



Antimicrobial activity of moringa peregrina seed oil: chemical composition and effect of extraction procedure

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ABSTRACT

Background and objective: The importance of new sources of oils has recently emerged, one such source being Moringa. There is an urgent need to choose the most appropriate extraction method regarding its impact on the properties and stability of the extracted oil. The objective of this research work is to study the impact of two extraction methods on the chemical and thermal properties as well as the antimicrobial activity of *Moringa peregrina* seed oil.

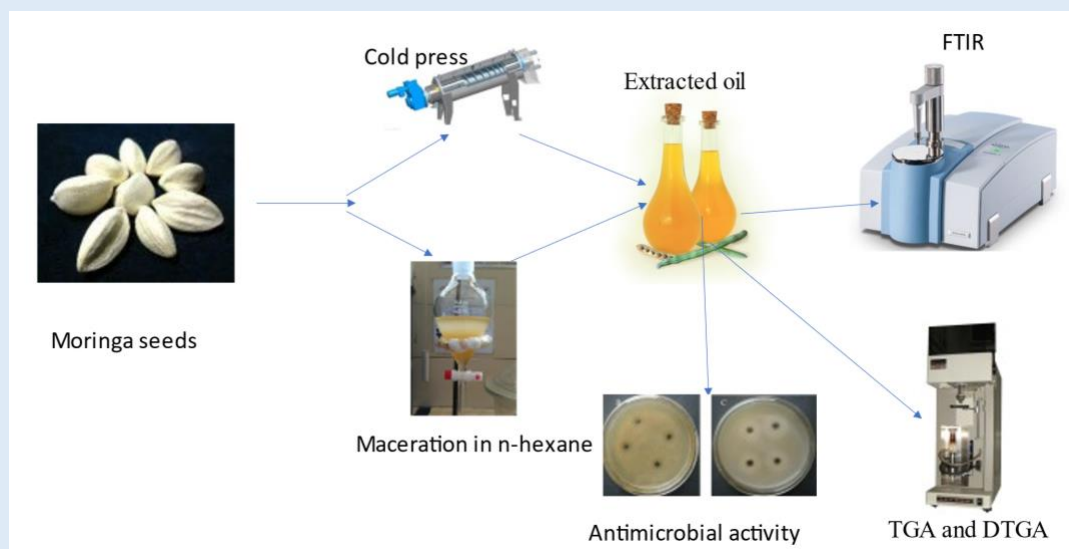
Methods: Moringa peregrina seeds were collected from their original growing locations in Sudan. Cold pressing (CP) and maceration in *n*-hexane (MH) was used to extract the oil, and their yields were found to be 9.12% and 21.87%, respectively. Chemical properties of the oil were studied and in each case triplicate analyses were completed. The mean and the standard deviation were then determined (mean±SD).

Results: Besides the peroxide value, the chemical properties of the oil extracted by the two methods remained relatively unchanged. Additionally, the results revealed a higher activity of the cold pressed oil against all tested organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*) compared to the one obtained by maceration method. Furthermore, the thermogravimetric analysis and derivative thermogravimetric

analysis (TGA and DTGA) demonstrated significant variations in the onset of degradation between the oil samples extracted by the two methods: 200°C (cold pressing) against 274°C (maceration). The maximum mass loss for the oil extracted by the two methods does not differ significantly, and the final degradation temperature was similar.

Conclusion: The differential scanning calorimeter (DSC) thermograms for the oil extracted by the two methods showed somewhat similar characteristics regarding the heating curves, while the cooling curves differed considerably.

Keywords: Antimicrobial activity; Cold press; Maceration; *Moringa peregrina*; Thermal properties.



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INTRODUCTION

Vegetable oils, apart from their exciting and well-known uses in foods, have recently drawn special attention as raw materials, ingredients or precursors for producing and synthesizing numerous materials such as biodiesel, polymers, cosmetics, medicines, and pharmaceutical products [1-3]. The yield, quality, and stability of vegetable oils depends highly on the extraction methods, in addition to some other factors. Hydraulic pressing, expeller pressing, direct and indirect ultrasonic extraction, and solvent extraction are the most common applied methods for extracting oil from seeds. In addition, a combination of the above-mentioned methods with enzymatic and ultrasonication treatments to facilitate the extraction/recovery and preserve the

quality of the oil have been reported in the literature [4-5].

The antimicrobial activity and thermal properties of vegetable (or seed) oils have been investigated by several authors [6-12]. Great variations in the antimicrobial activity of the vegetable oils have been noticed based on the type of the oil as well as on the extraction methods [8-11]. Regarding the thermal properties, both thermogravimetric analysis (TGA and DTGA) and differential scanning calorimetry (DSC) were used to determine the thermal properties of different types of vegetable oils [6, 7, 12]. In general, under air-flow, the TGA results revealed three steps of decomposition by almost all oil samples which were attributed to polyunsaturated, mono-unsaturated, and saturated fatty

acids. The temperature range of the decomposition varies greatly depending on the type of the oil. The lowest decomposition temperature was found to be around 200 °C whereas the highest temperature was around 800°C. On the other hand, DSC was used to determine the exothermic and the endothermic transitions, such as the onset of melting and crystallization, the melting and crystallization enthalpies, polymerization of fatty acid composition, and decomposition of unsaturated and saturated fatty acids. It could be concluded that the results have shown significant variations in the values of these transitions between the different vegetable oils.

There are about 13 species of Moringa trees in the family *Moringaceae*. It is one of the very valuable multipurpose trees for semiarid areas. Different parts of Moringa are good source of protein, vitamins, β -carotene, amino acids, minerals and various phenolics. The main product derived from *Moringa* tree is seed oil, which is used for cooking and in cosmetics and medicine as well. The oil contains a high percentage of oleic acid, which is beneficial from a nutritional point of view and imparts high stability during cooking and frying [13, 14]. *M. peregrina* is indigenous to Sudan as well as to some African (Ethiopia, Egypt and Somalia) and Easian (Syria, Yemen, Saudi Arabia, Jordan, Pakistan and Iran) countries [13, 14]. Although *M. peregrina* seed oil has fascinating characteristics [14], very few articles report on its antimicrobial activity [9], and we did not find any article that reports on the thermal properties nor the influence of extraction methods on its characteristics in the published literature.

The target of the present examination was to examine the influence of the extraction methods (cold pressing and maceration in n-hexane) on the thermal properties and the antimicrobial activity of the seed oil of *Moringa peregrina*.

MATERIALS AND METHODS

Sample collection and pretreatments: *Moringa peregrina* seeds were collected from their original growing locations (Wadi Alkasinger) in the Northern State of Sudan. The seeds were cleaned from any extraneous materials and dehulled using a hammer. The kernels were kept at 10°C before utilizing.

Cold pressing: 500g of the ground kernel seeds were exposed to water-vapor (boiled water) and pressed using a screw pressing machine. The extracted oil was collected and stored at 4°C for further analyses.

Maceration in n-Hexane: 300g of the ground kernel seeds were percolated in one liter of n-hexane and kept for four days with occasional shaking. The percolates were filtered and concentrated at 60°C under reduced pressure in a rotary evaporator.

Determination of chemical properties of the oil: Acid, peroxide, and saponification values of the oil were studied as per the standard techniques of AOAC [15]. In each case triplicate analyses were completed, and the mean and the standard deviation were determined (mean \pm SD).

Fourier Transform Infrared spectroscopy: FT-IR spectrum of the oil was obtained using an IR 300 model spectrometer (Thermo Nicolet). A drop of oil was placed between a pair of salt plates. The pair of the plates was inserted into a holder that fits into an infrared spectrophotometer. The scanning was carried out in the range between 4000 and 500 cm⁻¹. The number of scans was adjusted to 10 scans with resolution of 4 cm⁻¹.

Testing of antibacterial susceptibility: The paper disc diffusion technique was utilized to screen the antibacterial activity of plant extracts and performed by

utilizing Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [16]. Bacterial suspension was diluted with sterile physiological solution to 108cfu/ml (turbidity = McFarland standard 0.5). 100 microliters of bacterial suspension were swabbed consistently on the surface of MHA and was permitted to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were put on the surface of the MHA and soaked with 20 μ l of the oil sample. The inoculated plates were incubated at 37°C for 24 hours in the inverted position. The diameters (mm) of the inhibition zones were estimated.

Thermogravimetric analysis: 10 mg of the oil sample was heated to a temperature range of 30 °C to 700 °C. Analysis was done in the nitrogen atmosphere with a flux of 50 ml min⁻¹ and a heating rate of 10 °C min⁻¹. The thermal stability was measured by the analysis of TGA/DTGA curves that registered mass loss of oil during the heating time.

Differential scanning calorimetry: The DSC curves were obtained in a DSC apparatus in nitrogen (50 ml min⁻¹) with heating rates of 10 °C min⁻¹, and a sample mass of 10 mg with a temperature range of -80 °C to 30 °C. Samples of oil (8-10 mg) were weighed in aluminum pans; the covers

were then sealed into place and studied with DSC instrument. Oil samples were equilibrated at 30 °C for 5 min, cooled to -80 °C at a rate of 5 °C min⁻¹, equilibrated at -80 °C for 3 min, and then reheated to 30 °C at a rate of 5 °C min⁻¹. Dry nitrogen was purged in the DSC cell at 50 cm³ min⁻¹. Thermograms were analyzed and the enthalpy, onset, and offset temperatures of the transitions and peak temperatures were obtained.

Statistical analysis: All investigations were carried out in triplicate, with the mean standard deviation (SD, n = 3) calculated. SPSS statistics software was used to conduct all statistical tests (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp).

RESULTS

Oil content and chemical properties of seed oil of

Moringa peregrina: The oil content of the *Moringa peregrina* seeds, which was extracted by cold pressing (CP) and maceration in n-hexane (MH) methods, was found to be 9.12% and 21.87%, respectively. The chemical characteristics of the extracted oil of the two methods are shown in Table 1. Except the peroxide value, it can be noted from the table that no significant differences in the reported values are present between the two methods.

Table 1. Chemical characteristics of *Moringa peregrina* seed oil extracted by cold pressing (CP) and maceration in n-hexane (MH).

Oil sample	AV (mg KOH/g oil)	SV (mg KOH/g oil)	FFA (%)	PV (meq O ₂ /Kg oil)
CP	0.48±0.02	206.40 ±3.96	0.24±0.01	0.39±0.00
MH	0.44±0.01	203.24±6.24	0.22±0.01	-

*Acid value (AV), saponification value (SV), Free fatty acid (FFA) and peroxide value (PV).

FT-IR analyses: The FT-IR analyses were utilized to examine the variations in the structural features of the oil samples based on different extraction methods. As can be seen from Figure 1, the intense sharp peaks at 2924 cm⁻¹ and 2853 cm⁻¹ are ascribed to asymmetric and

symmetric stretching vibrations of C-H group (of sp³ hybridized system), respectively. Regarding Figure 2, almost identical absorption peaks were noticed for cold press extracted oil and there are no significant variations.

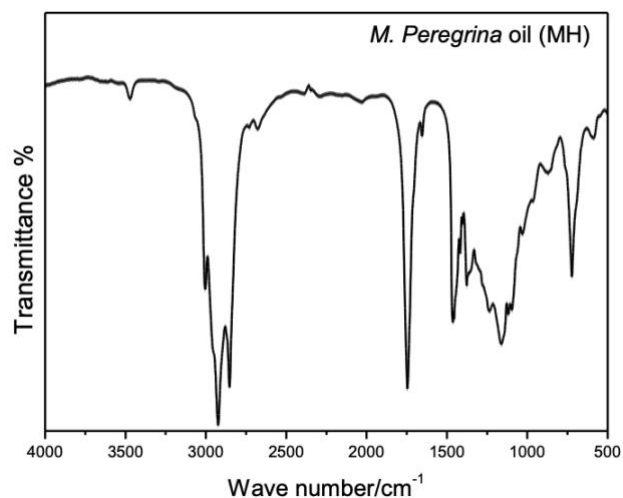


Figure 1. IR spectrum of *Moringa peregrina* seeds oil extracted by maceration method.

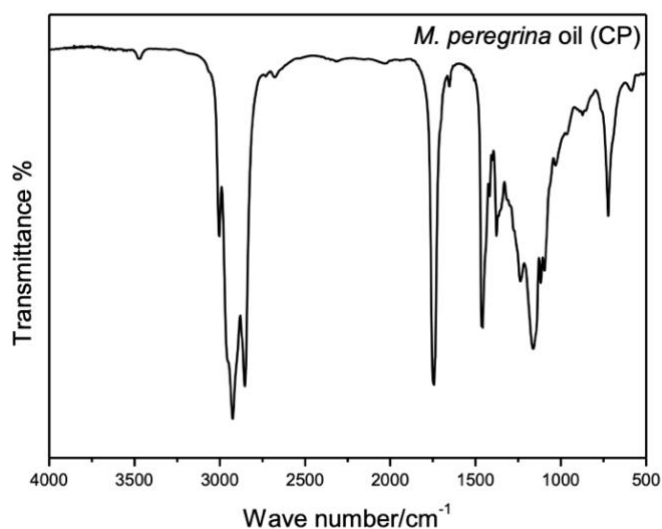


Figure 2. IR spectrum of *Moringa peregrina* seeds oil extracted by the cold press method.

Biological activity of oil: The antimicrobial activity of *Moringa peregrina* oil seeds extracted by cold press (CP) and maceration (MH) methods (Table 2) was assessed

against two-gram positive (+ve) bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), two gram negative (–ve) bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and one fungal microorganism (*Candida albicans*).

Table 2. The antimicrobial activity of *Moringa peregrina* oil seeds.

Microorganism		Inhibition zones for CP /mm	Inhibition zones for H /mm
Gram positive	<i>Bacillus subtilis</i> NCTC 8236	18	12
	<i>Staphylococcus aureus</i> ATCC 25923	10	-
Gram negative	<i>Escherichia coli</i> ATCC 25922	10	10
	<i>Pseudomonas aeruginosa</i> ATCC 27853	12	11
Fungal microorganism	<i>Candida albicans</i> ATCC 7596	18	12

Thermal stability of *Moringa peregrina* oil: The thermal stabilities of the extracted oils were investigated under a flow of nitrogen and the mass loss was plotted against

temperature. Figures 3 and 4 show the thermograms (TGA & DTGA) of *Moringa peregrina* oil, which was extracted by the cold press method.

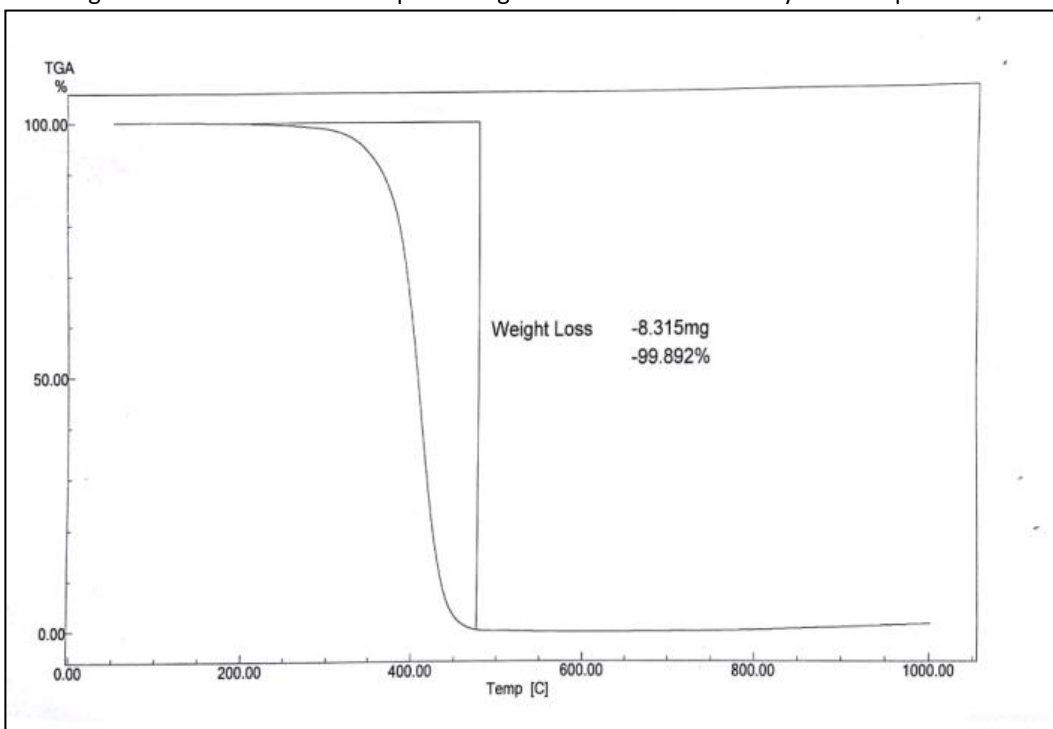


Figure 3. TGA curve of *Moringa peregrina* seeds oil extracted by cold press method.

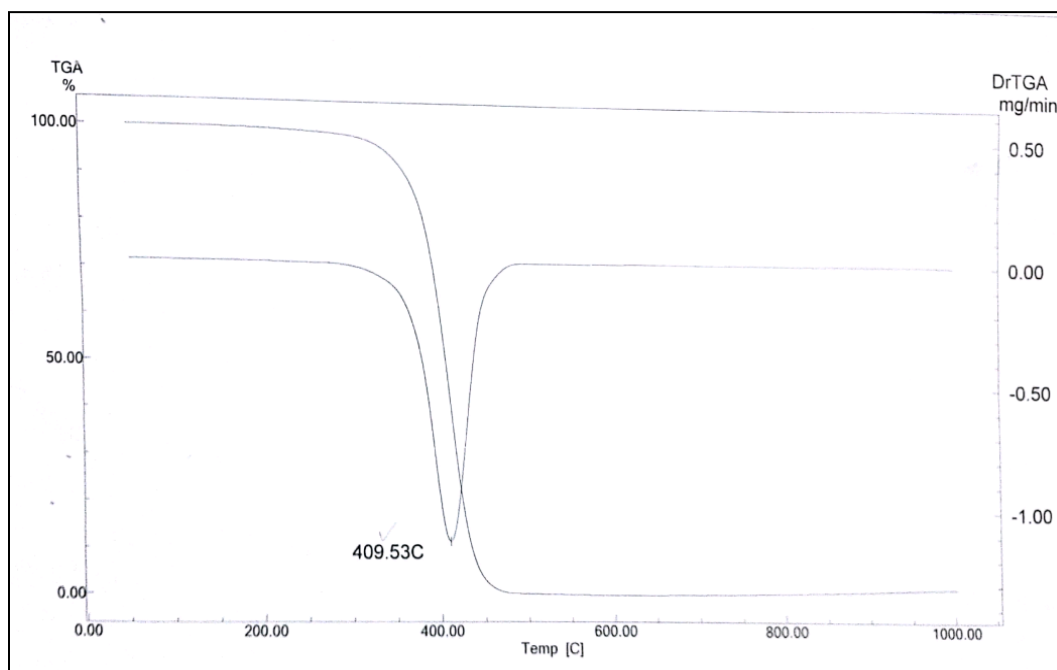


Figure 4. TGA/DTGA curve of oil extracted by cold press method.

Note: The DSC thermograms of *Moringa peregrina* seed oil extracted by cold press and maceration methods are shown in Figures 5 and 6, respectively.

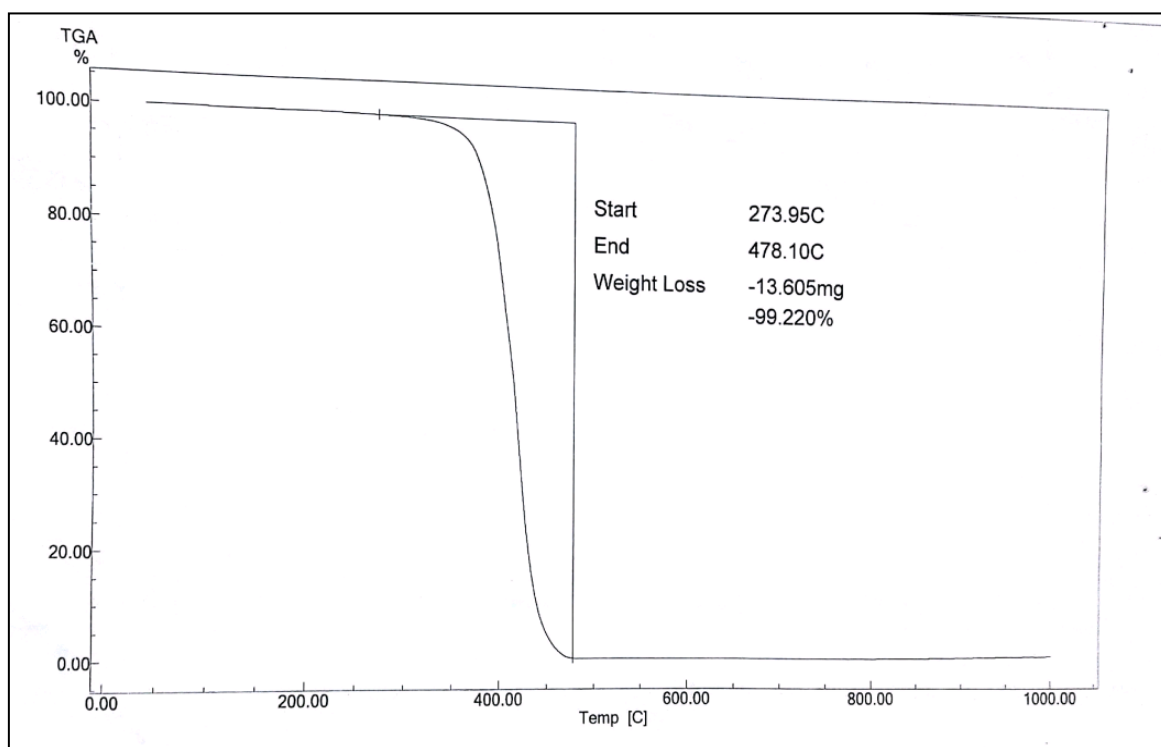


Figure 5. TGA thermogram of *Moringa peregrina* seed oil, extracted by maceration method.

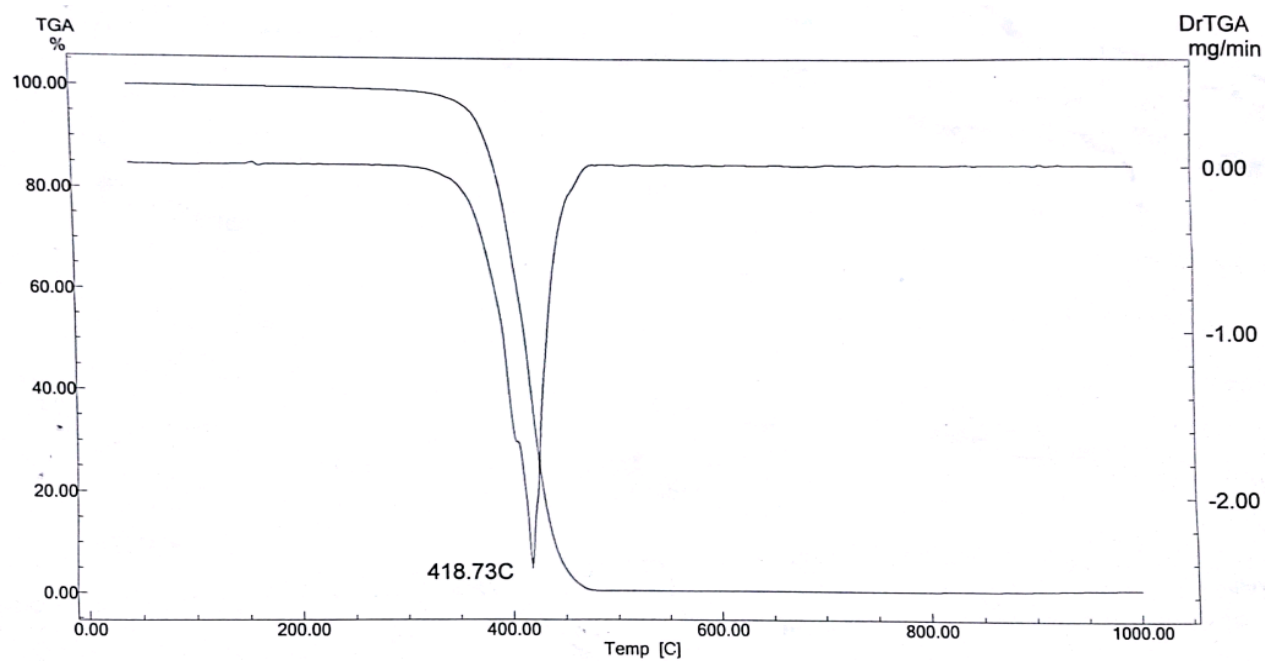


Figure 6. TGA/DTGA curve of oil extracted by maceration method.

Furthermore, the cooling thermogram of the oil extracted by maceration revealed two exothermic peaks at -56.61°C (11.28J/g) and -43.82°C (7.43J/g) (Figures 7 and 8). These findings revealed that the lowest onsets of

crystallization of cold pressed and macerated samples were -49.07°C and -56.61°C , whereas the highest onsets of melting were 10.31°C and 10.52°C , respectively.

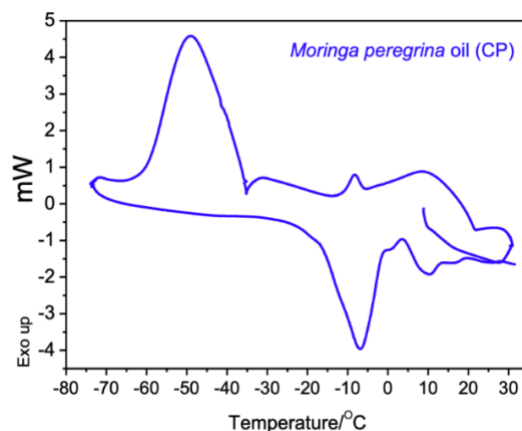


Figure 7. DSC curve of *Moringa peregrina* oil extracted by cold press method.

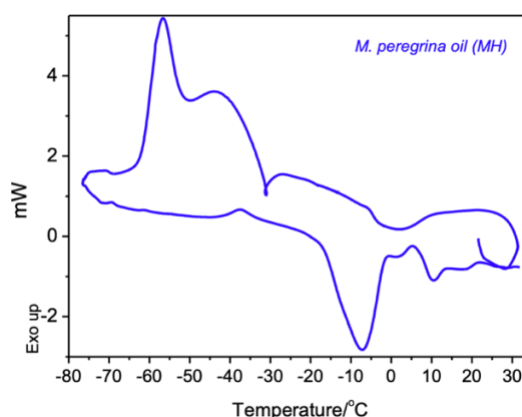


Figure 8. DSC curve of *Moringa peregrina* oil extracted by maceration method.

DISCUSSION

The oil content of the *Moringa peregrina* seeds was extracted by both cold pressing (CP) and maceration in n-hexane (MH) methods. The results revealed that both methods are less effective in the extraction of oil seeds compared to the Soxhlet method (using n-hexane) as reported by previous studies [17, 18]. However, the previous studies on other oil seeds extracted by cold press, maceration, and Soxhlet method (with n-hexane) have displayed similar trends, and express agreement with the above results concerning the efficiency of the extraction method and the oil content [17,19].

The chemical characteristics between the two methods of extraction (Table 1) showed no significant differences in the reported values except for peroxide

value. Furthermore, the chemical characteristics of the oil extracted by the two methods falls within the range of previously reported values [17, 14].

The FT-IR analyses showed an identical absorption peak for the oil samples that were extracted by the two methods, with no significant variations. Figures 1 and 2 showed an intense, sharp peak at 1745 cm^{-1} , which is due to carbonyl stretching vibration of the ester group (triglyceride). Furthermore, several peaks appear at 1456, 1373, 1231, 1161 and 715 cm^{-1} , which are due to -C-H bending vibrations (1456, 1373), C-O stretching vibration, C-H bending (1231, 1163), and C-H rocking (715 cm^{-1}). Very weak peaks are also noted at 3003 and 1656 cm^{-1} , which are due to C-H stretching vibration (of sp^2 hybridized system) and carbon double bond (C=C)

stretching vibration. Similar results for vegetable oils (canola, coconut, corn, olive, peanut, safflower, and soybean oils) were reported in the literature [20].

Table 2 shows the antimicrobial activity of *Moringa peregrina* oil seeds extracted by cold press (CP) and maceration (MH) methods, which was assessed against bacterial and fungal microorganisms. The oil sample extracted by the cold press method showed higher activity against tested organisms compared to the oil sample obtained via maceration method (Table 2). The magnitude of cold press extracted oil inhibition zones against the tested organisms was as follows: *Bacillus subtilis* (18 mm), *Staphylococcus aureus* (12 mm), *Escherichia coli* (10 mm), *Pseudomonas aeruginosa* (12 mm) and *Candida albicans* (18 mm), whereas for oil extracted by the maceration method was as follows: *Bacillus subtilis* (10 mm), *Staphylococcus aureus* (-), *Escherichia coli* (10 mm), *Pseudomonas aeruginosa* (11 mm) and *Candida albicans* (12 mm).

Escherichia coli proved the least resistant (10mm), while *Candida albicans* was the most resistant for both oil samples (Table 2). Lalas et al. [9] also reported *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Candida albicans*, *C. tropicalis* and *C. glabrata* to be sensitive to *Moringa peregrina* seed oil.

The thermal stabilities of the extracted oils using the two methods of extraction were reported in different figures. Figures 3 and 4 show the thermograms (TGA & DTGA) of *Moringa peregrina* oil, which was extracted by the cold press method. It can be noticed that the cold press extracted oil is stable up to 200°C and there was no significant degradation of the oil at this temperature. Furthermore, the TGA and DTGA curves revealed that 99.89% of the mass loss of the oil occurred in a single step that starts above 200 °C (Tonset temperature) and up to

470 °C (Toffset temperature). In addition, Figure 4 displayed that the temperature at which the maximum mass loss occurred for cold press extracted oil was 409.53 °C. On the other hand, Figures 5 and 6 demonstrate that 99.22% of the mass loss of the oil extracted by maceration occur in a single step between 273.95 °C (Tonset temperature) and 478.10 °C (Toffset temperature). The temperature at which the maximum mass loss occurred was 418.73 °C (Figure 3.6). It could be noted from above that the onset of degradation of the oil extracted by the two methods (cold press and maceration, respectively) varies significantly; 200 °C against 273.95 °C. This could be due to the extraction conditions that involve the exposure of the sample to water vapor in case of cold press prior to pressing. This process most likely raised the level of moisture in the oil and hence accelerated the oxidation process or rancidity. According to previous studies [21-23], the decomposition of vegetable oils under nitrogen atmosphere (thermal stability) takes place in one step, whereas in air atmosphere (oxidative stability), there are three steps. The single step decomposition of the vegetable oils in nitrogen atmosphere involves breaking down the oxygenated hydrocarbon present into volatile lower molecular hydrocarbons, carbon dioxide and carbon monoxide. On the other hand, the three distinct steps in the degradation of vegetable oils under oxygen atmosphere ascribed to the thermal analysis of the polyunsaturated fatty acids, mono-unsaturated fatty acids and saturated fatty acids respectively [24].

As can be seen from Figures 5-8, which represent the TGA/DTGA and DSC thermograms of *Moringa peregrina* seed oil extracted by both methods, the heating curve of the cold press extracted oil sample has two distinguished endothermic peaks at -6.92°C (-61.71J/g) and 10.31°C (-6.22J/g) (Figure 5). Additionally, the cooling curve displayed three exothermic peaks at -

49.07°C (2.47J/g), -8.29°C (13.57J/g) and 8.42°C (13.57J/g). The heating profile of the oil extracted by maceration method, on the other hand, also exhibited two peaks (Figure 6): at -7.26°C (-58.20J/g) and 10.52°C (-4.91J/g). The results from Figures 7 and 8 revealed that the lowest onsets of crystallization of cold pressed and macerated samples were -49.07°C and -56.61°C, whereas the highest onsets of melting were 10.31°C and 10.52°C, respectively.

CONCLUSION

In the present study, the results have shown that the method of extraction influences the properties and thermal stability of the extracted *Moringa peregrina* seed oil. Regarding the antimicrobial activity of the oil, cold pressing proves to be better than maceration in n-hexane for all tested micro-organisms. Additionally, the extraction method has observable effects on the onset of degradation of the oil and does not significantly affect the maximum as well as the final degradation temperatures. The results of DSC did not reveal any noticeable changes in the heating curves of the oils

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extracted by the two methods, while the cooling curves showed clear differences.

Abbreviations: AV, acid value; CP, cold pressing; DSC, differential scanning calorimeter; DTGA, derivative thermogravimetric analysis; FFA, free fatty acid; MH, maceration in n-hexane; MHA, Mueller Hinton agar; NCCLS, National Committee for Clinical Laboratory Standards; PV, peroxide value; SV, saponification value; TGA, thermogravimetric analysis.

Competing interests: No financial interests or personal relationships between the authors influence the research

Authors' contributions: Mariod, and Essa conceived and planned the presented idea, Nahla carried out the experiments, Nahla and Essa wrote the manuscript draft, Mohamedain and Mariod supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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