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Extraction, Physico-Chemical Characterization and Antimicrobial Studies of Seed Oil of Sofeda (*Manilkara zapota*)

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Abstract. *Manilkara zapota*, an evergreen tree with multiple varieties, is cultivated for its delectable fruit. Different parts of the tree have been used in traditional medicine to cure a wide range of ailments, including fever, dysentery, and diarrhea. Owing of this tree's medicinal potential, several portions of it have been the subject of investigation. *Manilkara zapota* (L.) seed oil extraction and purification were examined in this work. Standard techniques were used to evaluate the seed oil's physico-chemical characteristics. FT-IR was used to determine the presence of functional groups in alkane, carbonyl, alkene, and methyl ester. The oil's primary chemical constituents were also examined using ^{1}H and ^{13}C NMR spectroscopy. Utilizing GC–MS spectroscopy, the chemical composition of the oil extracted from *M. Zapota* seed was determined. The activities of oil against bacteria and fungi were also evaluated. Both Gram-positive and Gram-negative bacteria were resistant to the oil. It has a strong reaction to *S. typhi* while showing no reaction to *E. coli*. When the oil's antifungal activity was evaluated against *Aspergillus Niger* and *Aspergillus flavum*, excellent inhibitory results were obtained. The derived oil has significant antibacterial and antifungal properties, according to this study.

Keywords: *Manilkara zapota*, extraction, characterization, physico-chemical analysis, antimicrobial.

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Экстракция, физико-химическая характеристика и антимикробные исследования масла семян Софеды (*Manilkara zapota*)

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Аннотация. *Manilkara zapota*, вечнозеленое дерево с множеством разновидностей, выращивается из-за его восхитительных плодов. Различные части дерева использовались в традиционной медицине для лечения широкого спектра заболеваний, включая лихорадку, дизентерию и диарею. Из-за целебных свойств этого дерева несколько его частей были предметом исследования. В данной работе были рассмотрены вопросы экстракции и очистки масла из семян *Manilkara zapota* (L.). Для оценки физико-химических характеристик масла из семян были использованы стандартные методы. FT-IR использовали для определения присутствия функциональных групп в алкане, карбониле, алкене и метиловом эфире. Основные химические компоненты масла также были исследованы с помощью ЯМР-спектроскопии ^{1}H и ^{13}C. С помощью GC–MS спектроскопии был определен химический состав масла, извлеченного из семян *M. Zapota*. Также была оценена активность масла в отношении бактерий и грибков. Как грамположительные, так и грамотрицательные бактерии были устойчивы к маслу. Оно обладает сильной реакцией на *S. typhi*, но не проявляет никакой реакции на *E. coli*. Когда была оценена противогрибковая активность масла в отношении *Aspergillus Niger* и *Aspergillus flavum*, были получены отличные результаты ингибирования. Согласно этому исследованию, полученное масло обладает значительными антибактериальными и противогрибковыми свойствами.

Ключевые слова: *Manilkara zapota*, экстракция, характеристика, физико-химический анализ, антимикробное средство.

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1. Introduction

Manilkara zapota (L.) P. Royen is the scientific name for Sapodilla, which belongs to the Manilkara genus in the Sapotaceae family. The Sapotaceae family contains 58 genera and approximately 1250 species that occur in neotropical regions of the world [1]. Manilkara is a genus of plants that comprises a wide range of medicinal and culinary herbs. The best-known species from this genus are *M. bidentata* (A.DC.), *M. hexandra* (Roxb.), *M. zapota* (L.), *M. chicle*, *M. kauki* etc. Traditionally these species are used in wound healing, inflammation and fever and various parts of the plant, such as leaves, fruit and seeds which have possess bioactivities such as antioxidant, antimicrobial, antidiabetic, hypo-cholesterolemic, antinociceptive, anti-inflammatory, antidiarrheal, anthelmintic, antitumor, anti-arthritis, xanthine oxidase inhibitory activity and feeding deterrent activity of the plant [2]. All of these pharmacological values demand the wide research of *Manilkara zapota* tree.

There are three basic methods for extracting oils from seeds, nuts, plants, and other sources. solvent extraction, cold press, and expeller pressed. The final method uses a chemical to extract as much oil from the plant as feasible. The basic method for extracting vegetable oil from oleaginous materials is to use a Soxhlet based solvent extraction procedure. For the oil to be transported from the solid matrix to the fluid medium, crushed oil seeds are placed in a thimble that is in contact with pure solvent. The greatest leaching qualities of the desired solute are taken into account while selecting a solvent. [3]. The compositional quality of edible oils was monitored using a variety of physical and chemical measures [4, 5]. These physicochemical parameters include iodine value (IV), saponification value (SV), viscosity, density, peroxide value (PV), Acid Value (AV). Edible oils are one of the most important components of the diet for cooking. Several researchers studied the impact of temperature on the stability, viscosity, peroxide value, and iodine value to assess the quality and functionality of the oil [6].

This study is an experimental study of solvent extraction method followed by rotary evaporator using sofeda seed as raw material. The aim of the study was to determine the oil content, physico-chemical properties as well as antimicrobial and antifungal activities of sofeda seed oil.

2. Experimental

2.1. Materials and Methods

Seeds of sofeda (*Manilkara zapota*) were collected from Anderkilla, Chattogram. All the chemicals required in this study includes Ethyl alcohol, Acetone, Diethyl ether, Chloroform, Hanus solution, Potassium iodide, Sodium thiosulphate, Potassium dichromate, Starch indicator, Potassium hydroxide, Phenolphthalein, Hydrochloric acid, Sodium bicarbonate, Acetic anhydride, Calcium carbonate, Sulphuric acid (0.5N), Sodium hydroxide, Glacial acetic acid**,** Dimethyl sulfoxide (DMSO) from Sigma-Aldrich. FT-IR was recorded on 8400S Shimadzu IR spectrometer in Department of Chemistry, University of Chittagong, Chattogram. ^{1}H-NMR data were examined at room temperature on Bruker Avance 400MHz spectrometer at Wazed Miah Science Research Center (WMSRC), Jahangirnagar University, Dhaka, GC–MS spectroscopy of the oil sample is performed in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

2.2. Extractions of oil from Manilkara zapota (Sofeda) seed and Purification

The collected seeds of Sofeda were (about 500 g dry weight) first cleaned, dried, dehulled and crushed in a pestle. Acetone was used as solvent. 300 ml solvent was taken into a round bottom flask (500ml capacity) and the flask was connected to Soxhlet apparatus. The crushed seeds were placed in Soxhlet with a thimble (a cloth bag). The condenser was connected and multiple extraction was done. About 150g crushed seed was extracted repeating the process. Then filtration of extract was done using Whatman filter paper. The processes were repeated for two times. After filtration the solution was concentrated by using rotary evaporator. After concentration under vacuum, the resulting aqueous suspension was separated by using separating funnel (ethyl acetate: water = 2:1). The content of the funnel was shaken thoroughly and allowed to stand for a while until two distinct layers was separated. The aqueous layer was discarded. The organic layer was subjected to similar treatment once more. The ethyl acetate solution, taken in a pre-weighed ground joint flask was first concentrated in rotary evaporator at a temperature not exceeding 45 °C and then to complete dryness with nitrogen gas. The dryness was monitored until the flask's final weight remained constant. By deducting the weight of the empty flask from the final weight, total oil was calculated. The total amount of oil was determined gravimetrically [7].

$$\text{Oil yield(wt\%)} = \frac{\text{mass of oil extract (g)}}{\text{mass of ground seed (g)}} \text{X } 100$$

When the oil extraction was completed, the oil was taken in a vial for complete dryness with nitrogen gas. It was also noted to keep the sample covered with parafilm and aluminum foil to protect exposure to light, heat and oxygen.

2.3. Physico-chemical Characteristics

The physico-chemical characteristics including Specific Gravity, Viscosity, pH, Moisture content, Saponification Value, Acid Value, Percentage of Free Fatty Acid (FFA), Iodine value, Acetyl value, Reichert-Meissl value, Polenske value etc were analyzed by standard methods [7]. The physico-chemical characteristics of the oil are given below in Table 1 and Table 2 respectively.

Table 1. Physical characteristics of the oil

Physical Characteristics	Value
Color	Pale yellow
Odor	Mild sweet
Yeild (%)	18
Moisture Content	0.23
pH	5.6
Specific gravity (at 30 °C)	0.9186
Viscosity (mp, at 30 °C)	497.54
Activation Energy (K/mol)	6.554

Table 2. Chemical characteristics of the oil

Chemical Characteristics	Value
Saponification value	196
Saponification equivalent value	286.22
Iodine value	72
Acid value	6
Free fatty acids (%)	3
Ester value	190
Reichert- Meissl value	0.0385
Polenske value	0.105
Henher value	79.45
Peroxide value (meq/kg)	12
Acetyl value	15
Unsaponifiable matter (%)	1.13

Characterization

2.4. FT-IR Spectroscopy

FT-IR spectroscopy is an excellent tool for analysis as the intensities of the bands in the spectrum are proportional to concentration. Mid IR spectra have been used to characterize edible oils and fats because they differ in the intensity and the exact frequency at which the max absorbance or transmittance of the band appears. The oil composition affects the exact positions of the band and yields a shift when the proportion of fatty acids changes. FT-IR spectra of Examined Oil is shown in Fig. 1 and analytical evaluation of this spectrum is given in a Table 3.

From the Fig. 1 we can see that the sofeda seed oil show a maximum absorbance at ~ 3010.05 cm^{-1}. This is due to C-H stretching of olefinic double bonds attributed to unsaturated fatty acid, while bands centered at ~2928 and ~2854 cm^{-1} known as methylene absorbance peaks are associated with Symmetric

Table 3. Evaluation of FT-IR spectrum

Wavenumber (cm^{-1})	Group	Type of vibration	Remark
3010.5	C-H	str.	-CH=CH- (cis olefin)
2928.07	C-H	asym. str.	aliphatic ($–CH_2$)
2854.77	C-H	sym. str.	aliphatic ($–CH_3$)
1744.69	C=O	str.	(C=O) ester
1455.35	C-H	bending	aliphatic ($–CH_2$ & $-CH_3$)
1365.66	C-H	sym. bending	aliphatic ($–CH_2$)
1247.03	C-H	out of plane bend	aliphatic ($–CH_2$)
1163.13,1098.51	C=O	str.	(C=O) ester
975.08	C-H	out of plane bend	trans (–CH=CH-)
722.37	C-H	rocking	aliphatic ($–CH_2$)

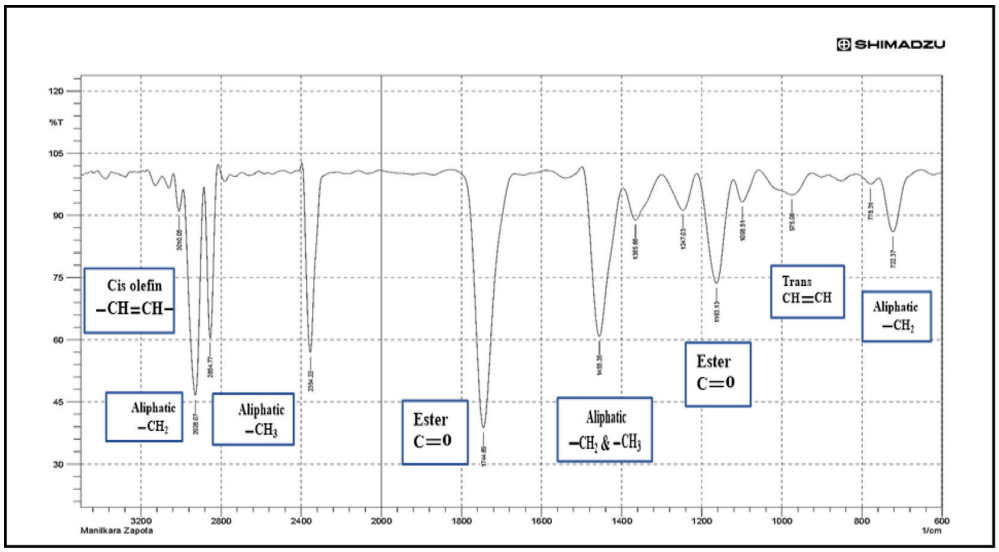


Fig. 1. FT-IR spectrum of the oil

and asymmetric stretching vibrations of aliphatic C-H in -CH_2 and terminal -CH_3 groups, respectively [9, 10]. In addition, sharp peak at ~1745cm^{-1} known as ester peak because of C=O stretching vibration of carbonyl groups of the triacyglycerols. Bands in the region of ~1455cm^{-1}, ~975 cm^{-1} are assigned to bending vibrations of -CH_2 and -CH_3 aliphatic groups as well as rocking Vibrations [11, 12]. Symmetric H-C-H bending at ~1365cm^{-1} could be attributed to glycerol group O-CH_2 (mono, di and triglycerides [12]. While at ~975cm^{-1} of CH=CH shows trans unsaturation [13]. Band at ~1163cm^{-1}and ~1098cm^{-1} wavenumber is due to the stretching vibration of C=O ester groups and -CH_2 wag. The last major peak located at ~722cm^{-1} could be associated with overlapping of the $(CH_2)_n$ rocking vibration and out of plane vibration (–CH wag.) of cis -di- substituted olefins [11].

2.5. NMR Spectroscopy

Fig. 2 and Fig. 3 shows the ^{1}H NMR and ^{13}C NMR Spectra of *M. zapota* seed oil and the following Table 4 summarize the assignments of all pertinent peak seen in the studied NMR spectra.

Fig. 2 and Fig. 3 represent ^{1}H NMR and ^{13}C NMR Spectra of *M. zapota* seed oil respectively. The ^{1}H spectra of any edible vegetable oil show at least nine signals of significant intensity. These Signals are due to protons of the main components, i.e.; of the triglycerides. In Fig. 2, methylic protons (–CH_3) of saturated, oleic and linoleic acyl groups give signal between 0.86 and 0.90 ppm, this is due to the overlapping of the triplets of the methylic proton signals. Signal appears at 126 ppm corresponding to methylenic protons of saturated acyl groups, a shoulder near 127 ppm corresponding to methylenic protons of oleic acyl groups, and peak at 131 ppm resulting from the overlapping of the methylenic protons of oleic, linoleic and linolenic acyl groups. Signals between 1.85~2.02 ppm is due to α-methylene proton related to a protons in α position in relation to a single double bond (–CH_2-CH=CH-), also named allylic protons. Signal occurred due to the methylene protons in α position related to carbonyl group (–OCO-CH_2-), and it appears at 2.27–2.33 ppm. Signal overlapping at 2.75–2.78 ppm, due to responses from α-methylene protons related to the

Table 4. ^{1}H NMR and ^{13}C NMR Spectra of oil

Chemical Shift		Functional groups	Assignments
^{1}H (ppm)	^{13}C (ppm)		
0.86–0.90	14.06, 14.07	$-CH_2CH_3$ (saturated, oleic, and linoleic acids)	All acyl chain except linolenic
2.004–2.072	34.01, 34.16	$-CH_2-COO-CH_3$	All acyl chains
1.26–1.31	29–31	$(CH_2)_n$ saturated aliphatic	All acyl chains
1.61	22.55, 22.66	$-CH_2-CH_2COOCH_3$	All acyl chains
1.85–2.02	26.1	$-CH_2CH=CHCH_2$	All unsaturated acyl chains
2.27–2.33	34.01, 34.16	$-CH=CHCH_2CH_3$	Linolenic chains
2.77	24.85	$CH=CHCH_2CH=CH$	Linolenic and linoleic chains
4.12–4.32	62.06–77.38	CH_2-OCOR	Triglycerides (glyceryl group)
5.29	126.99–131.99	CH=CH (olefinic group), CH–OCOR	All unsaturated fatty acids, Triglycerides (glyceryl group)
	172.74–173.18	C=O (carbonyl group)	All ester

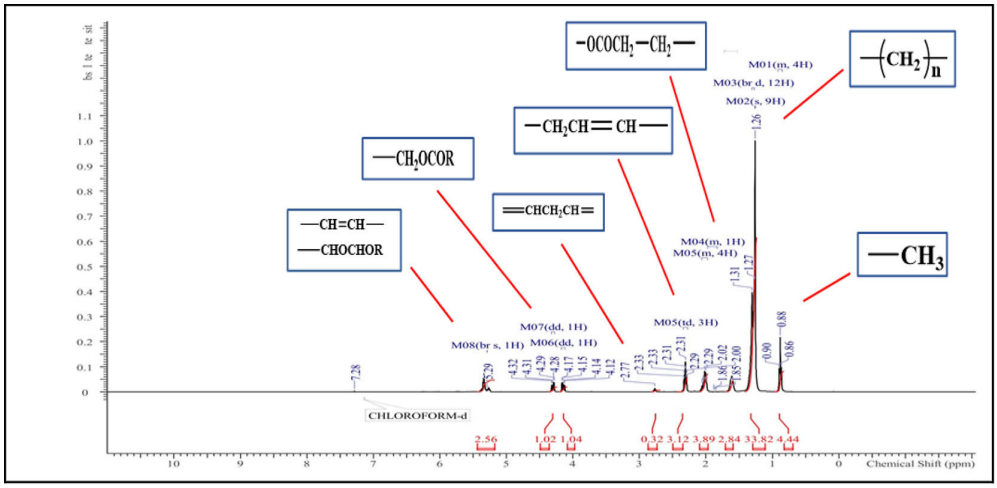


Fig. 2. ^{1}H NMR spectrum of the oil

double bonds (=CH-CH_2-CH=) (bi-allylic protons). Signals appear at 4.122–4.321 ppm, due to the protons on carbon atoms of the glyceryl group. Signals appear at 5.25–5.38 ppm, represents olefinic protons of different acyl groups. Fig. 3 shows the ^{13}C NMR spectrum of the oil under analysis with well-defined four distinct regions (A, B, C and D): A (14.07–34.16 ppm) due to methyl or alkyl functional groups for saturated acids, oleic, and linoleic acids; B (62.06–68.91 ppm) due to glyceryl C-atoms; C (127.87–130.13 ppm) due to the presence of unsaturated alkenes; and D (172.74–173.18) due to the presence of carbonyl groups. Both ^{1}H and ^{13}C NMR spectra of the oil are similar to those of known vegetable oils [15, 16].

2.6. GC–MS Analysis

Table 5 shows the composition result of *Manilkara zapota* seed oil. GC–MS analysis of the oils led to the identification of different Fatty acid compounds present in it.

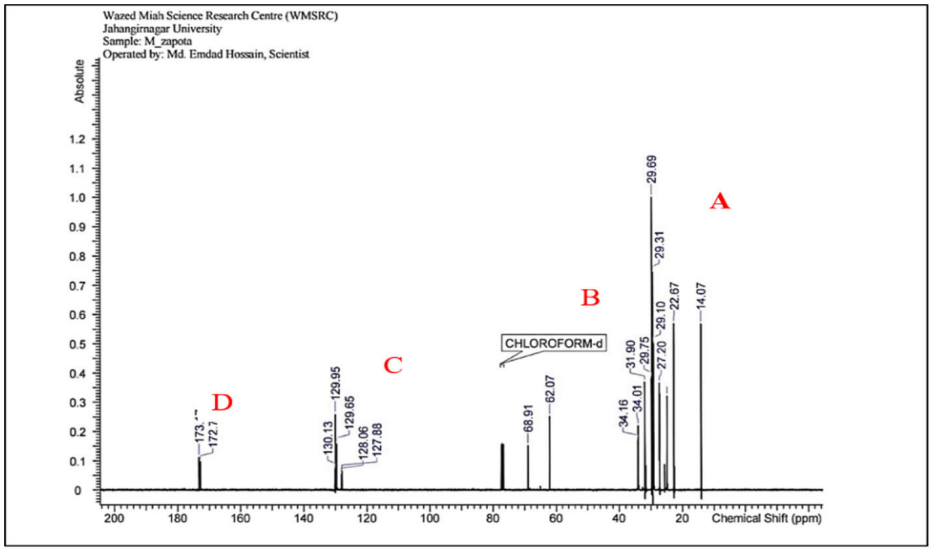


Fig. 3. ^{13}C spectrum of the oil

Table 5. Fatty acid composition of *Manilkara zapota* essential oil

SL No	Compound Name	Retention Time	Area	Area %
1	Methyl caprylate	1.586	456428	16.064
2	Methyl dodecanoate	1.875	1262322	44.428
3	Methyl tridecanoate	1.951	179839	6.329
4	Methyl myristoleate	2.113	177886	6.261
5	Methyl pentadecanoate	2.287	509852	17.944
6	Methyl palmitate	2.679	100617	3.541
7	Methyl oleate	3.756	36270	1.277
8	Methyl linoleate	4.205	35679	1.256
9	Methyl heneicosanoate	4.887	2748	0.097
10	Methyl behenate	5.139	1843	0.065
11	Methyl eicosatrienoate	5.265	55350	1.948
12	Methyl erucate	5.785	1165	0.041
13	Methyl arachidonate	6.257	1295	0.046
14	Methyl tricosanoate	6.729	1072	0.038
15	Methyl docosadienoate	7.082	18927	0.666

The oil mainly contained saturated fatty acids (88.012 %) and unsaturated fatty acids (11.988 %). The most abundant fatty acids of *M. zapota* seed oil were Methyl dodecanoate (44.428 %), Methyl pentadecanoate (17.944 %), Methyl caprylate (16.064 %) followed by Methyl tridecanoate (6.329 %) and Methyl myristoleate (6.261 %), which together comprised 91.026 % of the total fatty acid.

3. Discussion of oil Characterization

The found oil to be a light-yellow tint. From these oil extracts, the total oil content of Sofeda (*Manilkara zapota*) seed was calculated gravimetrically. It was estimated that the oil produced was 18 % yield (Table 1). The Sofeda seed oil yield should be considered as modest. Seed oil has a moisture level of 0.23 % (Table 1), according to investigation. The sofeda (*Manilkara zapota*) seed oil's low moisture content benefits shelf life. The seed oil's pH was determined to be 5.6 (Table 1), indicating that it has a somewhat acidic character. Since the specific gravity (sp. gr.) of fats and oils generally does not change significantly, this attribute can be used to identify one lipid (fat/oil) from another. At 30 °C, Sofeda seed oil was found to have a specific gravity of 0.9186 (Table 1).

At 30 °C, Sofeda seed oil had a viscosity of 497.54 m.p (Table 1). Each molecule in a liquid has an activation energy, which may be thought of as occupying roughly an equilibrium position. For a molecule to move to another equilibrium position in the direction of flow, it has to acquire a certain activation energy. Although other elements are also significant, in general, the higher this energy, the greater the viscosity of the liquid. Sofeda seed oil has an activation energy of 6.55 Kcal/mole, (Table 1) which is fairly high.

The amount of KOH in milligrams needed to completely saponify 1 gm of oil or fat is known as the saponification value. Sofeda seed oil has a saponification value of 196 (Table 2), the same as olive and soybean oils (190–195). So, this oil is suitable for use as cooking oil. The seed oil's equivalent saponification value is determined to be 286.22.

The amount of iodine that is combined with 100 grams of an oil or fat is known as the iodine value. The oil sample's iodine levels were determined to be 72 (Table 2), which is practically identical to olive oil (75–94). This result indicates that the oil is non-dying and fairly saturated. The amount of potassium hydroxide (KOH) needed to neutralize 1 gm of free fatty acids is known as the acid value. The sofeda seed oil's acid values were estimated to be 6 mg KOH/g (Table 2). The acid value of oil used for human consumption should not be more than 4 mg KOH/g. Here, the acid value is really high. There might be two causes. Due to the rotary evaporator's vacuum pump's limitations, some ester may break down into free fatty acids during the evaporation process. Again, during the storage process, oxidation or rancidity may occur.

It was determined that Sofeda seed oil had a percentage of free fatty acids (F.F.A. %) of 3 (Table 2), which was practically the same as mustard oil (3.2).

The ester value is defined as the number of mg. of KOH necessary to combine with the fatty acids which are in combination with glycerol in 1g of fat or oil. It is determined by subtracting the acid value (A.V.) from the saponification value (S.V.). The ester value of the seed oil was found to be 190 (Table 2) which was also near to the soyabean oil (188.73).

The Reichert- Meissl value (R.M.V) is defined as the number of milliliters of 0.1N-KOH solution required to neutralize 5g. of a fat or oil. The R. M.V of the seed oil was found to be 0.0385 (Table 2). R.M.V. is the measure of volatile water soluble lower fatty acids (butyric-C 4 to capric -C 10) present in the oil or fat. Relative lower R.M.V. of the oil is an indication of low content steam volatile fatty acids.

The Polenske value is the volume of 1N NaOH solution needed to neutralize the fatty acids that are volatile, water-insoluble but soluble in alcohol after being distilled from 5g of a fat or oil. The sofeda

seed oil's Polenske value was determined to be 0.1052 (Table 2), indicating that the oil has a relatively low concentration of volatile, alcohol-soluble but water-insoluble fatty acids. Low Reichert-Meissl and Polenske values denote a relatively low saponification value, which is caused by the oil's relatively greater fatty acid content and low saponification value.

The amount of water-insoluble fatty acids in an oil or fat is measured by the Henher value. The oil's Henher value, which was observed to be 79.45 % (Table 2), indicates that it has a larger percentage of water-insoluble fatty acids with high molecular weight.

The autoxidation of the double bonds found in the constituent fatty acids in fixed oils and fats causes oxygen from the air to be absorbed. The quantity of iodine released from potassium iodide by the peroxides in the oil or fat, measured in milliequivalents per kg or millimoles per kg, is known as the peroxide value. The sofeda seed oil was found to have a peroxide value of 12 (Table 2). The results indicate that the seed oil under investigation includes a little proportion of unsaturated fatty acids.

The acetyl value is the number of mg. of KOH required to neutralize the acetic acid obtained by saponifying 1g. of acetylated fat or oil. The acetyl value of the oil is found to be 15 (Table 2), which is almost same as cotton seed oil (0.7–12.2) and olive oil (10.04) and indicates low content of free hydroxyl groups in the oil. The percentage of unsaponifiable matter (U.S.M.%) of the investigated seed oil was found to be 1.13 (Table 2) which is almost same as the linseed oil (1–1.5). The Fat Analysis Committee of the American Chemical Society proposed that if the U.S.M.% exceeds 2 %, some type of foreign matter is probably present. The foreign matter may consist of a mineral or similar hydrocarbon oil, wax or fat, spermaceti of rosin oil. This result indicates that the oil sample contain a small amount of unsaponifiable matter such as sterols, tocopherols, vitamin A and D, hydrocarbons etc.

4. Antibacterial and antifungal activity investigation of Sofeda seed oil

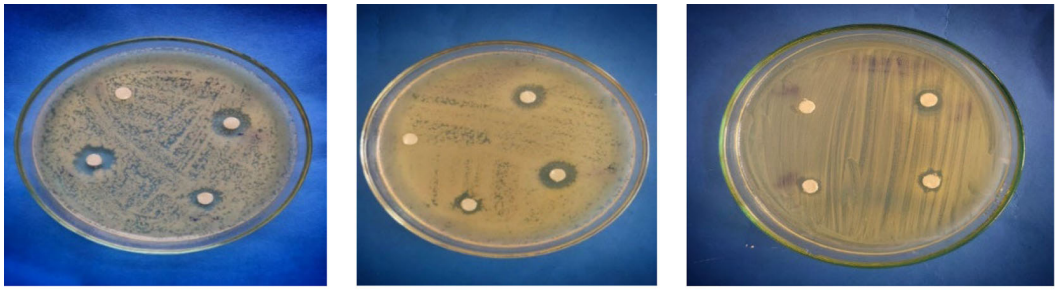
The antibacterial and antifungal activity of Sofeda (*Manilkara zapota*) seed oil was evaluated in different concentrations of acetone extract against targeted species. Dimethyl sulfoxide (DMSO) was used to prepare different concentrations of oil sample.

The antimicrobial activity of the extracted oil from *Manilkara zapota* seed was studied in different concentrations (650, 700, 750 and 800 µl/ml) against four pathogenic bacterial strains, two Gram-positive (*Bacillus cereus* ATCC 14574, *Acinetobacter baumannii* ATCC 17978) and two Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028), and two fungal strains (*Aspergillus niger* ATCC 16404, *Aspergillus flavum* ATCC 204304). These strains have been selected for the basis of its application purpose of further formulation study. The antibacterial and antifungal potential of the oil was assessed in terms of the zone of inhibition of bacterial growth [16]. The results of the antibacterial and antifungal activities are presented in Table 6 and Table 7 respectively.

From Table 6, we can see that the extracted oil does not show any activity against *E. coli.* The growth of maximum inhibition was found against *S. typhi*. In case of other bacteria, we found moderate inhibition. Small changes in concentration of the test oil sample were found to affect growth of the organism significantly.

Table 6. Antibacterial activity of *M. zapota* seed oil

Antibacterial activity (Zone of inhibition mm)						
Microorganism	*M.zapota* oil concentration (µl/ml)				Positive control	Negative control
	650	700	750	800	Amoxicillin 500 mg	DMSO
B. cereus	-	7	8	9	9	-
Acetobacter	-	6	7	8	14	-
E. coli	-	-	-	-		-
S. typhi	12	13	14	14.5	11	-

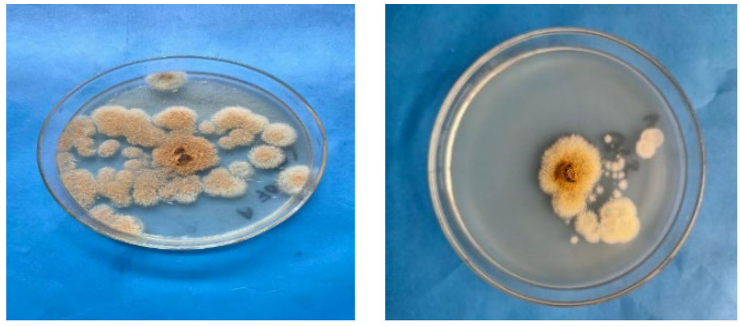


Susceptibility of *B. cereus* Susceptibility of *S. typhi* Susceptibility of *Acetobacter*

Fig. 4. Susceptibility pattern of different bacteria to extracted oil from *M. zapota* seed

Table 7. Antifungal activity of *M. zapota* seed oil

Antifungal activity (Zone of inhibition mm)		
Microorganism	M. zapota oil concentration (µl/ml)	
	600	750
Aspergillus niger	18	21
Aspergillus flavum	19	30



Susceptibility of *Aspergillus niger*

Fig. 5. Susceptibility of different fungus to extracted oil from M. zapota seed

Conclusion

One of the underutilized seeds that has the ability to provide a huge fortune as oil is the sofeda seed (*M. zapota*). In this study, we investigated the significant human consumption potential of sofeda seed oil. The oil is particularly appropriate for using as fuel as evidenced by the lower levels of unsaturated fatty acids. Since this oil's physical and chemical characteristics are nearly identical to those of other well-known oils like olive oil and soybean oil, it can be used as a substitute for such oils. Such that it may be utilized in the culinary industry as a flavoring ingredient, lubricant, and cosmetics as an emollient and scent. The oil has inhibitory effect against bacteria and fungus, according to the current study. The current research proved that there is a huge potential for using the oil as a new source of oil for biofuel, antifungal and bacterial agents.

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