1-1-2024

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Cogent Food & Agriculture

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/oafa20

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To cite this article: Sonu Kumar Mahawer, Manar Fawzi Bani Mfarrej, Ravendra Kumar, Himani Karakoti, Om Prakash, Satya Kumar, Dharmendra Singh Rawat, Aditi Kundu, Pieter Malan & Faheem Ahmad (2024) Phytochemistry of isolated essential oils from *Cautleya spicata* (Sm.) Baker for their pesticidal evaluation and *in-silico* approaches to reveal the mode of nematicidal action, Cogent Food & Agriculture, 10:1, 2401594, DOI: 10.1080/23311932.2024.2401594

To link to this article: https://doi.org/10.1080/23311932.2024.2401594

Published online: 16 Sep 2024.

COGENT FOOD & AGRICULTURE
2024, VOL. 10, NO. 1, 2401594
https://doi.org/10.1080/23311932.2024.2401594

FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

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Phytochemistry of isolated essential oils from *Cautleya spicata* (Sm.) Baker for their pesticidal evaluation and *in-silico* approaches to reveal the mode of nematicidal action

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ABSTRACT

This study aimed to assess the chemical composition and pesticidal activities of essential oils (EOs) from *Cautleya spicata* rhizomes and aerial parts. The EOs were assessed against root-knot nematodes (*Meloidogyne incognita*) by mortality and hatchability bioassays and the phytotoxicity of the EOs was determined against *Raphanus raphanistrum* subspp. *Sativus* (radish) and *Cichorium intybus* (chicory) seeds. *In-silico* screening of the primary constituents of EOs and five chosen target proteins of the nematode was conducted to predict the expected mechanism of action of these oils. *p*-Cymene and β-pinene were the key compounds in the EOs from the rhizomes and aerial parts. The EO from the aerial parts (LC_{50} value = 0.555 µL/mL for nematode mortality assay and IC_{50} value = 0.016 µL/mL for nematode egg hatchability after 96 h) exhibited significantly higher nematicidal activity than the rhizome EO. However, rhizome EO showed higher phytotoxic activity against radish (*R. raphanistrum*) with lower IC_{50} values (0.157 and 0.123 µL/mL for RLI and SLI, respectively) and chicory (*C. intybus*) seeds (100% inhibition at 1.0 µL/mL concentration). Docking studies revealed the strongest inhibitory actions of β-selinene against acetylcholinesterase (AChE) and cytochrome c oxidase (CytC), spathulenol against glutathione S-transferase (GST-1), and β-eudesmol against heat shock protein 90 (HSP90) and odorant response gene-3 (ODR3) proteins. The study unveiled the chemical diversity present in both the EOs and rhizomes of *C. spicata* along with their considerable potential as plant-based pesticidal agents effective against nematodes and weed species.

ARTICLE HISTORY
Received 3 March 2024
Revised 11 May 2024
Accepted 26 July 2024

KEYWORDS
Zingiberaceae; *plant-parasitic nematode*; phytotoxic activities; molecular docking; hydro-distillation; *in-vitro* activities

SUBJECTS
Agriculture & Environmental Sciences; Botany; Food Chemistry

1. Introduction

The world's population is about 7.8 billion, and it is estimated to reach nearly 9.7 billion by 2050 (United Nations, 2019). Sustainable food production is a major challenge for meeting food security. New approaches are needed to control various pests and diseases that drastically reduce global crop yields. Many insect pests, plant nematodes, plant pathogenic fungi and weeds have resisted the pesticides commonly used for their management. The continuous use of these chemical pesticides has also resulted in harmful impacts such as environmental pollution, health issues and biodiversity loss. The use of chemical pesticides has also affected the farmers involved in exporting farm production (Shabana et al., 2017; Lengai et al., 2020). Currently, researchers are searching for biorationals as

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alternatives to chemical pesticides. In this context, plant secondary metabolites, specifically essential oils (EOs), protect plants from harmful insect pests and diseases owing to their bioactive properties. Consequently, these compounds are the optimal choice for use as environment-friendly pesticides. EOs are mixtures of volatile organic compounds (VOCs), mainly phenylpropanoids and terpenoids, with a high vapor pressure at room temperature (Sadgrove et al., 2021). EOs, which are obtained from different plant parts via hydro-distillation, steam distillation, and cold expression, have traditionally been used in food preservation and perfumery. Plant EOs have been reported to possess excellent pesticidal activity (Keerthiraj et al., 2021; Kundu et al., 2020).

Cautleya spicata (Sm.) Baker, also known as Chinese Butterfly Ginger, is an important species in the *Cautleya* genus of the Zingiberaceae family. Only around three to four species of this genus are found in high-elevation (1800–2800) tropical and temperate Eastern Himalayan regions, of which *C. spicata* is one of them (Semwal et al., 2015). *Cautleya spicata* is distributed from the Himalayas to China (Yunnan). This species is cultivated as an ornamental garden plant. Sometimes, it is also grown on other plants such as epiphytes. Ethnopharmacologically, the rhizome juice of this plant is used to treat stomach disorders. The *C. spicata* extract has also been reported to have biological properties such as antimicrobial, anti-infertility and anti-inflammatory activities (Goel et al., 2002).

From the ethanolic extract of *C. spicata* rhizomes, eight compounds, i.e. astragalin, β-sitosterol β-D-glucoside, β-sitosterol, (*E*)-labda-8(17), 12-diene-15,16-dial, bergapten, kaempferol, zerumin A and quercetin have been isolated and reported (Semwal et al., 2015). However, to the best of our knowledge, there are no reports on the chemical and biological activities of EOs from different plant parts of *C. spicata*. The biological properties of EOs are directly related to their qualitative and quantitative compositions (Moumni et al., 2020). Therefore, it is crucial to study its chemical composition and establish its association with its biological properties. Keeping these facts in mind, this study was conducted to assess the chemical profile of *C. spicata* rhizome and aerial parts, correlating its pesticidal properties, *viz*., nematicidal and phytotoxic activities, and understanding the mechanisms of action *via in silico* analysis.

2. Materials and methods

2.1. Collection site and plant material

The aerial parts and rhizomes of *C. spicata* were obtained from Munsyari, Uttarakhand (latitude 30°04′17.56′ N; longitude 80°14′26.79″ E; altitude 2101 m), in October 2021. The plant material was validated by Dr. D.S. Rawat, a taxonomist, and deposited with voucher number (GBPUH-1547) in the Department of Biological Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

2.2. Essential oil extraction

Fresh *C. spicata* [rhizomes (950 g) and aerial parts (800 g)] were subjected to hydro-distillation using Clevenger's apparatus for 3 h to extract the EOs. Around 1 L of water was used for each parts' EO extraction. After 3 h the yielded EO volume was recorded in gradual tube of Clevenger's apparatus and yield (v/w) were computed in respect of weight of fresh plant material used. The yielded EOs from rhizome and aerial parts were collected and dried over anhydrous sodium sulfate to remove traces of water, and stored in amber color glass vials at 4 °C.

2.3. GC-MS analysis of essential oils

The compositional variability of the EOs was analyzed using a GC-MS instrument Agilent Technologies, 5975 C with a 60 m length HP-5MS column. The injection volume was 1 µL with carrier gas helium (flow rate 1 mL/min) at a head pressure of 10 psi. Starting at 40 °C, the temperature was increased up to 90 °C at a rate of 2 °C/min. The temperature was increased to 150 °C at a rate of 3 °C/min, and final temperature was maintained for 1 min. Furthermore, the temperature was increased to 250 °C at a rate of 10 °C. The MASS acquisition parameters were as follows: ion source temperature, 100 °C; electron ionization, 70 eV; full scan mode (50–550 mass units); transfer line temperature, 250 °C; solvent delay, 3 min; EM voltage, 1220. The constituents within these samples were identified by comparing their mass spectra with entries in the NIST14 library and cross-referencing the data with relevant literature sources, particularly Adams (2007). Further retention indices (RI) were computed according to Kovats (1978):

$$RI^{CAL} = 100 \times N + \left[\log(RT comp - v) - \log(RT - v)\right] / \left[\log(RT/a - v) - \log(RT sa - v)\right]$$

Here,

N = number of carbons in the smaller alkane, RT*comp* = retention time of the analyte, v = column void time, RT*la* = retention time of the larger alkane, and RT*sa* = retention time of the smaller alkane.

2.4. Fold change analysis

Fold-change analysis was conducted to assess alterations in compound concentrations in response to specific treatments. The fold change, which represents the ratio of the compound concentration between the experimental conditions, was calculated. The data, initially comprising compound concentrations across different treatments, were organized and processed using R Studio. The fold-change values were computed for each compound based on their concentrations, allowing for evaluation of the relative changes in abundance or activity under varying experimental conditions. This analysis provides insights into the relative differences in compound levels, which are essential for understanding the impact of treatments on the compounds studied.

2.5. Nematicidal bioassay

2.5.1. Nematode population collection and maintenance

Nematode eggs from *Meloidogyne incognita* were obtained from infected tomato (*Solanum lycopersicum*) roots at the Crop Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The Culturing was conducted in a controlled glasshouse environment at 25 ± 2 °C, and samples based on observable root-knot symptoms in the tomato plants. These mature egg masses were cultured in distilled water at constant conditions of temperature (25 °C) in a growth chamber. The resulting juveniles were collected and maintained at 5 °C for future use following Eisenback et al. (1985) and Latif's et al. (2014) methods.

2.5.2. Preparation of essential oil emulsions

EOs were directly used to prepare the emulsions. These emulsions were prepared using a 1.00% Tween 20 surfactant solution. A concentrated solution (2.00 µL/mL) was initially prepared in a 1.00% Tween 20-distilled water solution. This concentrated solution was then gradually diluted using a surfactant solution to create secondary concentrations ranging from 1.00 to 0.25 µL/mL.

2.5.3. Nematode mortality bioassay

The mortality bioassay for nematodes followed established procedures (Mahawer et al., 2023) under controlled laboratory conditions, with slight modifications. After adding 1.0 mL of nematode solution with 1.0 mL of EO emulsion to every gridded Petri plate, the resulting concentrations post-dilution were 1.0, 0.5 and 0.25 µL/mL. The control group consisted of nematodes suspended in a 1.00% Tween 20 aqueous solution. To ensure accuracy, all treatments were replicated thrice. The Petri dishes were consistently kept at 27 ± 1 °C during the experiment. Nematode mortality was observed at 24, 48, 72 and 96 h intervals using a stereoscopic microscope at 4X magnification. Deceased nematodes were identified by their lack of movement and straight bodies; any potential revival was checked, as previously described methods (Choi et al., 2007; Mahawer, et al., 2023). The mortality percentages were computed using Abbott's formula (Abbott, 1925). Furthermore, LC_{50} values, indicating the concentration at which 50% of the nematodes were killed, were determined using probit analysis based on the percent mortality.

The formula for mortality calculation is:

$$\text{Mortality}(\%) = 100 \times \left((\text{Mc} - \text{Mt}) / (100 - \text{Mc}) \right)$$

Where:

Mt = nematode mortality in treatment; Mc = nematode mortality in control

2.5.4. Nematode egg hatchability bioassay

The nematode egg hatchability assay was similar to that of the mortality assay, except that it used approximately 100 eggs per mL instead of nematode larvae. The control group consisted of a solution containing approximately 100 nematode eggs in 1.0 mL Tween 20 (1%) aqueous solution. Petri dishes were consistently kept at 27 ± 1 °C during the experiment. Observations of egg hatching were made at 24, 48, 72 and 96 h intervals using a stereoscopic microscope at 4X magnification. The Egg Hatching Inhibition Percentage (EHI %) was calculated using a formula to quantify the inhibitory effects on egg hatching following established protocols (Mahawer et al., 2023).

The formula for calculating EHI %:

$$\text{EHI}\% = \frac{\text{EHc} - \text{EHt}}{\text{EHc}} \times 100$$

where

EHI = Egg Hatching Inhibition

EHc = number of eggs hatched in control
EHt = number of eggs hatched in treatment.

Additionally, IC_{50} values were determined for percent EHI by utilizing nonlinear regression curves with GraphPad Prism 9 version 9.5.1(733) (GraphPad Software, La Jolla, CA).

2.6. Phytotoxicity bioassay

The *in vitro* evaluation of the EOs was evaluated in chicory (*Cichorium intybus*) and radish (*Raphanus raphanistrum* subsp. *sativus* (L.)) seeds to assess their effect on seed germination and seedling growth (root length and shoot length) using Petri dish bioassays. The radish seeds were procured from the Central Vegetable Research Centre (VRC) at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, whereas chicory seeds were obtained from the ICAR-Directorate of Weed Research, Jabalpur, India. For the phytotoxicity assessment, ten weed seeds were placed between two layers of filter paper within a 9 cm diameter Petri dish. Subsequently, 5.00 mL of different concentrations (0.25, 0.50 and 1.00 µL/mL) of EO was added to the Petri dish. The control solution comprised Tween 20 (1.00%) in water. Pendimethalin, a standard herbicide, was used as the positive control. Afterward, the plates were placed in incubators at temperatures of 32 °C/25 °C, following a 12-h light/12-h dark cycle. Each treatment was replicated thrice. Seed germination, root length and shoot length were recorded on day five. Utilizing the provided formulas, calculations were performed to ascertain the germination inhibition percentage, as well as the percentages of root length inhibition and shoot length inhibition.

$$\text{Seed germination inhibition \% (GPI\%)} = \frac{\text{SG in control} - \text{SG in sample}}{\text{SG in control}} \times 100$$

$$\text{Root length inhibition \% (RLI\%)} = \frac{\text{RL in control} - \text{RL in sample}}{\text{RL in control}} \times 100$$

$$\text{Shoot length inhibition \% (RLI\%)} = \frac{\text{SL in control} - \text{SL in sample}}{\text{SL in control}} \times 100$$

where
SG = Seed germinated (numbers)
SL = shoot length (cm)
RL = root length (cm)

Similar to the Nematode EHI assay, the IC_{50} values were computed from the herbicidal data.

2.7. In silico study

A molecular docking investigation was conducted using the seven major chemical constituents of *C. spicata* EOs. This study aimed to assess their interactions with five presumed target receptor proteins of *M. incognita*, with the goal of elucidating the mechanism responsible for nematicidal activity.

2.7.1. Selection of proteins

An *in-silico* investigation targeted five specific protein receptors found in *Meloidogyne incognita*: cytochrome c oxidase subunit 1 (CytC), acetylcholinesterase (AChE), heat shock protein 90 (Hsp90), odorant response gene-3 (ODR3) and glutathione S-transferase (GST-1) (Rajasekharan et al., 2018; Kundu et al., 2020). These proteins perform vital functions in the diverse biological processes in nematodes. CytC is essential for the oxidative phosphorylation pathway and is crucial for energy metabolism. AChE regulates synaptic transmission and movement. Similarly, Hsp90 collaborates with co-chaperones to ensure protein stability, refold denatured proteins during stress, and fold newly synthesized proteins. ODR3 manages chemosensory functions, whereas glutathione S-transferases (GSTs) act as detoxifying and antioxidant enzymes.

2.7.2. Homology modelling

The 3D structural models of these proteins were developed using the SWISS-MODEL web server (https://swissmodel.expasy.org/), utilizing protein sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database (Rajasekharan et al., 2018; Kundu et al., 2020). These modeled protein structures were utilized in docking assays conducted using PyRx software.

2.7.3. Ligand preparation

For docking analysis, compounds were selected as ligands from EOs with careful consideration, focusing on those with higher percentage compositions. The corresponding three-dimensional (3D) structures of these ligands were downloaded from the PubChem webserver (https://pubchem.ncbi.nlm.nih.gov/) in Structure Data File (SDF) format. Because the tested EOs demonstrated significant nematicidal activity, a molecular docking study was conducted to

gain deeper insights into the interactions between these compounds and the target proteins. The selected compounds included α-pinene (CID:6654), γ-terpinene (CID:7461), *p*-cymene (CID:7463), β-pinene (CID:14896), β-eudesmol (CID:91457), spathulenol (CID:92231) and β-selinene (CID:519361). The SDF-format structures of these ligands were imported into PyRx Software using the embedded Open Babel tool within PyRx. Subsequently, energy minimization procedures involving charge addition and universal force-field optimization were applied to the ligands. After the energy minimization phase, the ligands were changed to the AutoDock Ligand format (PDBQT) to enable further analysis and ensure a solid foundation for subsequent docking simulations.

2.7.4. Molecular docking

The chosen ligands were subjected to an *in-silico* study using PyRx software, specifically employing the Vina Wizard tool. This process involved selecting both the protein and multiple ligands within PyRx using Vina Wizard Control, aiming to identify various interactions between the ligands and proteins and determine their binding affinity for nematicidal activity. The docking process was initiated using the 'Run Vina' control. The resulting outcomes were analyzed through the 'Analyse Vina' tool (Dallakyan & Olson, 2015). Additionally, for visualization purposes, PyMOL version 2.5.7 (Schrödinger, Inc.) and LIGPLOT+version v.2.2.8 (EMBL-EBI, Cambridgeshire, UK) were used to observe the 3D and 2D interactions of the docking poses, respectively. For comparative analysis, the docking study also involved known inhibitors of target proteins, such as physostigmine (CID: 5983) (for AChE), ethacrynic acid (CID:3278) (for GST-1), albendazole (CID:2082) (for CytC), and geldanamycin (CID: 5288382) (for Hsp90).

2.8. Statistical analyses

All experiments were conducted in triplicates. Two-way Analysis of Variance (ANOVA) was employed to assess the significance of differences among treatment means using OriginPro2022b (SR1 9.9.5.171; student trial version). Statistical significance was considered when the p value was ≤0.05. Tukey's test was used to compare mean values. Additionally, principal component analysis (PCA) and matrix plots were generated for chemometric analysis using OriginPro (Mahawer et al., 2023). The Pearson correlation coefficients among the major chemical constituents and pesticidal activities of the EOs were also computed using OriginPro.

3. Results and discussion

3.1. Chemical composition

GC-MS analysis revealed that both the EOs obtained from *C. spicata* are largely composed of monoterpenes (Table 1). Specifically, the EO from the aerial parts (CSAEO) had a higher concentration of monoterpenes (88.12%) than the rhizome EO (CSREO, 77.94%). In both the EOs, the predominant compound was *p*-cymene, constituting 49.78% of the CSREO and 40.09% of the CSAEO. Additionally, β-pinene (22.43% and 21.36% in CSREO and CSAEO, respectively), α-pinene (5.73% and 6.28% in CSREO and CSAEO, respectively) and spathulenol (2.11% and 3.37% in CSREO and CSAEO, respectively) were the major compounds shared by both EOs. Notably, β-selinene (2.12%) was unique to CSREO, while γ-terpinene (19.08%), β-eudesmol (4.82%) and *o*-cymene (1.05%) were unique to CSREO among the major compounds.

3.2. Chemometric analysis

Heat map cluster analysis was conducted to visually represent the varying compositions of the EOs. This method used colors to indicate the relative percentage content of chemical constituents, with a dark maroon color denoting a higher content and a light cream color indicating lower percentages within the EOs (Figure 1(A)). Furthermore, a multivariate statistical method called PCA was used to distinguish the samples being studied, focusing on classes of compounds and major chemical constituents (>1.0%). The analysis focused on the first two principal components (PCs), which collectively explained approximately 100% of the overall data variance. The PCA results highlighted that CSREO and CSAEO were primarily distinguished by their monoterpenes (Figure 1(B)). EOs were segregated based on the presence of β-pinene and *p*-cymene as the primary chemical constituents (Figure 1(C)).

Based on a literature search, there are no reports available on the chemical composition of *C. spicata* EOs. In this study, *p*-cymene was found to be the main compound in both the rhizome and aerial part EOs of *C. spicata*. Several EOs from other Zingiberaceae plants, such as *A. malaccensis*, *A. allughas*, *A. speciosa*, *A. galanga*, *A. oblongifolia*, *Zingiber chrysanthum*, have been found to contain *p*-cymene, β-pinene, and α-pinene (Padalia et al., 2010; Thin et al., 2018; Palariya et al., 2019). *Cuminum cyminum* EO was also a source of *p*-cymene and β-pinene (Li & Jiang,

Table 1. Relative group composition (% TIC) of compounds from *Cautleya spicata* essential oils.

S. N	Compound	RI^{DB}	RI^{CAL}	CSREO	CSAEO
	Monoterpenes			**77.94**	**88.12**
1.	α-Thujene	930	931	–	0.15
2.	α-Pinene	939	937	5.73	6.28
3.	β-Pinene	979	979	22.43	21.36
4.	β-Myrcene	990	991	–	0.11
5.	*p*-Cymene	1024	1027	49.78	40.09
6.	*o*-cymene	1026	1026	–	1.05
7.	γ-terpinene	1059	1059	–	19.08
	Oxygenated monoterpenes			**2.89**	**0.31**
8.	1,8-Cineole	1031	1030	0.84	–
9.	*cis*-linalool oxide	1072	1068	0.35	–
10.	*trans*-linalool oxide	1086	1082	0.29	–
11.	*l*-linalool	1090	1091	0.51	–
12.	*l-trans*-pinocarveol	1139	1139	0.18	0.01
13.	Terpinen-4-ol	1177	1172	0.37	0.02
14.	Myrtenol	1195	1196	0.08	0.12
15.	2-Pinen-4-one	1205	1200	0.03	–
16.	Ascaridole	1237	1235	–	0.16
17.	*trans*-3-caren-2-ol	1727	1727	0.24	–
	Sesquiterpenes			**4.51**	**0.68**
18.	*trans*-caryophyllene	1419	1409	–	0.36
19.	Aromadendrene	1441	1438	0.61	0.27
20.	(-)-Alloaromadendrene	1460	1466	0.97	–
21.	β-Selinene	1490	1481	2.12	–
22.	β-Guaiene	1493	1490	0.43	–
23.	β-Bisabolene	1505	1503	0.38	0.05
	Oxygenated sesquiterpenes			**2.18**	**9.13**
24.	β-Elemene	1390	1384	–	0.10
25.	±-trans-nerolidol	1563	1563	–	0.14
26.	Apathulenol	1578	1578	2.11	3.37
27.	Globulol	1590	1581	0.07	–
28.	γ-Eudesmol	1632	1632	–	0.69
29.	Cubenol	1646	1638	–	0.01
30.	β-Eudesmol	1650	1655	–	4.82
	Not identified			**12.48**	**1.76**

CSREO: *Cautleya spicata* rhizome essential oil; CSAEO:= *Cautleya spicata* aerial part essential oil= '-'= not present; RI^{CAL}: retention indices (calculated); RI^{DB}: Retention indices from literature on a DB-5 MS column in literature (Adams, 2007) and (William & James, 2010).
Bold values = Class of compounds.

2004). *p*-Cymene have also been reported in several other *Alpinia* species of the Zingiberaceae plant family in high amount (Murakami et al., 2009; Kawai et al., 2021). It has also been reported in EOs of oregano and thyme plants of the Lamiaceae family in high amounts (Mutlu-Ingok, et al., 2021).

Based on the chemical constituents of *C. spicata* EOs, a Venn diagram was plotted to compare the compositional variability between CSREO and CSAEO (Figure 2). Based on the Venn diagram, it was found that a total of 10 compounds were unique in CSREO (1,8-cineole, *cis*-linalool oxide, *trans*-linalool oxide, *l*-linalool, 2-pinen-4-one, *trans*-3-caren-2-ol, (-)-alloaromadendrene, β-guaiene, β-selinene and globulol), whereas 11 compounds (β-myrcene, α-thujene, *o*-cymene, γ-terpinene, ascaridole, *trans*-caryophyllene, ±-*trans*-nerolidol, cubenol, β-eudesmol, γ-eudesmol and β-elemene) were unique. The remaining nine compounds, α-pinene, β-pinene, *p*-cymene, *l-trans*-pinocarveol, terpinen-4-ol, myrtenol, aromadendrene, β-bisabolene and spathulenol, were common to both EOs. The chemical structures of the major compounds are shown in Figure 3.

Fold changes in the relative expression outputs of various compounds were also assessed in this study. The analysis revealed diverse alterations across the compounds (Figure 4). Notably, α-pinene exhibited a slight increase, with a fold change of approximately 1.10, whereas β-pinene displayed a decrease, with a fold change of approximately 0.95. Compounds like β-myrcene, thujene, *o*-cymene, γ-terpinene, ascaridole, *trans*-caryophyllene, ±-*trans*-nerolidol, β-eudesmol, γ-eudesmol, and β-elemene exhibited fold changes marked as 'Infinity,' due to zero expression in the control sample (CSREO), rendering division by zero infeasible. Conversely, myrtenol displayed a notable increase in expression, with a fold-change of 1.50. Meanwhile, spathulenol showed the most significant change, with a fold change of approximately 1.60. These diverse alterations in relative expression outputs suggest complex regulatory mechanisms governing these compounds, warranting further investigation to elucidate their functional significance in the studied context.

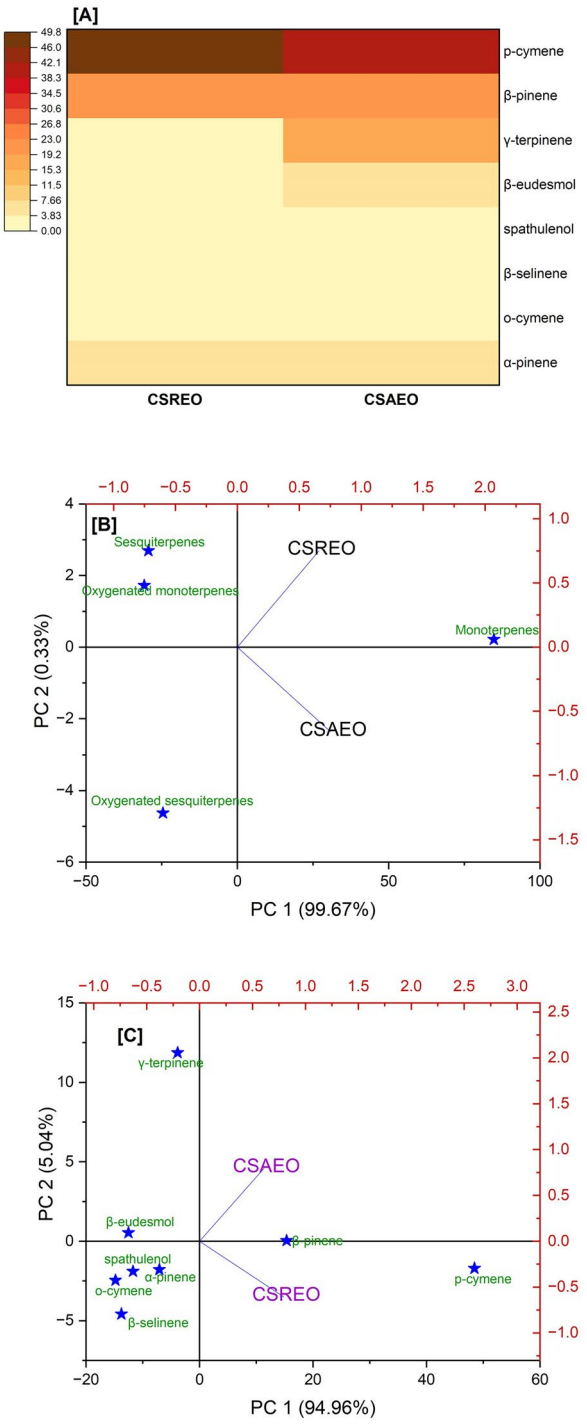

Figure 1. Chemometric plots. (A) Heat map clustering of the *C. spicata* essential oils based on their chemical constituents. (B) PCA for the class of compounds of *C. spicata* essential oils; (C) PCA for the individual chemical compounds of the *C. spicata* essential oils.

3.3. Nematicidal activity

3.3.1. Nematode mortality bioassay

The results of nematode mortality assay revealed that after 96 h, CSAEO showed significantly higher nematode mortality percentage at 1.0 µL/mL (64.67%) and at 0.5 µL/mL (61.33%). At 1.0 µL/mL concentration level, CSREO showed 25.00% nematode mortality after 96 h (Figure 5). When LC_{50} values were compared, CSAEO was more effective, with an LC_{50} value of 0.555 µL/mL, followed by CSREO (LC_{50} value = 7.845) after 96 h (Figure 6).

3.3.2. Nematode egg hatchability assay

The nematode EHI assay revealed that after 96 h, CSREO showed highest EHI (89.19%) at 1.0 µL/mL (Figure 7). However, at lower concentrations (0.25 and 0.5 µL/mL), CSAEO was more effective, with nematode EHI of 80.60% and 83.09%, respectively. Regarding IC_{50} values, CSAEO was found to be more effective with an IC_{50} value of 0.016 µL/mL than CSREO (IC_{50} value = 0.089 µL/mL) (Figure 8).

The higher nematicidal activity of both compounds might be the result of *p*-cymene, which was present in both EOs in high amounts. The *p*-cymene standards (99% and 97% pure) and *p*-cymene containing EOs have been previously reported to have significant nematicidal activity against J2 of *M. incognita* (Ntalli et al., 2010). However, the higher nematode mortality percentage for aerial part EO might be the result of γ-terpinene, which was 19.08% in aerial part EOs, and it was completely absent in the rhizome EO of *C. spicata*. The γ-terpinene standard (97% pure) was also reported to have significant nematicidal activity against *Bursaphelenchus xylophilus* (pine wood nematode) (Kong et al., 2007).

3.4. Phytotoxicity bioassay

The results of the phytotoxic bioassay revealed that neither EO had any effect on germination of radish seeds. However, the higher root length inhibition (65.18%) and shoot length inhibition (70.23%) was rescored for CSREO at 1.00 µL/mL (Figure 9). The IC_{50} values were also in a similar order. The IC_{50} values for RLI% were 0.157 and 0.750 µL/mL for CSREO and CSAEO, respectively. The IC_{50} values for SLI% were 0.123 and 0.418 µL/mL for CSREO and CSAEO, respectively (Figure 11).

Both EOs exhibited a significant impact on the SGI%, RLI% and SLI% of chicory seeds. The rhizome EOs exhibited 100% inhibition of all three parameters at 1.00 a concentration level. The EOs of the aerial parts also showed phytotoxic potential at par with CSREO and pendimethalin (Figure 10). However, the EO from the aerial part exhibited the lowest IC_{50} values for SGI% (0.369 µL/mL)

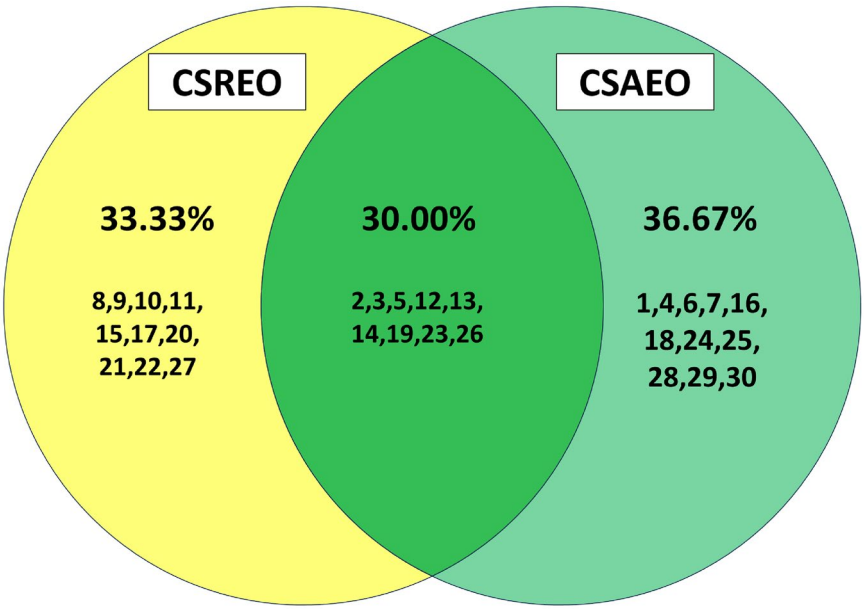

Figure 2. Venn diagram for chemical constituents of *C. spicata* essential oils.

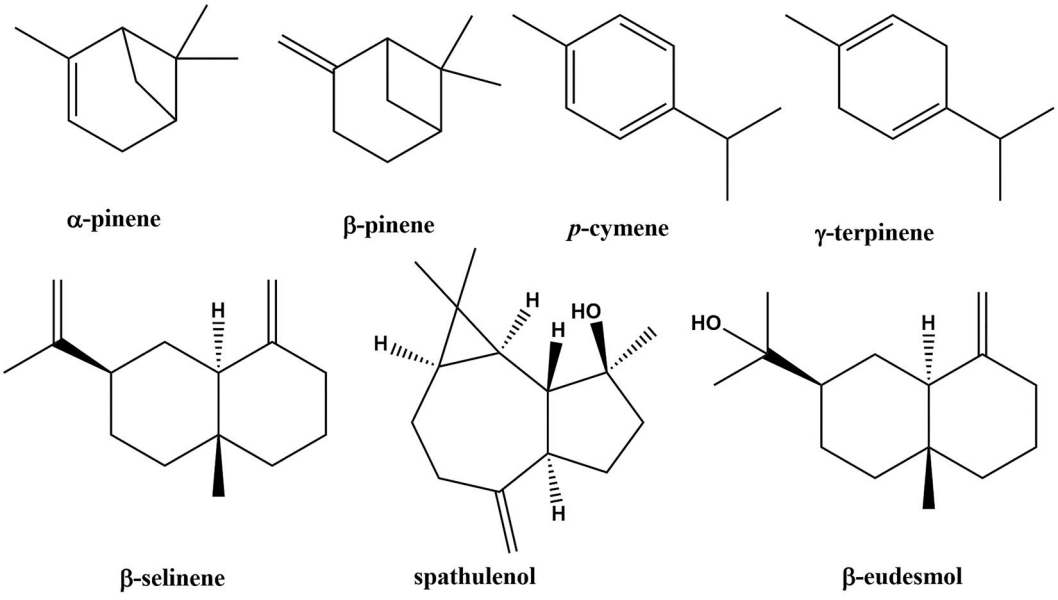

Figure 3. Chemical structure of the major chemical constituents (>2.0%) of *C. spicata* essential oils.

and RLI% (0.079 µL/mL), whereas the lowest IC_{50} value was recorded for CSREO (0.130 µL/mL) (Figure 11).

Both the EOs contained *p*-cymene as the main compound. However, *p*-cymene did not show any phytotoxicity against *Amaranthus retroflexus*, *Chenopodium album* and *Rumex crispus* (Kordali et al., 2008). Therefore, it can be inferred that the phytotoxic activity of *C. spicata* EOs might be due to the combined effect of more than one chemical constituent of the EOs (Caesar & Cech, 2019).

3.4.1. Pearson's correlation coefficient for the chemical constituents and pesticidal activities of Cautleya spicata essential oils

Pearson's correlation coefficient was computed for the chemical constituents and pesticidal properties of *C. spicata* rhizome and aerial part EOs (Figure 12). The correlation coefficients showed that α-pinene, *o*-cymene, γ-terpinene, spathulenol and β-eudesmol were highly correlated (r = 1) with nematode mortality percentage, root length inhibition and shoot length inhibition against radish

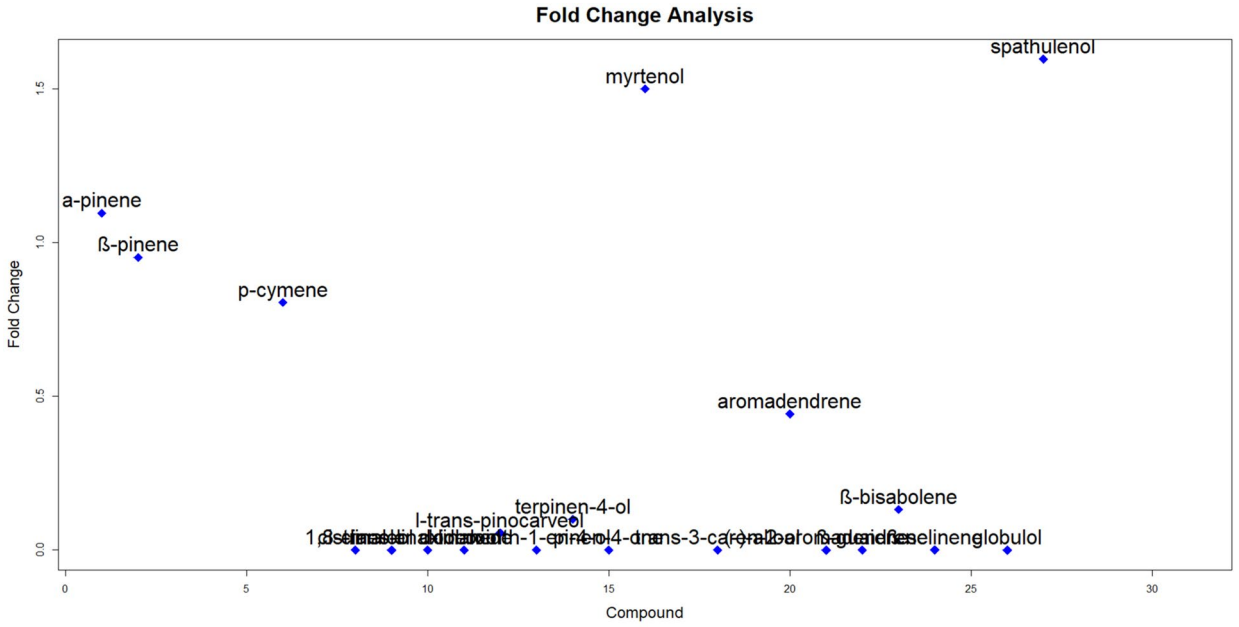

Figure 4. Fold change analysis diagram for *C. spicata* essential oils from rhizomes and aerial parts.

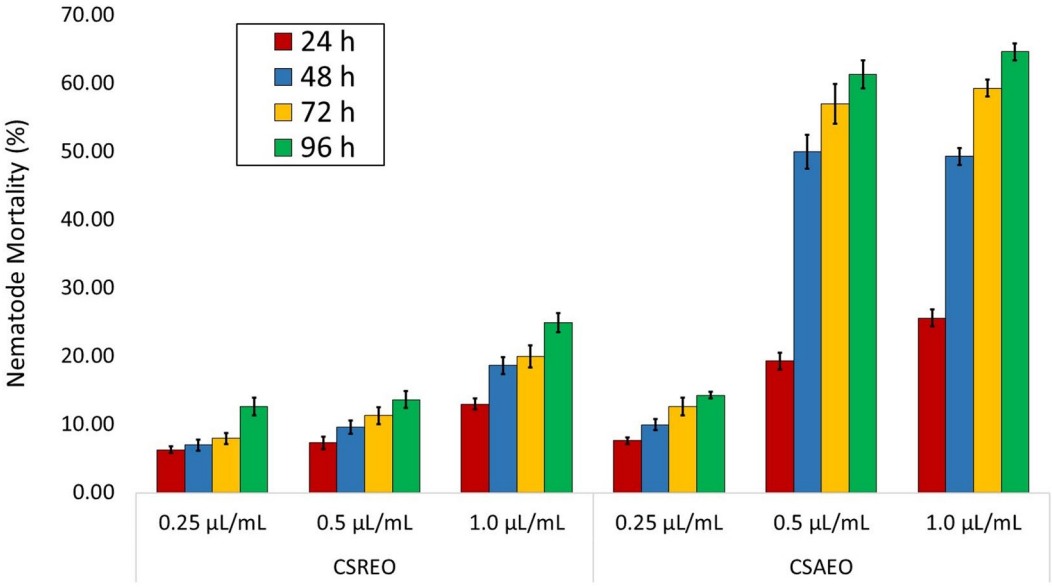

Figure 5. Nematode mortality from the effect of *C. spicata* essential oils from different plant parts against J2 larvae of *M. incognita*.

seeds. In contrast, β-pinene, *p*-cymene and β-selinene showed a direct positive correlation (r = 1) with nematode EHI percentage and all the herbicidal parameters against chicory seeds. A chord diagram was generated to represent the positive correlation between the chemical constituents and pesticidal properties of *C. spicata* EOs (Figure 13). The chord diagram shows the connections between the parameters, with correlation coefficients greater than 0.6.

3.5. Molecular docking studies

The docking results of the major components of the extracts in comparison with those of standard inhibitors are listed in Table 2. The findings of molecular docking study showed that the compounds, β-selinene (–7.6 kcal/mol) and spathulenol (–6.5 kcal/mol) received higher docking score than the standard inhibitor, physostigmine (–6.4 kcal/mol) with AChE. β-Selinene, which had the highest binding affinity, interacted with 11 amino acid residues

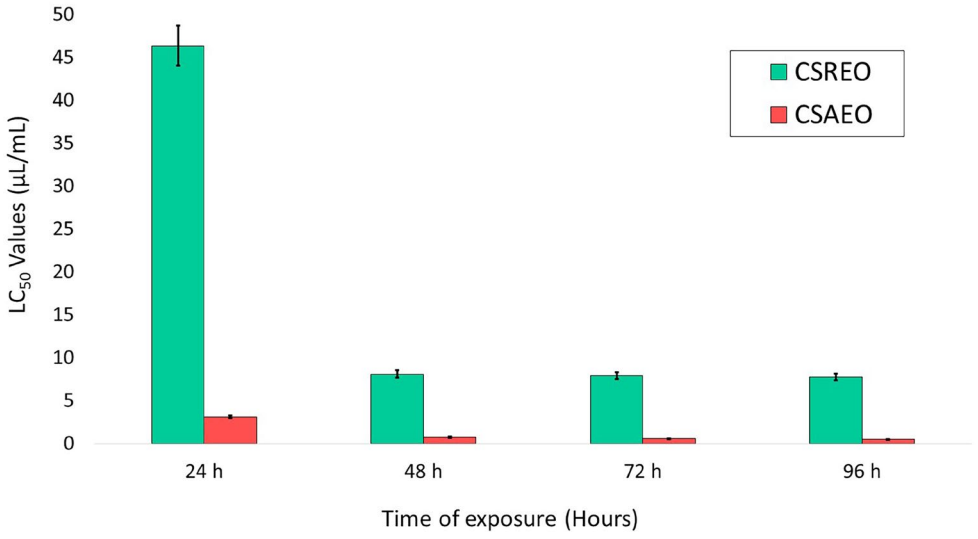

Figure 6. LC_{50} values of the *C. spicata* essential oils from different plant parts against J2 larvae of *M. incognita*.

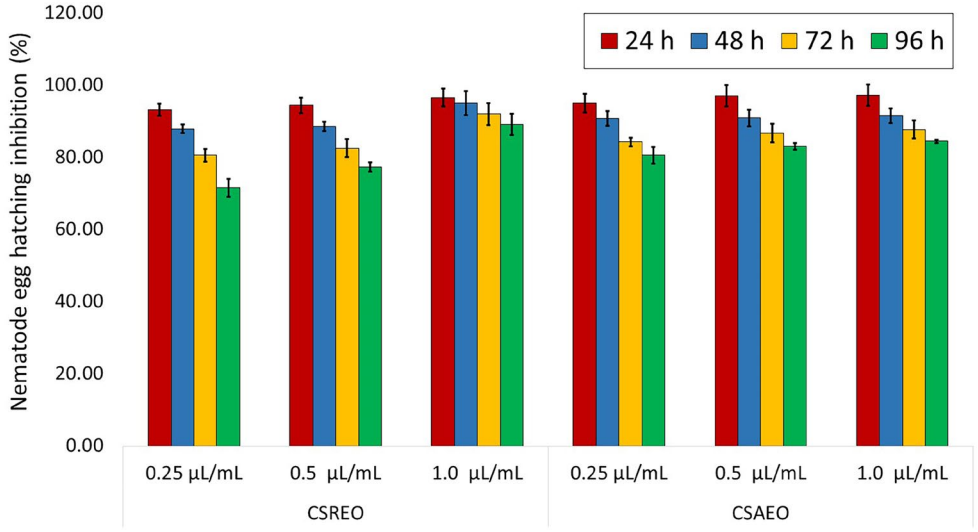

Figure 7. Nematode (*M. incognita*) egg hatchability percentage from the effect of *C. spicata* essential oils from different plant parts.

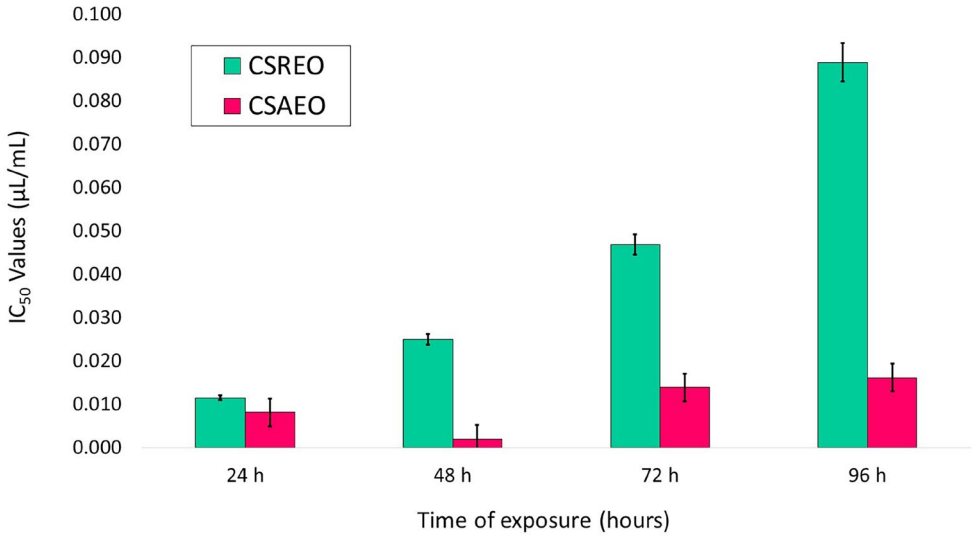

Figure 8. IC_{50} values of *C. spicata* essential oils against *M. incognita* egg hatching inhibition.

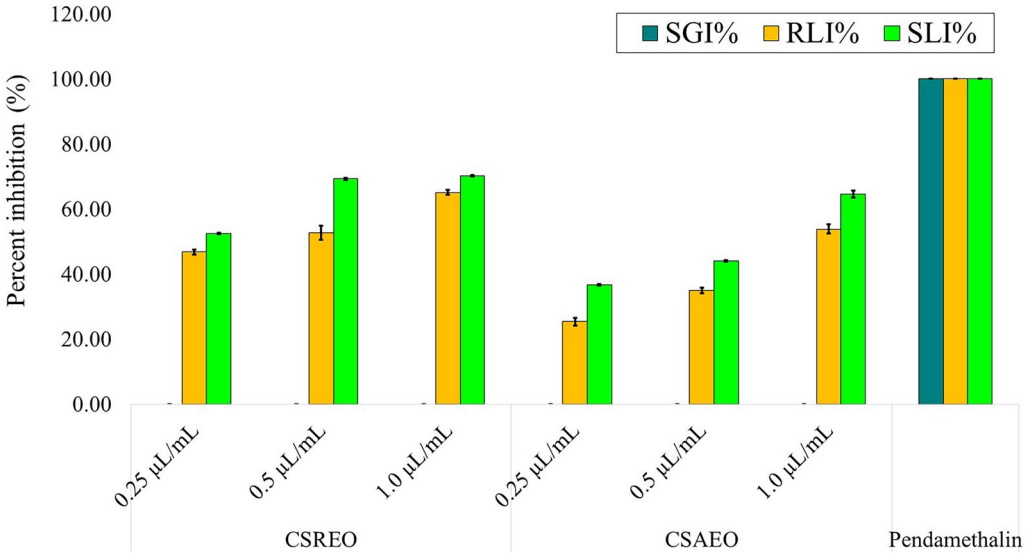

Figure 9. Phototoxic activity of *C. spicata* essential oils against radish seeds.

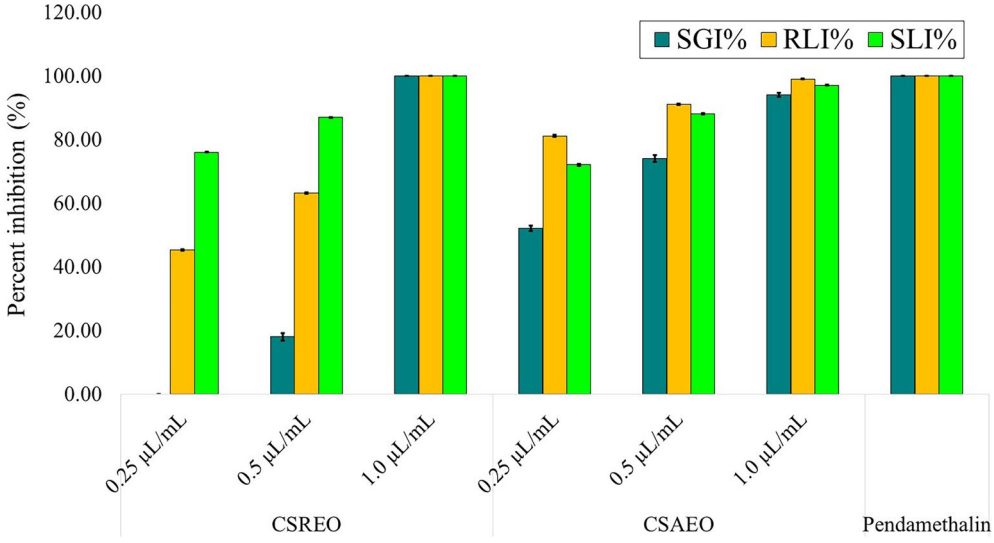

Figure 10. Phytotoxic activity of *C. spicata* essential oils against chicory seeds.

(GLY171, TYR173, TRP391, SER252, GLY170, GLU251, GLY169, TRP138, TYR390, HIS514 and LEU344) of AChE (Figure 14(A1 and A2). However, the standard inhibitor of AChE, physostigmine interacted with TYR448, HIS450, LEU451, PRO452, THR453, ARG459, ASP462, ARG466 and ALA447 amino acid residues. Among these, HIS450 interacted *via* H-bonding (Figure 14(B1 and B2)). Similarly, β-selinene (–7.3 kcal/mol) and β-eudesmol (–7.0 kcal/mol) showed higher binding affinity with Cyt C in comparison with standard inhibitor, albendazole (–6.9 kcal/mol). The amino acid residues ILE464, PHE17, PHE13, LEU16, ILE467, PHE388, PHE396, TYR392 and PHE20 were involved in the interaction between β-selinene (showing the highest affinity) and Cyt C (Figure 14(C1 and C2)). In contrast, albendazole interacted with the THR304, TYR293, GLY350, LEU353, HIS285, ASP359, HIS363, ASP364, SER354, HIS286, ARG433, VAL368, HIS371, HIS235, VAL282 and LIE302 amino acid residues of Cyt C, where HIS285, ASP359, HIS363 and ASP364 interacted *via* hydrogen bonding (Figure 14(D1 and D2)). With GST-1, spathulenol (–6.9 kcal/mol), β-selinene (–6.8 kcal/mol) and β-eudesmol (–6.6 kcal/mol) received higher binding score than the standard inhibitor, ethacrynic acid (–6.4 kcal/mol). Among these, spathulenol interacted with the PHE110, ILE106, PHE163, PHE98, ASN95, SER159, GLY13, HIS162 and MET166 amino acid residues of GST-1 (chain A) (Figure 14(E1 and E2)). However, ethacrynic acid interacted with LEU68, ALA17, HIS162, GLY13,

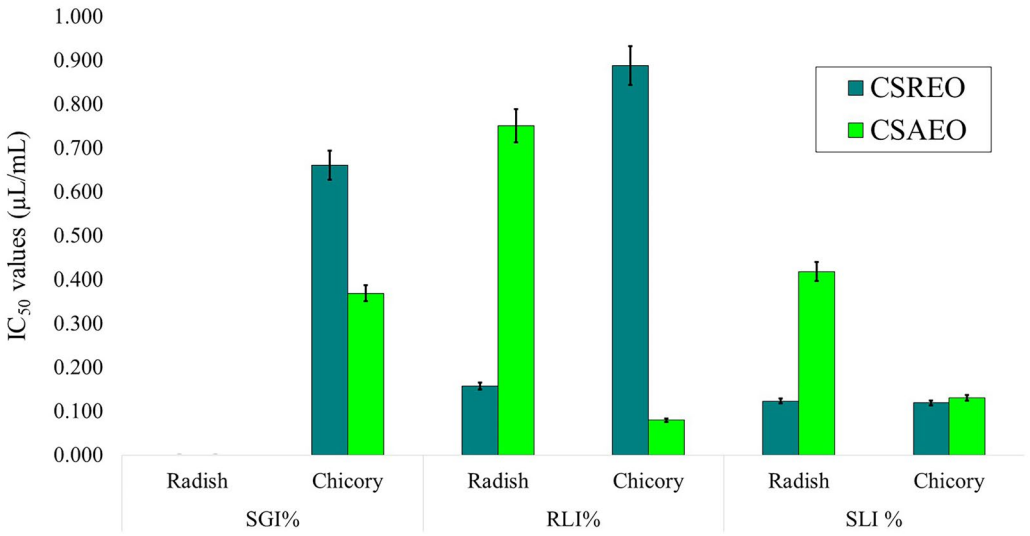

Figure 11. IC_{50} values of *C. spicata* essential oils for phytotoxic activity against radish and chicory seeds.

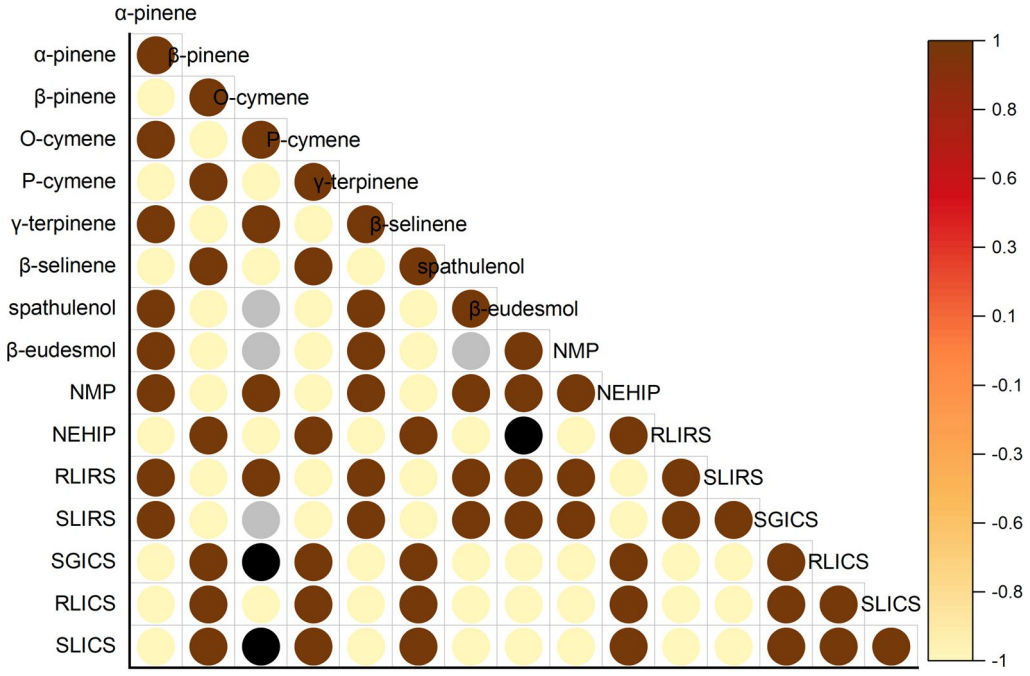

Figure 12. Pearson's correlation coefficient diagram among chemical constituents (>1.0%), and pesticidal properties of *C. spicata* essential oils. (NMP: nematode mortality percentage; NEHIP: nematode egg hatching inhibition percentage; SGIRS: seed germination inhibition in radish seeds; RLIRS: root length inhibition in radish seeds; SLIRS: shoot length inhibition in radish seeds; SGICS: seed germination inhibition in chicory seeds; RLICS: root length inhibition in chicory seeds; SLICS: shoot length inhibition in chicory seeds).

SER159, ASN95, PHE163, GLY99, ILE106, ARG14, GLY65 and ALA64 amino acid residues, where ALA64 interacted via hydrogen bonding (Figure 14(F1 and F2)). Ethacrynic acid interacted with the opposite chain of GST-1 (chain B) compared to spathulenol. With HSP90, all compounds showed lower binding affinity than the standard inhibitor geldanamycin (–7.4 kcal/mol). However, β-eudesmol (–7.0 kcal/mol) received the highest binding score among the tested ligands. This interacted with 12 amino acid residues of HSP90 (GLY106, GLY108, SER84, PHE109, THR86, ASN22, ASP25, LYS29, MET69, LEU78, ASN77 and LYS83), where LYS83 interacts with β-eudesmol (Figure 14(G1 and G2)). In contrast, geldanamycin interacted with six amino acid residues (LEU523, GLU524, LEU525, GLU527, ASN546 and PHE535) without any hydrogen

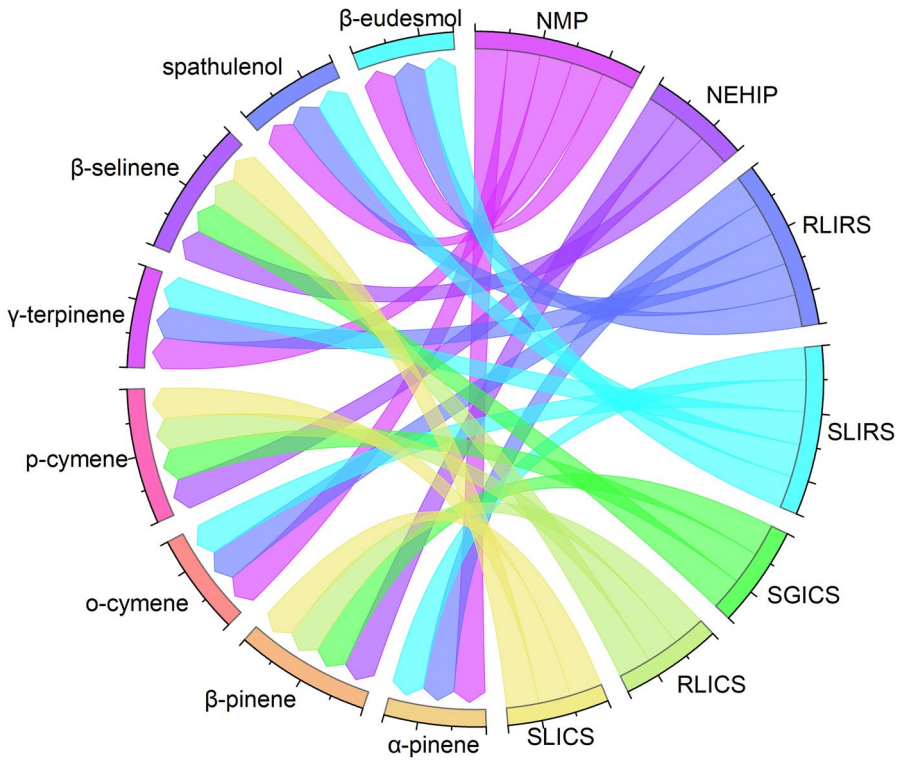

Figure 13. Chord diagram showing correlation among the chemical constituents (>1.0%), and pesticidal properties of *C. spicata* essential oils. The connecting chord represents a Pearson's correlation coefficient greater than 0.6. (NMP: nematode mortality percentage; NEHIP: nematode egg hatching inhibition; RLIRS: root length inhibition in radish seeds; SLIRS: shoot length inhibition in radish seeds; SGICS: seed germination inhibition in chicory seeds; RLICS: root length inhibition in chicory seeds; SLICS: shoot length inhibition in chicory seeds).

Table 2. Molecular docking Score for binding of docked ligands from *C. spicata* essential oils with different proteins for nematicidal activity.

	Docking score (kcal/mol)				
Ligand	AChE	Cyt C	GST-1	HSP90	ODR3
α-Pinene	−6.2	−6.1	−5.6	−5.3	−5.2
γ-Terpinene	−6.2	−6.5	−5.4	−5.7	−6.0
p-Cymene	−6.4	−6.5	−5.4	−6.3	−6.0
β-Pinene	−4.7	−6.4	−5.8	−5.2	−5.3
β-Eudesmol	−6.4	−7.0	−6.6	−7.0	−7.1
Spathulenol	−6.5	−6.7	−6.9	−6.6	−6.3
β-Selinene	−7.6	−7.3	−6.8	−6.7	−6.6
Physostigmine*	−6.4	–	–	–	–
Albendazole**	–	−6.9	–	–	–
Ethacrynic acid***	–	–	−6.4	–	–
Geldanamycin****	–	–	–	−7.4	–

*standard inhibitor of AChE; **standard inhibitor of Cyt C; ***standard inhibitor of GST-1; ****standard inhibitor of HSP90.

bonding (Figure 14(H1 and H2)). Similarly, β-eudesmol (−7.1 kcal/mol) showed the highest binding affinity with ODR3 among all the tested ligands. A total of 12 amino acid residues (LYS271, THR46, THR328, ALA327, ASN270, GLU41, ARG78, GLY43, ASP150, SER151, TYR176 and LEU175) were involved in interaction of β-eudesmol with ODR3 (Figure 14(I1 and I2)).

In this study, sesquiterpene compounds such as β-selinene inhibited AChE through strong interactions with crucial amino acid residues, potentially disrupting the function of the enzyme involved in neurotransmitter breakdown. Similarly, the strong binding of β-selinene to Cyt C suggests its potential role in modulating apoptotic pathways or electron transport, affecting cellular function. The interactions of the compound Spathulenol with GST-1 imply a possible role in interfering with the detoxification process, which GST-1 typically facilitates. Moreover, compounds such as 5 β-eudesmol binding to Hsp90 could affect its chaperone function, impacting protein folding and stability in cells. The strong binding

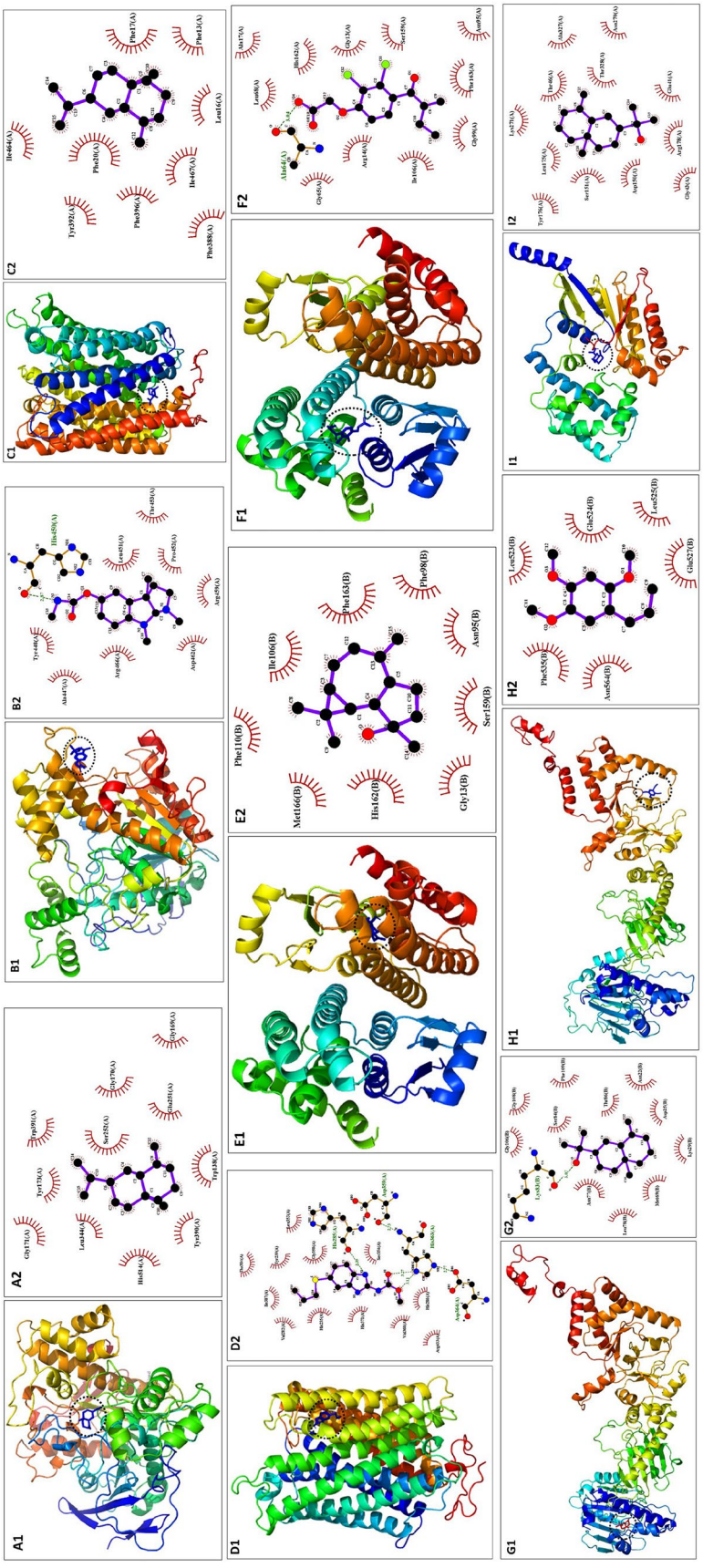

Figure 14. 3D diagram (A1–I1) showing interaction pocket for ligands and 2D diagram (A2–I2) showing interacted residues for (A) AChE-β-selinene; (B) AChE - physostigmine; (C) Cyt C-β-selinene; (D) Cyt C- albendazole; (E) GST-1- spathulenol; (F) GST-1- ethacrynic acid; (G) HSP90- β-eudesmol; (H) HSP90-geldanamycin and; (I) ODR3-β-eudesmol.

affinity of β-eudesmol for ODR3 might affect its function, potentially influencing signaling pathways or cellular responses related to ODR3. These interactions suggest that these compounds could serve as potential leads for targeting the respective proteins, possibly as inhibitors or modulators, based on their specific binding modes and affinities. However, further experimental validation is necessary to confirm its efficacy and therapeutic potential.

4. Conclusion

This research has provided valuable insights into the chemical composition and pesticidal activities of EOs extracted from *Cautleya spicata* rhizomes and aerial parts. GC-MS analysis revealed the presence of 31 compounds, with p-cymene and β-pinene identified as the main constituents in both rhizome and aerial part EOs. A comparative assessment of nematicidal and herbicidal activities against *M. incognita*, *R. raphanistrum* (Radish) and *C. intybus* (Chicory) seeds demonstrated distinct efficacy patterns for the EOs. The EO from the aerial parts exhibited higher nematicidal activity, whereas the rhizome EO displayed superior herbicidal potential. However, the efficacy of EOs is heavily influenced by the type and amount of key structural components; therefore, the major compounds can be chosen to develop environmentally friendly formulations. In the *in-silico* screening, molecular docking studies revealed the potential mechanisms of action, revealing the inhibitory effects of β-selinene on AChE and CytC, spathulenol on glutathione S-transferase (GST-1) and β-eudesmol on HSP90 and ODR3 proteins. These findings contribute to our understanding of the pesticidal properties of *C. spicata* EOs and highlight their potential applications in nematode control and weed management in sustainable agricultural production systems. However, the study lacks specificity in identifying individual bioactive compounds responsible for the biological activities of *C. spicata* EOs. Therefore, future research should focus on isolating and characterizing active compounds, especially p-cymene and β-pinene which are in high amount in the *C. spicata* EOs. Exploration of various advanced extraction techniques for enhanced compound yield, and investigating synergistic effects between these compounds or with other botanicals is also necessary for effective utilization of these EOs for future uses. Additionally, there is a need for comprehensive ecological impact assessments, extensive field trials to assess practical applicability, and comparative studies with synthetic pesticides to establish the efficacy and environmental sustainability of *C. spicata* EOs as plant protection agents. Addressing these limitations and pursuing these future research directions will significantly enhance the understanding and utilization of *C. spicata* as eco-friendly pest management solutions.

Acknowledgment

The authors gratefully acknowledge the G.B. Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India, for providing research facilities, opportunities for conducting this research and administrative support. Dr. Faheem Ahmad acknowledges the support and facility of the Department of Botany, Aligarh Muslim University.

Author contributions statement

Sonu Kumar Mahawer conducted the experiments, analyzed the data, and prepared the initial draft of the manuscript. Himani Karakoti conducted the molecular docking studies. Ravendra Kumar, Faheem Ahmad, and Om Prakash conceptualized and designed the experiments; provided samples, reagents, and materials; and contributed to manuscript writing and editing. Ravendra Kumar and Faheem Ahmad designed and provided the necessary facilities for the nematicidal activity experiments. Dharmendra Singh Rawat, Satya Kumar, Manar Fawzi Bani Mfarrej, and Pieter Malan identified the plant material and contributed to the manuscript review and editing. Aditi Kundu conducted the GC-MS analysis and performed the data analysis. All authors have reviewed the results and approved the final version of the manuscript.

Disclosure statement

The authors reported no potential conflict of interest.

Funding

No funding was received.

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Data availability statement

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

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