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Research paper

Identification of zingiber components by gas chromatograph/mass spectrometer and semi-empirical calculations

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Abstract

The important used of Zingiber in different fields such as medicine and foods, this leads to study the physical and chemical properties of its components. The chemical components in Zingiber officinale Roscoe were identified by gas chromatograph/ mass spectrometer (GC/MS) with electron ionization mode. The major components of Zingiber under investigation namely: (1) Pentadecanoic acid (2) 1,3-Dioxepane, 2-pentadecyl (3) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (4) gingerol. Electron ionization mass spectra of these compounds have been obtained and investigated. In addition, the semi-empirical (MNDO) method was used to calculate some physical and thermochemical properties for the structural of these compounds such as total energy, electronic energy, ionization energy, heats of formation, proton affinity and dipole moments which might to predict the activity and behavior of these compounds.

Keywords: *Zingiber; Gas Chromatography-Mass Spectrometry and Semi-Empirical Calculations.*

1. Introduction

Plants and leaves are as considered one of the main sources of biologically active compounds. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1].

Ginger (Zingiber officinale Rosc.) belongs to the family Zingiberaceae. It originated in South-East Asia and then used in many countries as a spice and condiment to add flavor to food [2]. Besides this, the rhizome of ginger has also been used in traditional herbal medicine. The health-promoting perspective of ginger is attributed to its rich phytochemistry.Ginger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. It also has anti-inflammatory and anti-oxidative properties for controlling the process of aging. Furthermore, it has antimicrobial potentialas well which can help in treating infectious diseases [3-6].

GC-MS is a technique used for screening/identification/quantification of many susceptible compounds in plant extracts. Gas chromatography (GC) is used to separate all volatile components that might be present in the sample.The retention time (RT) is an identifying characteristic of these components. The detector for the GC is the mass spectrometer (MS). The fragmentation pattern for a component is unique and therefore is an identifying characteristic of a component. The identification of a component by its retention time (GC) and fragmentation pattern (MS), along with sample specific information afforded to make GC-MS the foremost confirmation method for analyzing herbal extract [7]. Up to date, gas chromatography–mass spectrometry (GC–MS) has played the most important role in the identification of the chemical composition of medical plants [8-10].

Many analytical methods including gas chromatograph coupled with mass spectrometer (GC/MS), high-performance liquid chromatography (HPLC), and its coupling to mass spectrometry (LC/MS), nuclear magnetic resonance (NMR), thin layer chromatography (TLC) and capillary electrophoresis (CE), have been used to characterize gingerol- related compounds in ginger[11–16]. Among these methods, GC/MS has been used quite often to analyze ginger samples to predict the different components and its identification.

On the other hand, quantum chemical methods for the calculations of thermochemical data have developed beyond the level of just reproducing experimental data and can now make accurate predictions where the experimental data are unknown or uncertain [17] The semi-empirical molecular orbital (MO) methods of quantum chemistry [18-29] are widely used in computational studies of large molecules, particularly in organic chemistry and biochemistry. A great number of authors have been described the activity of the compounds using quantum chemical methods [30-35].

Much work have been done on Zingiber using GC-MS, LC-MS and many other analytical techniques as shown above. In the same time there have not found any work concern in quantum chemical calculations using semi-empirical (MNDO) method so far. So, the objective of the present work as follow:

1) Using GC-MS to identify the chemical composition record and investigate the mass spectra of Zingiber components by electron ionization mass spectrometry EI-MS.
2) Suggested the fragmentation pathways of the studied components, which was used to characterize the structures of these components.
3) Calculate and discussed some of thermochemical data physical properties of Zingiber components under investigation. The data present in this paper provided useful information on the different components of zingiber and can be used in the characterization of this important plant.

2. Materials and methods

2.1. Exteraction

Dried roots of Zingiber officinale L. (ginger; Family Zingiberaceae) were obtained from a local Herbs and Medicinal Plants market (Alexandria, ARE). The plant was authenticated by staff members of the Botany Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The method used to extract the different components of zingiber is a new simple method. An aqueous extract was prepared by soaking 100 g of the dry ground ginger root in 500 ml of distilled water at 40-50°C with daily shaking for 5 days; then, the extract was stored at 4°C. The infusions were filtered through double-layered gauze, the filtrate was centrifuged at 3000 rpm at 50 °C for 10 min, and the water was evaporated in a hot air oven at 50°C.

2.2. GC-MS analysis

GC-MS analysis was carried out on THERMO TRACE ULTRA GC coupled with THERMO ISQ (Gas Chromatograph–Mass Spectrometer instrument) employing the following conditions: column TG-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as a carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ^{o}C; ion-source temperature 280 ^{o}C. The oven temperature was programmed from 110 ^{o}C (isothermal for 2 min), with an increase of 10 ^{o}C/min, to 200^{o}C, then 5oC/min to 280^{o}C, with hold 9min. Mass spectra of the four components recorded at 70eV; scan interval time was 0.2 second and fragments from 40 to 450 Da. Total GC running time is 36min. min.

2.3. Identification of the components

The components of Zingiber were identified by comparing their relative retention times and mass spectra with those of WILEY and NIST 05 mass spectral database. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST and Wiley libraries. The retention time, molecular weight, molecular formula and composition percentage of the sample material were recorded and presented in Table 1.

3. Results and discussion

In Figure 1 Total Ion Chromatogram (TIC) measured for extracted Zingiber. The chemical composition of the studied components are listed in Table 1. The chemical structures with its mass spectra of the components are shown in Figures 2-5.

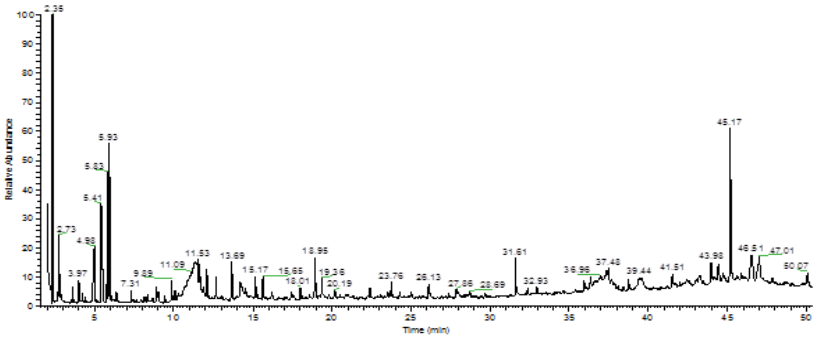


Fig. 1: Total Ion Chromatogram (TIC) Measured for Extracted Zingiber.

Table 1: The Chemical Composition of the Studied Components of Zingber

No.	Rt.	Compound	Molecular formula & Molecular weight	Compound nature and activity
1	11.41	Pentadecanoic acid	$C_{15}H_{30}O_2$ (MW:242)	Palmitic acid have antioxidant activity
2	12.69	1,3-Dioxepane, 2-pentadecyl	$C_{20}H_{40}O_2$(MW:312)	Bioactive compound have antiproliferative activity
3	18.95	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$ (MW:144)	Flavonoid compound have anti- microbial and anti-inflammatory effects.
4	45.19	Gingerol	$C_{17}H_{26}O_4$ (MW:294)	Bioactive compound have an antipyretic, hypotensive, cardiotonic,antiplatelet,antian-giogenic,anti-inflammatory and analgesic, cytotoxic, apoptotic and antitumor activities

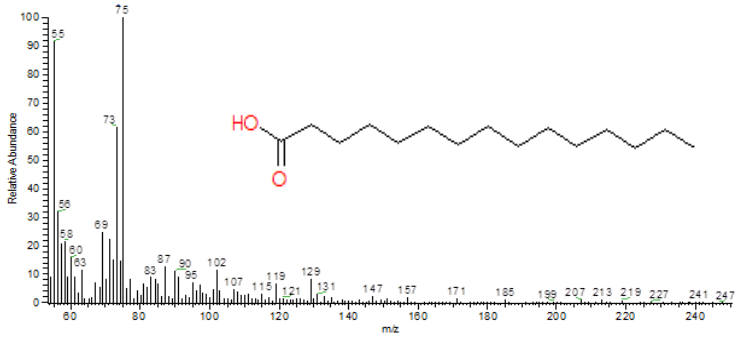
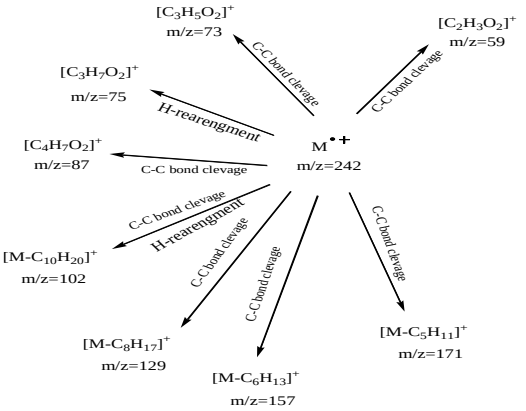


Fig. 2: The Mass Spectrum of Pentadecanoic Acid.



Scheme 1: Fragmentation Pathways of Pentadecanoic Acid Using Electron Ionization Technique.

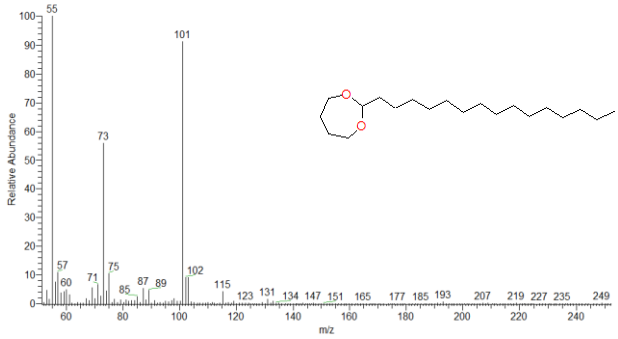
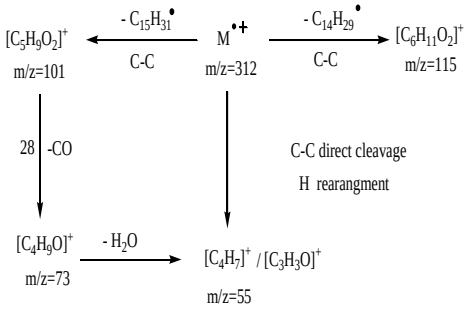


Fig. 3: The Mass Spectrum of 1, 3-Dioxepane, 2-Pentadecyl.



Scheme 2: Fragmentation Pathways of 1, 3-Dioxepane, 2-Pentadecyl Using Electron Ionization Technique.

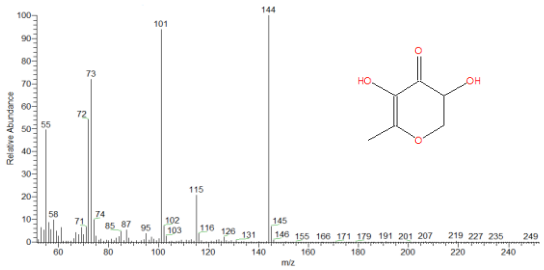
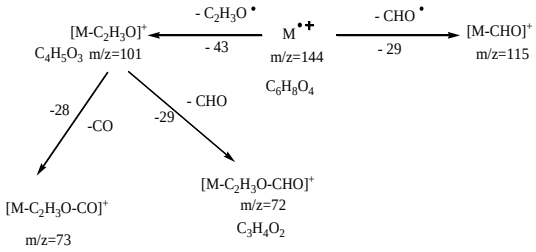


Fig. 4: The Mass Spectrum of 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl.



Scheme 3: Fragmentation Pathways of4h-Pyran-4-One, 2, 3-Dihydro-3, 5-Dihydroxy-6-Methylusing Electron Ionization Technique.

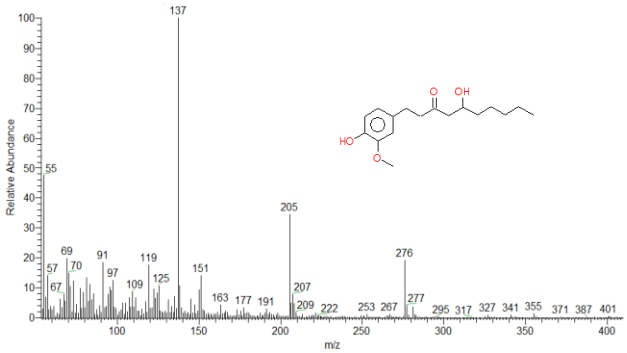
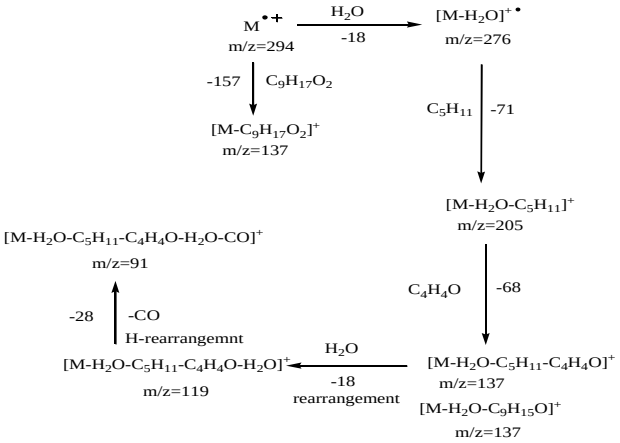


Fig. 5: The Mass Spectrum of Gingerol.



Scheme 4: Fragmentation Pathways of Gingerol Using Electron Ionization Technique.

Structures investigation of the studied components mass spectra:

The fragmentation pathways of the main fragment ions formed from molecular ions of the four studied compounds at 70 eV are interpreted through fragmentation Schemes 1 – 4.

1) The molecular ion of Pentadecanoic acid at m/z 242 has very low intensity in the EI mass this indicates that the molecular ion of this component thermally not stable. This molecular ion undergoes fragmentation by form $[C_2H_3O_2]^+$, $[C_3H_5O_2]^+$,$[C_3H_7O_2]^+$,$[C_4H_7O_2]^+$,$[M\text{-}C_8H_{17}]^+$$[M\text{-}C_6H_{13}]^+$and $[M\text{-}C_5H_{11}]^+$ at m/z 59,73,75,87,129,157 and 171 respectively as shown in Scheme 1.
2) The molecular ion of 1, 3-Dioxepane, 2-pentadecyl at m/z =312 is not observed in the EI mass spectrum this indicates the molecular ion of this component thermally not stable. Also, the relative intensities of the ions, especially in the high mass region are generally low. The presence of the most intensities feature of the spectrum involves a formation of the base peak at m/z 101 having formation of form $(C_5H_9O_2)^+$. The molecular ion of 1, 3-Dioxepane, 2-pentadecyl undergoes fragmentation to form the fragment ion $[C_6H_{11}O_2]^+$ m/z 115 and the fragment ion $[C_5H_9O_2]^+$ m/z101 which under fragmentation to form by loss of $CO^{\bullet}$ to form the fragment ion $[C_4H_9O]^+$ at m/z 73.
3) The molecular ion of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl at m/z 144 is observed in the EI mass spectrum which represents the base peak (RI = 100%) in the mass spectrum this indicates that the molecular ion of this component have high stability. The first fragmentation pathway for 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl is the formation of the fragment ion at m/z 101 which is certainly due to the ion $[M\text{-}C_2H_3O]^+$ this fragment ion undergoes fragmentation by losing CHO and CO to form the ions $[M\text{-}C_2H_3O\text{-}CHO]^+$(m/z 72) and $[M\text{-}C_2H_3O\text{-}CO]^+$(m/z 73), respectively. While the second fragmentation pathway is the formation of the fragment ion at m/z 115 which due to the loss of $CHO^{\bullet}$ to form the fragment ion $[M\text{-}CHO]^+$.
4) The molecular ion of gingerol at m/z =294 is not observed in the EI mass spectrum this indicates the molecular ion of this component thermally not stable. The first fragmentation pathway is the formation of the fragment ion at m/z 276 by loss H_2O to form $[M\text{-}H_2O]^+$ ion with relative intensity 20% this fragment ion undergoes fragmentation by loss of C5H11 radical to form the fragment ion $[M\text{-}H_2O\text{-}C_5H_{11}]^+$ at m/z 205. This fragment ion represent the second peak with relative intensity 35% undergoes fragmentation by loss C4H4O to form the $[M\text{-}H_2O\text{-}C_5H_{11}\text{-}C_4H_4O]^+$ at m/z 137 represent the base peak in gingerol mass spectrum which can produced from two processes $[M\text{-}H_2O\text{-}C_5H_{11}]^+$ then loss C_4H_4O and from the molecular ion by loss of $C_9H_{17}O_2$ as shown in Scheme 4. Undergoes fragmentation by loss of H_2O to form the fragment ion $[M\text{-}H_2O\text{-}C_5H_{11}\text{-}C_4H_4O\text{-}H_2O]^+$ at m/z 119 which also undergoes fragmentation by loss CO ion $[M\text{-}H_2O\text{-}C_5H_{11}\text{-}C_4H_4O\text{-}H_2O\text{-}CO]^+$ at m/z 91. The second fragmentation process of the molecular ion of gingerol is the formation of the fragment ion $[M\text{-}C_9H_{17}O_2]^+$ at m/z 137 by loss of $C_9H_{17}O_2$.

Semi-empirical molecular orbital calculations of the studied compounds:

Computational chemistry methods have been introduced that allow analysis of reaction mechanisms and prediction of the reactivity in synthetic chemistry. Therefore, computational chemistry used to predict the reactivates of a wide variety of organic molecules [36].

The optimized structures of the studied molecules shown in Table 2 using the MNDO method With HyperChem 7.5 program [37] for calculating the molecular properties of the studied molecules. Table 2. The values of these parameters like total energy, heats of formation, electronic energy, ionization energy, electron affinity and dipole moment of the studied molecules obtained using MNDO method at the ground and charged states while Table 3 show the proton affinities of the studied molecules at different oxygen atoms.

Table 2: Values of Calculated Total Energy, Electronic Energy, Heats of Formations, Dipole Moment, Ionization Energy and Electron Affinity Using MNDO Method of the Studied Molecules.

	Pentadecanoic acid	1,3-Dioxepane, 2-pentadecyl	4H-Pyran-4-one, 2,3dihydro-3,5-dihydroxy-6-methyl	Gingerol
Total energy (kcal mol^{-1})	-68989 (M) -68751 $(M)^{+1}$ -68983 $(M)^{-1}$	-87005 (M) -86805 $(M)^{+1}$ -86992 $(M)^{-1}$	-50033 (M) -49843 $(M)^{+1}$ -50059 $(M)^{-1}$	-88379 (M) -88184 $(M)^{+1}$ -88390 $(M)^{-1}$
Electronic energy (kcal mol^{-1})	-414628 (M)- 414113 $(M)^{+1}$ -414186 $(M)^{-1}$	-619308 (M) -596999 $(M)^{+1}$ -589254 $(M)^{-1}$	-216985 (M) -217339 $(M)^{+1}$ -216847 $(M)^{-1}$	-587706 (M) -578744 $(M)^{+1}$ -582991 $(M)^{-1}$
Heats of formation $\Delta H_f(M)$ (kcal mol^{-1})	-162	-163	-161	-183
Heats of formation $\Delta H_f(M)^{+\bullet}$ (kcal mol^{-1})	74	37	29	15
Heats of formation $\Delta H_f(M)^{-1}$ (kcal mol^{-1})	-158	-150	-188	-192
Dipole moment (D)	1.64	1.14	1.53	4.96
Ionization energy (eV)	10.2	8.7	8.2	8.5
Electron affinity (eV)	-0.17	-0.60	1.17	0.40

Table 3: Calculated Heats of Formation Values for Protonated Molecules $\Delta H_f(M+H)^+$ and Proton Affinities (PA) at Different Oxygen Atoms Sites Using MNDO Method.

	Pentadecanoic acid	1,3-Dioxepane, 2-pentadecyl	4H-Pyran-4-one, 2,3dihydro-3,5-dihydroxy-6-methyl	Gingerol
$\Delta H_f(M)$ (kcal mol^{-1})	-162	-163	-161	-180
$\Delta H_f(M+H)$ (kcal mol^{-1})	-159 at O1 -140 at O2	-161	-171 at O1 -161 at O2 -106 at O3 -158 at O4	-182 at O1 -184 at O2 -145 at O3 -152 at O4
$\Delta H_f(M+H)^+$ (kcal mol^{-1})	93 at O1 97 at O2	74	4 at O1 29 at O2 38 at O3 36 at O4	1 at O1 -14 at O2 2 at O3 -1.4 at O4
Proton affinity (PA) (kJ. mol^{-1})	464 at O1 448 at O2	540	837at O1 736at O2 699at O3 707at O4	774 at O1 837 at O2 770 at O3 784 at O4

It is interesting to calculate the physical parameters such as total energy, electronic energy, dipole moment and thermochemical quantities such as heats of formation $\Delta H_f(M)$, $\Delta H_f(M)^{-1}$ and $\Delta H_f(M)^{+\bullet}$ for the studied molecules using the MNDO method. Hence, one can calculate the IE value for these molecules as the difference between $\Delta H_f(M)^{+\bullet}$ and $\Delta H_f(M)$ and the electron affinity as the difference between total energies at neutral (M) and anion $(M)^{-1}$ states.

From the calculated data reported in Table 1, one can note that, the heats of formation of the molecules (1) Pentadecanoic acid (2) 1,3-Dioxepane, 2-pentadecyl and (3) 4H-Pyran-4-one, 2,3dihydro-3,5-dihydroxy-6-methyl at the neutral state are approximately have the same values (-162,-163 and -161 kcal/mol) respectively while the heats of formation of Gingerol molecule is -183 kcal/mol. These negative values of the heats of formation for the four molecules indicate their stability while for gingerol compound which has the highest one in stability as shown in Table 1. This conformed by the total ion chromatogram, hence gingerol represent the major compound and have the more intense peak as shown in fig.1.

Ionization potential and dipole moment are one of the main characteristics of atoms or molecules. They rely heavily on nature and strength of intermolecular bonds and chemical bonds in molecules [36]. The calculated values of ionization energy of the Pentadecanoic acid, 1, 3-Dioxepane, 2-pentadecyl: 4H-Pyran-4-one, 2, 3dihydro-3, 5-dihydroxy-6-methyl and gingerol molecules are 10.2, 8.7, 8.2 and 8.5eV as shown as in Table 1. are approximately have the same value except Pentadecanoic acid have ionization energy value equal to 10.2 eV as reported in Table 1.

The dipole moment for (1) Pentadecanoic acid (2) 1,3-Dioxepane, 2-pentadecyl (3) 4H-Pyran-4-one, 2,3dihydro-3,5-dihydroxy-6-methyl at the neutral state are approximately have the same values (1.643,1.141 and 1.534 D) respectively. This means that they have the same molecular polarity while gingerol molecule has the highest value of the dipole moment which equal to 4.96 D this means that, gingerol has highest molecular polarity. As shown in Table 1.

Proton affinities and heats of formation are important thermodynamic quantities that can be derived from a variety of experimental measurements. Modern composite computational methods provide the means to reliably estimate the same quantities with an accuracy that often rivals that of experiment [38]. In addition, these methods can provide information to complement results obtained experimentally and to examine problems that are not easily approached directly, such as site-specific proton affinities [38]. A case in point is the protonation of the molecules, which often can take place at more than one position. In principle, the protonation of these compounds may be protonated at the hetero atoms (oxygen atoms in these compounds). The most negative atoms in the four compounds are localized on oxygen atoms. The calculated data reported in Table 2. For the studied molecules have been done using MNDO method at different positions of oxygen atoms of the optimized structures Figure 6.

From the calculated thermochemical data of the studied molecules, one can note that the proton affinity for Pentadecanoic acid molecule at the site (O1─H) is 464 kJ. mol^{-1} and (O2─H) is 448 $kJ.mol^{-1}$. The proton affinity at the site (O2─H) is less than the value at (O1─H) site by 16 $kJ.mol^{-1}$ The calculated values of the proton affinity of 1,3-Dioxepane, 2-pentadecyl molecule at the two positions O1 and O2 have equal values 540 kJ. mol^{-1} . This due to the symmetry of the two Oxygen atoms positions.

4H-Pyran-4-one,2,3dihydro-3,5-dihydroxy-6-methyl molecule have four proton affinity positions, the most stable position is at (O1─H) since have the highest proton affinity (837 kJ. mol^{-1}) value than other three positions. This due to the carbonyl C═O group.

Finally , the gingerol molecule have four proton affinity positions, the most stable position is at (O2─H) since have the highest proton affinity (837 kJ. mol^{-1}) value than other three positions as in Table 2.

From the above discussion of the proton affinity, one can note that, 4H-Pyran-4-one,2,3dihydro-3,5-dihydroxy-6-methyl and gingerol compounds have the highest proton affinity value 837 kJ. mol^{-1}) at (O1─H) and (O2─H), respectively. The protonation at

carbonyl group is favored which consider as the more stable site of protonation in all studied molecules. Also, One can note that the most two stable protonation sites are occur at the double bond oxygen atom C═O. This means that, these sites are active side bond for biological activity of gingerol and 4H-Pyran-4-one,2,3dihydro-3,5-dihydroxy-6-methyl. This confirmed also by previous corresponding author Phd thesis [39].

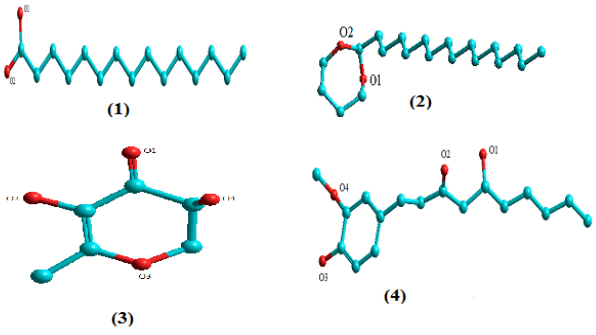


Figure 6. The different protonation positions of optimized structures of the studied molecules **(1-4)**

4. Conclusions

The experimental investigations show that the application of GC-MS can provide a rather detailed analysis of Zingiber Components by using the obtained chromatogram and mass spectra. The mass spectra of the studied components have been recorded and investigated, and the fragmentation mechanisms pathways have been suggested and discussed. The experimental data together with the theoretical quantum chemical calculations MNDO give us more information about the chemical behavior of the studied molecules which may be important for many chemical and medical applications. So MNDO method have been used to calculated these thermochemical and physical properties of the studied components and correlate these data with experimental part to understanding the chemical behavior of the studied compounds (these data are clear in Table2-3 and a. section).

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