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Seasonal Variations in the Composition, Antioxidant, and Antimicrobial Activities of *Zanthoxylum piperitum* (L.) DC. 'Odorum' Leaves Essential Oil

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Abstract

The current study aimed to show the seasonal fluctuations' impact on the composition and bioactivities of the essential oil (EO) of *Zanthoxylum piperitum* (L.) DC. 'Odorum' (the Odorum) leaves. The four seasons steam-distilled EOs were examined chemically using GC/MS, followed by molecular networking, and tested biologically for antioxidant and antimicrobial characteristics. The identified compounds (47) were molecularly docked against 11 antimicrobial targets. The highest yield was obtained in Winter (0.552 %). GC/MS analysis displayed monoterpenes, sesquiterpenes, and cinnamic acid derivatives as the major chemical classes in all seasons. Winter EO had the highest D-limonene level (66.37 %), and the strongestsignificant ($p<0.05$)antioxidant action in the assays of FRAP (41.81 ± 4.63 µM Trolox equivalent/ mg EO) and ORAC (438.00 ± 23.24 µM Trolox equivalent/ mg EO). E-methyl cinnamate predominated in EOs of summer (47.78%) and fall (45.91%). Each season exhibited a predominant bactericidal/fungicidal effect against certain GIT pathogens; Summer EO had the most potent action against *Bacillus subtilis* (MIC and MBC= 1.97 µg/ml), *Enterococcus faecalis* (MIC and MBC = 125 µg/ml), *Escherichia coli* (MIC and MBC= 15.6 µg/ml), and *Candida albicans* (MIC = 1.97 µg/ml) and (MBC=3.9 µg/ml); while fall EO was the most powerful against *Salmonella typhimurium* (MIC= 1.97 µg/ml) and (MBC=3.9 µg/ml); whereaswinter EO showed the utmost activity against *Helicobacter pylori* (MIC = 3.9 µg/ml) and (MBC =.7.8 µg/ml). Docking studies clarified 10 compounds with superior affinity than that of the native co-crystallized ligands mainly towards 3 target proteins. Hence, The Odorum EO could be strongly regarded as a promising natural food preservative.

Keywords: *Zanthoxylum piperitum* (L.) DC. 'Odorum'; GC-MS; GNPS; Antioxidant; Antimicrobial; Molecular Docking.

1. Introduction

Essential oils, extracted from aromatic plants, have diverse biological and medicinal properties besides their application in perfumes, food spices, deodorants, aromatherapy, and cosmetic purposes [1].

Zanthoxylum L. known as Fagara or Prickly ash is a large aromatic genus of the family Rutaceae, containing about 225 species distributed all over the world with the majority found in Asia. They are mostly used as food spices and in folk medicine for the treatment of various illnesses [2]. Essential oils from *Zanthoxylum* plants and their phytoconstituents have confirmed therapeutic potential in ethnobotanical research, making them promising sources for modern drug discovery and healthcare products [2]. *Zanthoxylum piperitum* (L.) DC., commonly known as Japanese pepper, is a well-

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known aromatic plant mostly distributed in Japanese islands, China, and the Korean peninsula and widely used as a condiment and traditionally used to treat digestive complaints [3]. Lots of studies have been issued on *Z. piperitum* essential oil regarding its chemical composition and biological activities showing its anti-oxidant, antifungal, anti-inflammatory, antinociceptive, insecticidal, and insect-repellent actions [4], as well as its wide antibacterial activity, especially against food pathogens. Consequently, it has the potential to be used as a food preservative from a natural origin.

Zanthoxylum piperitum (L.) DC. 'Odorum' is a successfully cultivated and growing aromatic cultivar of *Zanthoxylum piperitum*. Little published data on this cultivar were recorded [5], which triggered to the investigation of its chemical composition and biological effects. Thus, this study aims to analyze *Zanthoxylum piperitum* (L.) DC. 'Odorum' essential oil throughout the year seasons using GC/MS to assess the effect of seasonal change on the essential oil composition, and then the identified compounds were clustered by molecular networking (MN) generated by Cytoscape software. Additionally, the antioxidant activity was assessed by different techniques, and the antimicrobial action was tested against several micro-organisms including *Helicobacter pylori (H. pylori)*, the serious Gram-negative bacteria attacking human GIT.

Besides, knowing that computational chemistry studies like molecular docking constitute one of the most important methods to predict the mechanism of action for a particular drug candidate [6], molecular docking studies were conducted to investigate, discover, and propose the antimicrobial mode of action for the identified compounds.

Such information would be helpful to predict the best season to harvest this plant in terms of quality, yield, and biological efficiency of its essential oil for further recommendation for its use in pharmaceutical or potentially food industries.

2. Material and Methods:

2.1. Plant material:

Plant material of *Zanthoxylum piperitum* (L.) DC. 'Odorum' was collected over the year's four seasons from Mazher Botanical Garden, Cairo, Egypt. The plant was kindly identified by Mrs. Theresa Labib, consultant of taxonomy at the Ministry of Agriculture and the former director of El-Orman Botanical Garden (Taxonomist). A voucher specimen was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University under the number 10-2-2022-I.

2.2. Preparation of the essential oil samples:

In each season, fresh leaves (250 g) were collected and immediately extracted by hydro-distillation method using a Clevenger-type apparatus for about 4 hours to yield a colorless to pale-yellow oil.The obtained essential oil samples were dried over anhydrous sodium sulfate to remove any water and then stored indark airtight containers in the freezer at -5 ºC until further GC-MS analysis and biological studies.The yield in % (v/w) of the essential oils was calculated based on fresh plant weight.

2.3. General Experimental Procedures:

GC/MS analyses of the essential oil samples were performed using a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA), combined with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The injection volume was 1 µL of pure essential oil. The device was equipped with a TR-5 MS column (0.32 mm inner diameter and 0.25 µm filmthickness). Injections were made in the split mode ratio of (1:10). The initial oven temperature was kept at 60^{o}C for 1 min; then raised at 4^{o}C/min to 240 ^{o}C and held for 1 min. The injector and detector temperature were held at 210^{o}C. The carrier gas was helium at a 1.0 ml/ min flow rate. Mass spectra were obtained by electron ionization (EI) at 70 eV in a spectral range of *m/z* 40-450.

2.4. Essential oil analysis using GC-MS:

Analysis of all samples was performed via GC-MS as described previously [7]. The identification of essential oil chemical constituents was de-convoluted using AMDIS software and identified by their retention indices (relative to n-alkanes C8-C22), mass spectrum matching to (Wiley spectral library collection and NSIT library database).

2.5. Molecular networking of essential oil constituents:

Molecular networking of the compounds from the GC-MS results of the four Odorum essential oils was performed using the Global Natural Products Social Molecular Networking (GNPS) platform to visualize and interpret mass data. It was established by transforming the Thermo raw data files into the open format (mzML.) using MS Convert 3.0 open-source software (www.proteowizard.org), then uploaded to the open-access GNPS server (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp) using the open-access Winscp 5.21 cross-platform. For network generation, the parameters were set as previously mentioned [8]. The network spectra were then compared to GNPS GC-MS spectral libraries and Cytoscape 3.10.0 generated the molecular network which can be accessed via the following link (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=60bc6371f70e4d5ba6d79340dba44cbd.)

2.6. In vitro antioxidant activity of the essential oil:

2.6.1. Oxygen Radical Absorbance Capacity (ORAC) Assay:

The essential oil sample of each season was prepared in a concentration of 1 mg/mL in DMSO: methanol (10: 90 v/v). The assay was carried out along with the method of Liang et al.,[9] with minor modifications; 12.5 µL of the prepared essential oil sample was incubated with 75 µL fluorescein (10 mM) for 30 min. Background fluorescence measurement was accomplished for 3 cycles each cycle 90 sec., then 12.5 µL of freshly prepared 2,2-azobis (2-amidinopropane (AAPH) (240 mM) was added directly to each well. Then the same fluorescence measurement was continued for 2.5 hrs. (100 cycles, each 90 seconds). The antioxidant effect of the essential oil samples was displayed as micromoles of Trolox equivalent to milligrams of essential oil µM TE/ mg oil.

2.6.2. Ferric Reducing Antioxidant Power (FRAP) Assay:

The essential oil samples were prepared in a concentration of 2.5 mg/mL in DMSO. FRAP assay was carried out according to the previous method [7], with slight modifications to be carried out in microplates; 300 mM of acetate buffer (PH=3.6) was mixed with 10 mM of 2, 4, 6-tripyridyl-S-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v/v, respectively to freshly prepare TPTZ reagent. Then 190 µL of TPTZ reagent was mixed with 10 µL of the prepared essential oil sample in 96 wells plate. After 30 min. incubation in the dark at ambient temperature, the resultant blue color was observed at 593 nm. The antioxidant ferric-reducing ability of the essential oil samples was also expressed as µM TE/ mg oil.

2.7. Antimicrobial activity:

A group of bacterial and yeast strains available in the stock culture of Micro Analytical Center, Faculty of Veterinary Medicine, Cairo University, Egypt, including three Gram-positive bacteria; *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 10541), four Gram-negative bacteria were used.; *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 90274), *Salmonella typhimurium*, *Helicobacter pylori* was derived from Ain Shams Hospital as a clinical isolates. The strain was identified using The VITEK® 2 Compact system and confirmed by VITEK® MS in 57357 Hospital. Besides a yeast strain *Candida albicans* (ATCC 10221). The antimicrobial activity of the tested samples was determined using the agar well diffusion method as reported previously [10] and the zones of inhibition were measured and expressed in millimetres (mm). The positive control was standard antibiotics: Gentamycin (10 mg/ml; for both Gram-positive and negative strains), Fluconazole (10 mg/ml; as antifungal agent), and the triple antibiotics, (Amoxicillin 0.05 mg/ml, Clarithromycin 0.05 mg/ml, and metronidazole 0.8 mg/ml) for *H. pylori*. DMSO was used as a negative control. After 72 h of incubation at 37°C under a microaerophilic condition with humidity, the inhibition clear zone was determined in diameter (mm).

Relative potencies (%) were calculated for each essential oil by the following equation as declared in the previous study of El-hawary etal., [11]: (Micro-organism growth inhibition zone due to the essential oil / Micro-organism growth inhibition zone due to standard antimicrobial) x 100.

2.7.1. Minimal Inhibitory Concentration (MIC):

MIC of essential oil samples was determined by micro-dilution assay in a 96-well microplate [7]using Mueller-Hinton broth (Difco-BBL, Sparks, MD, USA) for both bacterial and *candida* strains. While for *H. pylori* the MIC of the tested essential oils was determined by using Mueller-Hinton broth with 2.5% lysed horse blood; The inocula were prepared with fresh microbial cultures in sterile 0.85% NaCl to match the turbidity of 1.0 McFarland standard, and 2 µl were added to the wells to achieve a final density of 3.0 ×10^6 CFU (colony forming units)/ml. After incubation at 35°C for 72 h under microaerophilic conditions (15% CO_2). MICs were evaluated visually as the lowest concentration of the essential oil showing complete growth inhibition of the reference strain. A positive control (containing inoculum without the essential oil) was used to create a baseline concentration of the micro-organism used. As well, a negative control was included on each microplate (containing the essential oil without inoculum) to make a baseline in order to eliminate turbidity due to any of the essential oils components. The turbidity was detected by the unaided eye or measured OD at 630 nm using BioTek 800 TS microplate reader to facilitate reading microdilution tests and recording of results may be used as long as there is no sample compromise in the ability to discern growth in the wells.

2.7.2. Minimal Bactericidal Concentration (MBC):

The MBC values of the essential oils were determined following the previously mentioned procedure [7]. MBC was established as the lowest concentration of essential oil able to cause total bacterial death, represented by the visible absence of colonies on the agar plates. The MBC/MIC ratio was used to conclude the antimicrobial activity; either bactericidal or bacteriostatic effect; of the Odorum essential oil from each season against each microbial

strain. When the MBC/MIC ratio was less than 2, the effect was considered to be bactericidal, whereas when the ratio was greater than 4, the effect was considered to be bacteriostatic [12]. Each experiment was repeated three times, and results were compared to observe the seasonal variations effect on the antimicrobial activity of the Odorum essential oil as shown in table (4).

2.8. In silico prediction of the mechanism of action by Molecular Docking studies:

Different eleven molecular docking studies were performed to investigate the antibacterial/antifungal mode of action for the 47 identified compounds. The target receptors were selected as follows: DNA gyrase B kinase, topoisomerase IV, penicillin-binding protein (PBP 1a), dihydrofolate reductase (DHFR), dihydropteroate synthase (DHPS), D-alanine ligase (Ddl), isoleucyl-tRNA synthetase (IARS), RNA polymerase enzyme, urease enzyme, secretory aspartate protease (SAP5), and β-1,3-glucan synthase (β-1,3-GS). The aforementioned receptors were extracted from the Protein Data Bank (PDB IDs: 6F86, 6YIG, 1MWT, 4LAE, 2VEG, 1EHI, 1JZS, 5UAH, 1E9Z, 5JWG, and 6PAL, respectively). They were prepared for docking by correction, 3D hydrogenation, and energy minimization [13]. On the other hand, PubChem was used to download the 47 identified compounds, besides Gentamycin, Amoxicillin, Clarithromycin, Fluconazole, and Metronidazole were used as reference standards. Moreover, the co-crystallized inhibitor in each receptor was used to compare the binding scores of the examined candidates. All the tested ligands were prepared by partial charges justification and energy minimization as well [14]. The AutoDock Vina [15] was used for molecular docking following the default methodology, while PyMOL [16] and Chimera [17] were used for the visualization steps.

2.9. . Statistical Analysis:

Statistical analysis was applied using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). The assays were carried out in triplicate (n=3) and their results were represented as means ± Standard deviation (SD). Data analysis was obtained through the analysis of variance (ANOVA), and by Tukey's HSD test with a significance level at 5% (p <0.05*).*

3. Results

3.1. Percentage yield and physical characters of the Odorum essential oils across the year:

All essential oil samples were pale yellow and had the same distinctive odour. Their percentage yield varied from(0.438 to 0.552 v/w) and can be arranged descending as follows; 0.552 % (winter), 0.54% (spring), 0.495 (summer), and 0.438% (fall). Their specific gravity ranged from 0.85 for the fall season to 0.9 for the summer, winter, and spring seasons.

3.2. GC-MS analysis of the Odorum essential oil:

The analysis of the chemical composition of the Odorum essential oil revealed that a total of 47 compounds were characterized in the four season's samples. The identified components present in the Odorum essential oils over the four seasons, their relative percentages, and their classes are shown in table (1).Investigation of the Odorum essential oil from the leaves collected through the year by GC-MS revealed the presence of 29, 38, 12, and 9 components in summer, fall, winter, and spring essential oil samples, constituting (95.16, 99, 98.93, and 99.7%, respectively), of the total essential oil composition.

Monoterpenes, sesquiterpenes, and cinnamic acid derivatives are the major chemical classes. a stacked bar chart (Suppl. Figure 1) expressing the chemical classes and their percentile detected over the four seasons. The four essential oils were characterized by a higher content (five to fifteen times) of total monoterpenes (37.94–76.28%) than total sesquiterpenes (4.06–8.36%), and a higher percentage of monoterpene hydrocarbons (32.35–75.01%) than oxygenated monoterpenes (1.22–9.11%) constituents over the four seasons. Winter essential oil had the highest concentration of both monoterpene hydrocarbons (75.01%) and sesquiterpene hydrocarbons (7.78%), whereas fall essential oil had the highest percentage of oxygenated monoterpenes (9.11%) and oxygenated sesquiterpenes (2.06%). The most common components in the samples of the Odorum essential oils were D-limonene, E-methyl cinnamate, α-terpinyl acetate, α-humulene, and germacrene B.

The main ingredient in the essential oils of summer and fall was E-methyl cinnamate (47.58 and 45.91%, respectively). D-limonene, however, was the main component in the winter and spring essential oils with concentrations of (66.37 and 53.98%, respectively). Only samples of essential oil from the winter and spring seasons included relatively high concentrations of β-myrcene (7.21 and 6.29%, respectively), and α-humulene (4.44 and 3.72%, respectively).Monoterpenes, sesquiterpenes, and cinnamic acid derivatives are the major chemical classes. a stacked bar chart (Suppl. Figure 1) expressing the chemical classes and their percentile detected over the four seasons. The four essential oils were characterized by a higher content (five to fifteen times) of total monoterpenes (37.94–76.28%) than total sesquiterpenes (4.06–8.36%), and a higher percentage of monoterpene hydrocarbons (32.35–75.01%) than oxygenated monoterpenes (1.22–

Table (1): The chemical composition of the essential oils of Odorum oil samples over the seasons as analysed by GC-MS

Peak No.	Kovat's Index	Compound Name	Relative percentages (%)			
			Summer Essential Oil	Fall Essential Oil	Winter Essential Oil	Spring Essential Oil
Monoterpene hydrocarbons						
1	991	*β*-Myrcene	____	____	7.21	6.29
2	1004	(-)-*β*-Pinene	____	0.36	____	-
3	1024	D-Limonene	32.35	32.28	**66.37**	**53.98**
4	1088	Terpinolene	____	____	1.43	0.86
Oxygenated monoterpenes						
5	1099	Linalool	0.73	1.16	0.41	____
6	1144	*cis*-*β*-Terpineol	____	1.02	____	____
7	1146	Isopulegol	____	1.19	0.27	____
8	1153	Citronellal	0.86	____	____	0.33
9	1161	*cis*-4-Thujanol	0.87	____	____	____
10	1177	Terpinen-4-ol	0.2	0.71	____	____
11	1189	*α* –Terpineol	0.6	0.74	____	____
12	1195	(Z)-Piperitol	____	0.2	____	____
13	1208	*trans*- Piperitol	0.37	____	____	____
14	1228	Citronellol	0.27	0.98	____	____
15	1261	Methyl Citronellate	____	0.37	____	____
16	1344	exo-2-Hydroxycineole acetate	0.23	0.33	____	____
17	1350	*α*-Terpinyl acetate	1.46	2.41	0.59	0.89
Sesquiterpene hydrocarbons						
18	1419	*β*-caryophyllene	2.07	2.35	1.94	-
19	1433	*γ* –Elemene	0.1	0.1	-	-
20	1454	*α*-Humelene	2.92	3.21	4.44	3.72
21	1473	*γ*-Gurjunene	-	-	0.98	-
22	1481	Germacrene D	-	0.08	-	-
23	1557	Geramcrene B	0.68	0.56	0.42	0.34
Oxygenated sesquiterpene						
24	1549	Elemol	0.67	0.7	-	-
25	1562	Dehydronerolidol	0.1	0.15	-	-
26	1564	*d*-nerolidol	-	0.08	-	-
27	1571	*cis*-Eudesm-6-en-11-ol	0.19	-	-	-
28	1581	Caryophyllene oxide	0.13	0.16	-	-
29	1606	Humulene epoxide II	0.15	0.13	-	-
30	1631	*γ*- eudesmol	0.11	0.25	-	-
31	1649	*β*-Eudesmol	0.14	0.42	-	-
32	1653	*α*-eudesmol	0.12	0.17	-	-
Cinnamic acid derivatives						
33	1379	(E)- Methyl Cinnamate	**47.78**	**45.91**	14.52	33.07
Oxygenated diterpenes						
34	1960	Sandaracopimaradiene	0.59	0.16	-	-
35	2114	Phytol	-	0.24	-	-
Acyl chlorides						
36	2133	Linoleoyl Chloride	0.24	0.24	-	-
Alkanes						
37	2700	Heptacosane	0.5	-	-	-
Fatty acid methyl esters						
38	1126	Methyl caprylate	-	-	0.35	0.22
39	1225	Methyl nonanoate	-	0.17	-	-
40	1277	Methyl 8-methyl-nonanoate	-	0.14	-	-
41	1926	Methyl hexadecanoate	0.15	0.34	-	-
42	2091	Oleic acid, methyl ester	0.24	0.52	-	-
43	2092	9,12-Octadecadienoic acid, methyl ester	-	0.76	-	-
44	2092	9,12-Octadecadienoic acid, methyl ester, (E,E)-	0.34	-	-	-
45	2128	Methyl octadecenoate	-	0.1	-	-
Fatty acids						
46	2153	Linolein, 1-mono	-	0.11	-	-
47	2707	Linolein, 2-mono	-	0.2	-	-
Identified components (%)			95.16	99	98.93	99.7
Unidentified compounds %			4.84	1	1.07	0.3
Monoterpenes hydrocarbons (%)			32.35	32.64	**75.01**	61.13
Sesquiterpenes hydrocarbons (%)			5.77	6.3	**7.78**	4.06
Total hydrocarbons (%)			38.12	38.94	82.79	65.19
Oxygenated monoterpenes (%)			5.59	**9.11**	1.27	1.22
Oxygenated sesquiterpenes (%)			1.61	**2.06**	-	-
Oxygenated diterpenes (%)			0.59	**0.4**	-	-
Total oxygenated compounds (%)			7.79	**11.57**	1.27	1.22
Total monoterpenes (%)			37.94	41.75	**76.28**	62.35
Total sesquiterpenes (%)			7.38	8.36	7.78	4.06
Total diterpenes (%)			0.59	0.4	-	-
Total % of cinnamic acid derivatives			47.78	45.91	14.52	33.07
Total % of acyl chloride			0.24	0.24	-	-
Total % of alkanes			0.5	-	-	-
Total % of total fatty acids			-	0.31	-	-
Total % of fatty acid methyl esters			0.73	2.03	0.35	0.22

9.11%) constituents over the four seasons. Winter essential oil had the highest concentration of both monoterpene hydrocarbons (75.01%) and sesquiterpene hydrocarbons (7.78%), whereas fall essential oil had the highest percentage of oxygenated monoterpenes (9.11%) and oxygenated sesquiterpenes (2.06%). The most common components in the samples of the Odorum essential oils were D-limonene, E-methyl cinnamate, *α*-terpinyl acetate, *α*-humulene, and germacrene B.

The main ingredient in the essential oils of summer and fall was E-methyl cinnamate (47.58 and 45.91%, respectively). D-limonene, however, was the main component in the winter and spring essential oils with concentrations of (66.37 and 53.98%, respectively). Only samples of essential oil from the winter and spring seasons included relatively high concentrations of *β*-myrcene (7.21 and 6.29%, respectively), and *α*-humulene (4.44 and 3.72%, respectively).

3.3. Molecular networking of essential oil constituents

Additionally, using the Global Natural Products Social (GNPS) networking program, the integrated molecular network (Figure 1) was investigated to indicate the degree of structural similarity between the identified components. The molecular network generated was composed of 322 nodes that were shown as a pie chart (Figure 1).

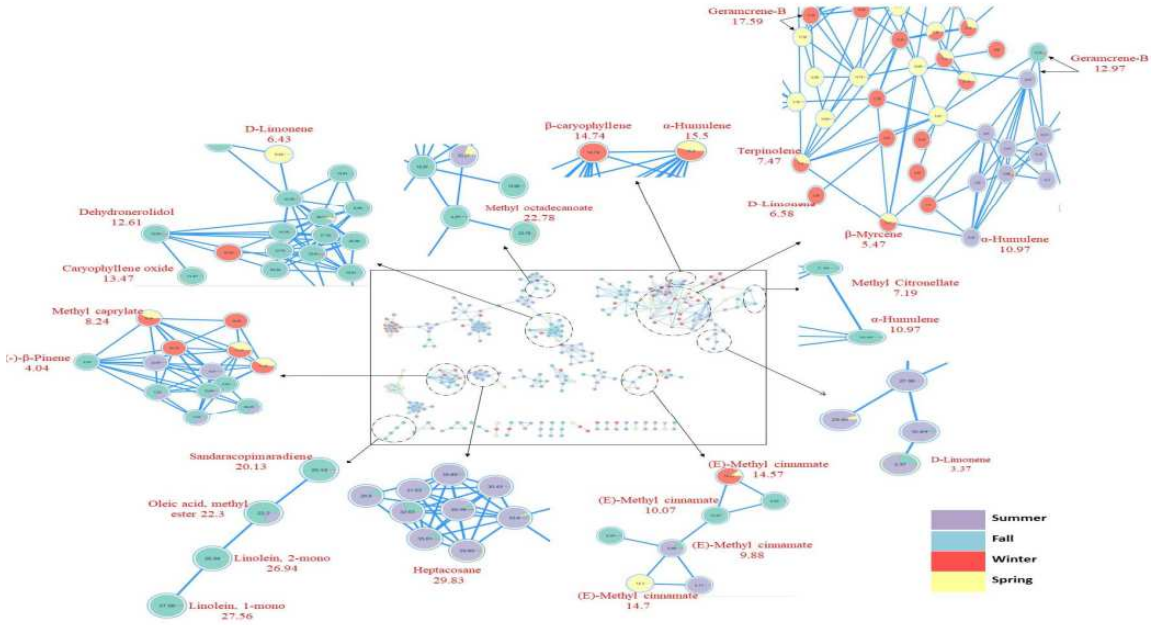

Figure 1: Molecular networking of the GC-MS analysis of the Odorum oil from each season.The network is displayed as a pie chart to reflect the relative abundance of each compound in the analyzed samples. Nodes are labeled with the retention time at which the compound emerged in the GC-MS chromatogram. The node colors range between four colors (purple, blue, red, and yellow), each corresponding to one season (summer, fall, winter, and spring, respectively.

3.4. In vitro antioxidant activity:

To determine the *in vitro* antioxidant activity of essential oil of each season, the ORAC assay, and FRAP assay were used, All data are represented as means ± SD in table (2).

In the FRAP experiment, the antioxidant activity is assessed depending on the compound's capacity for reduction of the ferric ions to ferrous form under acidic conditions through an electron transfer process. As presented in table (2), the four Odorum essential oils showed FRAP activity with the highest significant value ($p<0.05$) in winter essential oil (41.81 ± 4.63 µM TE/ mg oil), and the lowest activity was observed in summer essential oil (28.90 ± 1.55 µM TE/ mg oil), while fall and spring FRAP values were relatively close (34.08±1.68 and 34.43 ± 3.06 µM TE/ mg oil, respectively).

The ORAC assay is commonly used to assess the antioxidant properties of nutritional supplements and

foods. It is a hydrogen atom transfer reaction-based assay. In our present work, all the Odorum essential oils showed ORAC antioxidant activity; with the highest significant ($p<0.05$) activity observed in winter essential oil (438± 23.3 µM TE /mg oil), followed by spring essential oil (390.97 ± 3.98 µM TE /mg oil), then summer essential oil (303.61 ± 32.78 µM TE /mg oil), and the lowest activity was for fall essential oil (224.28 ± 22.39 µM TE /mg oil).

Table (2): *In vitro* Antioxidant activity of the Odorum essential oil over the seasons

Antioxidant Activity of The OdorumEssential Oil of Each Season	Summer Essential Oil	Fall Essential Oil	Winter Essential Oil	Spring Essential Oil
FRAP assay (µM TE/mg)	28.90 ± 1.55 [b]	34.08 ± 1. 68 [b]	41.81 ± 4.63 [a]	34.43 ± 3.06 [b]
ORAC assay (µM TE /mg)	303.61 ± 32.78 [ab]	224.28 ± 22.39 [b]	438.00 ± 23.24 [a]	390.97 ± 103.60 [a]

In ORAC and FRAP assay results of the oil samples are presented as µM TE/ mg oil.
Assays were done in triplicates (n=3), Values are expressed as mean ± SD. Values with the same letters in the row do not differ significantly in the Tukey test ($p<0.05$).

3.5. Antimicrobial activity:

Results of the antimicrobial activity were shown in tables (3-4). MIC and MBC are represented as µg/ml. The antimicrobial activity of the four Odorumessential oil samples through the seasons was assessed by agar well diffusion method against eight micro-organisms. The four essential oil samples exhibited potent antibacterial and antifungal activities in table (3) with high relative potencies, exceeding those of the standard drugs (Gentamycin, Amoxicillin, Clarithromycin, Metronidazole, and Fluconazole) in all the essential oil samples against all the examined Gram-positive, Gram-negative bacteria and yeast, except for the summer (90%) and spring (88%) essential oils against *H. pylori*.

According to Duarte et al., [18] EOs are classified as weak, moderate, or strong antimicrobial if their MIC is ≥1600, 600-1500, or ≤ 500 µg/ml, respectively. The results confirmed that all the tested Odorum essential oils over the year (having MIC < 500 µg/ml) can be considered as strong active antimicrobials in table (3).

Table (3): Results of antimicrobial activity produced by the tested Odorum oils in the agar well diffusion assay expressed as inhibition zones (mm), relative potency of standard drug (%), and its minimum inhibitory concentration (µg/ml)

Name of Micro-organism	Summer Essential Oil			Fall Essential Oil			Winter Essential Oil			Spring Essential Oil			Standards Antimicrobial Agents		
	Inhibition Zone (mm)	relative potency (%)	MIC (µg/ml)	Inhibition Zone (mm)	relative potency	MIC (µg/ml)	Inhibition Zone (mm)	relative potency (%)	MIC (µg/ml)	Inhibition Zone (mm)	relative potency (%)	MIC (µg/ml)	Gentamycin	Triple antibiotics	Fluconazole
Bacillus subtilis	42 ± 0.58	155.5	**1.97**	38 ± 1.0	140.7	7.8	33 ± 0.58	122.2	62.5	35 ± 1.0	129.6	15.6	27 ± 0	-----	-----
Staphylococcus aureus	37 ± 1.0	148	7.8	38 ± 0	152	3.9	34 ± 1.0	136	7.8	37 ± 0.58	148	**1.9**	25 ± 0.58	-----	-----
Enterococcus faecalis	28 ± 0.58	164	**125**	26 ± 1.0	152.9	250	25 ± 1.53	147	250	26 ± 1.73	152.9	250	17 ± 0.58	-----	-----
Helicobacter pylori	22.33± 1.0	91.7	62.5	25 ± 1.0	102.7	7.8	27.67±0.85	113.7	**3.9**	21.65± 0.58	88.98	15.6	-----	24.33 ± 0.52	-----
Escherichia coli	36 ± 0.58	163.6	**15.6**	32 ± 0.58	145.4	15.6	34 ± 1.0	154.5	62.5	36 ± 1.73	163.6	62.5	22 ± 0	-----	-----
Pseudomonas aeruginosa	39 ± 1.0	150	3.9	37 ± 0.58	142.3	7.8	42 ± 0.58	161.5	**1.97**	44 ± 0.58	169.2	**1.97**	26 ± 1	-----	-----
Salmonella typhimurium	32 ± 0.58	160	3.9	42 ± 1.0	210	**1.97**	31 ± 0.58	155	15.6	35 ± 1.0	175	15.6	20 ± 0.58	-----	-----
Candida albicans	43 ± 0.58	148	**1.97**	37 ± 0.58	127.5	7.8	36 ± 1.73	124.1	7.8	39 ± 1.73	134.4	3.9	-----	-----	29 ± 1

The results are the mean of 3 readings (n=3), expressed as mean ± SD.
The amount of oil used in agar well diffusion method in each season was 100 µl.
Values of inhibition zones are expressed as mean (mm) ± Standard deviation.
Triple antibiotics =Amoxicillin 0.05mg/ml, Clarithromycin 0.05mg/ml, and Metronidazole 0.8mg/ml (for *H.pylori*)

Table (4): Evaluation of MBC (µg/ml) and MBC/MIC index values of the Odorum oil over the seasons against susceptible pathogenic microorganisms

Tested Micro-organism	Summer Oil		Fall Oil		Winter Oil		Spring Oil	
	MBC (µg/ml)	MBC/MIC	MBC (µg/ml)	MBC/MIC	MBC (µg/ml)	MBC/MIC	MBC (µg/ml)	MBC/MIC
Bacillus subtilis	**1.97**	1	15.6	2	125	2	15.6	1
Staphylococcus aureus	15.6	2	15.6	4	62.5	8	**3.9**	2
Enterococcus faecalis	**125**	1	1000	4	1000	4	500	2
Helicobacter pylori	125	2	15.6	2	**7.8**	2	31.125	2
Escherichia coli	**15.6**	1	62.5	4	125	2	500	8
Pseudomonas aeruginosa	15.6	4	7.8	1	**1.97**	1	**1.97**	1
Salmonella typhimurium	62.5	16	**3.9**	1	15.6	1	125	8
Candida albicans	3.9	**1.97**	**15.6**	**2**	**7.8**	**1**	**7.8**	**2**

The results are the mean of 3 readings (n=3), expressed as mean ± SD

3.6. In silico prediction of the antimicrobial activity mechanism of action by molecular docking analysis:

To visualize deeply the probable molecular mechanism of the pharmacologically active compounds associated with the observed antimicrobial effect of the Odorum essential oil, molecular docking is frequently applied. The 47 compounds from the Odorum essential oil were evaluated for their ability to bind to 11 target proteins that were typically correlated with bactericidal/bacteriostatic effects, such as DNA gyrase B kinase (6F86), topoisomerase IV (5YIG), penicillin-binding protein (1MWT), dihydrofolate reductase (4LAE), dihydropteroate synthase (2VEG), D-alanine D-alanine ligase (1EHI), isoleucyl-tRNA synthetase (1JZS), RNA polymerase enzyme (5UAH), and urease enzyme (1E9Z), or correlated with fungicidal/fungistatic effect, such as secretory aspartate protease (5JWG) and β-1,3-glucan synthase (6PAL). Indeed, a comparison between the 47 essential oils 'components, the standard antimicrobial drugs (Gentamycin, Amoxicillin, Clarithromycin, Fluconazole, and Metronidazole), and the co-crystallized ligand in each protein against the 11 protein targets was carried out in terms of binding scores (kcal/mol) (Suppl. Table 1), with the highest numerical values indicating the highest affinity between the ligand and the target protein [19].

The binding interactions of the essential oil of Odorum components with the target receptor are exemplified in (Suppl. Table 2).

4. Discussion

The present study describes the seasonal variations that occur to the Odorum essential oil throughout the year, and the influence of these qualitative and quantitative variations on the essential oil's biological activities to obtain the essential oil with the finest quality and the most potent activity.

4.1. Percentage yield and physical characters of the Odorum essential oils across the year:

Winter season yielded the highest essential oil 0.552% among other seasons, The harvesting period is responsible for the fluctuation in essential oil yields; nevertheless, other parameters, such as geographical origin, extraction methods, temperature, and time of extraction, are constant.

4.2. GC-MS analysis of the Odorum essential oil:

The present GC-MS analysis of the Odorum essential oil in table (1) showed differences in chemical composition between seasons, according to various earlier research, the observed quantitative and

qualitative variance of the essential oil may be directly related to seasonal influence or climate change throughout the year [20, 21].

In Egypt, previous investigation on the essential oil of *Z. piperitum* leaves was performed by GC-MS analysis and revealed the presence of *β*-phellandrene, cyclohexanol, germacrene-D, *α*-pinene, geranyl acetate, and 1,8-cineole among the essential oil constituents [22]. Meanwhile, in Japan, a headspace GC-MS of the essential oil of *Z. piperitum* leaflets was studied and revealed the major constituents to be *β*-phellandrene (23.7%), D-limonene (15.8%) and citronellal (10.6%) and others [23], which were also present in our Odorum essential oils although in varying concentrations. Also, in another study in Japan, *Z. piperitum*essential oil was extracted by different methods and showed hydrocarbons such as D-limonene as the major constituent along with geraniol, *β*-phellandrene, caryophyllene, and others [24]. Furthermore, seasonal fluctuation research on *Z. piperitum*essential oil from China found that monoterpenes made up the majority of the essential oil, which is consistent with our findings, but their principal constituents were *β*-phellandrene (26.90%), citronellal (15.32%) and *β*-myrcene (3.24%) [25]. Essential oils of *Z. piperitum* grown in various nations (Korea and Ireland) [26], were found to have certain constituents such as *γ*-terpinene, *β*-phellandrene, piperitone, and sabinene.

Accordingly, the essential oils from *Z. piperitum* and its cultivar *Z. piperitum* (L.) DC. 'Odorum' are similar in the presence of major compounds such as D-limonene and *β*-myrcene but differ in the presence of other constituents such as methyl cinnamate.This ingredient heterogeneity may be related to varietal, genetic, and geographic factors, which have previously had an impact on essential oil composition. Consequently, the current study is the first to outline the impact of seasonal fluctuation on the essential oil from *Z. piperitum* (L.) DC. 'Odorum' leaves grown in Egypt.

4.3. Molecular networking of essential oil constituents

The molecular network of the four seasons (Figure 1). revealed that monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons represent the dominant skeleton in the network, which aligns with the present GC-MS findings in table (1). Besides, other clusters of alkane and fatty acids methyl ester derivatives were apparent in the network indicating chemical diversity in the Odorum essential oil.

A previous study reported the molecular network of MS spectral data from three *Zanthoxylum* species extracts' and showed that the flavonoids can be considered as metabolic markers for the three species [27]. However, to our knowledge, molecular networking on the volatile constituents of *Z. piperitum* essential oil has not been reported before.

4.4. In vitro antioxidant activity:

Finding potent antioxidants from herbal sources is becoming more and more popular to reduce the oxidative stress brought on by free radicals, which are responsible for many age and disease-related processes in the body, including cancer, heart and immune system diseases, and brain damage. Since essential oils are mixtures of various organic compounds, some of these compounds have either hydroxyl groups or conjugated carbon double bonds, which can contribute to a hydrogen atom transfer reaction (HAT reaction) or involve an electron transfer reaction (ET reaction), thereby restricting free radicals and reducing oxidative stress and playing a crucial role in disease prevention [28]. Consequently, this report investigates the antioxidant capacity of Odorum essential oil from each season using various *in vitro* approaches to evaluate its potential to scavenge free radicals and to comprehend the prominent effective antioxidant mechanism(s) by which its compounds exert this activity.

The different FRAP and ORAC antioxidant readings of Odorum essential oils over the four seasons may be attributed to the difference in constituents and their concentrations between the four seasons. Previous studies revealed that the higher the monoterpenes content, especially monoterpene hydrocarbons (e.g.; D-limonene, *β*-myrcene *α*-terpinene, *γ*-terpinene, and terpinolene), the higher the ability of the oil to exert antioxidant potential [29], as a result of the presence of structurally strong activated methylene groups. Meanwhile, sesquiterpene hydrocarbons and their oxygenated derivatives have been shown to have extremely little antioxidant activity [30], which is consistent with our findings. Winter essential oil, possessing the highest D-limonene (66.37%) content, showed the highest FRAP and ORAC activities, while fall and summer essential oils had the lowest D-limonene concentrations (32.28 - 32.35%, respectively) exhibited the lowest FRAP and ORAC activities. The highest concentration of total monoterpene hydrocarbons in the present study is in the order of winter (75.01%), spring (61.13%), fall (32.64%), and summer (32.35%). Likewise, the antioxidant activity measured using the FRAP or ORAC methodologies followed the same potency sequence, with the only difference being that summer essential oil held higher ORAC action than fall essential oil. This could be attributed to to the presence of some oxygenated compounds in summer essential oil sample (citronellal, *cis*-4-thujanol,*trans*-piperitol and *cis*-eudesm-6-en-11-ol), which does not exist in fall essential oil. This group of compounds was reported

to exhibit significant correlations with the ORAC assay, even at low concentrations [31].

These outcomes revealed that the essential oil of winter has the strongest antiradical activity compared to the essential oils collected from other seasons. These are the first FRAP and ORAC antioxidative assays on Odorum essential oil that have been published.

4.5. Antimicrobial activity:

Concerning the effect of essential oil samples against Gram-positive bacteria, summer essential oil gained the highest efficacy against *B. subtilis* and *E. faecalis* which was evidenced by its largest growth inhibition zones (42 ± 0.58 and 28 ± 0.58 mm), higher relative potency (155.5 and 164 %) and the lowest MIC values (1.97 and 125 µg/ml) against the two micro-organisms, respectively. On the other hand, spring essential oil was the strongest against *S. aureus* with a relative potency of (148%) and MIC of (1.9 µg/ml).

Regarding the effectiveness of the four essential oil samples against Gram-negative bacteria, summer essential oil, was the most potent against *E. coli* with an inhibition zone, relative potency , and MIC of (36 ± 0.58 mm, 163.,6%, and 15.6 µg/ml, respectively). Spring essential oil demonstrated the highest efficacy against *P. aeruginosa* as its growth inhibition zone was (44 ± 0.58 mm), with relative potency of (169.2%) and MIC of (1.97 µg/ml), while fall essential oil was the most potent among the four samples against *S. typhimurium* with growth inhibition zone of (42.01 ± 1 mm), very high relative potency (210%) and MIC of (1.97 µg/ml). Remarkably, *H. pylori* was susceptible to all the Odorum essential oils across the year (Suppl. Figure 2), however, fall and winter essential oils showed higher potency than the usual triple antibiotics used in its treatment, regarding their inhibition zones (25 ± 1 and 27.67 ± 0.8 mm), relative potency (102.7 and 113.7%), and MIC (7.8 and 3.9 µg/ml, respectively). Notably, this is the first report to examine the effect of Odorum essential oil against *H. pylori*, which attacks the human GIT and causes severe symptoms including peptic ulcer, gastritis, and gastric cancer, and requires a long-term regimen of multiple medications [32]. According to our current research, winter essential oil, which had a high D-limonene content (66.37%), was an excellent candidate for the treatment of *H. pylori* because it serves as both a powerful antioxidant and an antibacterial agent, and this may suggest further *in vivo* investigations on the winter essential oil effect against this GIT damaging pathogen. The powerful antioxidant function of limonene is essential in the treatment of *H. pylori* infection which causes harm to gastrointestinal tissues. Prior research identified limonene in particular to be responsible for the gastroprotective properties of citrus essential oil [33].

Furthermore, the MBC of the four essential oil samples against the eight tested micro-organisms was determined in table (4) to reveal the concentration at which the essential oil exerted its antimicrobial activity. The MICs and MBCs are in correlation to the inhibition zones, meaning that the smaller the MICs and MBCs the wider the diameter of the inhibition zones. The MBC range of the four essential oils was 1-1000 µg/mL. Summer essential oil had the lowest MBC against *B. subtilis*, *E. faecalis*, and *E. coli* (1.97, 125, and 15.6 µg/mL, respectively) suggesting a high potency against these three bacterial species. While fall essential oil showed the highest potency against *S. typhimurium* (MBC was 1.97 µg/ml). When tested against *H. pylori* and *P. aeruginosa*, winter essential oil showed the highest potency (MBC values of 7.8 and 1.97 µg/ml, respectively). Spring essential oil demonstrated the highest activity against *S. aureus* and *P. aeruginosa*, with MBC values of (3.9 and 1.97 g/ml, respectively).

Furthermore, the MBC/MIC ratio is an important metric that indicates if a compound has bacteriostatic or bactericidal potential. The MBC/MIC ratio for all seasons was equal to or less than 4 with few exceptions, revealing that these essential oils have potent bactericidal activity against all susceptible micro-organisms. The exceptions (MBC/MIC > 4) were for winter essential oil against *S. aureus* (MBC/MIC = 8), spring essential oil against *E. coli* (MBC/MIC = 8), and summer and spring essential oils against *S. typhimurium* (MBC/MIC= 16 and 8, respectively), which indicated their bacteriostatic activity.

This antibacterial activity of Odorum essential oil samples could be attributed to its lipophilic property, as the four examined essential oil samples according to the above-mentioned results of GC/MS analysis were composed mainly of hydrocarbons, which allows the essential oil to easily partition in the lipids of the bacterial cell membrane, disrupting its structure, making it more permeable, and causing loss of membrane integrity. This results in the leakage of vital components from the microbial cell and the interruption of the various cell actions, which leads to cell rupture and cell death [34].

D-limonene, a monoterpene hydrocarbon, carries out their antimicrobial effects by diffusing into cell membranes, which may account for the high antibacterial activity of Odorum's winter and fall essential oils against *H. pylori* and *P. aeruginosa* as these two essential oil samples were rich in D-limonene. On the other hand, the potent antimicrobial activity of both the summer and fall essential oils against *B. subtilis, S. aureus, E. faecalis, E. coli,* and

S. typhimurium may be attributed to their main component; E-methyl cinnamate [34].

Regarding the antifungal activity of Odorum essential oil, interestingly all the seasons' essential oil showed very powerful fungicidal activity against C. albicans, with the most powerful essential oil being the summer essential oil with inhibition zone, relative potency,and MIC of (43 ± 0.58 mm, 1.97 µg/ml, and 148%), respectively, when compared to Fluconazole. This strong fungicidal activity of the four essential oils can also be related to their major compounds; E- methyl cinnamate and D-limonene as they had been proven to show considerable antifungal activity against *C. albicans* in previous studies [34]. As well, D-limonene was considered one of the major constituents of four natural essential oils that showed strong antifungal activity against three types of fungi; *Aspergillusflavus, Penicilliumviridicatum*, and *Aspergilluscarbonarius*[35]. This demonstrated broad-spectrum antifungal activity of D-limonene against various fungal strains encourages further research into Odorum essential oil's antifungal effectiveness against other fungi.

So far, this is the first report on the influence of seasonal change on the antimicrobial activity of the cultivar Z*. piperitum* (L.) DC. 'Odorum'. Previous antimicrobial studies had been conducted on the volatile oil of *Z. piperitum* (having a relatively similar composition to Odorum cultivar) and supported our antimicrobial results as they showed potency against foodborne micro-organisms. Hence, the antimicrobial results of the Odorum essential oils encourage its use as a natural food preservative and antimicrobial agent against a wide spectrum of bacteria.

4.6. In silico prediction of the antimicrobial activity mechanism of action by molecular docking analysis:

According to the obtained docking scores (Suppl. Table 1), 10 of the analyzed compounds demonstrated superior affinity with higher binding scores than that of the native co-crystallized ligands mainly towards 3 target proteins (penicillin-binding protein, dihydrofolatereductase, and D-alanine D-alanine ligase). These 10 compounds were heptacosane, linolein 2-mono, linolein 1-mono, methyl octadecenoate, 9,12-octadecadienoic acid methyl ester, oleic acid methyl ester, linoleoyl chloride, 9,12-octadecadienoic acid methyl ester (E, E)-, phytol, and methyl hexadecanoate.

Regarding the set of target protein structures involved in antimicrobial activity, the results indicated an increased affinity of most docked structures towards the *β*-1,3-glucan synthase (6PAL). This *β*-1,3-glucan synthase (6PAL) is among the targets of clinically approved antifungal medicines and catalyzes the formation of the linear (1,3)-*β*-D-glucan in the fungal cell wall [36]. All the docked compounds showed higher binding scores to the target protein; *β*-1,3-glucan synthase (6PAL), than that recorded for the co-crystallized ligand, indicating a possible higher affinity for the target protein. Regarding the summer essential oil, most of its components (dehydronerolidol, linoleoyl chloride, heptacosane, methyl hexadecanoate, oleic acid methyl ester and 9,12-octadecadienoic acid methyl ester (E, E)-) showed higher binding scores (-5.366 & -7.341; -5.621 & -7.906; -6.377 & -9.654; -5.375 ; -8.376 & -5.746 & -8.497; -5.543 & -8.204), than that recorded for the antifungal drug Fluconazole (-4.788 and -6.194 kcal/mol), implying a potential increase in affinity for the target proteins *β*-1,3-glucan synthase (6PAL) and secretory aspartate protease (5JWG), respectively. The presence of hepatcosane, in summer essential oil, having the highest binding scores of (-6.377 and -9.654 kcal/mol, respectively), for the two target proteins (6PAL and 5JWG) compared to the co-ligand and Fluconazole could explain the powerful antifungal effect of the summer essential oil to other seasons' essential oil. The heptocosane (alkane class) structure was well accommodated in the 6PAL and 5JWG binding pocket by a hydrogen-pi interaction with Tyr45 and His 85, respectively, and these results were in accordance with our previous finding in minimal bactericidal concentration (MBC) in table (4). A recent review highlighted that the natural *β*-1,3-glucan synthase inhibitors could be classified as cyclic lipopeptides, glycolipids, and acidic terpenoids [37], but this study reveals that alkane class is one of the upcoming inhibitors on the same target. A previous study had correlated the improved antifungal effect of the other different oil components as limonene (monoterpene) and (E)- methyl cinnamate, the two major components in Odorum essential oil samples, to the existence of more double bonds in their structures [38]. These two major components indeed exerted their antifungal effect by increasing cell membrane permeability and inhibiting respiration via altering redox homeostasis in fungal cells [38] and both limonene and methyl cinnamate got stabilized through the formation of a hydrogen-pi bond with His85 and hydrogen bonds with Lys47 and Arg222. Therefore, the assumption that there is a synergistic antifungal effect between different classes of Odorum essential oil is highly plausible. Besides, summer essential oil was the most effective antibacterial agent against *B. subtilis*, *E. faecalis,* and *E. coli*, this was probably due to the presence of heptacosane which recorded the highest binding scores against 5 target proteins (penicillin-binding protein (1MWT), dihydrofolate reductase (4LAE), dihydropteroate synthase (2VEG), isoleucyl-tRNA synthetase (1JZS), and RNA polymerase enzyme (5UAH)) compared to the standard antibacterial drug Gentamycin. The heptacosane got structure stabilized in these 5 target proteins mostly through the

formation of a hydrogen-pi bond with His583 (1MWT), stabilized within the binding pocket of (4LAE) without need for bond formation indicating the high affinity, a hydrogen-pi bond with Phe151 (2VEG), a hydrogen-pi bond with His54 (1JZS), and again it was stabilized within the binding pocket of (5UAH) without need for bond formation indicating the high affinity as well.

As previously declared in MBC in table(4), the winter essential oil revealed the most powerful antibacterial effect against *H. pylori*, which is the main cause of peptic ulcer and gastric cancer, and the key target for its treatment is the urease enzyme [39]. All the docked compounds in winter essential oil showed moderate binding scores (-3.973 to -4.906 kcal/mol) to the target urease enzyme (1E9Z), compared to those recorded for the triplet antibiotic (-4.881 to -6.025 kcal/mol) and showed a good affinity for the target protein. Compared to other winter essential oil constituents, the *α*-terpinyl acetate and methyl caprylate had the highest binding energies (-4.906 and -4.832 kcal/mol) to urease enzyme (1E9Z), respectively, with the formation of one hydrogen bond with Lys212 and one hydrogen-pi bond with Tyr232, respectively. Other monoterpenes such as *β*-myrcene, D-limonene, terpinolene, linalool, and isopulegol, also exert their bactericidal effect by denaturing their cell walls, which allows vital nutrients to leak. Also, methyl cinnamate had interactions with the bacterial coating characteristics [40]. The two major oils' components; were limonene (-3.973 kcal/mol) which got stabilized within the binding pocket of urease without the need for bond formation indicating the high affinity, and methyl cinnamate (-5.081 kcal/mol) which formed one pi-hydrogen bond with Asp230. Although heptacosane had the highest binding score of (-6.673 kcal/mol) to the urease enzyme (1E9Z) and was detected only in the summer season, the summer essential oil showed a weak antibacterial effect (MBC = 125 µg/ml); in table (4) against *H. pylori*. This could be attributed to the weak penetration of the investigated compound which may be considered in future research.

In the fall season, the presence of certain constituents (fatty acids and their methyl esters) such as 9,12-octadecadienoic acid methyl ester, methyl octadecenoate, and linolein, 1-mono, which had the highest binding scores (-7.785, -7.663 and -9.945 kcal/mol) to the target proteins; DNA gyrase B kinase (6F86), topoisomerase IV (5YIG), and D-alanine D-alanine ligase (1EHI), respectively, compared to the standard antibacterial drug Gentamycin (-6.058, -6.580, and -7.267 kcal/mol, respectively). These three constituents got structure stabilized through the formation of one hydrogen bond with Asp73, one hydrogen bond with Gly86, and two hydrogen bonds with Ser186 and Asn318, respectively, and had assured the powerful antibacterial effect (MBC = 3.9 µg/ml); in table (4) of the fall season against *S. typhimurium*.

Similarly, several constituents in spring essential oil like methyl caprylate, geramcrene B, and *α*-terpinyl acetate showed moderate binding scores (-5.190 to -6.977 kcal/mol) to the 8 target proteins [DNA gyrase B kinase (6F86), topoisomerase IV (5YIG), penicillin-binding protein (1MWT), dihydrofolate reductase (4LAE), dihydropteroate synthase (2VEG), D-alanine D-alanine ligase (1EHI), isoleucyl-tRNA synthetase (1JZS) and RNA polymerase enzyme (5UAH)] compared to the standard antibacterial drug Gentamycin (-5.767 to -7.644 kcal/mol), clarifying its well-observed antibacterial effect against *S. aureus* and *P. aeruginosa* (MBC = 3.9 and 1.97 µg/ml); in table (4).

5. Conclusions

The findings of the present study have proven the importance of the Odorum essential oil as a natural effective broad-spectrum antimicrobial agent and encourage further studies to consider its potential usage as a replacement for artificial food preservatives in the food industry or a promising antibacterial agent of natural source.

6. Conflicts of interest

The authors declare no conflicts of interest.

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