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Original Research Article

Phytochemical Characterization and Neuroprotective Potential of *Ruta chalepensis* Essential Oil via Cholinesterase Inhibition

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ABSTRACT

Neurodegenerative diseases, including Alzheimer's disease, are an increasing global health issue, with cholinesterase inhibition serving as an important therapeutic approach to improve cholinergic neurotransmission. The current study aims to examine the chemical composition and cholinesterase inhibitory ability of the essential oil isolated from the aerial parts of *Ruta chalepensis*. . The anticholinesterase activity of the essential oil was evaluated using a colorimetric assay against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) at concentrations of 200, 100, 50, 12.5, and 6.25 µg/mL, with galantamine serving as the positive control. The essential oil was extracted using a hydro-distillation and analyzed by gas chromatography–mass spectrometry, identifying twenty-six components that comprised 92.99% of the total oil content. Ketones constituted the primary chemical class (43.45%), with 2-nonanone (34.20%) being the principal component. Fatty acids, aldehydes, and monoterpenes were identified. The evaluated essential oil exhibited moderate inhibitory effects relative to galantamine (reference drug), with IC_{50} values of 47.71 ± 0.95 µg/mL ($p<0.0001$) for AChE and 56.28 ± 1.92 µg/mL ($p<0.0001$) for BChE. The findings indicate that *Ruta chalepensis* essential oil contains bioactive compounds with potential anticholinesterase activity, requiring an *in vivo* investigation for future therapeutic applications in neurological disorders such as Alzheimer's disease.

Keywords: *Ruta chalepensis,* Essential oil, Cholinesterase inhibitors, Gas chromatography–mass spectrometry (GC–MS), Neurodegenerative diseases, Alzheimer's disease.

Introduction

Neurodegenerative diseases, particularly Alzheimer's disease (AD), represent a major global health challenge, with their incidence steadily increasing due to the aging population worldwide. [1] Cholinesterase enzymes, such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), hydrolyze the neurotransmitter acetylcholine, causing decrease in cholinergic neurotransmission which is an indicator of Alzheimer's disease.[2] Additionally, the current treatment for Alzheimer's disease primarily consists of cholinesterase inhibitors, which act by blocking these enzymes and improving cholinergic function.[3] The seek for safer, natural alternatives has been driven by the adverse effects associated with several synthetic inhibitors, such as galantamine [4], which can cause nausea, vomiting, diarrhea, dizziness, muscle weakness, bradycardia, and hepatotoxicity. Medicinal plants comprise a wide variety of phytochemicals, including steroids, alkaloids, flavonoids, tannins, saponins, glycosides, volatile oils, phenolic compounds, and many more. Essential oils are volatile secondary metabolites that plants produce in naturally occurring ways. Many plant groups are rich in essential oils, and essential oils in general are abundant in certain aromatic plant families.[5] *Ruta chalepensis* (*R. chalepensis*) L. (Rutaceae), is a fragrant subshrub that has a long history of usage in folk medicine throughout the Middle East and Mediterranean for a variety of medical conditions, including gastrointestinal, neurological, and inflammatory issues.[6]

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The essential oils found in its aerial portions are known to include bioactive components such flavonoids, phenolics, ketones, and monoterpenes.[7-9] Antioxidants,[6,9] cytotoxic,[6,8] antibacterial,[9] and neuroprotective actions [10] are only a few of the many pharmacological effects shown by these phytochemicals. This study examines the chemical composition and cholinesterase inhibitory potential of *R. chalepensis* essential oil, considering the increasing interest in plant-derived cholinesterase inhibitors. Gas chromatography–mass spectrometry (GC–MS) was employed to analyze the components of the oil, while an *in vitro* colorimetric assay was conducted to evaluate its inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The aim of this study is to investigate the efficacy of *R. chalepensis* essential oil as a natural neuroprotective agent and its potential as a candidate for the development of treatments for Alzheimer's disease.

Materials and Methods

Plant material collection

The aerial parts of *Ruta chalepensis* were collected during the spring/2024 from the campus of the Faculty of Sciences, University of Jordan, Amman/Jordan (32°00'48.5"N 35°52'26.4"E). The plant was taxonomically identified by Prof. Dr. Sawsan A. Oran, Department of Biological Sciences, University of Jordan. A voucher specimen (JU-GH-RC-13, greenhouse collection) was deposited in the herbarium of the Department of Biological Sciences, University of Jordan, Amman.

Essential oil extraction

The aerial parts of the identified plant were collected, air-dried at room temperature in the dark, and ground into a fine powder using an electric blender (HR2767/00, Philips, Netherlands). Fifty grams of dried powder was subjected to hydro-distillation using a Clevenger-type apparatus with 450 mL of distilled water for 3 hours, starting from the first drop of distillate until the essential oil yield stabilized. The essential oils that were extracted were dried using anhydrous sodium sulfate and then kept in dark vials at a temperature of 4 °C until they

were needed. [6] The essential oil yield (%) was calculated using the following formula [1]:

$$Essential\ oil\ yield\ (\%) = \frac{Net\ weight\ of\ extracted\ oil\ (g)}{Weight\ of\ dry\ plant\ material\ (g)}\ x100 \quad [1]$$

Gas chromatography–Mass spectrometry (GC/MS) analysis

The chemical composition of *R. chalepensis* essential oil was assessed using gas chromatography–mass spectrometry (GC–MS).[6] Five microliters of oil were dissolved in 1 mL of GC-grade n-hexane, and 1 µL of this solution was injected into a Varian Chrompack CP-3800 GC/MS/MS-200 system equipped with a DB-5 capillary column. The analysis used helium as the carrier gas and a linear temperature program ranging from 60°C to 246°C. Identification of components was achieved by comparing mass spectra with WILEY, NIST, and ADAMS libraries. Quantitative analysis was carried out using a GC-FID system with the same chromatographic conditions. The composition of each compound was expressed as a percentage of the total peak area. Each sample undergoes analysis in triplicate.

Assessment of acetylcholinesterase and butyrylcholinesterase inhibition

The anticholinesterase activity of *R. chalepensis* essential oil was evaluated using a spectrophotometric method to determine its inhibitory effects against two key enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), [11] with acetylthiocholine iodide and butyrylthiocholine chloride (Sigma-Aldrich, St. Louis, MO, USA) serving as substrates. The reaction was started by adding the substrates to a mixture containing sodium phosphate buffer (pH 8.0), the chromogenic reagent DTNB (5,5′-dithiobis (2-nitrobenzoic acid)), and the essential oil at various concentrations (200, 100, 50, 12.5, and 6.25 µg/mL). DTNB reacts with the thiocholine produced by enzymatic hydrolysis to form a yellow-colored 5-thio-2-nitrobenzoate anion, which was measured at 412 nm using a 96-well microplate reader.[11] The percentage inhibition (I%) of enzyme activity was calculated using the following equation [2] [11]:

$$I\% = \frac{(E - S)}{E}\ x100 \quad [2]$$

Where E is the enzyme activity without the sample, and S is the activity with the test sample.

All tests were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Galantamine at various concentrations (200, 100, 50, 12.5, and 6.25 µg/mL) was used as a positive control.

Statistical analysis

The data were presented as means ± standard deviation (SD). The One-Way ANOVA was employed to determine statistical significance, with Tukey's post-hoc test conducted using GraphPad Prism 10, where $p<0.05$ was considered significant.

Results and Discussion

Oil extraction and phytochemical analysis

The hydro-distilled dried aerial parts of *R. chalepensis* yielded 0.41 ± 0.02% essential oil (w/w). The chemical constituents of the extracted oil were analyzed using GC-MS. The identified compounds, including their retention times, area percentages, and chemical classifications, are shown in Table 1. The GC-MS chromatogram of the essential oil is illustrated in Figure 1. Twenty-six compounds were identified, accounting for 92.99% of the total oil content. Ketones were the most prevalent chemical class, accounting for 43.45% of the total, with 2-nonanone was the primary component (34.20%). Additional significant components included methyl palmitate, which accounted for 14.05% of the fatty acid group, and trans-2,4-hexadiena (5.2%) of the aldehyde class. Moreover, monoterpenes such as camphor (1.83%) and 1,8-cineole (2.86%) were detected. Phenolic and phenylpropanoid derivatives, such as methyleugenol (1.90%) and eugenol methyl ether (1.66%), were among the minor components.

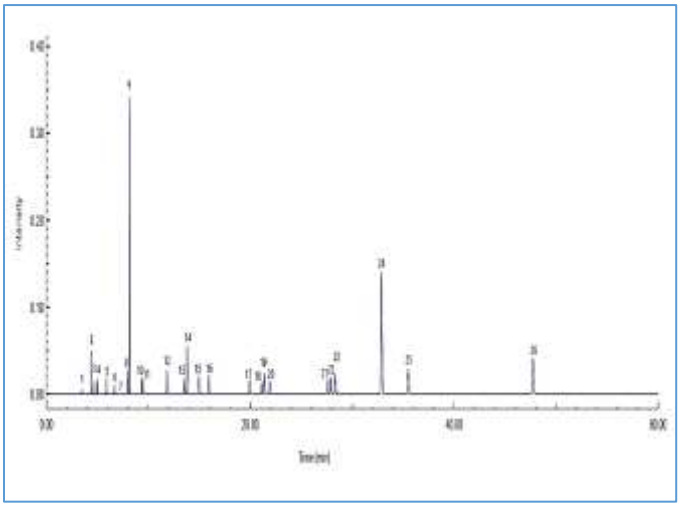

Figure 1: *Ruta chalepensis* essential oil chromatogram

These findings partially align with previous studies on *R. chalepensis*. In particular, Althaher *et al*.[6] reported a different chemical profile in which the major compound was 2-cyclohexen-1-one,3-[(2,3,4,9 tetrahydro-1H-pyrido[3,4-b]indole-1-yl)methyl] (45.97%), followed by 2-nonanone (19.45%). 2-Undecanone and 2-nonanone have been identified as the principal components of *R. chalepensis* essential oil in samples from various countries, including Turkey, India, Algeria, and Lebanon.[12-15] Particularly, in a Tunisian study, 2-undecanone alone makeup 87.18% of the total detected compounds in the essential oil. [7] In Djibouti, the essential oil from *R. chalepensis* contains 2-undecanone as the primary component (51.3%) of the total oil. [8] In *R. chalepensis* L. essential oil from Saudi Arabia, linoleic acid was identified as the predominant fatty acid, followed by oleic acid, palmitic acid, and linolenic acid. [9] This variation in chemical constituents can be linked to many factors, including the plant's geographical origin, harvesting season, extraction method, and environmental conditions. [6] However, 2-nonanone is consistently identified as a significant molecule in most investigations, highlighting its significance as a bioactive element in *R. chalepensis* essential oil.

Anticholinesterase activity of R. chalepensis essential oil

The potential anticholinesterase activity of the essential oil was assessed via its inhibitory effects on acetylcholinesterase (AChE) (Figure 2-A) and butyrylcholinesterase (BChE) (Figure 2-B). The activity was compared with galantamine (positive drug). The essential oil exhibited moderate inhibitory effects, with IC_{50} values of 47.71 ± 0.95 µg/mL for AChE and 56.28 ±1.92 µg/mL for BChE.

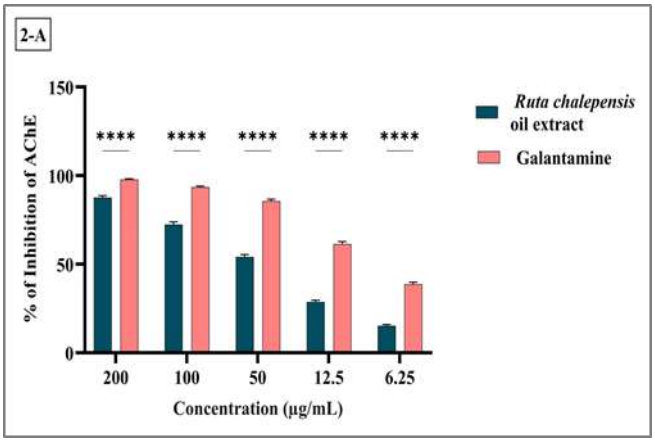

Figure 2: Inhibitory activity of *Ruta chalepensis* essential oil extract and galantamine (standard drug) against (A) acetylcholinesterase (AChE) and (B) butyrylcholinesterase (BChE).

Moreover, galantamine demonstrated significantly higher potency in comparison with the essential oil, yielding IC_{50} values of 8.84± 1.20 µg/mL for AChE ($p<0.0001$) and 13.68± 1.32 µg/mL for BChE ($p<0.0001$). These findings indicate that although *R. chalepensis* essential oil has potential cholinesterase inhibitory activity, its efficacy is significantly lower ($p<0.0001$) compared to the standard drug.

In this investigation, the essential oil showed higher inhibition of acetylcholinesterase (AChE) (IC_{50} = 47.71 ± 0.95 µg/mL) than butyrylcholinesterase (BChE) (IC_{50} = 56.28 ±1.92 µg/mL), which is particularly significant in the management of Alzheimer's disease. During the initial phases of the disease, AChE predominantly regulates acetylcholine levels; however, as the disease advances and AChE activity declines, and BChE acquires greater significance.[17] Therefore, medications that inhibit both enzymes, especially BChE in advanced stages, are therapeutically effective.

Comparative studies support the *Ruta* species' cholinesterase inhibitory ability. For example, Khadhri *et al*. [18] found that an ethanolic extract of *R. chalepensis* leaves inhibited AChE significantly (IC_{50} = 12.0 ± 1.10 µg/mL). The chloroform extract of *R. chalepensis* inhibited AChE and BChE with IC_{50} values of 41.14 ± 2.85 µg/mL and 79.56 ± 3.66 µg/mL, respectively. [19] While *R. tuberculata* acetonic extract effectively inhibited AChE (IC_{50} = 20.48 ± 0.20 µg/mL). [20] The chloroform extract of *R. montana* showed anticholinesterase activity, with an IC_{50} of 159.64 ± 0.49 µg/mL. [21]

Finally, *R. chalepensis* essential oil has a rich phytochemical profile, which supports its traditional usage in the treatment of neurological disorders. These findings provide a solid background for future pharmacological research.

Table 1: Chemical composition of *Ruta chalepensis* essential oil

No.	Compounds	Retention time (min)	% Area
1	trans-2-Hexen-1-ol	3.52	0.60
2	trans-2,4-Hexadienal	4.37	5.02
3	2-Methylheptan-4-one	4.73	1.50
4	Cumene	4.99	0.57
5	Unknown	5.01	1.51
6	Isopropylbenzene	5.90	1.67
7	tert-Butylbenzene	6.68	0.98
8	1,8-Cineole	7.95	2.86
9	2-Nonanone	8.20	34.20
10	Camphor	9.37	1.83
11	α-Terpineol	9.46	1.55
12	2-Decanone	11.84	2.65
13	1-Undecyne	13.54	1.77
14	Cyclohexanone, 2-(2-methylpropylidene)	14.97	1.98
15	Unknown	13.82	5.50
16	trans-2,4-Decadienal	15.95	2.13
17	Isoborneol	21.37	2.36
18	Nonadecan-2-one	19.94	1.63
19	Tetradecan-1-ol	21.13	1.38
20	Tridecan-2-one	21.92	1.49
21	Eugenol methyl ether derivative	27.61	1.66
22	Methyleugenol	27.92	1.90
23	(Z)-8-(3,5-Dimethyl-4-hydroxyphenyl)-2-octene	28.34	2.30
24	Methyl palmitate	32.88	14.05
25	(4-formyl-2,6-Dimethoxyphenyl)-1,3-benzodioxole-5-carboxylate	35.50	2.80
26	Methyl oleate	47.74	4.11
	Total identified		**92.99%**
	Chemical Classification		
	Alcohol		0.60%
	Aldehyde		7.15%
	Alkyne		1.77%
	Aromatic compounds		6.02%
	Monoterpenes		8.60%
	Ketone		43.45%
	Fatty acids		19.54%
	Phenolic compound		2.3%
	Phenylpropanoid		3.56%

Conclusion

The essential oil of *Ruta chalepensis* has a phytochemical profile that is characterized by monoterpenes, fatty acids, aldehydes, and ketones. The most prominent of these was 2-nonanone (34.20%). On the other hand, the essential oil had moderate inhibitory effects on the acetylcholinesterase and butyrylcholinesterase enzymes compared to galantamine (standard drug), highlighting its potential as a natural therapeutic agent for the treatment of neurological diseases. Moreover, further studies are required for isolation of its active constituents and conducting *in vivo* efficacy and safety studies.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Javaid SF, Giebel C, Khan MA, Hashim MJ. Epidemiology of Alzheimer's disease and other dementias: Rising global burden and forecasted trends. F1000Res. 2021;10:425.
2. Villeda-González JD, Gómez-Olivares JL, Baiza-Gutman LA. New paradigms in the study of the cholinergic system and metabolic diseases: Acetyl- and butyrylcholinesterase. J Cell Physiol. 2024;239(8):e31274.
3. Moreira NCS, Lima JEBF, Marchiori MF, Carvalho I, Sakamoto-Hojo ET. Neuroprotective effects of cholinesterase inhibitors: current scenario in therapies for Alzheimer's disease and future perspectives. J Alzheimers Dis Rep. 2022;6(1):177-193.
4. Bhat BA, Almilaibary A, Mir RA, Aljarallah BM, Mir WR, Ahmad F, Mir MA. Natural therapeutics in aid of treating Alzheimer's disease: a green gateway toward ending quest for treating neurological disorders. Front Neurosci. 2022;16:884345.
5. Abaza I, Aboalhaija N, Alsalman A, Talib W, Afifi F. Aroma profile, chemical composition and antiproliferative activity of the hydrodistilled essential oil of a rare *Salvia* species (*Salvia greggii*). J Biol Act Prod Nat. 2021;11(2):129-137.
6. Althaher AR, Oran SA, Bustanji YK. Phytochemical analysis, *in vitro* assessment of antioxidant properties and cytotoxic potential of Ruta *chalepensis* L. essential oil. J Essent Oil Bear Plants. 2020;23(6):1409-1421.
7. Lengliz O, Mejri J, Abderrabba M, Khalifa R, Mejri M. *Ruta chalepensis* L. essential oil: A new anti-sprouting agent for potatoes bio conservation. J Chem. 2018;2018 (1): 8547851.
8. Abdoul-Latif FM, Elmi A, Merito A, Nour M, Risler A, Ainane A, Bignon J, Ainane T. Essential oil of *Ruta chalepensis* L. from Djibouti: Chemical analysis and modeling of *in vitro* anticancer profiling. Separations. 2022;9(12):387.
9. Al Masoudi LM, Hamdi H, Zaid AA. Chemical composition, antifungal, antibacterial, antioxidant, antiviral and cytotoxic activities of *Ruta chalepensis* L. fixed oil from Saudi Arabia. J Biobased Mater Bioenergy. 2024;18(4):701-709.
10. Yücel YY, Karakoç ÖC, Servi H, Gücel S, Nalbantsoy A, Polatoğlu K. Fumigant and contact toxicity of *Ruta chalepensis* L. (Rutaceae) essential oil against five coleopteran stored product pests and its effects on cholinesterases. Plant Prot Bull. 2023;63(4):52-65.
11. Salihu AS, Salleh WMNHW, Ghani NA, Althaher AR, Abed SA. Chemical composition, anticholinesterase activity and cytotoxicity of the essential oil of *Luvunga scandens* (Roxb.) Wight from Malaysia. Z Naturforsch C. 2025;.*In press*
12. Baser KHC, Özek T, Beis S. Constituents of the essential oil of *Ruta chalepensis* L. from Turkey. J Essent Oil Res. 1996;8(4):413-414.
13. Bagchi GD, Dwivedi PD, Singh A, Haider F, Naqvi AA. Variations in essential oil constituents at different growth stages of *Ruta chalepensis* on cultivation at North Indian plains. J Essent Oil Res. 2003;15(4):263-264.
14. Haddouchi F, Chaouche TM, Zaouali Y, Ksouri R, Attou A, Benmansour A. Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. Food Chem. 2013;141(1):253-258.
15. Khoury M, Stien D, Ouaini N, Eparvier V, Apostolides NA, El Beyrouthy M. Chemical composition and antimicrobial activity of the essential oil of *Ruta chalepensis* L. growing wild in Lebanon. Chem Biodivers. 2014;11(12):1990-1997.
16. Aboalhaija N, Syaj H, Afifi F, Abu-Dahab R, Abusulieh S, Amro R. Phytochemistry and antiproliferative potential of a naturalized plant to Jordan: *Lavandula stoechas*. Chem Biodivers. 2025;e202500181.
17. Chen ZR, Huang JB, Yang SL, Hong FF. Role of cholinergic signaling in Alzheimer's disease. Molecules. 2022;27(6):1816.
18. Khadhri A, Bouali I, Belkhir S, Mokded R, Smiti S, Falé P, Araújo M, Serralheiro M . *In vitro* digestion, antioxidant and antiacetylcholinesterase activities of two species of *Ruta*: *Ruta chalepensis* and *Ruta montana*. Pharm Biol. 2017;55(1):101-107.
19. Gali L, Bedjou F. Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated *Ruta chalepensis*. S Afr J Bot. 2019;120:163-169.
20. Saidi A, Hambaba L, Bensaad MS, Kadi IE, Bensouici C, Sami R, Alharthi S, Elhakem A, . Alsharari ZD, Baty RS, Alruwais R S, Aljabri M D, Rahman M M, Shati A A, Elsaid F GPhytochemical analysis using cLC-DAD, nutritional importance and assessment of antioxidant, antidiabetic and anticholinesterase activities of *Ruta tuberculata* Forssk organic extracts. Sustainability. 2022;14(16):10451.
21. Slougui N, Rabhi NL, Achouri R, Bensouici C. Evaluation of the antioxidant, antifungal and anticholinesterase activity of the extracts of *Ruta montana* L., harvested from Souk-Ahras (North-East of Algeria) and composition of its extracts by GC-MS. Trop J Nat Prod Res. 2023;7(7): 3452-3461.