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Chemical composition and biological activity of lemongrass volatile oil and *n*-Hexane extract: GC/MS analysis, *in vitro* and molecular modelling studies

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Abstract

Lemon grass, formally identified as *Cymbopogon citratus*, is a plant that belongs to the Poaceae family. The present work aimed to examine the chemical composition by GC/MS analysis and assess the biological potential of *C. citratus* volatile oil and *n*-hexane extract. The volatile oil and *n*-hexane extract were evaluated for antioxidant potential and tested for their enzyme inhibition against tyrosinase, butyrylcholinesterase (BChE), acetylcholinesterase (AChE), *α*-amylase, and *α*-glucosidase. The chemical analysis of the lemongrass n-hexane extract (HE) and volatile oil (VO) revealed that the main constituents in the HE are aliphatic hydrocarbons (42.98%), triterpenoids (20.14%), and aromatic hydrocarbons (17.25%). Conversely, the main constituents of the (VO) are predominantly monoterpenes, namely *α*-citral (36.08%), *β*-citral (34.22%), and *β*-myrcene (13.84%). The oil showed more potent antioxidant potential in DPPH, ABTS, CUPRAC, FRAP, and phosphomolybdenum (10.18, 35.69 mg Trolox equivalent/g, 98.97 and 69.73 mg Trolox equivalent/g and 43.01 mmol Trolox equivalent/g). The HE displayed higher BChE (1.53 mg Galanthamine equivalent)/g), as well as *α*-amylase and *α*-glucosidase inhibitory activities (0.39 and 2.40 mmol Acarbose equivalent/g). The VO demonstrated more potent tyrosinase inhibitory activities (57.19 mg Kojic acid equivalent/g) along with acetyl and butyrylcholinesterase inhibition. Dominant compounds exhibited the ability to bind with high affinity to various target proteins, with a particular affinity for AChE and BChE. The volatile oil and *n*-hexane extract of *C. citratus* show significant promise as a viable choice for the advancement of novel therapeutic strategies aimed at addressing oxidative stress, neurodegeneration, and diabetes.

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1. Introduction

In public and primary healthcare, medicinal plants have evolved into the most prominent and safest alternative medications [1,2]. These therapeutic herbs are essential agents within the primary healthcare system, contributing to the overall well-being and maintenance of good health [3–5]. Notably, more than two-thirds of the world's population currently depends on medicinal plants as a fundamental source of therapeutic medications [6]. The rise in popularity of these natural therapies can be ascribed to their ubiquitous acceptability, compatibility, flexibility, and the negligible negative effects encountered when applied to the human body [7–9].

Cymbopogon is a genus within the Poaceae (Gramineae) family, comprising approximately 144 identified species. Its distribution spreads extensively across tropical and subtropical areas of Africa, Asia, and Central America. The abundance of essential oils in this plant has earned it a reputation for its widespread usage in fragrance, skin care products, and therapeutic applications [10]. One of the most renowned species in the *Cymbopogon* genus is *C. citratus*, typically known as Lemongrass and found worldwide [10]. It is well known for its lemon-like essence owing to the presence of citral as its major component [11]. Lemongrass remains a popular traditional remedy for addressing the plethora of ailments such as coughs, epithelial diseases, flu, pneumonia, malaria, headaches, gingivitis, leprosy, and vascular disorders. Additionally, in certain regions, it is employed to alleviate acne, pimples, and blackheads, as well as to combat lice and dandruff [11,12].

Lemongrass is a highly abundant reservoir of several secondary metabolites, including the volatile oil that constitutes citral, as well as polyphenolic components such as luteolin, apigenin, chlorogenic acid, *p*-coumaric acid, and caffeic acid [13–15]. Notably, monoterpenes are the main constituents of the hydrodistilled oil that is obtained from *Cymbopogon* species [16]. In addition, several pharmacological investigations on *C. citratus* have demonstrated its medicinal potential as an antioxidant, antibacterial, antiaging, hypoglycemic, cytotoxic, anti-inflammatory, and insect-repellent agent [17–20]. Various reports on the biological activity of various *Cymbopogon* species have been published [12]. For example, Rhimi et al. reported the antifungal activity of *C. citratus* and *C. proximus* known as (Halfbar) towards *Candida* spp [16]. The *C. citratus* methanolic extract exhibited antioxidant and α-glucosidase inhibitory properties [20]. Another investigation provided evidence that the ethyl extract of *C. citratus* is efficacious in the management of acne vulgaris [11]. *Cymbopogon citratus* is distinguished from other Cymbopogon species by its unique fragrant characteristics, diverse food and therapeutic uses, abundant phytochemical profile, and considerable economic significance [10,21].

Molecular docking was selected as the optimal computational technique to evaluate the underlying mechanism of inhibitory effect for the biologically active constituents, thereby elucidating the interactions between the enzyme and the principal secondary metabolites [22–24]. It involves predicting the preferred orientation and binding affinity of small molecules (ligands) to target proteins or receptors, which is essential for understanding molecular interactions [25]. The primary purpose of the present research is to provide insight into the differences in the phytochemical composition of the volatile oil and *n*-hexane extract of *C. citratus* by GC/MS analysis as the method of analysis. Additionally, various *in vitro* studies have been conducted to systematically evaluate the potential of *C. citratus* volatile constituents as antioxidant, anti-Alzheimer's, anti-diabetic, and anti-tyrosinase agents. Molecular modeling was conducted to investigate the interactions between the tested enzymes and major constituents. These findings present compelling opportunities for the potential use of *C. citratus* in managing various illnesses. The graphical abstract summarizes the chemical analysis, and the *in vitro* biological assays performed on *Cymbopogon citratus* volatile oil.

2. Materials and methods

2.1. Plant Material

The fresh leaves of lemon grass (*Cymbopogon citratus*) were acquired in February 2022 from a privately-owned farm placed in Shibin Al Kawm City, Al Minufiyah, Egypt. Professor Usama K. Abdel Hameed from the Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt, verified the authenticity of the plant. The Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt received a voucher specimen for the deposit, identified by the code PHG-P-CC-463.

2.2. Isolation of the volatile oil of lemongrass

Finely chopped fresh leaves weighing 500 gm of *C. citratus* were hydrodistilled using distilled water for 5 hours on a Clevenger apparatus. Upon completion of the distillation process, the light, yellow-coloured essential oil was gathered, measured, and dehydrated using anhydrous sodium sulphate. The mean hydrodistillation output was 0.25% (v/w). The sample was stored in sealed opaque glass vials at a temperature of -4 °C until it was subjected to GC/MS and subsequent examination.

2.3. Preparation of the n-hexane extract of lemongrass

In the extraction procedure, 100 gm of desiccated leaves of *C. citratus* were subjected to cold maceration in distilled analytical quality *n*-hexane (Nasr Pharmaceuticals, Cairo, Egypt) for 48 hours until complete exhaustion. After filtering and concentrating the extracts in vacuo at 45 °C until dry, the dried residue (3.1 g) was obtained with a yield of 3.1%. The yield was determined per 100 gm of lemon grass and reported as a percentage (w/w) [26]. For further investigation, the extract was kept in a tightly sealed container in the refrigeration unit.

2.4. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis was performed on a Kyoto, Japan-made Shimadzu GCMS-QP 2010 chromatograph with a DB-5 capillary column from Bellefonte, PA, USA. It has a 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness. The injection temperature was 250 °C. After 2 min of isothermal setup at 45 °C, the temperatures were raised to 300 °C at 5 °C/min and kept isothermal for 5 min. Helium was used as the inert carrier gas at 1.41 mL/min. Diluted samples (1% volume/volume) were injected at a 15:1 split ratio with a 1 μL injection volume. The study was conducted using the following MS operating parameters: the interface temperature was kept constant at 280 °C, the ion-source temperature was set at 220 °C, the electron ionisation (EI) mode was employed at 70 eV, and the scan range extended from 35 to 500 amu. The hexane sample was exposed to the same conditions, except the oven temperature. More precisely, the oven temperature was first adjusted to 50 °C and maintained for 3 minutes. Next, the temperature was raised at an average rate of 5 °C per minute until it reached 300 °C and then held at that level for 10 minutes [27–29]. To identify the essential oil components, the mass fragmentation data (MS) and Kovats indices (KI) were compared with the data found in the NIST Mass Spectral Library (2011) and the 4th edition of Adams [30] with the information provided in the 8th edition of the Wiley Registry of Mass Spectral Data, together with relevant details documented in the scientific literature [31–34].

2.5. Total phenolic and flavonoid content

To determine the total phenolic and flavonoid contents, the Folin-Ciocalteu and $AlCl_3$ tests were employed [35]. The findings of the studies were reported as gallic acid equivalents (mg

GAEs/g sample) and rutin equivalents (mg REs/g sample). All relevant experimental information can be found in the Supplementary Materials.

2.6. Antioxidant assays

The antioxidant activity of the samples under investigation was evaluated in triplicate using well-established techniques as previously described [36,37]. Positive controls in the antioxidant studies included trolox and EDTA. Quantification of the radical scavenging activity of DPPH and ABTS, together with CUPRAC and FRAP, was performed and reported as mg of Trolox equivalents (TE) per gram of the measured sample. The metal chelating assay (MCA) quantified the amount of ethylenediaminetetraacetic acid equivalents (EDTAE) per gram of the evaluated sample. Total antioxidant activity, measured by the phosphomolybdenum test (PM), was expressed as mmol TE/g extract [38,39].

2.7. Enzyme inhibition assays

An analysis was conducted to examine the inhibitory properties of the essential oils against AChE, BChE, tyrosinase, amylase, and glucosidase. Previous methodologies were employed to ascertain the activities in triplicate [36,37]. AChE and BChE inhibitory activities were quantified in mg of galanthamine equivalents (GALAE)/ g of the tested sample, tyrosinase in mg of kojic acid equivalents (KAE)/g of the tested sample, and amylase and glucosidase in mg of acarbose equivalents (ACAE)/g of the tested sample [38,39].

2.8. Molecular modeling

The X-ray crystal structures of the specifically chosen target proteins were obtained from the protein data bank available at (https://www.rcsb.org/) [40] α-amylase (PDB ID: 1B2Y) [41], AChE (PDB ID: 6O52) [42], BChE (PDB ID: 6EQP) [43]. A novel homology model of human tyrosinase was constructed by utilizing natural sequences (UniProt entries: P14679) and the three-D structure of tyrosinase from Priestia megaterium (PDB ID: 6QXD) [44].; and that of α-glucosidase was constructed using the 3D structure of Mus musculus glucosidase (PDB ID: 7KBJ) [45] as a template [46] from human sequence (UniProt entries: P0DUB6). All proteins have been created following the previously detailed procedure [47]. The 3D structures of the ligands were derived from the ChemSpider database (https://www.chemspider.com/) and then optimized using the UCSF Chimaera software [48]. Every docking grid file was generated using the cocrystal ligand of each protein using MGLTools 1.5.6 software. This software combined all hydrogen atoms and assigned gasteiger partial charges to all protein atoms. Computer simulation of docking was conducted using the Lamarckian genetic process in AutoDock 4.2.6 software available at https://autodock.scripts.edu [49] and adopting the docking protocol [50]. Binding energy scores for ligand poses were calculated and protein-ligand interaction was investigated using Biovia DS Visualizer v4.5 from BIOVIA in San Diego, CA, USA.

2.9. Statistical analysis

The experiments were performed in triplicate, and differences between the volatile oil and hexane extract were compared using the Multiple t-test ($p < 0.05$). GraphPad Prism 6.01 (GraphPad Inc., La Jolla, CA, USA).

3. Results and discussion

3.1. GC/MS analysis of the volatile oil and n-hexane extract of lemongrass

The abundance of bioactive phytochemicals found in plants is primarily responsible for their potential biological properties [4,8,9,51–53]. In the current study, The GC/MS analysis was

employed for both qualitative and quantitative analysis of the constituents in the volatile oil of the lemongrass and *n*-hexane extract (Fig 1). Table 1 provides the identified compounds, their percentages, and the retention indices. A total of fifty-three and twelve compounds were identified in the VO and HE of lemon grass accounting for 100% and 87.56%, respectively. On one hand, the VO was predominated with the oxygenated monoterpenes (84.95%) followed by monoterpene hydrocarbons (13.84%). Where the major constituents were found to be *α*-citral (36.08%), *β* -citral (34.22%) and *β*-myrcene (13.84%). On the other hand, the HE was predominated with aliphatic hydrocarbons (42.98%), triterpenoids (20.14%), and aromatic hydrocarbons (17.25%). Where, lupeol 16.23%) and betulinaldehyde (3.91%). Regarding the hydrocarbons, *n*-propylcyclohexane, octane, 2,6-dimethyl, 3-cyclohexylpropyl alcohol, hemellitol, and *n*-decane were the major identified components in the hexane extract. The chemical structures of the major identified constituents are represented in (Fig 2).

Prior investigations on the essential oil constituents of *C. citratus* leaves have shown that the primary recognizable constituents are found to be the two isomers of citral and *β*-myrcene [54]. Moustafa et al. showed that *C. citratus* leaves from El-Kanater El-Khairiya, Egypt, comprise the main oil components as *α*-citral and *β*-citral, accounting for 35.91% and 35% respectively [55]. Additionally, the oil of *C. citratus* leaves from Eastern Nepal showed a similar pattern, with neral (36.1%) and geranial (53.1%) monoterpene aldehydes as the predominant components, and smaller quantities of (*E*)-caryophyllene and caryophyllene oxide [56]. While the roots are rich in α-elemol, geranial, neral and *α*-eudesmol [56]. Meanwhile, the *n*-hexane extract of *C. citratus* stem and rhizomes from Malaysia predominated with fatty acids as linoleic acid, methyl palmitate, 7,10-octadecadienoic acid, methyl ester, in addition to stigmasterol [57]. According to another research conducted by Cortes-Torres et al., the essential oil from the leaves of *C. citratus* obtained from Puebla, Mexico revealed a significant presence of myrcene, *Z*-geranial, and *E*-geranial [58]. This work conducted by Guerrini et al. focuses on the detection of volatile components in cultivated plants of *C. citratus* growing in an Amazonian Ecuador region using two different approaches; the first method involved hydrodistillation of the oil followed by GC/MS analysis, which indicates that the primary constituents were oxygenated monoterpenes (89.05%), with geraniol, geranial, and neral being the most abundant. On the other hand, the headspace fractions analysis revealed a lower percentage of oxygenated monoterpenes (68.64%), with *cis*-Isocitral and *trans*-Isocitral being the major components [59]. Another study revealed nine components in *C. citratus* oil and eight in *C. proximus*. The main compounds in *C. citratus* were geranial and neral, while *C. proximus* had piperitone and α-terpinolene [16]. Based on the aforementioned findings, it can be deduced that citral, specifically neral and geranial, is consistently found in high concentrations in *C. citratus* from various sources and various geographical locations. Therefore, citral can serve as a crucial indicator for the identification and verification of *C. citratus* which was characterized in the hydrodistilled oil. Significantly, the chemical composition of the volatile oil obtained through hydrodistillation differs from that of the hexane extract, as the hydrodistillation method is more adept at extracting a greater variety of volatile compounds, particularly lighter and more volatile components [26]. Hexane extraction mainly extracts non-polar compounds, including lipids, fats, and hydrophobic compounds such as terpenes and certain aromatic compounds [27,29].

3.2. Total phenolic and flavonoid content of n-hexane extract of lemongrass

The increasing prevalence of natural antioxidants, particularly polyphenols, can be ascribed to their advantageous impact on the health of humans. Polyphenols possess the capacity to

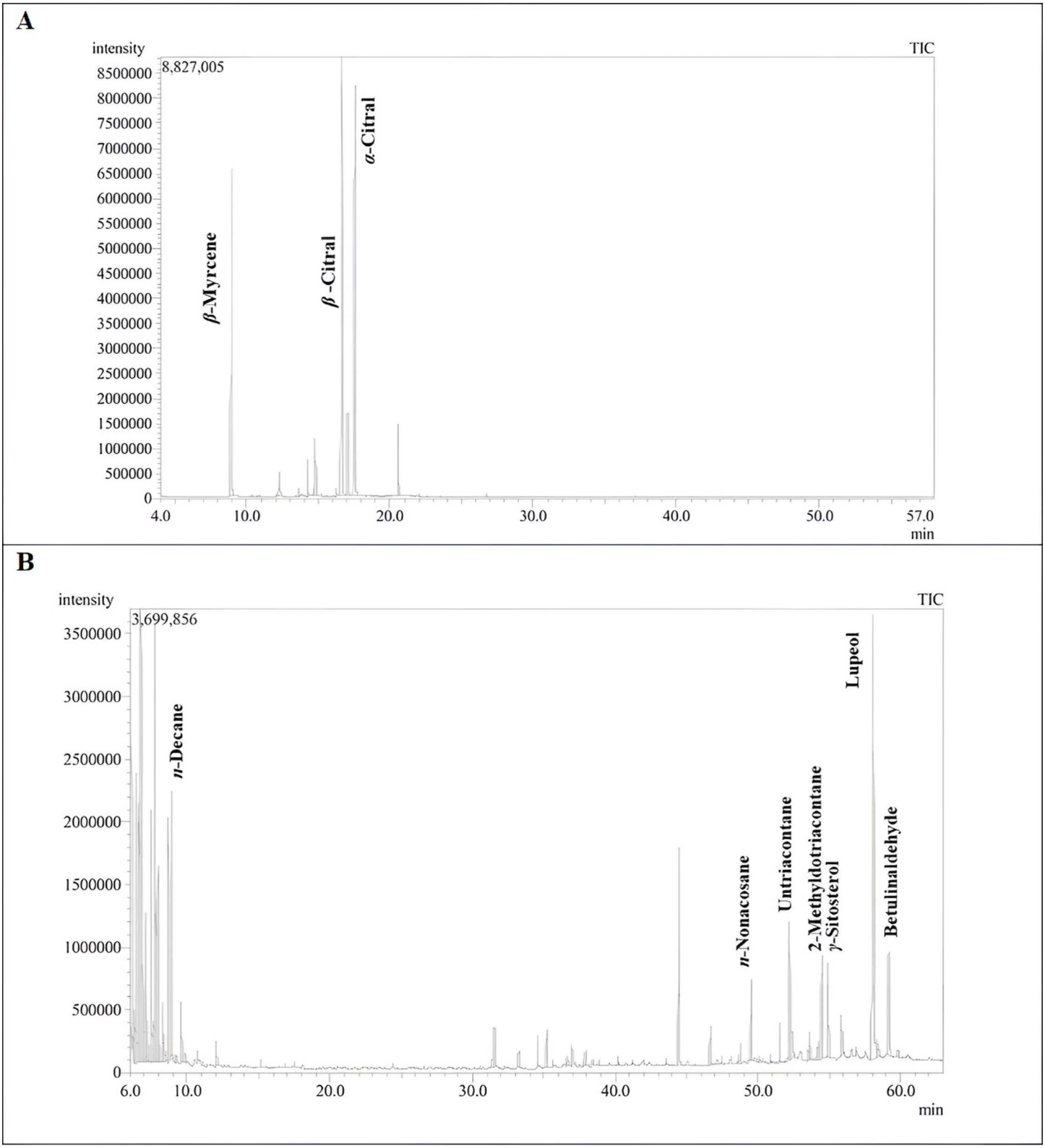

Fig 1. GC chromatogram of (A) Volatile oil and (B) *n*-Hexane extract of Lemongrass.

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Table 1. Chemical composition (%) of the volatile oil and *n*-hexane extract of lemongrass using GC/MS analysis.

No.	$Rt_{(min)}$	Compound	$RI_{Exp.}$[a]	RI_{Lit}[b]	Molecular Formula	Content (%)	
						HE	VO
1	6.08	1-Ethyl-4-methylcyclohexane	901	896	C_9H_{18}	2.82	–
2	6.16	Cyclohexane, 1-ethyl-1-methyl-	903	903	C_9H_{18}	1.32	–
3	6.43	**3-Cyclohexylpropyl alcohol**	912	–	$C_9H_{18}O$	**5.40**	–
4	6.60	**2-Phenylpropane**	918	919	C_9H_{12}	**3.65**	–
5	6.73	***n*-Propylcyclohexane**	923	924	C_9H_{18}	**6.27**	–
6	6.85	**Octane, 2,6-dimethyl**	927	933	$C_{10}H_{22}$	**4.26**	–
7	7.06	Heptane, 3-ethyl-2-methyl	934	940	$C_{10}H_{22}$	1.98	–
8	7.19	2,6-Dimethyloctane	938	935	$C_{10}H_{22}$	0.74	–
9	7.32	2,4,4,6-Tetramethyl-2-heptene	943	942	$C_{11}H_{22}$	0.25	–
10	7.70	*m*-Ethyltoluene	956	958	C_9H_{12}	6.19	–
11	7.76	Octane, 4,5-diethyl-	958	–	$C_{12}H_{26}$	3.49	–
12	7.91	Mesitylene; 1,3,5-Trimethylbenzene	963	964	C_9H_{12}	2.87	–
13	8.26	*o*-Ethylmethylbenzene	975	973	C_9H_{12}	0.83	–
14	8.40	*trans-p*-Menthane 1-Methyl-4-(1-methyl ethyl)-cyclohexane	979	978	$C_{10}H_{20}$	0.47	–
15	8.48	*cis*-Hydrindan	982	981	C_9H_{16}	0.17	–
16	8.68	**Hemellitol**	989	996	C_9H_{12}	**3.71**	–
17	8.79	Sulcatone (Methyl heptanone)	987	987	$C_8H_{14}O$	–	1.21
18	8.88	***n*-Decane**	996	1000	$C_{10}H_{22}$	**3.48**	–
19	8.94	***β*-Myrcene**	992	992	$C_{10}H_{16}$	–	**13.84**
20	9.21	2,5-Dimethylnonane	1007	1024	$C_{11}H_{24}$	0.10	–
21	9.58	5-Ethyl-2-methyl heptane	1018	1019	$C_{10}H_{22}$	0.96	–
22	9.85	Butylcyclohexane	1027	1030	$C_{10}H_{20}$	0.10	–
23	10.69	Isobutyl acetoacetate	1053	1060	$C_8H_{14}O_3$	0.13	–
24	12.03	*n*-Undecane	1096	1100	$C_{11}H_{24}$	0.48	–
25	12.26	*β*-Linalool	1099	1099	$C_{10}H_{18}O$	–	1.09
26	13.66	7-Methyl-3-methyleneoct-6-enal	1145	1146	$C_{10}H_{16}O$	–	0.33
27	14.26	Isoneral	1165	1183	$C_{10}H_{16}O$	–	1.59
28	14.60	Rose furan oxide	1176	1174	$C_{10}H_{14}O_2$	–	0.34
29	14.81	Isogeranial	1183	1184	$C_{10}H_{16}O$	–	2.27
30	16.26	Nerol	1231	1228	$C_{10}H_{18}O$	–	0.53
31	16.67	***β* -Citral (Neral)**	1246	1248	$C_{10}H_{16}O$	–	**34.22**
32	17.05	*trans*-Geraniol (Lemonol)	1259	1259	$C_{10}H_{18}O$	–	5.65
33	17.57	***α*-Citral (Geranial)**	1276	1277	$C_{10}H_{16}O$	–	**36.08**
34	20.58	Nerol acetate	1384	1381	$C_{12}H_{20}O_2$	–	2.85
35	31.36	Neophytadiene	1825	1830	$C_{20}H_{38}$	0.11	–
36	31.51	Hexahydrofarnesyl acetone	1832	1835	$C_{18}H_{36}O$	0.62	–
37	33.20	Palmitic acid, methyl ester	1920	1923	$C_{17}H_{34}O_2$	0.25	–
38	34.57	Palmitic acid, ethyl ester	1987	1990	$C_{18}H_{36}O_2$	0.45	–
39	35.16	Isopropyl palmitate	2017	2023	$C_{19}H_{38}O_2$	0.59	–
40	36.54	Linoleic acid, methyl ester	2088	2082	$C_{19}H_{34}O_2$	0.12	–
41	36.68	Linolenic acid, methyl ester	2096	2096	$C_{19}H_{32}O_2$	0.19	–
42	36.96	Stearic acid, methyl ester	2110	2109	$C_{19}H_{38}O_2$	0.52	–
43	37.69	Linoleic acid	2150	2152	$C_{18}H_{32}O_2$	0.11	–
44	37.79	Linoleic acid ethyl ester	2156	2155	$C_{20}H_{36}O_2$	0.21	–
45	37.93	Linolenic acid, ethyl ester	2163	2169	$C_{20}H_{34}O_2$	0.30	–

(Continued)

Table 1. (Continued)

No.	$Rt_{(min)}$	Compound	$RI_{Exp.}$[a]	RI_{Lit}[b]	Molecular Formula	Content (%)	
						HE	VO
46	40.20	*n*-Tricosane	2289	2300	$C_{23}H_{48}$	0.13	–
47	46.63	*n*-Heptacosane	2688	2700	$C_{27}H_{56}$	0.61	–
48	47.43	Palmitic acid, neryl ester	2742	2726	$C_{26}H_{48}O_2$	0.12	–
49	48.60	Squalene	2823	2823	$C_{30}H_{50}$	0.14	–
50	49.51	*n*-Nonacosane	2887	2900	$C_{29}H_{60}$	1.22	–
51	50.88	*n*-Triacontane	2986	3000	$C_{30}H_{62}$	0.11	–
52	51.49	*n*-Octacosanal	3032	3032	$C_{28}H_{56}O$	0.66	–
53	52.21	Untriacontane	3087	3100	$C_{31}H_{64}$	2.21	–
54	52.35	Octacosanol	3098	3110	$C_{28}H_{58}O$	0.94	–
55	53.51	Dotriacontane	3187	3200	$C_{32}H_{66}$	0.21	–
56	53.62	*β*-Sitosterol	3195	3197	$C_{29}H_{50}O$	0.54	–
57	54.08	Stigmasterol	3227	3248	$C_{29}H_{48}O$	0.27	–
58	54.20	Triacontanal	3236	3251	$C_{30}H_{60}O$	0.32	–
59	54.45	2-Methyldotriacontane	3253	3259	$C_{33}H_{68}$	2.36	–
60	54.93	*n*-Tritriacontane	3286	3300	$C_{33}H_{68}$	1.91	–
61	55.82	*γ*-Sitosterol	3342	3351	$C_{29}H_{50}O$	1.63	–
62	58.07	**Lupeol**	3554	3450	$C_{30}H_{50}O$	**16.23**	–
63	58.30	*γ*-Sitostenone	3570	3458	$C_{29}H_{48}O$	0.55	–
64	58.45	*n*-Hexatriacontane	3581	3600	$C_{36}H_{74}$	0.35	–
65	59.14	**Betulinaldehyde**	3629	3628	$C_{30}H_{48}O_2$	**3.91**	–
Monoterpene Hydrocarbons						**0.47**	**13.84**
Oxygenated Monoterpenes						–	**84.95**
Diterpenoids						**0.11**	–
Sesquiterpene Hydrocarbon						**0.14**	–
Oxygenated Sesquiterpene						**0.62**	–
Triterpenoids						**20.14**	–
Sterols						**2.99**	–
Fatty acids and fatty acids derivatives						**2.86**	–
Aromatic Hydrocarbons						**17.25**	–
Aliphatic Hydrocarbons						**42.98**	–
Others						–	**1.21**
Total identified compounds						**87.56**	**100**

[a]Retention index calculated experimentally on DB-5 column relative to n-alkane series (C8–C28).

[b]Reported retention indices. Compounds are listed in order of their elution on DB-5 GC column.

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alleviate the oxidative stress sequences linked to several diseases including cancer, diabetes, inflammation, wound healing, cardiovascular abnormalities, and neurodegenerative disorders [60–64]. Total phenolic and flavonoid content in the (HE) of lemongrass leaves was quantitatively determined. The findings showed high phenolic levels represented as 7.44 ± 0.34 mg GAE/g (gallic acid equivalent) and total flavonoid content of 1.91 ± 0.06 mg RE/g (rutin equivalent). The presence of phenolics in the hexane extract would add value to its antioxidant activity and mitigate various illnesses associated with oxidative stress such as neurodegeneration disorders [65,66]. In prior research on the total flavonoids and phenolics in various lemongrass extracts, a previous study on the ethanol extract of lemongrass leaves showed TPC equivalent to 67.28 mg GAE/g extract [67]. In another study, using the aqueous

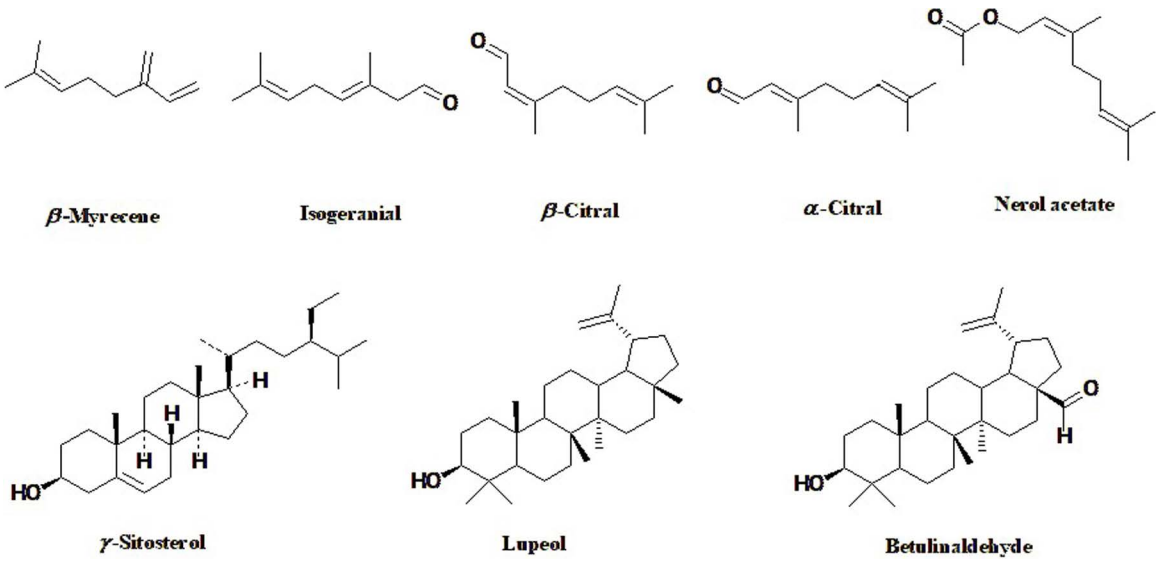

Fig 2. Chemical structures of the major constituents identified in the volatile and *n*-hexane extract of Lemongrass using GC/MS analysis.

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Table 2. Antioxidant potential of the volatile oil and *n*-hexane extract isolated from the Lemongrass.

Samples	DPPH	ABTS	CUPRAC	FRAP	MCA	PM
	(mg TE/g)	(mg TE/g)	(mg TE/g)	(mg TE/g)	(mg EDTAE/g)	(mmol TE/g)
Volatile oil	10.18 ± 0.43[a]	35.69 ± 0.31[a]	98.97 ± 1.12[a]	69.73 ± 1.10[a]	31.16 ± 0.88[a]	43.01 ± 0.06[a]
***n*-Hexane extract**	6.86 ± 0.81[b]	5.97 ± 0.33[b]	30.57 ± 3.44[b]	12.50 ± 0.14[b]	25.76 ± 0.56[b]	1.00 ± 0.04[b]

Values expressed as means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: Ethylenediaminetetraacetic acid equivalent; n.a.: not active. Different letters indicate significant differences in the extracts ($p < 0.05$).

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extract it showed TPC and TFC equivalent to 10.7 ± 0.2 mg GAE/g and 23.9 ± 0.3 mg quercetin/g, respectively [68]. Also, the concentrations of total phenolics and flavonoids in the ethyl acetate extract were 132.31 mg caffeic acid/g extract and 104.50 mg naringin/g extract, respectively [11].

3.3. The in vitro antioxidant potential of volatile oil and n-hexane extract of lemongrass

The present work used many assays to assess the in vitro antioxidant properties of the VO and HE of Lemongrass. By the phosphomolybdenum test, the total antioxidant capacity of the VO was determined to be 43.01 ± 0.06 mmol TE/g. Besides, the VO was found to possess notable reducing activity as evidenced by its activity in the CUPRAC and FRAP assays (98.97 ± 1.12 and 69.73 ± 1.10 mg TE/g, respectively). Similarly, it demonstrated notable radical scavenging potential in both the DPPH and ABTS assays (10.18 ± 0.43 and 35.69 ± 0.31 mg TE/g, respectively). It is noteworthy that the HE radical scavenging potential in the DPPH experiment exhibited a similar pattern, measuring 6.86 ± 0.81 mg TE/g. It was notable that the VO and HE of lemongrass showed a significantly different metal chelating activity of 31.16 ± 0.88 and 25.76 ± 0.56 mg EDTAE/g, respectively. Whereby the HE demonstrated lower reducing

Table 3. Enzyme inhibitory effects of the volatile oil and *n*-hexane extract isolated from the Lemongrass.

Samples	AChE Inhibition (mg GALAE/g)	BChE Inhibition (mg GALAE/g)	Tyrosinase Inhibition (mg KAE/g)	α-Amylase Inhibition (mmol ACAE/g)	α-Glucosidase Inhibition (mmol ACAE/g)
Volatile oil	1.45 ± 0.17	1.01 ± 0.23[a]	57.19 ± 5.87[a]	n.a.	n.a.
***n*-Hexane extract**	n.a.	1.53 ± 0.08[a]	45.71 ± 0.80[a]	0.39 ± 0.003	2.40 ± 0.005

Values expressed as means ± S.D. of three parallel measurements. GALAE: Galanthamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; n.a.: not active. Similar letters indicate non-significant differences in the extracts ($p < 0.05$).

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potential in CUPRAC and FRAP assays (30.57 ± 3.44 and 12.50 ± 0.14 mg TE/g, respectively). Moreover, the HE has a lower total antioxidant capacity of 1.00 ± 0.04 mmol TE/g revealed by the phosphomolybdenum assay (Table 2).

The volatile oil has shown relatively greater antioxidant activity in scavenging and reducing tests. The observed outcome can be ascribed to the high levels of bioactive constituents found in the VO as compared to the HE (Table 1). The presence of the major compounds; α-citral, β-citral and β-myrcene accounts for the volatile oil's possible antioxidant action. The findings of our study were consistent with those of prior investigations, the essential oil of lemongrass at a concentration of 100 μL/mL in ethanol showed DPPH inhibition by 76.30 ± 1.23% [58]. Interestingly, different plants with high percentages of oxygenated monoterpenes represented in α-citral and β-citral showed antioxidant potential. A previous study on *Cinnamomum bodinieri* revealed the high amount of citral isomers and recorded a high antioxidant activity with IC_{50} values of 6.887 ± 0.151 and 19.08 ± 0.02 mg/mL in DPPH and ABTS assays, respectively [58]. A recent study by Guerrini et al. in an Amazonian region of Ecuador examined the antioxidant potency of cultivated plants of *C. citratus*, the results revealed IC_{50} values of 2.270 ± 0.340 and 4.322 ± 0.651 mg/mL in DPPH and ABTS assays, respectively [59]. The petroleum ether fraction of lemon grass showed antioxidant activity by 0.12 ± 0.0082 and 0.042 ± 0.0045 mmol TE/g in DPPH and FRAP assays, respectively [11]. Regarding the major identified constituents, citral showed antioxidant due to the DPPH scavenging activity and hepatoprotective properties [69,70]. Gupta et al. showed that lupeol elevated the antioxidant levels and reduced the level of thiobarbituric acid-reactive oxygen species [71]. Also, lupeol showed reduction in the reactive oxygen species in the neurons acting as therapeutic agent in neurodegenerative disorders [72]. Moreover, lupeol isolated from *Ficus pseudopalma* exhibited antioxidant activity in DPPH and FRAP assays [73].

3.4. Enzyme inhibitory potential of the volatile oil and n-hexane extract of lemongrass

The application of enzyme inhibitors has important consequences in the management and treatment of diseases [74]. Consequently, extensive research efforts have been and continue to be undertaken to explore novel enzyme inhibitors that can enhance the effectiveness of therapies administered to patients. To examine the volatile oil and *n*-hexane extract efficiency as enzyme inhibitors, four important enzymes (acetyl-/butyryl-cholinesterase, tyrosinase, α-amylase, and α-glucosidase) were used. (Table 3) illustrates the findings.

The VO exhibited inhibitory effects on three examined enzymes: AChE, BChE, and tyrosinase, with values of 1.45 ± 0.17 mg GALAE/g, 1.01 ± 0.23 mg GALAE/g, and 57.19 ± 5.87 mg

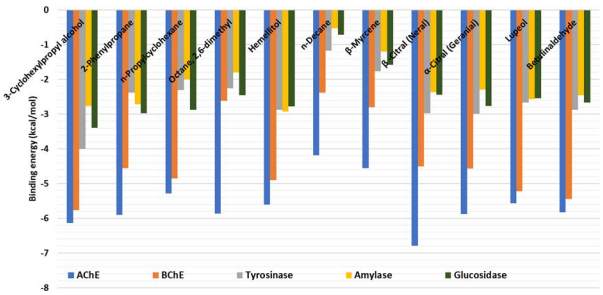

Fig 3. Docking score of main phytochemicals in the Lemongrass volatile oil and *n*-hexane extract.

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KAE/g, respectively. While the HE displayed BChE and tyrosinase inhibitory activities of 1.53 ± 0.08 mg GALAE/g and 45.71 ± 0.80 mg KAE/g, respectively. Moreover, the HE showed antidiabetic activity represented in inhibition of *α*-amylase and *α*-glucosidase equivalent to 0.39 ± 0.003 and 2.40 ± 0.005 mmol ACAE/g, respectively) by contrast the VO did not display any *α*-amylase or *α*-glucosidase inhibitory activities.The lack of inhibitory activity in the VO compared to the significant effects observed with the HE can be explained by differences in chemical composition, nature of inhibition, and potential synergistic effects among compounds present in each extract. The presence of lupeol as a major compound in the hexane extract, postponed carbohydrate digestion and absorption, thereby diminishing postprandial hyperglycaemia in rats as well as inhibiting α-amylase enzyme [75].

The enzyme acetylcholinesterase operates rapidly to degrade acetylcholine, a neurotransmitter, therefore terminating transmission between neurons at cholinergic synapses. Enzyme inhibitors are included in the therapeutic regimen for several neurological disorders and are presently the principal medication authorised by the FDA for the management of Alzheimer's disease (AD) [76,77]. Naturally occurring cholinesterase inhibitors, specifically from plant source, have gained significant interest in this regard, as they have shown effectiveness in the management of Alzheimer's disease (AD) [78,79]. A prior investigation conducted by Madi et al. demonstrated that the essential oil of *C. citratus* exhibits acetylcholinesterase inhibitory activity in various seasons. Specifically, the oil sample in winter demonstrated moderate AchE inhibitory activity with an IC_{50} value of 2.86 ± 0.17 mg/mL, which was comparatively to physostigmine (IC_{50} 0.012 mg/mL) [54]. The regulation of blood sugar to control hyperglycaemia in diabetic patients is based on the inhibition of carbohydrate-metabolizing enzymes; *α*-amylase and *α*-glucosidase [80]. The observed antidiabetic findings align with prior research on the petroleum extract that showed an IC_{50} value of 1.77 ± 0.55 *μ*g/mL towards *α* -glucosidase [11]. A previous study found that the methanol extract of *C. citratus* inhibits *α*-amylase with an IC_{50} value of 0.31 mg/mL. At a concentration of 1 mg/mL, the hexane extract inhibited *α*-glucosidase by 99% [81]. The promising tyrosinase inhibitory activity of lemongrass oil following previous reports regarding the potential effects of *n*-hexane extract of lemongrass on tyrosinase, elastase and collagenase enzymes with inhibitory percentages of 25.41 ± 0.68 and 49.05 ± 1.20, 72.89 ± 1.97%, respectively [11]. The interactions between catalytic residues and Cu^{2+} ions in tyrosinase are fundamental to its enzymatic function, influencing substrate binding, catalysis, and regulatory mechanisms [82]. The volatile constituents can indeed function as catalytic residues in various enzymatic reactions due to their ability to donate or accept electrons, participate in redox reactions, and stabilize reaction intermediates [83]. Citral the major constituent in lemongrass oil showed a non-competitive inhibitory effect against a fungal source of tyrosinase [84]. Lupeol showed *in vitro* anti-tyrosinase activity along with other skin-related enzymes such as elastase [85]. Regarding the cholinesterase activity, citral from *Cymbopogon flexuosus* showed AChE inhibitory activity [86,87]. Lupeol

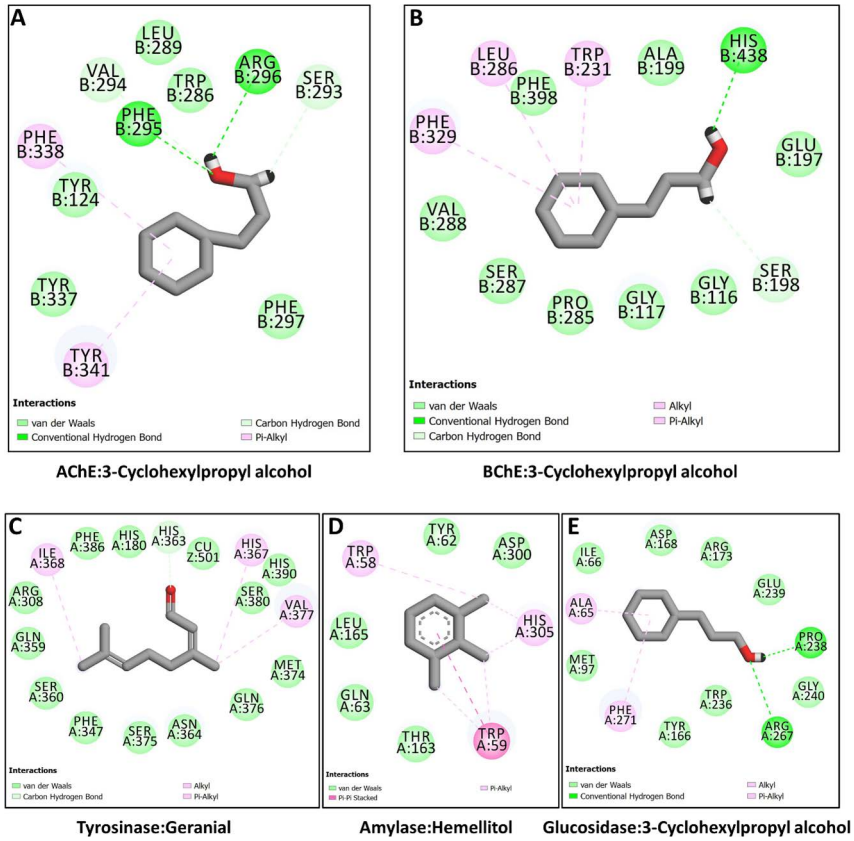

Fig 4. Protein-ligand interaction:(A) AChE and 3-Cyclohexylpropyl alcohol, (B) BChE and 3-Cyclohexylpropyl alcohol, (C) tyrosinase and geranial, (D) amylase and hemellitol, and (E) glucosidase and 3-Cyclohexylpropyl alcohol.

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exhibited inhibitory potential on the AChE and BChE in vitro assays and Alzheimer's model [72]. The findings of this research validate the potential of lemongrass extracts for utilization as ingredients in cosmetic skincare products either incorporated in antiaging, whitening or hypopigmenting preparations. Consequently, lemongrass emerges as a promising natural reservoir of easily accessible and cost-effective extracts abundant in antioxidant and enzyme inhibitory agents.

3.5. Molecular modelling

Molecular docking simulation was used to investigate the binding of bioactive components in lemongrass volatile oil and *n*-hexane extract. The docking binding energy scores are shown in (Fig 3). The dominating compounds exhibited promising binding affinity towards all the target proteins, with a particular affinity for AChE and BChE. For example, 3-cyclohexylpropyl alcohol bound strongly to both AChE (Fig 4A) and BChE (Fig 4B) via H-bonds, hydrophobic contacts, and a few van der Waals interactions with the enzymes' active site residues.The compound formed a critical H-bond with Phe295 in the AChE active site (Fig 4A). Phe295 is a member acyl-binding pocket (Phe295, Phe297, and Trp236) responsible for substrate selectivity by restricting access to bigger members of the choline ester series and stabilizing ACh's acetyl group [88]. Similarly, His438 formed an H-bond with cyclohexylpropyl alcohol, via the

hydroxyl group, with BChE (Fig 4B). Because His438 is a part of the catalytic triad of BChE (Ser198, Glu325, and His438), this interaction can play a major role in blocking the enzyme's activity [89].

Geranial also formed hydrophobic contacts but several van der Waals interactions with residues and catalytically Cu^{2+} ion in the active site of tyrosinase (Fig 4C). Among these interactions, a van der with His363 and a hydrophobic contact His367 may contribute to the inhibition of the enzyme since both residues are members of the seven conserved histidine residues essential for tyrosinase activity [90]. The interaction of amylase enzyme with hemellitol hydrophobic contacts and a few van der Waals interactions buried the ligand in the enzyme's catalytic pocket (Fig 4D). Most of these interacting residues, including Trp58 and Trp59 are also present in the crystal complex of amylase with acarbose [91].

Finally, like in the cases of AChE and BChE, the key interactions between 3-cyclohexylpropyl alcohol and glucosidase were H-bonds via hydroxyl group of the ligand and hydrophobic contacts with hydrophobic amino acid residues in the active site channel of the enzyme (Fig 4E). Blocking these residues, including Pro238 and Arg267, via H-bonds, may lead to inhibition because they play a role in the creation of glucose-binding cavities [92]. Therefore, they key interactions by which these compounds may block the activity of the selected enzymes were H-bonds, hydrophobic contacts, and van der Waals interactions.

4. Conclusions

The GC/MS analysis of lemongrass extracts showed that the VO was dominated by oxygenated monoterpenes (84.95%), primarily α-citral (36.08%), β-citral (34.22%), and β-myrcene (13.84%), while the HE was dominated by aliphatic hydrocarbons (42.98%), triterpenoids (20.14%), and aromatic hydrocarbons (17.25%). Besides, the hexane extract revealed considerable amounts of phenolics 7.44 mg GAE/g and flavonoids 1.91 mg RE/g. In the DPPH, ABTS, CUPRAC, FRAP and PM assays, the VO of lemongrass was more active and potent than the HE. In the metal chelating assay (MCA), the VO and HE showed comparable results of 31.16 and 25.76 mg EDTAE/g, respectively. Furthermore, for the AChE, BChE, and tyrosinase inhibitory activities, the VO showed more efficacy. That might be attributed to its high content of monoterpenes. While the hexane extract showed antidiabetic activity through inhibition of α-amylase and α-glucosidase enzymes. Molecular docking simulation was also used to study Lemongrass bioactive components and their binding sites to understand the mechanism. This study can assist in demonstrating the chemical composition and biological significance of lemongrass VO and HE. Additional *in-vivo* investigations are recommended for developing new candidates for oxidative stress diseases and diabetes.

Supporting information

S1 File. All experimental information regarding the determination of total phenolic and flavonoid content and determination of Antioxidant and Enzyme Inhibitory Effects can be found in the Supplementary Materials.
(DOCX)

Author contributions

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Writing – original draft: Shaza H. Aly, Abdullahi Ibrahim Uba, Nilofar Nilofar.

Writing – review & editing: Gokhan Zengin, Omayma A. Eldahshan.

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