Investigation of some bioactive compounds in oil and ethanol extracts of ginger (*Zingerbiene officlica*) using GC-MS

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ABSTRACT

Objective: The molecular characterization of some bioactive compounds in ginger interest because of their various pharmacological activities. To the best of our knowledge, we are isolate hydrocarbon (low molecular weight), alcohol, phenol, acid, ester from nonpolar (oil) and polar (ethanol) extracts using gas chromatography-mass spectrometry [GC-MS] technique.

Methods: Gas chromatography-mass spectrometry (GC-MS) analysis of the oil and ethanol extracts of ginger was carried out by using a GC-MS equipment.

Results: The GC-MS analysis has revealed the existence of different bioactive chemical compounds in the oil & ethanolic extracts of ginger. The major compounds of oil extract are beta-elemene (0.27%), curcumen (3.12%), zingerberene (10.86%) , bisabolene(3.75) , elemol(1.14%), germacrene(0.23%) , 7-epi trans sesquisabinene (1.82%), zingerberone (35.92%), ethyl palmitate (0.53%), pardol(3.97%). A total of 53 compounds identified representing of total ginger oil extract. While, the major compounds of ethanolic extract are elmene (0.51%), zingerbiene (4.43%), alloaromadentrene (0.42%), curcumene(21.83%), gama cadienene(3.24%)8-epi-.gama.-eudesmol(0.34). A total of 50 compounds identified representing 99.98% of total ginger ethanolic extract.

Conclusion: In this study, successful identification some of important bioactive compounds using GC-MS technique.

Keywords: Ginger, ethanol extract, GC-MS technique, zingeriberene, curcumene.

تفسير لبعض المركبات الفعاله بيولوجيا في مستخلصات الزنجبيل الزيتي والإيثانول (Zingerbiene officlica) باستخدام GC-MS

الهدف من الدراسة: التوصيف الجزيئي لبعض المركبات الفعاله حيوياً في الزنجبيل بسبب خواصها الفعالية الفارماكولوجيه. تم فصل الهيدروكربونات (ذات الاوزان و الجزيئيه الواطئه) ، والكحول ، والفينول ، والحامض ، والإستر من المستخلصات غير القطبية (الزيت) والقطبية (الإيثانول) باستخدام تقنية قياس الاستشراب الغازي لطيف الكتله.

طريقة العمل: تم إجراء تحليل الطيف الكتلي للكروماتوغرافيا الغازية لمستخلصات الزيت والإيثانول من الزنجبيل باستخدام تقنيةGC-MS.

النتائج: كشف تحليل GC-MS عن وجود مركبات كيميائية عديده فعاله حيويا في مستخلصات الزنجبيل والإيثانول. المركبات الرئيسية لمستخلص الزيت هي بيتا-إيلمين (0.27٪)، الكركمين (3.12٪)، زنجبرين (10.86٪)، بيسابولين (3.75) ، إليمول (1.14٪) ، جرمازرين (0.23٪) ، epi trans -7 sesquisabinene (1.82)٪ ، زنجبيرون (35.92٪) ، إيثيل بالميتات (0.53٪) ، باردول (3.97٪). تم تحديد إجمالي 53 مركب تمثل إجمالي خلاصة زيت الزنجبيل. في حين أن المركبات الرئيسية لمستخلص الإيثانول هي الإلمين (0.51٪)، الزنجبرين (4.43٪)، 0.42) alloaromadentrene٪)، الكركمين (21.83٪)، 3.24) gama cadienene٪0.34) 8-epi-.gama.-eudesmol (٪)). تم تحديد ما مجموعه 50 مركبًا تمثل 99.98 ٪ من إجمالي خلاصة الإيثانول بالزنجبيل.

الخلاصة: تم في هذه الدراسة الكشف عن بعض المركبات الحيوية الفعاله باستخدام تقنية GC-MS

الكلمات المفتاحيه: الزنجبيل، خلاصة الإيثانول، تقنية GC-MS، الزنجبرين، الكركمين.

Ginger is widely natural product used as an herbal medicine due their pharmacological properties. It belongs to the *Zingiberaceae* family, The target of our metabolomic medicinal ginger type dried rhizome of *(Zingerbiene officinale*)[1].

Ginger used in different shapes fresh, dried, powdered, or as an oil or juice, and is sometimes added to processed food. the main bioactive compound in ginger, responsible for much of its medicinal properties. It has powerful anti-inflammatory[2], antioxidant [3], antibacterial [4], antiemetic [5], hypoglycemic effects [6].

The chemical constituents of crude ginger which consists of a complex mixture of primary and secondary metabolites such as alkaloids,

saponins, flavonoids, carbohydrates, proteins and terpenoids [3]Figure 1.

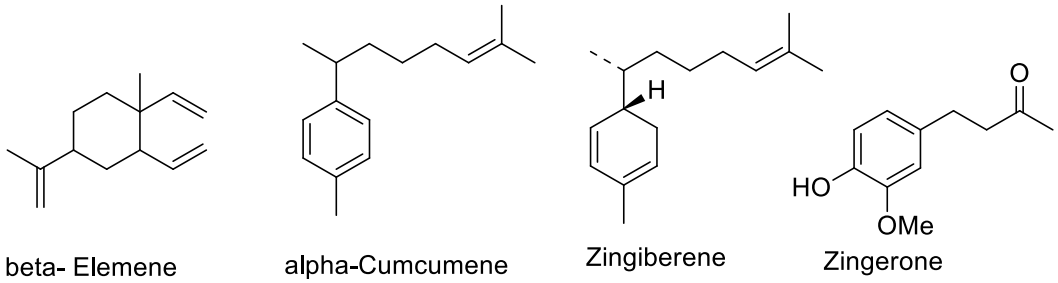

Figure 1: Chemical structure of some bioactive ginger extract.

Several analytical studies of ginger have been reported using different chromatographic (GC –MS) technique to identify active constituents [7]. Our study revealed several important differences in chemical composition between oil and ethanol gingers extracts via GC-MS.

Experimental

Apparatus

Clevenger apparatus, Electronic balance, pH-meter, Magnetic stirrer, GC-MS (Agilent, CA-USA).

Materials

All the chemicals and drying agent are used in the extracts were of analytical grade.

Preparation of oil extract [8].

The essential oils were isolated from the ginger parts of rhizome fresh plants by hydrodistillation using a Clevenger-type apparatus for the temperature was set to 80^{o}C. The extraction process was set for about 5h. The 500mL round bottom flask of the Clevenger apparatus was filled with about 250mL water, then 100g of the grinded fresh ginger was added into the flask. The quick fit Clevenger apparatus was set on a thermostatic heating mantle. This contain oil and water mixture and was separated by running off the water and reading the oil in the inbuilt calibrated tube, extraction process repeated

several times till reach 2 kg of fresh ginger have been used. The essential oils obtained were stored in a sealed container at −4° C until chromatographic analysis. The yield percentage was calculated as weight (g) of essential oils per 100 g of the plant.

Preparation of ethanol extract [9].

The fresh ginger wash, peel, pulverize then dry in shade at room temperature for 14 days. A 50g of dried ginger macerated in 500mL of distill water, chloroform, petroleum ether [40-60ºC], and ethanol for 72h, filter by Whatman No.1, all extracts were dried and precipitate as gum.

Gas chromatography- Mass spectrometry [GC-MS] analysis [10]

The phytochemical investigation of oil and ethanolic extracts were carried out on a GC-MS equipment, the conditions of GC-MS system were as follows: A quadruple detector and a capillary column (30 m×0.25 mm innerdiameter×0.25μm film thickness). Helium was used as the carrier gas with a constant flow of 1.2 mL/min. The initial temperature of oven temperature program was set at 40°C and continued for 4 min, rising by 5°C/min to 250°C, which continued for 10 min. The injector temperature was 250°C. The volume of injected sample was 1μL. Electron ionization (EI) was used in the MS and standard mass spectra with 70 ev ionization energy were recorded m/z from 0-500 at 60 minutes. Sample dissolved in methanol was run at a range of 40-500 m/z and the results were compared by using National Institute of Standards and Technology **by the U.S. Secretary of Commerce on behalf of the U.S.A** (NIST08), 2018 and chemical abstract services (CAS).

Results& Discussion

The bioactive constituents of yellow volatile oil of ginger was detected by GC-MS Chromatogram, electronic ionization (EI) is the classical ionization technique in mass

spectroscopy and is used as the standard method in all GC/MS instruments. This application of mass spectrometry is limited to volatile compounds of low molecular weight. Approximately only 53 volatile compounds in the oil extract is characterized on the basis of GC-MS evidence with different retention times [9] (Figure 2). The analysis of complex mixtures such as crude oil of the identified some lowmolecular weight compounds beta-elemene(0.27%), curcumen (3.12%), zingerberene (sesquiterpene)(10.86%) , bisabolene(3.75) , elemol(1.14%), germacrene(0.23%) , 7-epi trans sesquisabinene (1.82%), zingerberone (35.92%), ethyl palmitate (0.53%), pardol(3.97%). due to the reiteration of 5 compounds (Table 1). The large compound fragments compared to small compounds gave taller appearance of peaks at different m/z ratios during 56 minutes.

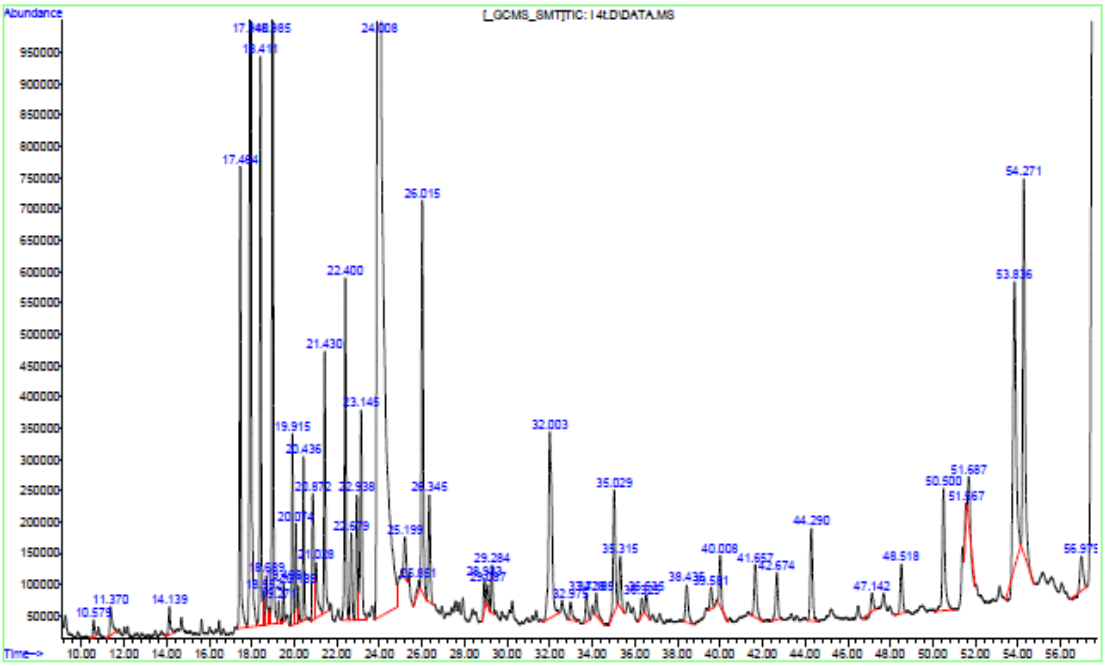

Figure 2: GC-MS chromatogram of ginger oil extract.

Table 1: List of some bioactive compounds in ginger oil extract identified by using GC-MS.

No.	Name	Retention Time (RT) min.	Molecular formula	Mass/ charge m/z	Base Peak (%)
1	beta.-Elemene	14.139	$C_{15}H_{24}$	204.5	93
2	Alpha -Curcumene	17.464	$C_{15}H_{22}$	202.5	119
3	Zingiberene	17.939	$C_{15}H_{24}$	204	119
4	Cubedol	18.687	$C_{15}H_{26}O$	222	161.1
5	gamma.-Bisabolene *	19.272	$C_{15}H_{24}$	204	93
6	Elemol	19.916	$C_{15}H_{26}O$	222	93
7	Germacrene	20.190	$C_{15}H_{24}$	204	121
8	7-epi-cis-Sesquisabinene hydrate	20.072	$C_{15}H_{26}O$	222.4	119
9	Zingerone	24.012	$C_{11}H_{14}O_3$	194.2	137
10	Ethyl palmitate	42.677	$C_{18}H_{36}O_2$	284.2	88
11	Paradol	54.268	$C_{17}H_{26}O_3$	278.1	137

* Mixture cis & trans isomers.

However, the identified of some bioactive compounds in the ethanol extract of ginger elmene (0.51%), zingerbiene (4.43%), alloaromadentrene(0.42%), curcumene(21.83%), gama cadienene(3.24%)8-epi-.gama.-eudesmol(0.34) are listed in (Table 2).

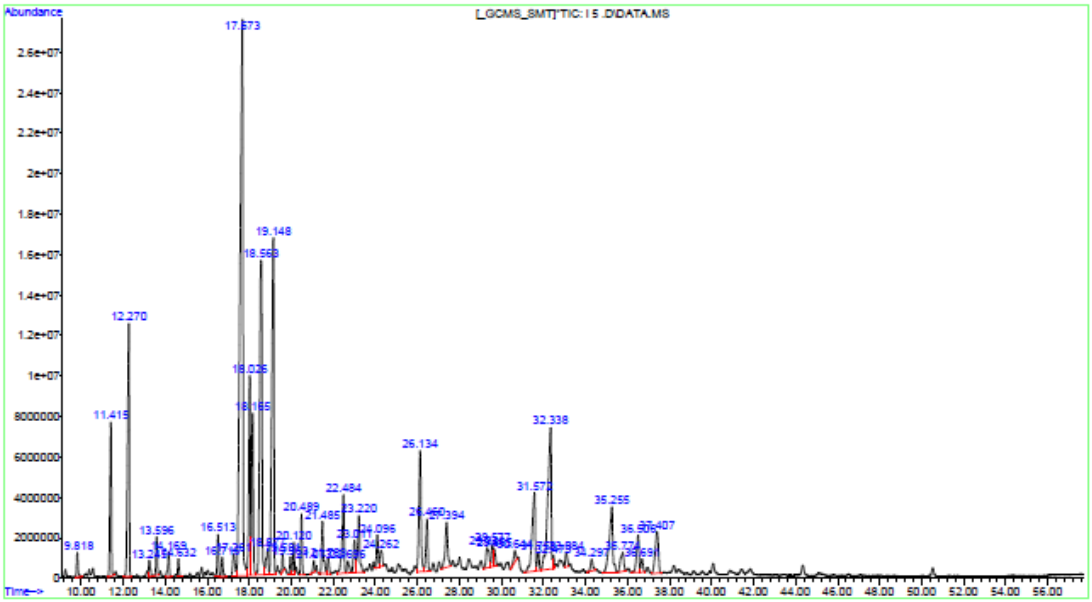

Figure 3: GC-MS chromatogram of ginger ethanol extract.

The GC-MS analysis of some common in nonpolar (oil) and polar (ethanol) extracts (Figures 4- 7) [11].

Table 2: List of some bioactive compounds in ginger ethanol extract identified by using GC-MS.

No.	Name	Retention Time (RT) min.	Molecular formula	Mass/ charge m/z	Peak Area (%)
E-1	Beta-Elemene	14.168	$C_{15}H_{24}$	204.2	93
E-2	Zingiberene	14.635	$C_{15}H_{24}$	204.1	119
E-3	Alloaromadendrene	16.716	$C_{15}H_{24}$	204.1	91
E-4	alpha.-Curcumene	17.679	$C_{15}H_{22}$	202.2	119.1
E-5	gamma.-Cadinene	19.168	$C_{15}H_{24}$	204.2	161
E-6	8-epi-.gama.-eudesmol	22.693	$C_{15}H_{26}O$	222.1	189.1

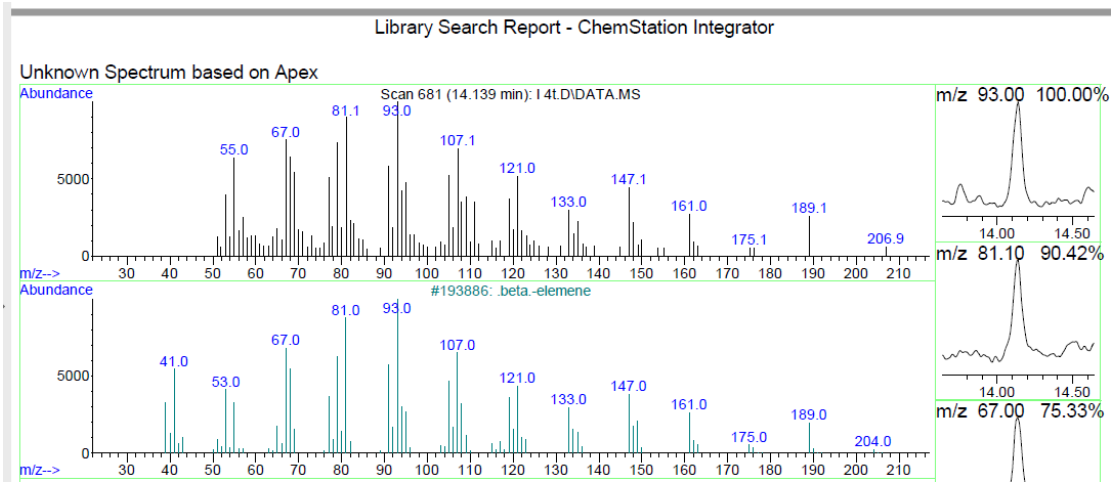

Figure 4 : GC-MS chromatogram of beta-Elemene base peak (m/z) 93.

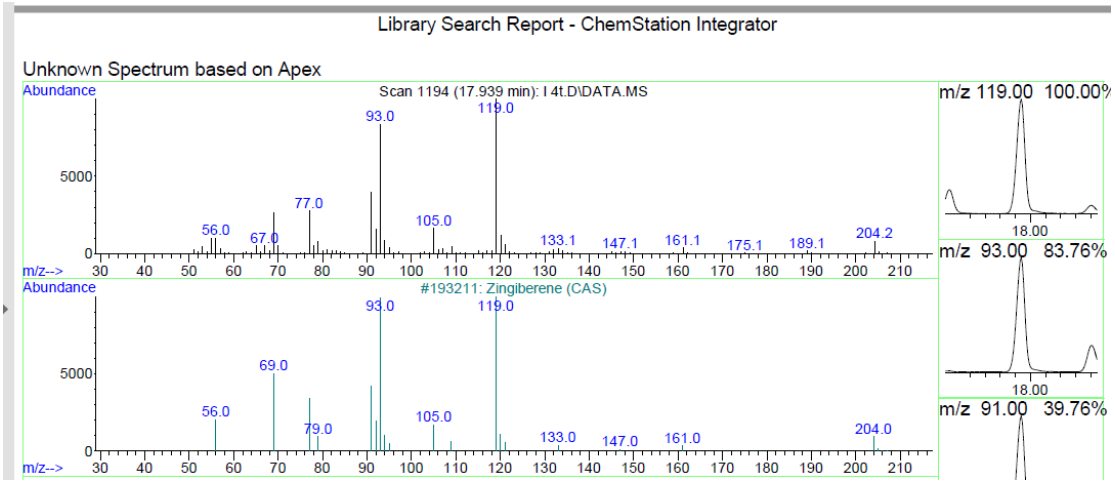

Figure 5 : GC-MS chromatogram of Zingibrene base peak (m/z) 119.1.

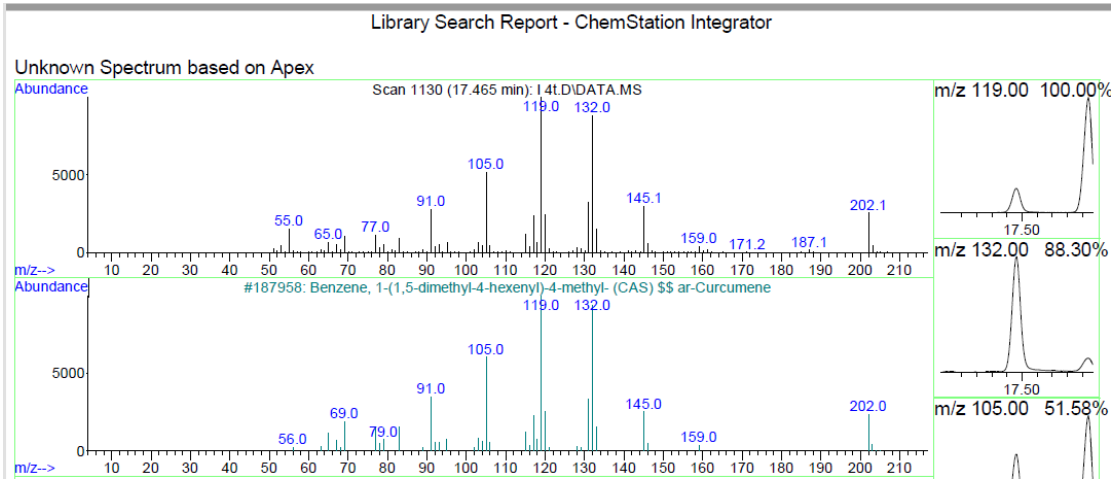

Figure 6 : GC-MS chromatogram of Curcumene base peak (m/z) 119.10.

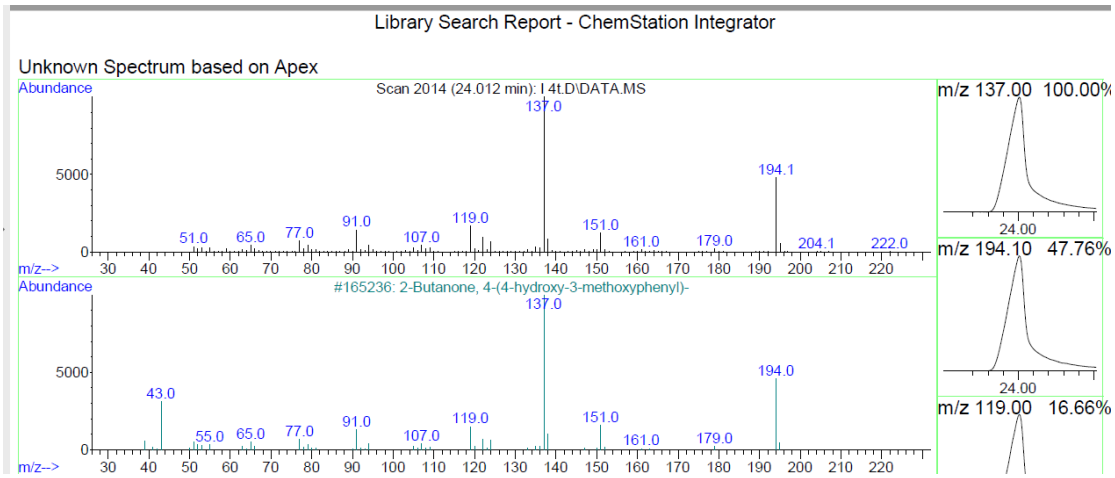

Figure 7: GC-MS chromatogram of Zingerone base peak (m/z) 137.

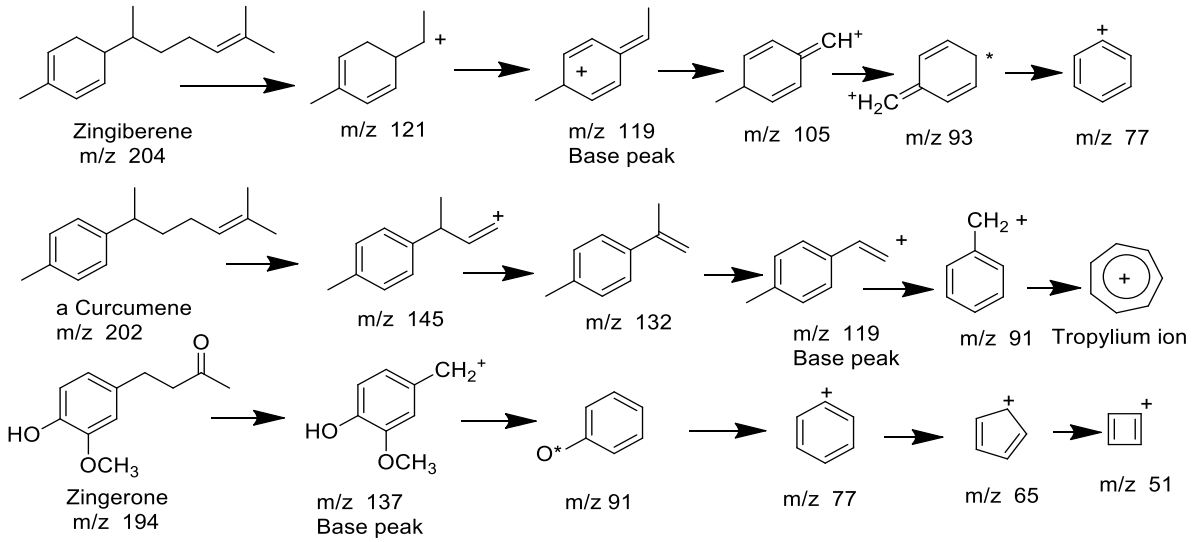

Figure 8: Some proposed fragment ions for zingiberene, curcumene and zingerone.

again the structural assignment for zingiberene, curcumene and zingerone were based primarily on mass spectral evidence from first principles, since the reference spectra were available until after the preliminary two structural assignments due to different mechanism. McLafferty rearrangement occurs in carbonyl compounds only if the gamma carbon contains hydrogen. The hydrogen from gamma carbon is transferred to an unsaturated site. The formula cyclohexatrienyl cation is an aromatic species with a $[C_7H_7]^+$ known as o

tropylium ion m/z 91as shown in the fragmentation of alpha- curcumene.

Geometric isomers Z- E isomers of bioactive organic compounds can be detected using GC-MS, they have the same m/z fragmentations with aid (NIST08) and CAS as shown with gamma.-Bisabolene (Figures 9) .

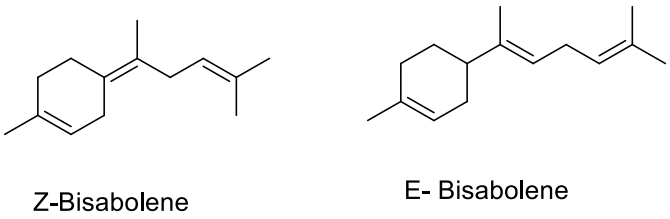

Figure 9: Chemical structures of Z-E isomers of Bisabolene.

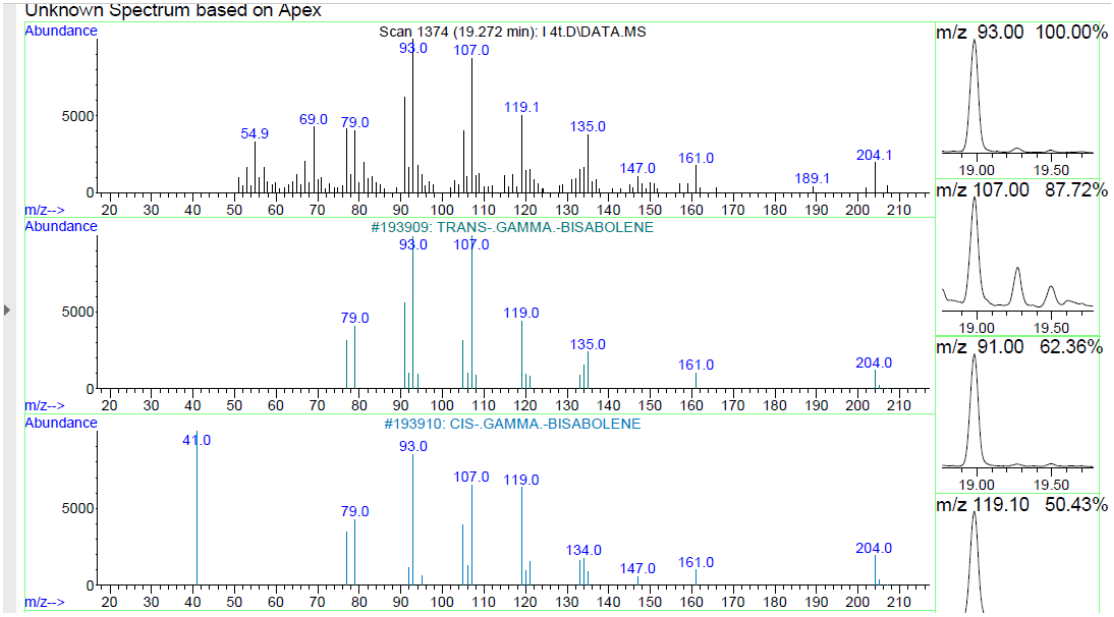

Figure 10: GC-MS chromatogram of Z-E isomers of Bisabolene, base peak m/z 93; E-isomer base peak m/z 107 ; Z isomer base peak m/z 119.

Table 3: Comparative studies of some bioactive compounds in ginger oil & ethanol extracts using GC-MS.

Type	Oil extract	Ethanol extract
Number of compound	A total of 53 compounds	A total of 50 compounds
Time consume (min)	56.98	40.75
Organic volatile compounds	Hydrocarbon (Terpenoid) Alcohol	Hydrocarbon Alcohol

	Acid Ester ketone	

The qualitative and quantitative analytical results are show the data in Table3. A total of 53 in oil extract in 56.98 min., while the total of 50 components in ethanol extract in 40.75 min. were identified by GC/MS. The chemical compositions of low molecular weight of oil extract are more than ethanol extract in ginger. Most of the isolated hydrocarbon with low molecular weight and occur predominantly as natural plant compounds. The production and types of the hydrocarbon (terpenes) can be linked naturally to external factors, such as differences in soil, light, temperature and water levels, because known as the largest group of plant natural products. The ginger oil, its potential health benefits because safe and effective, ginger product can be widely used as medicinal plant using GC-MS technique as a rapid, simple, low cost and solvent free method for low molecular weight organic analysis. Further studies of ginger using hexane, methanol and water extracts and identify their bioactive constituents.

Conclusion

This paper describes the identification of volatile components some major bioactive low molecular weight organic compounds of two different extracts of ginger (oil and ethanol) using gas chromatography – mass spectrometry [GC-MS].

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