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Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome

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Influence of various extraction techniques (solvent-solvent, cold maceration and soxhlet extraction) on the mineral content and antioxidant capacity of ginger oil was investigated. The minerals determined were magnesium, manganese, zinc, iron, calcium, potassium, sodium and phosphorus. Flavonoid and phenolic contents were determined alongside the antioxidant capacity of the oils which was assayed using DPPH radical scavenging activity and total reducing power. Extracted oil showed strong characteristic pungent gingerly odor and was liquids at room temperature. Highest oil yield was obtained by soxhlet extraction technique (8.04± 0.04%); followed by cold maceration technique (5.30±0.03%) and the lowest by solvent-solvent extraction technique (4.83±0.08%). The results of the mineral analysis showed that oil obtained by soxhlet extraction technique had the highest concentration of all minerals determined, except manganese (3.80±0.01 mg/100g) which was highest in the oil obtained by cold maceration. The oil obtained by solvent-solvent extraction showed lowest concentration of all minerals determined, except calcium (1.08±0.10 mg/100g) and zinc (0.63±0.11 mg/100g) which were lowest in oil obtained by cold maceration. However, the concentration of phosphorus was highest in all the oils when compared with other minerals. Oil obtained by soxhlet extraction showed the highest concentration of flavonoids (118.00±1.00 mgQE/g) and phenolics (217.33±1.53 mgGAE/g) as well as the highest average DPPH radical scavenging capacity (55.56±0.04%) and average total reducing power (0.88±0.002) while the least concentration of flavonoids (44.45±2.97 mgQE/g), phenolics (112.43±1.42 mgGAE/g), average DPPH radical scavenging capability (19.73±0.01%) and average total reducing power (0.55±0.030) were obtained for the oil obtained by solvent-solvent extraction technique. The results showed that heat and reflux condition involved in the soxhlet extraction technique enhanced the extraction of minerals and phytochemicals from ginger rhizome and also enhanced the antioxidant capacity of ginger rhizome oil.

Capsule Summary: Efficiency of extraction methods such as solvent-solvent extraction, cold maceration and soxhlet extraction affected the range of phytochemicals and minerals extracted from ginger oil and their antioxidant capacity.

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INTRODUCTION

Extraction technique as well as extraction solvent determine to a large extent the amount and class of bioactive compounds extracted from plants. Many techniques are available which are used in extracting bioactive compounds from medicinal plants. These techniques are broadly classified as conventional and non-conventional techniques. The conventional techniques include soxhlet extraction, maceration, hydro-distillation and solvent- solvent extraction. Due to the attendant limitations of the conventional techniques some of which include; lengthy extraction time, requirement of costly and high purity solvents, evaporation of the large amount of solvent, poor extraction selectivity and thermal decomposition of thermo labile compounds (Luque de Castro and Garcia-Ayuso, 1998), more promising extraction techniques referred to as the non-conventional techniques were developed which include ultrasound assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction (Azmir et al., 2013). According to Bushra et al., (2009) and Jakopic et al., (2009), extraction yields, polyphenolic contents as well as antioxidant activities of most plant materials strongly depends on the nature of extraction technique and solvent. Recovery of antioxidant compounds from plant materials can be accomplished through various extraction techniques taking into account their chemistry and uneven distribution in the plant matrix (Bushra et al., 2009). Also, the amount of the antioxidant components that can be extracted from a plant material is mainly affected by the vigor of the extraction procedure which may differ from one sample to another (Bushra et al., 2009).

Ginger botanically known as zingiber officinale is a popular spice, herb as well as a flavoring agent with well-known medicinal properties which is attributed to its strong antioxidant activities. Afzal et al., (2001) have reported ginger's medicinal properties against rheumatism, diabetes and digestive disorder. Ginger has also been reported by Langner et al., (1998) and White, (2007) for its use traditionally to treat gastrointestinal disorder such as stomach aches, abdominal spasm, nausea and vomiting as well as arthritis and motion sickness. Besides its use as a spice, in Nigeria, ginger is sometimes brewed in boiling water to make ginger tea and its oil is also used for medical purposes (Bode, 2003). Ginger is used as preparation aid for various foods and because of its aroma and flavor, it is also employed as flavoring agent in bread, tea, carbonated drinks, biscuits, pickles and some other confectionaries (Longe, 1986; Dairo and Ojekale, 2006). A number of pharmacological activities like cardiovascular protection, antioxidant, anti-inflammatory and glucose lowering activities have been reported for ginger (Shukla and Singh, 2007). According to Abitogun and Badejo, (2010), ginger has an oil yield of about 7% and antibacterial potential against *staphylococcus aureus*, *Klebsiella eshericha*, *Pseudominas aeruginosa* and *Escherichia coli* due to the presence of saturated fatty acids such as myristic, lauric and palmitic acids. The presence of volatile essential oils which are about 1-3% accounts for ginger's distinguishing spicy aroma while oleoresins; about 4-7.5% are responsible for its pungent flavor (Balachandarn et al*.,* 2006). Chemical analyses of ginger have shown the presence of various bio-active compounds such as curcumin, 6-gingerol, 6-shogoals, zingiberene, bisaboline and other forms of lipids which confer on it its pungent and stimulating medicinal properties (Yoshikawa et al., 1993; Bliddal et al*.,* 2000) which are in turn responsible for its numerous medicinal applications such as analgesic, antiulcer, antipyretic, antiemetic among others (Mascolo et al., 1989; Phillips et al., 1993).

This research work is therefore aimed to examine the influence of various extraction techniques (solvent-solvent, maceration and soxhlet extraction) on the mineral contents and antioxidant activity of edible oil extracted from ginger.

EXPERIMENTAL

Sample collection and pre-treatment

Healthy fresh ginger rhizomes were purchased from a local market in Ogun state, Nigeria and copiously washed with distilled water to remove dirt's. A portion of the fresh ginger was used for solvent-solvent extraction of ginger oil while the remaining portion was chopped into smaller sizes, oven dried at 55ºC for 48 hours and then pulverized with the use of laboratory blender (LEXUS MG-2053 OPTIMA). The pulverized sample was further divided into two (2) parts; one of which was used for oil extraction by maceration while the other was used for soxhlet extraction of the oil.

Solvent-solvent extraction

A 50 g of thoroughly washed fresh ginger roots were blended with 150 ml distilled water in a laboratory blender (LEXUS MG-2053 OPTIMA). The mixture was then filtered with a muslin clothe to obtain as much filtrate as possible. The residue was re-extracted with 50ml distilled water twice; making a total of about 250 ml filtrate. The edible oil in the filtrate was extracted with 50 ml n-hexane (5times) by using a 500 ml separating funnel. The extraction solvent was thereafter removed *in vacuo* using rotary evaporator (Eyela N-1001) at 40 ^{o}C to recover the ginger oil. The oil was placed on a water bath at 40 ^{o}C for about two hours to ensure complete removal of residual solvent after which it was stored in a glass bottle and the analysis was carried out on the freshly extracted oil

Cold maceration

A 50 g of dried pulverized ginger was macerated in 200 ml n-hexane and filtered after 48 hrs. The residue was re-macerated with 150 ml n-hexane (twice) for 24 hrs and the filtrates were combined. The extraction solvent was thereafter removed *in vacuo* using rotary evaporator (Eyela

N-1001) at 40°C to recover the ginger oil. The oil was placed on a water bath at 40°C for about two hours to ensure complete removal of residual solvent after which it was stored in a glass bottle and the analysis was carried out on the freshly extracted oil

Soxhlet extraction

Solvent extraction was carried out on 50 g of pulverized sample with soxhlet apparatus at 80°C for a period of 8 hours using n-hexane as the extraction solvent. The extraction solvent was thereafter removed *in vacuo* using rotary evaporator (Eyela N-1001) at 40°C to recover the ginger oil. The oil was placed on a water bath at 40°C for about two hours to ensure complete removal of residual solvent after which it was stored in a glass bottle and the analysis was carried out on the freshly extracted oil.

Determination of physical properties of oil

The oils extracted from ginger were analyzed for some physical characteristics viz: color, odor and state at room temperature. The color and state of the oil at room temperature were noted by visual inspection while the odor was perceived by smelling.

Total flavonoid content

Total flavonoid content of the oil sample was determined by Aluminum trichloride spectrophotometric method of Dewanto et al. (2002) using Quercetin as standard. The method was based on formation of a flavonoid-aluminum complex. 1 mL aliquot of oil sample in methanol (100µg/ml) or standard at various concentrations (2, 4, 6, 8 and 10µg/ml) was diluted with distilled water (4 ml) in a 10 ml volumetric flask. Then, 5% $NaN0_2$ solution (0.3 ml) was added to the volumetric flask. After 5 min, 10% $AlCl_3$ (0.3 ml) was added and at 6 min, 1M NaOH (2 ml) was added. Water (2.4 ml) was then added to the reaction flask and mixed thoroughly. Absorbance of the reaction mixture was read at 510 nm. Total Flavonoid Content was estimated from Quercetin calibration curve (R^2=0.9972) and results expressed as mg Quercetin Equivalent per gram (mgQE g^{-1}) of oil sample. The analysis was carried out in triplicates.

Total phenolic content

The total phenolic content of the oil was assayed by the method of Singleton and Rossi, 1965. The assay is based on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds present in the sample. 1 ml aliquot of oil sample (100 µg/ml) in methanol was diluted with distilled water (9 ml) in a standard volumetric flask. 1ml of Gallic acid standards of various concentrations (2, 4, 6, 8 and 10 µg/ml) in methanol was similarly prepared. Folin-Ciocalteu's reagent (1 ml) was added to the mixture and vortexed. After 5 min, 10 ml of sodium carbonate solution (7%) was added to the mixture, and then incubated for 90mins at room temperature. After incubation, the absorbance against the reagent blank was determined at 760nm. A reagent blank was prepared using distilled water instead of the oil solution. The amount of total phenolic component in the oil was estimated from Gallic acid calibration curve (R^2=0.9984) and results expressed as mg Gallic Acid Equivalent per gram (mgGAE g^{-1}) of oil sample. The analysis was carried out in triplicates.

DPPH radical scavenging activity

The antioxidant activity of the oil sample was evaluated spectrophotometrically through its free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical by the method of Mensor et al., (2001). A 2.5 mL of oil solution of various concentrations (10, 20, 40, 60, 80 and 100 µg/ml) prepared in methanol was added to 1.0 mL of methanolic solution of DPPH (0.3mM) and kept in the dark at room temperature for 30 min. The same procedure was carried out on Gallic acid at various concentrations (2, 4, 6, 8 and 10 µg/ml). The absorbance of the resulting mixture was read at 518 nm and converted to percentage inhibition using the equation 1. Where, A_c and A_s are the absorbance's of control and sample, respectively.

$$DPPH\ inhibition\ (\%) = \left\{\frac{A_c - A_s}{A_c}\right\} * 100 \qquad (1)$$

The analysis was carried out in triplicate for each concentration. Methanol (2.5 ml) plus 1 ml of 0.3mM DPPH was used as the control. The IC50 value representing the concentration of the compounds that caused 50% inhibition of radical formation was obtained by interpolation from linear regression analysis (Stoilova et al., 2007).

Total reducing power

The total reducing power of the oil was assayed according to the method of Yen & Duh, 1993 as described by Premanath & Lakshmidevi (2010). 1 ml of various concentration of the oil sample (10, 20, 40, 60, 80 and100µg/ml) was mixed with phosphate buffer (500µL 20mM, pH 6.6) and 1% potassium ferricyanide (500µL). It was incubated at 50°C for 20mins; after which 500µL of 10% Trichloroacetic acid was added, and the mixture centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300µL) and the absorbance was read at 700 nm. Gallic acid solutions of various concentrations (2, 4, 6, 8 and 10µg/ml) were analyzed likewise. Increase in the absorbance of the reactions mixture indicated increase in the reducing power of the sample. The sample concentration providing 0.5 of absorbance (IC50) was calculated from the graph of absorbance at 700 nm against sample concentration (Barros et al., 2007). The analysis was carried out in triplicates.

Determination of mineral contents of oil

Table 1: Physical characteristics of Ginger oils

Oil sample	Color	Odor	Room temperature	% Yield
SSE	Yellow	Pungent and gingerly	Liquid	4.83± 0.08
CME	Brownish yellow	Pungent and gingerly	Liquid	5.30± 0.03
SXE	Deep brownish yellow	Pungent and gingerly	Liquid	8.04± 0.04

Table 2: Flavonoid and phenolic contents of Ginger oils

Oil sample	Flavonoid content (mgQE/g)	Phenolic content (mgGAE/g)
SSE	44.45±2.97	112.43±1.42
CME	96.67±1.51	186.55±2.31
SXE	118.00± 1.00	217.33±1.53

Data are expressed as mean ± standard error of three replicates. SSE= solvent-solvent extraction, CME= Cold maceration extraction, SXE= soxhlet extraction

The oil samples were digested separately for mineral analysis by wet digestion method described by Oluremi et al., 2013. 0.5 g of the sample was weighed and transferred into 75 mL micro digestion tubes. Concentrated H_2SO_4 (4 ml) and H_2O_2 (2 ml) were added carefully. The tubes were heated in a block digester (pre-heated to 270 °C) for 30 minutes. They were then taken out and allowed to cool. Another portion of H_2O_2 (2 mL) was added and heated further to achieve complete digestion which was indicated by appearance of clear solution. Magnesium, manganese, zinc, iron and calcium were determined in the digest by using an Atomic Absorption Spectrophotometer (Buck scientific Model 2010), after the equipment had been calibrated using 100 mg/L of the standard solution of each element to be determined. Meanwhile, potassium and sodium were determined in the digest with the use of Flame Photometer (Jenway FP 160 model) while phosphorus was determined with the use of a Spectrophotometer (Spectro SC LabMed model).

RESULTS AND DISCUSSION

Physical characteristics of Ginger oils

The results of the physical characteristics of ginger oils as determined in this work are given in Table 1. Oils obtained by solvent-solvent extraction, cold maceration extraction and soxhlet extraction techniques were yellow, brownish yellow and deep brownish yellow in color respectively. The oils were liquids at room temperature and with strong characteristic pungent gingerly odor. From the results obtained, the soxhlet extraction technique gave the highest oil yield (8.04± 0.04%) followed by cold maceration extraction (5.30± 0.03%) while the least oil yield (4.83± 0.08%) was obtained by solvent-solvent extraction technique. The highest oil yield obtained by soxhlet extraction may be due to the high temperature coupled with the reflux condition involved in the extraction technique. This is in agreement with Bushra et al., (2009) who reported that regardless of the plant material and extraction solvent employed, better yields of extracts were obtained when extraction was done under reflux. However, the oil yields obtained by the three extraction techniques were low.

Total flavonoid and phenolic contents

The flavonoid and phenolic contents of ginger edible oils obtained by the different extraction techniques are given in Table 2. The Flavonoid content ranged from 44.45±2.97 to 118.00± 1.00mgQE/g while the phenolic content ranged from 112.43±1.42 to 217.33±1.53mgGAE/g. The highest Flavonoid and phenolic contents were obtained for the SXE oil while the lowest Flavonoid and phenolic contents were obtained for the SSE oil. This may imply that heat and reflux condition involved in soxhlet extraction technique enhanced the extraction of flavonoids and phenolics from ginger. Highest level of polyphenols has been reported in Vogel seeds for its extraction made under reflux condition with ethanol/water (70:30, v/v) when compared with other extraction methods viz; maceration, ultrasound and heating plate (Dutra et al., 2008). Antolovich et al., (2000) has also attributed the higher recovery of some bound phenolics to effective extractions which occur under reflux condition. Likewise, increase in flavonoids content have been reported for *Aloe barbadensis* leaves from 4.28 to 4.66 g CE/100 g of DW, when extracted with aqueous methanol using the reflux technique (Bushra et al., 2009);which was also involved in soxhlet extraction technique employed in this work.

DPPH radical scavenging activity

Table 3 represents the results of the DPPH radical scavenging strength of the oils. From the results obtained, the average percentage DPPH radical scavenging capacity of the oils ranged from 19.73±0.01% to 55.56±0.04% with the highest reported for SXE oil and the lowest reported for SSE oil. In addition, the IC50 value (representing the concentration of the compounds that caused 50% inhibition of free radical formation) was highest in SSE oil (132.96±0.21µg/ml)

Table 3: Percentage inhibition of DPPH free radical by Ginger oils

Oil sample	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	100 µg/ml	DPPH inhibition (%)	IC50 (µg/ml)
SSE	4.97±0.15	9.10±0.20	15.33±0.15	32.47±0.10	36.80±0.10	19.73±0.01	132.96±0.21
CME	14.95±0.10	28.97±0.10	50.47±0.10	66.01±0.14	67.20±0.10	45.52±0.04	57.98±0.06
SXE	24.37±0.09	43.39±0.09	59.22±0.20	73.18±0.04	77.64±0.05	55.56±0.04	39.60±0.09

Data are expressed as mean ± standard error of three replicates. SSE= solvent-solvent extraction, CME= Cold maceration extraction, SXE= soxhlet extraction

Table 4: Total reducing power (Absorbance) of Ginger oils

Oil sample	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	100 µg/ml	Absorbance	IC50 (µg/ml))
SSE	0.51±0.002	0.53±0.002	0.55±0.002	0.60±0.002	0.68±0.001	0.55±0.030	11.32±0.34
CME	0.50±0.003	0.55±0.003	0.73±0.002	0.85±0.003	0.94±0.020	0.71±0.010	5.00±1.09
SXE	0.53±0.002	0.62±0.002	0.86±0.001	1.06±0.002	1.34±0.002	0.88±0.002	4.44±0.05

Data are expressed as mean ± standard error of three replicates. SSE= solvent-solvent extraction, CME= Cold maceration extraction, SXE= soxhlet extraction, Average Total reducing power = Absorbance

Table 5: DPPH radical scavenging activity and Total reducing power (TRP) of Gallic acid

Standard	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	Average %	IC_{50} (µg/ml)
DPPH inhibition (%)	32.56±0.02	61.55±0.03	85.50±0.03	90.1±0.01	94.91±0.04	73.00±0.03	3.01±0.01
Absorbance	0.599±0.15	0.674±0.03	0.774±0.21	0.989±0.01	1.075±0.01	0.82±0.01	1.00±0.02

Data are expressed as mean ± standard error of three replicates, Total reducing power = Absorbance

followed by CME (57.98±0.06µg/ml) and lowest in SXE oil (39.60±0.09µg/ml); since the percentage inhibitory strength of medicinal plants against free radicals is inversely proportional to their IC50 values. The exhibition of highest DPPH radical scavenging capacity by SXE oil may be attributed to its possession of highest concentration of flavonoids and phenolics. Likewise, SSE oil which showed the lowest concentration of flavonoids and phenolics also showed the lowest DPPH radical scavenging capacity. This may imply that hot solvent systems under reflux condition are more efficient for the extraction of antioxidant phyto-constituents. In order words, the results showed that the higher the concentration of these phytochemicals, the higher the DPPH radical scavenging capability of ginger oil. This is in agreement with researches by several authors (Galvez et al., 2005; Shahidi and Wanasundara, 1992; Velioglu et al., 1998) who have reported the positive effect of flavonoids and phenolics on the antioxidant ability of many medicinal plants. According to Kessler et al., (2003), phenolic compounds act as free radical terminators and the mechanism of action of flavonoids is through scavenging or chelating process (Cook and Samman, 1996 & Bajpai et al., 2005). However, the antioxidant capacity shown by ginger rhizome oil may be due to much more phytochemicals other than the ones determined in this work which may be present in the oils and their synergistic effect too.

Total reducing power

The results of total reducing power of the oils are represented in Table 4. From the results obtained, SXE oil exhibited the highest reducing power with an average absorbance of 0.88±0.002 and lowest IC50 value of 4.44±0.05µg/ml while SSE oil exhibited the lowest reducing power with an average absorbance of 0.55±0.030 and the highest IC50 value of 11.32±0.34µg/ml. IC50 in this case is the sample concentration providing an absorbance of 0.5 at 700 nm. The same trend noticed in the DPPH scavenging capacity of the oils was also repeated in its total reducing power. The SXE oil which had the highest concentration of flavonoids and phenolics also showed the best reducing power while the SSE oil with the lowest concentration of flavonoids and phenolics showed the least reducing power. This may also be attributed to the heat and reflux-enhanced extraction of flavonoids and phenolics in SXE method. This also showed the dependence of the reducing power of ginger oil on the concentration of flavonoids and phenolics present in it.

Antioxidant activity of gallic acid

Table 5 shows the results of the DPPH radical scavenging capacity and reducing power of Gallic acid which was used as a positive control. The results show that the oils extracted by

Table 6: Mineral contents of Ginger oils

Oil	P	Ca	Mg	K	Na	Fe	Mn	Zn
	mg/100g							
SSE	10.73±0.21	1.65±0.10	1.68±0.15	5.28±0.02	6.81±0.11	0.96±0.15	0.17±0.01	0.96±0.01
CME	41.64±0.05	1.08±0.10	13.85±0.13	38.34±0.01	23.19±0.12	1.43±0.15	3.80±0.01	0.63±0.11
SXE	76.79±0.11	2.88±0.03	18.48±0.15	76.68±0.12	42.36±0.10	1.65±0.11	2.73±0.02	1.77±0.03

Data are expressed as mean ± standard error of three replicates. SSE= solvent-solvent extraction, CME= Cold maceration extraction, SXE= soxhlet extraction

the three methods showed lower antioxidant capacity than Gallic acid. Gallic acid showed an average percentage DPPH radical scavenging capability of 73% with an IC50 of 3.01±0.01µg/ml and total reducing power of 0.82 and IC50 of 1.00±0.02µg/ml

Mineral content of Ginger oils

Table 6 shows the results of the mineral contents of the oils as determined in this work. From the results obtained, SXE oil showed the highest concentrations of all the minerals determined except manganese (3.80±0.01 mg/100g) which was highest in CME oil. Likewise, SSE oil showed the lowest concentration of all minerals determined except calcium (1.08±0.10 mg/100g) and zinc (0.63±0.11 mg/100g) which were lowest in CME oil. Also, phosphorus occurred at the highest concentration in the three oils as compared with other minerals determined. However, the minerals with the lowest concentration in SXE oil, CME oil and SSE oil were iron (1.65±0.11 mg/100g), zinc (0.63±0.11mg/100g) and manganese (0.17±0.01mg/100g) respectively.

The results of the mineral analysis suggest that ginger oil irrespective of the extraction technique is rich in both micro and macro minerals which are essential for human health and can therefore be recommended for pharmaceutical purposes. However, in other to obtain adequate concentrations of both micro and macro minerals from ginger oil, a combination of extraction techniques may be required.

CONCLUSIONS

The oil extracted by soxhlet extraction technique in which high amount of heat as well as reflux condition were applied showed the highest concentrations of flavonoids, phenolics, all minerals determined except manganese (Mn) as well as the best antioxidant capacity. Likewise, the oil extracted by solvent-solvent extraction technique in which neither heat nor reflux was applied showed the lowest concentrations of flavonoids, phenolics, all minerals determined except calcium (Ca) and zinc (Zn) as well as the least antioxidant activities. The soxhlet extraction technique also gave the highest oil yield while the lowest oil yield was obtained for the solvent-solvent extraction technique. It is worthy of note that of all the minerals determined, phosphorus had the highest concentration in the oils obtained by the three methods.

The results obtained in this research suggest that heat coupled with refluxing enhanced the extraction of flavonoids and phenolics which may invariable have enhanced the antioxidant capacity of the ginger oil. Hence, in order to get the best medicinal and nutritional values from ginger oil, extraction with a method that involves relatively high amount of heat and reflux condition may be required. In addition, extraction of ginger oil with a combination of techniques may enhance the extraction of more phyto-chemicals and hence the usability of the oil for pharmaceutical or medicinal purposes. However, we have further works ongoing specifically to determine the chemical composition and antimicrobial strength of each of the oils obtained by the different extraction techniques employed in this present work. These would further expound the specific usability of oil extracted by the individual extraction technique.

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