STUDIA UBB CHEMIA, LXIX, 3, 2024 (p. 93-107)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2024.3.06

ESSENTIAL OIL FROM *KNEMA ELEGANS* WARB.: CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTI-ACETYLCHOLINESTERASE ACTIVITIES

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ABSTRACT. *Knema elegans* Warb. is an evergreen tree belonging to the Myristicaceae family, primarily native to Southeast Asia. This study examined the chemical composition and assessed the antioxidant and anti-acetylcholinesterase properties of the essential oil from the leaves of *K. elegans* in Vietnam. Gas chromatography/mass spectrometry analysis revealed the main compounds in the essential oil, including bicyclogermacrene (23.8%), germacrene D (17.1%), β-caryophyllene (15.4%), δ-cadinene (8.2%), and α-cadinol (7.9%). Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods, with half-maximum inhibitory concentration (IC_{50}) values of 93.11 ± 1.95 µg/mL and 114.95 ± 2.41 µg/mL, respectively, indicating moderate antioxidant potential. Additionally, the essential oil exhibited significant anti-acetylcholinesterase effects, with an IC_{50} value of 87.44 ± 2.52 µg/mL. These findings highlight the biological significance of *K. elegans* essential oil and suggest avenues for further investigation, particularly in elucidating its mechanisms of action in antioxidant and anti-acetylcholinesterase activities. Notably, this research represents the first comprehensive exploration of the chemical composition, antioxidant activity, and acetylcholinesterase inhibition effects of *K. elegans* essential oil from its leaves.

Keywords: *Knema elegans, sesquiterpenes, Myristicaceae, DPPH, ABTS, AChE*

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INTRODUCTION

Myristicaceae is a prominent family of flowering plants, commonly known as the nutmeg family, comprising approximately 20 genera and over 500 species [1]. These plants are primarily distributed in tropical regions around the world, with a significant presence in Africa, Asia, Pacific islands, and the Americas [2]. Among the genera within Myristicaceae, *Knema* stands out as a significant representative. This genus comprises approximately 60 species of small-medium trees distributed primarily in tropical regions of Asia, Africa, and Australia [3, 4]. Across various cultures in its native range, *Knema* species have been utilized for their medicinal properties, serving as essential components in traditional herbal remedies [5]. The plants are often harvested for their bark, leaves, fruits, and even roots, which contain bioactive compounds believed to have therapeutic effects such as alleviating fevers, digestive issues, and respiratory problems [4, 5]. Furthermore, scientific research has begun to explore the biological activities and potential therapeutic applications of *Knema* compounds. Studies have identified bioactive constituents such as phenolic lipids, flavonoids, and lignans in *Knema* extracts, exhibiting a range of pharmacological properties including antioxidative, antidiabetic, antimicrobial, anti-inflammatory, antimalarial, neuroprotective, and hepatoprotective activities [3-5]. These findings suggest the promising potential of *Knema* plants in modern pharmacology and drug discovery.

Essential oils, derived from various plants, have garnered significant attention due to their diverse therapeutic properties, including antioxidant and anti-acetylcholinesterase (anti-AChE) activities [6-8]. Antioxidants play a crucial role in neutralizing harmful free radicals, thus protecting cells from oxidative stress and reducing the risk of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [7]. Furthermore, anti-AChE activity is vital in the context of cognitive health, as it inhibits the breakdown of acetylcholine, a neurotransmitter essential for memory and cognitive function [9]. With the increasing interest in natural remedies and the demand for effective therapeutic agents, there is a burgeoning curiosity in exploring the potential applications of essential oils. These oils not only hold promising therapeutic potential but also offer sustainable alternatives to synthetic pharmaceuticals with fewer adverse effects [8]. Therefore, comprehending the biological activity of essential oils becomes paramount, propelling the quest for oils with heightened efficacy across various therapeutic applications.

Knema elegans Warb. is a flowering plant species belonging to the Myristicaceae family, originating from tropical regions of Southeast Asia [10]. To date, ethnobotanical information regarding *K. elegans* is somewhat

limited. However, several studies have demonstrated the presence of flavane derivatives and flavonoids in extracts of *K. elegans*, exhibiting antioxidant activity, α-glycosidase inhibition, DNA polymerase β inhibition, and DNA damage [11-13]. Furthermore, researchers have explored the chemical composition and biological activities of essential oils from various *Knema* species. Specifically, essential oils from *K. hookeriana* [14], *K. kunstleri* [15], *K. intermedia* [16], *K. malayana* [17], *K. angustifolia* [18], *K. pierrei* [19], and *K. globularia* [20], along with their antioxidant [15, 19, 20], antimicrobial [19], anti-AchE [14], anti-tyrosinase [16, 17], and lipoxygenase inhibition [15] activities, were evaluated. Despite the increasing interest in *Knema* plants, comprehensive information regarding the chemical composition and biological activities of *K. elegans* essential oil is still lacking. Motivated by this gap in knowledge, our study focuses on the chemical composition and evaluation of antioxidant and AChE inhibitory activities of essential oil extracted from *K. elegans* leaves collected in Vietnam. By conducting thorough analyses, we aim to contribute to a deeper understanding of the medicinal and pharmacological properties of *K. elegans*, shedding light on its potential applications in healthcare and beyond.

RESULTS AND DISCUSSION

Chemical composition of essential oil

The leaves of *K. elegans* were hydrodistilled to successfully obtain pure essential oil with a yield of 0.11% (v/w). *K. elegans* essential oil revealed a chemical composition with 30 constituents identified, rich in sesquiterpene hydrocarbons (77.8%) and oxygenated sesquiterpenes (16.3%) by GC/MS analyses. As presented in Table 1, bicyclogermacrene (23.8%), germacrene D (17.1%), β-caryophyllene (15.4%), δ-cadinene (8.2%), and α-cadinol (7.9%) were the major compounds of *K. elegans* essential oil. The structures of these major compounds are illustrated in Fig. 1. In addition, considerable amounts of α-humulene (4.2%), germacrene B (3.4%), caryophyllene oxide (2.6%), epi-α-cadinol (1.7%), and γ-muurolene (1.3%) were detected.

This research marks the initial exploration into the chemical composition of essential oil derived from *K. elegans*, thereby precluding direct comparisons with analogous samples from the same species. However, an extensive examination of essential oils from other *Knema* species, including *K. hookeriana* [14], *K. kunstleri* [15], *K. intermedia* [16], *K. malayana* [17], *K. angustifolia* [18], *K. pierrei* [19], and *K. globularia* [20], has been documented.

Table 1. Chemical compositions of *Knema elegans* essential oil

No.	Compound [a]	RT [b]	RI [c]	RI [d]	Area (%)
1	δ-Elemene	23.56	1338	1335	0.5
2	α-Copaene	24.94	1377	1374	0.2
3	β-Cubebene	25.50	1386	1387	0.7
4	β-Elemene	25.98	1393	1389	0.1
5	β-Caryophyllene	26.54	1421	1417	15.4
6	γ-Elemene	26.97	1435	1434	0.2
7	Aromadendrene	27.08	1440	1439	0.1
8	α-Humulene	27.55	1455	1452	4.2
9	9-*epi*-(*E*)-Caryophyllene	27.78	1465	1464	0.6
10	γ-Muurolene	28.17	1476	1478	1.3
11	Germacrene D	28.43	1480	1484	17.1
12	β-Selinene	28.59	1488	1489	0.8
13	Bicyclogermacrene	28.85	1498	1500	23.8
14	(*E*,*E*)-α-Farnesene	28.89	1504	1505	0.2
15	β-Bisabolene	28.98	1506	1505	0.1
16	γ-Cadinene	29.34	1514	1513	0.9
17	δ-Cadinene	29.55	1525	1522	8.2
18	Germacrene B	30.75	1559	1559	3.4
19	Spathulenol	31.36	1579	1577	0.6
20	Caryophyllene oxide	31.57	1583	1582	2.6
21	Globulol	31.62	1587	1590	0.5
22	Viridiflorol	31.67	1594	1592	0.7
23	Guaiol	31.83	1600	1600	0.9
24	Ledol	32.25	1605	1602	0.2
25	Humulene epoxide II	32.31	1612	1608	0.3
26	*epi*-α-Cadinol	33.11	1639	1638	1.7
27	*epi*-α-Muurolol	33.15	1645	1640	0.6
28	β-Eudesmol	33.47	1650	1649	0.1
29	α-Cadinol	33.53	1659	1652	7.9
30	Intermedeol	34.31	1667	1665	0.2
	Sesquiterpene hydrocarbons (No. 1-18)				77.8
	Oxygenated sesquiterpenes (No. 19-30)				16.3
	Total % of compounds identified				94.1

[a]Elution order on Equity-5 column; [b]Retention time (min); [c]Retention indices on Equity-5 column; [d]Literature retention indices.

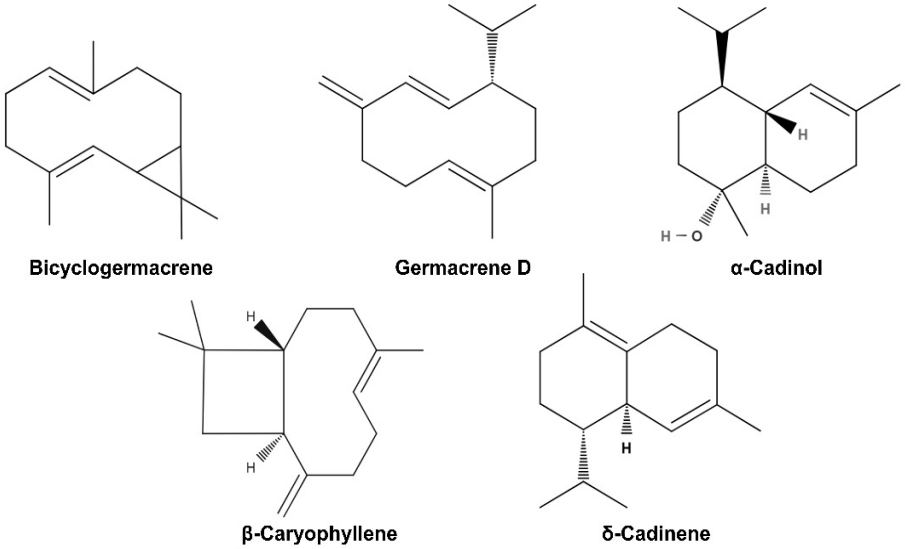

Figure 1. Chemical structures of main compounds in the essential oil of *Knema elegans.*

Previous studies have identified distinctive volatile compounds within these species, shedding light on the unique aromatic profiles of each. The major components of *K. elegans* essential oil compared with other *Knema* species are presented in Fig. 2. For instance, the essential oil from *K. hookeriana* displayed a predominant presence of β-caryophyllene (26.2%), germacrene D (12.5%), δ-cadinene (9.2%), germacrene B (8.8%), and bicyclogermacrene (5.5%) [14]. On the other hand, *K. kunstleri* essential oil exhibited β-caryophyllene (23.2%), bicyclogermacrene (9.6%), δ-cadinene (7.3%), α-humulene (5.7%), and germacrene D (4.3%) as major constituents [15]. Notably, β-elemene (25.48%) emerged as an abundant compound in *K. globularia* essential oil [20]. Further distinctions were observed in the major compounds of *K. malayana* and *K. pierrei* essential oils, where δ-cadinene (20.2% and 19.04%) took precedence [17, 19]. A significant amount of globulol was found in essential oils from the leaves (35.46%) and twigs (30.83%) of *K. angustifolia* [18], while τ-muurolol (20.1%) was demonstrated to be the major compound in *K. intermedia* essential oil [16]. Despite acknowledging sesquiterpene compounds as predominant in *Knema* genus essential oils from Vietnam and other regions globally, our findings reveal distinct differences in the main compounds present in *K. elegans* essential oil compared to other *Knema* species. The observed variations in essential oil chemical compositions may stem from factors such as plant age, climatic and environmental

conditions, selection of plant organs, and harvest timing [21, 22]. These factors potentially influence plant biosynthesis pathways, leading to diverse chemical compositions and content, thereby contributing to the development of distinct chemotypes [23].

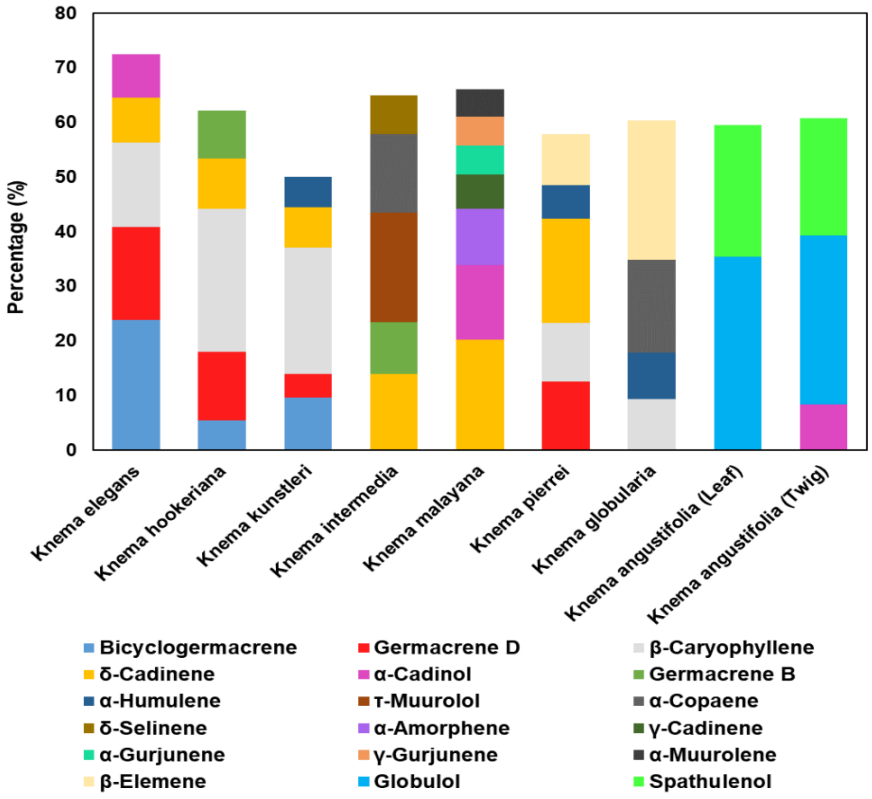

Figure 2. Comparison of major components in essential oils from different *Knema* species.

Antioxidant activity of essential oil

The essential oil of *K. elegans* showed moderate antioxidant activity based on both DPPH and ABTS methods compared to the ascorbic acid as a standard synthetic antioxidant (Fig. 3). The scavenging activity increased with the increment of essential oil concentration. At a concentration of 50 µg/mL of *K. elegans* essential oil, the DPPH and ABTS colors were reduced by 38.09% ± 1.74 and 32.14% ± 1.52, respectively, while ascorbic acid

showed a reduction of 95.05% ± 2.62 and 89.37% ± 2.95, respectively, at the same concentration. To calculate IC_{50}, standard curves of antioxidant activity in DPPH and ABTS assays were constructed (Fig. 4). According to the IC_{50} data, the *K. elegans* essential oil revealed IC_{50} values of 93.11 ± 1.95 µg/mL and 114.95 ± 2.41 µg/mL for DPPH and ABTS, respectively. The standard antioxidant, ascorbic acid, showed IC_{50} values of 11.86 ± 0.45 µg/mL and 16.40 ± 0.51 µg/mL, regarding DPPH and ABTS, respectively.

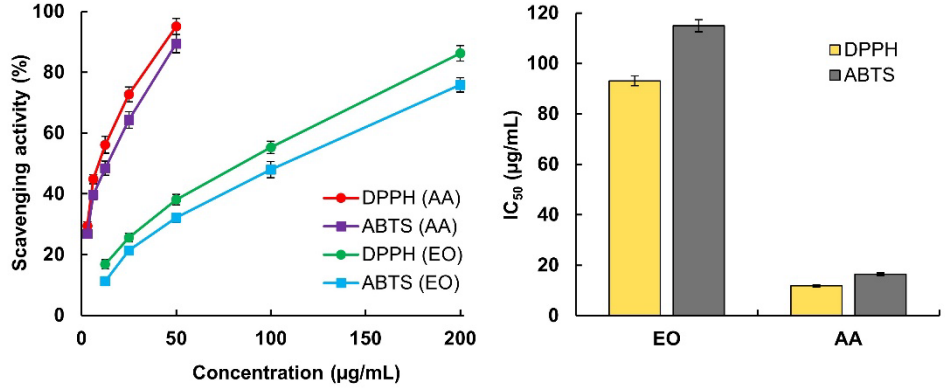

Figure 3. Antioxidant activity at different concentrations and half-maximum inhibitory concentration (IC_{50}) of *Knema elegans* essential oil (EO) and ascorbic acid (AA) based on the scavenging of DPPH and ABTS. Values are means (n = 3) ± standard deviation.

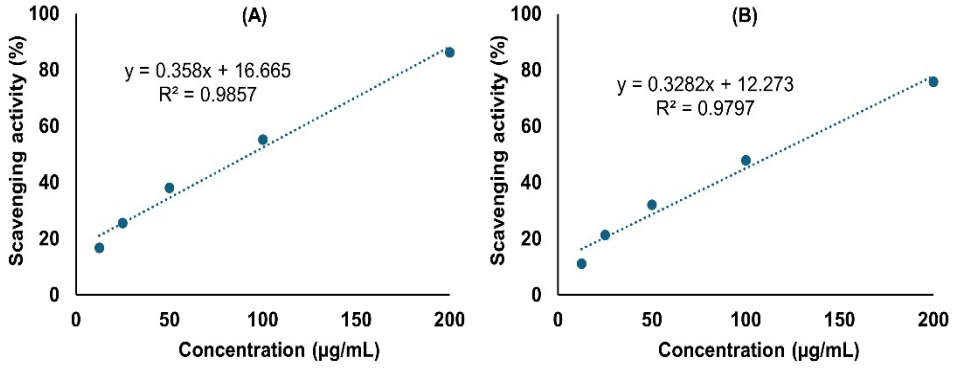

Figure 4. Standard curves of antioxidant activity in DPPH (A) and ABTS (B) assays for *Knema elegans* essential oil.

There are no reports in the literature regarding the antioxidant capacity of *K. elegans* essential oil. However, there are reports of this activity for essential oils obtained from other species of the genus *Knema*. For instance, the essential oil of *K. kunstleri*, composed mainly of β-caryophyllene (23.2%), bicyclogermacrene (9.6%), δ-cadinene (7.3%), α-humulene (5.7%), and germacrene D (4.3%), which presented potential antioxidant activity by the DPPH method, with an IC_{50} value of 80.5 µg/mL [15]. Similarly, the essential oil from the leaves of *K. pierrei*, mainly consisting of δ-cadinene (19.04%), germacrene D (12.57%), β-caryophyllene (10.74%), β-elemene (9.32%), and α-humulene (6.18%), showed antioxidant activity with IC_{50} values of 152.17 µg/mL by the DPPH method and 173.62 µmol Trolox/g by the FRAP method [19]. In another study by Thinh et al. [20], the essential oil of *K. globularia*, characterized by major compounds like β-elemene (25.48%), α-copaene (17.05%), β-caryophyllene (9.37%), and α-humulene (8.42%), exhibited antioxidant activity with IC_{50} values of 198.13 µg/mL by the DPPH method and 143.61 µg/mL by the ABTS method. It can be seen that *K. elegans* essential oil in this study demonstrated stronger antioxidant activity than *K. globularia* and *K. pierrei* essential oils. However, *K. elegans* essential oil showed weaker antioxidant activity than *K. kunstleri* essential oil. These variations can be attributed to differences in the content and presence of compounds in essential oils, as demonstrated earlier [24, 25].

The remarkable antioxidant activity exhibited by *K. elegans* essential oil can be attributed to its key sesquiterpene components, notably bicyclogermacrene, germacrene D, β-caryophyllene, and δ-cadinene. This assertion was supported by various studies focusing on essential oils from different plant sources [26, 27]. For instance, an investigation involved *Marrubium peregrinum* essential oil sourced from three distinct locations in Serbia and revealed robust antioxidant capabilities, with the primary constituents β-caryophyllene, bicyclogermacrene, and germacrene D demonstrating potent activity in neutralizing DPPH•, NO•, and O_2•− radicals, as evidenced by IC_{50} values that ranged from 8.81 to 16.41 µg/mL [28]. Similarly, the essential oil derived from the female leaf sample of *Baccharis punctulata*, featuring bicyclogermacrene, germacrene D, and β-caryophyllene as predominant components, exhibited noteworthy antioxidant efficacy, reaching 0.328 mg AAE/g in the DPPH test [29]. In another study, *Kundmannia sicula* essential oil, enriched in the sesquiterpene hydrocarbon germacrene D, showcased commendable ABTS free radical scavenging activity, with an IC_{50} value of 14.5 µg/mL [30]. Moreover, an independent study highlighted the antioxidant potential of δ-cadinene, the predominant compound in *Jatropha curcas* essential oil, with an IC_{50} value of 314 µg/mL in the DPPH test [31]. It is essential to underscore that the observed antioxidant activities in essential

oil may have arisen from the synergistic interactions among both major and minor components [24, 32, 33]. Overall, the diverse compositions of this essential oil contributed to its collective antioxidant efficacy.

Anti-acetylcholinesterase activity of essential oil

AChE is an enzyme crucial for terminating nerve impulses by catalyzing the breakdown of acetylcholine, a neurotransmitter [6, 9]. Dysregulation of AChE activity is associated with various neurological disorders, including Alzheimer's disease [6, 9]. In this study, we assessed the potential AChE inhibition of *K. elegans* essential oil by measuring its rate at five different concentrations, as presented in Fig. 5. The AChE inhibition activity increased with the increasing concentration of essential oil. At a concentration of 200 µg/mL, *K. elegans* essential oil and galantamine achieved AChE inhibition rates of 69.16% ± 2.14 and 89.05% ± 2.87, respectively. To calculate IC_{50}, standard curves of anti-AChE activity were constructed (Fig. 6). According to the IC_{50} data, the *K. elegans* essential oil exhibited significant inhibitory activity with an IC_{50} value of 87.44 ± 2.52 µg/mL, while galantamine showed an IC_{50} value of 15.57 ± 0.89 µg/mL. To date, there are no reports in the literature regarding the anti-AChE activity of *K. elegans* essential oil. However, there is one report on this activity for essential oil obtained from the genus *Knema*. Our findings are supported by Salihu et al., who reported that *K. hookeriana* essential oil showed high AChE inhibitory activity with an IC_{50} value of 70.5 µg/mL [14].

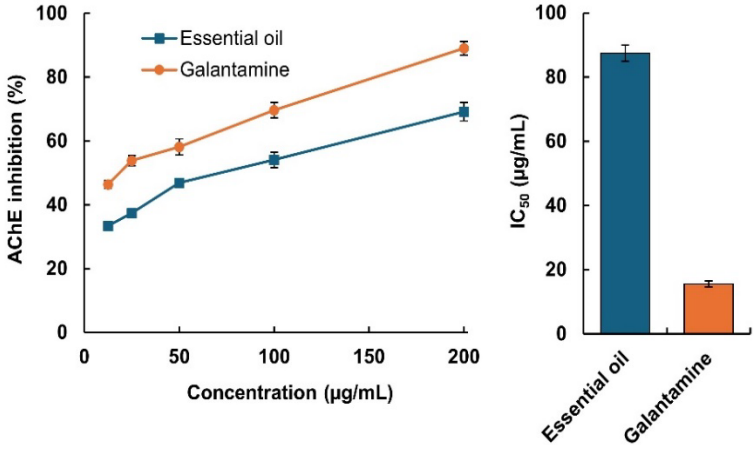

Figure 5. Anti-acetylcholinesterase activity at different concentrations and half-maximum inhibitory concentration (IC_{50}) of *Knema elegans* essential oil and galantamine. Values are means (n = 3) ± standard deviation.

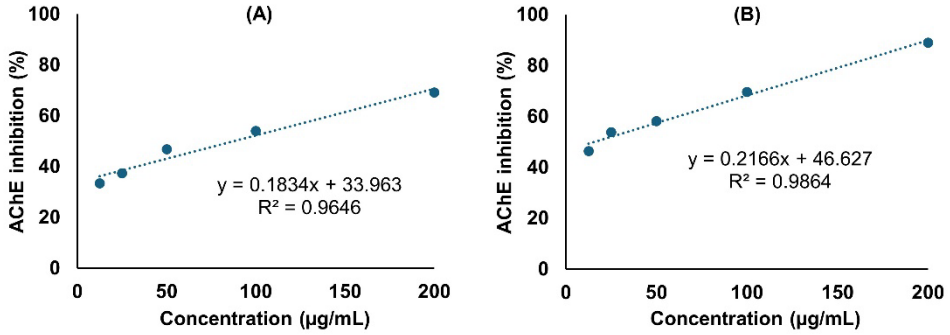

Figure 6. Standard curves of anti-acetylcholinesterase activity for *Knema elegans* essential oil (A) and galantamine (B).

Previous research has established that essential oils rich in sesquiterpenes possess anti-cholinesterase properties [34-36]. The anti-AChE activity of *K. elegans* essential oil, as indicated in the study, can be attributed to its main sesquiterpene compounds. This is consistent with findings from other studies, such as the one involving essential oil from *Psychotria poeppigiana* leaves, where germacrene D and bicyclogermacrene, the two main compounds, exhibited significant AChE inhibition [37]. Similarly, *Gynura bicolor* leaf essential oil, known for its richness in (*E*)-β-caryophyllene, demonstrated substantial AChE inhibition, reaching 53% at a concentration of 0.50 µg/mL, with an ID_{50} (50% inhibitory dose) value of 85 µg/mL [38]. The anti-AChE activity of essential oils is commonly associated with competitive inhibition of the enzyme. This inhibition occurs when bioactive molecules within the essential oil bind to the active site of the AChE enzyme [39]. While AChE inhibition is of particular interest in the context of studying treatments or interventions for Alzheimer's disease and other neurodegenerative conditions, it is difficult to attribute the anti-AChE activity of *K. elegans* essential oil only to one or several active compounds. This implies that the overall effect on AChE activity may be a result of the combined actions of various constituents in the essential oil, working together in a synergistic manner [39].

Conclusions

In summary, this research delved into the chemical composition of essential oil from *K. elegans* leaves in Vietnam and its antioxidant and anti-AChE properties. The moderate antioxidant activity and significant anti-AChE

effects suggest that this essential oil could be explored for its neuroprotective and cognitive-enhancing properties. Further studies could focus on elucidating the mechanisms behind these observed effects, exploring potential applications in neurodegenerative diseases, and optimizing extraction techniques to enhance the oil's bioactivity. Additionally, clinical trials may be warranted to validate the oil's safety and efficacy for potential pharmaceutical or nutraceutical use. Overall, these findings open avenues for continued research into the medicinal properties of *K. elegans* essential oil.

EXPERIMENTAL SECTION

Plant material

Leaves of *K. elegans* were collected at the Ba Vi National Park in Hanoi, Vietnam in August 2022. The identification and authentication of plant samples was performed by author Dau B. Thin from Hong Duc University, Vietnam. A voucher specimen was deposited in the herbarium of that university with code BV106. Fresh leaves were transported to the laboratory, chopped, and air-dried under shade for a week before essential oil extraction.

Extraction of essential oil

The essential oil of *K. elegans* was collected by hydrodistillation method. For this purpose, the air-dried leaves were submitted to hydrodistillation using a Clevenger-type apparatus for 4 h as described previously [40]. The resultant essential oil was collected in clean glass vials, dried with anhydrous sodium sulfate, and stored at 4°C until analyses.

Essential oil chemical analysis

The essential oil of *K. elegans* was analyzed using gas chromatography coupled to mass spectrometry (GC-MS) with a GCMS-QP2010 Plus apparatus from Shimadzu, Japan. The analysis employed a fused silica Equity-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium, flowing at a rate of 1.5 mL/min, served as the carrier gas. The column temperature program was initiated at 60°C for 2 min, followed by a 3°C/min increase to 240°C, and then a 5°C/min increase to 280°C, maintained for 40 min. The sample was injected with a split ratio of 10:1, and the injector and interface temperatures were set at 280°C. The mass spectrometer operated in electron impact mode at 70 eV, and the detector voltage was fixed at 0.82 kV. Mass spectra were

acquired through automatic scanning every 0.5 s, covering mass fragments in the 40–500 m/z range. Identifications relied on comparisons between the obtained spectra and those stored in the MS library and the retention index (RI) compared with literature data [41, 42]. The RI was calculated using the Van Den Dool and Kratz equation [43], determined by co-injecting a homologous series of linear *n*-alkanes. A semi-quantitative analysis was conducted to determine the relative amounts of each component in the essential oil. For quantitative data on essential oil constituents, the GC2010 equipment from Shimadzu, coupled with a flame ionization detector (FID), was used under conditions similar to the GC-MS system.

Antioxidant assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of *K. elegans* essential oil was conducted following previously established procedures [44]. Briefly, a 0.1 mM DPPH solution in methanol was prepared. Subsequently, 2 mL of this solution was added to 0.1 mL of various concentrations of essential oil dissolved in methanol for testing. The reaction mixture was thoroughly vortexed, placed in the dark at 25°C for 30 min, and then measured at 517 nm. The essential oil's ability to scavenge the DPPH radical was calculated as % inhibition using the following equation: % inhibition = (Ac − As) / Ac × 100, where Ac is the absorbance of the control, and As is the absorbance in the presence of essential oil. Ascorbic acid served as a positive control. The experiment was performed in triplicate. The inhibitory concentration (IC_{50}) of the essential oil, required to inhibit 50% of the DPPH radicals, was determined from the standard curve and compared to that of ascorbic acid.

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay of *K. elegans* essential oil was conducted following previously established procedures [44]. Briefly, the ABTS radical was generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate through incubation at room temperature in the dark for 16 h. The ABTS solution was then diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm. The ABTS reaction mixture comprised 2.6 mL of a diluted ABTS radical solution and 0.1 mL of essential oil at various concentrations, dissolved in methanol for testing. After incubation at 25°C for 6 min, the absorbance of the mixture solution was measured at 734 nm. Ascorbic acid served as a positive control. The experiment was performed in triplicate, and the percentage inhibition of the ABTS radical and the IC_{50} value by the essential oil were calculated, as described in the DPPH assay.

Anti-acetylcholinesterase assay

The anti-AChE assay of *K. elegans* essential oil was conducted following previously established procedures [45]. Briefly, the total reaction volume (200 µL) consisted of 150 µL of 0.1 M sodium phosphate buffer (pH 8.0), 20 µL of AChE (0.45 U/mL), 10 µL of different concentrations of essential oil dissolved in methanol, 10 µL of 5,5′-dithio-bis-[2-nitrobenzoic acid] (DTNB) (0.03 mM), and 10 µL of acetylthiocholine iodide (0.68 mM). This reaction mixture was incubated at room temperature for 20 min. Following incubation, the optical density was measured immediately at 412 nm. Galantamine was used as a positive control. The experiment was performed in triplicate. The inhibition rate (%) of AChE activity was calculated using the following equation: % inhibition = (Ac − As) / Ac × 100, where Ac is the absorbance of the control, and As is the absorbance of the test sample. An extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against extract solution concentrations.

Statistical analysis

All the experiments were performed in triplicate and results were given as the mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel 2016 for Windows (Microsoft, USA).

REFERENCES

1. M. J. Christenhusz; J. W. Byng; *Phytotaxa,* **2016**, *261*(3), 201-217.
2. B. Li; T. K. Wilson; Myristicaceae. In: Flora of China Online, Vol. 7, Science Press, Beijing, China, **2008**.
3. W. M. N. H. W. Salleh; F. Ahmad; *Pharm. Sci.,* **2017**, *23*(4), 249-255.
4. A. S. Salihu; W. M. N. H. W. Salleh; *Vietnam J. Chem.,* **2023**, *61*(4), 397-411.
5. N. Q. Hop; N. T. Son; *Curr. Pharm. Biotechnol.,* **2023**, *24*, 1524-1553.
6. A. Maggio; S. Rosselli; M. Bruno; *Curr. Pharm. Des.,* **2016**, *22*(26), 4011-4027.
7. M. G. Miguel; *Molecules,* **2010**, *15*(12), 9252-9287.
8. F. Bakkali; S. Averbeck; D. Averbeck; M. Idaomar; *Food Chem. Toxicol.,* **2008**, *46*(2), 446-475.
9. T. C. D. Santos; T. M. Gomes; B. A. S. Pinto; A. L. Camara; A. M. D. A. Paes; *Front. Pharmacol.,* **2018**, *9*, 1192.
10. P. H. Ho; An Illustrated the Flora of Vietnam, Vol. 1. Tre Publishing House, Ho Chi Minh, Vietnam, **1999**.
11. Y. X. Zhang; Z. Lu; W. C. Wu; Y. G. Chen; R. Zhan; *Phytochem. Lett*., **2021**, *42*, 121-124.

12. Z. Lu; W. C. Wu; M. Wang; J. Q. Zhang; Y. G. Chen; R. Zhan; L. D. Shao; *Biochem. Syst. Ecol*., **2020**, *88*, 103991.
13. J. Z. Deng; S. R. Starck; S. Li; S. M. Hecht; *J. Nat. Prod*., **2005**, *68*(11), 1625-1628.
14. A. S. Salihu; W. M. N. H. W. Salleh; T. H. Ogunwa; *Nat. Prod. Res*., **2024**, *14*, 2516-2521.
15. W. M. N. H. W. Salleh; M. Z. A. Anuar; S. Khamis; M. A. Nafiah; M. D. Sul'ain; *Nat. Prod. Res*., **2021**, *35*(13), 2279-2284.
16. A. S. Salihu; W. M. N. H. W. Salleh; W. N. Setzer; *Z. Naturforsch. C*., **2023**, *78*, 293-298.
17. A. S. Salihu; W. M. N. H. W. Salleh; *J. Essent. Oil Bearing Plants,* **2023**, *26*(2), 253-260.
18. R. Barman; J. Saikia; P. Sarmah; P. Konwar; M. Kumar; P. K. Bora; P. Bhattacharyya; S. P. Saikia; S. Haldar; D. Banik; *S. Afr. J. Bot*., **2023**, *159*, 617-626.
19. D. B. Thin; B. B. Thinh; J. O. Igoli; *Chem. Nat. Compd*., **2023**, *59*: 584-586.
20. B. B. Thinh; N. T. Khoi; R. V. Doudkin; D. B. Thin; I. A. Ogunwande; *Nat. Prod. Res*., **2023**, *37*(10), 1625-1631.
21. A. Barra; *Nat. Prod. Commun*., **2009**, *4*(8), 1147-1154.
22. A. C. Figueiredo; J. G. Barroso; L. G. Pedro; J. J. Scheffer; *Flavour Fragr. J*., **2008**, *23*(4), 213-226.
23. N. Verma; S. Shukla; *J. Appl. Res. Med. Aromat. Plants,* **2015**, *2*(4), 105-113.
24. R. Amorati; M. C. Foti; L. Valgimigli; *J. Agric. Food Chem*., **2013**, *61*(46), 10835-10847.
25. T. A. Misharina; E. S. Alinkina; L. D. Fatkulina; A. K. Vorobyova; I. B. Medvedeva; E. B. Burlakova; *Appl. Biochem. Microbiol*., **2012**, *48*, 102-107.
26. H. Bartikova; V. Hanusova; L. Skalova; M. Ambroz; I. Bousova; *Curr. Top. Med. Chem*., **2014**, *14*(22), 2478-2494.
27. H. Khan; A. L. Khan; J. Hussain; M. Adnan; I. Hussain; T. Khan; A. R. Khan; *Pak. J. Sci. Ind. Res. B: Biol. Sci*., **2008**, *51*(6), 343-350.
28. B. Kaurinovic; S. Vlaisavljevic; M. Popovic; D. Vastag; M. Djurendic-Brenesel; *Molecules,* **2010**, *15*(9), 5943-5955.
29. J. Ascari; M. S. de Oliveira; D. S. Nunes; D. Granato; D. R. Scharf; E. Simionatto; M. Otuki; B. Soley; G. Heiden; *J. Ethnopharmacol,* **2019**, *234*, 1-7.
30. S. Casiglia; M. Bruno; M. Bramucci; L. Quassinti; G. Lupidi; D. Fiorini; F. Maggi; *J. Essent. Oil Res*., **2017**, *29*(6), 437-442.
31. R. A. Babahmad; A. Aghraz; A. Boutafda; E. G. Papazoglou; P. A. Tarantilis; C. Kanakis; M. Hafidi; Y. Ouhdouch; A. Outzourhit; A. Ouhammou; *Ind. Crops Prod*., **2018**, *121*, 405-410.
32. L. D. do Nascimento; A. A. B. de Moraes; K. S. da Costa; J. M. P. Galúcio; P. S. Taube; C. M. L. Costa; J. D. Cruz; E. H. D. A. Andrade; L. J. G. de Faria; *Biomolecules,* **2020**, *10*(7), 988.
33. B. B. Thinh; D. B. Thin; I. A. Ogunwande; *Nat. Prod. Commun.,* **2024**, *19*(3), 1934578X241239477.
34. A. Arya; R. Chahal; R. Rao; M. H. Rahman; D. Kaushik; M. F. Akhtar; A. Saleem; S. M. A. Khalifa; H. R. El-Seedi; M. Kamel; G. M. Albadrani; M. M. Abdel-Daim; V. Mittal; *Biomolecules,* **2021**, *11*(3), 350.

35. H. Hajimehdipoor; M. Mosaddegh; F. Naghibi; A. Haeri; M. Hamzeloo-Moghadam; *An. Acad. Bras. Cienc.,* **2014**, *86*, 801-806.
36. B. B. Thinh; L. D. Chac; *J. Essent. Oil Bearing Plants,* **2024**, *27*(2), 574-583.
37. A. S. N. Formagio; W. Vilegas; C. R. F. Volobuff; C. A. L. Kassuya; C. A. L. Cardoso; Z. V. Pereira; R. M. M. F. Silva; D. A. D. S. Yamazaki; G. D. F. Gauze; J. Manfron; J. A. Marangoni; *J. Ethnopharmacol,* **2022**, *296*, 115220.
38. M. Miyazawa; H. Nakahashi; A. Usami; N. Matsuda; *J. Nat. Med.,* **2016**, *70*, 282-289.
39. J. Calva; M. Silva; V. Morocho; *Molecules,* **2023**, *28*(24), 8112.
40. B. B. Thinh; D. B. Thin; *J. Essent. Oil Bearing Plants,* **2023**, *26*(3), 653-663.
41. R. P. Adams; Identification of essential oil components by gas chromatography-mass spectrometry. 4th ed. Carol Stream (IL): Allured Publishing Corporation, **2007**.
42. NIST; Mass Spectral Library (NIST/EPA/NIH). National Institute of Standards and Technology, Gaithersburg, USA, **2018**.
43. H. Van Den Dool; P. Kratz; *J. Chromatogr. A*., **1963**, *11*, 463-471.
44. D. B. Thin; A. A. Korneeva; B. B. Thinh; I. A. Ogunwande, *Russ. J. Bioorg. Chem.*, **2023**, *49*(4), 815-822.
45. S. Bendjabeur; O. Benchabane; C. Bensouici; M. Hazzit; A. Baaliouamer; A. Bitam; *J. Food Meas. Charact*., **2018**, *12*, 2278-2288.