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COMPARATIVE STUDIES OF THE CHEMICAL CONSTITUENTS OF SUPERCRITICAL FLUID EXTRACTION (SFE) AND HYDRODISTILLATION EXTRACTION (HDE) OF OIL FROM THE *ZINGIBER OFFICINALE*

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Abstract

The essential oil from the rhizomes of Zingiber officinale was extracted using Hydro distillation (HD) and Supercritical Fluid Extraction (SFE) methods. Gas Chromatography-Mass Spectrometry (GC/MS) was employed to analyze the chemical compositions of the extracted oils, revealing differences in both the quantity and quality of the samples. The HD method isolated 24 compounds, while SFE isolated 10. HD yielded 0.08% of yellowish oil, whereas SFE produced 0.2% of yellowish-green oil. The primary compound in the SFE extract was α-Zingiberene (31.1%), followed by 6-gingerol (17.7%), sesquiphellandrene (12.0%), β-Bisabolene (9.6%), and α-curcumene (6.8%). In contrast, the HD extract's major component was α-Zingiberene (12.0%), with β-Phellandrene (10.3%), α-citral (9.5%), Camphene (9.0%), citral (7.0%), and Eucalyptol (6.6%). Ledene oxide-(I) is a new compound which was found for the first time in ginger. This comparative analysis suggests that SFE, with its shorter extraction time and higher yield, is more advantageous, particularly considering the importance of low operating costs and safety in achieving effective results.

INTRODUCTION

The progress in science and technology has made it possible to study the complex chemical components found in herbs. For thousands of years, herbs have served as an alternative to modern medicine, supporting a healthy lifestyle. The diverse chemical compounds synthesized by medicinal plants enable them to carry out essential biological activities [1]. Nowadays, there are growing concerns about food safety which led to greater emphasis on using naturally available antibacterial agents. The extracts of herbs and spices can act as natural food preservatives, as they not only add flavor but also offer antioxidant properties [2] and antimicrobial effects [3]. *Zingiber officinale* is one of the plant families that serve as a valuable natural resource, offering products useful in food and spices, medicine, dyes, perfumes, and aesthetics [4].

The *Zingiber officinale* rhizome, known as ginger, is a widely used species of ginger family (Zingiberaceae). It is abundant in Indo-Malaysia and includes over 1,200 plant species across 53 genera. The *Z. officinale* genus itself comprises around 85 species of aromatic herbs

native to East Asia and tropical Australia [5]. The ginger plant cultivation originated in Southeast Asia and gradually expanded to countries such as East Africa, India, China, Mexico, and other regions worldwide. In traditional Chinese medicine, ginger is essential for treating a variety of ailments, including nausea, stomach aches, respiratory issues, diarrhea, abdominal bloating, and rheumatism [6].

Z. officinale rhizome extract can be effectively used to develop new antibiotic drugs [7]. *Z. officinale* Roscoe is known as "ginger," which is cultivated throughout Africa, and the areas of Austria, China, Latin America, South-eastern and Asia Japan [8]. Ginger is used as a food, condiment, nutrition, spice, and herb [9]. Gingerols in ginger give it a specific odor and flavor. It also exhibits effective hypotensive, analgesic, and antipyretic effects [10]. Ginger contains various bioactive phytochemicals, responsible for diverse pharmacological activities [11]. The ginger has potential to lessen the risk of many diseases like colitis and cancer [12]. It imparts antimicrobial activity, adjusts cholesterol, [13] antioxidant, anti-inflammatory [14] anti-colitis, anti-influenza, anti-oxidant, anti-tumor and anti-tussive effects [7]. The use of a good diffusion assay found that aqueous peel extracts of Ginger possess anti-bacterial efficiency against both gram-negative and positive bacterial strains [15].

The SAR study of ginger phytochemicals revealed ginger to be a better candidate to treat neurodegenerative diseases [16]. The ginger essential oils could be used to develop improved antimicrobial agents i.e., bactericides and fungicides to suppress phytopathogen growth [17]. The clinical trials revealed that during early pregnancy; ginger use is effective in reducing nausea and vomiting [18]. The underlying mechanism of Ginger constituents involves cell cycle/DNA damage, apoptosis, cytoskeletal regulation and adhesion, chromatin/epigenetic regulation, inflammation and immunology to express biological activities [19]. Among herbal medicines, *Z. officinale* has been recognized for its effectiveness in treating various illnesses. It has been cultivated since ancient times and has been a vital ingredient in Chinese, Ayurvedic, and Tibb-Unani remedies, used to treat conditions such as constipation, rheumatism, catarrh, vomiting, and other digestive disorders [20].

In this study, the chemical compositions of Zingiber oil were examined, using two methods: hydro distillation and Supercritical Fluid Extraction (SFE) [21, 22]. The extraction yields were estimated, and their chemical composition was examined from each method. As far as we know, no studies have yet been published on the chemical composition of these oils.

Supercritical Fluid Extraction (SFE)

It is a selective extraction technique that uses CO_2 to safely and efficiently extract specific compounds from liquids and solids. In SFE, optimizing experimental conditions such as temperature and pressure in addition to the percentage of the modifier and extraction times [23] is crucial, as these factors significantly influence the extraction process. The sample is dried in an oven at 50 °C to remove moisture [24]. The optimal drying temperature ranges from 40 °C to 70 °C [25, 26], as higher temperatures could cause starch gelatinization, potentially affecting the extraction process and resulting in a lower yield of ginger oil [27].

Hydro distillation Extraction (HDE)

It is a solvent-free method for extracting essential oils and bioactive compounds from plants, involving hydrodiffusion, hydrolysis, and thermal decomposition. In Hydro Distillation (HDE), of *Z. officinale* [28] forms an azeotropic mixture with water, due to different densities and is separated by decantation after condensation. Hydro distillation is a straightforward setup that can extract large quantities of samples [29]. The active compound 6-Gingerol was found in a minimum concentration of 0.18%. Additionally, Ledene oxide-(I), a compound identified for the first time in *Z. officinale*. Ledene oxide-(I), also found in Polygala Javana DC, possesses anti-inflammatory, analgesic, antibacterial, fungicidal, and anti-tumor properties [30, 31]. The

comparison between SFE and HDE is done in Table 1.

GC and GC-MS analyses.

Table 1: Types of extraction method described.

Extraction methods	Supercritical Fluid (SFE)	Hydrodistillation (HDE)
Time	Shorter extraction time (90 mins) – more convenient and greener environment	Longer time required (8 hrs)
Colour of oil	Yellowish-green	Pale yellow
Character of oil	Pungent ginger smell	Pleasant ginger odour
Solvent recovery	Simple solvent recovery	Require extra steps in solvent recovery
Temperature	Low temperature can be employed by the extraction	High temperatures will be employed
Thermal	Thermally labile compounds can be extracted with minimal damage	Thermally labile compounds will be damage
%Yield of extraction	Higher percentage yield (0.22 %)	Lower percentage yield (0.08 %)

The conditions for the gas chromatography system [32] used to analyze *Z. officinale* are detailed in Table 2 [33]. The components were identified by comparing their mass spectra and retention indices with those in the literature and the Wiley Registry of Mass Spectral Data, 6th Edition. Due to the presence of several overlapping peaks in GC-MS analysis, which complicated accurate qualitative and quantitative analysis, chemometric methods were employed as an effective tool to address this issue [34].

Table 2: Conditions of GC/MS analysis [32].

Instrument	DB-1MS capillary column (30m × 0.25mm I.D.)
Carrier gas	Helium
Flow rate	1.0 mL/min
Inlet temperature	270°C
Interface temperature	250°C
Column temperature progress	50°C as initial temperature for 3 minutes
Programmed temperature	50°C to 150°C, 150°C to 190°C, 190°C to 250°C at the rate of 5°C/min, 2°C/min and 10°C/min, respectively. maintained for 3 min.

Experimental

Plant material

In February 2018, rhizomes of *Z. officinale* were sourced from a local market in Kuantan District, Pahang, Malaysia, and stored in a well-ventilated area. The voucher specimen SK 1545/14 was deposited at the Herbarium of the Institute of Bioscience (IBS), University Putra Malaysia. The plant was identified by the Resident Botanist of IBS.

Sample preparation

Initially, the *Z. officinale* rhizomes were thoroughly cleaned to remove any soil residue. Following it was divided into equal portions based on weight for subsequent hydro distillation extraction (HDE) and supercritical fluid extraction (SFE) processes. The *Z. officinale* sample was then cut into thin slices (5-6 mm) and placed in plastic bags, which were stored at room temperature.

Extraction Method

The two distinct methods: hydrodistillation extraction (HDE) and supercritical fluid extraction (SFE) were used. The objective was to identify and compare the chemical compounds present in *Z. officinale* using different extraction techniques. Additionally, the experiment aimed to optimize various parameters, including sample preparation, extraction duration, sample quantity, temperature, and pressure, to determine their impact on the extraction process and resulting chemical constituents.

The setup used is illustrated in Fig. 1 [35], consists of a carbon dioxide source cylinder connected to a deep tube, which draws CO_2 into the system. The CO_2 then passes through a dryer and a cooling device, causing it to condense. A double syringe pump feeds the condensed CO_2 into a 0.4-liter extractor vessel, which is placed in a temperature-controlled oven. The supercritical CO_2 exits the extractor through a micro metering valve, which regulates the pressure release in the system [36]. For the extraction process, a 20 g lavender sample was mixed with 5 ml ethanol and 10 g glass beads and then placed in the extractor.

The extractor was preheated for 60 minutes before extraction. The extraction conditions were: 90 minutes of static extraction followed by 30 minutes of dynamic extraction, at 45 °C, 200 bar, and a flow rate of 10 ml/min, extract was collected in a glass vial containing 4 ml EtOH.

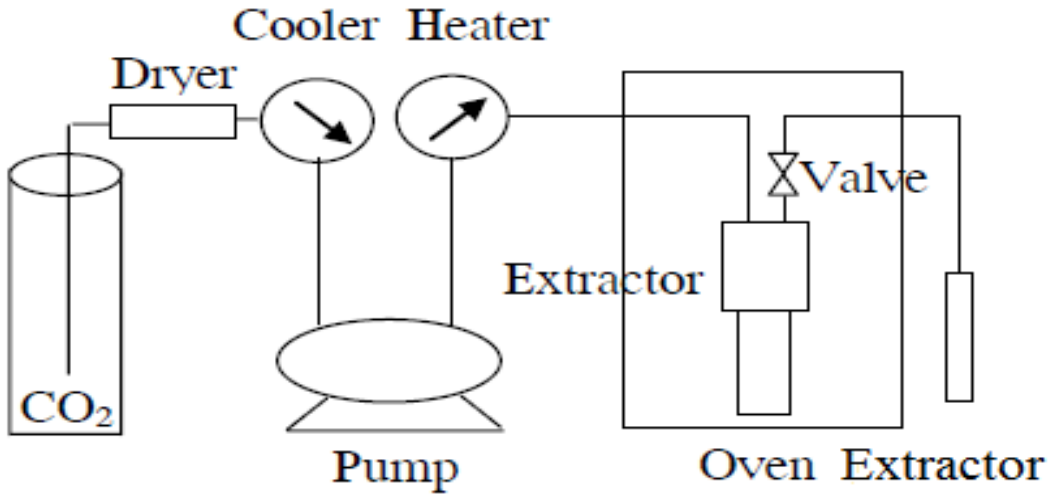


Fig. 1. Schematic diagram of supercritical extraction apparatus.

Supercritical Fluid Extraction (SFE) procedure

For the SFE of ginger, 500 g of dried ginger was loaded into the extractor vessel. The SFE conditions were: optimized temperature (55°C) and pressure (3700-4000 psi) for 90 minutes, with a proper flow rate of CO_2 and particle permissibility. The liquefied CO_2 solvent was pumped into the vessel, allowing the sample to equilibrate during static extraction.

During dynamic extraction, the CO_2 passed the extract into a collection vessel. Following depressurization, the extracted oil [37] was weighed.

Hydrodistillation extraction (HDE) procedure

Traditionally, essential oils are manufactured and extracted using the hydrodistillation method, which requires precise temperature control. To ensure optimal results, hydrodistillation is typically performed at room temperature and atmospheric

pressure, as these conditions are essential for the process. In this experiment, a Clevenger-type hydrodistillation apparatus was used to extract essential oils from *Z. officinale*. The process involved: Weigh 500 g of fresh ginger and cut 5-6 mm thick slices, place in a round-bottomed flask with 1500 ml of distilled water, and boil. Add 5 ml hexane to the Clevenger apparatus and conduct extraction for 9 hours. After that, collect oil in a vial. Removing excess water using sodium sulfate anhydrous. Purging the oil with nitrogen to remove the solvent. Weighing the resulting essential oil.

GC-MS Analysis

It was employed to investigate the qualitative and quantitative composition of *Z. officinale* rhizome. The study utilized an Agilent 7890A GC system equipped with a DB-1MS capillary column (30 m x 0.25 mm x 0.25 µm) and a stationary phase of 100 % dimethylpolysiloxane. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The inlet and interface temperatures were set at 270 °C and 250 °C, respectively. The GC method involved a temperature program with multiple ramps: 50-150 °C at 5 °C/min, 150-190 °C at 2 °C/min, and 190-250°C at 10 °C/min, with initial and final holds of 3 minutes each. The scan rate was 5 scans per second, covering a range from 35 m/z to 500 m/z, with a splitting ratio of 10:1. The MS operated at 70 eV ionization energy and a 10:1 splitting ratio.

Comparison between SFE and HDE

The results showed that Supercritical Fluid Extraction (SFE) yielded a higher extraction rate than Hydrodistillation Extraction (HDE), despite requiring a shorter extraction time of 90 minutes. SFE produced a 0.22% yield of yellowish-green oil, whereas HDE produced a 0.08% yield of pale-yellow oil with a pleasant ginger aroma from 500g of *Z. officinale*. For comparison, Sultan (2005) reported that HDE yielded 1.58% and 0.98% essential oil from ginger samples in Thailand and China, respectively [38, 39]. Additionally, Kumar Roy et al. (2013) reported that the essential oil yield extracted from fresh ginger using hydrodistillation was 0.23% for samples from Bangladesh and 0.21% for samples from China [40].

The variation in essential oil yield from *Z. officinale* plants from different locations may be attributed to factors such as loss of rhizome peels during transportation, as the peels are primarily responsible for the essential oil content. Furthermore, the Supercritical Fluid Extraction (SFE) method used in this study employed low temperature and high pressure to avoid thermal degradation during extraction, which may contribute to the higher yield and quality of the extracted oil [41]. Mesomo et al. (2012) compared the efficiency of different solvents in extracting ginger oil using Supercritical Fluid Extraction (SFE). They found that CO_2 extraction resulted in the highest yield of 3.21 %w/w, followed by propane with a yield of 2.75 %w/w. The superior performance of CO_2 was attributed to its optimal temperature and pressure conditions, which significantly enhanced the extraction yield. In contrast, propane's yield was positively influenced by pressure, but to a lesser extent than CO_2 [42].

Mesomo et al. (2013) also compared the extraction yields of HDE and SFE (CO_2) and found that HD gives a maximum yield of 1.79 %w/w, while SFE leads to a higher yield of 2.62 %w/w. Although the yields differed, their composition, analysed by GC, was found to be similar. In industrial applications, essential oils that are difficult to extract are often obtained using high pressures [43], but this increases the risk of decomposition due to high temperatures. Hydrodistillation at reduced pressure is a safer alternative, as it uses lower temperatures. In this experiment, the extraction was conducted at controlled temperatures and atmospheric pressure (1 atm) to ensure safety [44].

A comparison of extraction methods reveals that Supercritical Fluid Extraction (SFE) may achieve higher yields than hydrodistillation due to its high-pressure conditions. Additionally, SFE requires significantly less time than conventional Hydrodistillation Extraction (HDE).

Results and Discussion

Analysis of Components

GC/MS and TLC were employed to qualitatively analyze and identify the chemical compounds, to evaluate the efficacy of the extraction methods.

GC/MS Analysis

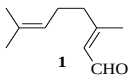
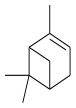
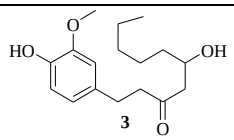
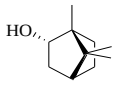
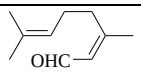
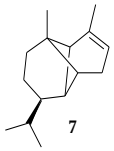
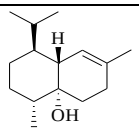
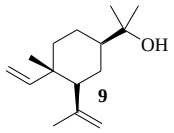
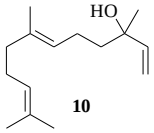
A valuable tool for measuring, identifying, and separating volatile chemicals. The chemical constituents were identified through NIST mass library searches, which provided a direct match for each compound's mass spectrum. In the case of SFE, ten chromatographic peaks were detected, and the corresponding mass spectra and Table 3 reveal the major compounds present in *Z. officinale*. The mass spectrum displays the relative intensity of ions with different mass-to-charge ratios, facilitating structure elucidation through isotope peaks. Notably, each compound's mass spectrum was unique, even when sharing the same molecular formula.

When analyzing hydrocarbon compounds using mass spectrometry, the dominant peak represents parent ions composed of primary carbon and hydrogen isotopes. It detects molecules, each compound's mass spectrum exhibits multiple peaks, beyond just the neutral molecule peak. This is because the molecular ion is unstable and breaks down into smaller cations and radical cations, which contain additional hydrogen atoms compared to the neutral molecule

The Table 4 shows compounds extracted using SFE, which include **12** (31.14%), **3** (17.70%), **14** (12.05%), **17** (9.66%), **18** (6.83%), **5** (0.19%), **24** (0.28%), **4** (0.60%), **6** (3.68%) and **7** (0.45%). Notably, **3** (17.07%) is the active compound responsible for ginger's spiciness, while Zingerone (0.45%) contributes to its spicy aroma in small amounts.

The Table 5 presents the constituents extracted using HDE, comprising **12** (12.04%), **24** (10.32%), **1** (9.50%), **5** (9.00%), **6** (7.03%), **2** (2.84%), **23** (1.43%), **4** (3.05%), **15** (1.30%), **24** (0.19%), **18** (3.99%), **19** (1.50%), **17** (2.28%), **14** (4.51%), **9** (1.32%), **10** (0.86%), **11** (0.72%), **13** (1.22%), **8** (1.38%), **22** (0.81%), and **16** (0.80%). Notably **3**, the active compound, was present in a minimal amount of 0.18%.

Table 3: Comparison between chemical components from SFE and HDE.

Chemical component	Structure	SFE (%)	HDE (%)	Chemical component	Structure	SFE (%)	HDE (%)
(*E*)-Citral	 1	3.68	9.50	1*R*-α-pinene	 2	-	2.84
6-Gingerol	 3	17.0	0.18	Borneol	 4	0.60	3.05
Camphene	 5	0.19	9.00	(Z)-Citral	 6	-	7.03
Copaene	 7	0.45	0.19	Cubenol	 8	-	1.38
Elemol	 9	-	1.32	(*E*)-Nerolidol	 10	-	0.86

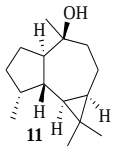
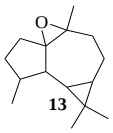
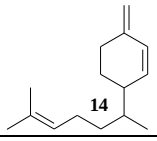
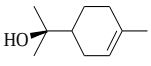
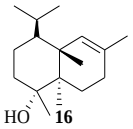
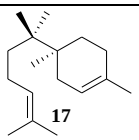
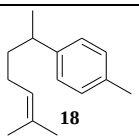
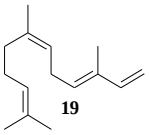
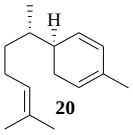
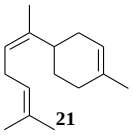
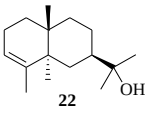
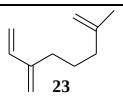
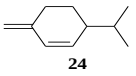
Epiglobulol	 11	-	0.72	Eucalyptol	 12	-	6.62
Ledene oxide-(I)	 13	-	1.22	Sesquiphellandrene	 14	12.05	4.51
Terpineol	 15	-	1.30	*α*-Cadinol	 16	-	0.80
α-Bisabolol	 17	-	1.26	*α*-Curcumene	 18	6.83	3.99
α-Farnesene	 19	-	1.50	*α*-Zingiberene	 20	31.14	12.04
β-Bisabolene	 21	9.66	2.28	*β*-Eudesmol	 22	-	0.81
β-Myrcene	 23	-	1.43	*β*-Phellandrene	 24	0.28	10.32

Table 4: Chemical constituents of *Z. officinale* oil by using SFE.

Compound (s)	Molecular weight	Molecular formula	Retention time (min)	Relative content (%)
Camphene **5**	136	$C_{10}H_{16}$	7.48	0.19
β-Phellandrene **24**	136	$C_{10}H_{16}$	9.77	0.28
Borneol **4**	154	$C_{10}H_{18}O$	13.75	0.60
(*E*)-Citral **1**	152	$C_{10}H_{16}O$	16.41	3.68
Copaene**7**	204	$C_{15}H_{24}$	19.61	0.45
α-Curcumene**18**	202	$C_{15}H_{22}$	22.02	6.83
α-Zingiberene **20**	204	$C_{15}H_{24}$	22.42	31.14
β-Bisabolene **17**	204	$C_{15}H_{24}$	22.75	9.66
Sesquiphellandrene **14**	204	$C_{15}H_{24}$	23.06	12.05
6-Gingerol **3**	294	$C_{17}H_{26}O_4$	47.71	17.07

Table 5: Chemical constituents of *Z. officinale* oil by using HDE.

Compound	Molecular weight & formula	Retention time (min)	Relative content (%)	Compound	Molecular weight & formula	Retention time (min)	Relative content (%)
1*R*-α-pinene **2**	136, $C_{10}H_{16}$	7.13	2.84	α-Farnesene **19**	204, $C_{15}H_{24}$	**22.67**	1.50
Camphene **5**	136, $C_{10}H_{16}$	7.49	9.00	β-Bisabolene **21**	204, $C_{15}H_{24}$	**22.75**	2.28
β-Myrcene **23**	136, $C_{10}H_{16}$	8.73	1.43	Sesquiphellandrene **14**	204, $C_{15}H_{24}$	**23.07**	4.51
β-Phellandrene **24**	136, $C_{10}H_{16}$	9.78	10.32	Elemol **9**	222, $C_{15}H_{26}O$	23.61	1.32
Eucalyptol **12**	154, $C_{10}H_{18}O$	9.87	6.62	*E*-Nerolidol **10**	222, $C_{15}H_{26}O$	23.94	0.86
Borneol **4**	154, $C_{10}H_{18}O$	13.76	3.05	Epiglobulol **11**	222, $C_{15}H_{26}O$	24.65	0.72
Terpineol **15**	154, $C_{10}H_{18}O$	14.42	1.30	α-Bisabolol **17**	222, $C_{15}H_{26}O$	25.26	1.26
(*Z*)-Citral **6**	152. $C_{10}H_{16}O$	15.66	7.03	Ledene oxide-(I) **13**	220, $C_{15}H_{24}O$	25.56	1.22
(*E*)-Citral **1**	152, $C_{10}H_{16}O$	16.47	9.50	Cubenol **8**	222, $C_{15}H_{26}O$	25.71	1.38
Copaene **7**	204, $C_{15}H_{24}$	19.61	0.19	β-Eudesmol **22**	222, $C_{15}H_{26}O$	26.18	0.81
α-Curcumene **18**	202, $C_{15}H_{22}$	22.03	3.99	α –Cadinol **16**	222, $C_{15}H_{26}O$	26.32	0.80
α-Zingiberene **20**	204, $C_{15}H_{24}$	22.44	12.04	6-Gingerol **3**	294, $C_{17}H_{26}O_4$	46.23	0.18

GC-MS analysis:

- Supercritical Fluid extraction (SFE) shown in Fig. 2
- Hydrodistillation extraction (HDE) shown in Fig. 3

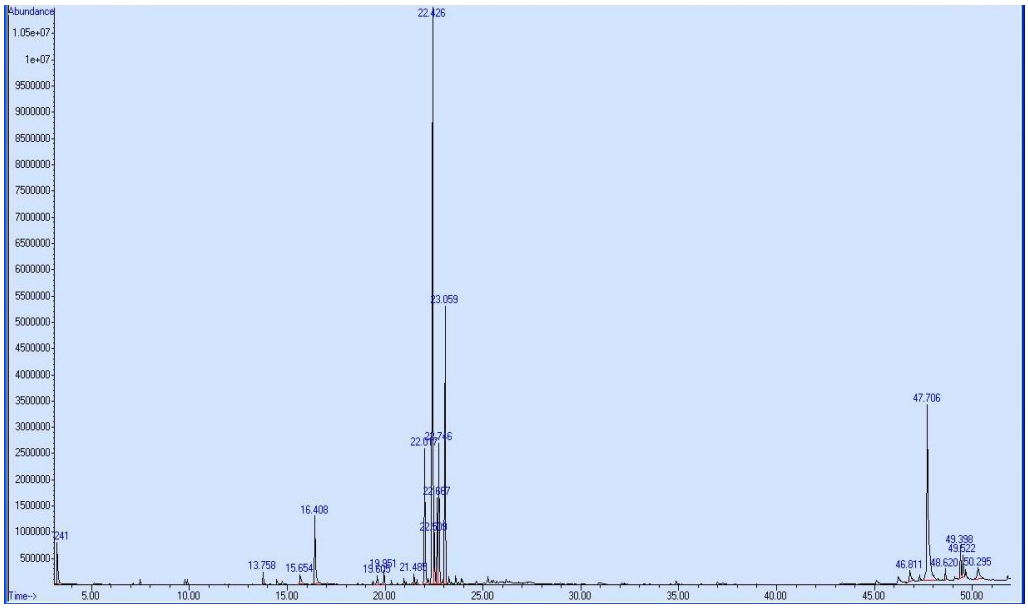


Fig. 2: GC-MS analysis of supercritical Fluid extraction (SFE).

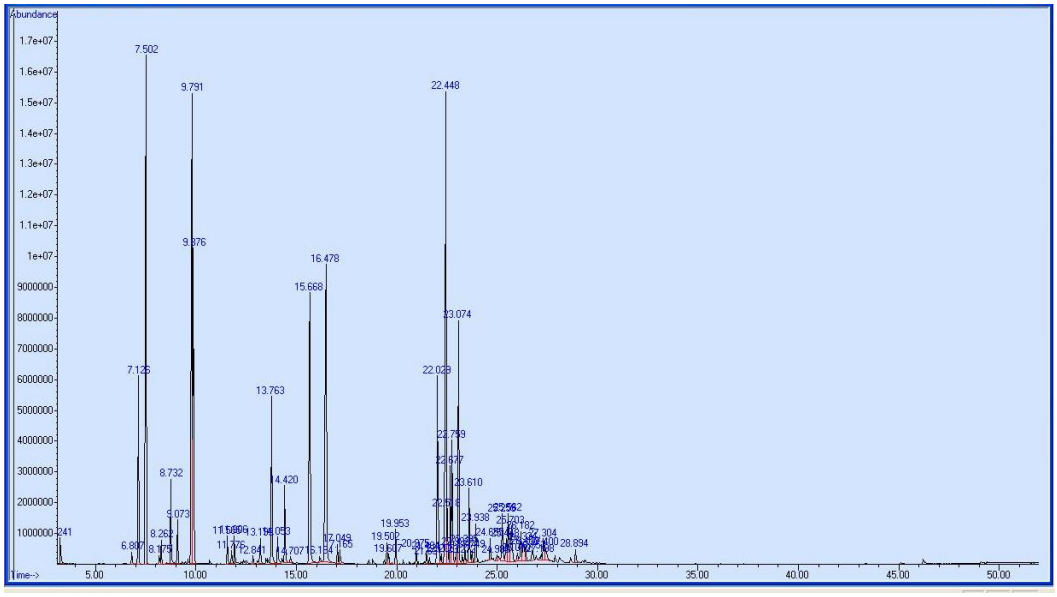


Fig. 3: GC-MS analysis of Hydrodistillation instrument (HDE).

Conclusion

The ginger (*Zingiber officinale*) imparts multiple bioactivities including anti-inflammatory, antinausea, antimicrobial, anticancer, respiratory protective, cardiovascular protective, anti-obesity, antioxidant, antidiabetic, and neuroprotective activities due to various bioactive compounds it possesses including polysaccharides, phenolic compounds (gingerols and shogaols), terpenes, organic acids, lipids, and raw fibers. At present, many extraction methods are being used, each having its own characteristics. With the progress in science and technology, there is a need to obtain higher extraction rate. In this experiment, essential oils of *Zingiber officinale* were extracted using the hydrodistillation (HD) and supercritical fluid extraction (SFE) techniques, followed by their analysis with GC/MS. The hydrodistillation (HD) and supercritical fluid extraction (SFE) techniques isolated 24 (0.08% yellowish oil) and 10 (0.2% yellowish-green oil) compounds, respectively. This comparative analysis reveals that SFE is more advantageous in

providing shorter extraction time, low operating costs and higher yield. And HD is more efficient in providing greater no of constituents.

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