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COMPARATIVE ANTIMICROBIAL ACTIVITY AND BIOACTIVE CONSTITUENTS OF OILS FROM RHIZOMES OF *ZINGIBER OFFICINALE* ROSCOE OBTAINED BY DIFFERENT EXTRACTION METHODS

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ABSTRACT: *Ginger, known for its rich array of bioactive compounds, holds significant therapeutic potential due to its diverse medicinal properties. This study investigated the antimicrobial activity and bioactive principles present in ginger rhizome oils obtained using three different extraction methods viz: liquid-liquid extraction, cold maceration, and soxhlet extraction. The oils' bioactive principles were identified by Gas Chromatography Mass Spectrometry (GC-MS) while their antimicrobial activity was determined by agar well diffusion technique. The GC-MS analysis revealed the presence of thirty compounds in each of the oils from liquid-liquid extraction and cold maceration, and fifty four compounds in the soxhlet-extracted oil. The most predominant compound in both liquid-liquid (31.13%) and cold maceration (16.99%) oils was oleic acid whereas the Soxhlet-extracted oil contained predominantly linoleic acid methyl ester (9.27%). Some bioactive compounds identified in these oils include δ-elemene, isoborneol, α-Bisabolol oxide, stearic acid, undecanone, palmitic acid, α-copaene, zingiberene, aromadendrene, farnesol, 2-methylhexane and farnesene which possess antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-ulcer, anti-cancer, hypolipidemic, mosquito repellant, antimicrobial, and antidiabetic activities. Additionally, the oils exhibited promising antimicrobial potential against the test organisms with Staphylococcus aureus showing the least susceptibility to all the samples. Staphylococcus aureus and Candida albicans were completely resistant to the oil obtained by Soxhlet extraction at all tested concentrations. Meanwhile, Klebsiella pneumoniae (20 mm), Proteus vulgaris (18 mm), and Pseudomonas aeruginosa (17 mm), were most susceptible to the oil obtained by liquid-liquid extraction, cold maceration and soxhlet extraction, respectively. The study highlighted the significance of extraction methods on the chemical composition and antimicrobial activity of ginger rhizome oils, underscoring the importance of choosing appropriate extraction techniques to optimize the oils' therapeutic properties for specific medicinal applications.*

KEYWORDS: Antimicrobial, Bioactive constituent, Extraction, GC-MS, Ginger oil.

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INTRODUCTION

Extraction stands as a pivotal phase in the retrieval of natural products, playing a vital role in the separation of bioactive elements from plants through the use of selective extraction solvents in accordance with established procedures (Zhang et al., 2018). This step is crucial in the phytochemical processing itinerary, facilitating the discovery of bioactive constituents from plant materials. The primary objective of extraction is to isolate soluble plant metabolites, leaving behind the insoluble cellular marc (Azwanida et al., 2015). Various extraction methods, including maceration, infusion, percolation, decoction, soxhlet extraction, microwave-assisted extraction, ultrasound-assisted extraction (sonication extraction), accelerated solvent extraction, and supercritical fluid extraction (Dhanani et al., 2017) and come with their distinct advantages and limitations (Azwanida et al., 2015). Hence, careful consideration is necessary in the choice of extraction method, as the recovery of bioactive compounds from natural products greatly depends on this selection. The appropriate extraction technique is also pivotal for the standardization of herbal products, enabling the removal of desirable soluble constituents while leaving out undesired elements with the aid of solvents (Dhanani et al., 2017).

Several factors, beyond the extraction method itself, influence the physical properties, chemical composition, and significantly, the biological activities of extracts from natural products. These factors include the extraction solvent, extraction duration, speed of agitation, pH of the extraction solvent, temperature, solvent-sample ratio, and particle size of the extracted material. Researchers have extensively reported on the effects of these factors on extract yield and the biological activities of extracts from natural products (Dieu-Hien et al., 2019). In a previous study by Adaramola and Onigbinde (2017), significant variations in mineral, flavonoid, and phenolic contents, as well as antioxidant capability, were observed in ginger oils obtained through different extraction methods. Similarly, Vongsak et al. (2013), in a comparison of maceration, soxhlet extraction, and percolation for extracting phenolics and flavonoids from Moringa oleifera, found that maceration with 70% ethanol at 1:40 w/v exhibited the highest phenolics and flavonoids content compared to soxhlet extraction and percolation using a similar solvent.

Among the myriad of plants yielding biologically active compounds, ginger (*Zingiber officinale*) has been a subject of significant extraction and identification efforts. Rich in antioxidant constituents, ginger has been employed as a curative medicine since ancient times (Shahrajabian et al., 2019). Scientific investigations have documented its compositions and diverse biological activities, including antimicrobial, antioxidant, and immune-enhancing properties (Saira et al., 2014). Bioactive compounds that have been identified in ginger include α-zingiberene, 6-gingerol, β-sesquiphellandrene, 6-shogaol, α-farnesene, β-bisabolene, and α-curcumene (Zhan et al., 2008), with reported percentages of 22.29%, 9.38%, 8.58%, 7.59%, 3.93%, 3.87%, and 2.63%, respectively. Ginger is reported to contain 9% lipids or glycolipids and 5-8% oleoresin (Chrubasik et al., 2005). Extensive studies have explored its extracts for various biological activities such as anti-tumor, anticonvulsant, anxiolytic, treatment of Parkinson's disease, anti-inflammatory, antibacterial, antidiabetic, and cholesterol-lowering effects (Yassen & Ibrahim, 2016; Toda et al., 2016; Fadaki et al., 2017).

This present study aims to investigate the chemical constituents and the antimicrobial potential of ginger oils obtained using different extraction methods. Liquid-liquid extraction (LLE), cold maceration extraction (CME), and soxhlet extraction (SXE) methods were employed to extract

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oils from the rhizomes of Zingiber officinale. The findings of this study would not only contribute to the understanding of ginger oil extraction but also provide insights into the potential medicinal applications of oils extracted through these methods.

MATERIALS AND METHODS

Chemicals

N-hexane was obtained from BDH Limited, Poole England. Nutrient agar was obtained from Lab M Limited, 1 Quest Park, Moss Hall Road, Heywood, Lancashire BL9 7JJ, United Kingdom while Potato Dextrose agar was purchased from Rapid Labs Ltd, Unit 2 Hall Farm Church Road, Little Bentley, Colchester ESSEX CO7 8SD United Kingdom. Ciprofloxacin and Fluconazole were obtained from Sigma-Aldrich Co Ltd, United Kingdom. All chemicals and reagents used were analytical grade.

Sample Collection and Preparation

Sample collection and oil extractions were carried out according to the methods previously described by Adaramola and Onigbinde (2017). Fresh, healthy and mature rhizomes of ginger were harvested from a farm where it was cultivated in Ilisan Remo, Ogun state, Nigeria. These were rinsed with copious amount of distilled water in order to remove dirt. A portion of it was used for liquid-liquid extraction while the remaining portion was chopped into smaller bits, oven dried at 50 ^{o}C for 48 h and then pulverized with laboratory blender (LEXUS MG-2053 OPTIMA). The pulverized sample was subsequently divided into two (2) parts. One part was used for oil extraction by maceration while the other part was used for soxhlet extraction of the oil.

Extraction of Ginger Oils

Liquid-liquid Extraction

Fifty grams of the thoroughly washed fresh ginger rhizomes were blended with 150 mL distilled water in a laboratory blender (LEXUS MG-2053 OPTIMA). The resulting mixture was filtered with a muslin cloth to obtain the filtrate. The residue was further re-extracted with about 50 mL distilled water twice to obtain about 250 mL filtrate. Fifty milliliters (50 mL) of n-hexane was added to the filtrate (5 times) in a 500 mL separating funnel and the mixture was shaken vigorously. After the layers were separated, the n-hexane layer was removed. The n-hexane was thereafter removed *in vacuo* using rotary evaporator (Eyela N1001) at 40 ^{o}C to recover the extracted ginger oil. The oil was placed on a water bath at 40 ^{o}C for about 2 h for complete removal of residual solvent after which it was stored in a glass bottle for analysis and labeled as LLE oil.

Cold Maceration

Fifty grams of the dry and pulverized ginger rhizome was extracted by maceration in 200 mL n-hexane with intermittent shaking for 48 h after which the resulting mixture was filtered with a Whatman No. 1 filter paper. The residue was re-macerated in 150 mL n-hexane (x 2) for 24 h and filtered. The filtrates were combined and extraction solvent was thereafter removed *in vacuo* using a rotary evaporator (Eyela N-1001) at 40 ^{o}C to recover the oil. The oil was placed

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on a water bath at 40ºC for about 2 h to ensure complete removal of residual solvent after which it was stored in a glass bottle for analysis and labeled as CME oil.

Soxhlet Extraction

Oil was extracted from 50 g of pulverized sample with soxhlet apparatus at 80^{O}C for a period of 8 h with n-hexane as the extraction solvent. The extraction solvent was thereafter removed *in vacuo* using a rotary evaporator (Eyela N-1001) at 40ºC to recover the ginger oil. The oil was placed on a water bath at 40 ºC for about 2 h to ensure complete removal of residual solvent after which it was stored in a glass bottle for analysis and labeled as SXE oil.

Antimicrobial Assay

The antimicrobial activity of ginger oil extracts against clinical isolates of Gram negative and Gram positive bacteria and a fungus was carried out using agar diffusion method as previously described by Olajuyigbe and Afolayan (2012). The test organisms including *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATTC 10702, *Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* ATCC 6830, *Pseudomonas aeruginosa* ATCC 19582 and *Candida albicans* were obtained from Babcock University Teaching Hospital Laboratory, Ilisan-Remo, Ogun State Nigeria. The test bacteria were grown in nutrient broth for 24 h while the fungus was grown in a potato dextrose broth for 5 days. The inocula of the test organisms were prepared using the colony suspension method. Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transferring 0.1 mL of the bacterial suspension to 9.9 mL of sterile nutrient broth before being used. Sterile Mueller Hinton agar (MHA) (Oxoids Ltd, Basingstoke, Hampshire, UK) plates were swabbed with the resultant saline suspension of each adjusted bacterial strain while sterile potato dextrose agar plates were swabbed with the adjusted *Candida albicans*. Wells were then bored into the agar medium using a heat sterilized 5 mm cork borer. The wells were filled with 100 µL of each extract concentration along with 100 µL of ciprofloxacin (2.5 µg/mL) and Fluconazole taking care not to allow spillage of the solutions onto the surface of the agar. The culture plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of these solutions before being incubated at 37°C for 24 h for the bacterial isolates and 3-5 days for the fungus. Dimethyl sulfoxide (DMSO) served as the blank while Ciprofloxacin (for bacteria) and Fluconazole (for fungi) were used as standard drugs. After the incubation period, the diameter of each zone of inhibition was recorded and expressed in millimeters. Minimum inhibitory concentration of the extract on the organisms was carried out at lower concentrations as described above. The entire microbial assay was conducted under strict aseptic conditions and all analyses were carried out in triplicates.

GC-MS Analysis of Ginger Oils

Bioactive principles in the ginger oil extracts were identified by GCMS-QP2010 PLUS SHIMADZU, JAPAN according to a previously described method by Fapohunda et al. (2017). Briefly, a fused-silica capillary column (30 m x 0.25 mm x 1.00 µm), a split injector and an ion-trap mass spectrometer detector were used for the GC-MS analysis. The column temperature was programmed from 80ºC to 280ºC at 3.0ºC/min. The temperature of the injector and detector was kept at 250ºC. Helium was used as a carrier gas at a flow rate of 1.58 mL/min. Recording of mass spectra was done in electron impact mode (70 eV), scanning from 40 to 600

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m/z. Identification of components was done by their identical GC retention times and retention indices relative to *n*-alkanes and by computer-aided matching of their spectra with spectra of known compounds as published by the National Institute of Standards and Technology (NIST) (NIST, 2009). Fragmentation patterns of eluted compounds were identified by comparing with known data from the database. Meanwhile, the relative percentage of each identified compound was obtained by comparing its average peak area to the total area.

RESULTS

In this study, oil from ginger rhizome was extracted with n-hexane but with three different methods of extraction viz; liquid-liquid extraction (LLE), cold moderation (CME) and soxhlet extraction (SXE). The resulting oil extracts obtained from the different extraction methods were comparatively assayed for their antimicrobial activity against selected bacteria (*Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris*) and a fungus (*Candida albicans*). Also, the bioactive composition of each extracted oil was determined with GC-MS. This was in order to determine the influence of extraction methods on the antimicrobial ability and bioactive constituents of ginger rhizome oil.

The susceptibility of the isolates to the different oils extracted showed that most of the isolates were susceptible to different concentrations of the oils to produce inhibition zones 09 ± 0.23 and 20 ± 0.23 mm. However, while *Proteus vulgaris* was susceptible at 4 mg/mL and 5 mg/mL to LLE oil and *Staphylococcus aureus* was susceptible to LLE oil and CME oil at 5 mg/mL, both *Candida albicans* and *Staphylococcus aureus* were not susceptible to SME oil as shown in Table 1. The results showed that ginger oils obtained by the different methods exhibited considerable antimicrobial properties at varying degrees and in considerably concentration dependent fashion. Of all the bacteria, *Staphylococcus aureus* was the most resistant isolate to all the oil samples at the test concentrations. However, it was inhibited at a concentration of 5 mg/mL with zones of inhibition of 12 ± 0.03 mm and 11 ± 0.03 mm by the oils obtained by LLE and CME at 5 mg/mL, respectively while it was resistant to the SXE oil. The results showed that *Klebsiella pneumoniae* was most susceptible to LLE oil with 20 ± 0.23 mm zone of inhibition at 5 mg/mL, *Proteus vulgaris* was the most susceptible to CME oil with 18 ± 0.04 mm zone of inhibition at 5 mg/mL while *Pseudomonas aeruginosa* was the most susceptible to SXE oil with 17 ± 0.22 mm zone of inhibition at 5 mg/mL. Though *Candida albicans* was resistant to SXE oil at all the test concentrations, the fungus was, however, highly susceptible to LLE oil with a zone of inhibition of 18± 0.04 mm and CME oil with a zone of inhibition of 16± 0.03 mm at 5 mg/mL concentration. While the minimum inhibitory concentrations of LLE oil against the microbial isolates ranged between 0.10 ± 0.01 mg/mL and 4.50 ± 0.03 mg/mL, those of CM oil ranged between 0.30 ± 0.01 mg/mL and 4.50 ± 0.10 mg/mL and MICs for SXE oil ranged between 0.20 ± 0.01 mg/mL and 5.00 ± 0.10 mg/mL. Although *S. aureus* had the highest MICs of 4.50 ± 0.03 mg/mL for LLE oil and CME oil and 5.00 ± 0.10 mg/mL for SXE oil, *Klebsiella pneumoniae* was susceptible at 0.10 ± 0.01 mg/mL, 0.40 ± 0.01 mg/mL and 0.30 ± 0.01 for LLE oil, CME oil and SXE oil, respectively, as shown in Table 2.

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Table 1: Susceptibility of test microbes to ginger oils

S/N	Microorganism	Extracts	Diameter zone of inhibition (mm)					Ciprofloxacin/Fluconazole
			1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL	(2.5 µg/mL)
1	*Staphylococcus aureus*	LLE Oil CME Oil SXE Oil	NI NI NI	NI NI NI	NI NI NI	NI NI NI	12 ± 0.03 11 ± 0.03 NI	21 ± 0.10
2	*Enterococcus faecalis*	LLE Oil CME Oil SXE Oil	10 ± 0.03 10 ± 0.11 12 ± 0.01	12 ± 0.13 11 ± 0.22 13 ± 0.11	12 ± 0.05 12 ± 0.03 13 ± 0.12	12 ± 0.01 12 ± 0.21 14 ± 0.24	14 ± 0.13 14 ± 0.23 15 ± 0.03	21 ± 0.21
3	*Bacillus cereus*	LLE Oil CME Oil SXE Oil	12 ± 0.13 10 ± 0.23 09 ± 0.23	12 ± 0.12 12 ± 0.11 11 ±0 .03	13 ± 0.04 13 ± 0.03 11 ± 0.22	13 ± 0.31 13 ± 0.22 12 ± 0.31	17 ± 0.16 15 ± 0.33 13 ± 0.34	22 ± 0.14
4	*Pseudomonas aeruginosa*	LLE Oil CME Oil SXE Oil	13 ± 0.04 12 ± 0.03 14 ± 0.01	13 ± 0.33 13 ± 0.13 14 ± 0.03	13 ± 0.02 13 ± 0.02 14 ± 0.13	13 ± 0.03 13 ± 0.03 14 ± 0.23	15 ± 0.33 16 ± 0.22 17 ± 0.22	19 ± 0.13
5	*Klebsiella pneumoniae*	LLE Oil CME Oil SXE Oil	13 ± 0.04 12 ± 0.11 11 ± 0.13	13 ± 0.03 12 ± 0.14 12 ± 0.04	13 ± 0.14 13 ± 0.05 12 ± 0.03	17 ± 0.21 13 ± 0.22 13 ± 0.04	20 ± 0.23 14 ± 0.03 13 ± 0.13	21 ± 0.06
6	*Proteus vulgaris*	LLE Oil CME Oil SXE Oil	NI 12 ± 0.13 11 ± 0.03	NI 13 ± 0.04 12 ± 0.02	NI 14 ± 0.13 12 ± 0.12	12 ± 0.05 15 ± 0.04 13 ± 0.03	16 ± 0.31 18 ± 0.04 14 ± 0.03	21 ± 0.21
7	*Candida albicans*	LLE Oil CME Oil SXE Oil	10 ± 0.03 10 ± 0.03 NI	13 ± 0.01 12 ± 0.01 NI	13 ± 0.02 15 ± 0.01 NI	14 ± 0.11 15 ± 0.12 NI	18 ± 0.04 16 ± 0.03 NI	20 ± 0.23

Data are expressed as mean ± standard deviation of three replicates. LLE- liquid-liquid extraction, CME- cold maceration extraction, SXE- soxhlet extraction, NI- no inhibition

Table 2: Minimum inhibitory concentrations of ginger oils against microbes

S/N	Microorganisms	LLE oil (mg/mL)	CME oil (mg/mL)	SXE oil (mg/mL)
1	*Staphylococcus aureus*	4.50 ± 0.03	4.50 ± 0.10	5.00 ± 0.10
2	*Enterococcus faecalis*	0.40 ± 0.01	0.40 ± 0.01	0.30 ± 0.01
3	*Bacillus cereus*	0.30 ± 0.01	0.60 ± 0.01	0.80 ± 0.02
4	*Pseudomonas aeruginosa*	0.50 ± 0.02	0.50 ± 0.01	0.20 ± 0.01
5	*Klebsiella pneumoniae*	0.10 ± 0.01	0.40 ± 0.01	0.30 ± 0.01
6	*Proteus vulgaris*	3.50 ± 0.10	0.30 ± 0.01	0.90 ± 0.22
7	*Candida* species	1.00 ± 0.12	0.50 ± 0.01	5.00

LLE- liquid-liquid extraction, CME- cold maceration extraction, SXE- soxhlet extraction

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This present study also investigated variation in the chemical constituents of ginger rhizome oil extracted by LLE, CME and SXE methods. The chromatograms obtained from the GC-MS analysis of the ginger oils from the different extraction methods are presented in Figures 1.3. The chemical constituents present in the oil samples and their biological activities as reported in the cited literatures are presented in Tables 3 and 4, respectively. The most and least predominant constituents of the oil samples were oleic acid (31.13 %) and (2E)-2-ethyl-2-pentanal (0.26 %) for LLE, oleic acid (16.99 %) and octanoic acid methyl ester (0.36 %) for CME and linoleic acid methyl ester (9.27 %) and tridecane (0.45 %) for SXE. Of the 82 chemical compounds identified in the three oil smaples, the results showed that oils extracted by both liquid-liquid extraction and cold maceration methods had 30 compounds each, while the oil extracted by soxhlet extraction method had fifty three (53) compounds. The major chemical compounds in LLE oil with percentage composition greater than 2% include Oleic Acid (31.13%), Palmitic acid (9.38%), Stearic acid (6.01%), Stearidonic acid (5.60%), Linoleic acid methyl ester (4.91%), 6-Gingerol (4.11%), 2-Ethyl-2-hexenal (2.08%), Pentadecanoic acid, 14-methyl-, methyl ester (4.02%), 10-Octadecenoic acid, methyl ester (3.97%), 13,16-Docosadienoic acid methyl ester (3.92%), Adrenic acid (3.51%), 2-Methyl-Z,Z-3,13-Octadecadienol (3.16%) and 13, 16 – Docosadienoic acid (2.91%). Of the 30 chemical compounds identified in the CME oil, Oleic Acid (16.99%), Linoleic acid methyl ester (14.23%), Pentadecanoic acid, 14-methyl-, methyl ester (11.23%), 10-Octadecenoic acid, methyl ester (11.08%), α-copaene (2.95%), Stearic acid, methyl ester (2.77%), Stearic acid (3.17%), Caryophyllene oxide (4.64%), Linoleic acid ethyl ester (3.07%), 13,16-Docosadienoic acid methyl ester (3.00%) and 3,4,5,6-Tetramethyl-2,5-octadiene (2.18%) had relatively high concentration above 2%. Likewise, of the 53 chemical compounds identified in the Soxlet extracted oil, 6-Octadecenoic acid, methyl ester (7.42%), Palmitic acid, methyl ester (7.21%), 1-ethyl-2-methyl Benzene (4.47%), Stearidonic acid (4.23%), γ-Muurolene (2.87%), 4,4-Diallyl-cyclohexanone (2.69%), α-Muurolene (2.62%), Adrenic acid (2.50%), 2-butyl-2-Octenal (2.02%), Farnesene (2.05%), Patchulane (2.14%), Linoleic acid ethyl ester (2.34%), 1-Bromoundecane (2.41%) were the most prominent compounds with percentages greater than 2 %. While these chemical compounds are present at different concentrations and were eluted at different retention time; Hexanoic acid methyl ester (LLE = 0.54%, CME = 0.74%, SXE = 0.83%) < 1-Isopropyl-2-methylbenzene (LLE = 0.74, CME = 0.91, SXE = 0.79) < Isoborneol (LLE = 1.01, CME = 0.85, SXE = 0.99) < Decanoic acid methyl ester (LLE = 0.60%, CME = 1.63%, SXE = 1.69%) < Stearic acid, methyl ester (LLE = 1.00%, CME = 2.77%, SXE = 1.78%) < Stearidonic acid (LLE = 5.60%, CME = 1.07%, SXE = 4.23%) < Linoleic acid methyl ester (LLE = 4.91%, CME = 27.30%, SXE = 11.61%), identified around the same retention time were chemical compounds present across the three oils. Although Oleic Acid and Linoleic acid methyl ester with 31.13%, 16.99% and 9.27% of the chemical compositions in LLE, CME and SXE, respectively were chemical compounds with the highest percentage composition in the respective oils, all the identified chemical compounds belong to different groups as shown in Table 3.

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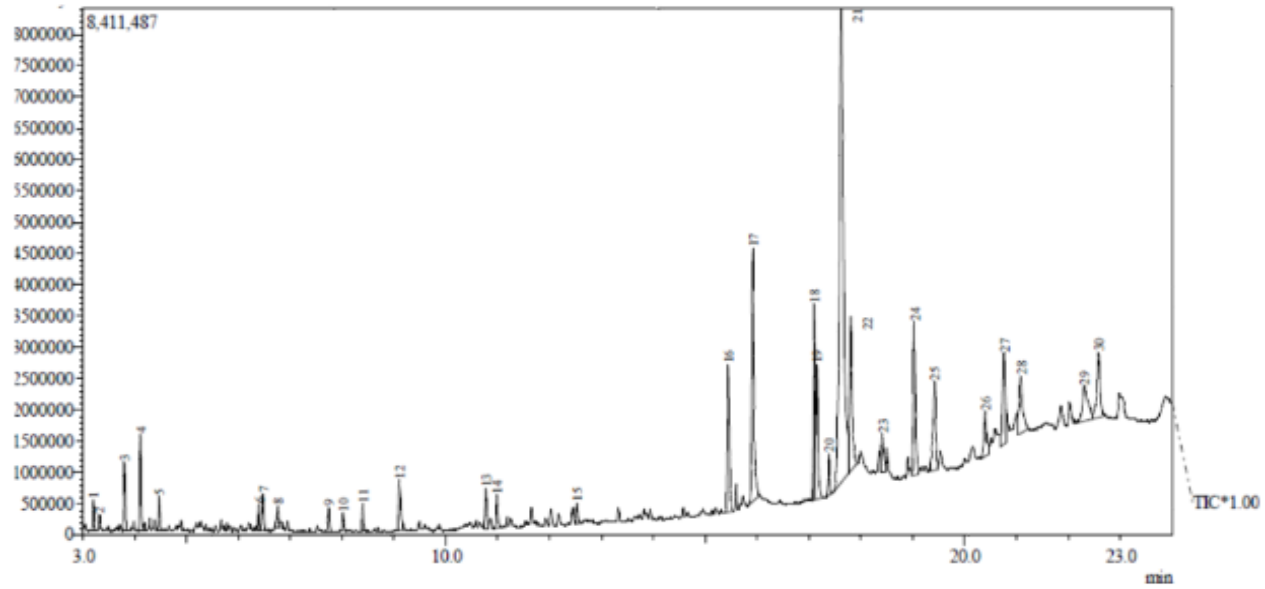

Figure 1: GC-MS Chromatogram of Ginger oil obtained by Liquid-Liquid Extraction

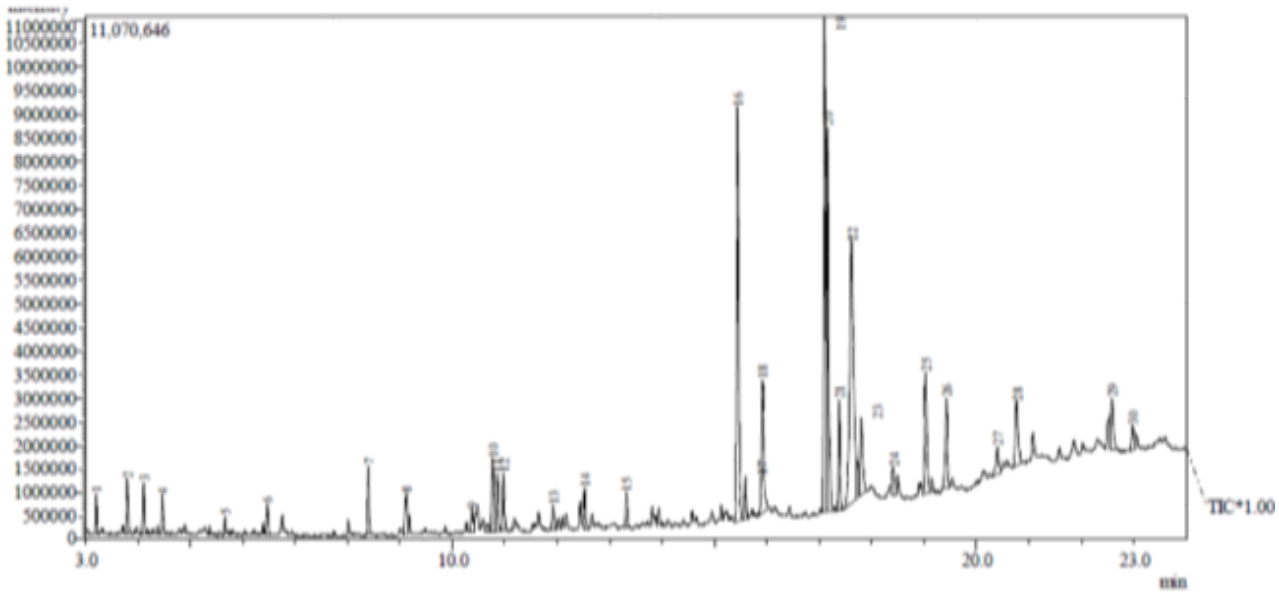

Figure 2: GC-MS Chromatogram of Ginger oil obtained by Cold Maceration

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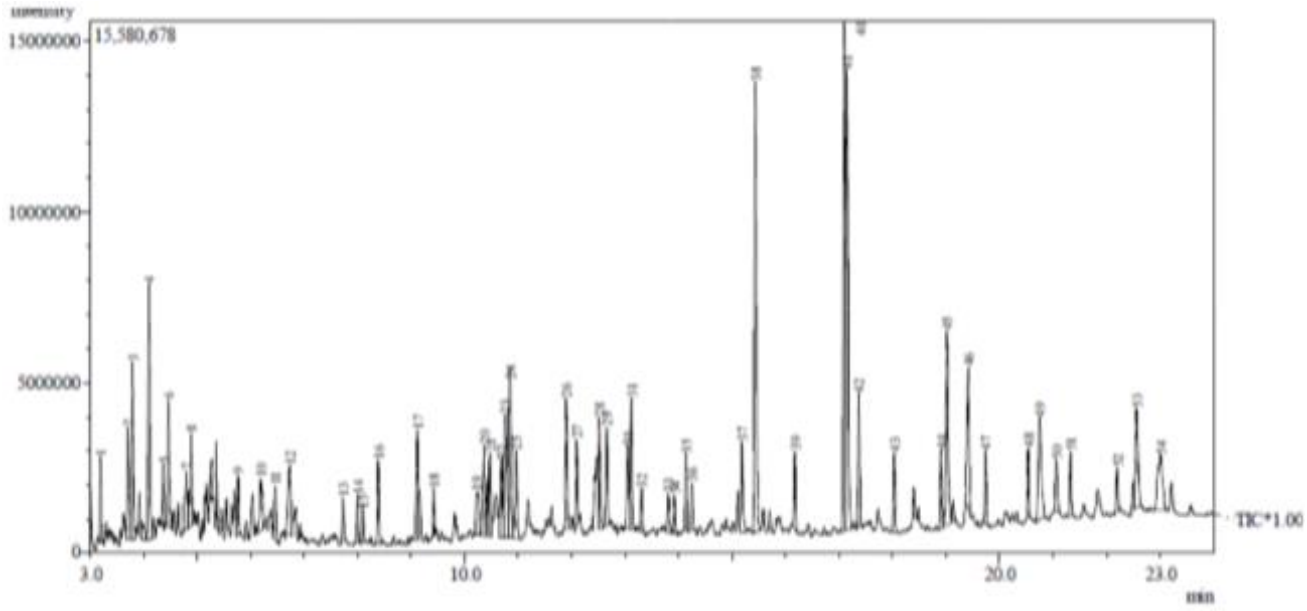

Figure 3: GC-MS Chromatogram of Ginger oil obtained by Soxhlet Extraction

DISCUSSION

Medicinal plants have one or more of their parts which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs**.** Donaldson et al. (2005) tested the activity of ginger oil against *Candida* species and found that all oils were active against it. This was observed in this study with the exception of SXE oil to which the *Candida* species was resistant. It was reported that ginger essential oil and oleoresin contained considerable amounts of phenolic compounds (Ding et al., 2012) and the marked antioxidant and antimicrobial activity of essential oil and oleoresin from spices and herbs are believed to be due to phenolic compounds of the essential oils. The antimicrobial activity of ginger extracts may be attributed to the fact that it contains antimicrobial substances such as zingiberol, zingiberene and bisabolene (Melvin et al., 2009). The ginger oil obtained by these different extraction methods showed varied chemical compounds and the antimicrobial activities exhibited by the oil samples could be due to differences in number, concentration and class of pharmacologically active compounds extractable with the different extraction methods (Singh et al., 2008). It has been reported that extraction methods as well as extraction solvent determine to a large extent the amount and class of bioactive compounds extractable from plants (Adaramola and Onigbinde, 2017).

From this study, the ability of the SXE method to extract more compounds could be due to the high temperature coupled with refluxing that accompany the method. This is consistent with Sultana et al. (2009) who stated that, irrespective of the plant material and extraction solvent used, better yield of extracts are obtained when extraction was done under reflux. Antolovich et al. (2000) attributed higher recovery of some bound phenolic compounds to effective extraction which occurred under reflux conditions. The majority of compounds identified in the oils extracted by LLE, CME and SXE were hydrocarbons (terpenes) and oxygenated

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hydrocarbons (terpenoids) reportedly possessing good antibacterial activity (Zengin and Baysal, 2014).

Hexanoic acid methyl ester, 1-Isopropyl-2-methylbenzene, Isoborneol, Decanoic acid methyl ester, Stearic acid, methyl ester, Stearidonic acid and Linoleic acid methyl ester were present in the three oils at different concentrations, implying that these chemical compounds would be present in ginger oil sample regardless of any of these three methods employed in its extraction. Furthermore, results from this study showed that the oils extracted by these three methods of extraction contain omega-3 fatty acid which is an essential fatty acid needed by human bodies. Fatty acids are usually present as three main classes of esters viz: triglycerides, phospholipids and cholesterol esters in organisms. Any of these forms are important dietary sources of fuel for animals and are essential structural components for cells. Furthermore, the oils contain long chain fatty acids which are "conditionally essential" for growth and development (Newton et al., 2007)**.** Presence of these long chain fatty acids in the ginger oil samples suggested that the oils could be used industrially to produce soap and could be converted to fatty alcohols and fatty amines which are precursors to surfactants, detergents, and lubricants (Kumar et al., 2016). Other applications include their use as emulsifiers, texturizing agents, wetting agents, antifoam agents or stabilizing agents. Presence of long chain fatty alcohols is an additional factor that makes the ginger oil samples good for the production of detergents and surfactants, cosmetics and as components in food and industrial solvents.

Since many fragrances are essential oils (Zielinska et al., 2019; Zielinska et al., 2020), oils from ginger could be used as co-emulsifiers, emollients and thickeners in cosmetics and food industry. The ginger oils also contain a sizable amount of aromatic hydrocarbons which are solvents to remove or thin out oil- or grease-based compounds and as starting materials to make other chemicals such as dyes and plastics. Since the highest number of compounds was present in the oil obtained by the SXE method, SXE could be considered the most suitable method to extract bioactive constituents from ginger rhizomes in comparison with the other two (LLE and CME). Although to the best of our knowledge, the pharmacological activities for some of the compounds have not been reported, some of the activities reported for the identified compounds included but not limited to antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, hypolipemic, antidiabetic, anti-irritant and anti–proliferative. Antimicrobial activities of the oils could be attributed to the presence of compounds such as alcohols, aldehydes, alkenes, esters and ethers found in the ginger rhizome oil samples. Based on the various pharmacological activities reported in literature for some of the compounds identified in the ginger rhizome oils, the strong antimicrobial activity displayed by the oils could be attributed to the presence of the identified chemical compounds and/or the synergistic action of the different compounds detected in the oils. Furthermore, the presence of these chemical compounds confirmed the pharmaceutical importance of ginger rhizome and its use in the management and treatment of various diseases. Meanwhile, further studies on the isolation, characterization and biological evaluation of these identified compounds, especially those for which no pharmacological activities have been reported, is imperative in order to ascertain their potential benefits.

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Table 3: Chemical constituents identified in oil obtained by LLE, CME and SXE oil samples

Peak No	Compound	LLE = 30	CME = 30	SXE = 54	Nature of Compound	Retention Time (min)	Molecular Formula	% Composition (LLE, CME, SXE)
		Present/Absent				(LLE, CME, SXE)		(LLE, CME, SXE)
1.	Hexanoic acid methyl ester	+	+	+	Fatty acid ester	3.201, 3.20, 3.19	$C_7H_{14}O_2$	0.54, 0.74, 0.83
2.	(2E)-2-Ethyl-2-pentenal	+	-	-	Aldehyde	3.311, 0.00, 0.00	$C_7H_{12}O$	0.26, 0.00, 0.00
3.	1,2,3-Trimethylbenzene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 3.70	C_9H_{12}	0.00, 0.00, 1.75
4.	1,2,4-Trimethylbenzene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 3.792	C_9H_{12}	0.00, 0.00, 1.99
5.	Hexanal dimethyl acetal	-	+	-	Acetal	0.00, 3.8, 00.00	$C_8H_{18}O_2$	0.00, 1.03, 0.00
6.	1,1-Dimethoxyhexane	+	-	-	Acetal	3.803, 0.00, 0.00	$C_8H_{18}O_2$	1.31, 0.00, 0.00
7.	1-ethyl-2-methyl Benzene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 4.1	C_9H_{12}	0.00, 0.00, 4.47
8.	(S)-1-piperideine-6-carboxylate	-	+	-	Alpha amino acid	0.00, 4.108, 0.00	$C_6H_8NO_2$	0.00, 1.19, 0.00
9.	2-Ethyl-2-hexenal	+	-	-	Aldehyde	4.107, 0.00, 0.00	$C_8H_{14}O$	2.08, 0.00, 0.00
10.	(3E)-3,7-Dimethyl-1,3,7-octatriene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 4.37	$C_{10}H1_6$	0.00, 0.00, 0.64
11.	1,4-diethyl-Benzene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 4.47	$C_{10}H_{14}$	0.00, 0.00, 1.91
12.	1-Methyl-3-propylbenzene	-	-	+	Aromatic hydrocarbon	0.00, 0.00, 4.79	$C_{10}H_{14}$	0.00, 0.00, 1.04
13.	1-Isopropyl-2-methylbenzene	+	+	+	Cumene	4.468, 4.467, 5.76	$C_{10}H_{14}$	0.74, 0.91, 0.79
14.	trans-3-Caren-2-ol	-	-	+	Alcohol	0.00, 0.00, 4.89	$C_{10}H_{16}O$	0.00, 0.00, 1.48
15.	Octanoic acid, methyl ester	-	+	+	Fatty acid ester	0.00, 5.658, 8.39	$C_9H_{18}O_2$	0.00, 0.36, 1.14
16.	1,1-Dimethoxyoctane	+	-	-	Acetal	6.397, 0.00, 0.00	$C_{10}H_{22}O_2$	0.56, 0.00, 0.00
17.	Isoborneol	+	+	+	Bicyclic monoterpenoid	6.472, 6.467, 6.46	$C_{10}H_{18}O$	1.01, 0.85, 0.99
18.	(Z)-2-Phenyl-2-butene	-	-	+	Alkene	0.00, 0.00, 6.18	$C_{10}H_{12}$	0.00, 0.00, 1.39
19.	4-(N-Benzoyl-aminomethyI)-2,3-dihydro-2-methyllH-Isoindole	-	-	+	Pyrrole	0.00, 0.00, 6.73	$C_{10}H_{12}N$	0.00, 0.00, 1.79
20.	5-Phenyl-4-pentenyl-1-alcohol	-	-	+	Alcohol	0.00, 0.00, 7.73	$C_{11}H_{14}O$	0.00, 0.00, 0.58
21.	Octenol	+	-	-	Alcohol	6.755, 0.00, 0.00	$C_8H_{16}O$	0.72, 0.00, 0.00
22.	Tridecane	-	-	+	Alkane	0.00, 0.00, 8.1	$C_{13}H_{28}$	0.00, 0.00, 0.45
23.	2-Butyl-2-octenal	-	+	+	Aldehyde	0.00, 9.12, 9.12	$C_{12}H_{22}O$	0.00, 1.55, 2.02
24.	Hexadecane	-	-	+	Alkane	0.00, 0.00, 16.18	$C_{16}H_{34}$	0.00, 0.00, 1.64
25.	Dodecane	-	-	+	Alkane	0.00, 0.00, 10.23	$C_{12}H_{26}$	0.00, 0.00, 1.17

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26.	β-cubebene	-	+	-	Sesquiterpenoid	0.00, 10.77, 0.00	$C_{15}H_{24}$	0.00, 0.99, 0.00
27.	α-copaene	-	+	-	Sesquiterpenoid	0.00, 10.77, 0.00	$C_{15}H_{24}$	0.00, 2.95, 0.00
28.	1-Ethyl-4-isobutylbenzene	+	-	-	Alkyl benzene	7.735, 0.00, 0.00	$C_{12}H_{18}$	0.55, 0.00, 0.00
29.	2-Undecanone	+	-	+	Ketone	8.014, 0.00, 8.00	$C_{11}H_{22}O$	0.42, 0.00, 0.63
30.	Decanoic acid methyl ester	+	+	+	Fatty acid ester	8.399, 8.40, 10.97	$C_{11}H_{22}O_2$	0.60, 1.63, 1.69
31.	Farnesene	-	-	+	Sesquiterpene	0.00, 0.00, 10.37	$C_{15}H_{24}$	0.00, 0.00, 2.05
32.	Germacrene	-	-	+	Sesquiterpenoid	0.00, 0.00, 10.48	$C_{15}H_{24}$	0.00, 0.00, 1.12
33.	Farnesol	-	-	+	Acyclic sesquiterpene alcohol	0.00, 0.00, 10.68	$C_{15}H_{26}O$	0.00, 0.00, 1.19
34.	Zingiberene	-	+	-	Monocyclic sesquiterpene	0.00, 10.85, 0.00	$C_{15}H_{24}$	0.00, 1.46, 0.00
35.	Dodecanoic acid methyl ester	-	+	-	Fatty acid ester	0.00, 10.98, 0.00	$C_{13}H_{26}O_2$	0.00, 1.62, 0.00,
36.	1,1,3,3-tetramethoxyPropane	+	-	-	Acetal	9.100, 0.00, 0.00	$C_7H_{16}O_4$	1.69, 0.00, 0.00
37.	δ-Elemene	+	-	-	Sesquiterpene	10.762, 0.00, 0.00	$C_{15}H_{24}$	1.18, 0.00, 0.00
38.	γ- Muurolene	-	-	+	Sesquiterpenoid	0.00, 0.00, 10.76	$C_{15}H_{24}$	0.00, 0.00, 2.87
39.	α- Muurolene	-	-	+	Sesquiterpenoid	0.00, 0.00, 10.85	$C_{15}H_{24}$	0.00, 0.00, 2.62
40.	Tridecanoic acid, methyl ester	+	-	-	Fatty acid ester	10.979, 0.00, 0.00	$C_{14}H_{28}O_2$	0.98, 0.00, 0.00
41.	Patchulane	-	+	+	Sesquiterpene	0.00, 11.92, 11.9	$C_{15}H_{26}$	0.00, 0.63, 2.14
42.	Aromadendrene	-	-	+	Sesquiterpenoid	0.00, 0.00, 12.09	$C_{15}H_{24}$	0.00, 0.00, 1.17
43.	Bisabolol oxide	+	+	-	Monocyclic sesquiterpene alcohol	12.527, 12.53, 0.00	$C_{15}H_{26}O_2$	0.54, 1.12, 0.00
44.	α-Bisabolol Oxide	-	-	+	Monocyclic sesquiterpene alcohol	0.00, 0.00, 12.52	$C_{15}H_{26}O_2$	0.00, 0.00, 1.59
45.	Pentadecanoic acid	-	+	+	Fatty acid	0.00, 13.32, 12.66	$C_{15}H_{30}O_2$	0.00, 0.85, 1.55
46.	2,6,10,14-tetramethyl-Pentadecane	-	-	+	Alkane	0.00, 0.00, 13.12	$C_{19}H_{40}$	0.00, 0.00, 1.96
47.	Myristic acid, methyl ester	-	-	+	Fatty acid ester	0.00, 0.00, 13.3	$C_{15}H_{30}O_2$	0.00, 0.00, 0.71
48.	Geranyl isopentanoate	-	-	+	Fatty alcohol ester	0.00, 0.00, 13.93	$C_{15}H_{26}O_2$	0.00, 0.00, 0.58
49.	Elixene	-	-	+	Sesquiterpene	0.00, 0.00, 13.05	$C_{15}H_{24}$	0.00, 0.00, 1.42
50.	Palmitoleic acid	-	-	+	Fatty acid	0.00, 0.00, 14.13	$C_{16}H_{30}O_2$	0.00, 0.00, 1.03
51.	Pentadecane	-	-	+	Alkane	0.00, 0.00, 14.25	$C_{15}H_{32}$	0.00, 0.00, 0.73
52.	Pentadecanoic acid, 14-methyl-, methyl ester	+	+	-	Fatty acid ester	15.443, 14.44, 0.00	$C_{17}H_{34}O_2$	4.02, 11.23, 0.00
53.	1,2-Hexadecanediol	-	+	+	Alcohol	0.00, 15.59, 15.18	$C_{16}H_{34}O_2$	0.00, 1.13, 1.37
54.	Palmitic acid	+	+	-	Fatty acid	15.914, 15.91, 0.00	$C_{16}H_{32}O_2$	9.38, 5.22, 0.00

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55.	Palmitic acid, methyl ester	-	-	+	Fatty acid ester	0.00, 0.00, 15.43	$C_{17}H_{34}O_2$	0.00, 0.00, 7.21
56.	Linoleic acid methyl ester	+	+	+	Fatty acid ester	17.10, 17.10, 17.1	$C_{19}H_{34}O_2$	4.91, 14.23, 9.27
57.	Linoleic acid ethyl ester	-	+	+	Fatty acid ester	0.00, 19.43, 19.41	$C_{20}H_{36}O_2$	0.00, 3.07, 2.34
58.	Eicosane	-	-	+	Alkane	0.00, 0.00, 19.73	$C_{20}H_{42}$	0.00, 0.00, 0.97
59.	Octadecane	-	-	+	Alkane	0.00, 0.00, 20.54	$C_{18}H_{38}$	0.00, 0.00, 0.97
60.	4,4-Diallyl-cyclohexanone	-	-	+	Ketone	0.00, 0.00, 20.74	$C_{12}H_{18}O$	0.00, 0.00, 2.69
61.	10-Octadecenoic acid, methyl ester	+	+	-	Fatty acid ester	17.153, 17.16, 0.00	$C_{19}H_{36}O_2$	3.97, 11.08, 0.00
62.	6-Octadecenoic acid, methyl ester	-	-	+	Fatty acid ester	0.00, 0.00, 17.15	$C_{19}H_{36}O_2$	0.00, 0.00, 7.42
63.	Heptacosane	-	-	+	Alkane	0.00, 0.00, 18.9	$C_{27}H_{56}$	0.00, 0.00, 1.35
64.	Stearic acid, methyl ester	+	+	+	Fatty acid ester	17.38, 17.38, 17.37	$C_{19}H_{38}O_2$	1.00, 2.77, 1.78
65.	Oleic Acid	+	+	-	Fatty acid	17.621, 17.62,0.00	$C_{18}H_{34}O_2$	31.13, 16.99, 0.00
66.	Stearic acid	+	+	-	Fatty acid	17.80, 17.80, 0.00	$C_{18}H_{36}O_2$	6.01, 3.17, 0.00
67.	4-Ethyl-2-methoxyphenol	+	-	-	Methoxyphenolic	18.416, 0.00, 0.00	$C_9H_{12}O_2$	1.28, 0.00, 0.00
68.	2-Methyleicosane	-	-	+	Alkane	0.00, 0.00, 18.03	$C_{21}H_{44}$	0.00, 0.00, 1.08
69.	Stearidonic acid	+	+	+	Fatty acid	19.03, 18.42, 19.01	$C_{18}H_{28}O_2$	5.60, 1.07, 4.23
70.	Caryophyllene oxide	-	+	-	Epoxide	0.00, 19.03, 0.00	$C_{15}H_{24}O$	0.00, 4.64, 0.00
71.	6-Gingerol	+	-	-	Polyphenolic	19.420, 0.00, 0.00	$C_{17}H_{26}O_4$	4.11, 0.00, 0.00
72.	Z-10-Pentadecen-1-ol	+	-	-	Alcohol	20.394, 0.00, 0.00	$C_{15}H_{30}O$	1.93, 0.00, 0.00
73.	cis-9-Hexadecenal	-	+	+	Aldehyde	0.00, 20.39, 13.8	$C_{16}H_{30}O$	0.00,1.25, 0.61
74.	13,16- Docosadienoic acid methyl ester	+	+	-	Fatty acid ester	20.754, 20.77, 0.00	$C_{23}H_{42}O_2$	3.92, 3.00, 0.00
75.	13, 16 – Docosadienoic acid	+	-	+	Fatty acid	21.08, 0.00, 21.06	$C_{22}H_{40}O_2$	2.91, 0.00, 0.99
76.	3,4,5,6-Tetramethyl-2,5-octadiene	-	+	-	Alkene	0.00, 22.58, 0.00	$C_{12}H_{22}$	0.00, 2.18, 0.00
77.	Tetracosane	-	-	+	Alkane	0.00, 0.00, 21.33	$C_{24}H_{50}$	0.00, 0.00, 0.93
78.	2,6-Dimethylheptadecane	-	-	+	Alkane	0.00, 0.00, 22.2	$C_{19}H_{40}$	0.00, 0.00, 0.73
79.	2-Methyl-Z,Z-3,13-Octadecadienol	+	-	-	Alcohol	22.31, 0.00, 0.00	$C_{19}H_{36}O$	3.16, 0.00, 0.00
80.	Adrenic acid	+	-	+	Fatty acid	22.583, 0.00, 22.57	$C_{22}H_{36}O_2$	3.51, 0.00, 2.50
81.	3,7-Dimethyl-2,6-nonadien-1-ol	-	+	-	Alcohol	0.00, 22.98, 0.00	$C_{11}H_{20}O$	0.00, 1.10, 0.00
82.	1- Bromoundecane	-	-	+	Alkyl halide	0.00, 0.00, 23.01	$C_{11}H_{23}Br$	0.00, 0.00, 2.41

+ = present; - = absent

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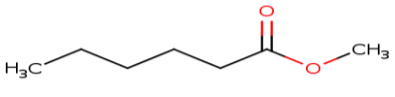
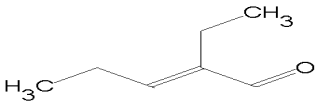
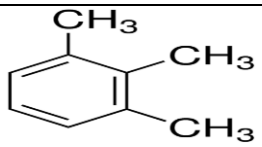
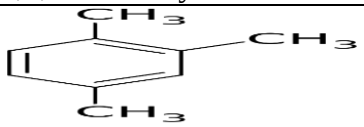
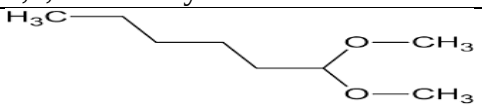
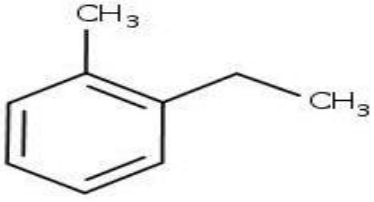
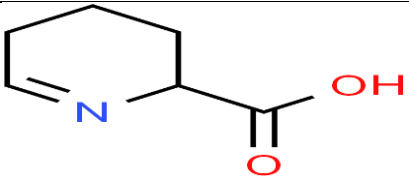
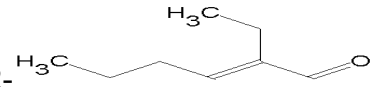
Table 4: Biological activities and structures of compounds identified in the oil samples

Peak No	Compound	Biological Activity/ Reference
1.	 Hexanoic acid methyl ester	Not found in literature
2.	 (2E)-2-Ethyl-2-pentenal	Not found in literature
3.	 1,2,3-Trimethyl Benzene	Not found in literature
4.	 1,2,4-Trimethyl Benzene	Not found in literature
5.	 Hexanal dimethyl acetal	Not found in literature
6.	 1,1-Dimethoxyhexane	Not found in literature
7.	 1-ethyl-2-methyl Benzene	Not found in literature
8.	 (S)-1-piperideine-6-carboxylate	Not found in literature
9.	 2- Ethyl-2-hexenal	Not found in literature

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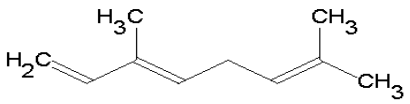
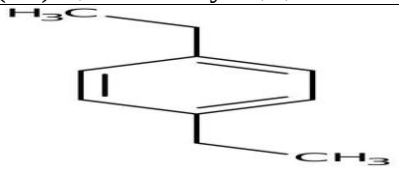
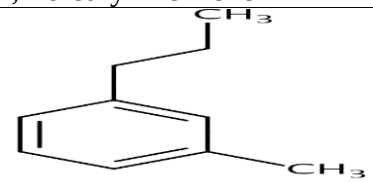
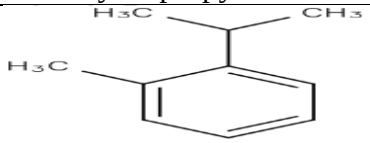
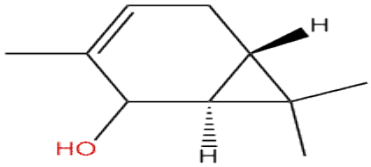
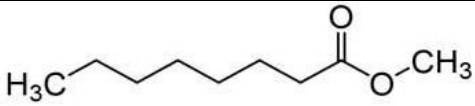
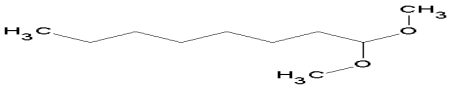
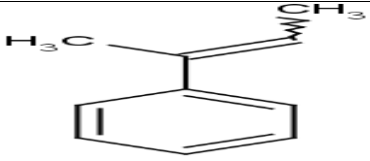
10.	 (3E)-3,7-Dimethyl-1,3,7-octatriene	Not found in literature
11.	 1,4-diethyl-Benzene	Not found in literature
12.	 1-Methyl-3-propylbenzene	Not found in literature
13.	 1-Isopropyl-2-methylbenzene	Not found in literature
14.	 trans-3-Caren-2-ol	Not found in literature
15.	 Octanoic acid, methyl ester	Not found in literature
16.	 1,1-Dimethoxyoctane	Not found in literature
17.	 Isoborneol	Antibacterial, antiviral (Aguiar et al., 2014)
18.	 (Z)-2-Phenyl-2-butene	Not found in literature
19.	4-(N-Benzoyl-aminomethyI)-2,3-dihydro-2-methyllH-Isoindole	Not found in literature

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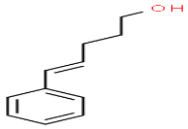
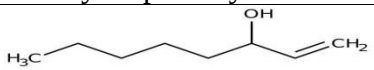
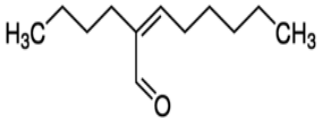
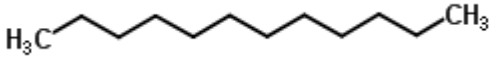
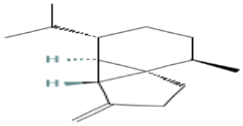
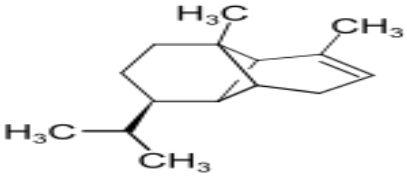
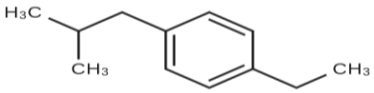
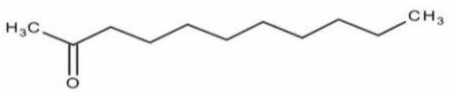
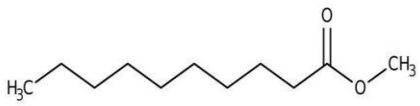
20.	 5-Phenyl-4-pentenyl-1-alcohol	Not found in literature
21.	 Octenol	Mosquito attractant (Kline, 1994)
22.	 Tridecane	Not found in literature
23.	 2-Butyl-2-octenal	Not found in literature
24.	 Hexadecane	Antibacterial, antioxidant (Yogeswari et al., 2012)
25.	 Dodecane	Not found in literature
26.	 β-cubebene	Not found in literature
27.	 α-copaene	Antioxidant, activity, anti–proliferative activity (Turkez et al., 2014)
28.	 1-Ethyl-4-isobutylbenzene	Not found in literature
29.	 2-Undecanone	Anti-inflammatory (Jing et al., 2014)
30.	 Decanoic acid methyl ester	Antibacterial, antifungal activities (Kumar et al., 2011)

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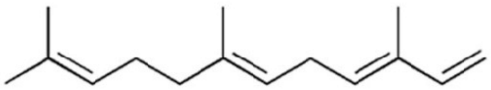
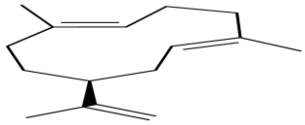
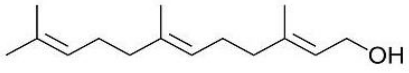
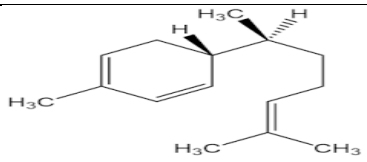
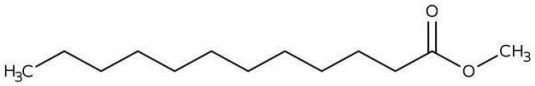
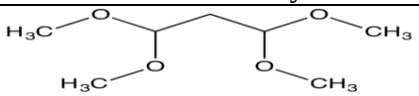
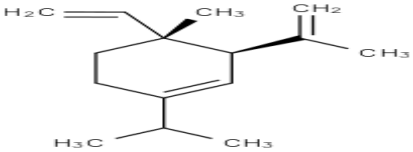
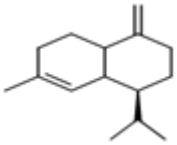
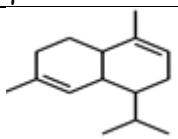
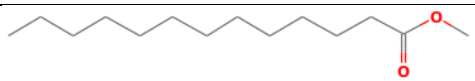
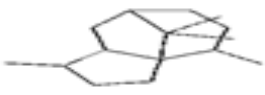
31.	 Farnesene	Antimicrobial (Adorjan and Buchbauer, 2010)
32.	 Germacrene	Anticarcinogenic, anti-inflammatory, and antibacterial (Swamy and Sinniah, 2015)
33.	 Farnesol	Antibacterial and antifungal (Langford et al., 2010)
34.	 Zingiberene	Antiviral and anticancer effects (Bou et al., 2013)
35.	 Dodecanoic acid methyl ester	Antibacterial, antiviral, antifungal (Ozcelik et al., 2005)
36.	 1,1,3,3-tetramethoxypropane	Not found in literature
37.	 δ-Elemene	Antiectoparasitic activity, antifungal, antioxidant, and antitumor activity (Birkeff et al., 2008; Xie et al., 2009)
38.	 γ- Muurolene	Not found in literature
39.	 α- Muurolene	Not found in literature
40.	 Tridecanoic acid, methyl ester	Antibacterial, antifungal (Chandrasekaran et al. 2011)
41.	 Patchulane	Not found in literature

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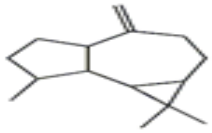
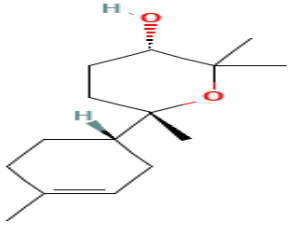
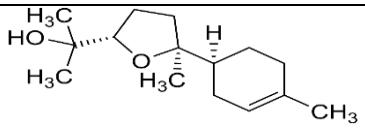
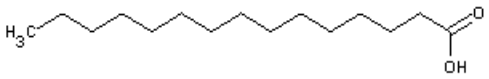
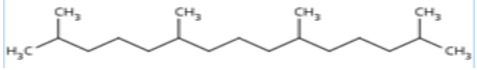
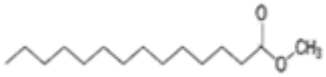
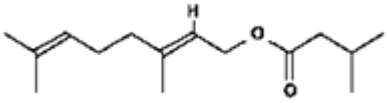
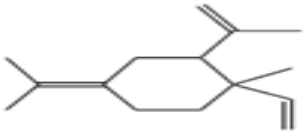
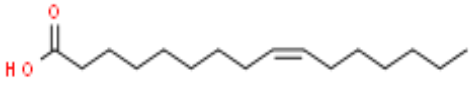
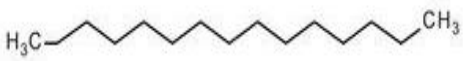
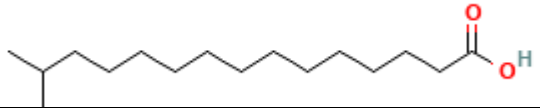
42.	 Aromadendrene	Antibacterial (Mulyaningsih et al., 2010)
43.	 Bisabolol oxide	Not found in literature
44.	 α-Bisabolol Oxide	Antimicrobial, anti-inflammatory, anti-irritant, antibacterial and non-allergenic properties, antiviral, hypolipemic, antidiabetic (Duke, 2002; Riju et al., 2009)
45.	 Pentadecanoic acid	Antioxidant (Duke, 2007)
46.	 2,6,10,14-tetramethyl-Pentadecane	Not found in literature
47.	 Myristic acid, methyl ester	Not found in literature
48.	 Geranyl isopentanoate	Anti-inflammatory, antioxidant and anti-viral (Hameed et al., 2016)
49.	 Elixene	Not found in literature
50.	 Palmitoleic acid	Antioxidant, antibacterial (Maedler et al., 2003)
51.	 Pentadecane	Antibacterial (Yogeswari et al., 2012)
52.		Not found in literature

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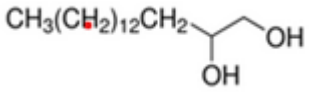
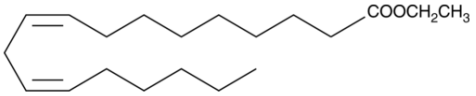
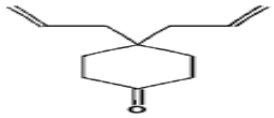
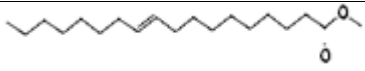
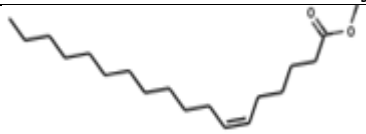
	Pentadecanoic acid, 14-methyl-, methyl ester	
53.	 1,2-Hexadecanediol	Not found in literature
54.	 Palmitic acid	Anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, hemolytic, 5-Alpha reductase inhibitor (Kumar et al., 2010; Aparna et al., 2012)
55.	 Palmitic acid, methyl ester	Antimicrobial, antioxidant, antiandrogenic (Mudiganti et al., 2016)
56.	 Linoleic acid methyl ester	Antimicrobial (Godwin et al., 2015)
57.	 Linoleic acid ethyl ester	Antiarthritic, antiacne, antimicrobial, inhibits lipopolysaccharide-induced pro-inflammatory cytokine production (Park et al., 2014)
58.	 Eicosane	Antimicrobial, antitumor (Nandhini et al., 2015)
59.	 Octadecane	Antimicrobial and antifungal (Abubacker and Kamala, 2015)
60.	 4,4-Diallyl-cyclohexanone	Not found in literature
61.	 10-Octadecenoic acid, methyl ester	Antioxidant, antimicrobial (Duke, 2007)
62.	 6-Octadecenoic acid, methyl ester	Not found in literature

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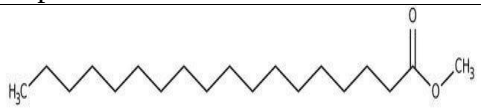
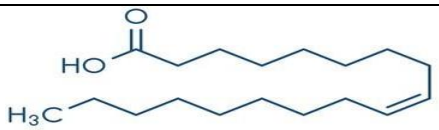
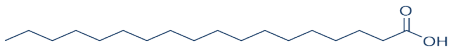
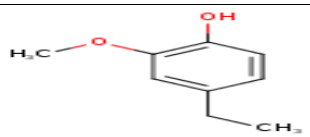
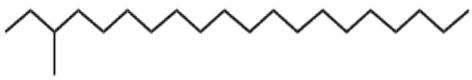
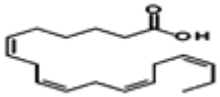
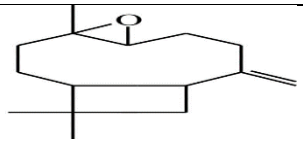
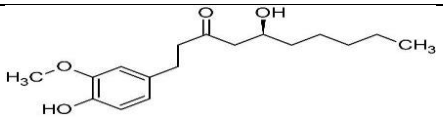
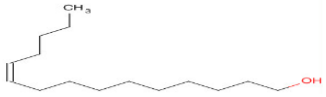
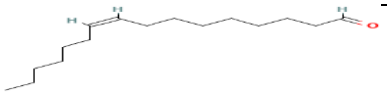
63.	 Heptacosane	Antibacterial, antioxidant activity (Mihailovi et al., 2011)
64.	 Stearic acid, methyl ester	Antifungal, antimicrobial, antibacterial, antiviral, antioxidant (Sudharsan et al., 2010)
65.	 Oleic Acid	Antitumor (Carrillo et al., 2012)
66.	 Stearic acid	Antifungal, antimicrobial, antibacterial, antiviral, antioxidant (Sudharsan et al., 2010)
67.	 4-Ethyl-2-methoxyphenol	Not found in literature
68.	 2-Methyleicosane	Antioxidant (Mudiganti et al., 2016)
69.	 Stearidonic acid	Anti-inflammatory (Sung et al. 2017)
70.	 Caryophyllene oxide	Antioxidant, antiviral, and analgesic properties (Singh et al., 2014; Hammami et al., 2015)
71.	 6-Gingerol	Anti-cancer (Kumara et al., 2017)
72.	 Z-10-Pentadecen-1-ol	Not found in literature
73.	 cis-9-Hexadecenal	Antibacterial and anti-inflammatory (Hoda et al.. 2019; Hoda et al., 2020)

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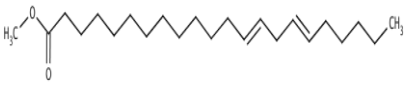
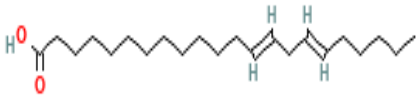
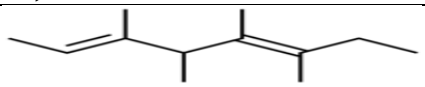
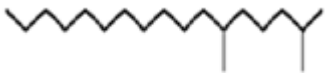
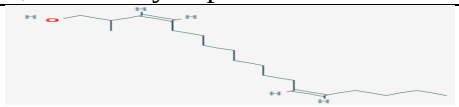
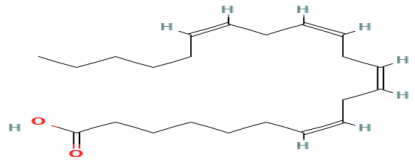
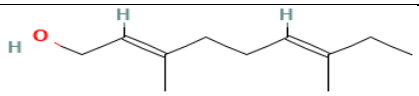
74.	 13,16- Docosadienoic acid methyl ester	Not found in literature
75.	 13, 16 – Docosadienoic acid	Not found in literature
76.	 3,4,5,6-Tetramethyl-2,5-octadiene	Not found in literature
77.	 Tetracosane	Not found in literature
78.	 2,6-Dimethylheptadecane	Not found in literature
79.	 2-Methyl-Z,Z-3,13-Octadecadienol	Not found in literature
80.	 Adrenic acid	Not found in literature
81.	 3,7-Dimethyl-2,6-nonadien-1-ol	Not found in literature
82.	 1- Bromoundecane	Not found in literature

CONCLUSION

This study concluded that ginger oil samples from the different methods of extraction showed variation in the chemical composition and their antimicrobial activities. Although the oils extracted by LLE and CME had the same number of compounds, most of the compounds identified in them were different. The SXE method was the most efficient extraction method. It extracted the highest number of components spanning across several classes of chemical compounds. Although combination of different extraction methods may be the most suitable way to extract more chemical components from a sample, the ginger oil from each of the three methods showed promising antimicrobial properties at varying degrees. The pharmacological activities of the oils could be attributed to the presence of important bioactive compounds such

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as isoborneol, δ-Elemene, α-Bisabolol oxide, pentadecanoic acid-14-methyl methyl ester, linoleic acid methyl ester, stearic acid methyl ester, zingiberene, farnesene, germacrene, farnesol, palmitic acid methyl ester, heptacosane, eicosane and octadecane in the oils. This study therefore showed that the antimicrobial potential of ginger rhizome oil, amongst other factors, is dependent largely on the method employed in the extraction of the oil.

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