



Mass Spectrometric Analysis of Isotopic Abundance Ratio in Biofield Energy Treated Thymol

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Abstract: Thymol is a natural monoterpenoid phenol possessing various pharmacological activities such as antimicrobial, antioxidant, etc. The stable isotope ratio analysis has drawn attention in numerous fields such as agricultural, food authenticity, biochemistry, metabolism, medical research, etc. An investigation of the effect of the biofield energy treatment (The Trivedi Effect®) on the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in thymol using gas chromatography - mass spectrometry was attempted in this study. The sample, thymol was divided into two parts - one part was denoted as control and another part was referred as biofield energy treated sample that was given Mr. Trivedi's unique biofield energy. T1, T2, T3, and T4 were represented to different time interval analysis of the biofield treated thymol. The GC-MS spectra of the both control and biofield treated thymol indicated the presence of molecular ion peak [M^+] at m/z 150 (calculated 150.10 for $C_{10}H_{14}O$) along with the similar pattern of fragmentation. The relative intensities of the parent molecule and other fragmented ions of the biofield treated thymol were enhanced as compared to the control thymol. The percentage change of the isotopic abundance ratio of P_{M+1}/P_M in the biofield treated thymol at T1, T2, T3 and T4 was increased by 3.25, 6.31, 96.75, and 140.25%, respectively as compared to the control thymol. In addition, the percentage change of the isotopic abundance ratio of P_{M+2}/P_M was increased in the biofield treated thymol at T1, T2, T3, and T4 by 5.33, 8.00, 101.33, and 140.00%, respectively with respect to the control sample. In summary, ^{13}C , 2H , and ^{17}O contributions from $(C_{10}H_{14}O)^+$ to m/z 151 and ^{18}O contribution from $(C_{10}H_{14}O)^+$ to m/z 152 in the biofield treated thymol were significantly increased gradually with respect to the time and was found that biofield energy treatment has time dependent effect on it. Hence, the biofield energy treated thymol might display altered isotope effects such as physicochemical and thermal properties, binding energy and the reaction kinetics with respect to the control sample. So, biofield energy treated thymol could be advantageous for designing the synthetic scheme for the preparation of pharmaceuticals through its kinetic isotope effects. Besides, biofield treated thymol might be useful to overcome the problems associated with thymol for e.g. pungent flavor, high dose requirement for the activity through understanding its isotope effects and the determination of its pharmacokinetic profile, bioavailability.

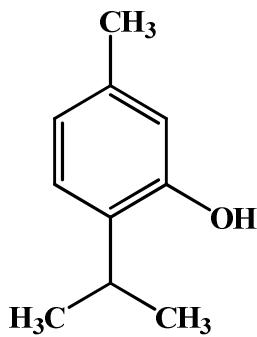
Keywords: Biofield Energy Treatment, The Trivedi Effect®, Thymol, Gas Chromatography - Mass Spectrometry, Isotopic Abundance Ratio, Isotope Effects, Kinetic Isotope Effect

1. Introduction

Thymol or chemically known as 2-isopropyl-5-methylphenol (IPMP) is a natural monoterpenoid phenol and is one of the major components of the essential oils isolated from several plants, such as *Thymus vulgaris*, *Origanum*

vulgare, etc. [1-4]. It is the derivative of cymene (Figure 1), $C_{10}H_{14}O$ and isomeric with carvacrol [5, 6]. Thymol possesses potent antimicrobial, antioxidant, anti-inflammatory, molluscicidal, antifeedant, and insecticidal activity [1-6]. In addition, thymol is also used in various consumer products like pharmaceutical preparations,

cosmetics, food preparations, oral rinses, etc. But it's uses are limited due to its unpleasantly pungent flavor [3, 7]. On the other hand, the antioxidant activity of thymol depends on the phenolic hydroxyl group. The major disadvantages of thymol for therapeutic uses are its poor water solubility and the requirement of high concentrations to achieve a therapeutic effect [8]. Literature reported that chemical modification of thymol was done to modify its biological activities [4, 8].



Chemical Formula: C₁₀H₁₄O
Exact Mass: 150.10
Molecular Weight: 150.22

Figure 1. Structure of thymol.

Analysis of natural abundance variations in the stable isotopes (or also known as stable isotope ratio analysis, SIRA) is a potential tool for the measurement of the flow of materials and energy both within and among the organisms. This method is widely used in agricultural, food authenticity, biochemistry, metabolism, medical research, environmental pollution, archaeology, etc. [9-11]. The variation in isotopic abundance ratio between isotopic forms of the molecule causes isotope effects *i.e.* the differences in physical and chemical properties of the molecule [12, 13]. Among of the other technique like infrared spectroscopy, nuclear magnetic resonance spectroscopy, and neutron activation analysis, mass spectrometry (MS) technique such as GC-MS has the major choice for isotope ratio analysis with sufficient precision [14, 15]. But when the molecules have molar isotope enrichments at below 0.1%, specialized instruments, such as isotope ratio mass spectrometer (IRMS), multiple-collector inductively coupled plasma mass spectrometry are basically used [10, 11].

The human biofield is an energy matrix that surrounds the human body, which emits continuously electromagnetic waves as biophotonic form. Healing practitioner has the capability to harness the energy from the environment, the "universal energy field" and can be transmitted the biofield energy into any living or non-living object (s) around the Globe in the useful manner. This method is called as biofield energy treatment [16, 17]. Mr. Trivedi is one of the eminent healing practitioners and has notable capability to alter the characteristic properties of several organic compounds [18-20], pharmaceuticals [21, 22], nutraceuticals [23], metals and ceramic in materials science

[24, 25], culture medium [26, 27] and improve the overall productivity of crops [28, 29] as well as to modulate the efficacy of the various living cells [30-33]. Literature demonstrated that biofield energy treatment (also called as The Trivedi Effect®) has the remarkable capability for alteration of the isotopic abundance ratio in the organic compounds [34-38]. Spectroscopic and thermal analysis of thymol concluded that the physicochemical, structural and thermal properties of thymol were significantly altered due to the biofield energy treatment. It was proposed that the altered crystallite size and thermal stability might improve the rate of the reaction and product yield during the production of pharmaceuticals [39]. For this reason, gas chromatography-mass spectrometric analysis was conducted in this study to determine the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in both of the control and biofield treated thymol.

2. Materials and Methods

2.1. Chemicals and Reagents

Thymol was purchased from S D Fine Chemicals Ltd., India. All the other chemicals and reagents used in this experiment were analytical grade procured from local vendors.

2.2. Biofield Energy Treatment

The sample thymol was divided into two parts: one was referred as control where no treatment was provided. The other part of the sample which denoted as biofield energy treated sample was handed over to Mr. Trivedi for the biofield energy treatment in a sealed condition. The biofield energy treatment was provided by Mr. Trivedi (also known as The Trivedi Effect®) through his unique energy transmission process to the test product in a sealed pack under laboratory conditions for 5 minutes without touching the sample. After treatment, control and the biofield treated samples were preserved at standard laboratory condition and analyzed by GC-MS. The biofield treated thymol was characterized in different time intervals denoted as T1, T2, T3, and T4 in order to understand the impact of the biofield energy treatment on isotopic abundance ratio with respect to the time.

2.3. Gas Chromatograph - Mass Spectrometry (GC-MS)

Perkin Elmer/Auto system XL with Turbo mass, USA was used here for GC-MS analysis. The GC-MS method was followed by previously published work [34]. It was done in a silica capillary column equipped with a quadrupole detector with pre-filter, one of the fastest, widest mass ranges. The mass spectrometer was operated in an electron ionization (EI) positive/negative, and chemical ionization mode at the electron ionization energy of 70 eV. Mass range: 10-650 Daltons (amu), stability: ± 0.1 m/z mass accuracy over 48 hours. The identification of analytes was done by retention time and by a comparison of the mass spectra of identified substances with references.

2.4. Method for the Calculation of Isotopic Abundance Ratio from the GC-MS Spectra

The isotopic abundances of the elements are principally categorized into three types: *A elements* having only one natural isotope in appreciable abundance; *A + 1 elements* (For e.g. C, N and H) containing two isotopes – one isotope is one nominal mass unit heavier than the most abundant isotope, and *A + 2 elements* (For e.g. O, Cl, S, Si, and Br) having an isotope that has two mass unit heavier than the most abundant isotope. The values of the natural isotopic abundance of some elements are obtained from literature and presented in the Table 1 [10, 40-42]. The peak height (*i.e.* relative intensity) in the mass spectra is directly proportional to the relative isotopic abundance of the sample [43-45]. Hence, the following method was adopted for the determination of the isotopic abundance ratio of the molecule:

P_M stands for the relative peak intensity of the parent molecular ion $[M^+]$ expressed in percentage. In other way, it indicates the probability to have *A elements* (for e.g. ^{12}C , 1H , ^{16}O , ^{14}N , etc.) contributions to the mass of the parent molecular ion $[M^+]$.

P_{M+1} represents the relative peak intensity of the isotopic molecular ion $[(M+1)^+]$ expressed in percentage

$$= (\text{no. of } ^{13}C \times 1.1\%) + (\text{no. of } ^{15}N \times 0.40\%) + (\text{no. of } ^2H \times 0.015\%) + (\text{no. of } ^{17}O \times 0.04\%)$$

i.e. the probability to have *A + 1 elements* (for e.g. ^{13}C , 2H , ^{15}N , etc.) contributions to the mass of the isotopic molecular ion $[(M+1)^+]$

P_{M+2} represents the relative peak intensity of the isotopic molecular ion $[(M+2)^+]$ expressed in the percentage

$$= (\text{no. of } ^{18}O \times 0.20\%) + (\text{no. of } ^{37}Cl \times 32.50\%)$$

i.e. the probability to have *A + 2 elements* (for e.g. ^{18}O , ^{37}Cl , ^{34}S , etc.) contributions to the mass of isotopic molecular ion $[(M+2)^+]$

Isotopic abundance ratio for *A + 1 elements* = P_{M+1}/P_M

Similarly, isotopic abundance ratio for *A + 2 elements* = P_{M+2}/P_M

Percentage (%) change in isotopic abundance ratio = $[(IAR_{Treated} - IAR_{Control})/ IAR_{Control}] \times 100$,

Where, $IAR_{Treated}$ = isotopic abundance ratio in the treated sample and $IAR_{Control}$ = isotopic abundance ratio in the control sample.

Table 1. The isotopic composition (*i.e.* the natural isotopic abundance) of the elements.

Element	Symbol	Mass	% Natural Abundance	<i>A+1</i> Factor	<i>A+2</i> Factor
Hydrogen	1H	1	99.9885		
	2H	2	0.0115	0.015n _H	
Carbon	^{12}C	12	98.892		
	^{13}C	13	1.108	1.1n _C	
Oxygen	^{16}O	16	99.762		
	^{17}O	17	0.038	0.04n _O	
	^{18}O	18	0.200		0.20n _O
Nitrogen	^{14}N	14	99.60		
	^{15}N	15	0.40	0.40n _N	
Chlorine	^{35}Cl	35	75.78		
	^{37}Cl	37	24.22		32.50n _{Cl}

A represents element, n represents the number of the element (*i.e.* C, H, O, N, etc.)

3. Results and Discussion

3.1. GC-MS Analysis

The GC-MS spectra of the control and biofield treated thymol are shown in the Figures 2-4. The GC-MS spectrum of the control thymol (Figure 2) displayed the presence of molecular ion peak $[M^+]$ at m/z 150 (calculated 150.10 for $C_{10}H_{14}O$) along with seven major fragmented peaks in lower m/z region at the retention time (R_t) of 12.43 min. The fragmentation pattern of thymol as shown in the Figure 2 was well accorded with the literature [46]. The fragmented peaks at m/z 135, 115, 107, 91, 77, 65 and 39 might be due to $C_{10}H_{15}^+$, $C_7H_{15}O^+$, $C_7H_7O^+$ or $C_8H_{11}^+$, $C_7H_7^+$, $C_6H_5^+$, $C_5H_5^+$, and $C_3H_3^{..+}$ ions, respectively as shown in Figure 2.

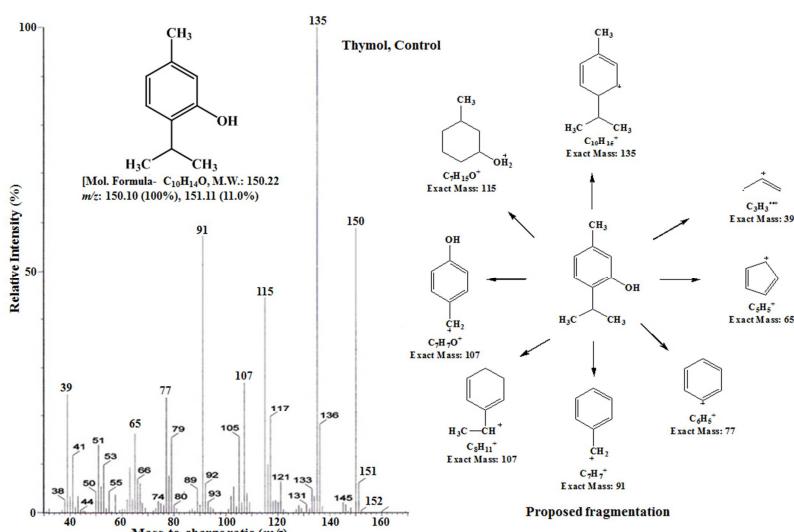
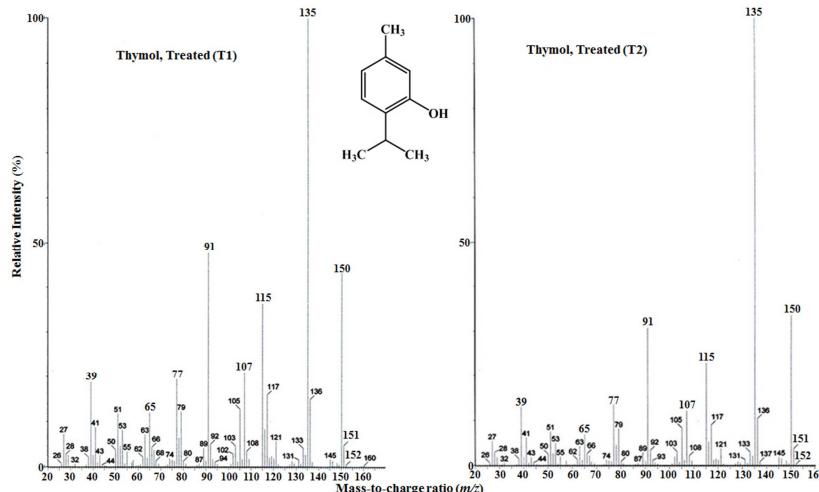


Figure 2. C-MS spectrum and proposed fragmentation of the control thymol.

The GC-MS spectra of the biofield treated thymol at T1, T2, T3, and T4 (Figures 3 and 4) revealed molecular ion peak [M^+] at m/z 150 at R_t of 12.39, 12.41, 12.44, and 12.44 min, respectively. So, the biofield treated thymol disclosed similar R_t and an identical pattern of fragmentation as observed in the control sample. The relative peak intensities of the parent molecule and its major fragmented ions of the control and biofield treated thymol are presented in the Table 2. It clearly

showed that the fragmented ion peak at m/z 135 was due to *p*-cymene ion ($C_{10}H_{15}$)⁺ and it exhibited 100% relative intensity (base peak). Table 2 displayed the relative intensities of the parent molecule at m/z 150 and other fragmented ions at m/z 115, 107, 91, 77, 65 and 39. It indicated that the relative intensities of the biofield treated thymol were significantly altered as compared to the control thymol.



From the above calculation, it has been found that ^{13}C has major contribution to m/z 151.

In the similar approach, P_{M+2} can be calculated as follow:

$$P(^{18}\text{O}) = [(1 \times 0.20\%) \times 58.89\%] / 100\% = 0.12\%$$

So, P_{M+2} i.e. ^{18}O contribution from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 152 = 0.12%.

P_M , P_{M+1} , P_{M+2} for the control and biofield energy treated thymol at m/z 150, 151 and 152, respectively were accomplished from the observed relative peak intensities of $[\text{M}^+]$, $[(\text{M}+1)^+]$, and $[(\text{M}+2)^+]$ peaks in the GC-MS spectra, respectively and are presented in the Table 3.

Table 3. Isotopic abundance analysis result of the control and biofield energy treated thymol.

Parameter	Control thymol	Biofield energy treated thymol			
		T1	T2	T3	T4
P_M at m/z 150 (%)	58.89	43.13	33.53	81.88	81.15
P_{M+1} at m/z 151 (%)	6.16	4.66	3.73	16.85	20.39
P_{M+1}/P_M	0.1046	0.1080	0.1112	0.2058	0.2513
% Change of isotopic abundance ratio (P_{M+1}/P_M)		3.25	6.31	96.75	140.25
P_{M+2} at m/z 152 (%)	0.44	0.34	0.27	1.24	1.46
P_{M+2}/P_M	0.0075	0.0079	0.0081	0.0151	0.0180
% Change of isotopic abundance ratio (P_{M+2}/P_M)		5.33	8.00	101.33	140.00

T1, T2, T3, and T4: Biofield energy treated sample analyzed at different time intervals; P_M = the relative peak intensity of the parent molecular ion $[\text{M}^+]$; P_{M+1} = the relative peak intensity of the isotopic molecular ion $[(\text{M}+1)^+]$; P_{M+2} = the relative peak intensity of the isotopic molecular ion $[(\text{M}+2)^+]$; and M = mass of the parent molecule.

The experimental values (Table 3) for the control sample and the above calculated theoretical values suggest that ^{13}C and ^{18}O might have major contributions from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 151 and 152, respectively. The percentage change of the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) in the biofield treated thymol with respect to the control thymol is presented in Table 3 and Figure 5. The isotopic abundance ratio of P_{M+1}/P_M in the biofield treated thymol at T1, T2, T3 and T4 was increased by 3.25, 6.31, 96.75, and 140.25%, respectively with respect to the control thymol. Consequently, the percentage change of the isotopic abundance ratio of P_{M+2}/P_M was enhanced in the biofield treated thymol at T1, T2, T3, and T4 by 5.33, 8.00, 101.33, and 140.00%, respectively with respect to the control sample. Thus, ^{13}C , ^2H , and ^{17}O contributions from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 151 and ^{18}O contribution from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 152 in the biofield treated thymol were significantly increased gradually with respect to the time (Figure 5). Hence, the biofield energy treatment showed time dependent effect on the isotopic abundance ratio in thymol.

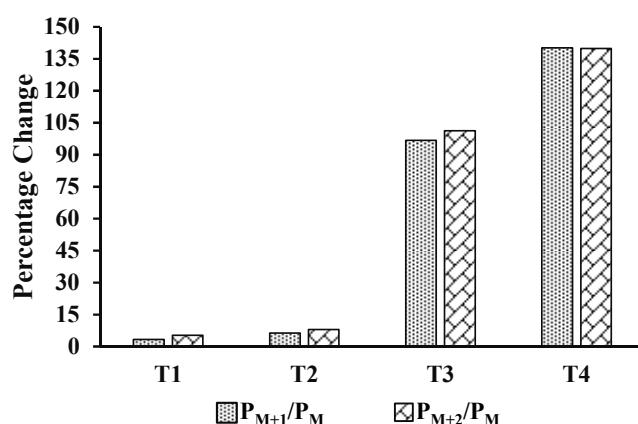


Figure 5. Percent change of the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the biofield energy treated thymol as compared to the control sample.

The neutrinos coming from the Sun have a potential impact on the isotopic composition of the materials. Neutrinos are the most probable transporter of the hidden mass in the Universe. They can pass through large distances in the matter without being affected by the electromagnetic forces and induce the fission reactions within a heavy nuclei. Consequently, neutrinos affect the natural abundance of isotopes of the element [48-49]. The biofield energy can freely move between human and environment that leads to the continuous movement or matter of energy [50]. It can be hypothesized that Mr. Trivedi's unique biofield energy treatment might have the ability for introduction of the neutrino fluence into the both of the living and nonliving substances that might interact with protons and neutrons in the nucleus. This interaction might change the neutron to proton ratio in the nucleus that might be responsible for modifying the behavior at atomic and molecular level. Based on this hypothesis, it is assumed that the possible reason for the alteration of the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) in the biofield treated thymol might be due to the involvement of a neutrino flux through biofield energy treatment.

The alteration of the isotopic abundance ratio of the molecule significantly affects the vibrational energy of the compound whether the electronic, translational, and rotational energies of the molecule remain unaffected. The relation between the vibrational energy and the reduced mass (μ) for a diatomic molecule is expressed as below [14, 51]:

$$E_0 = \frac{h}{4\pi} \sqrt{\frac{f}{\mu}}$$

Where E_0 = the vibrational energy of a harmonic oscillator at absolute zero or zero point energy

f = force constant

μ = reduced mass = $\frac{m_a m_b}{m_a + m_b}$, m_a and m_b are the masses of the constituent atoms.

The possible isotopic bond formation in the thymol molecule and their effect on the vibrational energy are presented in the Table 4. The change of the isotopic abundance ratios of $^{13}\text{C}/^{12}\text{C}$ for C-H bond and $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for O-H bond (not shown in the Table 4) has been found to have very little effect on the reduced mass. But when the alteration in the isotopic abundance ratios of $^{13}\text{C}/^{12}\text{C}$ for C-O, $^2\text{H}/^1\text{H}$ for C-H and O-H bonds and $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for C-O bond has much more effect on the ground state vibrational energy of the molecule due to the higher reduced mass (μ) as shown in the Table 4 that causes the isotope effects of the molecule. The isotopic abundance ratio analysis in thymol exposed that the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M were increased in the biofield treated thymol as compared to the control sample. So,

Table 4. Possible isotopic bond and their effect in the vibrational energy in thymol molecule.

Entry No.	Probable isotopic bond	Isotope type	Reduced mass (μ)	Zero point vibrational energy (E_θ)
1	$^{12}\text{C}-^{12}\text{C}$	Lighter	6.00	Higher
2	$^{13}\text{C}-^{12}\text{C}$	Heavier	6.26	Smaller
3	$^1\text{H}-^{12}\text{C}$	Lighter	0.92	Higher
4	$^2\text{H}-^{12}\text{C}$	Heavier	1.04	Smaller
5	$^{12}\text{C}-^{16}\text{O}$	Lighter	6.86	Higher
6	$^{13}\text{C}-^{16}\text{O}$	Heavier	7.17	Smaller
7	$^{12}\text{C}-^{17}\text{O}$	Heavier	7.03	Smaller
8	$^{12}\text{C}-^{18}\text{O}$	Heavier	7.20	Smaller
9	$^{16}\text{O}-^1\text{H}$	Lighter	0.94	Higher
10	$^{16}\text{O}-^2\text{H}$	Heavier	1.78	Smaller

Various reasons like radiogenic nuclides, interaction between cosmic rays and terrestrial matter, extraterrestrial materials, anthropogenic effects, etc. might show the variations in the natural isotopic composition of the molecules [14]. So, the current results indicate that the biofield treatment is an economic method for alteration of the natural isotopic abundance ratio of the compounds. The altered physicochemical and thermal properties, different rate of the reaction, selectivity and binding energy of the biofield energy treated thymol might be helpful for the studying the reaction mechanism during the synthesis of pharmaceuticals through its kinetic isotope effects. The current results were well correlated with our previous findings [39]. Biofield treated thymol might overcome the problems associated with thymol such as unpleasantly pungent flavor, high requirement for achieving the therapeutic efficacy by understanding its isotope effects as well as through the determination of the pharmacokinetic profile or mode of action, bioavailability of thymol and also its release profile from the drug delivery systems.

4. Conclusions

The current research work inferred that biofield energy treatment might be potential approach for altering the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in thymol. The GC-MS spectra of the both control and biofield treated thymol indicated the presence of molecular ion peak [M^+] at m/z 150 (calculated 150.10 for $\text{C}_{10}\text{H}_{14}\text{O}$) along with the

biofield treated thymol might display different physical and chemical properties like lower diffusion velocity, mobility, evaporation, higher binding energy with respect to the control sample. Isotope effects have a vital role in the thermal decomposition of the molecules [52, 53]. The alteration in the isotopic abundance ratio of one of the atoms in the reactants causes changes in the rate of a chemical reaction that is known as kinetic isotope effect (KIE). KIE is a very powerful method to study the reaction mechanism, to stabilize the transition state of the rate-determining step of the reaction and for understanding the enzymatic transition state and all aspects of enzyme mechanisms that is helpful for designing extremely effective and specific inhibitors [14, 51, 54, 55].

same pattern of fragmentation. In addition, the relative intensities of the parent molecule and other fragmented ions of the biofield treated thymol were changed with respect to the control thymol. The isotopic abundance ratio of P_{M+1}/P_M in the biofield treated thymol at T1, T2, T3 and T4 was increased by 3.25, 6.31, 96.75, and 140.25%, respectively with respect to the control thymol. Consequently, the percentage change of the isotopic abundance ratio of P_{M+2}/P_M was enhanced in the biofield treated thymol at T1, T2, T3, and T4 by 5.33, 8.00, 101.33, and 140.00%, respectively with respect to the control sample. In summary, ^{13}C , ^2H , and ^{17}O contributions from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 151 and ^{18}O contribution from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 152 in the biofield treated thymol were significantly increased gradually with respect to the time and was found that biofield energy treatment has time dependent effect on it. The biofield energy treated thymol might display isotope effects due to the increased isotopic abundance ratio with respect to the control sample. The biofield treated thymol might have the altered physicochemical and thermal properties, binding energy and the reaction kinetics as compared to the control sample. Thus, the biofield energy treated thymol could be valuable for designing the synthetic scheme for the preparation of pharmaceuticals through its kinetic isotope effects. Biofield treated thymol might be suitable to overcome the problems associated with thymol for e.g. pungent flavor, high dose requirement through understanding its isotope effects and the determination of its pharmacokinetic profile, bioavailability.

Abbreviations

A: Element; GC-MS: Gas chromatography-mass spectrometry; KIE: Kinetic isotope effect; M: Mass of the parent molecule; m/z : Mass-to-charge ratio; n: Number of the element; P_M : The relative peak intensity of the parent molecular ion [M^+]; P_{M+1} : The relative peak intensity of isotopic molecular ion [$(M+1)^+$]; P_{M+2} : The relative peak intensity of isotopic molecular ion [$(M+2)^+$]; R_t : Retention time.

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