and 1675 cm^{-1} represent C=C stretching vibrations indicative of alkenes and aromatics. Furthermore, the absorption peaks between 698-900 and 1420-1510 cm^{-1} indicate mono-and polycyclic and substituted aromatic groups (Table 9).

Table 9. Functional groups of nutshell and oil using FTIR.

Wavenumber, (cm^{-1})	Chemical bond	Compounds
4000–3400	O-H	Phenols
3200–2900	C-H	CH$_4$
1800–1700	C=O	Carboxylic acid, aldehyde, ketone
1700–1500	C=C	Aromatics
1550–650	C-O, C-C	Alkanes, alcohols, ethers, lipids
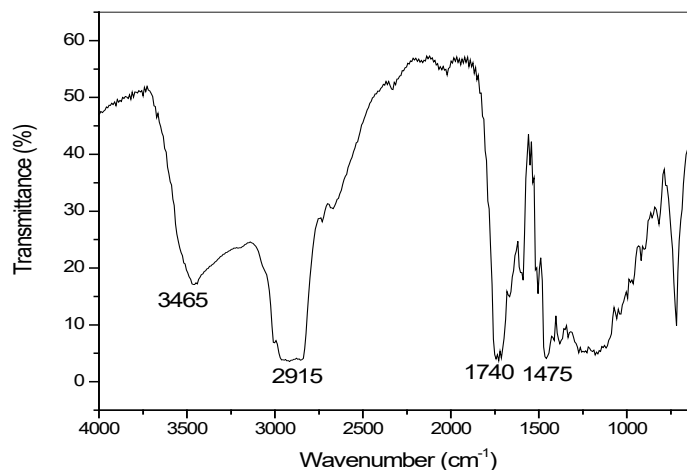
Fig. 3. FTIR of oil extracted from Pistachio shell.

Conclusion

The waste material can be reused or extract oil for humankind's benefit and learned nothing to be thrown in the environment. After eating pistachio, most people throw the nutshell into the garbage but can extract oil using the proposed procedure. The percentage yield of all samples was calculated. The results were satisfactory and can be considered as commercial production of oil.   The FTIR spectra indicate the presence of phenolic compounds and alcohol. The extraction method was simple and can be used for the extraction of oil from different waste materials.

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