Archives of Pharmaceutical Sciences Ain Shams University 2025; Vol. 9(1): 21-35

OPEN ACCESS

Pharmacognosy

Original Article

Phytochemical Profiling and Anticholinesterase Activity of *Alpinia zerumbet*: A Potential Source of Natural Therapeutics

Esraa A. Shahat[a], Iriny M. Ayoub[b], Riham O. Bakr[a], Haidy A. Gad[b,c], Omayma A. Eldahshan[b,d*], Abdel Nasser B. Singab[b,d*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt*

[b]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt*

[c]*Department of Pharmacognosy, Faculty of Pharmacy, King Salman International University. South Sinai, Egypt*

[d]*Centre for Drug Discovery Research and Development, Ain Shams University, Cairo 11566, Egypt*

ABSTRACT

Alpinia zerumbet is an economically important species that belongs to the family Zingiberaceae. It is widely used in traditional medicines and nutraceuticals. This study explores the phytochemical constituents of the extracts of both leaves and rhizomes of *A. zerumbet* together with their *in-vitro* anticholinesterase effect. Using the LC/MS-MS technique, both leaves and rhizomes of *A. zerumbet* were analyzed leading to the annotation of **24** constituents in the methanolic extract of the leaves and **23** constituents in the rhizomes extract encompassing various classes including phenolic acids, flavonoids, kavalactones, diarylheptanoids, gingerols and terpenoids. Both extracts demonstrated promising anticholinesterase activity, with the rhizomes exhibiting a stronger effect (IC_{50} = 2.229 ± 0.077 µg/mL) compared to the leaves (IC_{50} = 3.573 ± 0.123 µg/mL). Tacrine served as the standard for anticholinesterase activity, with an IC_{50} = 0.428 ± 0.015 µg/mL. The results suggest that *A. zerumbet*, particularly its rhizomes, holds potential as a natural source of anticholinesterase agents, warranting further investigation for therapeutic applications.

Keywords: *Alpinia zerumbet; leaves; rhizomes; anticholinesterase; LC-MS-MS; diarylheptanoid; kavalactones; gingerols.*

***Correspondence** | Abdel Nasser B. Singab and Omayma A. Eldahshan; Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt. Email: Dean@pharma.asu.edu.eg oeldahshan@pharma.asu.edu.eg

Citation | Shahat EA, Ayoub IM, Bakr RO, Gad HA, Eldahshan OA, Singab AB, 2025. Phytochemical Profiling and Anticholinesterase Activity of *Alpinia zerumbet*: A Potential Source of Natural Therapeutics. Arch Pharm Sci ASU 9(1): 21-35

DOI: 10.21608/aps.2024.322990.1198

Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.

Received 02 October 2024. **Accepted** 30 October 2024.

Copyright: ©2025 Shahat et *al.* This is an open-access article licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Published by: Ain Shams University, Faculty of Pharmacy

1. Introduction

Neurodegenerative diseases affect millions of people globally. Parkinson's and Alzheimer's diseases are among the most widespread neurodegenerative disorders **[1]**. According to the WHO, more than 55 million people have dementia around the world nowadays, and over 60% of them are living in the low-and middle-income countries. This number increases by 10 million cases yearly.

The hippocampus and several parts of the central nervous system (CNS) collaborate to form the memory where the mission of the hippocampus is controlled by cholinergic neurons that come from many brain regions. Different studies have shown that people with Alzheimer's disease (AD) and other age-related memory loss are distinguished by a specific loss of cholinergic neurons in the hippocampus and a considerable decrease in Acetylcholine (ACh) levels. The first

known neurotransmitter and the chief executor of the cholinergic nervous system is Acetylcholine (ACh) which plays a vital role in memory and learning **[2]**. Acetylcholinesterase (AChE) is a cholinergic enzyme mainly found at postsynaptic neuromuscular junctions, mainly in the nerves and muscles **[3],** and is predominantly found in the brain **[4]** Its major role is to stop synapses from transmitting neural information and sending signals to avoid the dispersal and activation of nearby receptors by the ACh **[3]**. Disturbances in AChE levels are associated with the pathogenesis and progression of many neurodegenerative diseases and neuropsychiatric disorders such as Alzheimer's disease and hepatic encephalopathy **[5]**. Consequently, inhibiting the degradation of ACh in the CNS by hindering acetylcholinesterase (AChE) activity is expected to enhance the ACh level in the brain, enhance brain function, and improve learning disabilities and memory damage.

Family Zingiberaceae (the Ginger family) is one of the flowering plant families that comprises about 53 genera and 1600 species of aromatic rhizomatous perennial herbs **[6]** and is widely distributed across the globe **[7]**. Nearly all organs of Zingiberaceae plants are utilized in traditional medicine, as food sources (spices and flavoring agents), and for producing natural dyes. Their rhizomes, such as those of *Curcuma longa*, *Zingiber officinale*, and *Alpinia galanga*, are particularly cherished for their nutritional, therapeutic, and pharmacological qualities and are commonly used to treat ailments like flatulence, diarrhea, and stomachache **[8]**.

Despite the common use of Zingiberaceae plant organs as edible ingredients, there is limited research on their nutritional composition and bioactivities.

Alpinia zerumbet (Pers.) Burtt. et Smith. is also identified as shell ginger **[9]**. It is a herbaceous plant with an aromatic odor, a rhizomatous stem, long sharpened leaves, and semi-pendent inflorescences. *Alpinia zerumbet* grows naturally in East Asia and has been introduced and naturalized in several regions of the world, such as the north and northeast regions of Brazil **[10, 11]**. It has been widely used in folk and traditional medicine, for treating several health disorders and diseases **[12]**.

In the literature, *A. zerumbe*t was reported to have numerous bioactive secondary metabolites, including gingerols, kava lactones like dihydro-5,6-dehydrokawain and 5,6-dehydrokawain as well as flavonoids, namely quercetin, kaempferol-3-*O*-deoxy hexosyl hexoside (15), and kaempferol-3-*O*-hexouronide (16), among others.

A wide spectrum of biological actions has been reported for *A. zerumbet* such as antidepressant and anxiolytic-like effects, anti-nociceptive, antioxidant, anti-inflammatory, cytotoxic, antiaging, antihypertensive, and antibacterial properties **[11]**. Besides, studies have proven the neuropharmacological and analgesic effects of the methanolic extract of *A. zerumbet* seeds **[13]**.

Several plants have been used in the treatment of many diseases **[14]** and proved to be neuroprotective agents **[15]**. Therefore, this research aims to explore the phytoconstituents of *Alpinia zerumbet* leaves and rhizomes and their correlation with *in vitro* anticholinesterase activity, which may aid in the management of cognitive disorders like Alzheimer's disease, dementia, memory loss, and related conditions.

2. Materials and methods

2.1. Plant material

Leaves and rhizomes of *Alpinia zerumbet* were collected from El Orman Botanical Garden, Egypt. Mrs. Therese Labib, former Taxonomy Specialist, at Orman Botanic Garden, Giza, Egypt identified the plant. Voucher specimens (PHG-P-

AZ-4) were placed in the Pharmacognosy Department's Herbarium at the Faculty of Pharmacy, Ain Shams University.

2.2. Preparation of the plant extracts

The air-dried powder of both rhizomes and leaves of *A. zerumbet* (0.65 & 0.20 kg) was first defatted using hexane then the defatted samples were extracted by MeOH, maceration till complete exhaustion. Then the extracts were filtered and concentrated using a rotary evaporator to yield dark solid residues (33 & 14 g), respectively.

2.3. Liquid chromatography-tandem mass spectrometry (LC-MS-MS) characterization

At the Faculty of Pharmacy, Ain Shams University, the LC-MS-MS was carried out by using a Xevo TQD triple quadrupole system (Waters Corporation, Milford, MA 01757, USA) equipped with an electrospray ESI source (electrospray voltage, 3.0 kV; sheath gas, nitrogen; capillary temperature, 440 °C) in both positive and negative mode.

The ion trap MS system was combined with a UPLC apparatus with a reversed-phase C-18 column (ACQUITY UPLC BEH C18 column, 1.7 µm particle size, 2.1 × 50 mm column). A gradient mobile phase (A: H_2O containing 0.1% formic acid, B: Acetonitrile, also acidified with 0.1% formic acid) was used for mobile phase elution at a flow rate of 0.2 mL/min. The MS spectra were obtained in both positive and negative modes (between *m/z* 100 and 1000) with a starting collision-induced dissociation energy of 20 eV. For processing the spectra, MassLynx 4.1 software was used. The compounds were tentatively recognized by matching retention times (Rt) and mass spectra to already reported literature **[16]**.

2.4. Determination of anti-cholinesterase activity using colorimetric inhibition kit

A colorimetric inhibition kit was used to measure the ability of the AChE enzyme to decompose the colored substrate and then measure the absorbance of the resulting yellow chromophore at 412 nm, followed by comparing it with reversible AChE inhibitors such as donepezil, tacrine, and rivastigmine **[17]**. The kit components included an AChE assay buffer, AChE substrate, probe mix, and a reversible inhibitor (Donezepil).

The residual extracts were dissolved in DMSO (Merck), to obtain different concentrations. The sample mixture was further diluted with the AChE assay buffer. Next, 10 µl of diluted test inhibitors were transferred into appropriate wells of a 96-well plate with a clear and flat bottom. The reaction mixtures were added to the test wells of a 96-well microtiter plate. For each well, 40 µL reaction mix was prepared containing 10 µL of diluted AChE substrate, 5 µL of probe mix, and 25 µL of AChE assay buffer were then added to test inhibitors (S), Inhibitor Control [IC], Enzyme Control [EC] and Background Control [BC] wells. The total reaction volume for each well was 200 µL. Enzyme Control (No Inhibitor) [EC] and Background Control [BC] were prepared by the addition of 100 µL of assay buffer to the designated well(s). Donezepil was used as a positive control. Finally, they were mixed well and incubated at room temperature for 10-15 min far from light. The absorbance (OD) was measured at 412 nm in kinetic mode for 40 min at room temperature. The inhibitory effect of the samples was calculated compared to the negative control:

$$\% \text{ Relative Inhibition} = \frac{(Slope\ of\ [EC] - slope\ of [S])}{Slope\ of\ [EC]}\ x\ 100$$

$$\% \text{ Relative Activity} = \frac{Slope\ of\ [S]}{Slope\ of\ [EC]}\ X\ 100$$

Where EC is the enzyme control and S is the sample.

The inhibition of enzyme activity was expressed in terms of IC_{50} (the concentration of the sample required to inhibit 50% of the enzyme) which was calculated by linear regression analysis.

3. Results and discussion

3.1. Phytoconstituents in *A. zerumbet* leaves and rhizomes via LC-MS/MS

Phytoconstituents of the methanol extracts from rhizomes and leaves of *A. zerumbet* cultivated in Egypt were tentatively identified employing LC-MS-MS analysis in both positive and negative ionization modes **(Fig. 1),** depending on fragmentation patterns that were compared with the previous literature. The structures of the assigned metabolites are presented in **Fig. 2-5,** together with their fragmentation patterns that helped in their assignments.

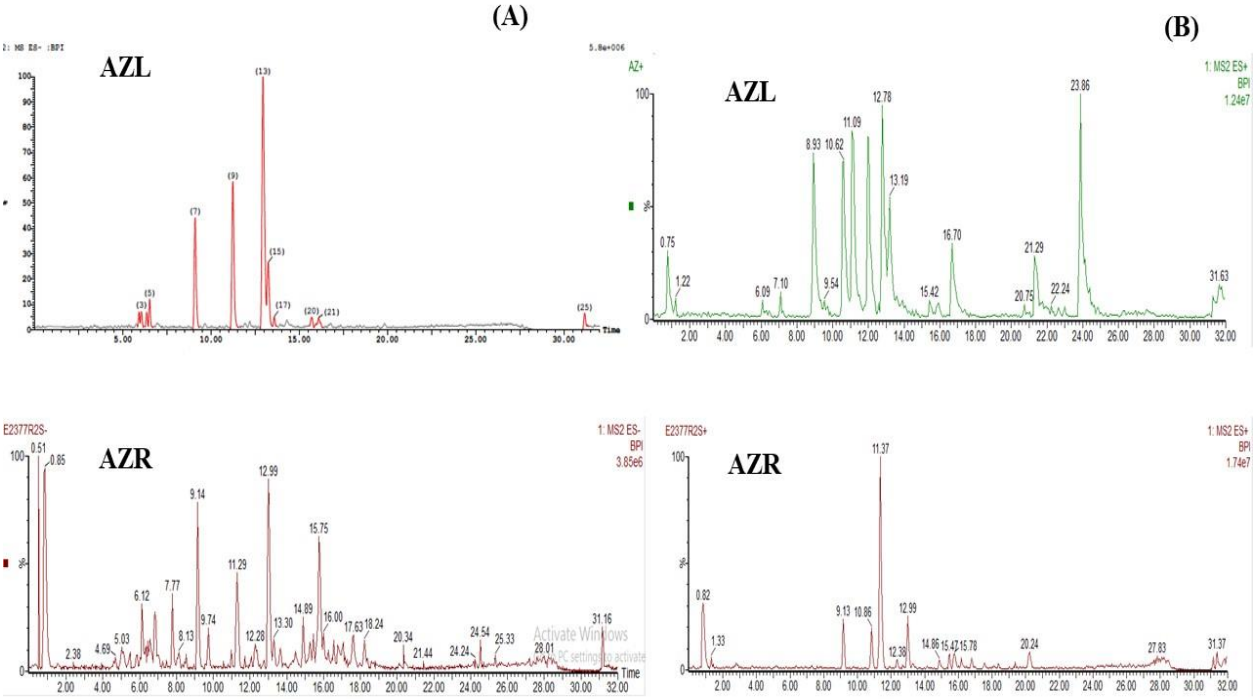


Fig. 1. Base Peak chromatograms of the methanol extracts of *A. zerumbet* leaves (AZL) and rhizomes (AZR) in negative ESI mode (A) and positive ESI mode (B).

A total of 23 constituents were tentatively recognized in the methanol extract of *A. zerumbet* rhizomes in the positive and/or negative ionization mode along with 24 constituents in the leaves. The identified phytoconstituents belonged to different phytochemical classes such as phenolic acids (7), kavalactones (2), gingerols (2), diarylheptanoids (3), diarylheptanoid-sesquiterpene (1), diterpenoids (2), and flavonoids (14) including chalcones (2), dihydrochalcone (1). The identified constituents are listed in **(Table 1)**.

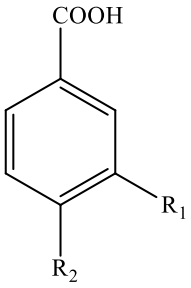
Table 1. Tentatively assigned metabolites in the methanol extract from *A. zerumbet* leaves and rhizomes employing LC-MS/MS in negative/positive ionization modes.

No.	Rt	Identified Compound	$[M-H]^-$	$[M+H]^+$	MS^2 ions (m/z)	Leaves	Rhizomes	Reference	Class
1	0.397	Citric acid	191		147, 129	-	+	**[18]**	Organic acid
2	0.085	Cinnamic acid	147		103	-	+	**[12]**	Phenolic acid
3	0.735	Piperidine alkaloid		342	324, 306, 288	-	+	**[19]**	Alkaloid
4	0.77	Caffeoyl hexoside	341		179, 119, 89, 71	-	+	**[20]**	Phenolic acid
5	1.55	Protocatechuic acid	153		109, 97, 91, 81	-	+	**[12]**	Phenolic acid
6	2.207	Hydroxybenzoic acid	137		92	-	+	**[21] [22]**	Organic acid
7	2.41	Quinic acid	191		179, 173, 169,146, 137,127, 73	-	+	**[12]**	Phenolic acid
8	3.2	Coumarin		147	103, 91	+	-	**[23]**	Coumarin
9	5.05	*p*-Coumaric acid	163		119, 93	-	+	**[12]**	Phenolic acid
10	5.30	Vanillic acid	167		152, 108	-	+		Phenolic acid
11	5.65	Benzoic acid		123	94, 77, 43	-	+	**[21]**	Organic acid
12	5.84	Quercetin hexouronide	477		301, 225, 179, 163, 151	+	-		Flavononol
13	5.88	Quercetin		303	229, 153, 137	+	-	**[24]**	Flavonol
14	6.108	Hexonic acid		197	179, 91	+	-	**[25]**	Sugar derivative
15	6.22	Kaempferol deoxy hexosyl hexoside	593		285	+	-	**[12]**	Flavonol
16	6.36	Kaempferol hexouronide	461	463	+ 287, 113, 85 _285, 270, 113	+	-	**[26]**	Flavonol
17	6.42	Kaempferol		287	213, 165, 153, 121, 93	+	-	**[27] [28]**	Flavonol
18	7.003	Hydroxy gallic acid	187		125, 97	-	+	**[29]**	Phenolic acid
19	8.62	Hydroxylated cardamon	285		269, 165, 119	+		**[30]**	Chalcone
20	8.9	Cardamonin	269	271	(+) 271, 167, 152, 124 (-) 254, 227, 177, 165, 139, 122	+	+	**[30][31]**	Chalcone
21	9.025	Alpinetin	269	271	(+) 167, 131, 193 254, 226, 165, 97 (-)	+	+	**[21]**	Flavanone
22	9.093	Apigenin	269		227, 225, 183, 151, 149, 107	+	+	**[12] [32]**	Flavone
23	**10.02**	α-Curcumene		203	147, 133, 119, 105, 91, 67	+	-	**[4]**	Sesquiterpene
24	10.83	Dihydro-5,6-dehydrokawain (DDK)		231	91, 105, 133, 203	+	+	**[33]**	Kava lactone
25	11.3	5,6-Dehydrokavain (Dk)		229	141, 131	+	+	**[33][34]**	Kavalactone
26	11.24	Pinocembin	255	257	213, 151, 123, 107, 103	+	+	**[12, 27]**	Flavanone
27	12.9	Genistein	269		227, 225, 197, 183	+	+	**[12, 32]**	Isoflavone
28	13.08	Uvangoletin	271		239, 165, 139, 124	+	+	**[21]**	Dihydrochalcone
29	13.15	Naringenin	271		229, 171, 153, 151, 123	+	-		Flavanone
30	14.44	Zedoalactone H	265		169, 96	-	+	**[27]**	Guaiane-type sesquiterpene
31	14.41	6-Shogaol		277	149, 135, 123, 121, 107	+	-	**[4]**	Gingerol (Shogaol)
32	15.2	6-Paradol		279	261,201, 149, 137,135, 123, 121	+	-	**[4]**	Gingerol (Paradol)
33	16.3	Alpininoid E	483		255, 181, 147	+	-	**[35]**	Diarylheptanoid-sesquiterpene
34	16.6	Zerumbetol		301	233, 231, 219, 203, 177, 137	+	+	**[36]**	Diterpenoid
35	16.88	3,5-Dihydroxy-1-(3,4,5-tri methoxyphenyl)− 7-(3,4-methoxyphenyl)heptane	433		171, 153	-	+	**[27]**	Diarylheptanoid

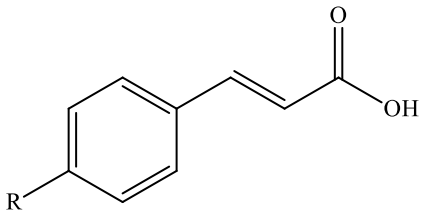
36	17.18	Isorhamnetin	315		271, 269, 147	-	+	**[24]**	Flavonoid
37	17.39	Villosin		301	218, 177	+		**[21]**	Diterpenoid
38	17.49	3-Hydroxy-1-4-hydroxyphenyl)-5-methoxy-7- phenyl-6-heptene	325		183		+	**[27]**	Diarylheptanoid
39	23.74	3,5-Dihydroxy-1-(4-hydroxy-3,5-dimethoxy phenyl)-7-(4-hydroxy-3-methoxyphenyl) heptane		407	193, 167	+		**[37]**	Diarylheptanoid

3.2. Phenolic acids

From the results, a number of phenolic acids were identified in the methanol extract of *A. zerumbet* rhizomes such as cinnamic acid **(2)**, protocatechuic acid **(5)**, hydroxybenzoic acid **(6)**, quinic acid **(7),** coumaric acid **(9)**, vanillic acid (**10**) and benzoic acid **(11) (Fig. 2)**. **Protocatechuic acid (Peak 5)** was tentatively identified at $[M-H]^-$ 153 *m/z* and a fragment ion at *m/z* 109 $[M-H-44]^-$ referring to the loss of CO_2 [12]**. Quinic acid (Peak 7)** showed a precursor ion at *m/z* 191 and a fragment ion at *m/z* 173 $[M-H-H_2O]^-$. While ***p*-coumaric acid** (**Peak 9)** displayed $[M-H]^-$ at *m/z* 163 and a fragment ion at *m/z* 119 $[M-H-44]^-$ due to the loss of CO_2. Besides, **vanillic acid (Peak 10)** was tentatively identified at *m/z* 167 $[M-H]^-$ showing a fragment ion at *m/z* 152 $[M-H-CH_3]^-$, and *m/z* 108 $[M-H-CH_3-CO_2]$[12]**.**



	R_1	R_2
(5) Protocatechuic acid	OH	OH
(6) Hydroxybenzoic acid	H	OH
(10) vanillic acid	OCH_3	OH
(11) Benzoic acid	H	H



	R
(2) Cinnamic acid	H
(9) Coumaric acid	OH

Fig. 2. Phenolic acids identified in the methanol extract of *A. zerumbet* rhizomes and leaves.

3.3. Flavonoids and chalcones

Flavonoids are a class of compounds with strong antioxidant properties that ameliorate many diseases associated with oxidative stress **[38]**. A total of fourteen flavonoids belonging to

various subclasses (flavones, flavonols, isoflavones, flavanones, and chalcones) were potentially identified in the methanolic extract of *A. zerumbet* **(Fig. 3)**. They are exemplified by apigenin **(22)** as a flavone, quercetin **(13)** and kaempferol **(17)** as flavonols, alpinetin **(21),** pinocembrin **(26)** and naringenin **(29)** as flavanones, in addition to their glycosides as quercetin hexouronide **(12)**, kaempferol deoxy hexosyl hexoside **(15)** and kaempferol hexouronide **(16)**. Three chalcones were also tentatively identified such as cardamonin **(20)**, hydroxycardamonin **(19),** and uvangoletin **(28)**. In addition, one isoflavone was identified as genistein **(27)**.

Flavanones were annotated (**Peaks 21**, **26, and 29**) which showed precursor ions $[M-H]^-$ at *m/z* 255. Pinocembrin **(Peak 26)** was previously identified and isolated from the leaf methanolic extract **[12, 40].** Fragments appeared at *m/z* 213 $[M-H-C_2H_2O]^-$, and *m/z* 151 for $[^{1,3}A]^-$ **[41]**. **Peaks 28 & 29** showed precursor ions at *m/z* 271 $[M-H]^-$ identified as uvangoletin and naringenin, respectively. Uvangoletin displayed characteristic fragment ions at *m/z* 124, 139, and 165 in a pattern similar to cardamonin and was previously isolated from *A. zerumbet* pericarp **[21],** while naringenin exhibited fragment ions at *m/z* 151, 123 and 229 referring to $^{1,3}A^-$, $^{1,4}A^-$ and $[M-H-CO_2]^-$ **[32]**.

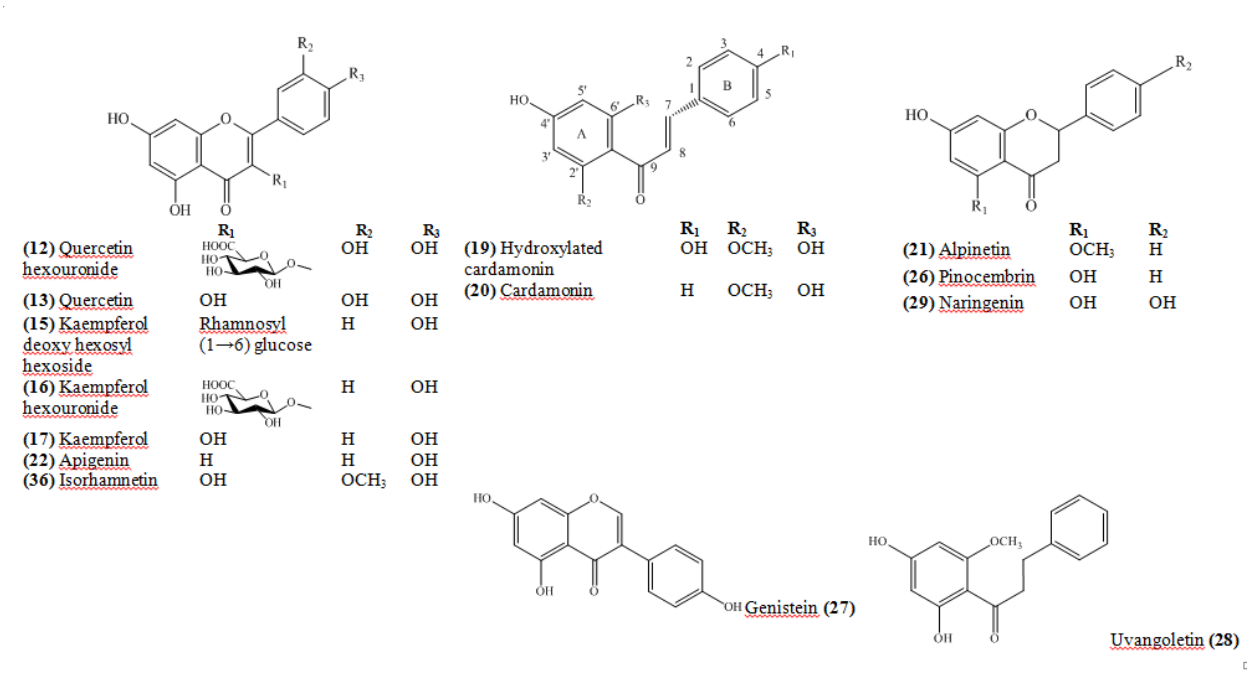


Fig. 3. Flavonoids and chalcones identified in the methanol extract of *A. zerumbet* rhizomes and leaves

Peaks 13 and 17 showed protonated ions at *m/z* 303 and 287 respectively with a characteristic fragmentation pattern of flavonol aglycone appearing at *m/z* 153 and 165 referring to $^{1,3}A^+$ and $^{0,2}A^+$, respectively **[24]**. **Peak 13** contains an additional fragment at *m/z* 137, which corresponds to the $^{0,2}B^+$ ion. **Peaks 13 and 17** were assigned as quercetin and kaempferol, respectively **[28]**.

Kaempferol deoxy hexosyl hexoside (**Peak 15)** showed a deprotonated ion $[M-H]^-$ at *m/z* 593 with fragment ions at *m/z* 431 and 285 indicating a loss of a hexosyl moiety $[M-H-162]^-$ then loss of a deoxyhexosyl moiety $[M-H-162-146]^-$.

Peaks 12 and 16 were tentatively identified as quercetin hexouronide and kaempferol hexouronide. The precursor ions [M-H]$^-$ were detected at *m/z* 477 and 461, respectively. A neutral loss of a hexouronide moiety (176 ppm) was confirmed from fragment ions observed at *m/z* 301 and *m/z* 285 indicating quercetin and kaempferol aglycones, respectively.

Four peaks were detected exhibiting deprotonated molecular ions at *m/z* 269. Chalcone is a characteristic subclass of *Alpinia* species **[39]**. **Peak 20** was assigned as cardamonin chalconoid with a molecular ion peak [M-H]$^-$ at *m/z* 269 and fragment ions at 254 and 227 *m/z* because of the loss of CH_3 and CH_2+CO respectively along with the characteristic fragment ion at *m/z* 165 as a result of the cleavage between C8 and C9 followed by serial neutral losses from the fragment ion at *m/z* 165 producing another two fragment ions at *m/z* 139 and 122 (165-CO-CH_3·), respectively. Another fragment ion was detected at *m/z* 177 which represents part I of the cardamonin structure **[30]**. Cardamonin showed a base peak at *m/z* 167 in the positive ion mode **[31]**. Cardamonin was previously isolated from *A. zerumbet* rhizomes, seeds, and pericarp **[21]**. These proposed fragmentation patterns of cardamonin in the negative ion mode are shown in **(Fig. 4)**.

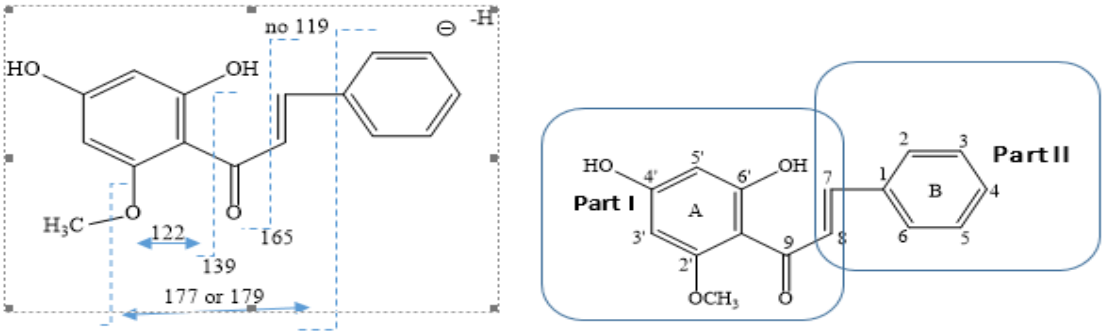


Fig. 4: Fragmentation of cardamonin (20)

Moreover, **Peak 22 and Peak 27** displayed precursor ions at *m/z* 269 [M-H]. They were identified as apigenin and genistein, respectively. Both isomers displayed common fragment ions at *m/z* 225 (-CO_2) and *m/z* 183 but interesting differences were also noted. Apigenin reveals various RDA fragments, particularly the noticeable ion at *m/z* 149 attributed to a $^{1,4}B^-$+2H fragment, and *m/z* 107 referred to $^{1,3}A^-$ - CO_2 **[32]**. Both apigenin and genistein were previously identified in the methanolic extract of *A. zerumbet* leaves **[12]**. **Peak 21** showed a precursor ion at *m/z* 269 [M-H] and was assigned as alpinetin classified as flavanone. Other flavanones were annotated (**Peaks 21**, **26 and 29**) which showed precursor ions [M-H]$^-$ at *m/z* 255. Pinocembrin **(Peak 26)** was previously identified and isolated from the leaf methanolic extract **[12, 40]**. Fragments appeared at *m/z* 213 [M-H-C_2H_2O]$^-$, and *m/z* 151 for [^{1,3} A]$^-$ **[41]**. **Peaks 28 & 29** showed precursor ions at *m/z* 271 [M-H]$^-$ identified as uvangoletin and naringenin, respectively. Uvangoletin displayed characteristic fragment ions at *m/z* 124, 139 and 165 in a pattern similar to cardamonin and was previously isolated from *A. zerumbet* pericarp **[21],** while naringenin exhibited fragment ions at *m/z* 151, 123 and 229 referring to $^{1,3}A^-$, $^{1,4}A^-$ and [M-H-CO_2]$^-$ **[32]**.

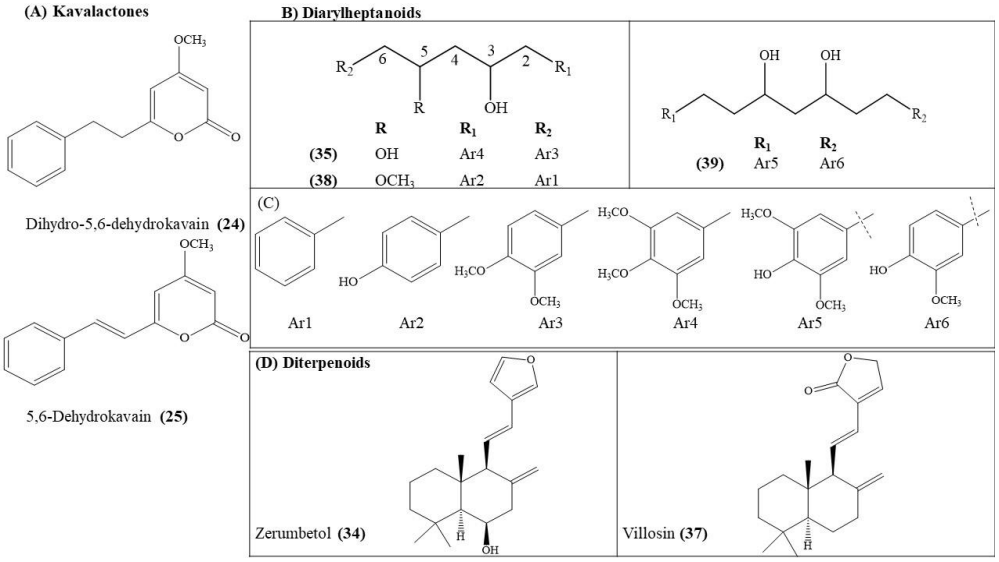


Fig. 5. Representative compounds identified in the methanol extract of *A. zerumbet* leaves or/and rhizomes using LC-MS/MS; (A) kavalactones, (B) diarylheptanoids, (C) possible aryl-substitutions, (D) Diterpenoids.

3.4. Kavalactones

Peaks 24 and 25 were identified as dihydro-5,6-dehydrokavain (DDK) and 5,6-dehydrokavain (DK) exhibiting precursor ions $[M+H]^+$ at *m/z* 231 and 229, respectively (**Fig. 5)**. Those were previously isolated from *A. zerumbet* leaves as major compounds **[34]**.

3.5. Terpenoids

Alpha-curcumin (**Peak 23)** displayed a protonated ion at *m/z* 203 and fragment ions at *m/z* 147 attributed to $[M+H- 2CH_3- C=CH_2]^+$ followed by a serial loss of CH_2 appearing at *m/z* 133, 119, 105 and a fragment ion at *m/z* 91 representing the tropylium cation. Curcumin is a sesquiterpene that was previously identified in the rhizomes of *Zingiber officinalis* **[4] (Fig. 6A)**.

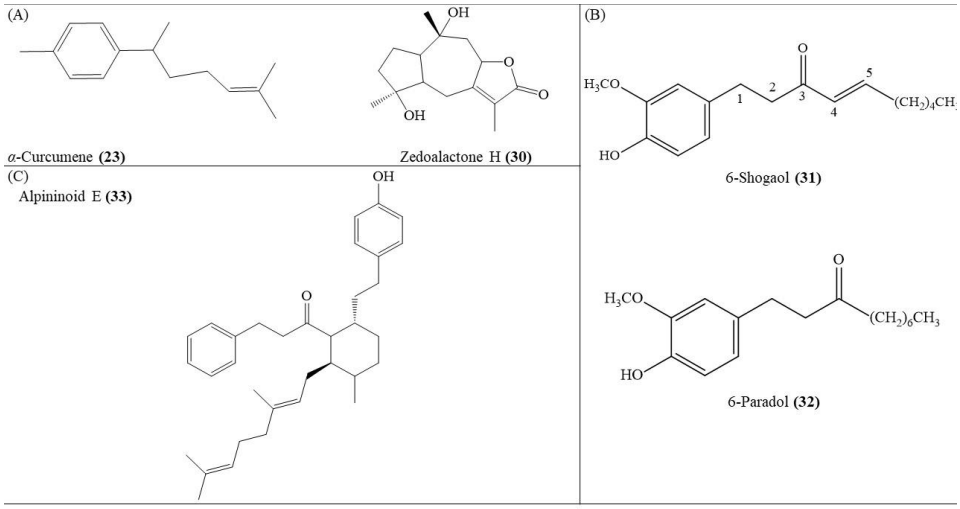


Fig. 6. (A) Sesquiterpenes, (B) Gingerols, (C) Diarylheptanoid-sesquiterpene identified in the methanol extract from *A. zerumbet* rhizomes and leaves.

3.6. Gingerols

Gingerol is one of the phenolic compounds that has a 3-methoxy-4-hydroxyphenyl functional group **[4]**. Two gingerols were identified in the methanolic extract of *A. zerumbet* leaves including one shogaol and one paradol **(peaks 31 and 32, respectively) (Fig. 6B). Peak 32** showed a protonated molecular ion peak $[M+H]^+$ at *m/z* 279 being 2 units greater than **peak 31**. The presence of a fragment ion at *m/z* 201 suggested that compound (**35**) could be 6-paradol, missing the double bond in 6-shogaol (peak 31) between carbons 4 and 5 **[4]**.

3.7. Diarylheptanoids

The Zingiberaceae family is rich in diarylheptanoids which are bioactive phenolics **[27]**. Diarylheptanoids are a class of natural chemicals with a 1,7-diarylheptane skeleton such as curcuminoids which are characteristic medicinal compounds of turmeric **[37]**. Three diarylheptanoids **(peaks 35, 38 & 39)** were identified in the methanol extracts of both rhizomes and leaves of *A. zerumbet* in addition to a diarylheptanoid-sesquiterpene **(peak 33) (Fig. 5)**. **Peak 33** was tentatively identified as alpininoid E; a diarylheptanoid-sesquiterpene that was previously isolated from *Alpinia officinarum* **[35]**. **Peak 39** was identified as 3,5-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane with a protonated ion at *m/z* 407 $[M+H]^+$ showing the two 3,5-dihydroxy moieties on the heptane skeleton which were previously isolated from ginger, in addition to producing two more characteristic ions at *m/z* 167 (product ion A) and 193 (product ion B) **[37] (Fig. 7)**.

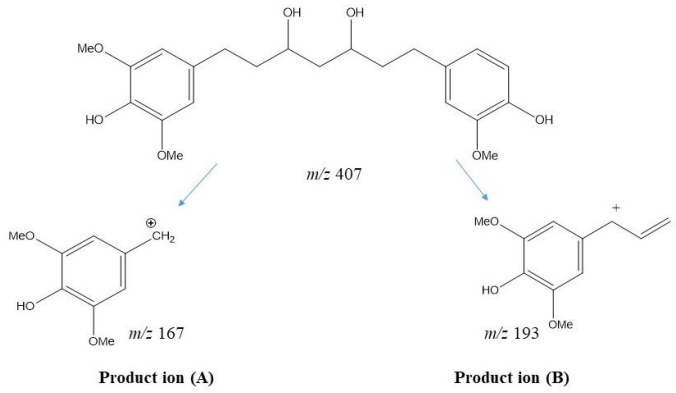


Fig. 7. Proposed fragmentation pattern of compound (43)

3.8. Acetylcholine esterase inhibitory activity of the defatted methanolic extracts of *A. zerumbet* leaves and rhizomes

Acetylcholinesterase (AChE) is important in the termination of impulse transmission by rapid degradation of the crucial neurotransmitter ACh. Disturbances in its levels are linked with the pathogenesis of many neurodegenerative and depressive disorders. Acetylcholine esterase has an important role in oxidative stress, inflammatory response, apoptosis, and adhesion of pathological proteins **[42]**.

Several studies have demonstrated that the AChE is found to be linked with the pathogenesis and development of Alzheimer's disease (AD) and that people with age-related AD and memory loss are considered with a loss of cholinergic neurons in the hippocampus and a drastic decrease in ACh levels. It is thought that increasing ACh levels in the CNS improves learning and memory **[2]**. Therefore, inhibiting ACh degradation in the CNS by inhibiting AChE

activity is expected to relieve learning disabilities and memory impairment by enhancing the ACh level in the brain. This is an important therapeutic strategy for treating some symptoms of this disease resulting in an increase in both the level and duration of the neurotransmitter action and improving cholinergic function in the brain **[43]**.

Clinically relevant AChEI, such as tacrine, donepezil, galanthamine, and rivastigmine are universally approved for the treatment of AD and applied in neurodegenerative disorders treatment but they have been limited for clinical use because of their short half-lives and antagonistic side effects. Thus, looking for new AChEIs with higher efficacy and safety from other alternatives such as natural products is the goal of nowadays investigators **[17]**.

The acetylcholine esterase inhibitory activity of defatted methanol extracts from rhizomes and leaves of *A. zerumbet* was compared to that of donepezil, tacrine, and rivastigmine which inhibited the acetylcholinesterase with IC_{50} = 0.197 ± 0.007, 0.428 ± 0.015 and 4.736 ± 0.163 respectively. The methanol extract of *A. zerumbet* rhizomes exerted a stronger inhibitory activity against acetylcholine esterase compared to the leaves with IC_{50} = 2.229 ± 0.077 and 3.573 ± 0.123 µg/mL respectively. A previous study reported the anticholinesterase of the methanol extract from *A. galanga* Willd. Rhizome where 100 µg/mL induced 16.98 ± 0.37 % inhibition **[44]**. Another study proved that the leaf extract of *A. zerumbet* could inhibit the activity of cholinesterase from pig serum evidently **[45]**. The anticholinesterase effect of both extracts from *A. zerumbet* leaves and rhizomes may be due to the presence of chalcones such as cardamonin together with flavonoids such as apigenin and pinocembrin. Cardamonin has been previously proven to have a neuroprotective effect through different mechanisms **[46]** and different flavonoids have been reported to be potent AChE inhibitors with outstanding efficacy **[47]**. The stronger effect of the rhizome extract might be because of the synergistic effect of phenolic acids where hydroxybenzoic acid and protocatechuic acid have been previously proven to exert high anticholinesterase activity **[48]**. No studies were traced regarding the anticholinesterase activity of methanol extracts from *A. zerumbet* cultivated in Egypt **(Fig. 8)**.

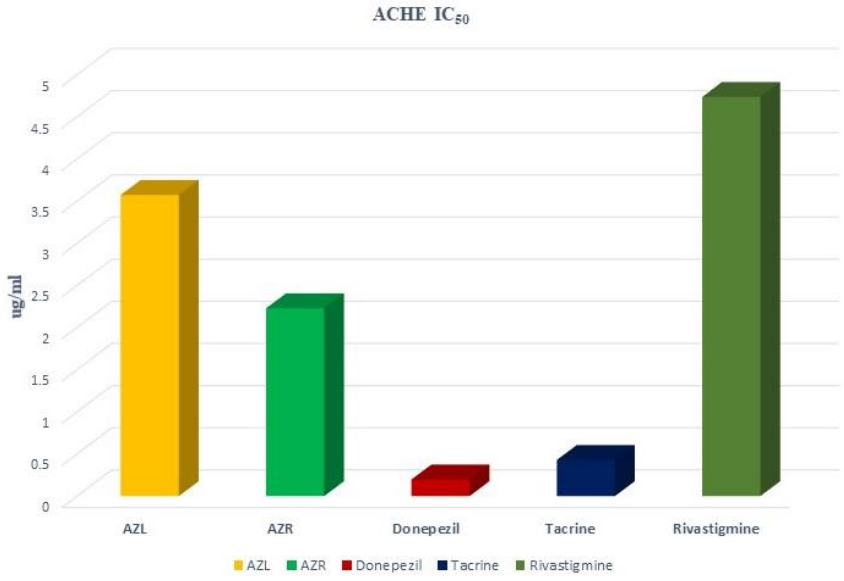


Fig. 8. The anti-acetylcholinesterase activity of the methanol extracts from *A. zerumbet* leaves (AZL) and rhizomes (AZR).

Conclusion

The current study is considered a comprehensive analysis of the phytochemical constituents and anticholinesterase activity of the methanol extracts from the leaves and rhizomes of *A. zerumbet*. The LC-MS/MS analysis revealed a rich diversity of bioactive compounds, including phenolic acids, flavonoids, kavalactones, diarylheptanoids, and terpenoids. The rhizome extract, in particular, exhibited significant anticholinesterase activity, outperforming the leaf extract. These findings suggest that *A. zerumbet*, especially its rhizomes, could serve as a valuable natural source of anticholinesterase agents. The study underscores the potential of *A. zerumbet* in the development of novel therapeutic strategies for neurodegenerative diseases, warranting further investigation into its bioactive compounds and their mechanisms of action.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent to Participate

Not applicable.

Consent for publication

Not applicable.

Availability of the data and Material

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that there is no conflict of interest.

Funding

The authors declare that no funds were received and this study was self-funded.

Author contribution

Esraa A. Shahat: Methodology, Investigation, Data curation, validation, visualization, Writing - review & editing. Iriny M. Ayoub: Methodology, Investigation, Data curation, validation, review & editing. Riham O. Bakr: Methodology, Investigation, Data curation, validation, review & editing. Haidy A. Gad: Methodology, Investigation, Data curation, validation, formal analysis, review & editing. Omayma A. Eldahshan: Conceptualization, Project administration, review & editing. Abdel-Nasser B. Singab Conceptualization, project administration, review & editing

4. References

1. Lamptey RNL, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. International Journal of Molecular Sciences 2022;23:1851.

2. Huang Q, Liao C, Ge F, Ao J, Liu T. Acetylcholine bidirectionally regulates learning and memory. Journal of Neurorestoratology. 2022;10:100002–100002.

3. Trang A, Khandhar PB. Physiology, Acetylcholinesterase. StatPearls Publishing; 2023.

4. Li MQ, Hu XY, Wang YZ, Zhang XJ, Li JP, Song ZM, et al. Qualitative analysis on chemical constituents from different polarity extracted fractions of the pulp and peel of ginger rhizomes by ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2021;35:e9029.

5. Abdelghffar EAR, El-Nashar HAS, Fayez S, Obaid WA, Eldahshan OA. Ameliorative effect of oregano (Origanum vulgare) versus silymarin in experimentally induced hepatic encephalopathy. Sci Rep. 2022;12:17854.

6. Mans DRA, Djotaroeno M, Friperson P, Pawirodihardjo J. Phytochemical and pharmacological support for the traditional uses of zingiberacea species in Suriname - A review of the literature. Pharmacognosy Journal.

2019;11:1511–25.

7. Saha K, Sinha RK, Sinha S. Distribution, Cytology, Genetic Diversity and Molecular phylogeny of selected species of Zingiberaceae – A Review. Feddes Repert. 2020;131:58–68.

8. Rachkeeree A, Kantadoung K, Suksathan R, Puangpradab R, Page PA, Sommano SR. Nutritional Compositions and Phytochemical Properties of the Edible Flowers from Selected Zingiberaceae Found in Thailand. Front Nutr. 2018;5:3.

9. Xiao T, Huang J, Wang X, Wu L, Zhou X, Jiang F, et al. Alpinia zerumbet and Its Potential Use as an Herbal Medication for Atherosclerosis: Mechanistic Insights from Cell and Rodent Studies. Lifestyle Genom. 2020;13:138–45.

10. Luz JGR, Nogueira JN, Alves CMG, Videira MN, Canuto KM, Castro KNC, et al. Essential oil of Alpinia zerumbet (Zingiberaceae) has anthelmintic efficacy against monogenean of Colossoma macropomum (Characiformes: Serrasalmidae). Aquac Res. 2021;52:5340–9.

11. Chan EWC, Wong SK, Chan HT. Alpinia zerumbet, a ginger plant with a multitude of medicinal properties: An update on its research findings. Journal of Chinese Pharmaceutical Sciences. 2017;26:775–88.

12. Ghareeb MA, Sobeh M, Rezq S, El-Shazly AM, Mahmoud MF, Wink M. HPLC-ESI-MS/MS Profiling of Polyphenolics of a Leaf Extract from Alpinia zerumbet (Zingiberaceae) and Its Anti-Inflammatory, Anti-Nociceptive, and Antipyretic Activities In Vivo. Molecules. 2018;23:3238.

13.Chauhan VS. Investigation of Neuropharmacological Activity of Seeds of Alpinia Zerumbet. Int J Appl Biol Pharm. 2014;5:36–41.

14. Singab AN, Bahgat D, Al-Sayed E, Eldahshan O. Saponins from genus Albizia: phytochemical and biological review. Med Aromat Plants S. 2015;3(001).

15. Mostafa NM, Eldahshan OA, Singab AB. The Genus Jacaranda (Bignoniaceae): An Updated Review. Pharmacognosy Communication. 2014;4:31–9.

16. Abdelghffar EAR, Mostafa NM, El-Nashar HAS, Eldahshan OA, Singab ANB. Chilean pepper (Schinus polygamous) ameliorates the adverse effects of hyperglycemia/dyslipidemia in a high-fat diet/streptozotocin-induced type 2 diabetic rat model. Ind Crops Prod. 2022;183:114953.

17. Imran M, Ullah F, Ayaz M, Sadiq A, Shah MR, Jan MS, et al. Anticholinesterase and antioxidant potentials of Nonea micrantha Bioss. & Reut along with GC-MS analysis. BMC Complement Altern Med. 2017;17:1–12.

18. Hassan HA, Ayoub IM, Ragab TIM, Afifi SM, El-Gendy AENG, Farrag ARH, et al. Metabolomics approach of Symphyotrichum squamatum ethanol extract and its nano-Ag formulation protective effect on gastric ulcer via bio-chemical and pathological analyses. Biomarkers. 2023;28:190–205.

19. Pivatto M, Crotti AEM, Lopes NP, Castro-Gamboa I, De Rezende A, Viegas C, et al. Electrospray ionization mass spectrometry screening of piperidine alkaloids from Senna spectabilis (Fabaceae) extracts: fast identification of new constituents and co-metabolites. J Braz Chem Soc. 2005;16 6 B:1431–8.

20. El-Shazly MAM, Hamed AA, Kabary HA, Ghareeb MA. LC-MS/MS profiling, antibiofilm, antimicrobial, and bacterial growth kinetic studies of Pluchea discords extracts. Acta Chromatogr. 2021;34:338–50.

21. Nishidono Y, Tanaka K. Phytochemicals of Alpinia zerumbet: A Review. Molecules. 2024;29:2845.

22. Ali A, Wu H, Ponnampalam EN, Cottrell JJ, Dunshea FR, Suleria HAR. Comprehensive profiling of most widely used spices for their phenolic compounds through lc-esi-qtof-ms2 and their antioxidant potential. Antioxidants. 2021;10:721.

23. Ren Z, Nie B, Liu T, Yuan F, Feng F, Zhang Y,

et al. Simultaneous Determination of Coumarin and Its Derivatives in Tobacco Products by Liquid Chromatography-Tandem Mass Spectrometry. Molecules. 2016;21:1511.

24. Bakr RO, Shahat EA, Elissawy AE, Fayez AM, Eldahshan OA. Evaluation of the hepatoprotective activity of Pulicaria incisa subspecies candolleana and in silico screening of its isolated phenolics. J Ethnopharmacol. 2021;271:113767.

25. Vuković N, Vukić M, Đelić G, Kacaniova M, Cvijović M. The investigation of bioactive secondary metabolites of the methanol extract of eryngium amethystinum. Kragujevac Journal of Science. 2018;40:113–29.

26. Ye LH, He XX, Yan MZ, Chang Q. Identification of in vivo components in rats after oral administration of lotus leaf flavonoids using ultra-fast liquid chromatography with tandem mass spectrometry. Analytical Methods. 2014;6:6088–94.

27. Rasheed DM, Farag MA, Khattab AR, Rahman MFA, El-Haddad AE. A comparative MS-based metabolomics study and in-vitro antidiabetic assay of galangals, turmeric, and ginger aided by molecular networking and chemometrics. Ind Crops Prod. 2023;205:117438.

28. Satheeshkumar N, Shantikumar S, Komali M. Identification and Quantification of Aldose Reductase Inhibitory Flavonoids in Herbal Formulation and Extract of Gymnema sylvestre Using HPLC-PDA and LC-MS/MS. Chromatography Research International. 2014;2014:518175.

29. Sayed SMA, Alseekh S, Siems K, Fernie AR, Luyten W, Schmitz-Linneweber C, et al. Identification of a Hydroxygallic Acid Derivative, Zingibroside R1, and a Sterol Lipid as Potential Active Ingredients of Cuscuta chinensis Extract That Has Neuroprotective and Antioxidant Effects in Aged Caenorhabditis elegans. Nutrients. 2022;14:4199.

30. He YQ, Liu Y, Zhang JW, Tang J, Su J, Li YY, et al. Characterization of cardamonin metabolism by P450 in different species via HPLC-ESI-ion trap and UPLC-ESI-quadrupole mass spectrometry. Acta Pharmacologica Sinica 2009;30:1462–70.

31. Dong F, Wang S, Yang A, Li Q, Wang Y, Dai L, et al. Systematic screening and characterization of cardamonin metabolites using UHPLC-Q-Exactive Orbitrap MS after oral administration to rats. Arabian Journal of Chemistry. 2020;13:8768–82.

32. Fabre N, Rustan I, De Hoffmann E, Quetin-Leclercq J. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography-electrospray ion trap mass spectrometry. J Am Soc Mass Spectrom. 2001;12:707–15.

33. Tarbah F, Barguil Y, Müller C, Rickert A, Weinmann W, Nour M, et al. c Société Française de Toxicologie Analytique. Annales de Toxicologie Analytique. 2013;25:109–19.

34. Xuan TD, Teschke R. Dihydro-5,6-dehydrokavain (DDK) from Alpinia zerumbet: Its Isolation, Synthesis, and Characterization. Molecules. 2015;20:16306.

35. Liu H, Wu ZL, Huang XJ, Peng Y, Huang X, Shi L, et al. Evaluation of Diarylheptanoid-Terpene Adduct Enantiomers from Alpinia officinarum for Neuroprotective Activities. J Nat Prod. 2018;81:162–70.

36. Yuanying W, Yanfang C, Xuemei X. A new diterpenoid from Alpinia zerumbet. Acta Bot Sin. 1997;39:983–4.

37. Jiang H, Timmermann BN, Gang DR. Characterization and identification of diarylheptanoids in ginger (Zingiber officinale Rosc.) using high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry. 2007;21:509–18.

38. Mostafa NM, Abd El-Ghffar EA, Hegazy HG, Eldahshan OA. New Methoxyflavone from Casimiroa sapota and the Biological Activities of Its Leaves Extract against Lead Acetate Induced Hepatotoxicity in Rats. Chem Biodivers.

2018;15:e1700528.

39. Youn I, Han AR, Piao D, Lee H, Kwak H, Lee Y, et al. Phytochemical and pharmacological properties of the genus Alpinia from 2016 to 2023. Nat Prod Rep. 2024;41:1346–67.

40. Natsume N, Yonezawa T, Woo JT, Teruya T. Effect of pinocembrin isolated from Alpinia zerumbet on osteoblast differentiation. Cytotechnology. 2021;73:307–17.

41. Mohamed SA, Abo-Elghiet F, Ahmed SF, Haleem ENA Al, El-Tantawy WH, Yasin NAE. Melodorum fruticosum Lour. Leaves Methanolic Extract Ameliorates Gentamicin-Induced Renal Toxicity in Rats via Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Pathways. Egypt J Chem. 2024;67:547–67.

42. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase Inhibitors in the Treatment of Neurodegenerative Diseases and the Role of Acetylcholinesterase in their Pathogenesis. Int J Mol Sci. 2021;22:9290.

43. Aazza S, El-Guendouz S, Miguel M da G. Screening for acetylcholinesterase inhibition, lipid peroxidation inhibition and antioxidant activity of medicinal plants from Morocco. Bol Latinoam Caribe Plantas Med Aromat. 2023;22:1–18.

44. Adewusi EA, Moodley N, Steenkamp V. Medicinal plants with cholinesterase inhibitory activity: A Review. Afr J Biotechnol. 2010;9:8257–76.

45. Zhuang QC, Wu SC, Chen KJ, Zhao G, Qian F. Preliminary research on Influence of Several Kinds of Plants Including *Alpinia zerumbet* on Activity of Cholinesterase from Pig Serum. Advanced Material Engineering. 2015;615–21.

46. Barber K, Mendonca P, Soliman KFA. The Neuroprotective Effects and Therapeutic Potential of the Chalcone Cardamonin for Alzheimer's Disease. Brain Sci. 2023;13:145.

47. Khan H, Marya, Amin S, Kamal MA, Patel S. Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and prospects. Biomedicine & Pharmacotherapy. 2018;101:860–70.

48. Budryn G, Majak I, Grzelczyk J, Szwajgier D, Rodríguez-Martínez A, Pérez-Sánchez H. Hydroxybenzoic Acids as Acetylcholinesterase Inhibitors: Calorimetric and Docking Simulation Studies. Nutrients. 2022;14:2476.