

Phytochemical and antioxidant analysis of bioactive compound extract from *Nelumbo nucifera* against cancer proteins: in silico spectroscopic approach

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Abstract

Nelumbonucifera nucifera, an aquatic crop cultivated throughout Asian countries, belongs to the Nelumbonaceae family and has been widely used in traditional medicines with key pharmacological activities such as anti-viral, antipyretic, antioxidant, anti-steroid, anti-inflammatory, anti-arrhythmia, anti-obesity, and anti-aging properties. The present study aims to explore and assess the phytochemical composition, GC-MS profiling, antioxidant efficacy, and the major phytoconstituent phytol subjected to theoretical spectroscopic characterization using the DFT method. The phytochemical profiling of *N. nucifera* reveals the presence of alkaloids, carbohydrates, saponin, phenol, and flavonoids. The antioxidant efficacy of *N. nucifera* extract against DPPH and ABTS radicals increased concentration-dependent manner, with an IC₅₀ value of 222.84 µg and 52.67 µg, respectively. The *in silico* HOMO-LUMO band gap, MESP, Mulliken, and NBO analysis highlights phytol chemical reactivity and stability. In addition, the molecular docking was performed against breast cancer SMAD proteins to confirm its antagonist property with a binding energy of -3.64 kcal/mol (60M2), -5.49 kcal/mol (1U7F), -5.05 kcal/mol (1U7V), and -3.73 kcal/mol (6FX4).

1. Introduction

Nelumbo nucifera botanically *Nelumbium Speciosa*, *Nelumbium nelumbo*, *Nymphaea nelumbo*, and *Nelumbium speciosum*, an aquatic crop cultivating throughout the Asian countries belongs to the Nelumbonaceae family [1]. *N. nucifera* and its byproducts are widely used in traditional medicines with key pharmacological activities. *N. nucifera* exhibits anti-viral, antipyretic, antioxidant, anti-steroid, anti-inflammatory, anti-arrhythmia, anti-obesity, and anti-aging properties [2–3]. The seeds of *N. nucifera* are rich in unsaturated fatty acids, vitamins, carbohydrates, phosphorus, amino acids, trace elements, secondary metabolites, and superoxide dismutase, traditionally used as food and medicine worldwide [5–6]. *N. nucifera* exhibits anti-obesity effects *in vivo* and *in vitro* by downregulating lipase and α -amylase followed by activating mRNA expression of uncoupled protein 3 [6]. Furthermore, the anti-wrinkle properties of *N. nucifera* extracts from seeds, leaves, and flowers are attributed to their ability to inactivate elastase, an enzyme responsible for the degradation of collagen and elastin [7]. The ethanolic seed extract of *N. nucifera* exhibits carbon tetrachloride-induced hepatoprotective in rat hepatocytes in a dose-dependent manner [8]. The polar and nonpolar seed extracts of *N. nucifera* exhibit cytoprotective activity against nitric oxide-induced DNA damage in RAW 264.7 macrophage cell lines [9]. In addition, neferine, a bioactive isoquinoline alkaloid isolated from the seed embryo of *N. nucifera*, restrains thrombus formation and platelet aggregation in both *in vivo* and *in vitro* models [10].

Phytol chemically 3,7,11,15-tetramethylhexadec-2-en-1-ol aliphatic diterpene alcohol with a molecular formula and mass of C₂₀H₄₀O and 296.5 g/mol. From the structural standpoint, phytol comprises four methyl groups stemming from carbon atoms, a single, double bond, hydrogen bond donor, acceptor, and thirteen rotatable bonds naturally extracted from photosynthetic organisms encompassing plants, bacteria, and algae [11]. Phytol biosynthesis occurs through two major pathways: the mevalonic acid pathway and the non-mevalonic acid (2-C-methylerythritol-4-phosphate) pathways. Phytol has cytotoxic,

anti-inflammatory, anti-convulasant, anti-diabetic, anti-mutagenic, and anxiolytic properties [12–13]. The *in vitro* cytotoxic property of phytol has been documented against different carcinoma cell lines, including MCF-7 and MDA-MB-231 (breast cancer), HT-29 (colon cancer), A-549 (lung cancer), PC-3 (prostate cancer), Hs294T (melanoma cells), and MRC-5 (lung fibroblasts) with IC₅₀ values of $8.79 \pm 0.41 \mu\text{M}$, $69.67 \pm 2.99 \mu\text{M}$, $34.82 \pm 1.66 \mu\text{M}$, $56.98 \pm 2.68 \mu\text{M}$, $77.85 \pm 1.93 \mu\text{M}$, $65.15 \pm 2.91 \mu\text{M}$, and $124.84 \pm 1.59 \mu\text{M}$, respectively. In addition, the derivatives of phytol, such as phytanyl chloride, phytanyl manoside, and phytanyl amide, have potent immune adjuvants [14].

A comprehensive review of existing literature highlights in-depth theoretical and spectroscopic characterization of phytol has yet to be documented. Supporting this observation, our present investigation presents the ethanolic leaf extract of *N. nucifera* subjected to a GC-MS analysis, antioxidant, and phytochemical profiling. In addition, the peak compound phytol has undergone detailed structural (optimized geometry), biological (molecular docking), and spectroscopic (FT-IR, FT-Raman, UV-Vis) characteristics using the theoretical DFT method.

2. Materials and methods

2.1. Plant collection and processing:

N.nucifera leaves were picked to Padur Lake, Thiruvallur, Poonamallee (13.05562° N latitude and 80.00806° E longitude) Chennai, Tamilnadu, India. After adequately washing the gathered plants with running tap water to remove any stuck dirt and soil particles, they washed again using sterile distilled H₂O. The leaves of *N.nucifera* were separated and shade dry for 15 days at ambient room temperature (37°C). Next, the dried leaves are grind well as a fine powder and the sample underwent successive extraction by soxhlation using ethanol. Whatman filter paper with a pore size of 1 μm was employed to filter the extracts, and only refined ethanolic extracts were utilized in subsequent research.

2.2. Gas Chromatography - Mass Spectrometry Analysis:

The GC-MS profiling of *N.nucifera* ethanolic extract was performed with a Shimadzu (GCMS-QP2020) furnished with a silica capillary fused column (SH-Rxi-5Sil MS) dimension 30 meters, 0.25 millimeters, and 250 micrometers of length, diameter, and film thickness, respectively. Helium was utilized as carrier gas at a constant 1 ml/min flow rate. The temperature in the injector was sustained at 280°C, and a 1 μl sample injection (split ratio 10:5) was applied. The mass spectra fragments were analyzed from 50 to 600 Da. The temperature in oven was initially whispered at 50°C for 3 min, then amplified at a rate of 15°C /min to 180°C, followed by a rise of 10°C /min to 280°C. The identification of phytochemicals in the extract was conducted by comparing the mass spectra, peak area, height, and retention time (RT) with the spectrum databases and phytochemical composition from the NIST collection.

2.3. DPPH assay

The antioxidant efficacy of *N. nucifera* was spotted utilizing the DPPH method proposed by Manzocco *et al.* 1998 [15]. Ethanolic extract of *N. nucifera* leaves were prepared at concentrations ranging from 20–100 µM. The procedure involved mixing 1 ml of 0.1 mM DPPH in a ethanolic solution with 3ml of the *N. nucifera* extract. The combination was incubated in the dark at 37 °C for 30 min. After incubation, the absorbance was measured at 517 nm using a spectrophotometer. The % of inhibition was derived using the equation below.

$$DPPH \text{ scavenging } (\%) = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4. ABTS assay

The antioxidant efficacy of the ethanolic extract of *N. nucifera* leaves was assessed using the ABTS method defined by Seeram *et al.* 2008 [16]. ABTS was dissolved in ethanol at a concentration of 0.007% from the stock solution, and 0.1% of the K₂S₂O₈ solution was prepared. To conduct the assay, 2ml of the ABTS was mixed with 3ml of ethanolic *N. nucifera* extract. The combination was thoroughly mixed and incubated in the dark at 37 °C for 30 min. After incubation, the colored solution was recorded at 734 nm utilizing a spectrophotometer. The percentage inhibition of free radical scavenging efficacy was calculated by the theoretical equation as follows

$$ABTS \text{ scavenging efficacy} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.5. Phytochemical Investigation

The ethanolic extract of *N.nucifera* through soxhlet extraction was subjected to qualitative phytochemical investigation to determine the bioactive compounds or phytochemicals such as Alkaloids, Carbohydrates, Glycoside, Saponin, Protein, Amino acid, Phenol, Flavanoid, Terpenoid, and Steroids using methods described by Arumugam *et al* [17].

2.6. Computational details

The theoretical simulation of phytol was employed with DFT/B3LYP along with the 6-31G(d,p) method to calculate structural parameters, electronic properties, Mulliken, and MEP analysis [18–21]. The TD-DFT approach, with the 6-31G(d,p) basis set, was used to forecast electronic and FMO characteristics of phytol [22–25]. In addition, NBO, MEP, and Mulliken findings of phytol were also done using the same basis set. AutoDock software accomplished the molecular docking of phytol with the target protein [26].

3. Results and discussions

3.1. Phytochemical profiling

The phytochemicals have been a critical focus in modern medicine due to their increased therapeutic potential and biological activities. Phytochemicals are bioactive compounds synthesized in plants that are categorized using chemical structures and functions that have significant roles in plants against environmental challenges and human health benefits. The ethanolic extract of *N.nucifera* leaves was subjected to qualitative screening of phytochemicals. The phytochemicals profile of *N.nucifera* leaf extract was tabulated in Table 1 and the corresponding pictorial representation was illustrated in Fig. 1. In the present findings, the leaf extract of *N.nucifera* highlights the presence of alkaloids, carbohydrates, saponin, phenol, and flavonoids. Phytochemicals in the leaf extract confer biological activity such as antioxidants, antimicrobials, and antiproliferative activities. Krubha et al., [27] have identified the phytochemicals viz., saponins, phlobotannins, flavonoids, tannins, sterols and phenols from *N,nucifera* plant. Shad et al., [28] were investigated plant metabolites viz., tannins, saponins, HCN phenolic acids and phosphorus from *N,nucifera* plant. Yamini et al., [29] investigated the bioactive compounds viz., carbohydrate, protein, fat, phenols, tannins, flavanoids, alkaloids, steroids, terpenoids, cardiac glycosides, coumarin, quinone from *N,nucifera* plant. In addition, the absence of terpenoids, steroids, amino acids, glycosides, and proteins limits its biological activities in hormonal regulation, muscle repair, and enzyme synthesis.

Table 1
Phytochemical characterization of
ethanolic leaf extract of *Nelumbo nucifera*

Test	Presence
Alkaloids	++
Carbohydrate	++
Glycoside	-
Saponin	++
Protein	-
Amino acid	-
Phenol	+
Flavanoid	+
Terpenoid	-
Steroid	-
- Absent, + present, ++ strongly present	

3.2. DPPH and ABTS assay

The DPPH and ABTS assays confirmed the free radical scavenging efficacy of *N.nucifera* leaf extract. The phenols and flavonoids in the extract donate electrons to neutralize the unpaired electrons in DPPH, leading to the decoloration of stable radical from deep violet color to yellow, followed by increased absorbance at 517 nm. The amount of DPPH has been reduced with increasing antioxidant activity. In ABTS, the blue-green chromophore was reduced with antioxidants in the sample to a colorless neutral form. The samples with different concentrations within 20–100 µg have been subjected to antioxidant assay (DPPH and ABTS). The percentage of inhibition has been increased with increased concentration due to phytoconstituents in the extract responsible for antioxidant activity. The DPPH and ABTS radical scavenging efficacy of *N.nucifera* leaf extract was presented in Table 2 and illustrated in Fig. 2a, 2b. In addition, the IC₅₀ value of ethanolic extract was felled at 222.84 µg for DPPH and 52.67 µg for ABTS assay. Krubha et al.,[27] have reported IC50 value 590 µg/mL for DPPH radical scavenging activity observed from *N,nucifera* plant extract. Trang et al.,[30] identified the IC50 value 1.036 µg/mL for the DPPH radical radical scavenging activity isolated to *N.nucifera* plant extract. Laoung-On et al., [31] investigated the IC50 value 173.89 µg/mL from the ABTS radical scavenging activity identify from *N.nucifera* plant extract. Chen et al., [32] optimized the IC50 value 495 µg/mL from ABTS radical scavenging activity observed from *N,nucifera* plant.

Table 2
Radical scavenging activity of ethanolic leaf extract of *Nelumbo nucifera*

DPPH		ABTS	
Concentration	Inhibition (%)	Concentration	Inhibition (%)
control	0	control	0
20	50.69447338	20	52.81104
40	51.81623932	40	56.87803
60	56.03635724	60	63.93546
80	62.07280005	80	67.04551
100	73.55817794	100	69.31824

3.3. GC-MS analysis

The volatile and semi-volatile phytochemicals comprised in the ethanolic extract of *N.nucifera* leaves were profiled using the GC-MS analytical technique. The spectrum of unknown phytochemicals has been compared with the spectrum of existing compounds in the NIST library. The GC-MS profile of the leaf extract of *N.nucifera* revealed significant phytochemicals characterized by molecular formula, retention time, composition, and molecular weights, which have a crucial role in biological and pharmaceutical activities. Table 3 elaborates on the list of phytochemicals and retention time of *N.nucifera* leaf extract. The major phytochemicals were identified viz., 3,7,11,15-Tetramethyl-2-

hexadecen-1-ol, 2(1H)-naphthalenone, 4a,5,8,8a-tetrahydro-4a-methyl, silver trifluoromethanesulfonate, and silane, [2-(ethenylsulfonyl) ethoxy]trimethyl with area percentage of 13.37, 9.60, 7.07, and 6.85 respectively (Fig. 3). Krubha et al.,[27] have reported the *N.nucifera* identified natural compounds dodecanoic acid methyl ester (RT-6.21min), tridecanoic acid 12-methyl methyl ester (RT- 7.2min), hexadecanoic acid methyl ester (RT- 8.1min), 9-octadecanoic acid [Z] methyl ester (8.89min), octadecanoic acid methyl ester (8.97min). Trang et al., [30] has investigated the *N.nucifera* identified secondary metabolites methyl-alpha-D-galactopyranoside (RT- 15.030min), furaneol (RT- 6.287min), thymine (RT- 6.623min), diethylnitroamine (RT- 7.357min), 3,5dihydroxyl-2-methyl-5,6-dihdropyran-4-one (RT- 7.539min), 5-oxymethyl furfurole (RT- 8.771min), methyl palmitate (RT- 17.082min), palmitic acid (RT- 17.483min), methyl linoleate (RT- 18.702min), methyl oleate (RT- 18.764min), methyl linolelaidate (RT- 19.129min), alpha-monopalmitin (RT- 22.478min), plastochromanol (RT- 38.336min) and delta-5-ergosterol (RT- 40.150min). Abdelhamid et al., [33] has reported *N.nucifera* lupeol (RT- 40.43min), octadecatrienoic acid-2- [(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy] methyl]ethyl ester (Z,Z,Z) (RT- 33.04min), 2,3-dihydroxypropyl-cis-13-docosenoate (RT- 29.91min), oleanolic acid(RT- 39.17min), tris(trimethylsilyl) ether derivative of 1,25-dihydroxyvitamin D2 (RT- 15.28min), trimethylsilyl derivative of 2-monoolein (RT- 27.21min), lucenin 2 (RT- 35.04min) and lupanol (RT- 29.16min), tetraeurin-A-diol (RT- 24.27min), carotene-1,1',2,2'-tetrahydro-1,1'- dimethoxy (RT- 33.44min),1-oxo-forskolin (RT- 30.79min), oleic acid-3- hydroxypropyl ester (RT- 23.41min), oleic acid trimethylsilyl ester (RT- 19.24min), betulin (RT- 32.78min), à-amyrin-trimethylsilyl ether (RT- 40.05min), stigmast-5-en-3-ol-(3á,24S)(RT- 33.78min), flavone-4'-OH,5-OH,7-di-O-glucoside (RT- 31.27min). On compared with other phytocompounds, phytol highlights a significant contribution in the extract of *N.nucifera* leaves, has potent anti-inflammatory, anti-convulasant, anti-diabetic, anti-mutagenic, and *in vitro*, cytotoxic properties against various cancer cells has been subjected to in silico spectroscopic characterization using DFT method.

Table 3
GC-MS analysis of ethanolic leaf extract *Nelumbo nucifera*

Peak	Retention Time	Area %	Name
1	4.389	3.46	Silane, (2-methoxycyclohexyl)trimethyl-, cis
2	8.191	2.60	Decanal
3	12.019	5.98	Methylmalonic acid, 2TBDMS derivative
4	14.474	7.07	Silver trifluoromethanesulfonate
5	15.302	4.75	Acetaldehyde, (2,4-dinitrophenyl)hydrazone
6	15.353	9.60	2(1H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4a-methyl-,trans-
7	18.154	13.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
8	18.225	3.29	25,26,27,28-Tetrahydroxycalix[4]aren
9	18.274	5.75	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidiny)-2-butynyl]-N',N'-bis(trifluoroacetyl)
10	18.626	3.40	2-Azatricyclo[4.3.1.1(4,8)]undecane
11	22.424	4.82	cis-1-Chloro-9-octadecene
12	23.195	4.79	(t-Butyl-dimethylsilyl)[2-methyl-2-(4-methyl-pent-3-enyl)-cyclopropyl]
13	23.320	1.57	Undec-10-ynoic acid, isobutyl ester
14	23.485	5.65	2-Octenoic acid
15	24.389	4.30	o-Chlorophenylsuccinic acid
16	24.460	3.41	1,2-Bis(trimethylsilyl)benzene
17	24.535	2.91	1,2-Bis(trimethylsilyl)benzene
18	24.660	6.85	silane, [2-(ethenylsulfonyl)ethoxy]trimethyl-
19	24.881	5.41	Silane, diethoxymethyl-
20	24.904	1.02	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, tris-(O-trimethylsilyl)-

3.4. Theoretical simulations

3.4.1. Optimized geometry

The crystal structure of phytol comprises sixty-one atoms that give rise to sixty and one hundred fifteen bond distances and angles, respectively. The titled compound phytol was optimized utilizing the DFT/B3LYP method with the 6-31G (d,p) basis set has been illustrated in Fig. 4. The bond distances

encompassing one (O_1-C_{21} and O_1-H_{61}), nineteen (C-C), and thirty-nine (C-H). Similarly, bond angles such as one ($C_{21}-O_1-H_{61}$ and $O_1-C_{21}-C_{20}$), two (O-C-H), twenty-two (C-C-C), sixty-six (C-C-H), and twenty-three (H-C-H). The structural (bond distances and angles) parameters of phytol were tabulated in Table 4.

Table 4
Theoretical and experimental geometrical parameters of phytol

B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)
O ₁ -C ₂₁	1.466	C ₇ -H ₃₁	1.100	C ₁₄ -C ₁₇	1.542
O ₁ -H ₆₁	0.979	C ₈ -C ₁₂	1.540	C ₁₄ -H ₄₆	1.102
C ₂ -C ₃	1.552	C ₈ -H ₃₂	1.101	C ₁₅ -C ₁₈	1.521
C ₂ -C ₇	1.549	C ₈ -H ₃₃	1.098	C ₁₅ -H ₄₇	1.098
C ₂ -C ₁₁	1.543	C ₉ -C ₁₀	1.541	C ₁₅ -H ₄₈	1.101
C ₂ -H ₂₂	1.103	C ₉ -H ₃₄	1.101	C ₁₆ -H ₄₉	1.099
C ₃ -C ₅	1.542	C ₉ -H ₃₅	1.097	C ₁₆ -H ₅₀	1.097
C ₃ -H ₂₃	1.101	C ₁₀ -C ₁₄	1.548	C ₁₆ -H ₅₁	1.095
C ₃ -H ₂₄	1.101	C ₁₀ -H ₃₆	1.101	C ₁₇ -H ₅₂	1.098
C ₄ -C ₆	1.549	C ₁₀ -H ₃₇	1.102	C ₁₇ -H ₅₃	1.097
C ₄ -C ₈	1.553	C ₁₁ -H ₃₈	1.097	C ₁₇ -H ₅₄	1.097
C ₄ -C ₁₃	1.543	C ₁₁ -H ₃₉	1.097	C ₁₈ -C ₁₉	1.514
C ₄ -H ₂₅	1.103	C ₁₁ -H ₄₀	1.095	C ₁₈ -C ₂₀	1.347
C ₅ -C ₆	1.541	C ₁₂ -C ₁₅	1.552	C ₁₉ -H ₅₅	1.099
C ₅ -H ₂₆	1.098	C ₁₂ -H ₄₁	1.098	C ₁₉ -H ₅₆	1.098
C ₅ -H ₂₇	1.100	C ₁₂ -H ₄₂	1.098	C ₁₉ -H ₅₇	1.093
C ₆ -H ₂₈	1.100	C ₁₃ -H ₄₃	1.097	C ₂₀ -C ₂₁	1.497
C ₆ -H ₂₉	1.101	C ₁₃ -H ₄₄	1.097	C ₂₀ -H ₅₈	1.089
C ₇ -C ₉	1.541	C ₁₃ -H ₄₅	1.097	C ₂₁ -H ₅₉	1.103
C ₇ -H ₃₀	1.101	C ₁₄ -C ₁₆	1.542	C ₂₁ -H ₆₀	1.098
B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)
C ₂₁ -O ₁ -H ₆₁	109.7	C ₆ -C ₅ -H ₂₆	109.6	H ₄₃ -C ₁₃ - H ₄₅	107.4

B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)
O ₁ -C ₂₁ -C ₂₀	107.1	C ₆ -C ₅ -H ₂₇	108.9	H ₄₄ -C ₁₃ - H ₄₅	107.8
O ₁ -C ₂₁ -H ₅₉	109.6	C ₅ -C ₆ -H ₂₈	109.4	C ₁₆ -C ₁₄ -C ₁₇	110.4
O ₁ -C ₂₁ -H ₆₀	110.6	C ₅ -C ₆ -H ₂₉	108.6	C ₁₆ -C ₁₄ -H ₄₆	108.0
C ₃ -C ₂ -C ₇	111.9	H ₂₆ -C ₅ -H ₂₇	106.4	C ₁₄ -C ₁₆ -H ₄₉	110.6
C ₃ -C ₂ -C ₁₁	111.8	H ₂₈ -C ₆ -H ₂₉	105.8	C ₁₄ -C ₁₆ -H ₅₀	110.8
C ₃ -C ₂ -H ₂₂	106.1	C ₉ -C ₇ -H ₃₀	108.5	C ₁₄ -C ₁₆ -H ₅₁	112.1
C ₂ -C ₃ -C ₅	116.2	C ₉ -C ₇ -H ₃₁	109.0	C ₁₇ -C ₁₄ -H ₄₆	107.9
C ₂ -C ₃ -H ₂₃	108.4	C ₇ -C ₉ -C ₁₀	112.7	C ₁₄ -C ₁₇ -H ₅₂	110.7
C ₂ -C ₃ -H ₂₄	108.4	C ₇ -C ₉ -H ₃₄	109.1	C ₁₄ -C ₁₇ -H ₅₃	111.3
C ₇ -C ₂ -C ₁₁	112.3	C ₇ -C ₉ -H ₃₅	109.6	C ₁₄ -C ₁₇ -H ₅₄	111.2
C ₇ -C ₂ -H ₂₂	106.9	H ₃₀ -C ₇ -H ₃₁	106.3	C ₁₈ -C ₁₅ -H ₄₇	109.4
C ₂ -C ₇ -C ₉	114.6	C ₁₂ -C ₈ -H ₃₂	108.4	C ₁₈ -C ₁₅ -H ₄₈	108.6
C ₂ -C ₇ -H ₃₀	108.5	C ₁₂ -C ₈ -H ₃₃	108.9	C ₁₅ -C ₁₈ -C ₁₉	115.3
C ₂ -C ₇ -H ₃₁	109.5	C ₈ -C ₁₂ -C ₁₅	113.0	C ₁₅ -C ₁₈ -C ₂₀	120.3
C ₁₁ -C ₂ -H ₂₂	107.3	C ₈ -C ₁₂ -H ₄₁	110.2	H ₄₇ -C ₁₅ - H ₄₈	106.7
C ₂ -C ₁₁ -H ₃₈	111.4	C ₈ -C ₁₂ -H ₄₂	109.9	H ₄₉ -C ₁₆ - H ₅₀	107.7
C ₂ -C ₁₁ -H ₃₉	110.8	H ₃₂ -C ₈ -H ₃₃	105.7	H ₄₉ -C ₁₆ - H ₅₁	107.9
C ₂ -C ₁₁ -H ₄₀	111.5	C ₁₀ -C ₉ -H ₃₄	109.1	H ₅₀ -C ₁₆ - H ₅₁	107.6
C ₅ -C ₃ -H ₂₃	108.6	C ₁₀ -C ₉ -H ₃₅	109.6	H ₅₂ -C ₁₇ - H ₅₃	107.8
C ₅ -C ₃ -H ₂₄	108.6	C ₉ -C ₁₀ -C ₁₄	115.0	H ₅₂ -C ₁₇ - H ₅₄	107.7

B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)	B3LYP	Bond lengths (Å)
C ₃ -C ₅ -C ₆	112.3	C ₉ -C ₁₀ -H ₃₆	108.6	H ₅₃ -C ₁₇ - H ₅₄	108.0
C ₃ -C ₅ -H ₂₆	109.6	C ₉ -C ₁₀ -H ₃₇	109.4	C ₁₉ -C ₁₈ -C ₂₀	124.4
C ₃ -C ₅ -H ₂₇	109.9	H ₃₄ -C ₉ -H ₃₅	106.4	C ₁₈ -C ₁₉ -H ₅₅	111.5
H ₂₃ -C ₃ - H ₂₄	106.2	C ₁₄ -C ₁₀ -H ₃₆	108.4	C ₁₈ -C ₁₉ -H ₅₆	110.4
C ₆ -C ₄ -C ₈	113.9	C ₁₄ -C ₁₀ -H ₃₇	108.8	C ₁₈ -C ₁₉ -H ₅₇	112.9
C ₆ -C ₄ -C ₁₃	110.4	C ₁₀ -C ₁₄ -C ₁₆	112.4	C ₁₈ -C ₂₀ -C ₂₁	128.1
C ₆ -C ₄ -H ₂₅	106.8	C ₁₀ -C ₁₄ -C ₁₇	110.5	C ₁₈ -C ₂₀ -H ₅₈	118.8
C ₄ -C ₆ -C ₅	115.0	C ₁₀ -C ₁₄ -H ₄₆	107.5	H ₅₅ -C ₁₉ - H ₅₆	106.6
C ₄ -C ₆ -H ₂₈	109.6	H ₃₆ -C ₁₀ - H ₃₇	106.2	H ₅₅ -C ₁₉ - H ₅₇	107.2
C ₄ -C ₆ -H ₂₉	108.0	H ₃₈ -C ₁₁ - H ₃₉	107.8	H ₅₆ -C ₁₉ - H ₅₇	107.9
C ₈ -C ₄ -C ₁₃	112.0	H ₃₈ -C ₁₁ - H ₄₀	107.6	C ₂₁ -C ₂₀ -H ₅₈	113.1
C ₈ -C ₄ -H ₂₅	106.1	H ₃₉ -C ₁₁ - H ₄₀	107.5	C ₂₀ -C ₂₁ -H ₅₉	110.1
C ₄ -C ₈ -C ₁₂	116.1	C ₁₅ -C ₁₂ -H ₄₁	109.4	C ₂₀ -C ₂₁ -H ₆₀	112.1
C ₄ -C ₈ -H ₃₂	107.9	C ₁₅ -C ₁₂ -H ₄₂	107.9	H ₅₉ -C ₂₁ - H ₆₀	107.4
C ₄ -C ₈ -H ₃₃	109.3	C ₁₂ -C ₁₅ -C ₁₈	114.9	C ₄ -C ₁₃ -H ₄₃	110.8
C ₁₃ -C ₄ -H ₂₅	107.1	C ₁₂ -C ₁₅ -H ₄₇	108.8	C ₁₂ -C ₁₅ -H ₄₈	108.1
C ₄ -C ₁₃ -H ₄₄	111.2	H ₄₁ -C ₁₂ - H ₄₂	106.2	H ₄₃ -C ₁₃ - H ₄₄	107.9
C ₄ -C ₁₃ -H ₄₅	111.6	-	-	-	-

In phytol, the bond distances of O_1-C_{21} and O_1-H_{61} were simulated at 1.466 and 0.979 Å, respectively. Similarly, the bond angles of $C_{21}-O_1-H_{61}$ and $O_1-C_{21}-C_{20}$ were calculated at 109.7 and 107.1°, respectively. The simulated bond angles of $O_1-C_{21}-H_{59}$ and $O_1-C_{21}-H_{60}$ were simulated at 109.6 and 110.6°, respectively. The C-H bond distances in CH_3 (methyl) groups were calculated between 1.093–1.099 Å. In the same way, the C-H bond distances in CH_2 (methylene) groups were simulated within 1.097–1.103 Å. Moreover, the H-C-H bond angles in CH_3 (methyl) and CH_2 (methylene) span within 106.6–108.0° and 105.8–107.4°, respectively. Furthermore, the C-C-C and C-C-H bond angles were spanned between 110.4–128.1° and 106.1–114.9°, respectively. The bond distances of C_4-C_8 (1.553 Å) and O_1-H_{61} (0.979 Å) have increased and decreased bond distances as compared to the remaining distances, confirming the weakest and strongest bond distances in the crystal structure of phytol. The bond angle of $C_{18}-C_{20}-C_{21}$ has an increased value of 128.1° compared to other bond angles due to the impact of the oxygen (O_1) atom in the hydroxyl group, confirming its electronegativity.

3.4.2. Vibrational analysis

The crystal structure of phytol comprises sixty-one atoms, giving rise to 177 vibrational modes, interpreted as $3N-6$, along with C_1 symmetry. The simulated wave numbers of phytol are illustrated in Table 5, and the agreeing spectrum (FT-IR and FT-Raman) is presented in Fig. 5. The wavenumbers are scaled with a scaling factor of 0.96.

Table 5
Simulated (FT-IR and FT-Raman) wave numbers (cm^{-1}) and assignments of phytol

Modes	Theoretical wave numbers (cm^{-1})				Vibrational assignments
	Unscaled	Scaled	I_{IR}	I_{Raman}	
1.	3630	3485	1.58	755.50	ν OH
2.	3173	3046	16.38	246.32	ν CH
3.	3137	3012	28.15	212.65	ν_{as} CH ₃
4.	3122	2997	39.40	210.54	ν_{as} CH ₃
5.	3119	2994	58.62	312.12	ν_{as} CH ₃
6.	3112	2988	51.89	405.07	ν_{as} CH ₃
7.	3111	2987	45.77	194.12	ν_{as} CH ₃
8.	3109	2985	66.05	304.65	ν_{as} CH ₃
9.	3108	2984	44.65	299.41	ν_{as} CH ₃
10.	3106	2982	77.62	389.76	ν_{as} CH ₃
11.	3099	2975	8.63	50.86	ν_{as} CH ₃
12.	3092	2968	100.09	26.99	ν_{as} CH ₂
13.	3088	2964	64.70	52.33	ν_{as} CH ₂
14.	3084	2961	103.58	21.81	ν_{as} CH ₂
15.	3082	2959	22.45	457.70	ν_{as} CH ₃
16.	3071	2948	36.83	75.01	ν_{as} CH ₂
17.	3062	2940	2.44	453.72	ν_{as} CH ₂
18.	3050	2928	51.16	271.82	ν_{as} CH ₂
19.	3047	2925	19.32	281.18	ν_{as} CH ₂
20.	3046	2924	50.24	337.67	ν_{as} CH ₂
ν_{s} - symmetric stretching; ν_{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
21.	3042	2920	20.76	143.61	ν _s CH ₂ , ν _s CH ₃
22.	3042	2920	18.54	1092.86	ν _s CH ₂ , ν _s CH ₃
23.	3041	2919	29.51	262.86	ν _s CH ₂ , ν _s CH ₃
24.	3039	2917	16.59	678.11	ν _{as} CH ₂ , ν _s CH ₃
25.	3036	2915	25.92	236.41	ν _s CH ₂ , ν _s CH ₃
26.	3035	2914	30.47	906.45	ν _s CH ₃
27.	3031	2910	6.04	580.03	ν _s CH ₂ , ν _s CH ₃
28.	3030	2909	32.27	98.56	ν _s CH ₂ , ν _s CH ₃
29.	3029	2908	35.58	49.06	ν _s CH ₃
30.	3022	2901	14.25	429.46	ν _s CH ₂ , ν CH
31.	3018	2897	69.08	230.56	ν _{as} CH ₂
32.	3014	2893	58.36	700.62	ν _s CH ₂
33.	3011	2891	39.43	403.16	ν _s CH ₂
34.	3010	2890	3.95	98.12	ν _s CH ₂ , ν CH
35.	3007	2887	31.58	180.96	ν _s CH ₂ , ν CH
36.	3003	2883	7.29	22.25	ν _s CH ₂ , ν CH
37.	2997	2877	4.85	513.34	ν _s CH ₂ , ν CH
38.	2990	2870	17.91	307.33	ν _s CH ₂ , ν CH,
39.	2989	2869	13.76	254.59	ν _{as} CH ₂ , ν CH
40.	2975	2856	55.95	511.25	ν _s CH ₂
41.	1750	1680	9.10	849.59	ρ CH ₂ , β CH, β _{as} CH ₃ , ν CC
ν _s - symmetric stretching; ν _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
42.	1557	1495	16.67	38.60	⊗ CH ₂ , β _s CH ₃
43.	1556	1494	3.16	53.22	⊗ CH ₂ , β _s CH ₃
44.	1555	1493	5.99	115.55	⊗ CH ₂ , β _s CH ₃
45.	1553	1491	10.24	333.99	⊗ CH ₂ , β _s CH ₃
46.	1552	1490	6.89	114.72	⊗ CH ₂
47.	1550	1488	4.53	453.99	β _s CH ₃
48.	1549	1487	3.04	320.31	β _s CH ₃
49.	1547	1485	1.80	186.29	⊗ CH ₂ , β _s CH ₃
50.	1545	1483	4.95	164.50	β _{as} CH ₃
51.	1545	1483	4.57	289.74	β _{as} CH ₃
52.	1541	1479	2.42	18.60	⊗ CH ₂ , β _s CH ₃
53.	1537	1476	8.98	370.72	⊗ CH ₂ , β _s CH ₃
54.	1536	1476	1.07	913.25	⊗ CH ₂ , β _s CH ₃
55.	1535	1474	4.14	151.03	⊗ CH ₂
56.	1535	1474	0.78	50.79	⊗ CH ₂ , β _s CH ₃
57.	1532	1471	4.67	284.88	⊗ CH ₂ , β _s CH ₃
58.	1530	1469	2.71	481.29	⊗ CH ₂ , β _s CH ₃
59.	1528	1467	0.78	504.23	⊗ CH ₂ , β _s CH ₃
60.	1526	1465	1.08	435.54	⊗ CH ₂ , β _s CH ₃
61.	1523	1462	2.57	432.59	⊗ CH ₂ , β _s CH ₃
62.	1466	1407	7.23	145.72	⊗ CH ₂ , β _s CH ₃
υ _s - symmetric stretching; υ _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
63.	1464	1405	7.11	59.22	ω CH ₂ , β _s CH ₃
64.	1458	1400	7.65	40.96	β _s CH ₃
65.	1456	1398	7.26	74.97	β _s CH ₃
66.	1454	1396	14.50	202.64	ω CH ₂ , β _s CH ₃ , β CH
67.	1447	1389	7.33	86.41	β _s CH ₃
68.	1438	1380	1.41	140.91	ω CH ₂ , β CH
69.	1432	1375	6.22	36.94	ω CH ₂ , β CH
70.	1418	1361	0.94	82.57	ω CH ₂ , β CH
71.	1414	1357	1.57	23.06	ω CH ₂ , β CH
72.	1409	1353	6.12	103.36	τ CH ₂ , β CH
73.	1408	1352	2.17	230.23	ω CH ₂ , β _{as} CH ₃
74.	1407	1351	2.30	67.03	ω CH ₂ , β CH
75.	1405	1349	5.43	76.16	ω CH ₂ , β CH
76.	1397	1341	4.67	186.94	τ CH ₂ , β CH
77.	1391	1335	1.39	107.28	τ CH ₂ , β CH
78.	1382	1327	0.46	317.53	ω CH ₂ , β CH
79.	1377	1322	2.20	162.91	ω CH ₂ , β CH
80.	1364	1309	2.94	175.64	τ CH ₂ , β CH
81.	1361	1307	1.39	571.35	τ CH ₂
82.	1353	1299	0.40	234.41	τ CH ₂ , β CH
83.	1351	1297	1.98	536.46	τ CH ₂
υ _s - symmetric stretching; υ _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
84.	1348	1294	0.53	491.73	ρ CH ₂ , β CH
85.	1333	1280	0.92	52.29	τ CH ₂ , β CH
86.	1327	1274	0.04	127.84	τ CH ₂ , β CH
87.	1317	1264	0.89	61.84	ω CH ₂
88.	1294	1242	0.68	113.79	ω CH ₂ , β CH
89.	1282	1231	9.65	371.72	τ CH ₂ , β _{as} CH ₃ , β OH
90.	1273	1222	12.43	382.48	τ CH ₂ , β OH
91.	1260	1210	4.59	53.76	τ CH ₂ , β _{as} CH ₃ , β CH
92.	1251	1201	5.55	128.47	τ CH ₂ , β _{as} CH ₃ , β CH, β OH
93.	1239	1189	59.45	392.55	τ CH ₂
94.	1230	1181	2.59	48.68	τ CH ₂ , β _s CH ₃
95.	1223	1174	4.95	115.07	τ CH ₂ , β _{as} CH ₃ , β CH
96.	1220	1171	1.27	211.21	τ CH ₂ , β _{as} CH ₃ , β CH
97.	1215	1166	6.92	121.28	τ CH ₂ , β _{as} CH ₃ , β CH
98.	1189	1141	0.76	308.61	τ CH ₂ , β _{as} CH ₃ , β CH
99.	1177	1130	4.50	117.98	τ CH ₂ , β _{as} CH ₃ , β CH
100.	1168	1121	2.71	36.64	τ CH ₂ , β _{as} CH ₃ , β CH
101.	1151	1105	1.37	127.10	ρ CH ₂ , β _{as} CH ₃ , β CH
102.	1131	1086	0.02	92.82	ρ CH ₂ , β _{as} CH ₃
103.	1114	1069	19.91	18.96	τ CH ₂ , β _{as} CH ₃ , β CH
104.	1091	1047	1.61	185.53	τ CH ₂ , β _{as} CH ₃ , β CH
ν _s - symmetric stretching; ν _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
105.	1088	1044	1.7	149.59	τ CH ₂ , β _{as} CH ₃ , β CH
106.	1078	1035	1.51	41.26	τ CH ₂ , β CH
107.	1073	1030	3.35	318.55	ω CH ₂ , β CH, β _{as} CH ₃
108.	1071	1028	0.64	308.59	τ CH ₂ , β _{as} CH ₃ , β CH
109.	1052	1010	0.69	377.08	τ CH ₂ , β _{as} CH ₃ , β CH
110.	1046	1004	6.30	231.74	ρ CH ₂ , β _{as} CH ₃ , β CH
111.	1042	1000	0.92	146.60	ρ CH ₂ , β _{as} CH ₃ , β CH
112.	1019	978	0.46	70.27	ρ CH ₂ , β _{as} CH ₃ , γ CH
113.	1012	972	0.91	161.91	ρ CH ₂ , β _{as} CH ₃ , γ CH
114.	1010	970	0.16	102.24	ρ CH ₂ , β _{as} CH ₃
115.	1006	966	3.48	165.72	ρ CH ₂ , β _{as} CH ₃ , γ CH
116.	998	959	0.03	174.17	τ CH ₂ , β _{as} CH ₃ , γ CH
117.	991	951	0.04	237.97	β _{as} CH ₃ , γ CH
118.	982	943	100.83	627.64	ρ CH ₂ , β _{as} CH ₃ , γ CH, β OH, υ CO
119.	972	933	3.10	255.22	ρ CH ₂ , β _{as} CH ₃ , γ CH
120.	965	926	0.25	146.52	τ CH ₂ , β _{as} CH ₃ , γ CH
121.	962	924	3.70	229.74	τ CH ₂ , β _{as} CH ₃ , γ CH, β OH
122.	959	921	1.43	44.31	β _{as} CH ₃
123.	953	915	2.60	343.44	β _{as} CH ₃
124.	907	871	3.99	157.68	ρ CH ₂ , β _{as} CH ₃ , γ CH
υ _s - symmetric stretching; υ _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
125.	883	848	0.32	392.49	ρ CH ₂ , β _{as} CH ₃ , γ CH
126.	871	836	0.50	33.32	ρ CH ₂ , β _{as} CH ₃ , γ CH
127.	858	824	1.30	62.61	ρ CH ₂ , β _{as} CH ₃ , γ CH
128.	851	817	0.21	697.96	ρ CH ₂ , β _{as} CH ₃ , γ CH
129.	820	787	1.92	173.70	ρ CH ₂ , β _{as} CH ₃ , γ CH
130.	800	768	2.08	38.42	ρ CH ₂ , β _{as} CH ₃ , γ CH
131.	786	755	7.34	355.72	ρ CH ₂ , γ CH
132.	771	740	5.49	22.15	ρ CH ₂
133.	767	736	1.77	47.74	ρ CH ₂
134.	758	728	2.74	60.34	ρ CH ₂
135.	613	588	3.48	129.89	ρ CH ₂ , β _{as} CH ₃
136.	598	574	6.10	238.37	ρ CH ₂ , β _{as} CH ₃
137.	554	532	0.24	90.77	ρ CH ₂ , β _{as} CH ₃
138.	496	476	9.67	436.27	ρ CH ₂ , β _{as} CH ₃ , γ OH
139.	494	474	2.32	82.29	ρ CH ₂ , β _{as} CH ₃
140.	485	466	10.93	169.21	ω CH ₂ , β OH
141.	465	446	0.39	500.10	ω CH ₂ , β _{as} CH ₃
142.	450	432	0.20	220.33	ω CH ₂ , β _{as} CH ₃
143.	426	409	0.06	97.28	ρ CH ₂ , β _{as} CH ₃
144.	415	398	0.33	86.90	ρ CH ₂ , β _{as} CH ₃
145.	386	371	1.56	257.95	ρ CH ₂ , β _{as} CH ₃ , γ CH
ν _s - symmetric stretching; ν _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
146.	383	368	0.16	47.21	ρ CH ₂ , β _{as} CH ₃
147.	377	362	0.44	195.17	ρ CH ₂ , β _{as} CH ₃ , γ CH
148.	351	337	3.07	177.14	ρ CH ₂ , β _{as} CH ₃ , γ OH
149.	299	288	28.08	568.20	ρ CH ₂ , γ OH
150.	292	280	8.21	133.54	ρ CH ₂ , β _{as} CH ₃ , γ OH
151.	282	271	0.68	59.75	ρ CH ₂ , β _{as} CH ₃
152.	265	254	73.90	1670.56	ρ CH ₂ , β _{as} CH ₃ , γ OH
153.	262	252	16.73	523.11	β _{as} CH ₃ , γ OH
154.	246	236	0.20	11.15	β _{as} CH ₃
155.	241	231	0.23	228.62	β _{as} CH ₃
156.	230	221	12.85	456.77	ρ CH ₂ , β _{as} CH ₃
157.	229	220	2.38	127.12	β _{as} CH ₃
158.	222	213	3.73	440.61	β _{as} CH ₃
159.	203	195	2.06	180.25	ρ CH ₂ , β _{as} CH ₃
160.	187	180	1.32	1022.00	ρ CH ₂ , β _{as} CH ₃ , γ OH
161.	166	159	3.32	164.36	β _{as} CH ₃
162.	159	153	0.14	28.77	β _{as} CH ₃ , γ CH
163.	138	132	0.04	39.66	ρ CH ₂ , β _{as} CH ₃
164.	128	123	3.23	118.50	β _{as} CH ₃
165.	125	120	0.02	139.74	ρ CH ₂ , β _{as} CH ₃
166.	114	109	0.23	99.34	ρ CH ₂ , β _{as} CH ₃
ν _s - symmetric stretching; ν _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

Modes	Theoretical wave numbers (cm ⁻¹)				Vibrational assignments
	Unscaled	Scaled	I _{IR}	I _{Raman}	
167.	103	99	0.42	91.44	ρ CH ₂ , β _{as} CH ₃
168.	86	83	0.05	123.68	ρ CH ₂ , β _{as} CH ₃
169.	70	67	1.29	2072.59	ρ CH ₂ , β _{as} CH ₃ , γ OH
170.	59	57	0.36	388.23	β _{as} CH ₃
171.	57	55	3.5	7596.12	ρ CH ₂ , β _{as} CH ₃
172.	43	41	0.26	3026.80	ρ CH ₂ , β _{as} CH ₃ , γ OH
173.	33	32	1.68	9824.31	ρ CH ₂ , β _{as} CH ₃ , γ OH
174.	28	27	0.06	2733.66	ρ CH ₂ , β _{as} CH ₃
175.	21	20	0.56	37394.32	ρ CH ₂ , β _{as} CH ₃ , γ OH
176.	16	15	0.01	17165.97	ρ CH ₂ , β _{as} CH ₃ , γ OH
177.	9	9	0.11	83472.16	ρ CH ₂ , β _{as} CH ₃ , γ OH
υ _s - symmetric stretching; υ _{as} - asymmetric stretching; δ - bending / deformation; β – in-plane bending; γ – out-of-plane bending; χ - scissoring; ω - wagging; τ - twist; ρ – rocking,					
Scaling factor 0.96 for all vibrations.					

3.4.2.1. Stretching vibrations

The molecular structure of phytol has a single hydroxyl (OH) group, giving rise to stretching vibrations. The OH group stretching is anticipated to fall between 3700 – 3550 cm⁻¹ [34]. Following this observation, the OH band was simulated at 3485 cm⁻¹ as a weak (FT-IR) and moderate peak (FT-Raman) spectrum. Phytol encompassing five methyl (CH₃) groups linked with aliphatic carbons have asymmetric and symmetric stretching wavenumbers at 3012 – 2975 and 2920 – 2909 cm⁻¹. The stretching frequencies of CH are highly characteristic bands, showing prominent peaks between 3100 – 3000 cm⁻¹ [34]. According to this literature, the bands anticipated at 3046 cm⁻¹ agree with the CH stretching mode. The CH₂ asymmetric and symmetric wavenumbers are generally observed within 3100 – 3000 and 3000 – 2900 cm⁻¹ [35]. In phytol, the asymmetric modes are simulated between 2968 and 2924, and symmetric modes are salvaged between 2920 and 2856 cm⁻¹.

3.4.2.2. Bending vibrations

The in-plane (OH) deformations and out-of-plane (OH) deformations bands are characterized between 1250 to 1150 and 710 to 517 cm^{-1} [36]. In phytol, the in-plane (OH) deformations were simulated at 1231, 1201 cm^{-1} , and out-of-plane (OH) deformation was calculated at 476 cm^{-1} . The methylene (CH_2) has four (scissoring, rocking, twisting, and wagging) deformations were simulated below 1500 cm^{-1} . Moreover, the electron-donating CH_3 deformations symmetric and asymmetric fell between 1465 to 1440 and 1390 to 1370 cm^{-1} [37]. Alligned with this observation, the symmetric deformations bands were simulated at 1495 to 1396 and asymmetric deformation at 1352 cm^{-1} . The methine in-plane deformation and out-of-plane deformations are anticipated to be observed between 1450 – 1000 and 1000 – 750 cm^{-1} [38]. The in-plane methine deformations of phytol were simulated between 1380 to 1000 and out-of-plane between 978 to 755 cm^{-1} .

3.4.3. Electronic properties

The peak compound in GC-MS phytol was subjected to theoretical simulation on electronic characteristics using the B3LYP/6–31 (d,p) basis set in polar solvent ethanol. The simulated electronic spectrum of phytol was presented in Fig. 6, and the agreeing findings were tabulated in Table 6. In line with these findings, phytol comprises three peaks at 167 (weak), 174 (prominent), and 188 nm (moderate), with a major contribution of $\text{H}\rightarrow\text{L}$, $\text{H-1}\rightarrow\text{L}$, and $\text{H}\rightarrow\text{L} + 1$ with 58, 58, and 96%, respectively. In addition, the HOMO-LUMO energy gap of phytol in ethanolic solvent was also simulated using the same basis set. The HOMO-LUMO surface map and the corresponding FMO characteristics of phytol were illustrated in Fig. 7 and presented in Table 7. The energy gap of phytol was simulated at 5.8820 eV, and the corresponding chemical softness and hardness fell at 5.8820, and 0.0850 eV confirms the compound's stability and reactivity (chemical). The DOS spectrum of phytol is presented in Fig. 8. The green and red peaks refer to the occupied and unoccupied molecular orbitals, which support the energy gap analysis.

Table 6 Experimental and calculated wavelengths (λ), excitation energies (E), oscillator strengths (f), and major contributions of phytol

TDDFT /B3LYP/ 6-31 G (d,p)					
Methanol	λ (nm)	E (eV)	f	Major contributions	Minor contributions
	188	6.5859	0.2021	$\text{H}\rightarrow\text{L}$ (58%), $\text{H-1}\rightarrow\text{L}$ (39%)	-
	174	7.0992	0.3439	$\text{H-1}\rightarrow\text{L}$ (58%), $\text{H}\rightarrow\text{L}$ (36%)	-
	167	7.3972	0.0135	$\text{H}\rightarrow\text{L}+1$ (96%)	-

Table 7
Frontier molecular analysis of phytol

B3LYP/6-31G (d, p)	Formula	Methanol
E_{HOMO} (eV)	-	-6.4044
E_{LUMO} (eV)	-	-0.5224
$E_{\text{HOMO-LUMO}}$ gap(eV)	-	5.8820
Ionization potential (I)	$-E_{\text{HOMO}}$	6.4044
Electron affinity (A)	$-E_{\text{LUMO}}$	0.5224
Electronegativity (χ)	$(I + A)/2$	3.4634
Chemical potential (μ)	$-\chi$	-3.4634
Chemical hardness (η)	$I-A$	5.8820
Chemical softness (s)	$1/2\eta$	0.0850
Global Electrophilicity (ω)	$\mu^2/2\eta$	1.0196
Maximum electron charge (ΔN_{max})	$-(\mu/\eta)$	0.5888

3.4.4. NBO

In theoretical chemistry, NBO analysis offers a comprehensive understanding of bonding interactions in a molecule by converting the intricate molecular orbitals from quantum chemical computations into a set of localized orbitals, which enhances the knowledge of the compound's physical properties, reactivity, and molecular structure. The compound phytol was subjected to NBO analysis using the DFT/B3LYP6-31G(d,p) basis set. The simulated NBO findings are tabulated in Table 8 provides a comprehension of inter- and intramolecular interactions within the crystal structure of phytol. Intramolecular interactions stabilize the molecular structure of phytol, the electron donating $\text{C}_{20}\text{-H}_{58}$ to anti-bonding $\text{C}_{18}\text{-C}_{19}$ with the transition of $\sigma\text{-}\sigma^*$ with an intramolecular stabilization of energy is 7.09 KJ/mol shows a prominent contribution in the stabilization of phytol. The lone pair (O_1) oxygen atom donates electrons to anti-bonding $\text{C}_{21}\text{-H}_{60}$ and has the second-highest intramolecular stabilization energy of 5.83 kJ/mol with the transition of $\text{LP}(2)\text{-}\sigma^*$. In addition, the crystal structure also stabilized by electron-donating C-H and C-C groups to acceptor C-C and O-C with stabilizing energy between 3.00-7.09 KJ/mol.

Table 8
Second order perturbation theory of Fock matrix in selected NBO basis for phytol

Donor (i)	Type	Acceptor (j)	Type	Transition	E(2) ^a (KJ/mol)	E(j)-E(i) ^b (a.u)	F(i,j) ^c (a.u)
C ₂ -H ₂₂	σ	C ₃ -C ₅	σ*	σ- σ*	3.86	0.85	0.051
C ₃ -H ₂₃	σ	C ₂ -C ₇	σ*	σ- σ*	3.33	0.85	0.048
C ₃ -H ₂₄	σ	C ₂ -C ₁₁	σ*	σ- σ*	3.39	0.85	0.048
C ₄ -H ₂₅	σ	C ₈ -C ₁₂	σ*	σ- σ*	3.93	0.85	0.052
C ₆ -H ₂₉	σ	C ₄ -C ₈	σ*	σ- σ*	3.66	0.85	0.050
C ₇ -H ₃₀	σ	C ₂ -C ₁₁	σ*	σ- σ*	3.36	0.85	0.048
C ₈ -H ₃₂	σ	C ₄ -C ₆	σ*	σ- σ*	3.65	0.85	0.050
C ₈ -H ₃₃	σ	C ₄ -C ₁₃	σ*	σ- σ*	3.23	0.85	0.047
C ₁₀ -H ₃₆	σ	C ₁₄ -C ₁₆	σ*	σ- σ*	3.40	0.85	0.048
C ₁₁ -H ₃₉	σ	C ₂ -C ₇	σ*	σ- σ*	3.18	0.86	0.047
C ₁₁ -H ₄₀	σ	C ₂ -C ₃	σ*	σ- σ*	3.02	0.87	0.046
C ₁₂ -H ₄₂	σ	C ₁₅ -C ₁₈	σ*	σ- σ*	3.49	0.89	0.050
C ₁₃ -H ₄₃	σ	C ₄ -C ₈	σ*	σ-σ*	3.16	0.86	0.047
C ₁₅ -C ₁₈	σ	C ₁₈ -C ₂₀	σ*	σ -σ*	3.10	1.28	0.056
C ₁₅ -C ₁₈	σ	C ₂₀ -C ₂₁	σ*	σ- σ*	4.52	1.03	0.061
C ₁₅ -H ₄₇	σ	C ₁₈ -C ₁₉	σ*	σ- σ*	3.86	0.89	0.052
C ₁₅ -H ₄₈	σ	C ₈ -C ₁₂	σ*	σ- σ*	3.34	0.86	0.048
C ₁₅ -H ₄₈	σ	C ₁₈ -C ₂₀	σ*	σ- σ*	3.74	0.55	0.041
C ₁₆ -H ₅₀	σ	C ₁₀ -C ₁₄	σ*	σ- σ*	3.19	0.86	0.047
C ₁₈ -C ₁₉	σ	C ₁₈ -C ₂₀	σ*	σ- σ*	3.00	1.29	0.056
C ₁₈ -C ₂₀	π	O ₁ -C ₂₂	σ*	π- σ*	3.97	0.49	0.040
C ₁₈ -C ₂₀	π	C ₁₂ -C ₁₅	σ*	π- σ*	3.18	0.62	0.040

Donor (i)	Type	Acceptor (j)	Type	Transition	$E(2)^a$ (KJ/mol)	$E(j)-E(i)^b$ (a.u)	$F(i,j)^c$ (a.u)
C ₁₉ -H ₅₅	σ	C ₁₈ -C ₂₀	π^*	$\sigma-\pi^*$	3.93	0.56	0.042
C ₁₉ -H ₅₆	σ	C ₁₈ -C ₂₀	π^*	$\sigma-\pi^*$	3.07	0.56	0.038
C ₁₉ -H ₅₇	σ	C ₁₅ -C ₁₈	σ^*	$\sigma-\sigma^*$	3.40	0.91	0.050
C ₂₀ -C ₂₁	σ	C ₁₅ -C ₁₈	σ^*	$\sigma-\sigma^*$	3.60	1.04	0.055
C ₂₀ -C ₂₁	σ	C ₁₈ -C ₂₀	σ^*	$\sigma-\sigma^*$	3.19	1.30	0.058
C ₂₀ -H ₅₈	σ	C ₁₈ -C ₁₉	σ^*	$\sigma-\sigma^*$	7.09	0.90	0.071
C ₂₁ -H ₅₉	σ	C ₁₈ -C ₂₀	π^*	$\sigma-\pi^*$	3.73	0.58	0.042
O ₁	LP(2)	C ₂₁ -H ₅₉	σ^*	LP(2)- σ^*	3.77	0.71	0.046
O ₁	LP(2)	C ₂₁ -H ₆₀	σ^*	LP(2)- σ^*	5.83	0.72	0.058

3.4.5. Mulliken charge distribution

Mulliken distribution of charges forecasts the distribution of electron density around the atoms. In Mulliken charge distribution, the positive and negative potential indicates the deficient and increased electron distributions, respectively. In phytol, all hydrogen atoms H₂₂-H₆₁ have a net positive distribution. Compared with other hydrogens, hydrogen H₆₁ in the hydroxyl (O-H) group has more positive potential by the influence of the oxygen atom. The molecular structure of phytol has a single oxygen atom with increased negative potential, confirming its electron density around the atom. All the carbon atoms, except for C₁₈, have a negative charge distribution. The carbon atom C₁₈ linked with a methyl group (CH₃) has increased positive potential due to the electron-donating nature of CH₃, which makes the electrons move away from the carbon atom. The mulliken population graph and theoretical findings are presented in Fig. 9 and Table 9. These results are in good agreement with the MEP analysis.

Table 9
Simulated Mulliken population analysis of
phytol

Atom	Charges	Atom	Charges
O ₁	-0.598179	H ₃₂	0.115144
C ₂	-0.085067	H ₃₃	0.131646
C ₃	-0.238925	H ₃₄	0.119224
C ₄	-0.090731	H ₃₅	0.133933
C ₅	-0.262091	H ₃₆	0.117028
C ₆	-0.234462	H ₃₇	0.111082
C ₇	-0.239155	H ₃₈	0.131776
C ₈	-0.234329	H ₃₉	0.12779
C ₉	-0.251689	H ₄₀	0.132722
C ₁₀	-0.225983	H ₄₁	0.129885
C ₁₁	-0.411902	H ₄₂	0.132649
C ₁₂	-0.251251	H ₄₃	0.127845
C ₁₃	-0.405873	H ₄₄	0.125686
C ₁₄	-0.088609	H ₄₅	0.132446
C ₁₅	-0.315792	H ₄₆	0.116535
C ₁₆	-0.401393	H ₄₇	0.129241
C ₁₇	-0.397829	H ₄₈	0.130777
C ₁₈	0.159088	H ₄₉	0.124465
C ₁₉	-0.488964	H ₅₀	0.127196
C ₂₀	-0.114651	H ₅₁	0.133136
C ₂₁	-0.092447	H ₅₂	0.124882
H ₂₂	0.113299	H ₅₃	0.127782
H ₂₃	0.115246	H ₅₄	0.128094

Atom	Charges	Atom	Charges
H ₂₄	0.114136	H ₅₅	0.159987
H ₂₅	0.112076	H ₅₆	0.143148
H ₂₆	0.13762	H ₅₇	0.142833
H ₂₇	0.124558	H ₅₈	0.121496
H ₂₈	0.118251	H ₅₉	0.124908
H ₂₉	0.115407	H ₆₀	0.124615
H ₃₀	0.117972	H ₆₁	0.350271
H ₃₁	0.123448	-	-

3.4.6. MEP analysis

MEP is a helpful tool in quantum chemical calculations for categorizing electrophilic and nucleophilic reactive regions using surface-colored maps [39]. The MEP surface graph of phytol has been presented in Fig. 10. The simulated MEP potential range of phytol spanned between -6.594×10^{-2} to 6.594×10^{-2} e.s.u. in the gas phase. The iso-surface map encompasses diverse colors, such as blue, white, and red, confirming the phytol's nucleophilic, neutral, and electrophilic regions. In phytol, the red color covers the oxygen (O₁) atom in the hydroxyl (OH) group, representing the electron-rich region and acting as a nucleophilic reactive site. In contrast, the blue region covers the hydrogen (H₆₁) in the hydroxyl (OH) group, confirming the electron-poor region and acting as an electrophilic reactive site.

3.5. Molecular docking

Molecular docking offers a better insight into the interaction between the ligand and receptors in drug development and discovery [40–41]. From the findings of this work, the interaction between phytol (ligand) extracted from ethanolic leaf extract of *N.nucifera* and SMAD (PDB. ID. 1U7V, 1U7F, 6OM2, and 6FX4) proteins has been made. The interactions of proteins with ligand shown in Fig. 11(a, b, c, d) and presented in Table 10. The phytol shows the binding affinity of -3.64, -5.49, -5.05, and -3.73 Kcal/mol for 6OM2, 1U7F, 1U7V, and 6FX4, respectively. The interactions are stabilized with hydrogen bondings and hydrophobic interactions within ligand and target proteins. The polar (hydrogen bonding) and nonpolar (hydrophobic) interactions have stemmed from amino and carboxyl groups of amino acids and electronegative (oxygen) atoms and hydrogen atoms. In 6OM2, the affinity is stabilized by creating a hydrogen bonding between the amino acid isoleucine 161 and hydrophobic interactions between amino acids valine, serine, phenylalanine, tyrosine, lysine, arginine, and aspartate. Similarly, in IU7F, hydrogen bonding has been noted between the amino acids glutamate 246 and lysine 340 with a bond distance of 2.87 and 3.11 Å. In contrast, the hydrophobic interaction was felled between the amino acids leucine, threonine, serine, cysteine, proline, phenylalanine, histidine, arginine, and asparagine. Furthermore, the

hydrogen bonding simulated between amino acids threonine 289 and histidine 291 confirms the affinity between phytol and IU7V protein. In addition, the ligand (phytol) polar interactions with amino acids glutamate 31 and lysine 8 with a bond length of 3.19 and 2.66 Å confirm the antagonist property against the 6FX4 protein. Liu et al., [42] have using these SMAD proteins 6OM2, 1U7F, 1U7V, and 6FX4 investigated molecular docking analysis on that binding percentage on 4.95, 6.79, 6.46, and 4.27 with using sweroside compound from *swertia* plant extract. These characteristic findings facilitate the researcher's future investigation in the field of drug discovery to develop novel anti-tumor drugs.

Table 10

PDB ID, Residues, Bond distance, nature of interactions, and binding energy of phytol

Compound	PDB. ID	Binding energy	Hydrogen bonding	Bond distance (Å)	Hydrophobic interactions
Phytol	6OM2	-3.64	IsoLeucine 161	3.35	Valine 98, 280, 226
					Serine 160, 225, 279
					Phenylalanine 278, 159
					Tyrosine 407, 224
					Lysine 253
					Arginine 99
					Aspartate 162
	1U7F	-5.49	Glutamate 246	2.87	Leucine 274, 273
			Lysine 340	3.11	Threonine 247
					Serine 266, 325
					Cysteine 270
					Proline 263
					Phenylalanine 269, 248
					Histidine 530
					Arginine 268
					Asparagine 265
	1U7V	-5.05	Threonine 289	2.73	Alanine 272
			Histidine 291	2.89, 2.90	Leucine 316, 315
					Serine 306
					Proline 305
					Phenylalanine 273, 290, 311
					Histidine 291
					Glutamate 288
					Asparagine 443
	6FX4	-3.73	Glutamate 31	3.19	Alanine 16
			Lysine 8	2.66	Valine 23
					Leucine 13
					Threonine 11, 17

4. Conclusion

The comprehensive examination of the ethanolic leaf extract of *Nelumbo nucifera* revealed a variety of phytochemicals with a notable presence of alkaloids, carbohydrates, and saponins. Phenols and Flavanoids are likely in moderate amounts and are responsible for ethanolic extracts' potential medicinal properties. The GC-MS analysis identified 20 chemicals, and 3,7,1,15-Tetramethyl-2-hexadecan-1-ol (synonym word of phytol) shows the highest peak, indicating the extract's prominence. Phytol, a diterpene alcohol crucial for chlorophyll, plays a pivotal role in plant photosynthesis due to its unique structure. The DPPH and ABTS assays indicate the ethanolic extract of *Nelumbo nucifera* exhibited significant radical scavenging activity, which increased with higher concentrations, confirming its antioxidant potential. The phytol compound to be involved Optimized geometry, Vibrational properties, Electronic properties, NBO, Mulliken charge distribution, MEP analysis, and Molecular docking. A molecular docking study indicates that phytol interacts effectively with SMAD proteins, forming hydrogen bonds and hydrophobic interactions, which suggests its potential as an anticancer agent. The binding affinities observed in the docking study support the hypothesis that phytol could be a promising compound for drug development.

Declarations

Ethical Approval

This article contains no studies with human participants or animals performed by any of the authors.

Consent to Participate

Not Applicable

Consent to Publish

Not Applicable

Authors Contributions

Renganathan Vijayan: Conceptualization, Formal analysis, Writing – Original draft.

Chinnaperumal Kamaraj: Data Curation, Visualization, Validation.

Ponnurengam Malliappan Sivakumar: Writing – Review & Editing, Resources, Conceptualization.

Selcuk Hazir: Supervision, Investigation, Visualization.

Ramalingam Karthik Raja: Conceptualization, Methodology, Writing – Review & Editing, Validation, Investigation.

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Declaration of Competing Interest

All authors declare no competing interests

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures

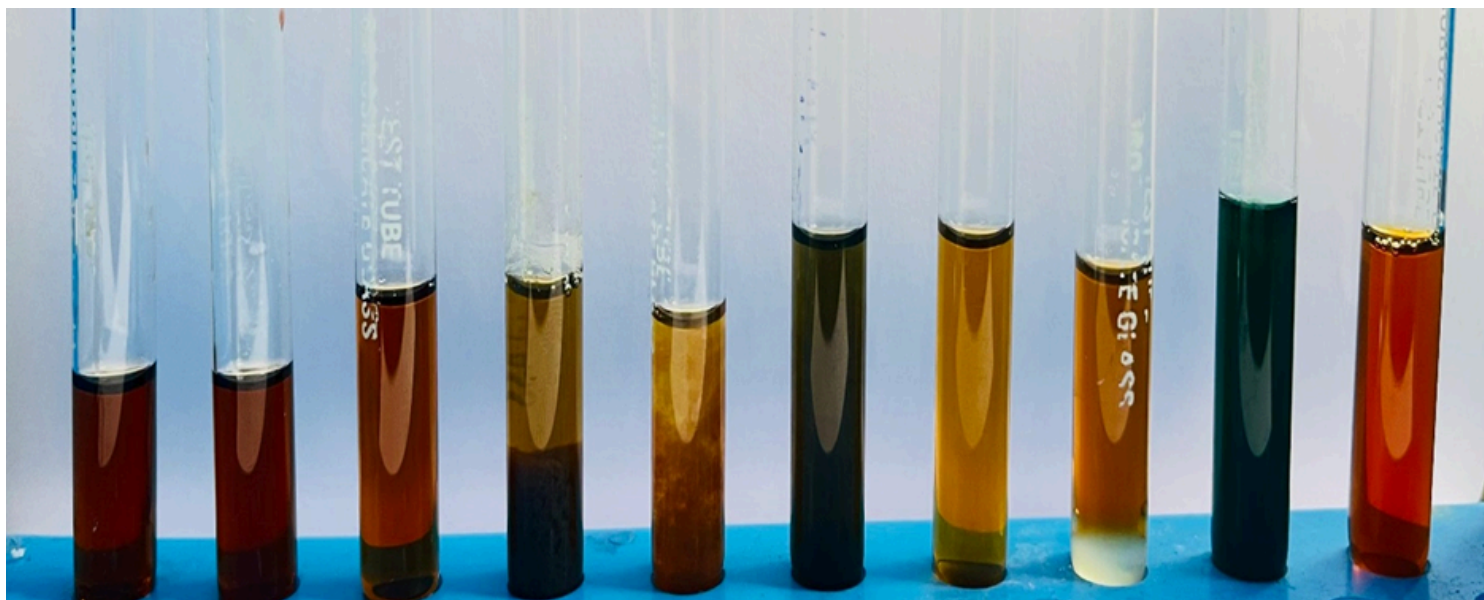
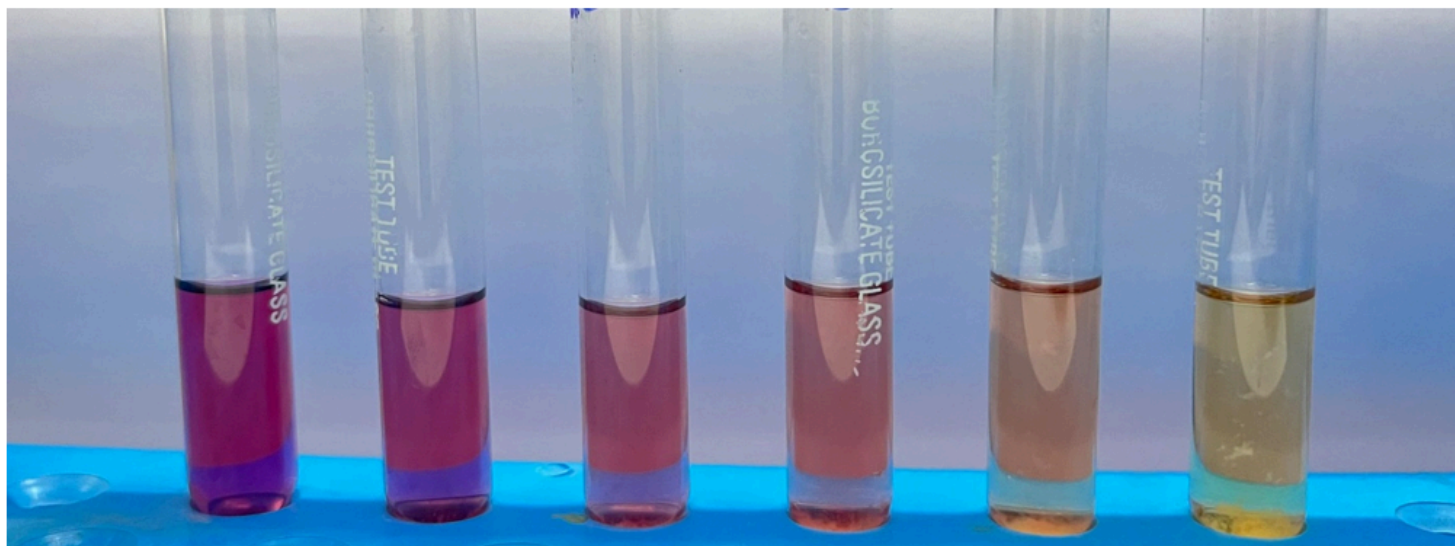
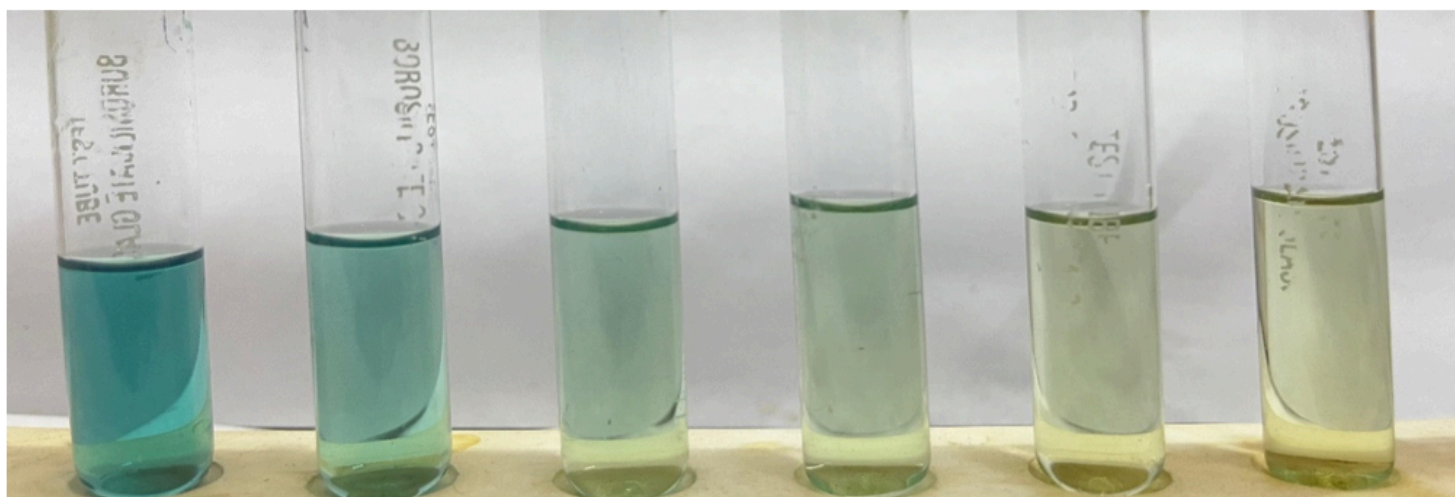


Figure 1

Phytochemical screening of leaf extract of *Nelumbo nucifera*



a



b

Figure 2

a. DPPH activity ethanolic leaf extract of *Nelumbo nucifera*

b. ABTS activity ethanolic leaf extract of *Nelumbo nucifera*

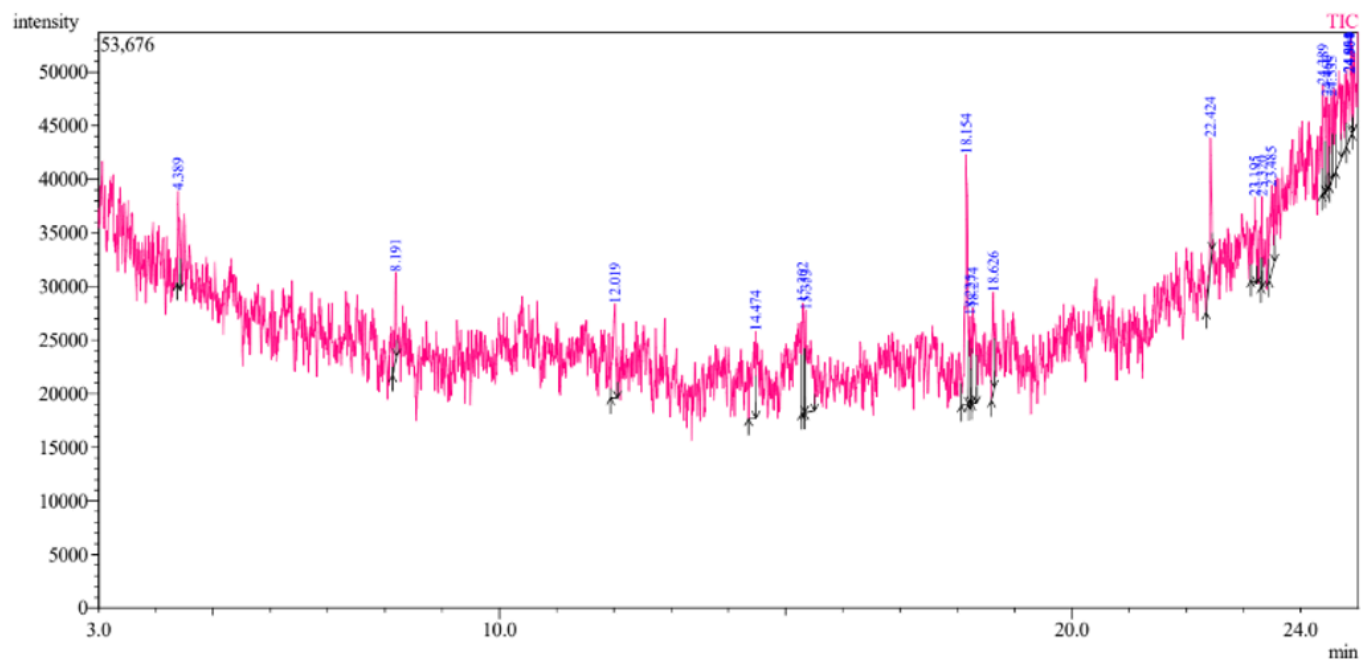


Figure 3

GCMS analysis of ethanolic leaf extract of *Nelumbo nucifera*

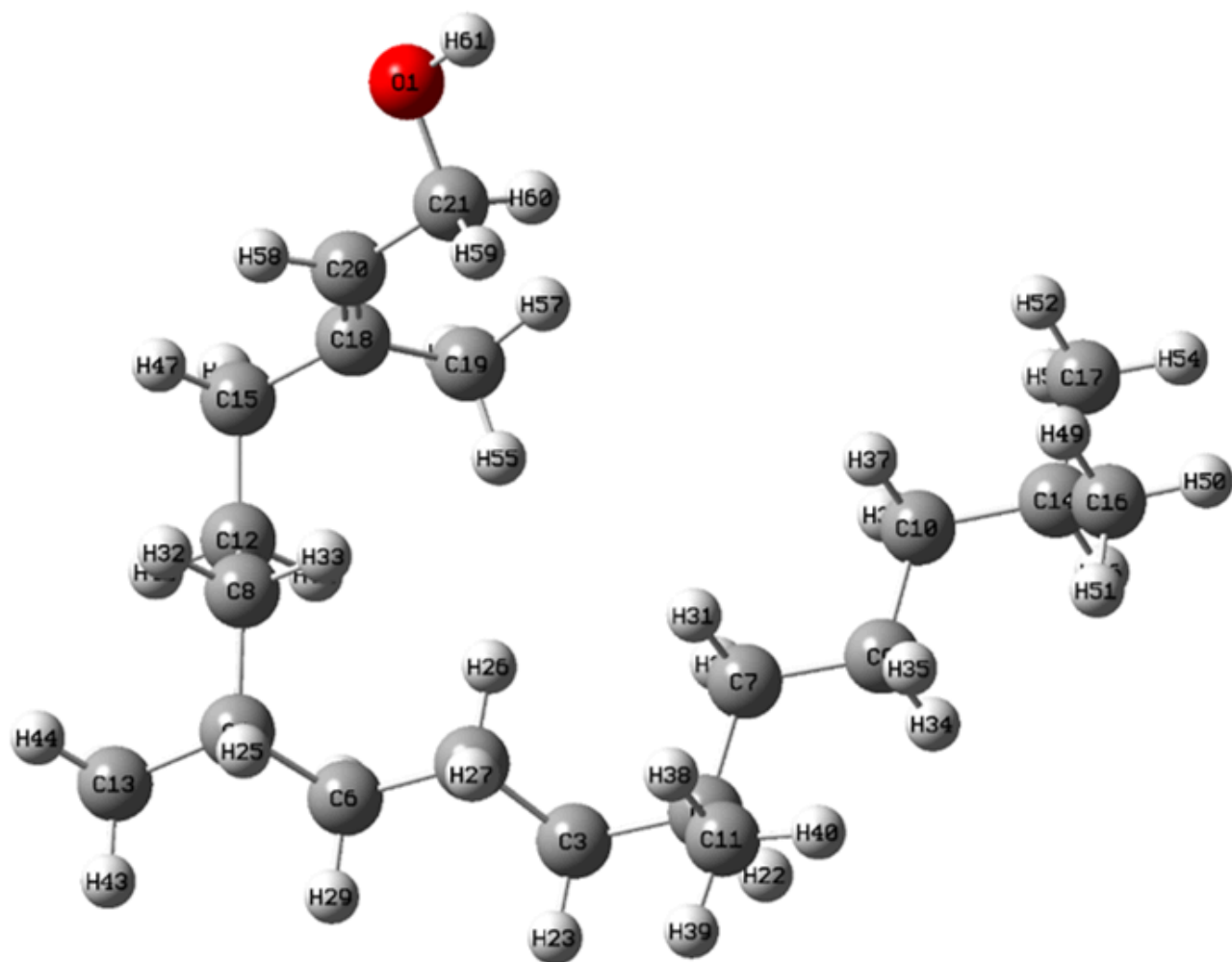


Figure 4

Optimized molecular structure of phytol

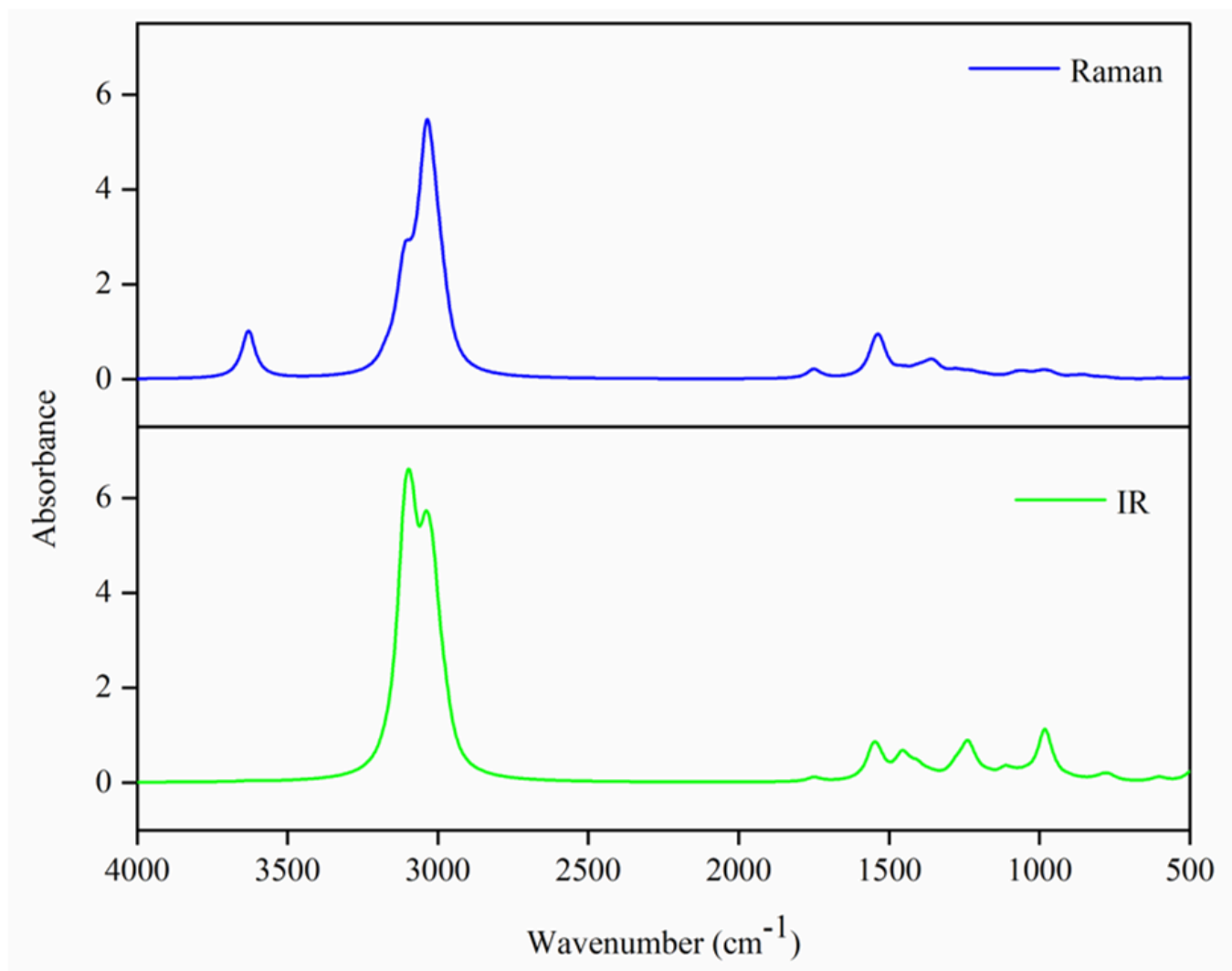


Figure 5

Simulated FT-IR and FT-Raman vibrational spectra of phytol

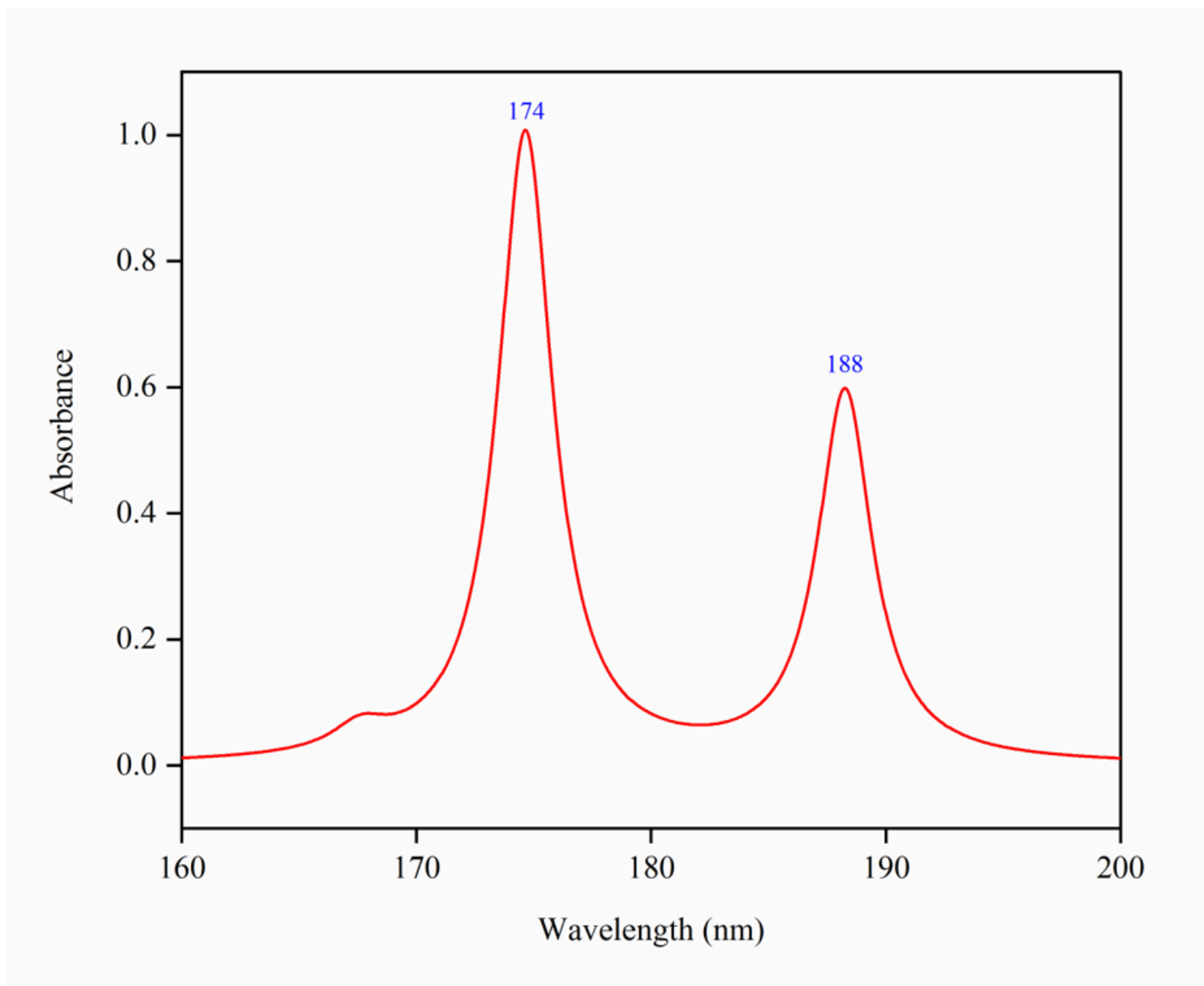
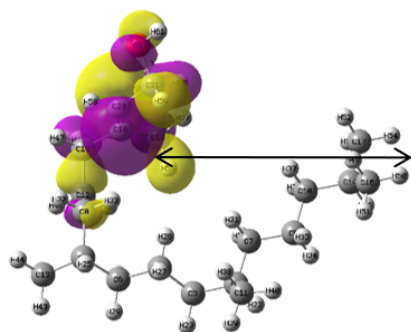


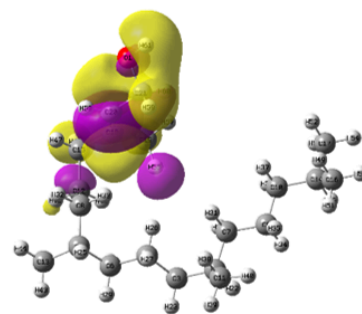
Figure 6

Simulated electronic spectra of phytol



$$E_{\text{HOMO}} = -6.4044 \text{ eV}$$

$$\Delta E_{\text{HOMO-LUMO}} = 6.0214 \text{ eV}$$



$$E_{\text{LUMO}} = -0.5224 \text{ eV}$$

Figure 7

HOMO-LUMO energy gap of phytol

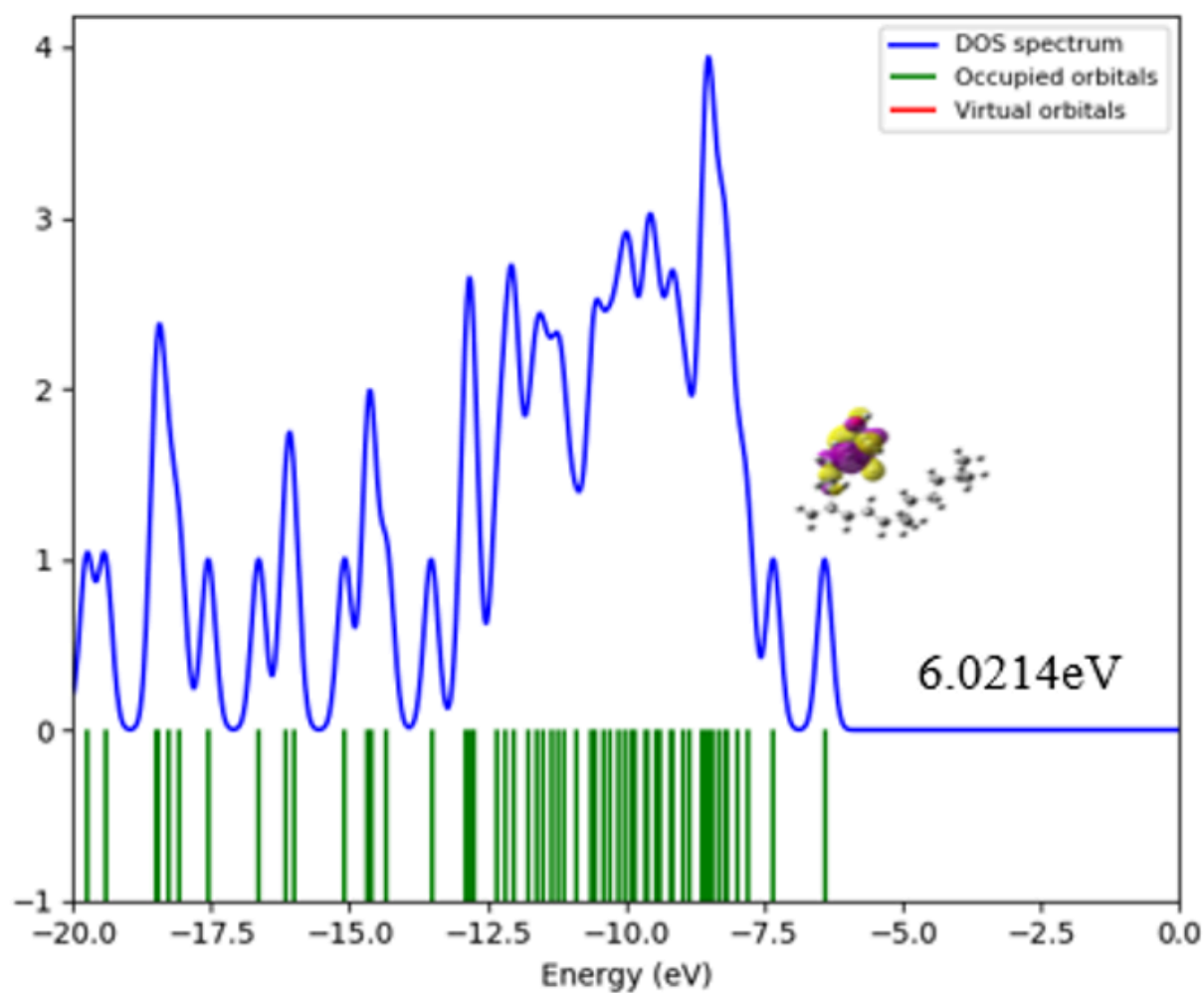


Figure 8

Simulated DOS spectrum of phytol

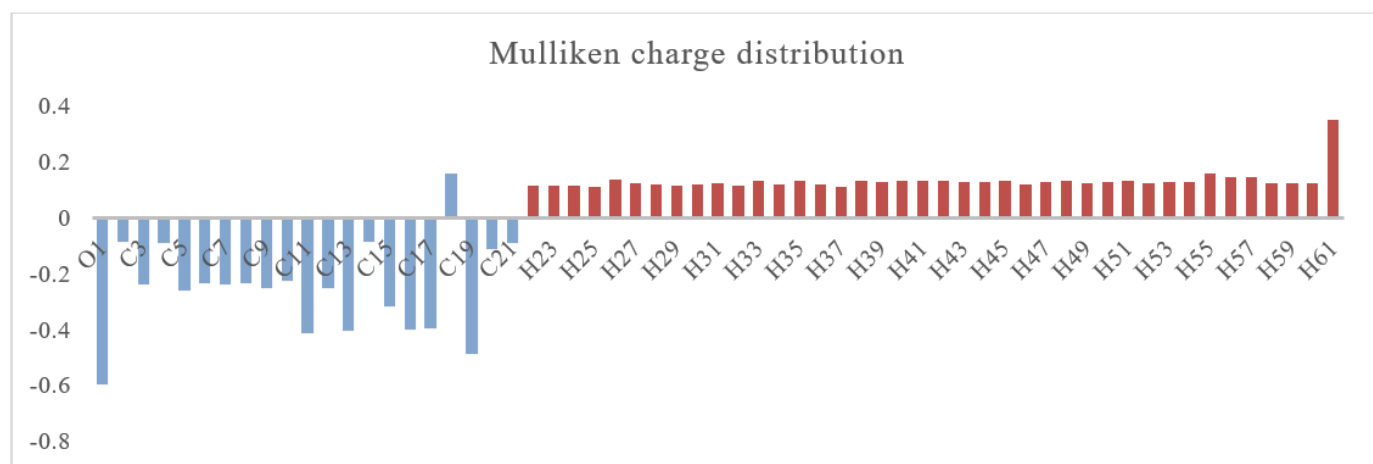


Figure 9

Simulated mulliken charge distribution of phytol

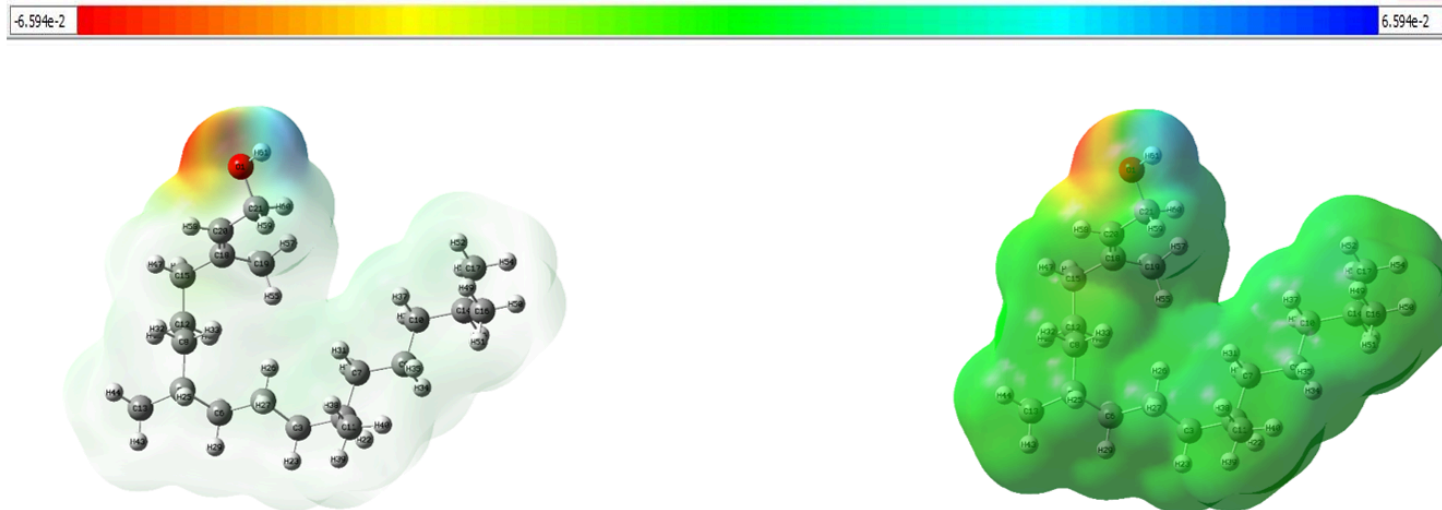


Figure 10

Simulated MEP surface map of phytol

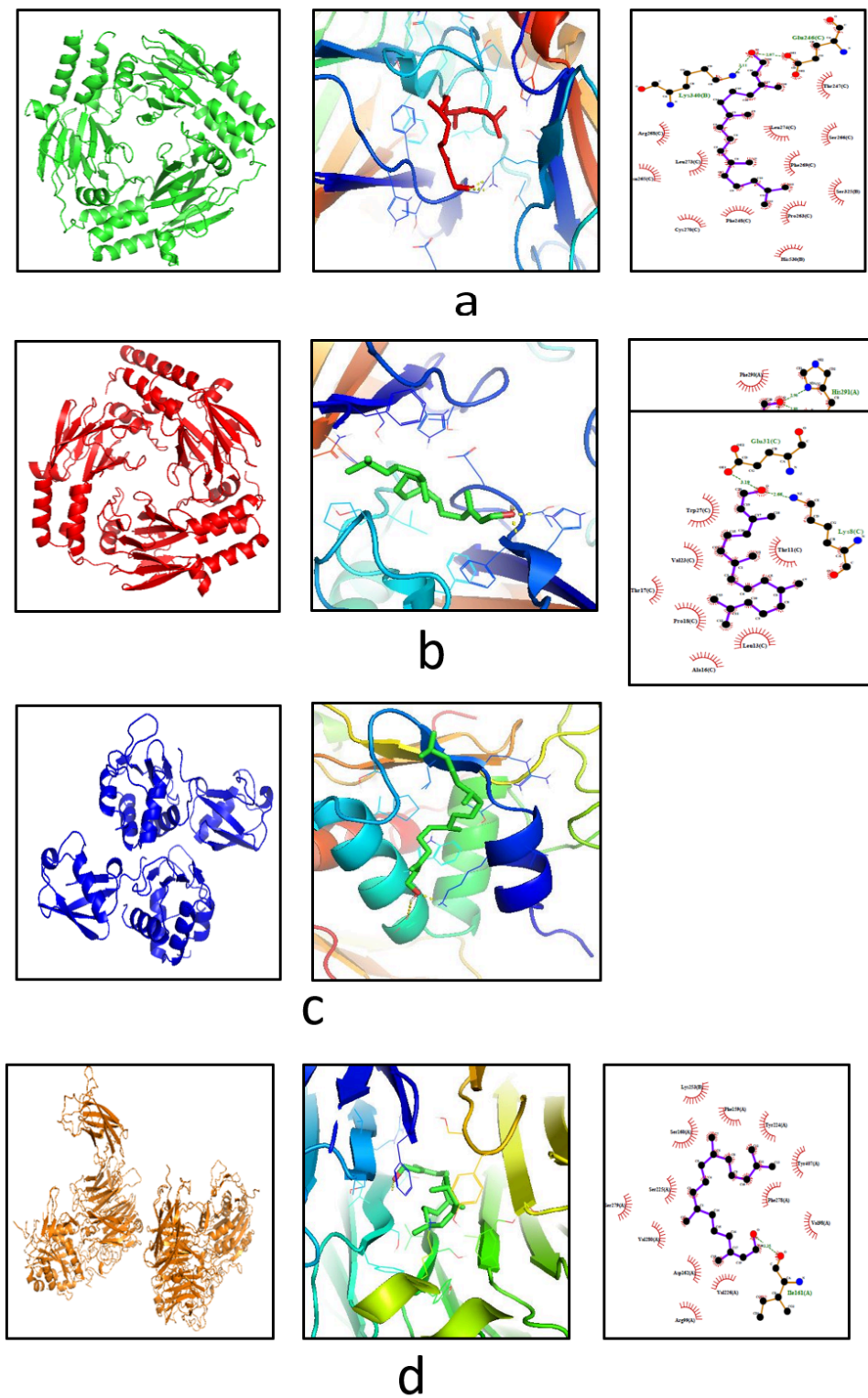


Figure 11

(a). The 3D PyMOL and 2D LigPlot⁺ view of phytol against 1U7F protein

(b). The 3D PyMOL and 2D LigPlot⁺ view of phytol against 1U7V protein

(c). The 3D PyMOL and 2D LigPlot⁺ view of phytol against 6FX4 protein

(d). The 3D PyMOL and 2D LigPlot⁺ view of phytol against 6OM2 protein