Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

Experimental evaluation of the antioxidant and antitumor activities of thyme and basil essential oils and their phenolic constituents: theoretical antioxidant evaluation

Khaled M. A. Ramadan[1,2*], Hossam S. El-Beltagi[3,4], Eslam S. A. Bendary[2] and Hussein M. Ali[2*]

Abstract

Background: Identifying specific biological activities of natural products are of the main concerns worldwide for the use in safe functional food manufacture; essential oils and their components are good candidates in this respect. The present work aims to evaluate the biological activities of basil and thyme oils as well as their phenolic constituents. Using computational methods to predict biological activities are currently effective tools in minimizing and explaining experimental works.

Results: Chemical composition of thyme and basil oils were determined using GC–MS. The identified phenolic components were thymol (28.21%) and carvacrol (0.47%) in thyme oil and eugenol (11.37%) in basil oil. The antioxidant activity of both oils and their phenolic constituents as expressed by EC50 value were 535.01, 134.37, 176.57, 407.89 and 2.29 µg/mL against DPPH and 131.95, 56.65, 57.15, 82.71and 32.80 µg/mL against hydrogen peroxide, respectively. The order of activity is basil oil > thyme oil while phenolic compound order is eugenol > thymol > carvacrol; reducing power showed the same order. Basil oil showed also higher and good antitumor activity where it reduces the surviving fraction to 38.4% of brain tumor cells (U251) and 61.3% of liver tumor cells (HEPG2) at concentration 10 µg/mL. The antioxidant activity were evaluated theoretically according to the main three mechanisms, Hydrogen-Atom-Transfer (HAT), Single Electron Transfer–Proton Transfer (SET-PT) and the Sequential Proton Loss Electron-Transfer (SPLET); the results proved the experimental order of antioxidant and biological activities, and explained the remarkably higher activities of basil oil and its main phenolic component, eugenol.

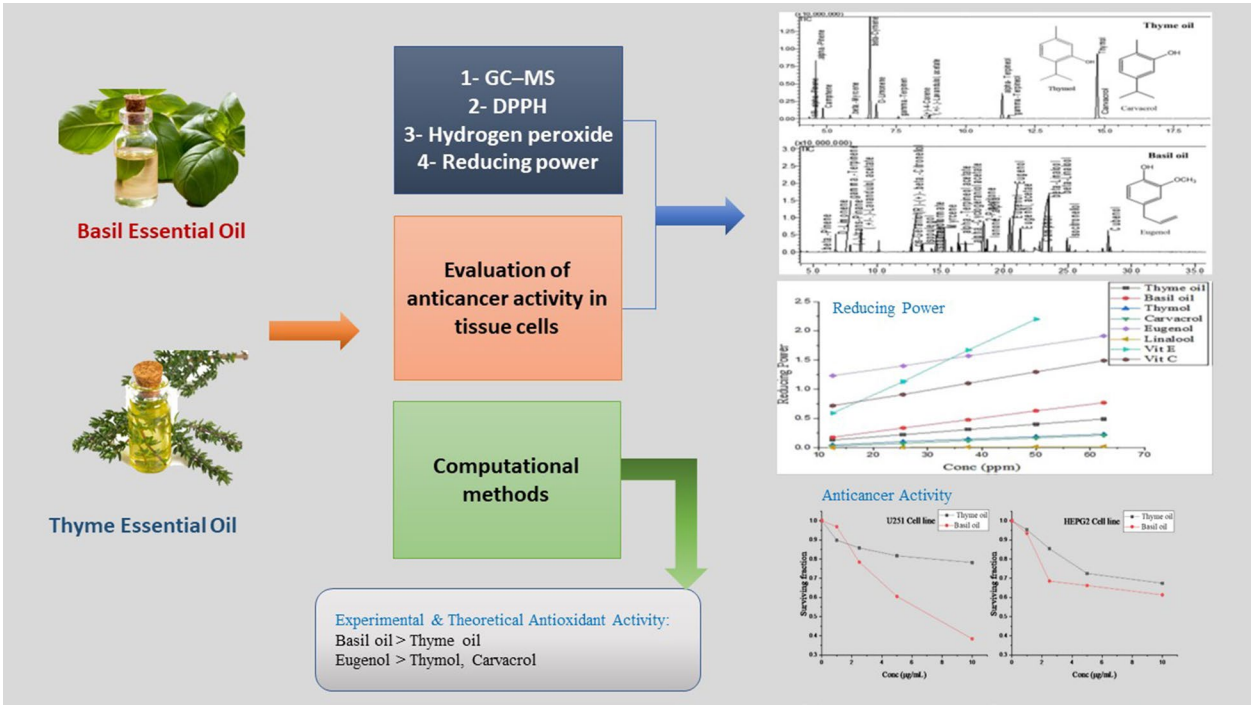
Conclusion: Theoretical calculation can be used successfully to explain and predict the experimental biological activity results. Basil oil and its main phenolic component, eugenol, were found effective as antioxidants. Basil oil was also efficient in reducing the surviving fraction of liver and brain cancer cells where it reduces brain cells even lower than cells treated by doxorubicin, a known anti-cancer agent; thus, basil oil and its main phenolic components, eugenol, can be used safely in food preservation and functional food production.

Keywords: Thyme oil, Basil oil, Eugenol, Thyme, Cavacrol, HAT mechanism, SET-PT, SPLET

*Correspondence: kramadan@kfu.edu.sa; hussein_galaleldeen@agr.asu.edu.eg

[1] Central Laboratories, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[2] Biochemistry Department, Faculty of Agriculture, Ain Shams University, Cairo 11241, Egypt
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Oxidative stress has gained global interest in the past decades because of its diverse implications on health concern [1]; it was considered responsible for aging [2] and various chronic diseases including cancer [3], atherosclerosis [4], liver fibrosis [5], kidney dysfunction [6], DNA damage [7] and neurodegenerative disorders such as Alzheimer, Huntington and Parkinson diseases [8]. Reactive-oxygen species (ROS), e.g. superoxide radical, hydrogen peroxide and hydroxyl radical are the main harmful products of oxidative stress. Superoxide radical is formed in mitochondria during the electron transport chain whereas hydrogen peroxide is produced from superoxide radical by the action of superoxide dismutase or from some biological processes, e.g. xanthine oxidase reactions [9]. The harmful effect of hydrogen peroxide is not only because of its oxidative effect but also through Fenton reaction where the resulted hydroxyl radical is considered the most reactive and harmful radical in cells [10].

Accordingly, searching for natural and safe antioxidants is in the focus of world interest. Essential oils are good candidates and found many pharmaceutical and food additive applications [11, 12]. Thyme oil was found to express antioxidant, antibacterial, antifungal, antilipidemic and antitumoral activities [13, 14]. Basil oil showed also various pharmaceutical and food applications as previously reviewed [15, 16].

Antioxidant activity of phenolic compounds is usually elucidated by three different mechanisms [17–19]. The Hydrogen-Atom Transfer (HAT) mechanism which takes place in only one step in which the hydroxyl hydrogen is transferred to the target radical. The other mechanisms come about in two consecutive steps; in the Single Electron Transfer-Proton Transfer (SET-PT) mechanism, the phenolic oxygen donates an electron to form radical cation in the first step then proton in the second step. Oppositely, in the Sequential Proton Loss Electron-Transfer (SPLET) mechanism, the phenolic proton is lost first to form phenolic anion then an electron is lost in the second step as depicted by the following equations.

HAT Mechanism

$$\mathrm{ArO\text{-}H} \longrightarrow \mathrm{ArO}^{\cdot} + \mathrm{H}^{\cdot} \quad (1)$$

SET-PT Mechanism

$$\mathrm{ArO\text{-}H} \longrightarrow \mathrm{ArO}^{+\cdot}\mathrm{H} + \mathrm{e}^{-} \quad (2)$$

$$\mathrm{ArO}^{+\cdot}\mathrm{H} \longrightarrow \mathrm{ArO}^{\cdot} + \mathrm{H}^{+} \quad (3)$$

SPLET Mechanism

$$ArO\text{-}H \longrightarrow ArO^{-} + H^{+} \quad (4)$$

$$ArO^{-} \longrightarrow ArO^{\cdot} + e^{-} \quad (5)$$

The present study identifies the thyme and basil oil compositions and their antioxidant activities. Antioxidant activity of the main phenolic components found in both oils, thymol, carvacrol and eugenol, were also determined against DPPH radical and hydrogen peroxide. Cytotoxicity against brain and liver tumor cells of thyme and basil oils were examined. In addition, to explain the biological activities of both oils, computational analysis of their phenolic components, according to the three postulated mechanisms, were performed to evaluate the theoretical indexes of their antioxidant activity.

Materials and methods

Chemicals and essential oils

The essential oil (EO) of basil was purchased from KATO Aromatic Co., Giza, Egypt. Thyme EO was purchased from Canada Essential Oils Co. LTD, Canada. Both oils were prepared by hydro-distillation. The major components, thymol, euganol, linalool and carvacrol, were delivered from Sigma–Aldrich Chemicals. α-Tocopherol (vit E) and ascorbic acid (vit C) were obtained from Fluka Chemical company. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Aldrich chemical company. Other chemicals were pure reagent grade.

GC–MS identification and quantification

Separation of essential oil components has been carried out in 1 µL of sample solution (10 µg/mL) in hexane:diethyl ether (ratio 1:1) by GC–MS (Shimadzu-QP-2010S plus) instrument equipped with [AOC−20i + s] autosampler autoinjector and a capillary column (Rtx-1 30 m × 0.25 mm I.D., 0.25 µm). The oven temperature program was adjusted for an initial temperature of 60 °C for 1 min followed by a 4 °C/min temperature ramp to 260 °C. The final temperature was maintained for 1 min. Injector and mass interface temperatures were adjusted at 260 °C. The initial head pressure of the helium carrier gas (He) was 51.4 kPa and injection mode was split (ratio 1:10); the column flow was 2.62 mL/min with linear velocity 58.7 cm/sec and purge flow 4.1 mL/min. The mass parameters were set as following: ion source temp. 210 °C, solvent cut time 5.00 min, MS detector (EI mode) with start time 5.0 min and end time 51.3 min; the compounds were acquired by scan mode ACQ start m/z 75 and end m/z 600. Auto injector was set to be pre-rinsed with washing solvent for 3 times and post rinsed 3 times then rinsed with samples two times with rinse volume 6 µL in high plunger washing speed. Injector port Dwell time was 0.3 s. The integration was performed by Lab Solution software 4.1. Samples were injected in triplets to test the stability and reproducibility of the column. Identification was based on both comparing spectra against NIST 11 s library and interpreting spectra.

Determination of DPPH Scavenging activity

DPPH radical scavenging activity was determined according to the method of Brand-Williams et al. [20] with some modifications. The reaction mixture contained 0.1 mL of DPPH solution (5 mM) and different concentrations of tested samples ranged from 1 to 200 ppm. The total volume of the reaction mixture was 3 mL (final DPPH concentration 65.85 µg/mL). Absorption of DPPH radical at 517 nm was measured at different intervals up to reaching a plateau/steady state for each reaction against a blank solution (contains no DPPH). Methanol was used instead of sample in control. The results were calculated as follows:

$$\%\ \text{DPPH radical scavenging activity} = ((Ac - As)/Ac) \times 100.$$

where As and Ac are absorbance of sample and control, respectively. Time required to reach the steady-state (T_{EC50}) is determined for each compound by plotting time vs. % scavenged DPPH.

The efficient concentration of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (EC_{50}) was calculated from means of three determinations of each concentrations using nonlinear- sigmoidal regression for dose/response four-parameter correlation implemented in OriginPro 2019b. Finally, the EC_{50} and T_{EC50} values were used to calculate the Antiradical Efficiency (ARE), expressed as mg antioxidant/g DPPH at the steady-state, as follows [21]:

$$ARE = 1/(EC_{50} \times T_{Ec50})$$

To standardize DPPH results, the antioxidant activity index (AAI), proposed by Scherer and Godoy [22] was calculated as follows:

$$AAI = [\text{DPPH concentration in reaction mixture } (\mu g/mL)/EC_{50}(\mu g/mL)].$$

Antioxidant potency is classified according to AAI as poor (AAI < 0.5), moderate (0.5 < AAI < 1.0), strong (1.0 < AAI < 2.0) and very strong (AAI > 2.0) antioxidant activity.

Determination of hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was determined according to the reported method [23] with some modification. The reaction mixture is composed of 1 mL hydrogen peroxide solution (35.4 mM) and different concentrations of samples ranged from 12.5 to 62.5 ppm with total volume of the reaction mixture 3 mL. Absorption of hydrogen peroxide at 230 nm was determined after 3 min against a blank solution without hydrogen peroxide while methanol substituted the sample in the control experiment.

The percentage of scavenging activity was calculated as follows:

$$\% \text{ of scavenging of } H_2O_2 = (1 - (As/Ac) \times 100$$

As and AC are absorbance of sample and control, respectively.

Reducing power method (RP)

The reducing power was determined according to the method of [24]. A volume of 0.4 mL of ethanolic solution of each sample with different concentrations, 12.5, 25, 37.5, 50 and 62.5 ppm was added to 1 mL potassium ferricyanide (1%) and 1 mL phosphate buffer (0.2 M, pH 6.6). The reaction mixture was incubated at 50 °C for 20 min then 1 mL trichloroacetic acid (10%) was added to the mixture and centrifuged at 650 ×*g* for 10 min. Two mL of the supernatant was mixed with 2 mL distilled water and 0.4 mL ferric chloride (0.1%) then absorbance was read at 700 nm. All experiments were performed in triplicates. Absorbance is used as reducing power indicator where higher absorbance of the reaction mixture indicates greater reducing power.

Evaluation of anticancer activity in tissue cells

Cytotoxicity of the thyme and basil oils was tested by sulforhodamine B (SRB) assay using the method of Skehan [25]. Tumor cell lines were U251 (Brain tumor cell line) and HEPG2 (liver carcinoma cell line) in addition to THLE2 normal liver cell line which is used as negative control. Doxorubicin (DOX) was used as positive control. Cells were plated in 96-multiwell plate (104 cell/well) for 24 h before treatment with the essential oils to allow attachment of cell to the plate wall. Different concentrations of the essential oils or DOX (0, 1, 2, 5, and 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the essential oils for 48 h at 37 °C under atmosphere of 5% CO_2. After 48 h cells were fixed with 50% trichloroacetic acid (TCA) for one hour then washed five times with tap water; plates were air dried and then stored until use then stained with 20% SRB stain. Excess stain was washed with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relationship between surviving fraction and drug concentration is plotted to get the surviving curve of each tumor cell line with essential oil.

Computational methods

All calculations were performed at the level of DFT/B3LYP with a basis dataset 6-311-G (d,p) using the Gaussian 09 package. HOMO energy was generated by Huckel calculations. Calculations of parent compounds were executed at restricted closed shell level while calculations of radicals were accomplished at unrestricted open shell level. Harmonic vibrational frequencies were computed for thermochemical correction of electronic thermal enthalpies of all species. Bond dissociation energy (BDE), ionization potential (IP), proton dissociation energy (PDE), proton affinity (PA), electron transfer energy (ETE) were calculated by subtracting the electronic thermal enthalpy of the products from those of reactants according to Eqs. 1, 2, 3, 4, 5, respectively. Electron enthalpy was used as calculated by Fermi–Dirac statistics [26]. Correction factor is considered as suggested by the program manual to eliminate systematic error in thermal energies of frequency calculations. Spin density and charges were calculated by NBO calculations.

Statistical analysis

Analysis of variance one-way ANOVA using Duncan's multiple range test at significant level $p \leq 0.05$ was computed by SPSS statistics package version 22.0.

Results and discussion

Chemical composition of thyme and basil oils

GC–MS analysis showed that both thyme and basil oils are rich in phenolic compounds as presented in Table 1 and the chromatograms in Fig. 1. Thyme oil contains thymol (28.21%) and carvacrol (0.47%); this result is in agreement with that reported by Masada [27] who reported that both phenolic compounds constitute 20–55% of thyme oil. Chemical composition of basil oil was reported to vary considerably depending on the plant species and method of distillation; the major components can be two or more of linalool, eugenol, methyl eugenol, estragole, chavicol and methyl cinnamate [28]. The basil oil used in this study showed that the main components are ß-linalool (43.54%), eugenol (11.37%), 2-piperitone (7.46%) and eugenyl acetate (4.24%). Accordingly, the phenolic contents of thyme and basil oils were attributed to the presence of thymol, carvacrol and eugenol.

Table 1 Chemical composition of thyme and basil oils

Compound	Rt	Area (%)	m/z
Thyme oil			
α-Pinene	4.596	15.12	136 (M^+), 93 (Base, M-C_3H_7)
Camphene	4.854	2.72	136 (M^+), 93 (Base, M-C_3H_7)
ß-Myrcene	5.838	0.84	136 (M^+), 41 (Base, C_3H_5)
ß-Cymene	6.552	35.02	134 (M^+), 119 (Base, M-15)
D-Limonene	6.782	4.33	136 (M^+), 68 (Base, C_5H_8)
γ-Terpinene	7.573	0.74	136 (M^+), 93 (Base, M-C_3H_7), 43 (C_3H_7)
4-Carene	8.421	0.7	136 (M^+), 93 (Base, M-C_3H_7), 41 (C_3H_5)
Lavandulol, acetate	8.689	1.27	196 (M^+), 69 (Base, C_4H_5O)
α-Terpineol	11.32	9.73	154 (M^+), 59 (Base, CMe_2OH)
γ-Terpineol	11.545	1.32	154 (M^+), 121 (Base, M-Me-H_2O)
Thymol	14.743	28.21	150 (M^+), 135 (Base, M-15), 39 (C_3H_3)
Carvacrol	14.97	0.47	150 (M^+), 135 (Base, M-15)
Basil oil			
ß-Pinene	5.442	0.06	136 (M^+), 93 (Base, M-C_3H_7)
D-Limonene	6.778	1.58	136 (M^+), 68 (Base, C_5H_8)
γ-Terpinene	7.556	0.06	136 (M^+), 93 (Base, M-C_3H_7), 43 (C_3H_7)
trans-Pinane	7.917	0.76	138 (M^+), 55 (Base, C_4H_7)
Lavandulol, acetate	8.776	3.95	196 (M^+, absent), 69 (Base, C_5H_9)
cis-Geraniol	12.647	0.64	154 (M^+), 69 (Base, C_5H_9)
ß-Citronellol	12.73	0.88	156 (M^+), 69 (Base, C_5H_9)
Isopulegol	13.539	1.24	154 (M^+), 41 (Base, C_3H_5)
Linaloal, formate	13.621	0.81	182 (M^+), 69 (Base, C_5H_9)
Isoborneol	14.354	0.12	154 (M^+), 95 (Base, C_7H_{11})
Myrcene	15.329	3.89	136 (M^+), 41 (Base, C_3H_5)
α-Terpineol, acetate	16.418	2.13	196 (M^+), 43 (Base, CH_3CO)
α-Cyclogeraniol, acetate	16.504	0.69	196 (M^+, absent), 43 (Base, CH_3CO or C_3H_7)
2-Piperitone	18.392	7.46	152 (M^+), 82 (Base, M-$Me_2CHCHCH_2$), 110 (M-C_3H_6)
α-Ionone	18.666	1.57	192 (M^+), 121 (Base, M-($Me_2CH_2CH_2$, H)
Eugenol	20.452	11.37	164 (M^+, base), 149 (M-15)
Eugenol, acetate	21.274	4.24	206 (M^+), 164 (Base, eugenol)
Camphor	22.819	1.34	152 (M^+), 95 (Base, C_7H_{11} or C_5H_7O)
ß-linalool	23.454	43.54	154 (M^+), 71 (Base, C_3H_7O)
Isocitronellol	24.944	1.58	156 (M^+), 83 (Base, C_6H_{11})
Cubenol	28.171	2.85	222 (M^+), 119 (Base, M-[$Me_2CHCHCH_2CH_2$, H_2O, H)], 110 ($C_7H_{10}O$)

Antioxidant activities

Antioxidant activities of both thyme and basil oils in addition to their main active phenolic constituents (thymol, carvacrol and eugenol) were examined against scavenging DPPH and hydrogen peroxide. It should be noted that other factors can also contribute to the antioxidant activity of oils e.g. benzylic C–H dissociation [29] and synergistic effect but these factors were not examined in the present work. DPPH is a free radical and hydrogen acceptor frequently used to measure the antioxidant activity of phenols due to its stable color [30] while hydrogen peroxide is a natural reactive oxygen species that is produced mainly from superoxide radical in the mitochondria during the electron transport chain [31, 32]. Linalool was also examined but found has no activity towards both species (DPPH and H_2O_2). Scavenging activities of the studied oils and their components against DPPH and hydrogen peroxide in comparison with vitamins C and E are presented in Table 2. The results showed that basil oil is much more reactive than thyme oil against both DPPH (EC50 134.37 and 535.01 µg/mL, respectively) and H_2O_2 (EC50 56.65 and 131.95 µg/mL,

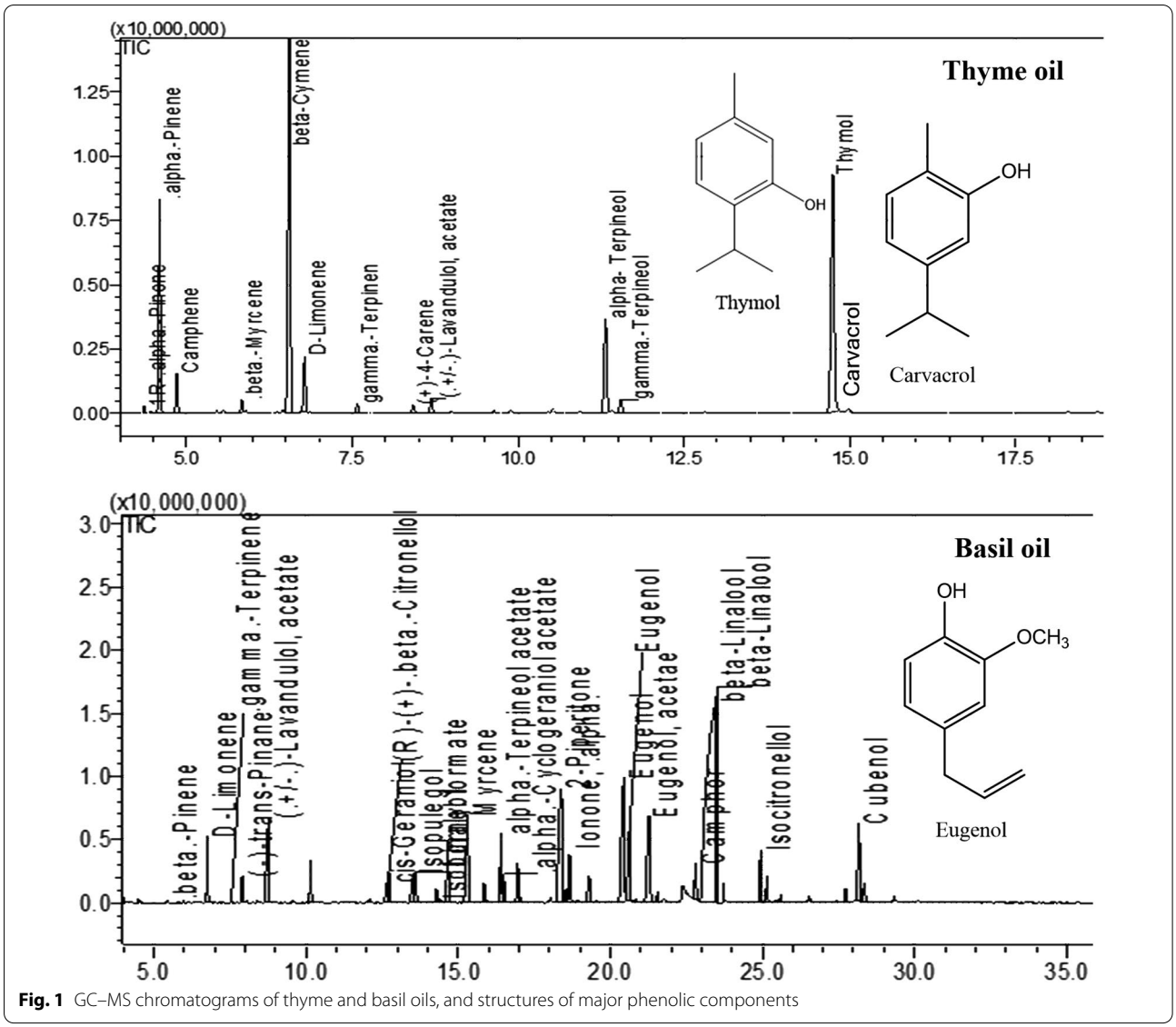


Fig. 1 GC–MS chromatograms of thyme and basil oils, and structures of major phenolic components

Table 2 Scavenging activity of examined oils and compounds against DPPH and H_2O_2

Sample	DPPH					H_2O_2		
	EC20	EC50	EC80	ARE	AAI	EC20	EC50	EC80
Thyme oil	134.61	535.01	2126.32	0.00019	0.123	45.31	131.95	384.30
Basil oil	38.20	134.37	472.67	0.00074	0.490	20.39	56.65	157.42
Thymol	2.13	176.57	14658.18	0.00057	0.373	16.27	57.15	200.67
Carvacrol	18.90	407.89	407.89	0.00025	0.161	37.87	82.71	180.61
Eugenol	1.66	2.29	3.17	0.04367	28.755	21.28	32.80	50.56
Vit E	7.61	12.06	19.13	0.00829	5.460	ND	ND	ND
Vit C	2.80	4.84	8.37	0.02066	13.605	12.79	19.36	29.29

respectively). According to the EC50, the components carvacrol, thymol and eugenol showed increasing order of activity against DPPH radical (EC50 407.89, 176.57 and 2.29 µg/mL, respectively) and towards H_2O_2 (EC50 82.71, 57.15 and 32.80 µg/mL, respectively); similar order against DPPH was previously observed [33]. Anti-radical Efficiency (ARE) and antioxidant activity index (AAI) showed the same order of activity with eugenol has remarkably higher activity while according to AAI scale eugenol is considered very strong antioxidant. The much higher antioxidant activity of eugenol compared to that of thymol and carvacrol can account for the higher antioxidant activity of basil oil compared to that of thyme oil where the major phenolic component of basil oil is eugenol (11.37%) while thyme oil contains the less active phenols (28.21% thymol and 0.47% carvacrol).

Reducing power

Reducing power was also determined and results are presented in Table 3; all examined samples showed concentration dependent as illustrated in Fig. 2. Again, basil oil showed higher reducing power than that of thyme oil while linalool showed very weak activity. Eugenol expressed remarkable reducing power compared to other phenolic components, thymol and carvacrol. Its activity was even higher than that of vitamins C at all concentrations and higher than that of vitamin E at concentrations < 35 µg/mL as presented in Fig. 2.

Cytotoxicity against brain and liver tumor cells

Cytotoxicity of thyme and basil oils against brain (U251) and liver (HEPG2) tumor cells were examined. Cytotoxicity of both oils was tested against all cell line concentrations (0–10 µg/mL). Normal liver cells (THLE2) and a standard synthetic anthracycline anti-cancer agent, doxorubicin (DOX), were used as negative and positive controls, respectively at (10 µg/ml). There is only few reports on the anticancer activity of basil oil [15] while no reports about brain antitumor activity of both oils. The results indicated that both thyme and basil oils have no cytotoxic effect on normal liver cells (THLE2) where cell viability was 99.4% and 99.1%, respectively at concentration 10 µg/mL. The effect of DOX drug on liver cancer cells (HEPG2), human brain glioblastoma multiforme (U251) and normal liver cells (THLE2) at 10 µg/mL was 48.2%, 61.0% and 99.81%, respectively. The results presented in Fig. 3 indicated that thyme oil had low activity towards brain tumor cells at all concentrations. On the other hand, basil oil showed much higher effectiveness where their activity at concentration 10 µg/mL were 78.2 and 38.4%, respectively suggesting that eugenol might be even more effective in brain cancer treatment. Though thyme oil showed little higher activity towards liver than brain tumor cells but still surviving fraction (67.4%) higher than that of basil oil (61.3%) at 10 µg/mL. These results differ from those reported previously, that basil oil has no effect on HEPG2 cells at low concentrations (1–10 µg/mL) but effective at higher concentrations (10–100 µg/mL) with IC50 40 µg/mL [34]. The difference in the results is attributed to the difference in basil oil composition where the previous report found the major components were methyl chavicol (44%), geranial (19%) and neral (15%) while contains no linalool and only 0.5% methyl eugenol. In contrary, our basil oil

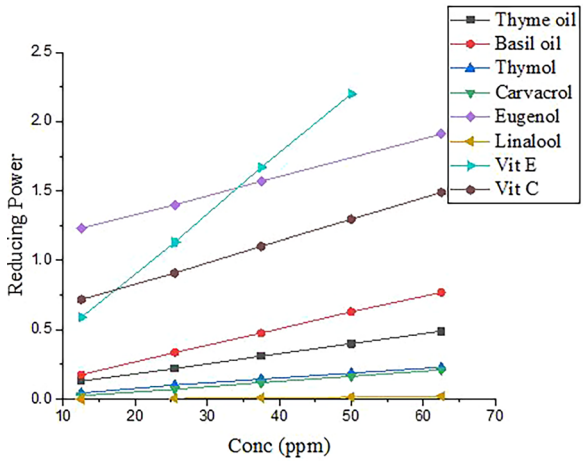


Fig. 2 Reducing power of thyme and basil oils, their components and vitamins E and C

Table 3 Reducing power of thyme oil, basil oil, thymol, Carvacrol, linalool, Eugenol, vit E and vit C

Conc (µg/mL)	Thyme oil	Basil oil	Thymol	Carvacrol	Linalool	Eugenol	Vit E	Vit C
12.5	$0.13^{e} \pm 0.005$	$0.176^{e} \pm 0.003$	$0.044^{e} \pm 0.003$	$0.025^{e} \pm 0.009$	0.0^{e}	$1.23^{e} \pm 0.287$	$0.59^{e} \pm 0.028$	$0.718^{e} \pm 0.017$
25.5	$0.22^{d} \pm 0.002$	$0.336^{d} \pm 0.005$	$0.102^{d} \pm 0.005$	$0.07^{d} \pm 0.014$	$0.004^{d} \pm 0.001$	$1.4^{d} \pm 0.262$	$1.131^{d} \pm 0.024$	$0.91^{d} \pm 0.056$
37.5	$0.31^{c} \pm 0.002$	$0.476^{c} \pm 0.005$	$0.144^{c} \pm 0.009$	$0.118^{c} \pm 0.079$	$0.009^{c} \pm 0.0009$	$1.57^{c} \pm 0.243$	$1.67^{c} \pm 0.007$	$1.103^{c} \pm 0.001$
50.0	$0.4^{b} \pm 0.002$	$0.63^{b} \pm 0.011$	$0.188^{b} \pm 0.011$	$0.164^{b} \pm 0.015$	$0.014^{b} \pm 0.005$	$1.74^{b} \pm 0.207$	$2.2^{b} \pm 0.005$	$1.295^{b} \pm 0.06$
62.5	$0.49^{a} \pm 0.002$	$0.77^{a} \pm 0.002$	$0.23^{a} \pm 0.068$	$0.210^{a} \pm 0.026$	$0.02^{a} \pm 0.009$	$1.911^{a} \pm 0.200$	–	$1.49^{a} \pm 0.065$

Data are means ± SD of three Absorbance (A) determinations. Values with different letter in each column are significantly different ($p \leq 0.05$)

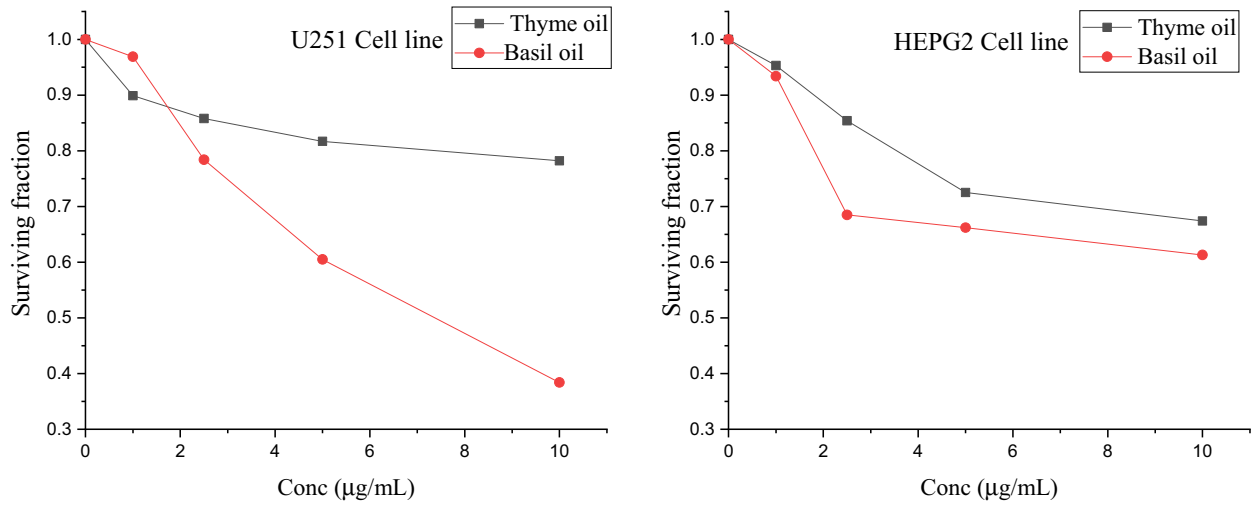


Fig. 3 Surviving fraction of brain and liver cells upon treatment with thymol and basil oils

contains linalool (42%) and eugenol (11%); Shiwakoti et al. [28] found also high content of linalool, methyl eugenol and eugenol which their percentage varies significantly depending on the basil species and method of distillation. It should be noted that linalool showed also anticancer activity against HEPG2 cells [35].

Doxorubicin is an anthracycline based glycoside with potent antitumor activity against a variety of cancer cells e.g. leukemia, soft tissue, neuroblastoma, bone sarcoma, breast carcinoma and ovarian carcinoma [36, 37]. DOX mode of action is retarding DNA replication through inhibiting topoisomerase II involved in DNA strand segregation; in addition, it induces oxidative stress resulting in DNA degradation [38]. The effect of DOX on liver cancer cells (HEPG2), human brain glioblastoma multiforme (U251) and normal liver cells (THLE2) at 10 µg/mL was 48.2%, 61.0% and 99.81%, respectively. Despite the activity of DOX against many types of cancer cells, DOX inadequate penetration of blood–brain barrier constrains its activity against brain cancer [37] which explains its less effectiveness against brain cancer cells (61.0%) than that of basil oil (38.7%) at 10 µg/mL.

The action of the tested essential oils on the in vitro cancer cells could be explained by the pro-oxidant effect of their phenolic constituents that affects mitochondrial and cell membranes in eukaryotic cells [39]. Phenolic components of essential oils, in contact with reactive oxygen species (ROS), produce phenoxyl radicals which are activated in the presence of transition metal ions in cells e.g. Fe^{2+} and Cu^{2+} causing cell destruction [40]. In addition, the lipophilic nature of phenolic compounds permeabilizes the mitochondrial membranes where transition metal ions (Fe^{2+} and Cu^{2+}) are sequestered in the inter membrane space and provokes a leakage of these ions and ROS from mitochondria [41]. Accordingly, more research studies are required to explain, on the molecular basis, the effectiveness of basil and thyme oils and their constituents as anticancer agents especially against brain tumor cells.

Theoretical investigation

Many natural phenolic compounds are well known potent antioxidants. Diverse biological properties of phenolic-rich plant extracts including antitumor, antiinflammatory and antiaging activities are mainly attributed to their phenolic contents [42]. Therefore, to understand better the antioxidant activity of the phenolic constituents of the essential oils, theoretical investigation of their phenolic constituents, thymol, carvacrol and eugenol, were carried out. The bond dissociation energy of the phenolic O–H group ($BDE_{O\text{-}H}$) is frequently used as indicator for the antioxidant activity according to HAT mechanism [43, 44]. In SET-PT mechanism, IP and PDE were used to model first and second steps, respectively whereas PA and ETE can index the two steps of SPLET mechanism, respectively [17, 18, 43]. HOMO energy also indicates the compound ability to donate electron to form radical cation in SET-PT mechanism [45]. Distribution of the radical electron and charge rather than localization on one atom is a good indicator of radical stability and antioxidant activity [43, 46]; it was found that decreasing spin density and charge on the electron donating atom are inversely correlated with the antioxidant activity and indicate better spin and charge distribution [46].

Table 4 Theoretical thermodynamic and electronic antioxidant indexes of major phenolic components

Phenol	BDE_{O-H} Kcal/mol	IP Kcal/mol	PDE Kcal/mol	PA Kcal/mol	ETE Kcal/mol	HOMO eV	ORSD	ORCSD	ORCC
Thymol	78.64	175.03	214.00	343.75	44.56	− 11.542	0.402	0.145	− 0.567
Carvacrol	78.35	175.97	212.77	344.28	43.74	− 11.566	0.400	0.160	− 0.547
Eugenol	75.91	171.32	214.97	339.84	45.73	− 10.043	0.382	0.140	− 0.593

The latter three parameters are unitless

IP Ionization potential, *BDEO–H* Bond dissociation Energy of phenolic O–H bond, *PDA* Proton Dissociation Energy, *PA* Proton Affinity, *ETE* Electron Transfer Energy, *HOMO* Highest Occupied Molecular Orbital, *ORSD* Oxygen Radical Spin Density, *ORCSD* Oxygen Radical Cation Spin Density, *ORCC* Oxygen Radical Cation Charge

Therefore, spin density on oxygen radicals or radical cations (ORSD and ORCSD, respectively) and charge on oxygen radical cations (ORCC) were also computed.

The calculated thermodynamic and electronic antioxidant activity parameters are presented in Table 4. Results of calculations support and explain the experimental antioxidant activities where the order of reactivity is eugenol > thymol > carvacrol as observed experimentally. The BDE in HAT mechanism as well as the first step models in SET-PT and SPLET mechanisms (IP and PA, respectively) of eugenol are lower by 4–5 kcal/mol than those of thymol and carvacrol. While the second step indexes of the latter two mechanisms (PDA and ETE, respectively) are higher for eugenol than those of other phenols by only 1–2 kcal/mol. In addition, HOMO energy of the three compounds showed the same order for electron donating ability. The higher HOMO energy and lower IP of eugenol explains its observed higher reducing power compared to other examined phenols while its low BDE_{O-H} accounts for its much higher DPPH scavenging activity. Unpaired electron delocalization as expressed by ORSD (HAT mechanism) showed better delocatization of eugenol radical (0.382) than the thymol (0.402) and carvacrol (0.400) which were almost similar. The unpaired electron and charge delocalization (ORCSD and ORCC, respectively) of radical cations (SET-PT mechanism) showed better stability of the same order (eugenol > thymol > carvacrol).

Conclusion

Basil oil and its main phenolic compound, eugenol, were found more potent antioxidant than thyme oil and its phenolic constituents, thymol and carvacrol. Basil oil exhibited also efficient antitumor activity especially against brain cancer cells where it reduced the surviving cell fraction to 38.4% compared to doxorubicin, a known anti-cancer agent (61.0%) at 10 µg/mL. Accordingly, it can be used safely in food preservation and functional food production. Theoretical calculation are used successfully to explain and predict the experimental biological activity results according to the antioxidant mechanisms, HAT, SET-PT and SPLET. Computational parameters were found to match experimental results where basil oil showed higher activity while eugenol was more active than thymol and carvacrol according to all the three examined mechanisms which matches the experimental results.

Abbreviations

HAT: Hydrogen-atom transfer; SET-PT: Single electron transfer-proton transfer; SPLET: Sequential proton loss electron transfer; DPPH 1: 1-Diphenyl-2-picrylhydrazyl; HOMO: Highest occupied molecular orbital; BDE: Bond dissociation energy; IP: Ionization potential; PDE: Proton dissociation energy; PA: Proton affinity; ETE: Electron transfer energy; AAI: Antioxidant activity index; ARE: Antiradical efficiency; ORSD: Oxygen radical spin density; ORCSD: Oxygen radical cation spin density; ORCC: Oxygen radical cation charge; ROS: Reactive oxygen species; EO: Essential oil; Vit E: α-Tocopherol; Vit C: Ascorbic acid.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research and Innovation, the Ministry of Education in Saudi Arabia, for funding this research work through the project number [INST067].

Author contributions

All authors have contributed to all research steps; however, the main contributions of each individual are: HMA (Computational work, interpreting results writing MS), KMAR and ESB (design and performing the antioxidant activities and reducing power) and HSE-B (design and executing the cancer cell line experiment). All authors read and approved the final manuscript.

Funding

This work was supported by the Deputyship for Research and Innovation, the Ministry of Education in Saudi Arabia, for funding this research work through the project number [INST067].

Availability of data and materials

Data support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no any competing interests regarding the present work.

Author details

[1]Central Laboratories, King Faisal University, Al-Ahsa 31982, Saudi Arabia. [2]Biochemistry Department, Faculty of Agriculture, Ain Shams University, Cairo 11241, Egypt. [3]Agricultural Biotechnology Department, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia.

[4]Biochemistry Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.

Received: 13 July 2022 Accepted: 1 December 2022
Published online: 27 December 2022

References

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017. https://doi.org/10.1155/2017/8416763.
2. Liguori I, Russo G, Curcio F. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757–72. https://doi.org/10.2147/CIA.S158513.
3. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. Cancer Cell. 2020;38:167–97. https://doi.org/10.1016/j.ccell.2020.06.001.
4. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. Curr Atheroscler Rep. 2017. https://doi.org/10.1007/s11883-017-0678-6.
5. Ramos-Tovar E, Muriel P. Molecular mechanisms that link oxidative stress, inflammation, and fibrosis in the liver. Antioxidants. 2017;9:1–21. https://doi.org/10.3390/antiox9121279.
6. Ling XC, Kuo KL. Oxidative stress in chronic kidney disease. Ren Replace Ther. 2018;4:975–91. https://doi.org/10.1186/s41100-018-0195-2.
7. Halliwell B, Adhikary A, Dingfelder M, Dizdaroglu M. Hydroxyl radical is a significant player in oxidative DNA damage: in vivo. Chem Soc Rev. 2021;50:8355–60. https://doi.org/10.1039/d1cs00044f.
8. Salim S. Oxidative stress and the central nervous system. J Pharmacol Exp Ther. 2017;360:201–5. https://doi.org/10.1124/jpet.116.237503.
9. Pacher P, Nivorozhkin A, Szabó C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev. 2006;58:87–114. https://doi.org/10.1124/pr.58.1.6.
10. Cheng Z, Ren J, Li Y, Chang W, Chen Z. Study on the multiple mechanisms underlying the reaction between hydroxyl radical and phenolic compounds by quantitative structure and activity relationship. Bioorg Med Chem. 2002;10:4067–73. https://doi.org/10.1016/s0968-0896(02)00267-5.
11. Baptista-Silva S, Borges S, Ramos OL, Pintado M, Sarmento B. The progress of essential oils as potential therapeutic agents: a review. J Essent Oil Res. 2020;32:279–95. https://doi.org/10.1080/10412905.2020.174669.
12. Zeng Z, Zhang S, Wang H, Piao X. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J Anim Sci Biotechnol. 2015. https://doi.org/10.1186/s40104-015-0004-5.
13. Khafaji SSO. Subject review: pharmacological application of thyme. Adv Anim Vet Sci. 2018;6:366–71. https://doi.org/10.17582/journal.aavs/2018/6.9.366.371.
14. Asprea M, Leto I, Bergonzi MC, Bilia AR. Thyme essential oil loaded in nanocochleates: Encapsulation efficiency, in vitro release study and antioxidant activity. LWT-Food Sci Technol. 2017;77:497–502. https://doi.org/10.1016/j.lwt.2016.12.006.
15. da Silva WMF, Kringel DH, de Souza EJD, Zavareze EDR, Dias ARG. Basil essential oil: methods of extraction, chemical composition, biological activities, and food applications. Food Bioprocess Technol. 2022;15:1–27. https://doi.org/10.1007/s11947-021-02690-3.
16. Poonkodi K. Chemical composition of essential oil of *Ocimum basilicuml* (Basil) and its biological activities-an overview. J Crit Rev. 2016;3:56–62. https://doi.org/10.31838/jcr.03.01.09.
17. Khargharia S, Rohman R, Kar R. Hybrid molecules of hydroxycinnamic and hydroxybenzoic acids as antioxidant and potential drug: a DFT study. ChemistrySelect. 2022;7(1–11):e202201440. https://doi.org/10.1002/slct.202201440.
18. Anitha S, Krishnan S, Senthilkumar K, Sasirekha V. Theoretical investigation on the structure and antioxidant activity of (+) catechin and (−) epicatechin—a comparative study. Mol Phys. 2020. https://doi.org/10.1080/00268976.2020.1745917.
19. Lu L, Qiang M, Li F, Zhang H, Zhang S. Theoretical investigation on the antioxidative activity of anthocyanidins: a DFT/B3LYP study. Dye Pigment. 2014;103:175–82. https://doi.org/10.1016/j.dyepig.2013.12.015.
20. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.
21. Alvares JJ, Furtado IJ. Kinetics of DPPH• scavenging by bacterioruberin from *Haloferax alexandrinus* GUSF-1 (KF796625). J Anal Sci Technol. 2021. https://doi.org/10.1186/s40543-021-00293-3.
22. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2, 2-diphenyl1-picrylhydrazyl method. Food Chem. 2009;112:654–8. https://doi.org/10.1016/j.foodchem.2008.06.026.
23. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and Inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989. https://doi.org/10.1093/carcin/10.6.1003.
24. Oyaizu M. Studies on products of Browning Reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44:307–15. https://doi.org/10.5264/eiyogakuzashi.44.307.
25. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990;82(13):1107–12. https://doi.org/10.1093/jnci/82.
26. Bartmess JE. Thermodynamics of the electron and the proton. J Phys Chem. 1994;98:6420–4. https://doi.org/10.1021/j100076a029.
27. Masada Y. Analysis of oil by gas chromatography and mass spectrometry. New York: John Wiley and Sons; 1976.
28. Shiwakoti S, Saleh O, Poudyal S, Barka A, Qian Y, Zheljazkov VD. Yield, composition and antioxidant capacity of the essential oil of sweet basil and holy basil as influenced by distillation methods. Chem Biodiv. 2017;14:e1600417. https://doi.org/10.1002/cbdv.201600417.
29. Boulebd H. DFT study of the antiradical properties of some aromatic compounds derived from antioxidant essential oils: C–H bond vs. O–H bond. Free Radic Res. 2019. https://doi.org/10.1080/10715762.2019.1690652.
30. Nishizawa M, Kohno M, Nishimura M, Kitagawa A, Niwano Y. Nonreductive scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) by peroxyradical: a useful method for quantitative analysis of peroxyradical. Chem Pharm Bull. 2005;53:714–6. https://doi.org/10.1186/s12964-015-0118-6.
31. Brand MD. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. Free Radic Biol Med. 2016;100:14–31. https://doi.org/10.1016/j.freeradbiomed.2016.04.001.
32. Lennicke C, Rahn J, Lichtenfels R, Wessjohann LA, Seliger B. Hydrogen peroxide–production, fate and role in redox signaling of tumor cells. Cell Commun Signal. 2015;13:39. https://doi.org/10.1186/s12964-015-0118-6.
33. Pérez-rosés R, Risco E, Vila R, Peñalver P, Cañigueral S. Biological and nonbiological antioxidant activity of some essential oils. J Agric Food Chem. 2016;64:4716–24. https://doi.org/10.1021/acs.jafc.6b00986.
34. Shirazi MT, Gholami H, Kavoosi G, Rowshan V, Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. Food Sci Nutr. 2014;2:146–55. https://doi.org/10.1002/fsn3.85.
35. Rodenak-Kladniew B, Castro A, Stärkel P, De Saeger C, de García Bravo M, Crespo R. Linalool induces cell cycle arrest and apoptosis in HepG2 cells through oxidative stress gen-eration and modulation of Ras/MAPK and Akt/mTOR pathways. Life Sci. 2018;199:48–59. https://doi.org/10.1016/j.lfs.2018.03.006.
36. Cagel M, Grotz E, Bernabeu E, Moretton MA, Chiappetta DA. Doxorubicin: nanotechnological overviews from bench to bedside. Drug Discov Today. 2017;22:270–81. https://doi.org/10.1016/j.drudis.2016.11.005.
37. Hau P, Fabel K, Baumgart U, Rümmele P, Grauer O, et al. Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. Cancer. 2004;100:1199–207. https://doi.org/10.1002/cncr.20073.
38. Meredith AM, Dass CR. Increasing role of the cancer chemotherapeutic doxorubicin in cellular metabolism. J Pharm Pharmacol. 2016;68:729–41. https://doi.org/10.1111/jphp.12539.
39. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol. 2008;46:446–75. https://doi.org/10.1016/j.fct.2007.09.106.
40. Shamim U, Hanif S, Ullah MF, Azmi AS, Bhat SH, Hadi SM. Plant polyphenols mobilize nuclear copper in human peripheral lymphocytes leading to oxidatively generated DNA breakage: implications for an anticancer mechanism. Free Radic Res. 2008;42:764–72. https://doi.org/10.1080/10715760802302251.

41. Mehta R, Templeton DM, O'Brien PJ. Mitochondrial involvement in genetically determined transition metal toxicity: II. Copper toxicity. Chem Biol Interact. 2006;163:77–85. https://doi.org/10.1080/10715760802302251.
42. Vuolo MM, Lima VS, Junior MRM. Phenolic compounds: structure, classification, and antioxidant power. In: Campos MRS, editor. Bioactive compounds. Cambridge: Woodhead Publishing; 2019. p. 33–50 (**10.1016/B978-0-12-814774-0.00002-5**).
43. Messaadia L, Bekkar Y, Benamira M, Lahmar H. Predicting the antioxidant activity of some flavonoids of *Arbutus* plant: a theoretical approach. Chem Phys Impact. 2020;1:100007. https://doi.org/10.1016/j.chphi.2020.100007.
44. Ali HM, Ali IH, Ali HM. Structure-antioxidant activity relationships, QSAR, DFT calculation, and mechanisms of flavones and flavonols. Med Chem Res. 2019;28:2262–9. https://doi.org/10.1007/s00044-019-02452-z.
45. Mendes APS, Borges RS, Silva ABF. The basic antioxidant structure for flavonoid derivatives. J Mol Model. 2012;18:4073–80. https://doi.org/10.1007/s00894-012-1397-0.
46. Ali HM, Ali IH. A DFT and QSAR study of the role of hydroxyl group, charge and unpaired-electron distribution in anthocyanidin radical stabilization and antioxidant activity. Med Chem Res. 2017;26:2666–74. https://doi.org/10.1007/s00044-017-1964-0.

Publisher's Note