scientific reports

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Assessment of antibacterial, nematicidal and herbicidal activities of *Cautleya spicata* (Sm.) Baker extracts, with chemical profiling using GC-MS

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The pesticidal activity of the rhizome and aerial parts of *Cautleya spicata* (Sm.) Baker was investigated, revealing significant findings. The GC-MS analysis revealed significant compositional variability among the extracts. β-Sitosterol was identified as the dominant compound in all extracts prepared with hexane, acetone, and methanol, while α-humulene was prominently present in the rhizome extracts. The methanolic extracts of *C. spicata* demonstrated substantial nematicidal activity against *Meloidogyne incognita* and herbicidal activity against seeds of *Raphanus raphanistrum* subsp. *Sativus* (L.) (Radish) and *Cichorium intybus* L. (Chicory). Additionally, the extracts exhibited effective antibacterial activities against four bacterial strains, including *Salmonella enterica* serotype typhi, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. In-silico molecular docking studies identified stigmasterol as exhibiting the best binding affinities with the studied proteins. These results highlight the potential of *C. spicata* extracts as promising candidates for the development of botanical pesticides and antibacterial agents.

Keywords *Cautleya spicata*, Plant extract, GC-MS, Antibacterial, Nematicidal activity, Herbicidal activity

Presently, the world's population is around 7.8 billion, with a projected increase of 1.9 billion by 2050[1]. Sustainable food production is the major challenge to meet the growing food requirement and to ensure food security. There is a great need for new approaches to control an array of insect pests and diseases, responsible for a drastic reduction in crop yields globally, threatening food security and agricultural sustainability. Numerous chemical pesticides are available on the market; however, many insect pests, plant parasitic nematodes, plant pathogenic fungi, bacteria, and weeds have developed resistance to commonly used pesticides, making their management increasingly challenging. The persistent utilization of these chemical pesticides has led to detrimental consequences, including environmental pollution, adverse health effects, and a decline in biodiversity. Moreover, the reliance on chemical pesticides has adversely impacted farmers engaged in the export of agricultural products[2,3].

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Currently, researchers showing interest in searching for biorationals as an alternative to chemical pesticides. In this particular context, plant secondary metabolites, renowned for their biological properties and their role in protecting plants against pathogens, emerge as the most favourable choice for biorational pesticides.

Cautleya spicata (Sm.) Baker, also known as 'Chinese Butterfly, is a significant species of the Zingiberaceae family, genus *Cautleya*. Out of the numerous species within this genus, only a limited number (approximately 3 to 4) have been identified in the elevated (1800–2800) zones of the tropical and temperate Eastern Himalaya and *C. spicata* is one of them[4]. *Cautleya spicata* is distributed in the Himalayas through to China (Yunnan). This plant species is cultivated as an ornamental garden plant[5]. Sometimes it also grows on other plants as an epiphyte[6]. In ethnopharmacology, the rhizome juice of this plant is used to treat stomach disorders. *Cautleya spicata* extract has also been reported to possess various biological properties, including antimicrobial activity, anti-infertility properties, and anti-inflammatory effects[7].

Previously, eight compounds i.e., astragalin, bergapten, (*E*)-labda-8(17), 12-diene-15,16-dial, β-sitosterol, β-sitosterol β-D-glucoside, kaempferol, zerumin A, and quercetin have been isolated from ethanolic extract of *C. spicata* rhizomes[4]. Recently we reported the chemical profiling of *C. spicata* rhizome and aerial part essential oils[8]. The results showed that *p*-cymene and β-pinene were the key compounds in the studied *C. spicata* essential oils. It was also recorded that these essential oils showed promising anti-nematic and phytotoxic activities. However, to the best of our knowledge, no prior research on the comparative chemical composition and biological functioning of various solvent extracts derived from different plant parts of *C. spicata* has been reported. The biological properties of plant extracts are directly related to their qualitative and quantitative composition. Therefore, exploring the chemo profile and its correlation with biological attributes is critical and noteworthy. Hence, the present research aimed to assess the chemo profile of extracts of *C. spicata* rhizomes as well as aerial parts in three different solvents, correlating its pesticidal properties viz., nematicidal, herbicidal, and antibacterial activities. In-silico study was also performed for the selected major chemical constituents of the extracts with the five different proteins of *M. incognita* and one protein for each bacterial strain under investigation to establish the expected mode of action for the nematicidal and antibacterial properties of those compounds.

Results

Chemical composition

The compositional variability of extracts was determined by GC-MS analysis. A wide variability was observed in the extracts from different plant parts in different solvents (Table 1). It was observed that sesquiterpenoids were presented only in rhizome extracts. A high amount of sterols and triterpenoids was found in the acetone and hexane extracts. Whereas, a good yield of aliphatic acids and esters was found in methanolic extracts. Among the chemical constituents, unidentified polyprenol (24.49%) and β-sitosterol (14.51%) were found as main constituents in the CSREH. β-Sitosterol was also recorded as the main constituent in CSREA (10.44%), CSAEA (33.67%), and CSAEH (25.07%). Whisky lactone (17.05%) was the main component in the methanolic extract of *C. spicata* rhizome whereas, methyl palmitate (28.30%) and methyl linolenate (10.14%) were the main components in the methanolic extract of *C. spicata* aerial part. Other major constituents (> 5.0%) were ethyl linoleate (7.16%), α-humulene (5.23%), and ethyl palmitate (5.10%), in CSREA; methyl linoleate (8.79%), methyl palmitate (7.54%), methyl linolenate (6.97%), β-sitosterol (6.93%), α-humulene (6.29%), and palmitic acid (5.65)% in CSREM; phytol (7.70%), and stigmasterol (7.27%), in CSAEA; phytol (8.76%), stigmasterol (8.12%), and phytyl palmitate (5.04%) in CSAEH and; phytol (7.46%), β-sitosterol (6.62%), and methyl linoleate (6.36%) in CSAEM.

Chemometric analyses

A colored matrix diagram was created based on 6 samples and their 30 (> 1.0%) chemical components to show relative content differences. The red to yellow color shows gradual chemical concentration from high to low, respectively (Fig. 1A). The quality and amount of the chemical constituents in the *C. spicata* extracts differed depending on the plant part and solvent used. Based on HCA, the tested extracts were grouped into four main clusters based on compositional similarity (Fig. 1A). In the first cluster, there were two extracts viz., CSREA and CSREM, whereas, in the second cluster only CSREH was there. The third cluster comprises CSAEA and CSAEH. The fourth cluster consisted of only CSAEM.

Principal component analysis (PCA) was also carried out to identify the chemical components that accounted for the majority of the variability within the plant extracts subject to study. There were two principal components (PCs) based on PCA analysis as the first two PCs collectively contributed to about 74.04% of the total variability (Fig. 1B). Contribution of PC1 was about 50.24% to the total variability whereas, PC2 contributed about 23.80%. Phytol and methyl palmitate governed the total contribution of PC1 whereas PC2 was associated with campesterol, stigmasterol, unidentified phytoprenol, and β-sitosterol. The HCA results also supported the grouping of extract in PCA.

Biological assay

Nematode mortality assay

Against *M. incognita* J2, the extracts of different solvents showed varied mortality results. For all the extracts, time-dependent mortality results were observed except CSREH and CSREA with respect to LC_{50} values. Because of high values in µg/mL unit, C_{50} values have been represented in mg/mL. The results revealed that, after 96 h, the LC_{50} value was lowest for the methanolic extract of *C. spicata* aerial part (CSAEM; 0.33 mg/mL) among all the extracts followed by CSREM (0.77 mg/mL), CSAEH (1.08 mg/mL), CSAEA (2.54 mg/mL), CSREH (7.08 mg/mL), and CSREA (23.60 mg/mL) (Fig. 2).

S. No	Groups	CAS	RI^{CAL}	RI^{DB}	CSREA	CSREH	CSREM	CSAEA	CSAEH	CSAEM
Monoterpenoids. including:					5.60	1.84	0.78	2.57	2.54	1.83
1.	β- pinene	127-91-3	978	979	1.74	1.01	0.11	1.22	0.64	1.48
Sesquiterpenoids. including:					25.00	25.88	11.9	-	-	-
2.	α-humulene	6753-98-6	1452	1454	5.23	3.75	6.29	-	-	-
3.	β-bisabolene	495-61-4	1509	1511	1.51	1.94	0.31	-	-	-
4.	δ-cadinene	483-76-1	1523	1527	1.33	1.63	0.45	-	-	-
5.	Spathulenol	77171-55-2	1576	1580	1.07	1.52	0.63	-	-	-
6.	β-eudesmol	473-15-4	1648	1651	1.68	2.16	1.31	-	-	-
Diterpenoids. including:					2.00	4.78	0.78	14.5	13.78	7.46
7.	Neophytodiene (two isomers)		1864	1865	-	-	-	3.13	1.67	-
8.	Phytol	150-86-7	2114	2114	-	-	-	7.70	8.76	7.46
Sterols & triterpenoids. including:					25.51	20.99	8.37	59.09	55.74	9.37
9.	Stigmasterol	83-48-7	3250	3249	0.49	0.72	0.32	7.27	8.12	1.00
10.	Campesterol	474-62-4	3216	-	0.69	1.16	0.68	3.35	3.90	0.67
11.	β-sitosterol	83-47-5	3311	3308	10.44	14.51	6.93	33.67	25.07	6.62
12.	Stigmast-4-en-3-one	1058-61-3	3442	3447	0.57	1.44	-	1.32	1.91	-
Aromatics. including:					1.25	0.55	1.04	1.65	0.29	9.41
13.	*p*-cymene	99-87-6	1024	1025	1.25	0.55	0.07	0.75	0.29	t
Aliphatic acids & esters. including:					27.66	13.72	51.62	10.17	8.76	69.59
14.	Palmitic acid	57-10-3	1968	1962	-	1.64	5.65	3.53	-	-
15.	Linolenic acid	463-40-1	2145	2143	0.42	0.27	3.45	t	-	-
16.	Linoleic acid	60-33-2	2139	2139	0.12	0.25	3.95	t	-	-
17.	Methyl palmitate	112-39-0	1928	1923	0.38	0.24	7.54	1.45	0.29	28.30
18.	Ethyl palmitate	628-97-7	1995	1993	5.10	0.42	2.28	0.65	0.74	-
19.	Methyl linolenate	301-00-8	2101	2102	-	0.32	6.97	-	0.23	10.14
20.	Ethyl linolenate	1191-41-9	2169	2169	5.82	0.47	3.00		-	-
21.	Methyl linoleate	112-63-0	2095	2092	0.63	0.32	8.79	0.42	-	6.36
22.	Ethyl linoleate	544-35-4	2163	2162	7.16	0.60	3.64	-	0.19	-
23.	Methyl stearate	112-61-8	2129	2128	-	-	-	-	-	3.97
24.	Ethyl stearate	111-61-5	2196	2194	1.11	-	0.42	t	0.17	t
25.	Phytyl palmitate	N/A	3561	-	-	-	-	1.45	5.04	-
27	Phytyl stearate	62172-54-7	3760	3751	-	-	-	1.96	0.91	-
Tocopherols. including:					0.72	0.91	-	2.83	0.91	-
Alkane & alkene					-	0.16	-	-	0.26	-
Other. including:					3.77	24.49	17.40	4.83	8.99	2.34
28	Hexahydrofarnesyl acetone	502-69-2	1846	1844				1.95	4.78	0.83
29	Farnesyl acetone	1117-52-8	1919	1920	-	-	-	0.28	0.28	-
30	Unidentified polyprenol	-	> 4000	-	-	24.49	-	-	-	-
31	Whisky lactone*	39212-21-0	1488	-	-	-	17.05	-	-	-
Not identified					8.41	6.68	8.13	4.36	8.73	1.18

Table 1. Relative group composition (% TIC) of compounds (> 1.0%) of extracts from *Cautleya spicata*. *CSREH C. spicata* rhizome extract in hexane, *CSREA C. spicata* rhizome extract in acetone,*CSREM C. spicata* rhizome extract in methanol, *CSAEH C. spicata* aerial part extract in hexane, *CSAEA C. spicata* aerial part extract in acetone, *CSAEM C. spicata* aerial part extract in methanol, *t* Trace (below 0.01% of TIC); * = Identification for the compound is not clear; - = not present; *CAS* chemical abstracts service number, RI^{CAL} calculated retention indices, RI^{DB} Retention indices from literature on a DB-5 MS column in reference Adams[9] and NIST web book[10])

Nematode egg hatchability Inhibition assay

After 96 h, all tested plant extracts showed more than 50% egg hatching inhibition at the minimum concentration of 0.25 µg/mL, whereas egg hatching inhibition exceeded 70% at the maximum concentration of 1000 µg/mL (Table 2). At the highest concentration, a similar trend was observed as in the nematode mortality assay for nematode egg hatching assay. Upon incubating for 96 h at 1000 µg/mL, the strongest egg hatching inhibition was recorded for the methanolic extract of *C. spicata* aerial part (CSAEM; 95.91 ± 1.25%) followed by CSREM (95.56 ± 2.05%), CSREH (94.39 ± 3.27%), CSAEH (90.85 ± 2.16%), CSREA (75.19 ± 2.05%) and CSAEA (71.24 ± 3.30%). However, there was no significant difference between the CSAEM and CSREM regarding nematode egg hatching inhibition.

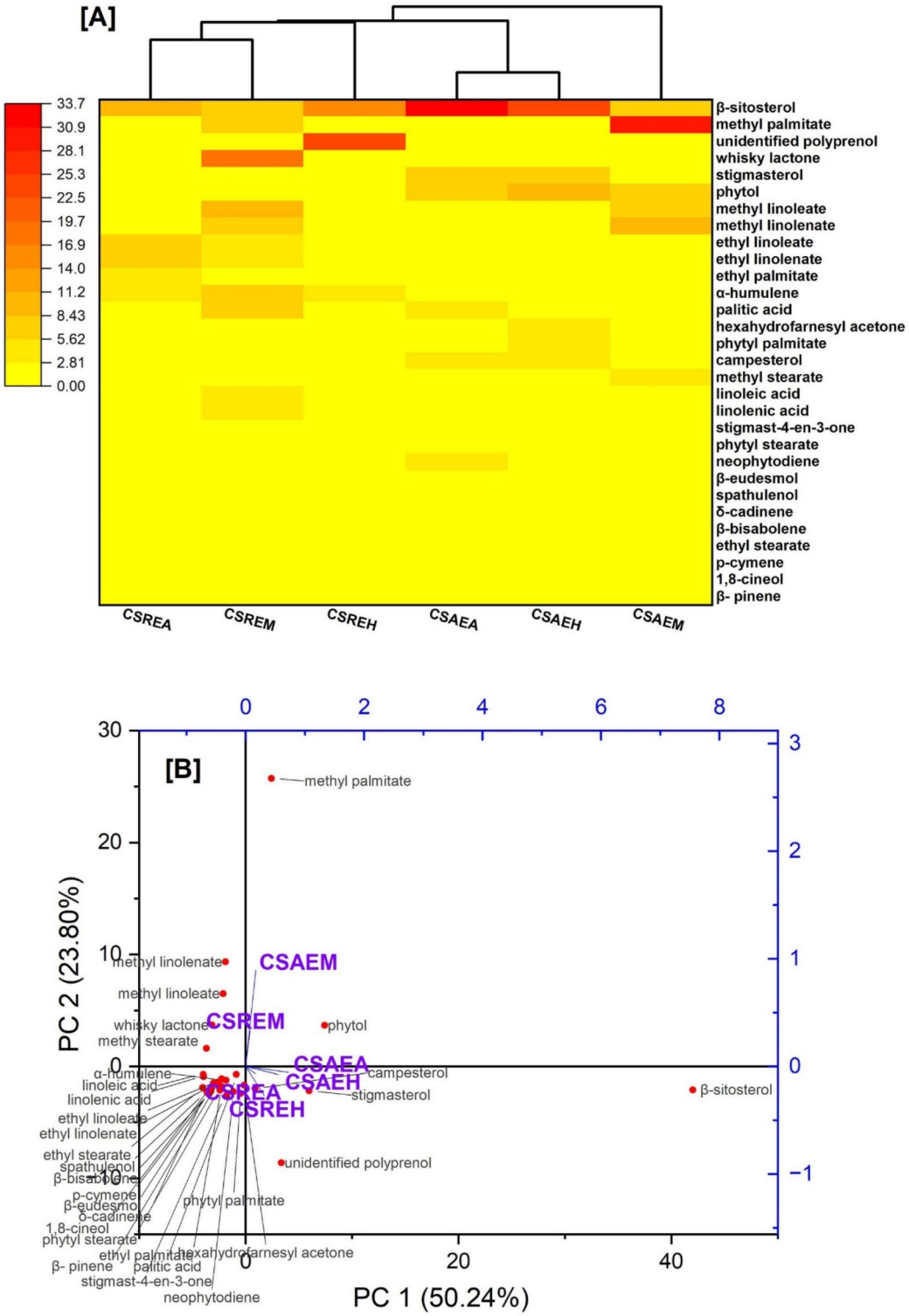

Fig. 1. (**A**) Matrix plot with HCA; and (**B**) PCA of the *C. spicata* extracts determined by the chemical makeup.

Herbicidal assay

In the case of radish, the extracts were found to be slightly effective for seed germination inhibition (SGI). However, significantly higher seed germination inhibition was observed for CSAEA (18.0%) and CSAEM (18.0%) among the extracts at the highest concentration (1000 µg/mL) (Table 3). Root length inhibition (RLI) against radish seeds was recorded in the range of 58.82–79.29%. However, no significant difference was recorded between the extracts and their concentrations. The shoot length inhibition (SLI) was observed from 57.21 to

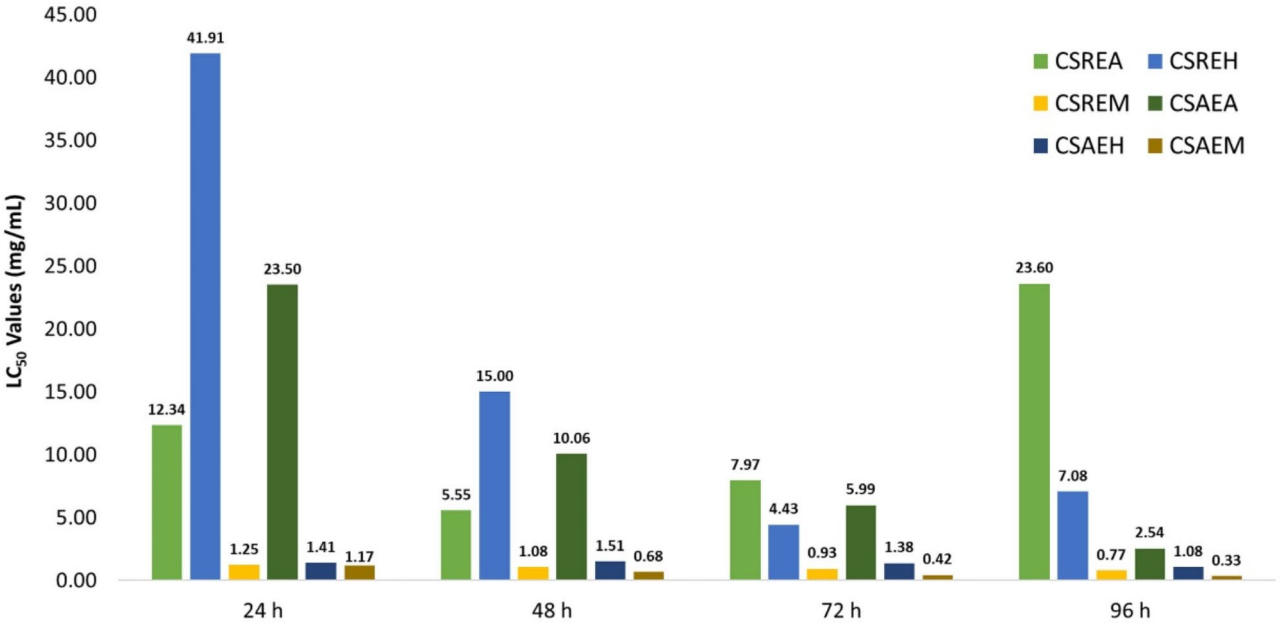

Fig. 2. LC_{50} values of different solvent extracts of *C. spicata* against Nematode (*M. incognita*) J2 in mortality assay.

Extract	Concentration (µg/mL)	Nematode egg hatching inhibition (%) (Mean ± SD)			
		24 h	48 h	72 h	96 h
CSAEA	250	89.48 ± 1.25^{a}	79.44 ± 1.63^{a}	67.16 ± 3.30^{a}	55.86 ± 2.62^{ab}
	500	90.15 ± 1.70^{a}	83.58 ± 2.62^{b}	71.39 ± 2.49^{b}	56.76 ± 2.94^{b}
	1000	92.15 ± 1.70^{b}	84.58 ± 1.41^{b}	77.94 ± 3.40^{c}	71.24 ± 3.30^{d}
CSAEH	250	100.00 ± 0.00^{f}	97.50 ± 2.05^{fg}	92.53 ± 2.87^{f}	88.15 ± 2.45^{h}
	500	99.63 ± 0.47^{f}	97.29 ± 1.70^{f}	93.66 ± 2.94^{fg}	88.57 ± 2.16^{h}
	1000	100.00 ± 0.00^{f}	99.00 ± 0.47^{gh}	97.60 ± 2.62^{i}	90.85 ± 2.16^{i}
CSAEM	250	95.56 ± 0.82^{d}	91.08 ± 1.25^{e}	88.23 ± 2.49^{e}	85.24 ± 2.45^{g}
	500	99.19 ± 1.70^{f}	96.86 ± 1.70^{f}	94.93 ± 2.45^{gh}	94.39 ± 1.63^{jk}
	1000	99.93 ± 0.47^{f}	99.64 ± 2.36^{h}	96.41 ± 1.63^{hi}	95.91 ± 1.25^{k}
CSREA	250	89.48 ± 1.25^{a}	80.87 ± 2.49^{a}	65.33 ± 2.45^{a}	54.54 ± 2.05^{ab}
	500	96.00 ± 2.94^{d}	90.86 ± 2.62^{e}	77.59 ± 2.94^{c}	65.28 ± 2.45^{c}
	1000	93.70 ± 0.94^{c}	87.72 ± 1.25^{c}	81.68 ± 1.70^{d}	75.19 ± 2.05^{e}
CSREH	250	96.00 ± 2.16^{d}	89.29 ± 2.94^{d}	78.93 ± 2.87^{c}	75.88 ± 3.27^{ef}
	500	95.63 ± 1.25^{d}	92.08 ± 0.82^{e}	90.13 ± 0.47^{e}	88.15 ± 2.16^{h}
	1000	98.96 ± 1.25^{f}	97.43 ± 2.16^{f}	95.77 ± 2.94^{hi}	94.39 ± 3.27^{jk}
CSREM	250	96.37 ± 1.25^{d}	86.51 ± 2.16^{c}	81.61 ± 3.27^{d}	77.20 ± 2.62^{f}
	500	97.56 ± 1.63^{e}	96.36 ± 1.63^{f}	94.79 ± 2.87^{gh}	92.65 ± 2.87^{ij}
	1000	100.00 ± 0.00^{f}	100.00 ± 0.00^{h}	96.76 ± 1.70^{hi}	95.56 ± 2.05^{k}

Table 2. Nematode (*M. incognita*) egg hatching Inhibition (%) by different solvent extracts of *C. Spicata*. (*CSREA C. Spicata* rhizome extract in acetone, *CSREH C. Spicata* rhizome extract in hexane, *CSREM C. Spicata* rhizome extract in methanol, *CSAEA C. Spicata* aerial part extract in acetone, *CSAEH C. Spicata* aerial part extract in hexane, *CSAEM C. Spicata* aerial part extract in methanol, *SD* standard deviation; the Tukey's test ($p < 0.05$) indicates that there is no statistically significant difference between mean values in a column that is followed by the same letter.)

94.42%. The hexane extract of *C. spicata* rhizome showed significantly higher shoot length inhibition (94.42%) at the highest concentration level (1000 µg/mL) (Table 3).

In the case of chicory seed, all the extracts showed significant herbicidal activity (100%) except at lower dose levels (250 µg/mL and 500 µg/mL) of hexane extract of *C. spicata* rhizome (CSREH). About 20.00 and 28.00%

Extract	Concentration (µg/mL)	Phytotoxicity parameters (Mean ± SD)		
		SGI%	RLI%	SLI%
CSREA	250	0.00 ± 0.00^{ab}	68.24 ± 1.20^{a}	73.95 ± 0.63^{ab}
	500	10.00 ± 0.71^{c}	66.59 ± 0.76^{a}	85.12 ± 0.41^{cd}
	1000	14.00 ± 0.55^{c}	73.41 ± 0.73^{a}	92.09 ± 0.11^{abc}
CSREH	250	8.00 ± 0.45^{abc}	63.06 ± 0.54^{a}	67.44 ± 0.59^{cd}
	500	6.00 ± 0.55^{bc}	66.82 ± 0.71^{a}	85.12 ± 0.35^{abc}
	1000	6.00 ± 0.89^{bc}	79.29 ± 0.75^{a}	94.42 ± 0.05^{a}
CSREM	250	14.00 ± 0.89^{c}	68.71 ± 0.84^{a}	92.56 ± 0.04^{abcd}
	500	0.00 ± 0.00^{abc}	71.29 ± 0.98^{a}	68.37 ± 0.69^{abc}
	1000	0.00 ± 0.00^{ab}	78.35 ± 0.56^{a}	87.44 ± 0.27^{ab}
CSAEA	250	4.00 ± 0.55^{c}	68.24 ± 0.62^{a}	91.63 ± 0.15^{d}
	500	8.00 ± 0.45^{c}	61.88 ± 0.81^{a}	91.63 ± 0.36^{d}
	1000	18.00 ± 0.45^{a}	58.82 ± 0.51^{a}	72.56 ± 0.72^{ab}
CSAEH	250	0.00 ± 0.00^{c}	62.82 ± 1.11^{a}	77.21 ± 0.39^{abcd}
	500	0.00 ± 0.00^{c}	64.47 ± 0.55^{a}	83.72 ± 0.35^{abc}
	1000	0.00 ± 0.00^{c}	71.29 ± 0.93^{a}	91.63 ± 0.25^{ab}
CSAEM	250	0.00 ± 0.00^{bc}	58.82 ± 0.59^{a}	57.21 ± 0.15^{ab}
	500	0.00 ± 0.00^{abc}	64.00 ± 1.11^{a}	67.44 ± 0.47^{ab}
	1000	18.00 ± 0.45^{a}	79.06 ± 0.50^{a}	90.7 ± 0.25^{bcd}
Pendimethalin		100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 3. Herbicidal activity of different solvent extracts of *C. Spicata* against *R. raphanistrum* sub Sp. *sativus* (L.) (*GPI* germination percent Inhibition , *SLI* shoot length Inhibition, *RLI* root length Inhibition, *CSREA C. Spicata* rhizome extract in acetone, *CSREH C. Spicata* rhizome extract in hexane, *CSREM C. Spicata* rhizome extract in methanol, *CSAEA C. Spicata* aerial part extract in acetone, *CSAEH C. Spicata* aerial part extract in hexane; *CSAEM C. Spicata* aerial part extract in methanol, *SD* standard deviation; the Tukey's test ($p < 0.05$) indicates that there is no statistically significant difference between mean values in a column that is followed by the same letter.)

seed germination inhibition, 77.89% and 73.16% root length inhibition, and about 86.00 and 76.00% shoot length inhibition recorded at 250 and 500 µg/mL of CSREH, respectively (Table 4).

Antibacterial activity

Considering the emergence of multi-antibiotic resistance among bacterial pathogens, searching for new antibacterial molecules has become a critical endeavour[11]. One such effort in this research is focused on the use of natural products isolated from medicinal plants, which are widely accessible resources, have little to no side effects, are less expensive, and have demonstrated good antimicrobial properties (Fig. 3). Thus, the application of plant based natural products as substitutes to antibiotics to counteract resistant bacterial pathogens has become major concern in scientific community worldwide[12,13]. Results of the antibacterial bioassay revealed that the *C. spicata* extracts in different solvents exhibited varying degrees of response to the tested pathogens (Fig. 3). The rhizome of *C. spicata* showed higher antibacterial activity against all the tested bacteria in methanol extract followed by acetone extract (Table 5). However, the aerial part extracts of *C. spicata* in any solvent did not exhibit inhibition zones.

During the primary screening study, it was observed that Gram-negative bacteria were found susceptible to acetone and methanolic rhizome extract of *C. spicata*. Therefore, these extracts were evaluated for their performance in terms of minimum inhibitory concentration (MIC). The findings revealed that Gram-negative bacteria were most sensitive to methanolic extract and MIC was found in the range of 800 – 400 mg/mL (Table 6). However, Gram-positive bacteria are more resistant to the plant extracts with MIC in the 1600 – 800 mg/mL which corroborated the results of preliminary screening. As per MIC results, the most sensitive strain to methanolic extract was *S. typhi* (400 mg/ mL) followed by *E. coli* ATCC 25,922 (800 mg/mL), whereas MIC for *L. monocytogenes* and *S. aureus* ATCC29213 were recorded to be 800 mg/mL and 1600 mg/mL.

Pearson's correlation coefficient

The Pearson's correlation coefficient was computed among chemical constituents and biological properties of *C. spicata* rhizome and aerial part extracts prepared in different solvents. The biological activity at the highest concentration level (1000 µg/mL) at 96 h exposure time was considered for correlation coefficient estimation. Though, the herbicidal activity against chicory seeds was 100% for all the extracts at the highest concentration, therefore it was not considered for correlation coefficient computation. Several chemical constituents were found positively correlated with biological activities reported in the current study (Table 7). The nematode mortality activity was found positively correlated ($r = > 0.6$) with methyl palmitate, methyl linolenate, ethyl linolenate, methyl linoleate and methyl stearate. In the contrary, nematode egg hatching inhibition activity was positively correlated ($r = > 0.6$) only with methyl linolenate. Among the herbicidal activity parameters, only seed

Extract	Concentration (µg/mL)	Phytotoxicity parameters (Mean ± SD)		
		SGI%	RLI%	SLI%
CSREA	250	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	500	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
CSREH	250	20.00 ± 0.71^{a}	77.89 ± 0.15^{b}	86.00 ± 0.08^{b}
	500	28.00 ± 0.84^{b}	73.16 ± 0.08^{a}	76.00 ± 0.11^{a}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
CSREM	250	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	500	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
CSAEA	250	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	500	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
CSAEH	250	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	500	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
CSAEM	250	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	500	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
	1000	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
Pendimethalin		100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 4. Herbicidal activity of different solvent extracts of *C. Spicata* against *C. intybus* (*GPI* germination percent Inhibition, *RLI* root length Inhibition, *SLI* shoot length Inhibition, *CSREA C. Spicata* rhizome extract in acetone; *CSREH C. Spicata* rhizome extract in hexane, *CSREM C. Spicata* rhizome extract in methanol, *CSAEA C. Spicata* aerial part extract in acetone, *CSAEH C. Spicata* aerial part extract in hexane, *CSAEM C. Spicata* aerial part extract in methanol, *SD* standard deviation, the Tukey's test ($p < 0.05$) indicates that there is no statistically significant difference between mean values in a column that is followed by the same letter.)

germination inhibition for radish seeds showed a positive correlation with β- pinene (r = > 0.6). Antibacterial activity against all the studied bacterial strains found positively correlated with α-humulene, β-eudesmol, palitic acid, linolenic acid, linoleic acid, and whisky lactone (r = > 0.6). Additionally, antibacterial activity against Gram-positive bacteria i.e., *L. monocytogenes*, *S. aureus* was also showed a positive correlation with spathulenol among the major chemical constituents of *C. spicata* extracts.

A chord diagram was also generated to represent a positive correlation between chemical constituents, and biological properties of *C. spicata* extracts (Fig. 4). The chord diagram showed the connections among the parameters which have a correlation coefficient of more than 0.6.

Molecular docking study

From the in vitro assays, it was found that the extracts showed potent nematode egg-hatching inhibition, mortality assay and antibacterial activity. Further, it was examined whether the major phytoconstituents from the extracts physically bind with the nematode and bacterial proteins. In this context, the docking was performed with 5 nematode protein receptors. The docking scores for the proteins, AChE, Cyt C, GST 1, HSP 90, and ODR3 ranged from (–4.4 to − 8.1 kcal/mol), (–4.9 to − 8.4 kcal/mol), (–4.4 to − 8.6 kcal/mol), (–4.1 to − 8.1 kcal/mol), and (–4.2 to − 8.3 kcal/mol), respectively (Fig. 5). Through the visualization of multiple docked poses, the best-docked pose was chosen based on having the lowest binding energy. The 2D interactions of the compounds and inhibitors with the highest docking scores were visualized with the help of Biovia Discovery Studio-2021 Client, and are shown in Fig. 6.

The docking study of the selected compounds was also performed with bacterial proteins and for comparison purposes, penicillin G was taken as standard antibacterial drug. The docking scores for the proteins, 1AOD, 1BXY, 1JIJ, and 5V2W ranged from (–4.5 to − 7.4 kcal/mol), (–5.4 to − 9.9 kcal/mol), (–4.6 to − 8.1 kcal/mol), and (–4.0 to − 6.9 kcal/mol), respectively. Results showed the highest docking score of stigmasterol for all the proteins as shown in Fig. 7. Figure 8A1 shows the recognized binding modes and molecular orientations of the most active compound stigmasterol with 1AOD. Eight key amino acid residues in the binding pocket of 1AOD interact with stigmasterol via Van der Waals, one amino acid (Tyr A206) via pi-sigma interaction, and others alkyl and pi-alkyl interactions. Similarly, for 1BXY, the best binding affinity of stigmasterol may be due to van der Waal interactions with 14 amino acid residues and other hydrophobic interactions such as pi-sigma, alkyl, and pi-alkyl (Fig. 8B1). Stigmasterol showed an analogous binding mode with 1JIJ and 5V2W except for some differences in different amino acid residues and their respective interactions (Fig. 8C1 and Fig. 8D1). However, on comparing the docking scores of ligands with the standard, it was found that compounds including stigmasterol, and β-sitosterol showed better docking scores for all the proteins. Figure 8A2, B2, C2, C3 shows the interaction of penicillin G with the proteins i.e., 1AOD, 1BXW, 1JIJ, and 5V2W, respectively via hydrogen bond, van der Waal, and other hydrophobic interactions.

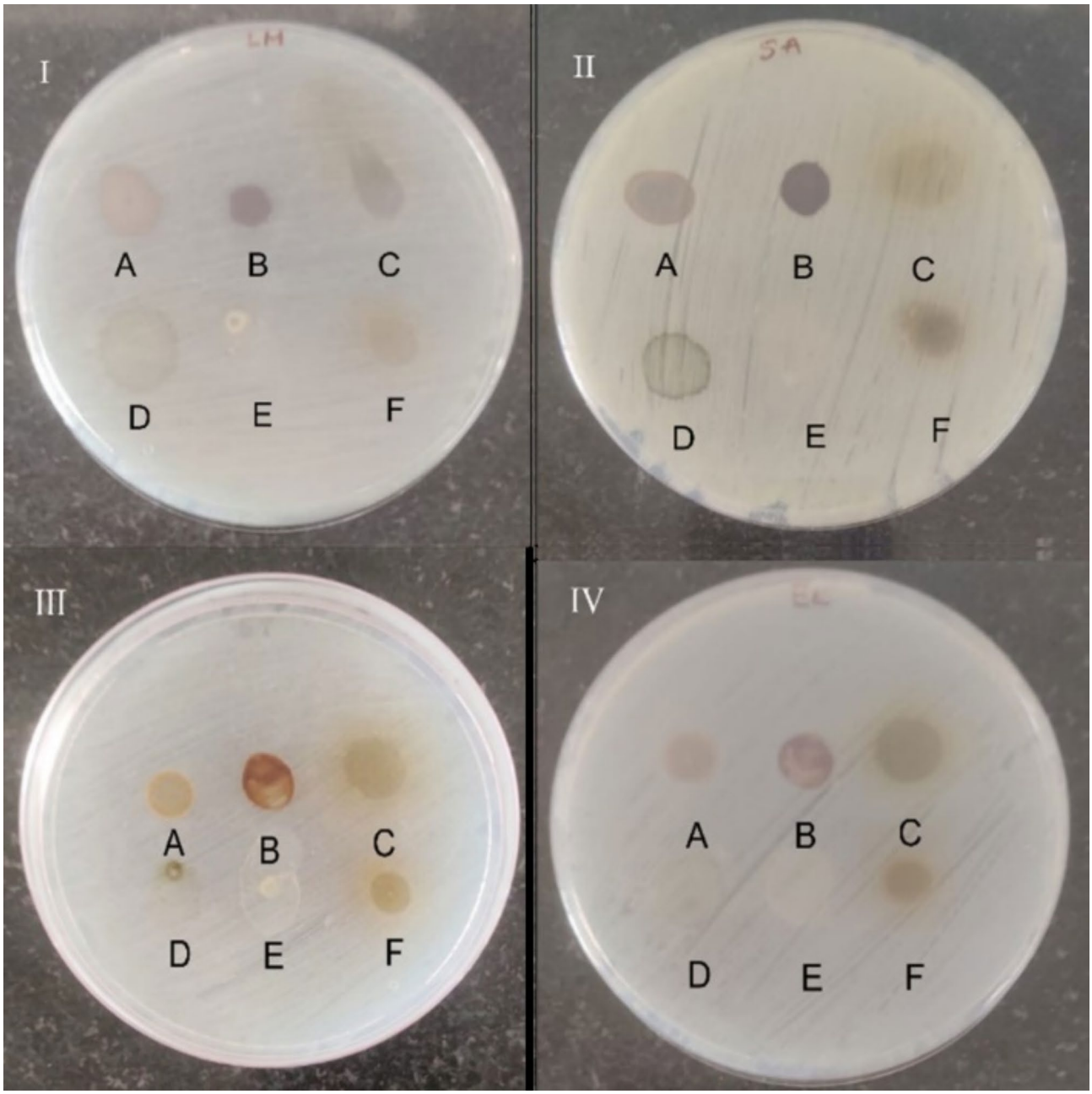

Fig. 3. Antibacterial activity against Gram-positive (*S. aureus* ATCC29213 and *L. monocytogenes*) and Gram-negative (*S. typhi* and *E. coli* 25922) bacteria using spot on lawn assay.

Discussion

The chemical composition of plants' extracts is influenced by several factors such as solvent used, temperature of extraction, plant part used, extraction time, extraction method, etc[14,15]. In the previous studies, the yields were reported in the sequence of methanol > acetone > hexane for Olive, *Allium cepa*, *Apium graveolens*, *Coriandrum sativum*, and *Petroselinum crispum*[16,17], however, in the current study, the yields are in the sequence of acetone > methanol > hexane. This discrepancy might be attributed to differences in the extraction sequence, solvent polarity, and plant matrix composition. Results in a similar trend to the current study were recorded for cinnamon extract where the yield was highest in ethyl acetate (moderately polar) followed by methanol (polar) and hexane (non-polar)[18]. Such variability suggests that extraction efficiency is highly dependent on solvent polarity and the solubility of target phytochemicals. Besides, the solvent and extraction conditions, the composition of plant extracts also may vary with different growing conditions, light intensity, edaphic conditions, differences in the physiology of accumulating secondary metabolites in different plant parts differently, etc[19–21].

GC-MS analysis in this study revealed significant variability in the chemical profiles of different plant parts extracted with various solvents. Sesquiterpenoids were found exclusively in rhizome extracts, while sterols and

Plant extract	Zone of inhibition (mm)			
	Gram-positive		Gram-negative	
	L. monocytogenes	S. aureus	S. typhi	E. coli
CSREH	-	-	-	-
CSREA	8.00 ± 0.10	8.50 ± 0.20	8.50 ± 0.10	8.50 ± 0.10
CSREM	9.00 ± 0.10	9.50 ± 0.10	11.0 ± 0.20	12.0 ± 0.10
CSAEH	-	-	-	-
CSAEA	-	-	-	-
CSAEM	-	-	-	-

Table 5. Antibacterial activity of *C. Spicata* rhizome and aerial part extracts in different solvents. (*CSREH C. Spicata* rhizome extract in hexane, *CSREA C. Spicata* rhizome extract in acetone, *CSREM C. Spicata* rhizome extract in methanol, *CSAEH C. Spicata* aerial part extract in hexane, *CSAEA C. Spicata* aerial part extract in acetone; *CSAEM C. Spicata* aerial part extract in methanol)

Plant extract	Minimum inhibitory concentration (MIC) (mg/mL)			
	Gram-positive		Gram-negative	
	L. monocytogenes	*S. aureus*	*S. typhi*	*E. coli*
CSREA	1600	1600	1600	1600
CSREM	800	1600	400	800

Table 6. Minimum inhibitory concentration (MIC) of the acetone and methanolic rhizome extract of *C. Spicata* against tested bacteria. (*CSREA C. Spicata* rhizome extract in acetone *CSREM C. Spicata* rhizome extract in methanol)

triterpenoids were abundant in extracts from the aerial parts. This variation is likely due to different growing conditions such as light intensity, external environment, and physiological factors, which differ for above-ground (aerial parts) and below-ground (rhizomes) plant parts. Additionally, in previous studies, the fresh dried plant material was used for extraction whereas, in the present study, the extraction was done in sequences (starting from hexane followed by acetone and finally with methanol) with the same plant material. This approach may have influenced both the yield and composition of the extracts, explaining the observed differences compared to prior research. Based on the literature search, there is only one study reported previously on the chemical composition of *C. spicata*[4]. The compound β-sitosterol was isolated from ethanolic extract of *C. spicata* rhizomes in their study, which is also found in high amount in hexane and acetone extracts of *C. spicata* aerial part and rhizome extracts in the current investigation. Other major compounds such as methyl palmitate and wiskey lactone were also reported in the other species of Zingiberaceae and other plant families. Methyl palmitate was the main compound in the extracts of *Alpinia pahangensis*, *Globba sessiliflora* and *A. scabra*[22–24]. Similarly, Whisky lactone was reported to be present in the oak wood (*Quercus* species)[25].

Methyl palmitate was found as the main chemical constituent in the methanolic extract of *C. spicata* arial part (CSAEM; 28.30%) which showed the lowest LC_{50} values for nematode mortality. The significantly lower nematode mortality after CSAEM was recorded for the methanolic extract of *C. spicata* rhizome (CSREM) which contains a sufficient amount of methyl palmitate (7.54%). It is inferred that the nematode mortality of these two extracts might be a result of methyl palmitate. Previously, methyl palmitate was reported to have significant mortality against *M. incognita* in bananas under in vitro as well as pot experiments[26]. An oil isolated from *Annona crassiflora* Mart. leaf was found to have methyl palmitate as the main component (48.14%) and the oil showed a significant mortality assay against *Caenorhabditis elegans*[27]. The polar nature of methanol likely facilitated the extraction of water-soluble nematicidal compounds, explaining the higher nematode mortality compared to hexane and acetone extracts. Hexane, being non-polar, extracted more lipophilic compounds that may have limited bioavailability due to poor water solubility[28]. Further, hexane extract from the aerial part exhibited significantly higher nematode mortality than rhizome extract in hexane. It might be due to that hexane extracted more potential nematicidal compounds from aerial parts but not from rhizome parts. Or it might be happened that a smaller number of compounds with nematicidal potential are present in rhizomes of *C. spicata*. This fact can be justified by the results of nematode mortality data, where rhizome extracts in each and every solvent shower higher LC_{50} values than the aerial part extracts. Regarding the nematode egg hatchability inhibition assay, the sequence of effectiveness was in the manner of: methanol > hexane > acetone. Because of the complexity of nematode egg physiology and the phytochemistry of plant extracts, it was suggested that the compounds bound the egg surface via compound-protein interaction or by penetrating the egg surface. It may also make some coating of the egg surface which prevents the oxygen supply for larval development and may also restrict the J2 from penetrating the egg surface during the hatching process, etc[29,30].

The herbicidal potential of *C. spicata* extracts may be attributed to major constituents like β-sitosterol, methyl palmitate, methyl linolenate, methyl linoleate, phytol, and stigmasterol, which have been previously reported

Compounds	Pearson's correlation coefficient value								
	NMP	NEHIP	SGIRS	RLIRS	SLIRS	ABALM	ABASA	ABAST	ABAEC
β- pinene	-0.41	-0.64	0.85	-0.18	0.01	-0.65	-0.65	-0.69	-0.71
α-humulene	-0.47	-0.09	-0.38	0.47	0.34	0.68	0.68	0.70	0.70
β-bisabolene	-0.80	-0.30	-0.06	0.38	0.52	0.40	0.40	0.35	0.31
δ-cadinene	-0.79	-0.28	-0.11	0.41	0.52	0.46	0.46	0.41	0.38
spathulenol	-0.71	-0.17	-0.22	0.48	0.51	0.61	0.61	0.57	0.54
β-eudesmol	-0.67	-0.14	-0.28	0.50	0.50	0.67	0.67	0.64	0.62
neophytodiene	0.09	-0.35	0.21	-0.94	-0.82	-0.47	-0.47	-0.46	-0.46
phytol	0.57	0.14	0.27	-0.51	-0.40	-0.70	-0.70	-0.70	-0.69
stigmasterol	0.16	-0.11	-0.05	-0.77	-0.52	-0.52	-0.52	-0.52	-0.52
campesterol	0.10	-0.09	-0.14	-0.74	-0.46	-0.44	-0.44	-0.44	-0.44
β-sitosterol	-0.09	-0.37	0.11	-0.89	-0.67	-0.40	-0.40	-0.42	-0.43
stigmast-4-en-3-one	-0.30	-0.13	-0.25	-0.46	-0.08	-0.19	-0.19	-0.23	-0.26
p-cymene	-0.92	-0.93	0.36	-0.43	-0.12	-0.31	-0.31	-0.33	-0.35
palitic acid	0.21	0.10	-0.28	-0.15	-0.52	0.64	0.64	0.68	0.71
linolenic acid	0.19	0.32	-0.54	0.35	0.02	0.72	0.71	0.77	0.80
linoleic acid	0.26	0.38	-0.55	0.34	-0.01	0.73	0.72	0.78	0.81
methyl palmitate	0.72	0.46	0.40	0.41	0.11	-0.16	-0.16	-0.14	-0.13
ethyl palmitate	-0.69	-0.59	0.00	0.04	0.18	-0.05	-0.05	-0.02	-0.01
methyl linolenate	0.77	0.60	0.10	0.54	0.13	0.16	0.15	0.19	0.22
ethyl linolenate	-0.63	-0.49	-0.02	0.19	0.25	0.08	0.08	0.11	0.13
methyl linoleate	0.65	0.55	-0.13	0.51	0.05	0.41	0.41	0.47	0.50
ethyl linoleate	-0.64	-0.49	-0.04	0.19	0.26	0.07	0.07	0.10	0.12
methyl stearate	0.63	0.37	0.50	0.36	0.16	-0.32	-0.32	-0.31	-0.31
ethyl stearate	-0.65	-0.53	-0.02	0.10	0.27	-0.11	-0.11	-0.08	-0.07
phytyl palmitate	0.16	0.17	-0.40	-0.40	-0.06	-0.41	-0.41	-0.41	-0.41
phytyl stearate	0.08	-0.38	0.25	-0.95	-0.85	-0.46	-0.46	-0.45	-0.45
hexahydrofarnesyl acetone	0.28	0.17	-0.26	-0.45	-0.15	-0.52	-0.52	-0.51	-0.51
Farnesyl acetone	0.13	-0.17	-0.03	-0.82	-0.59	-0.50	-0.50	-0.49	-0.49
polyprenol	-0.33	0.16	-0.19	0.37	0.39	0.58	0.58	0.50	0.46
whisky lactone	0.30	0.39	-0.54	0.31	-0.04	0.69	0.68	0.74	0.78

Table 7. Pearson's correlation coefficient among chemical constituents (> 1.0%) and biological activities of *C. spicata* extracts. (*NMP* nematode mortality percentage, *NEHIP* nematode egg hatching Inhibition percentage, *SGIRS* seed germination Inhibition against radish seeds , *RLIRS* root length Inhibition against radish seeds, *SLIRS* shoot length Inhibition against radish seeds, *ABALM L. monocytogenes*, *ABASA S. aureus*, *ABAST S. typhi*, *ABAEC E. coli*)

for their herbicidal properties[31–34]. It was also inferred that the herbicidal potential of extracts might be the interaction effect of more than one chemical constituent of the extracts[35,36]. So far, several mechanisms have been suggested to explain the herbicidal effects in plant species. These potential toxic mechanisms of plant species on weeds include: (a) accumulation of lipid globules in the cytoplasm and a reduction in organelle size, such as mitochondria, possibly due to DNA synthesis inhibition or membrane disruption; (b) structural breakdown and absence of intact organelles due to volatility, leading to poor root growth; (c) inhibition of mitosis in growing cells; (d) generation of reactive oxygen species, resulting in lipid peroxidation and membrane disintegration; and (e) a decrease in the total chlorophyll content and respiratory activity[37–40]. In the current investigation, the extracts of *C. spicata* exhibited herbicidal effects on radish seeds and chicory seeds especially on their seed germination and growth parameters in varying degrees. Therefore, the specific mechanism of extracts for their particular herbicidal effect is very complex to identify. However, it can be inferred that different chemical constituents of the extracts might cause the herbicidal effect by following one or more mechanisms from the above mentioned. Therefore, a significant variability was observed in different herbicidal parameters and both the weed seeds under investigation. However, there is a need to conduct further studies on compound isolation and their in-depth investigation to identify the actual mode of action of the extracts or their compounds. In *C. spicata* rhizomes extracted in acetone (CSREA), β-sitosterol was the main compound. The β-sitosterol has previously been reported to have antibacterial properties against *S. typhi*[41]. It was reported that it showed 20 mm of zone of inhibition against *S. typhi*. In the current study, the zone of inhibition for CSREA was 8.50 mm against *S. typhi*. β-sitosterol was also reported effective against *L. monocytogenes*, *S. aureus* and *E. coli*[42,43]. Alkaline substituted lactones such as whiskey lactones, have also been advocated to have antibacterial potential against several Gram-positive and Gram-negative bacteria[44]. The plant extracts are a complex mixture

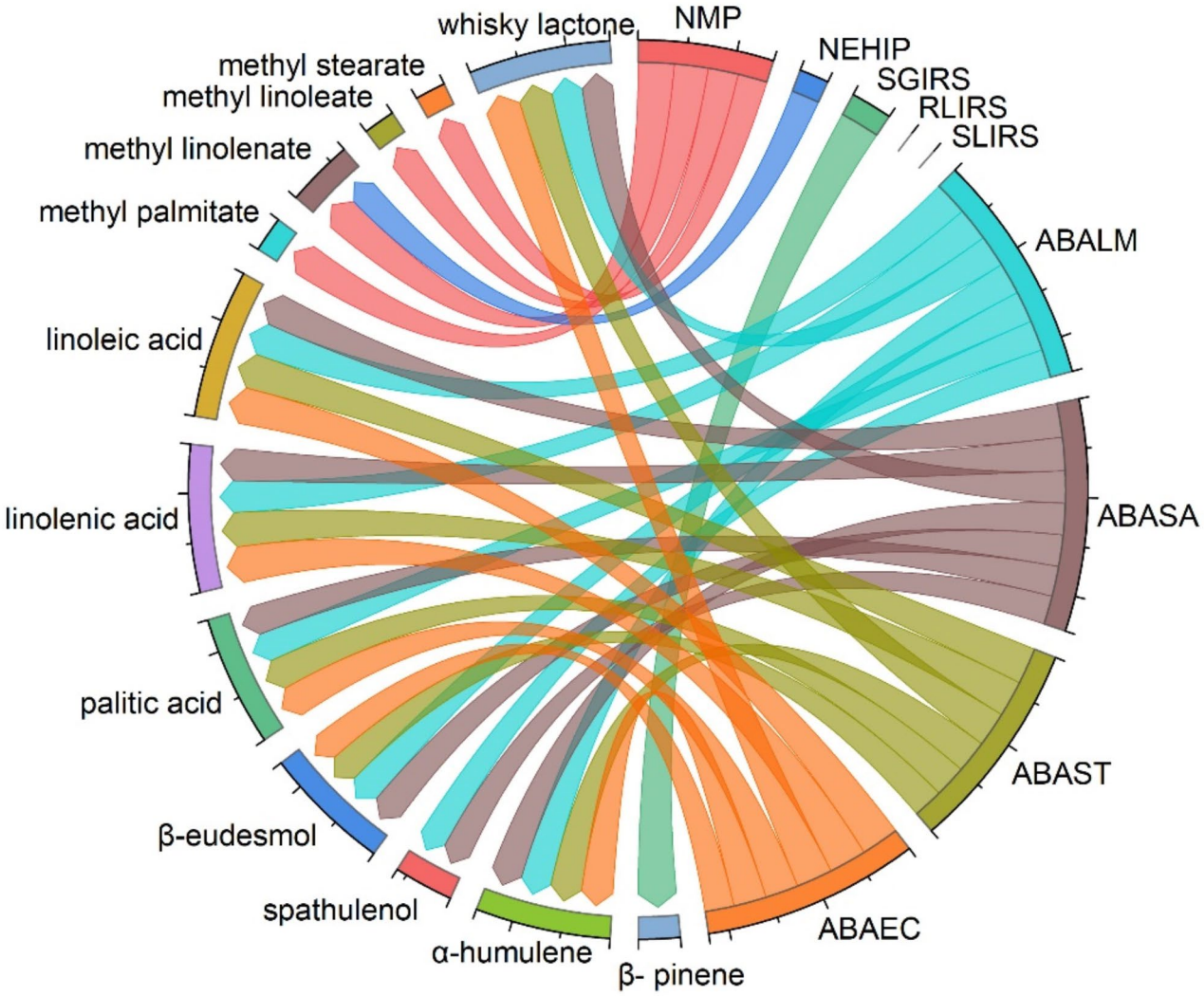

Fig. 4. Chord diagram displaying the relationships between the chemical components and the biological effects of *C. spicata* rhizome and aerial parts extracts prepared using various solvents. The connecting chords in the diagram indicate a significant positive correlation with a Pearson's correlation coefficient exceeding 0.6. (*NMP* nematode mortality percentage; *NEHIP* nematode egg hatching inhibition percentage, *SGIRS* seed germination inhibition against radish seeds, *RLIRS* root length inhibition against radish seeds, *SLIRS* shoot length inhibition against radish seeds, *ABALM L. monocytogenes*, *ABASA S. aureus*, *ABAST S. typhi*, *ABAEC E. coli*)

of several compounds that may interact with each other and can give synergistic or antagonistic action[45]. in the current study, only acetone and methanol extract of *C. spicata* rhizomes showed activity against bacterial strains. The plant extract CSREA shows higher MIC values (1600 mg/mL) for all tested bacteria compared to CSREM, which has lower MIC values (400–1600 mg/mL). In contrast, standard antibiotics typically exhibit much lower MIC values. For instance, gentamicin sulfate, a standard antibiotic showed MIC values 2, 4, and 4 µg/ml against *E. coli*, *L. monocytogenes*, and *S. aureus*[46]. Against *S. typhi*, multiple antibiotics including cefepime, ceftazidime, imipenem cilastatin, colimycin, vancomycin, amoxicillin, piperacillin, ceftriaxone, and ampicillin showed MIC values ranging from 0.002 mg/mL to 0.085 mg/*mL*[47]. *It* was inferred that in the extract of *C. spicata* rhizomes (in acetone and methanol), several other compounds were also there which might contribute to the enhanced antibacterial activity. Whereas, this might be an antagonistic or indifferent interaction in the case of other extracts. Usually, Gram positive bacteria are supposed to be more susceptible to plant extracts or antibiotics due to the absence of an outer membrane which acts as a permeability hurdle in Gram negative bacteria[48,49]. In the present investigation, the results are inverse, therefore, it can be suggested that the outer membrane is not involved in the mechanism of plant extracts under investigation. The varying MIC response of different *C. spicata* extracts to Gram-positive and Gram-negative bacteria are due to differences in the structure and composition of the membrane[50]. The findings suggest that *C. spicata* rhizome extract has the ability to fight a variety of pathogenic bacteria. The correlation analysis conducted in this study between the chemical constituents and biological activities of *C. spicata* extracts also reveals significant associations that support the in-vitro study findings.

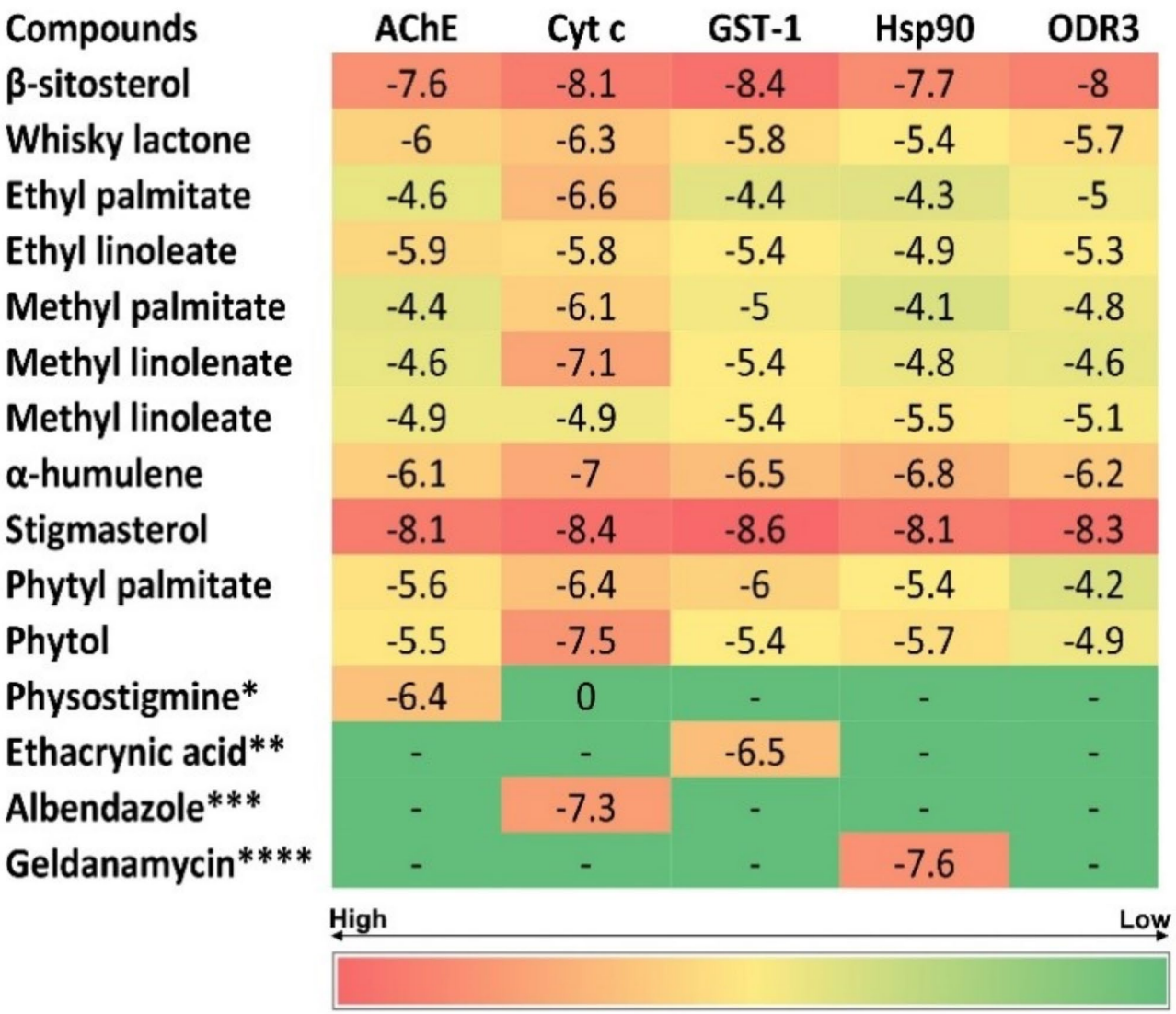

Fig. 5. The binding energy (kcal/mol) for ligand and protein interaction under investigation for nematicidal activity. (*AChE* Acetylcholinesterase, *Cyt c* cytochrome c oxidase, *GST-1* Glutathione S- transferase, *Hsp90* heat shock protein 90, *ODR 3* odorant receptor gene 3; *= inhibitor of AChE; **= inhibitor of GST1; ***= inhibitor of Cyt c; ****= inhibitor of Hsp90)

Strong positive correlations were found between specific compounds and biological activities, such as nematode mortality and egg hatching inhibition, as well as herbicidal and antibacterial effects. Compounds like methyl palmitate, methyl linolenate, and spathulenol showed notable correlations with targeted activities, suggesting their potential as key contributors to the observed bioactivity. These findings provide valuable insights for future research aimed at harnessing the therapeutic and pesticidal potential of *C. spicata* in combating nematodes, weeds, and bacterial pathogens.

The findings of the In-silico study suggest that for nematode proteins, the advantageous docking and binding energy exhibited for the stigmasterol-AChE complex. These interactions were supposed to be due to hydrophobic and Van der Waal's interactions between docked molecules and protein residues. Specifically, the Pi alkyl interaction with Phe434 and the alkyl interaction with Val338 are believed to contribute to the favourable binding energy values. Similarly, by comparing the binding scores of compounds with the known inhibitors, it was found that for all other proteins, stigmasterol showed the best binding affinity with docking score (least negative binding energy) more than the known inhibitors. The possible interactions between receptors and ligands were hydrophobic, covalent, and/or noncovalent bonds. The present study indicates the promising effect of sterol compounds against the nematicidal protein which may suggest their potent inhibitory effect. Results of the current study are constant with previously reported in-silico studies revealing the inhibitory effect of β-sitosterol and stigmasterol against AChE receptors and other proteins[51–53]. For antibacterial activity, the most significant internecion was for β-sitosterol – 1BXW and stigmasterol-1BXW complex.

The convergence of findings from molecular docking and in-vitro studies provides vigorous evidence confirming the nematicidal and antibacterial efficacy of compounds found in *C. spicata* extracts. Molecular

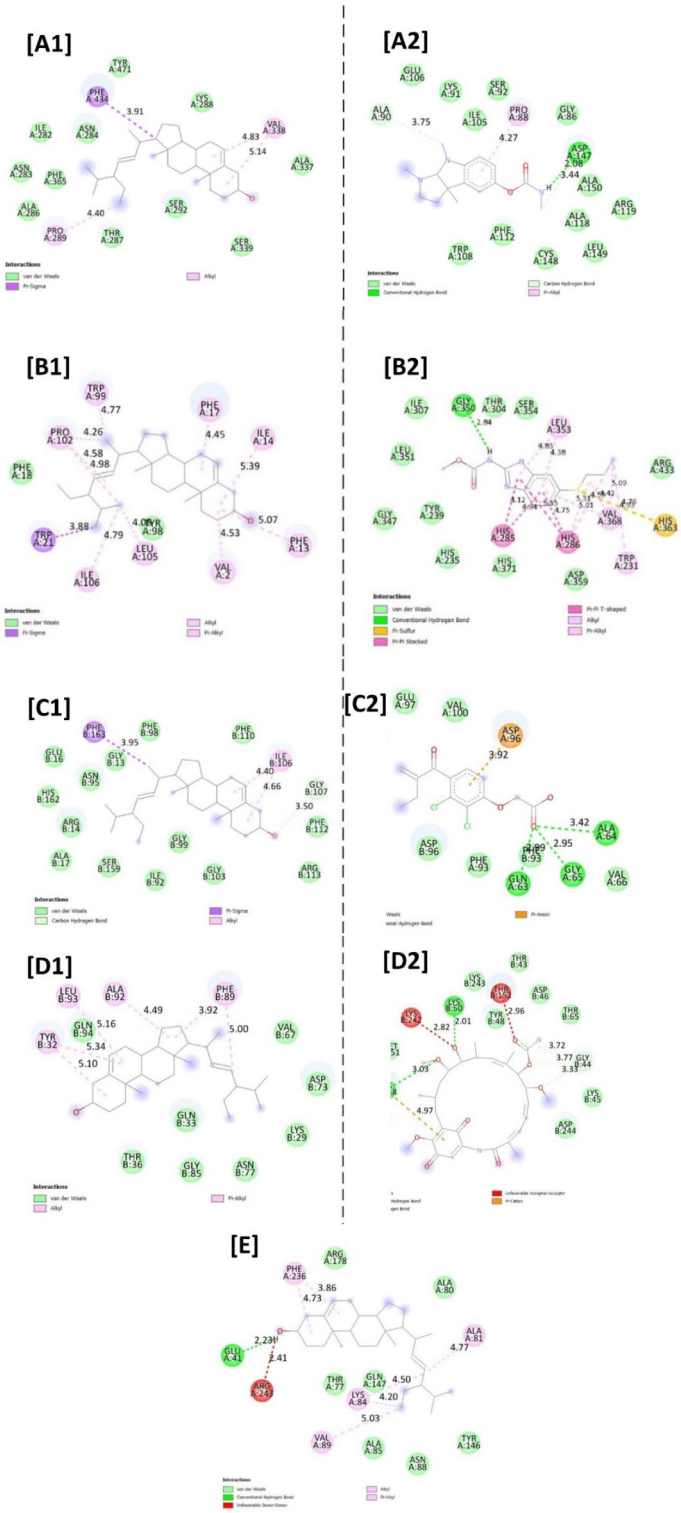

Fig. 6. 2D representation of ligand-protein interaction for the ligands having the highest binding energy for nematicidal activity. (**A1** and **A2**) interaction of stigmasterol and Physostigmine with AChE, respectively; (**B1** and **B2**) interaction of stigmasterol and Albendazole with Chy c, respectively, (**C1** and **C2**) interaction of stigmasterol and Ethacrynic acid with GST-1, respectively; (**D1** and **D2**) interaction of stigmasterol and Geldanamycin with Hsp90, respectively; (**E**) interaction of stigmasterol with ODR3.

Compounds	1AOD	1BXW	1JIJ	5V2W
β-sitosterol	-7.1	-9.7	-7.3	-6.7
whisky lactone	-4.5	-6.4	-5.9	-4.4
ethyl palmitate	-4.6	-5.4	-5.7	-3.8
ethyl linoleate	-5.1	-6.1	-5.2	-4.4
methyl palmitate	-4.5	-5.6	-5.1	-3.9
methyl linolenate	-5	-6.2	-5.6	-4.8
methyl linoleate	-5	-6	-5.6	-4.4
α-humulene	-5.3	-6.3	-7.2	-6.3
stigmasterol	-7.4	-9.9	-8.1	-6.9
phytyl palmitate	-5.2	-5.8	-4.6	-4
phytol	-5.3	-5.9	-5.4	-4.7
Penicillin G*	-6.8	-8.4	-8	-6

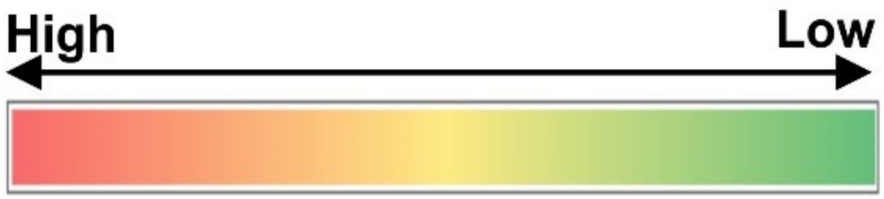

Fig. 7. The binding energy (kcal/mol) for ligand and protein interaction under investigation for antibacterial activity. (* = standard inhibitor for antibacterial activity)

docking explains potential mechanisms underlying these activities, offering insights into how specific compounds interact with target proteins. This synergy between computational and experimental approaches enhances our understanding of the biological activities of *C. spicata* extracts, facilitating the development of novel strategies against nematodes and bacterial infections.

Conclusions

The results of the present study explained the phytochemical variability in three different solvent extracts of *Cautleya spicata* rhizome and aerial parts. The GC-MS analysis showed that all extracts were dominated by β-sitosterol as the main constituent, and α-humulene was also found in good quantity in rhizome extracts with β-sitosterol. Data on pesticidal assays revealed the potential of these extracts towards nematode as well as weed control. Methanolic extracts of *C. spicata* showed higher potential for nematicidal actions against *M. incognita*. The *C. spicata* extracts were wound more active against chicory seeds in comparison with radish seeds. Though the results showed the chemo profiling variability and different extent of nematicidal and herbicidal actions. Furthermore, in-depth studies are required to find the actual pesticidal compounds in *C. spicata*. The in vivo studies are needed to explore the actual capacity of the plant toward nematode and weed control in field conditions. In silico studies predicted the interaction of docked chemical compounds at the molecular level. However, to determine the precise mechanism of action of the plant's chemical components and their purported pesticidal properties, further experimental studies are required.

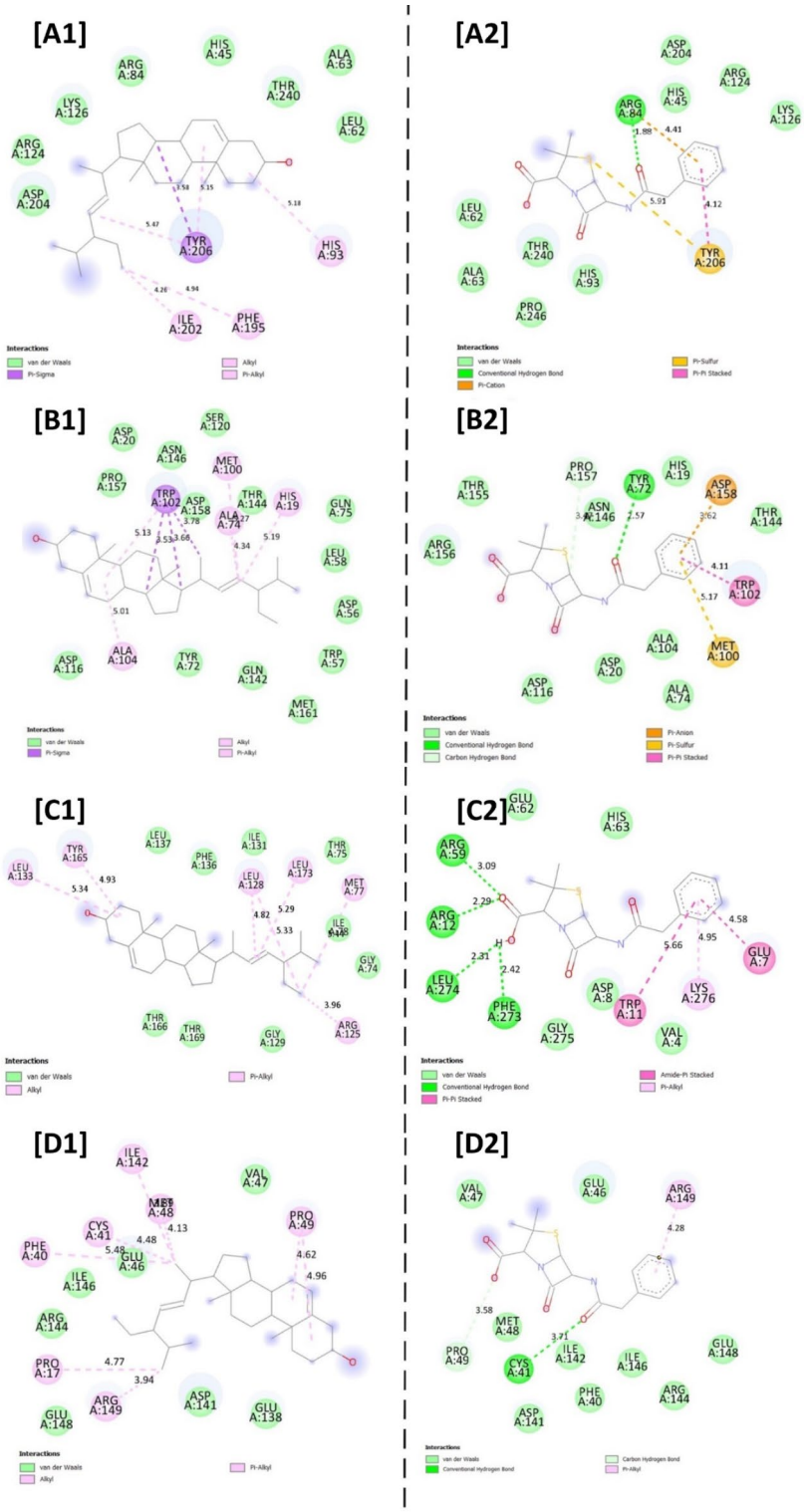

Fig. 8. 2D representation of ligand-protein interaction for the ligands having the highest binding energy for antibacterial activity. (**A1** and **A2**) interaction of stigmasterol and penicillin G with 1AOD, respectively; (**B1** and **B2**) interaction of stigmasterol and penicillin G with 1JIJ respectively, (**C1** and **C2**) interaction of stigmasterol and penicillin G with 1BXW, respectively; (**D1** and **D2**) interaction of stigmasterol and penicillin G with 5V2W, respectively.

S. no.	Plant part	Solvent used	Sample code	Extract yield (% DW)
1.	Rhizome	Hexane	CSREH (*C. spicata* rhizome extract in hexane)	0.68
2.	Rhizome	Acetone	CSREA (*C. spicata* rhizome extract in acetone)	2.23
3.	Rhizome	Methanol	CSREM (*C. spicata* rhizome extract in methanol)	0.91
4.	Arial part	Hexane	CSAEH (*C. spicata* aerial part extract in hexane)	0.79
5.	Arial part	Acetone	CSAEA (*C. spicata* aerial part extract in acetone)	3.78
6.	Arial part	Methanol	CSAEM (*C. spicata* aerial part extract in methanol)	1.42

Table 8. Sample details of different solvent extracts of *C. spicata* (*DW* dry weight).

Materials and methods

Sample and collection site

The aerial parts and rhizomes of *C. spicata* were obtained from the unreserved wild habitat of Munsyari, Uttarakhand (latitude 30°04'17.56" N; longitude 80°14'26.79" E; altitude 2101 m) in October 2021. The plant material (GBPUH-1547) was verified and deposited in the Department of Biological Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Plant extract preparation

Fresh plant material of *C. spicata* was subjected to hydro distillation through a Clevenger-type apparatus to isolate the essential oils. The distillation process was carried out for a duration of 3 h. The leftover material was shade dried and ground finely. The extract of rhizome (290 g) and aerial part (200 g) was prepared in three different solvents i.e., hexane, acetone, and methanol subsequently using cold percolation method[12]. The plant material was extracted in a series of organic solvents with increasing polarity (Table 8). The plant material and solvent were used in a 1:2.5 ratio, which was sufficient to completely immerse the plant material. The whatman filter paper was used to filter the resulting supernatant, and the residue left was used for the subsequent extraction steps. Following the third extraction, the crude extract was produced by evaporating filtrates using a rotary evaporator at reduced pressure at 50 °C.

The yield was calculated employing the formula given below and the dried extracts were stored in glass vials for future analysis.

$$Percent\ yield\ (\%DW) = \frac{Weight\ of\ dried\ extract}{Weight\ \ of\ dried\ plant\ material} \times 100$$

GC-MS analyses of extracts

In 220 µL of pyridine, an amount of 5–6 mg of dried rhizomes and seed extract was dissolved[54]. The GC-MS analysis of the prepared solution was carried out in the HP-5ms capillary column of the HP7890A gas chromatograph with a 5975 C VL MSD Triple-Axis Detector (Agilent Technologies, USA), operated in 1:20 split mode at 280 °C with a 1 mL min^{-1} constant helium flow rate. An Agilent 7693 A autosampler was used to inject a 1.00 µL sample. The initial operating temperature of the column had been fixed at 50 °C, and increased gradually by 3 °C per minute to 320 °C. The final temperature was maintained for a duration of 10 min.

The following acquisition parameters were established for the MSD detector: Transfer line, source, and quad temperatures – 280 °C, 230 °C, 150 °C, respectively. Ionization was done using 70 eV ionization energy with an ion source temperature of 230 °C to obtain the electron impact mass spectra (EIMS). For detection, a full scan mode was employed, covering the mass range from 41 to 650 atomic mass units (a.m.u.).

Using these conditions, the hexane solution containing C10-C40 n-alkanes was separated, and retention index values (RI^{Cal}) were calculated based on the mixture's separation results and the rhizome and seed extract solutions. Subsequently to integration, and the fraction of separated components in the total ion current (TIC) was calculated by applying the given below Eq. [55]:

$RI^{Cal}=100[n + (RT_x - RT_n)/(RT_{n+1} - RT_n)]$

Where, RT_x is the retention time of the analyte, RT_n is the retention time of n-alkane eluting directly before the analyte.

Assessment of biological activities of *Cautleya spicata*

Nematicidal activity

Collection of nematodes Cultured root-knot nematode [*Meloidogyne incognita* (Kofoid & White)] *eggs* were collected from infected tomato plants grown inside a glass house. The collected eggs were allowed to hatch at 27 ± 1 °C in a Patri-plate containing distilled water and a wired mesh cover with soft tissue. The hatched second-stage juveniles of nematodes travelled in the distilled water below mesh from soft tissue. The nematode solution was collected and diluted to prepare a stock solution containing ~ 100 nematodes per mL for further assays.

Preparation of extract emulsion A 1.0% aqueous Tween 20 solution was used to prepare an emulsion of diluted crude extracts in the respective solvent.

Initially, a primary Tween 20 aqueous stock solution of 2000 µg/mL concentration was prepared, which was further serially diluted to 1000–250 µg/mL using a Tween 20 surfactant solution.

Nematode *in vitro* mortality study Nematode in vitro mortality studies were conducted in accordance with the previously reported protocol[56] with a few minor modifications. In short, 1.00 mL of nematode solution (~100 J2 per mL) was put in the graded Petri plate containing 1.00 mL of double-strength extract emulsion to keep the final desired concentration of 250, 500, and 1000 µg/mL after diluting with nematode solution and incubated at 27 ± 1 °C.

The dead nematodes were observed and recorded for 96 h with every 24 h time interval at 4X magnification via a stereoscopic microscope (Model: C243606; Olympus). Each experiment was conducted and recorded in triplicates. The 1.0 mL J2 solution (~100 J2) containing the 1.0 mL Tween 20 aqueous solution (1.0%) without any extract was used as a negative control. The percent mortality against negative controls was determined using Abbott's formula[57].

$$Mortality\ (\%) = (Mt - Mc)/(100 - Mc) \times 100$$

For nematode mortality assay, LC_{50} values were computed from percent mortality data using probit analysis via OPSTAT, an online web portal for statistical analyses[58].

Hatchability testing of nematode eggs The egg hatching inhibition assay was conducted following a similar procedure as the nematode mortality assay, with a slight modification. Instead of using a solution containing J2 of nematode, a 1 mL solution containing approximately 100 nematode eggs was used. Petri plates containing the solutions were then placed in an incubator (temperature = 27 ± 1 °C). The 1.0 mL Tween 20 aqueous solution (1.0%) containing 1.0 mL nematode egg solution (~100 eggs) was served as control. All the treatments were performed in three replications. The hatching of the eggs was observed and recorded for 96 h with every 24 h time interval at 4X magnification via a stereoscopic microscope.

Percent inhibition in Egg hatching was calculated based on given formula:

$$Egg\ hatching\ inhibition\ (\%) = \frac{no.\ of\ eggs\ hatched\ in\ control - no.\ of\ egg\ hatched\ in\ the\ sample}{no.\ of\ eggs\ hatched\ in\ control} \times 100$$

Herbicidal assay The herbicidal potential of extracted essential oils was determined on the seeds of Radish (*Raphanus raphanistrum* sub sp. *sativus* L.) and Chicory (*Cichorium intybus* L.) in terms of rate of seed germination and growth of seedlings in a Petri dish bioassay[59,60].

The mature seeds of radish and chicory were obtained from the Vegetable Research Centre (VRC), G.B.P.U.A. & T., Pantnagar, Uttarakhand, India, and the ICAR-Directorate of Weed Research, Jabalpur, India, respectively. To determine herbicidal activity, ten weed seeds were placed in a 9 cm Petri plate between two sheets of filter paper. Afterward, 4 ml of extract at various concentrations (250, 500, and 1000 g/mL) were added to the Petri plate. The 1.0% aqueous solution of Tween 20 was taken as a control. The standard herbicide, pendimethalin was the positive control. The incubation temperatures for the treatment plates were 32 °C and 25 °C, with 12 h of darkness and 12 h of light, respectively. In triplicate, each treatment was carried out.

After five days of incubation, data on germination and the length of seedlings (root and shoot length) were recorded.

Percent inhibition in germination, root length growth, and shoot length growth were calculated by using the following formulae.

$$Germination\ percentage\ inhibition\ (GPI\%) = \frac{SG\ in\ control - SG\ in\ sample}{SG\ in\ control} \times 100$$

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

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

Where, SG is the number of seeds that germinated, SL is the growth of shoot length, and RL is the growth of root length.

Antibacterial bioassay

Spot on lawn antibacterial assay *Cautleya spicata* extracts were tested qualitatively against four bacterial strains including two Gram-positive bacterial pathogens i.e., *Listeria monocytogenes* and *Staphylococcus aureus* ATCC29213, and two Gram-negative bacterial pathogens i.e., *Escherichia coli* 25,922 and *Salmonella enterica serovar Typhi* using spot plate assay. Overnight grown cultures of bacteria were sub-cultured in Brain Heart Infusion (BHI) and Luria Bertani (LB) broth, respectively, until $OD_{600\ nm}$ reached 0.2. Each culture (100 µL) was separately swabbed on BHI and Muller Hinton (MH) agar plates. Then, 10 µL aerial part and rhizome plant extract solution were spotted onto BHI and MH agar plates and kept overnight at 37 °C. The antibacterial activity was assessed by measuring the inhibition zone diameter (cm) around each spot after incubation.

Minimum inhibitory concentration (MIC) determination The plant extracts (acetone and methanolic rhizome extract) that demonstrated antibacterial activity against bacterial pathogens in a spot-on lawn assay were then tested for minimum inhibitory concentration (MIC). The susceptibility of tested bacterial cells to plant extracts was determined using the micro broth dilution method as per Clinical and Laboratory Standards Institute (CLSI) guidelines[61]. In brief, overnight-grown bacterial cells were grown to mid-logarithm phase ($OD_{600\ nm}$

of 0.4) in BHI broth (for Gram-positive bacteria) and MH broth (for Gram-negative bacteria). Following that, each bacterial cell was diluted 1000 times to obtain an inoculum that contained 10^5 colony-forming units (CFU). The plant extracts (100 mg/mL) were diluted by 2-fold derail dilution in BHI and MH broth and mixed with an equal volume (100 µL:100 µL) of previously diluted bacterial cells to give a final volume of 200 µL. Untreated microorganisms in their respective broth served as a positive control, while the BHI/MH broth medium devoid of microorganisms served as a negative control. The 96-well plate was incubated for 14 h under a static condition at 37 °C. After the incubation period, plates were read at 600 nm using a microplate reader (Synergy-H1 microplate, BioTek USA). The lowest concentration of plant extract which inhibits 90% of the microbial growth compared with positive control growth in experimental conditions was considered as MIC and determined based on the plate readings. All the experiments were replicated thrice.

In -silico study Eleven major chemical components of the extracts were used for a molecular docking study with 5 and 4 putative target receptor proteins for establishing the mechanism of action for nematicidal and antibacterial activity, respectively.

Protein selection and preparation Five target protein receptors of *M. incognita* reported earlier i.e., cytochrome c oxidase subunit 1 (Cyt c), acetylcholinesterase (AChE), heat shock protein 90 (Hsp90), odorant response gene-3 (ODR3), and glutathione S transferase (GST-1) were used for in-silico study[62,63].

Cytochrome c oxidase subunit 1 is a vital component of the eukaryotic electron transport chain involved in ATP production in mitochondria. Acetylcholinesterase (AChE) is involved in the regulation of synaptic transmission and locomotion processes. Hsp90 must be fully functional to carry out its coordinating function in conjunction with other co-chaperones. It is essential for the stability and refolding of denatured proteins under stress as well as the folding of freshly produced proteins. ODR3 controls chemosensory processes.

Glutathione S-transferases (GSTs) are enzymes that detoxify various exogenous and endogenous toxic compounds and act as antioxidants. The protein sequence of selected protein was retrieved from the National Center for Biotechnology Information (NCBI) GenBank database[62,63]. 3D homology model of selected proteins was generated using SWISS-MODEL (https://swissmodel.expasy.org/). The modelled proteins were used for docking assay by using PyRx software.

Regarding the antibacterial activity of selected phytochemicals, phosphatidylinositol-specific phospholipase C from *L. monocytogenes* (PDB ID: 1AOD), tyrosyl-tRNA synthetase from *S. aureus* (PDB ID: 1JIJ), OmpA transmembrane domain from *E. coli* (PDB ID: 1BXW), and LuxS (5V2W), a lyase protein from *S. Typhi* were selected. These proteins were previously reported for in silico study of phytochemicals against these bacterial strains[64–68]. Protein crystal structures in three dimensions (3D) were downloaded in PDB format from the RCSB Protein Data Bank (https://www.rcsb.org/).

Ligand preparation For the docking studies, compounds were chosen from the extracts based on their higher percentage contents. The corresponding 3D structures of the ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the SDF (structure data file) format. The selected ligands were β-sitosterol (CID:222284), whisky lactone (CID:62900), ethyl palmitate (CID:12366), ethyl linoleate (CID:5282184), methyl palmitate (CID:8181), methyl linolenate (CID:5319706), methyl linoleate (CID:5284421), α-humulene (CID:5281520), stigmasterol (CID:5280794), phytyl palmitate (CID:6437053), and phytol (CID:5280435). The ligand structures (SDF format) were imported into PyRx Software using the embedded Open Babel tool within PyRx. Energy minimization, which involved charges addition and the universal force field optimization, was performed on ligands. Subsequently, ligands were changed into the AutoDock Ligand format (PDBQT) for further analysis.

Molecular docking

In silico study of the chosen ligands was conducted using PyRx software, specifically utilizing the Vina Wizard tool. To identify various ligand-protein interactions and determine the binding affinity for nematicidal and antibacterial activities, both the protein and multiple ligands were selected in PyRx using the Vina Wizard Control. The docking process was initiated by selecting the "Run Vina" control. The obtained results were analysed using the "Analyse Vina" tool[67].

For visualization purposes, Biovia Discovery Studio visualizer (v21.1.0.20298) was utilized to observe the interactions of the docking poses. For comparison purposes, the docking study was also performed with the known inhibitors available for the target proteins i.e., physostigmine CID: 5983 (for AChE), ethacrynic acid CID: 3278 (for GST-1), albendazole CID:2082 for (Cyt c), and geldanamycin CID: 5,288,382 (for hsp90). The standard antibacterial drug, Penicillin G (CID: 5904) was used to compare interactions of ligands with the bacterial proteins.

Statistics

All the experiments for lab study were carried out by following a completely randomized design (CRD) of the experiment. In vitro, bioassays were carried out in three duplicates. A two-factor analysis of variance (ANOVA) was performed using OriginPro 2023 (64-bit) 10.0.0.154 (students' trial version). Wherever required, data transformation was done using the square root transformation method. A significant difference between the treatment means was regarded as existing when the p-value was 0.05 or lower via Tukey's test. The chemometric analyses and Pearson's correlation coefficient were performed using OriginPro.

Data availability

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

Received: 21 November 2024; Accepted: 21 February 2025
Published online: 28 February 2025

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Acknowledgements
The authors acknowledge the Govind Ballabh Pant University of Agriculture and Technology for providing the necessary administrative and laboratory facilities for conducting the experiments.

Author contributions
S.K.M. performed experimental work, analysed data and prepared manuscript draft. H.K. conducted the molecular docking study. R.K and O.P. conceptualized and designed the experiments, contributed samples/reagents/materials, and contributed to manuscript editing. P.K. and S.C. designed the antibacterial activity assays and contributed to manuscript editing. S.K. and S.R. designed and provided facilities for the nematicidal activity experiments and contributed to manuscript editing. D.S.R. identified the plant material and contributed to manuscript editing. J.M. and V.A. I. performed the GC-MS analysis and analyzed the resulting data. P. Koli and Y. R. edited the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
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