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CHEMICAL SCIENCES

Chemical composition, pesticidal activities and *in-silico* investigation of *Hedychium spicatum* Sm. chloroform extract

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Abstract: The present study aimed to identify the bioactive constituents in the chloroform extract of *H. spicatum* rhizomes (HS-RCLE), further evaluated for its *in-vitro* pesticidal activities validating *via* molecular docking techniques. GC/MS analysis of HS-RCLE identified 14 compounds contributing 84.1 % of the total composition. The extract was dominated by oxygenated sesquiterpenes (43.1 %) with curcumenone (25.2 %) and coronarin *E* (14.8 %) as the major compounds. The extract recorded 89.4 % egg hatchability inhibition and 82.6 % immobility of *Meloidogyne incognita,* 66.7 % insecticidal activity on *Spodoptera litura*, 100 % phytotoxic activity on *Raphanus raphanistrum* seeds, and 74.7 % anti-fungal activity on *Curvularia lunata* at the respective highest dose studied. The biological activities were furthermore validated by using docking studies on certain proteins/enzymes namely acetylcholinesterase (PBD ID: IC2O), carboxylesterase (PDB ID: 1CI8), acetohydroxyacid synthase (PBD ID: 1YHZ) and trihydroxy naphthalene reductase (PBD ID: 3HNR). The bioactivity of the major constituents of the extract was predicted with the help of *in silico* PASS studies. HS-RCLE was observed to be a viable alternative source of natural pesticidal agents and paves the way for further studies on its mechanistic approaches and field trials to ascertain its pesticidal studies.

Key words: *Curvularia lunata, Hedychium spicatum, in silico, Meloidogyne incognita, Raphanus raphanistrum, Spodoptera litura.*

INTRODUCTION

Plants are an abundant source of bioactive compounds. Essential oils and extracts from these aromatic and herbal plants are considered promising bioactive metabolites because of their significant biological activities and chemical diversification (Zhelev et al. 2022). The *Hedychium* genus includes 98 species worldwide including India, China, Thailand, Indonesia, Laos, Vietnam, and Africa. The *Hedychium* genus is one of the largest genera of the family Zingiberaceae in the flora of India, where it is represented by 32 species out of which 18 are endemic (CoL 2021). The plants belonging to the *Hedychium* genus are well known for the presence of biological metabolites such as alkaloids, terpenoids, flavonoids, steroids, and iridoids (Rawat et al. 2019). Several traditional uses of *Hedychium* species worldwide have been documented, such as blood purification, stomachic and carminative properties, along with in the treatment of disorders like bronchitis, indigestion, eye disease, inflammations, and diarrhea (Gao et al. 2008, Jugran et al. 2011).

Hedychium spicatum Sm. belongs to the Zingiberaceae family and is a perennial herb with a strong camphoraceous odor. The species is widely native to the subtropical regions, like India, Bhutan, Nepal, Japan, Pakistan, China,

Myanmar, Thailand, Mauritius, Seychelles and Madagascar (Sirirugsa 1999, Chettri et al. 2008, Rawat et al. 2018). Due to wide traditional applications, various investigations on bioactive ingredients and pharmacological activities on *H. spicatum* have been reported. Modern pharmacological studies indicated that the herb exhibits diverse biological activities such as anti-inflammatory, anti-asthmatic, anti-allergic, analgesic, ulcer protection, hepatoprotective, antihyperglycemic, anticancer, and cytotoxic, tranquilizing, antioxidant, and antimicrobial properties (Dixit & Varma 1979, Joshi et al. 2008, Bisht et al. 2006, Reddy et al. 2009a, b, c, Prakash et al. 2010, 2016, Rawat et al. 2021, 2022).

The widespread use of chemical insecticides at ecotoxicological, environmental, and social levels has led to finding eco-friendly alternatives to synthetic chemicals in the reduction of pests. The use of plant extracts-based pesticides is attracting extensive attention from both farmers and agriculturists (Basile et al. 2022). Nevertheless, despite the growing interest in the search for natural products-based pesticides, many valuable plants, and their metabolites have not yet been explored; therefore, it is essential to conduct new studies on various wild species to evaluate their deterrent and pesticidal properties (Miresmailli et al. 2006, Dayan et al. 2009). In this regard, the use of bioactive metabolites from plants as natural pesticides is growing enormously, thanks to their high biodegradability and wide bioactivities. The metabolites present in the different herbal plants possess multiple properties due to single action or synergistic action on insect pests (Pathak et al. 2008). Although the mechanistic action is not perfectly known, several studies have shown that the greatest toxicity is caused by the interaction of the components with the nervous system of insects mediated by the inhibition of acetylcholinesterase or carboxylesterase (Ware & Whitacre 2004, Thapa et al. 2020).

A lot of documentation on the pharmacological properties of *H. spicatum* has been reported therefore to study the pesticidal potential activity of the chloroform extract of *H. spicatum* rhizomes the present study was designed. As part of continuous investigations on novel and bioactive compounds from *H. spicatum*, several studies on intriguing cytotoxic and anti-hyperglycemic activity have been reported in the labdane diterpenoids isolated from the chloroform extract of *H. spicatum* rhizomes (Reddy et al. 2009a, b, c). However, there is no information pertaining to its chemical composition. Thus, the present study was performed to investigate the chemical constituents in the chloroform extract from *H. spicatum* rhizomes and analysis of different pesticidal activities. The results were validated using molecular docking techniques. The identified constituents were tested for different biological activities using *in-silico* PASS studies.

MATERIALS AND METHODS

Plant material and extract preparation

The rhizomes (about 1 kg) of *H. spicatum* were collected from the Tarai region of Pantnagar (29° 02' 12'' N latitude, 79° 47' 21'' E longitude, altitude 243 m), Uttarakhand in July 2019. The species was identified by Dr. D.S. Rawat, Assistant Professor/ Plant Taxonomist, College of Basic Sciences, Department of Biological Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (Voucher Specimen No.: GBPUH-986). The rhizomes were cleaned in running water to remove unwanted debris and soil, shade-dried for almost 20 days, and ground by an electric grinder (Dolatabadi et al. 2011). A total of 760 g of ground powder was soaked in 2000 mL chloroform for 24 h with

sporadic shaking. The soaked plant materials were filtered through a Whatman filter paper (grade No: 42). The process was repeated three times and the combined extracts were concentrated under a vacuum.

GC/MS analysis

To determine the bioactive components, the prepared chloroform extract was analyzed by GC/MS using a Perkin Elmer gas chromatograph model GC Clarus SQ 8C coupled with a single quadrupole mass spectrometer model MS SQ8. The column conditions were as follows: PE-5 capillary column, 30 m × 0.25 mm I.D × 0.25 µm, working in electron influence method at 60 eV. A fixed stream of 1.32 mL/min and an additional volume of 1 µL of helium gas was used as carrier gas. The injection volume was 0.02 µL with a split ratio of 1:30. The ion source and injector temperatures were adjusted to 210 °C and 250 °C, respectively. The control of the oven temperature was as follows: First, the oven temperature started from 60 °C with a rise of 20 °C/min to 310 °C/min and then finished with isotherm for 10 min at 310 °C. MS spectra were recorded at 60 eV, with a scan value of 30-1100 m/z. The results obtained were compared with those of the spectral data obtained from the Wiley Library (Adams 2007).

Evaluation of nematicidal activity

Isolation, extraction, and identification of nematodes

Roots of tomato plants infected with root-knot nematodes (*Meloidogyne incognita*) were collected from the cultivated experimental fields at the Vegetable Research Centre, GBPUA&T, Pantnagar. Roots infested with root-knot nematodes were divided into small segments and inserted in a bottle containing 1.0 % NaOCl suspension. The bottle was hand-shaken for 5.0 min and suspension was poured through the sieve. After washing with tap water for 1 min, the residue was collected from top to bottom sieves 100-mesh and then 400-mesh and transferred into the 250 mL beaker. The suspension of the solution was observed with the help of a counting chamber prepared in a number of eggs/juveniles per mL (Hussey & Barker 1973). The species was identified by careful study of female perineal patterns.

Hatching and mortality test

A 100 mL suspension of eggs containing 50 eggs per mL was prepared in distilled water from the fresh roots of the tomato plant infected with root-knot nematodes (*M. incognita*). Five mL of egg suspension (50 eggs/mL) with 1.0 mL of each concentration at 0.25, 0.5, and 1.0 µL/mL of HS-RCLE was shifted into blocks of cavity glass (2.5 cm diameter) individually in triplicates. The data was recorded for 24-, 48-, 72- and 96-h respectively. The blocks of cavity glass containing 2.0 mL of egg suspension with 1.0 mL water were placed in control (Manilal et al. 2009). After the 96-h exposure, the number of eggs hatched was counted under a stereoscopic optical microscope (Olympus CX3) microscope (x40). The activity of HS-RCLE and the effect of concentrations and time interval were observed as the percentage (mean %) of the egg hatchability inhibition.

For mortality rate, eggs of *M. incognita* were placed in distilled water and were actively resumed at room temperature (26±2 °C) for 24 h. A solution of freshly hatched juveniles (J_2) was prepared in deionized water containing (50 J_2/mL). 2.0 mL of hatched juvenile's suspension and 1.0 mL of each concentration (0.25, 0.5, and 1.0 µL/mL) of HS-RCLE were placed in the block of glass cavity (diameter 2.5 cm) and placed at lab temperature. The experiment was repeated in triplicates. The block of glass cavity

containing 1.0 mL nematode solution and 1.0 mL of deionized water was treated as a control. After 72 h of exposure, the number of dead juveniles was calculated under a light stereo-binocular microscope (Olympus CX3) (x6). The immobilization of J_2 nematode larvae against HS-RCLE was calculated as the percentage (mean %) of deceased nematodes. The persistence of immobility even after their immersion in water was assumed to confirm nematode mortality (Cayrol et al. 1989).

Evaluation of insecticidal activity

Insects

Spodoptera litura eggs lying on castor leaves were collected from Crop Research Centre, Pantnagar, Uttarakhand, and were confirmed by Dr. R.M. Srivastava (College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand). The eggs were artificially subcultured and purified for 2 to 3 generations in a dark incubator at 28-30 °C, with relative humidity maintained at 70-80 %. Newborn larvae hatched from eggs were maintained in sterile glass chambers (20 cm × 15 cm × 6 cm) and fed with a fresh artificial diet (120 g soybean powder, 96 g wheat germ, 40 g yeast powder, 32 g agar, 16 g casein, 9.6 g ascorbic acid, 6.0 g potassium sorbate, 2.0 g methylparaben,1.2 g choline chloride, 0.4 g cholesterol, 0.24 g inositol, 0.08 g vitamin B complex, and 1.280 L H_2O). The larvae were individually transferred to sterile glass tubes (10 cm high and 2 cm diameter) after 5 days, fed on a fresh artificial diet, and raised at room temperature (28-30 °C) until to be pupated. After transformation from the pupal stage, male and female adults were paired and reared with honey water (15 %, w/v) in new containers (40 cm × 30 cm × 10 cm). The eggs of mated adults were collected on oiled papers that had been placed in the containers. The eggs were treated again to produce the subsequent generation of larvae. The rearing conditions were maintained with a photoperiod of 14 L:10D h, at 27 ± 0.5 °C and relative humidity (RH) of 75 ± 5 %. Third-instar larvae were used for this study (El-Aswad et al. 2003).

Insecticidal activity via contact activity

The drip method was used for the process of contact activity. From the raised adults, 5.0 healthy adults with good activity and consistent growth were selected (regardless of gender). They were placed in an activity test glass bottle (5.5 cm high, 2.5 cm in diameter). The extracts were dissolved in 1.0 % tween 20 water solution to prepare a serial testing solution, with 1.0 % tween 20 water solution as the negative control. According to the results of preliminary experiments, four concentrations of the extract (10 to 50 µL/mL) were determined in formal experiments. Each treatment and control of different concentrations was replicated five times. The death/survival of the test insects was observed and recorded 24 h later, and abnormal activity of the insects was regarded as death (El-Aswad et al. 2003).

Phytotoxic activity

Fresh fungal-treated seeds of *Raphanus raphanistrum* var *sativus* (radish) purchased from collected from Vegetable Research Centre, Pantnagar, Uttarakhand was used to investigate the phytotoxic effect exhibited by the EO. Seeds were stored in paper bags for a span of four weeks at room temperature. The viability of the seeds and their germination ability were checked prior to the experiments. Surfaces of seeds were sterilized through a two-step procedure (rinse for 30 s with 70 % ethyl-alcohol and subsequent treatment for 20 min with 10 % sodium hypochlorite solution), then washed three times with sterile distilled water, and finally, air-dried

in aseptic conditions under a laminar hood. Ten seeds of the weed were placed in Petri dishes surfaced with two layers of filter paper (Whatman No. 2). To make exact concentrations of extract in water (250, 500, 750, 1000 µL/mL), first a stock of extract in dimethyl sulfoxide (DMSO)/water (1.0 %, v/v) was prepared. Ultimately, 10 mL of each concentration was poured into the Petri dishes. In the controls, 1.0 % DMSO in water was used. Each treatment had five replicates, and all the experiments were replicated twice. The Petri dishes containing seeds were sealed by plastic paraffin film tape. Then, Petri dishes were kept in a germinator set at 25 °C with a 16-h photoperiod. In this experiment, germination percentage along with root and shoot lengths were measured (Cutler et al. 2002).

Antifungal activity

Fungal isolates

Post-harvest fungal pathogen isolate of *Curvularia lunata* was obtained from the fungal culture collection of the Department of Plant Pathology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The pathogenicity of fungal isolates was confirmed. Fungal isolates were grown in potato dextrose agar (PDA) medium at 25 ± 2 °C. Only actively growing colonies were used in bioassays.

In-vitro mycelial growth inhibition

The antifungal activity of HS-RCLE was evaluated through the poison food medium method. Different concentrations of HS-RCLE (50, 100, 250, 500 µL/mL) separately were prepared in Tween 20 distilled water solution (1.0 %, v/v) and aseptically added to sterile, cooled, molten potato dextrose agar (PDA Merck, Darmstadt, Germany) medium (45 °C). The resulting mixture was instantly dispensed onto sterilized glass Petri plates (90 mm diameter, 20 mL each) and allowed to solidify under aseptic conditions. A mycelial disk (6 mm diameter) of the tested fungi, taken from the margins of the actively growing cultures, was placed upside-down at the center of the Petri plates. Inoculated Petri plates were incubated in darkness at 25 ± 2 °C. Tween 20 distilled water solution (1.0 %, v/v) was used as a control. Each treatment in the experiment was used as triplicates (Cakir et al. 2004). The antifungal activity of the extract was measured considering the percentage of mycelial growth inhibition, calculated as per the formula:

% Mycelial growth inhibition = (dc - dt) dc × 100

where dc was the colony growth diameter in the control;

dt represented the diameter of colony growth in the treatment

Molecular docking studies

All the pesticidal activities were validated using molecular docking techniques. The X-ray crystal structure of acetylcholinesterase enzyme (PDB ID: IC2O), carboxylesterase; CaE (PDB: 1CI8), acetohydroxyacid synthase, AHAS (PDB: 1YHZ) and melanin biosynthesizing enzyme trihydroxy naphthalene reductase (PDB: 3HNR) was downloaded from RCSB protein data bank. The molecular docking studies of the major constituents on these proteins were performed using AutoDock4.2 with Discovery Studio and Cygwin64 Terminal tool to find out the binding energy, visualization of docking poses, and know the various ligand-target receptor interactions responsible for the pesticidal activity of HS-RCLE (Anza et al. 2021).

Acetylcholinesterase (AChE), (PDB ID: 1C2O) the target for the action of organophosphates and carbamate pesticides terminates nerve impulses by hydrolyzing the neurotransmitter acetylcholine (ACh) to acetic acid and choline

at the synapses and neuromuscular junction in most vertebrates, insects, and nematodes. Thus, the inhibition of AChE leads to the dysfunction of the nervous system and the death of the nematodes (Andrade-Jorge et al. 2021).

Certain compounds extracted from plants have the ability to affect the enzymatic profile of insect pests. For instance, among them, proteinaceous inhibitors have the ability to inhibit proteolytic activity and lead to disturbed growth and development. 3-D structure of the protein-ligand could serve as a new way to predict the toxic effects of chemical constituents of oils on *S. litura* and its binding affinity with detoxifying enzyme carboxylesterase (CaE) (PDB ID: 1CI8) found in the head capsule of *Spodoptera litura* larvae (Badawy et al. 2022).

Acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS), (PDB ID: 1YHZ) is the target of numerous commercial herbicides (apply to rice, corn, wheat, and cotton crops). Pesticides as AHAS inhibitors have three features: low application rates, high crop selectivity, and low toxicity in animals. In plants, AHAS inhibitor has an indirect effect on protein synthesis by suppressing the generation of branched-chain amino acids (isoleucine, leucine, and valine, also called BCAAs), and the root cause is AHAS enzyme did not complete the conversion either nor 2-ketobutyrate and pyruvate. (Wu et al. 2021).

Thefungalenzyme1,3,8-trihydroxynaphthalene reductase (PDB ID: 3HNR) found in the cell wall of *Curvularia lunata* is a key enzyme involved in melanin biosynthesis, that plays a crucial role in the process of fungi invasion. This enzyme is the target of some chemical fungicides, but the problem of resistance against these molecules requires the search for new molecules that are both effective and environment-friendly (Aamir et al. 2018).

In-silico PASS prediction study

The online application PASS (http://www.pharmaexpert.ru/passonline/) was used to evaluate the pesticidal activity spectra of selected major components of HS-RCLE. The structures of the components were downloaded from PubChem and then converted to SMILES format using SwissADME online tools (www.swissadme.ch), which are capable of generating pesticidal spectra using the PASS server. This server can predict > 4000 types of pesticidal function, together with drug and non-drug activity, suggesting the best potential drug-like compounds with 90 % validity. PASS calculation outcomes are expressed as Pa (probability of active molecule) and Pi (probability of inactive molecule). Pa and Pi scores range from 0.00 to 1.00, and usually, Pa + Pi = 1, since these probabilities are calculated independently. Biological activities for which Pa > Pi are considered probable only for a selected drug molecule (Islam et al. 2021).

Statistical analysis

Experimental results were the means ± standard deviation of three parallel measurements. The mean values and standard deviation were calculated statistically. The experiment of nematicidal activity, insecticidal activity, herbicidal activity, and antifungal activity was arranged in a Completely Randomized Design with three replicates for three to five concentrations in all the samples. Raw data were analyzed using 2-factor and 3-factor CRD (ANOVA); the mean values and standard deviation (SD) were calculated by Statistical Analysis. Percentage data were subjected to angular transformation (Snedecor & Cochran 1968).

RESULTS

Chemical composition of HS-RCLE

The yield (%, v/m) of the chloroform extract of *H. spicatum* rhizomes was 0.041 %. The chloroform extract of *H. spicatum* rhizomes analyzed by GC/MS was abbreviated as HS-RCLE (*H. spicatum* rhizome chloroform extract). Over 20 compounds out of which 14 constituents contributed 84.1 % of the total chloroform extract composition could be identified. Among the identified constituents, curcumenone (25.2 %) was the major component followed by coronarin *E* (14.8 %), α-selinene (8.4 %), germacrene-*D* (6.9 %), curzerene (5.3 %), *trans*-bergamotol (4.8 %), linderazulene (4.4 %), valerenic acid (3.8 %) and isovelleral (2.6 %) dominated the extract composition. The oxygenated sesquiterpenes were present in the highest amount accounting for 43.1 % of the extract followed by hydrogenated sesquiterpene (16.6 %) and oxygenated diterpene (14.8 %). 9.6 % of non-terpene compounds including n-alkyl hydrocarbon (1.7 %), saturated fatty acid (2.4 %), psoralen (1.1 %), and azulenoid (4.4 %) were also identified in the extract. The detailed qualitative and quantitative chemical composition of HS-RCLE is tabulated in table I. The GC chromatogram is given in fig. 1(a) while structures of the major compounds identified are given in fig. 1(b).

In-vitro nematicidal activity

Nematicidal activity of the used concentrations of *H. spicatum* extract comparable to untreated control (water) on the egg hatchability and larval immobilization of *M. incognita* was investigated. The results revealed a significant ($p < 0.05$) inhibitory effect on egg mass hatchability after 24-, 48- and 72-h exposure to treatments. Results in table II show that HS-RCLE had an inhibitory effect on the egg hatchability of *M. incognita* with % inhibition from 58.2 % to 89.4 % as a function of selected dose levels from 0.25 µL/mL to 1.0 µL/mL respectively. HS-RCLE after 36 h was found to show IC_{50} values of 2.5±1.0 µL/mL. HS-RCLE was found to be highly active against *M. incognita* (J_2). The % mortality was observed from 21.5 % to 82.6 % when the dosage was increased from 0.25 µL/mL to 1.0 µL/mL respectively with LC_{50} values of 1.5±0.4 µL/mL. No nematodes died in blank solvent and distilled water (Table III). Synthetic chemicals reportedly show negative ecological impacts therefore HS-RCLE may prove to be an environmentally benign alternative to destroy the growth of nematodes. To the best of my knowledge, the nematicidal activity of HS-RCLE has not been reported.

Molecular docking studies were also performed using acetylcholinesterase enzyme (PDB ID: IC2O) to corroborate the experimental results of the nematicidal activity. Acetylcholinesterase (AChE), (PDB ID: 1C2O) is considered the potential target for the action of organophosphates and carbamate pesticides terminating the nerve impulses by hydrolyzing the neurotransmitter acetylcholine (ACh) to acetic acid and choline at the synapses and neuromuscular junction in most vertebrates, insects, and nematodes. Thus, the inhibition of AChE leads to the dysfunction of the nervous system and the death of the nematodes (Andrade-Jorge et al. 2021)

The binding energies of the major constituents of HS-RCLE were found to be in the range of -5.51 to -8.79 kcal/mol indicating moderate to good inhibition of the enzyme (Table IV). Curcumenone strongly bonded with Leu528, Trp524, Pro403, Cys402, Asn230, Glu306, Asp397, Asn525, and His362 amino acid residues with van der Waals forces, His398, Pro232 with pi-alkyl whereas His406 with pi-sigma interactions using a binding energy of -8.04 kcal/mol. Coronarin *E* strongly formed van der Waals interactions with Tyr334, Phe330, His440, Gly118, Ser122, Asn85, Tyr121, Asp72, Ser81, Tyr442

Table I. Chemical composition of HS-RCLE.

S.N.	Compound	Class	Formula	K.I.	% composition	Method of identification (M.F.P.)
1.	dodecane	n-alkyl hydrocarbon	$C_{12}H_{26}$	939	1.7	M^+ = 170, m/z; 141, 127, 113, 98, 85, 71, 57 (100%), 43, 41
2.	δ-elemene	HS	$C_{15}H_{24}$	1002	1.3	M^+ = 204, m/z; 189,176,161,148,136, 121(100%),105,93,77,67,53,41
3.	**trans-bergamotol**	**OS**	**$C_{15}H_{24}O$**	**1031**	**4.8**	**M^+ = 220, m/z; 202,187,173,159,145,132,119,107,93(100%),79,68,41**
4.	**germacrene-*D***	**HS**	**$C_{15}H_{24}$**	**1091**	**6.9**	**M^+ = 204, m/z; 189,161(100%),147, 133,119,105,91,79,67,55,41**
5.	**curzerene**	**OS**	**$C_{15}H_{20}O$**	**1146**	**5.3**	**M^+ = 216, m/z; 201,187,173,159,148,133,119,108(100%),91,79,53,41**
6.	**α-selinene**	**HS**	**$C_{15}H_{24}$**	**1149**	**8.4**	**M^+ = 204, m/z; 189(100%),175,161,147,133,121,107,93,81,67,55**
7.	**furanodiene**	**OS**	**$C_{15}H_{20}O$**	**1159**	**1.4**	**M^+ = 216, m/z; 203,175,161,150,135,121,107(100%),93,77,67,53,41**
8.	isovelleral	OS	$C_{15}H_{20}O_2$	1160	2.6	M^+ = 232, m/z; 204,189,175,161,147,133,119,105,91(100%),77,69, 55,41
9.	**curcumenone**	**OS**	**$C_{15}H_{22}O_2$**	**1169**	**25.2**	**M^+ = 234, m/z; 219,191,176,161,149,133,121,107,91,79, 68 (100%), 43**
10.	n-hexadecanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	1188	2.4	M^+ = 256, m/z; 213,199,185,171,157,143,129,115,98,85,73, 60(100%),43
11.	valerenic acid	OS	$C_{15}H_{22}O_2$	1208	3.8	M^+ = 234, m/z; 219,189,173,161,147,133,122,107,91(100%),79,65, 55,41
12.	**linderazulene**	**azulenoid**	**$C_{15}H_{14}O$**	**1216**	**4.4**	**M^+ = 210, m/z; 209(100%),195,178, 165, 152, 139, 115, 105, 89, 76, 63, 51, 28**
13.	**coronarin E**	**OD**	**$C_{20}H_{28}O$**	**1243**	**14.8**	**M^+ = 284, m/z; 269,227,213,199, 187, 160, 147 (100%), 131, 115, 105, 91, 81, 69, 55, 41**
14.	trioxsalene	psoralen	$C_{14}H_{12}O_3$	1338	1.1	M^+ =228, m/z; 228 (100%),213, 199, 185, 171, 155, 141, 128, 115, 102, 91, 77, 67, 51, 39
Hydrogenated Sesquiterpene					16.6	
Oxygenated Sesquiterpene					43.1	
Sesquiterpene					**59.7**	
Oxygenated Diterpene					14.8	
Diterpene					**14.8**	
n-alkyl hydrocarbon					1.7	
Saturated fatty acid					2.4	
Psoralen					1.1	
Azulenoid					4.4	
Others					**9.6**	
Total					**84.1**	

HS-RCLE: H. spicatum rhizome chloroform extract, K.I.: Kovatt indices, M.F.P.: Mass fragmentation pattern, HS: Hydrogenated sesquiterpene, OS: Oxygenated sesquiterpene, OD: Oxygenated diterpene.

amino acid residues, Ile439 formed pi-alkyl interactions while Trp432, Trp84 formed pi-pi stacked interactions using effective binding energy of -8.77 kcal/mol. α-selinene had shown binding energy of -8.51 kcal/mol with target amino acid residues Tyr442, His440, Ile439, Ser81, Gly80, Tyr334, Asp72, Asn85 forming van der Waals interactions, Trp84, Phe330 with pi-alkyl interactions and Trp432 with pi-sigma interactions. Germacrene-*D* had shown binding energy of -5.51 kcal/mol with target amino acid residues Asn525, His406, Asn230, Cys402, Pro232, His398, Trp524, His362, Leu528, Pro529 forming van der Waals interactions and Pro403 as pi-alkyl interactions. Standard drug carbofuran was observed to show binding energy of -6.45 kcal/mol showing van der Waals interactions with Phe290, Tyr121, His440, Gly119, Phe331, Gly118, Ser200, Gly117, Tyr130, pi-pi stacked interactions with Trp84, pi-anion bonding with Glu199 whereas pi-sigma interactions with Phe330. Carbofuran was observed to show binding interactions with many amino acids as compared to the tested ligands. Curcumenone, coronarin *E,* and α-selinene identified in HS-RCLE showed greater binding energy as compared to the standard drug (Fig. 2). Further studies are needed to evaluate the safety of the extract for humans, after proper clinical trials.

In-vitro insecticidal activity

The maximum insect mortality up to 66.7 % was observed in HS-RCLE at 50 µL/mL dose level against *S. litura*. The detailed results are given in table V. The insecticidal activity in terms of LC_{50} values was observed to be 52.7 µL/mL. No reports exist on the insecticidal activity of HS-RCLE in the literature survey. The results are in agreement with the studies of previous researchers. These findings suggest that the extract has the potential for the development of novel insecticidal compounds for the control of insects and stored pests.

Molecular docking analysis for the major components of HS-RCLE against carboxylesterase; CaE (PDB: 1CI8) found in the head capsule of *S. litura* larvae was performed using AutoDock4.2. Curcumenone showed residual interactions with atoms of amino acid residues namely Gly118, Ser200, His440, Gly441, Tyr121, Phe290, and Glu199 forming van der Waals interactions, Trp84, Tyr442, Phe331 forming pi-alkyl contacts and Phe330, Tyr334 forming pi-sigma bonding with an average binding

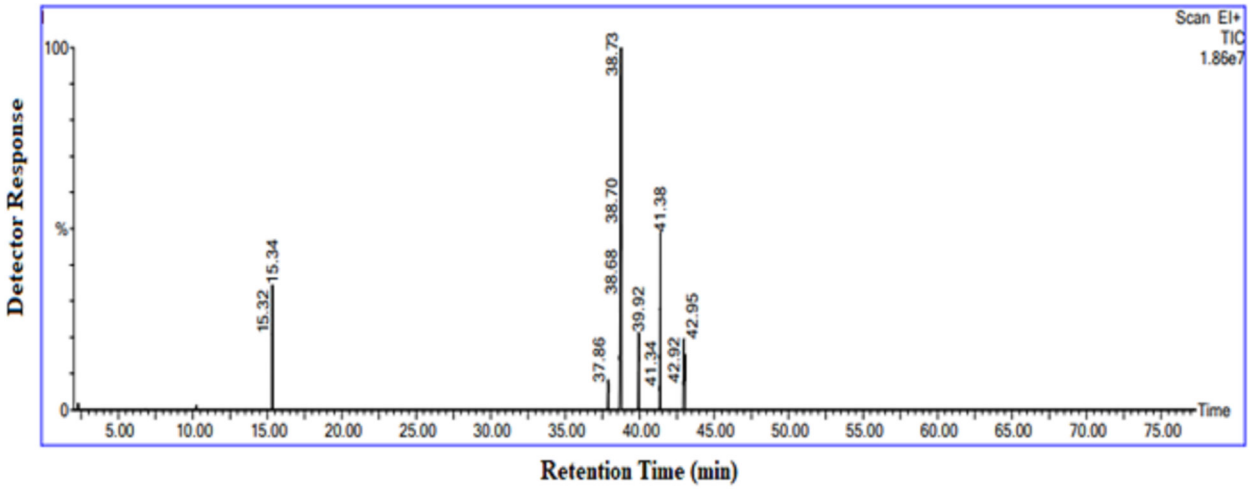

Figure 1(a). Ion chromatogram of HS-RCLE.

energy of length -6.55 kcal/mol. Coronarin *E* had shown binding energy of -8.52 kcal/mol with target amino acid residues Tyr121, Tyr70, Ser286, Arg289, Phe288, and Phe290 forming van der Waals interactions, Trp279, Phe331, Phe330, Ile287forming pi-alkyl contacts, and Phe330 as pi-pi T shaped interactions. In α-selinene amino acid residues Trp84, Ser81, Asn85, Asp72, and Tyr121 form van der Waals interactions while Phe330 and Tyr334 form pi-alkyl contacts with a binding energy of -7.63 kcal/mol. Germacrene-*D* had shown binding free energy of -7.45 kcal/mol, an O atom of the hydroxyl group at C-3 position showed H-bonding with O atom and N-atom of amino acid residue Tyr121, Phe330, Ile287, Phe288, Arg289 and Trp279 forming van der Waals interactions while Tyr334 and Phe331 forming pi-alkyl contacts (Table VI). Standard drug permethrin was observed to show binding energy of -8.72 kcal/mol showing van der Waals interactions with Gly117, Phe330, Phe331, Tyr130, Phe290, Phe288, Arg289, Gly123, Ser122, Gly118, Gly119, pi alkyl bonding with Tyr70, pi-pi stacked interactions with Trp84 whereas pi-sigma interactions with Trp279. The hydrogen bonds formed, and hydrophobic and ion pair interactions observed between the ligand and active site residues of the target are seen to play a key role in the accommodation of the small molecule into the catalytic domain of the target protein. Also, permethrin was observed to show binding interactions with many amino acids as compared to the tested ligands. No compounds showed greater binding energy as compared to the standard drug. The 2-dimensional and 3-dimensional binding interaction between HS-RCLE and carboxylesterase enzyme is presented in fig. 3.

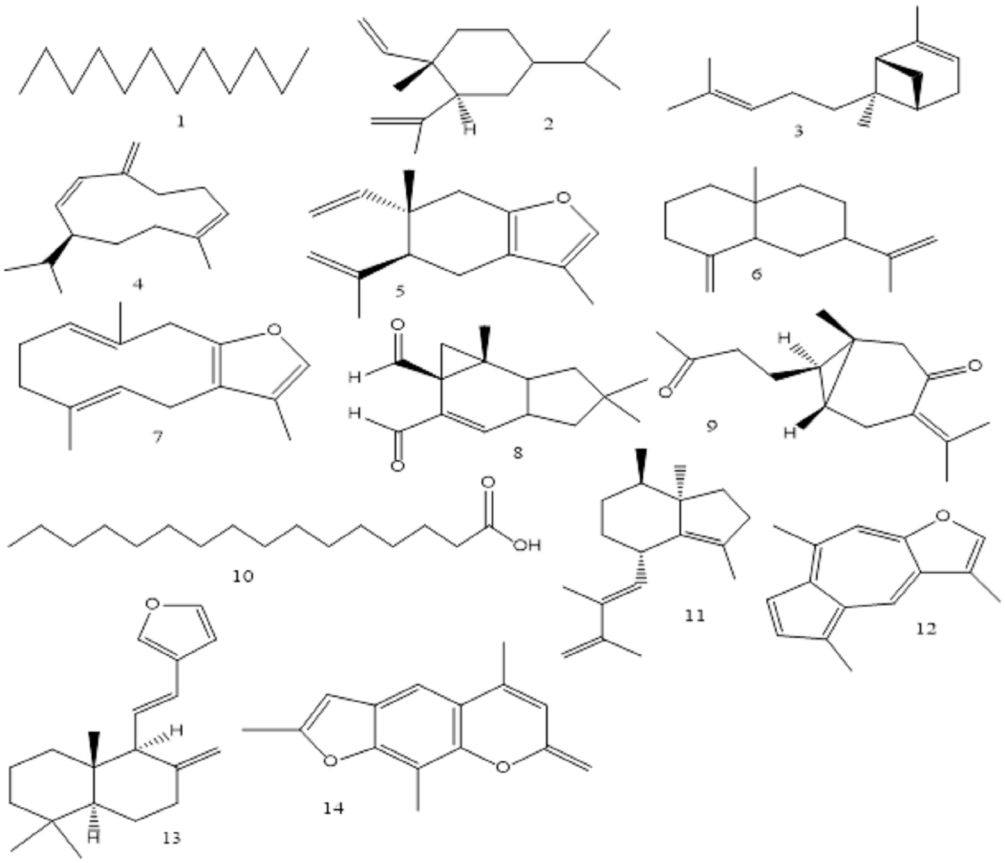

Figure 1(b). Structure of compounds in HS-RCLE. 1: dodecane; 2: δ-elemene; 3: trans-bergamotol; 4: germacrene-D; 5: curzerene; 6: α-selinene; 7: furanodiene; 8: isovelleral; 9: curcumenone; 10: n-hexadecanoic acid; 11: valerenic acid; 12: linderazulene; 13: coronarin E; 14: trioxsalene.

Table II. % egg hatchability inhibition in HS-RCLE against *M. incognita.*

Dose (µL/mL)	Number of eggs hatched after different time interval				Mean egg hatchability	% egg hatchability inhibition
	24 h	48 h	72 h	96 h		
0.25	49.0	51.3	76.0	87.0	65.8±18.6	**58.2**
0.50	32.3	36.6	44.0	51.6	41.2±8.4	**73.8**
1.00	8.0	12.6	19.6	26.0	16.6±7.8	**89.4**
Control	106.0	143.0	173.6	207.6	157.5±43.3	**0.0**
Carbofuran	0.0	0.0	0.0	0.0	0.0	**100.0**
S.E.M	0.5	0.5	0.4	1.1		
C.D. 1%	1.6	1.4	1.2	3.2		
C.D. 5%	15.7					
C.V.	**16.1**					

HS-RCLE: ***H. spicatum*** **rhizomes chloroform extract, S.E.M.: Standard error mean, C.D.: Critical Difference, C.V.: Coefficient of Variance.**

Table III. % immobility of *M. incognita* (J_2) against HS-RCLE.

Dose (µL/mL)	Number of larvae immobilized in different intervals of time			Mean larval immobilization	% larval immobility
	24 h	48 h	72 h		
0.25	9.0	19.3	21.0	16.4±6.5	**21.5**
0.50	19.3	32.6	36.3	29.4±8.9	**33.8**
1.00	33.0	44.3	49.0	42.1±8.2	**82.6**
Control	2.0	2.6	5.6	3.4±1.9	**0.0**
S.E.M	1.5	1.6	2.7		
C.D. 1%	6.4	6.2	11.1		
C.D. 5%	4.7	4.7	8.1		
C.V.	**16.1**				

HS-RCLE: ***H. spicatum*** **rhizomes chloroform extract, S.E.M.: Standard error mean, C.D.: Critical Difference, C.V.: Coefficient of Variance.**

Table IV. Docking results of major compounds in HS-RCLE with target IC2O by AutoDock 4.2.

Compounds/Ligand (PubChem SID)	ΔG_b	Ki	I.E.	B.E.	R.M.S.D.	Residual Interactions		
						Van der Waals	Pi-alkyl	Pi-sigma
curcumenone (153845)	-7.19	5.36	-8.09	-8.04	79.32	Leu528, Trp524, Pro403, Cys402, Asn230, Glu306, Asp397, Asn525, His362	His398, Pro232	His406
coronarin *E* (9971144)	-8.19	984.14	-8.79	-8.77	77.43	Tyr334, Phe330, His440, Gly118, Ser122, Asn85, Tyr121, Asp72, Ser81, Tyr442	Ile439	Trp432, Trp84 (both pi-pi stacked)
α-selinene (10856614)	-8.23	930.45	-8.53	-8.51	95.86	Tyr442, His440, Ile439, Ser81, Gly80, Tyr334, Asp72, Asn85	Trp84, Phe330	Trp432
germacrene-*D* (5317570)	-5.23	147.75	-5.52	-5.51	70.13	Asn525, His406, Asn230, Cys402, Pro232, His398, Trp524, His362, Leu528, Pro529	Pro403	-
carbofuran (2566)	-5.96	43.02	-6.55	-6.45	93.06	Phe290, Tyr121, His440, Gly119, Phe331, Gly118, Ser200, Gly117, Tyr130	Trp84, Glu199 (pi anion)	Phe330

IC2O: PBD ID for crystal structure of enzyme acetylcholinesterase from the gut of *Meloidogyne incognita* larvae, ΔG_b: Free energy of binding (kcal/mol), Ki: Estimated inhibition constant (µM) at temperature 298.15 K, I.E.: Final intermolecular energy (kcal/mol), B.E.: Binding energy (kcal/mol), Residual Interactions: H-bonds + Hydrophobic, R.M.S.D.: Root mean square distance.

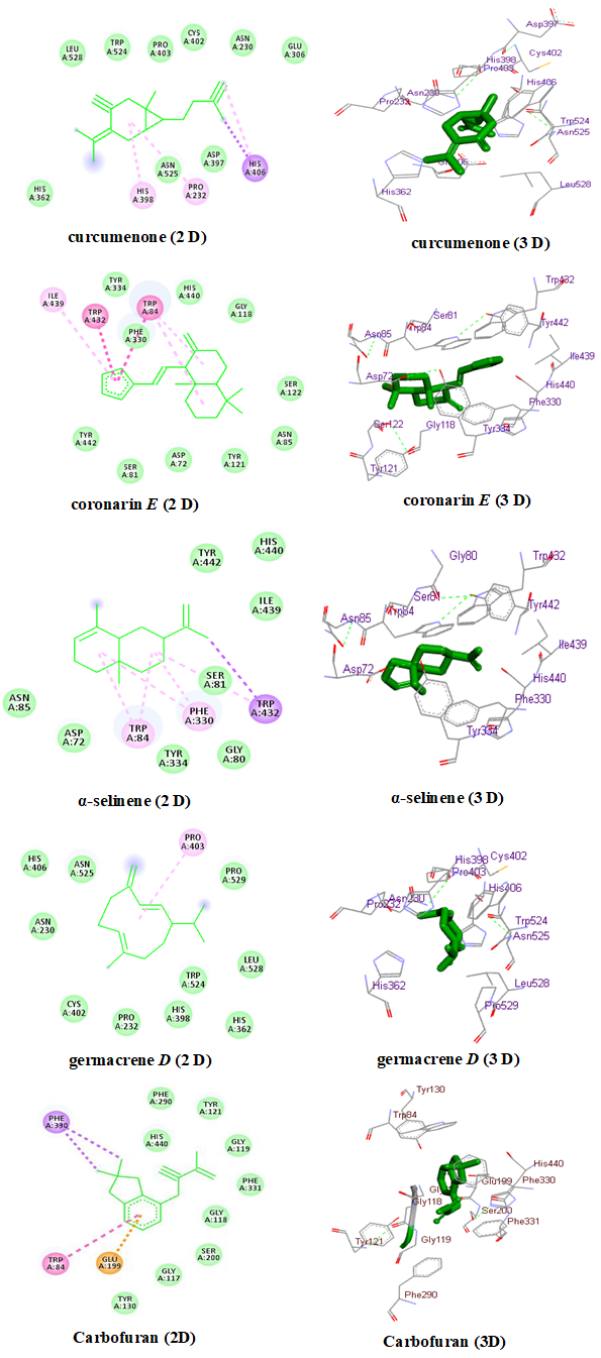

Figure 2. 2D and 3D interactions of major compounds in HS-RCLE with target IC2O. IC2O: PBD ID for crystal structure of enzyme acetylcholinesterase from the gut of *Meloidogyne incognita* larvae, amino acid residues in green rings are showing van der Waals interactions, amino acid residues in pink rings are showing pi-alkyl interactions, amino acid residues in purple rings are showing pi-sigma interactions, amino acids in red rings are showing unfavorable bumps.

In-vitro phytotoxic activity

The effect of HS-RCLE on weed germination indices is presented in table VII. The final germination percentage varied significantly ($p < 0.01$) among the different extract concentrations used. HS-RCLE was found to be highly active on *R. raphanistrum* with the % herbicidal inhibition from 80 % to 100 % as a function of dosage (50 µL/mL to 200 µL/mL). The IC_{50} value for herbicidal activity was observed to be 53.2±1.7 µL/mL.

HS-RCLE exhibited substantial herbicidal activity against the germination, seedling root, and shoot growth of *R. raphanistrum* in a dose-dependent manner. Statistically significant differences ($p < 0.01$) among treatments were also observed in the seedling length of the weeds. It was clearly observed that the inhibition in seedling growth was more than germination. The % root length inhibition was observed to be 97.9 % at 50 µL/mL and 100.0 % at 200 µL/mL for HS-RCLE. The % shoot growth inhibition was observed to be 98.4 % at 50 µL/mL and 100.0 % at 200 µL/mL for HS-RCLE. The detailed results pertaining to root and shoot length are mentioned in table VII.

The herbicidal activities shown by HS-RCLE were then validated using molecular docking software. Energetically favorable docking predictions (*i.e.*, those with calculated negative values for binding free energy) were analyzed to assess the binding interactions between the residues in the protein models of acetohydroxyacid synthase, AHAS (PDB: 1YHZ). Curcumenone showed residual interactions with atoms of amino acid residues namely Phe330, Phe290, Asp72, Phe288, Arg289, Leu282, Ile287, and Ser286 formed van der Waals interactions, while Tyr121, Trp279, Tyr70, and Tyr334 forming pi-alkyl contacts with an average binding energy of length -8.24 kcal/mol. Coronarin *E* had shown binding energy of -8.40 kcal/mol with target amino acid residues Asn525, His398, Trp524,

Table V. % mortality of *S. litura* against HS-RCLE in laboratory conditions.

Dose (µL/mL)	Mortality of insects at different time intervals			Mean insect mortality	% mortality
	12 h	24 h	36 h		
10	5.0	5.0	5.0	5.0±0.0	**0**
20	5.0	5.0	4.6	4.8±0.1	**2.3**
30	5.0	4.3	4.3	4.5±0.3	**8.9**
40	4.6	4.3	4.0	4.3±0.3	**13.4**
50	2.3	2.0	0.6	1.6±0.8	**66.7**
Control	5.0	5.0	5.0	5.00±0.0	**0**
Permethrin	0.0	0.0	0.0	0.0	**100.0**
S.E.M	0.1	0.2	0.3		
C.D. 1%	0.4	0.6	1.1		
C.D. 5%	0.3	0.5	0.8		
C.V.	11.5				

HS-RCLE: *H. spicatum* rhizomes chloroform extract, S.E.M.: Standard error mean, C.D.: Critical Difference, C.V.: Coefficient of Variance.

Asp397, Cys231, Leu305, Asn230, Glu306 forming van der Waals interactions while Pro232, His406, Pro403, Cys402 forming pi-alkyl contacts. In α-selinene amino acid residues Tyr121, Asp72, Ser81, Gly80, Tyr442, and Ile439 form van der Waals interactions, Phe330, Leu333, and Met436 form pi-alkyl contacts while Tyr334, Trp432, Trp84 forming pi-sigma contacts with a binding energy of -7.87 kcal/mol. Germacrene-*D* had shown binding free energy of -7.36 kcal/mol, an O atom of the hydroxyl group at C-3 position showed H-bonding with O atom and N-atom of amino acid residue Ser81, Tyr334, Asp72, His440, Gly80 forming van der Waals interactions, Ile439, Tyr442, Trp84 forming pi-alkyl contacts while Trp432, Phe330 forming pi-sigma contacts (Table VIII). The 2-dimensional and 3-dimensional binding interaction between different major components and acetohydroxyacid synthase enzyme is presented in fig. 4. Standard drug pendimethalin was observed to show binding energy of -7.50 kcal/mol showing van der Waals interactions with Phe290, Ala201, Phe288, Phe331, Gly119, Ser200, Gly118, Gly117, His440, Tyr130, Glu199, Gly441, Ser122, Phe330, pi donor hydrogen bond bonding with Tyr121 whereas pi-sigma interactions with Trp84. Also, pendimethalin was observed to show binding interactions with many amino acids as compared to the tested ligands. The major compounds of HS-RCLE *viz;* curcumenone, coronarin *E* and α-selinene showed greater binding energy as compared to the standard drug.

In-vitro mycelial growth inhibition activity

In the present study, the antifungal potential of HS-RCLE was evaluated. The antifungal activity was calculated by measuring the mean mycelial growth after 8 days of the *in-vitro* experiment. The mean mycelial growth area was observed to decrease as the dose level of the extract was increased. The mean mycelial growth of 6.4±1.6 cm was observed at 50 µL/mL which decreased to 4.9±1.2 cm, 4.1±1.1 cm, and 1.8±1.2 cm for the dose levels of 100, 250, and 500 µL/mL respectively. HS-RCLE at 50 µL/mL dose was found to show a percent mycelial growth inhibition of 10.8 %,

Table VI. Docking results of major compounds in HS-RCLE with target 1CI8 by AutoDock 4.2.

Compounds/Ligand (PubChem SID)	ΔG_b	Ki	I.E.	B.E.	R.M.S.D.	Residual Interactions		
						Van der Waals	Pi-alkyl	Pi-sigma
curcumenone (153845)	-5.96	42.69	-6.86	-6.55	75.38	Gly118, Ser200, His440, Gly441, Tyr121, Phe290, Glu199	Trp84, Tyr442, Phe331	Phe330, Tyr334
coronarin *E* (9971144)	-7.94	1.52	-8.53	-8.52	76.77	Tyr121, Tyr70, Ser286, Arg289, Phe288, Phe290	Trp279, Phe331, Phe330, Ile287	Phe330 (pi-pi T shaped)
α-selinene (10856614)	-7.34	4.20	-7.63	-7.63	91.59	Trp84, Ser81, Asn85, Asp72, Tyr121	Phe330, Tyr334	-
germacrene-*D* (5317570)	-7.17	5.57	-7.47	-7.45	92.18	Tyr121, Phe330, Ile287, Phe288, Arg289, Trp279	-	Tyr334, Phe331
permethrin (40326)	-6.65	13.38	-8.78	-8.72	76.56	Gly117, Phe330, Phe331, Tyr130, Phe290, Phe288, Arg289, Gly123, Ser122, Gly118, Gly119	Tyr70, Trp84 (pi pi stacked)	Trp279

1CI8: PDB ID for crystal structure of enzyme carboxylesterase from the head capsule of *Spodoptera litura* larvae, ΔG_b: Free energy of binding (kcal/mol), Ki: Estimated inhibition constant (μM) at temperature 298.15 K, I.E.: Final intermolecular energy (kcal/mol), B.E.: Binding energy (kcal/mol), Residual Interactions: H-bonds + Hydrophobic, R.M.S.D.: Root mean square distance.

while 74.7 % at 500 μL/mL was observed. The detailed results are given in table IX.

Energetically favorable docking predictions (*i.e.*, those with calculated negative values for binding free energy) were analyzed to assess the binding interactions between the residues in the protein models of melanin biosynthesizing enzyme trihydroxy naphthalene reductase (PDB: 3HNR) were compared. The docking analysis revealed that the investigated compounds had a high affinity for the active sites (target enzyme) of trihydroxy naphthalene reductase. Curcumenone showed residual interactions with atoms of amino acid residues namely Phe290, Phe330, Tyr121, Tyr70, Phe288, Ser286, Ile287, and Gly335 forming van der Waals interactions, while Phe331 and Tyr334 formed pi-alkyl contacts and Trp279, Arg289 (C-H) bonds with an average binding energy of length -8.32 kcal/mol. Coronarin *E* had shown binding energy of -9.94 kcal/mol with target amino acid residues Trp279, Tyr70, Asn85, Phe330, Phe288, Gly335 forming van der Waals interactions while Phe331, Tyr334 forming pi-alkyl contacts and Asp72 (pi-anion) and Tyr121 (pi-H donor) bonding. In α-selinene amino acid residues Gly118, Ser122, Gly123, Leu127, Gly117, Tyr130, Ile444, Gly441, and Glu199 form van der Waals interactions, His440, Phe330 forming pi-alkyl contacts while Trp84 forms pi-sigma contacts with a binding energy of -7.80 kcal/mol. Germacrene-*D* had shown binding free energy of -7.64 kcal/mol, an O atom of the hydroxyl group at C-3 position showed H-bonding with O atom and N-atom of amino acid residue Phe290, Tyr121, Gly119, Ser200, Phe331, Gly118, Gly117, Glu199, Tyr130, Ile444, Gly441 forming van der Waals interactions, His440 forming pi-alkyl contacts while Trp84, Phe330 forming pi-sigma contacts (Table X). Standard drug fluconazole was observed to show binding energy of -7.05 kcal/mol showing van der Waals interactions with Tyr130, Leu122, Tyr70, Asp72, Asn85, Gly441, Gly118 and Gly123, pi-alkyl interactions with His440, Phe330, Gly117 (pi-pi T-shaped), pi-sigma

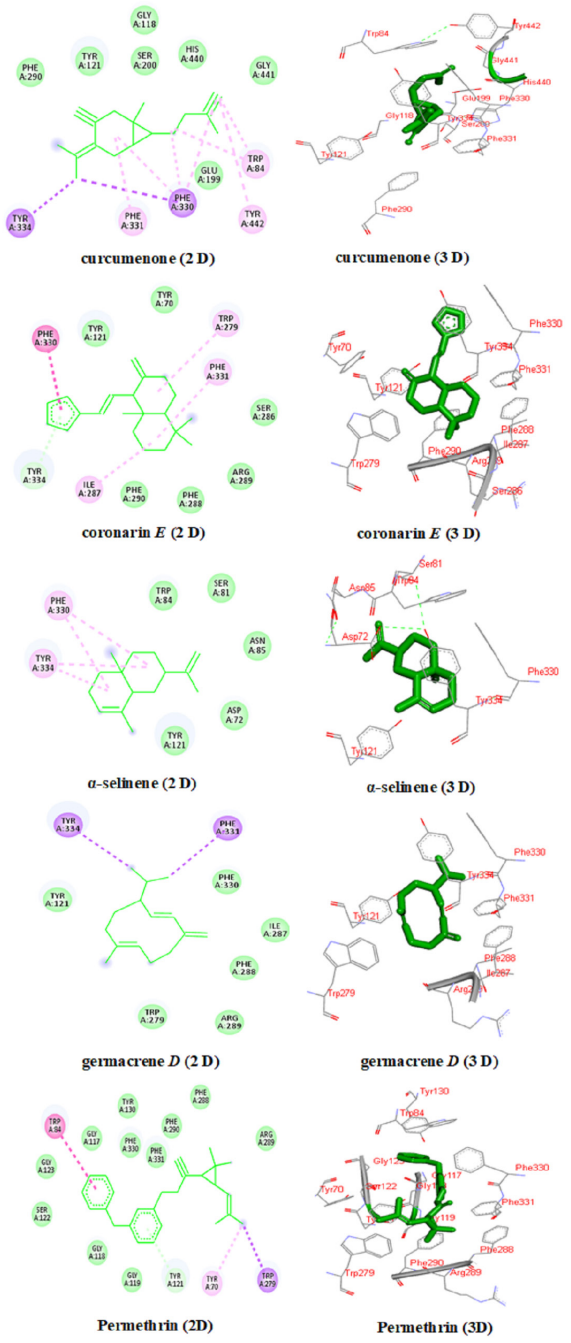

Figure 3. 2D and 3D interactions of major compounds in HS-RCLE with target 1CI8. 1CI8: PDB ID for crystal structure of enzyme carboxylesterase from the head capsule of *Spodoptera litura* larvae, amino acid residues in green rings are showing van der Waals interactions, amino acid residues in pink rings are showing pi-alkyl interactions, amino acid residues in purple rings are showing pi-sigma interactions, amino acids in red rings are showing unfavorable bumps.

interactions with Trp84 and Ser122 (pi donor H bond). All the major compounds of HS-RCLE *viz;* curcumenone, coronarin *E*, α-selinene, and germacrene-*D* showed greater binding energy as compared to the standard drug. The 2-dimensional and 3-dimensional binding interaction between different major components and trihydroxy naphthalene reductase enzyme is presented in fig. 5.

In silico PASS studies of major compounds of HS-RCLE

Prediction of activity spectra for substances (PASS) is a free online cheminformatic software that helps to predict the biological activities of bioactive chemical components based on the structure-based similarity to the largely complied database of these active molecules. The bioactivity score is calculated in terms of Pa and Pi values. A compound is possibly predicted to be active if its Pa (chances to be active) value is more than the Pi (chances to be inactive) value. The major components of HS-RCLE are predicted to exhibit diverse bioactivities (Pa > 0.5) such as antioxidant, anti-amylase, anti-inflammatory and anti-microbial, etc. The Pa values for predicted bioactivities lie between 0.737 and 0.112 (Table XI). The compounds showed significant anti-fungal activity in the range of 0.655 for δ-elemene to 0.238 for curcumenone. Similarly, the compounds also showed significant results in the case of insecticidal activities.

DISCUSSION

A recent study reported the presence of medicinal phytochemical constituents like phenolics, flavonoids, and alkaloids in different extracts of *H. spicatum*. Chemical analysis of *H. spicatum* rhizome methanolic oleoresin revealed the presence of curzerene (14.7 %), coronarin E (13.3 %), curdione (10.2 %), and linderazulene

Table VII. % phytotoxic activity of HS-RCLE against *R. raphanistrum* seeds in laboratory conditions.

Dose (µL/mL)	Number of weed germinated in different time intervals					Mean weed germinated	% Growth inhibition	% Root growth inhibition	% Shoot growth inhibition
	24 h	48 h	72 h	96 h	108 h				
50	1.3	1.6	2.0	2.0	2.0	1.8±0.3	**80.0**	**97.9**	**98.4**
100	0.3	0.6	1.6	2.0	2.0	1.3±0.7	**85.2**	**98.8**	**98.9**
150	0.0	0.6	1.0	1.0	1.0	0.7±0.4	**91.8**	**100.0**	**100.0**
200	0.0	0.0	0.0	0.0	0.0	0.0±0.0	**100.0**	**100.0**	**100.0**
Control	7.0	8.0	10.0	10.0	10.0	9.0±1.4	**0.0**	**0.0**	**0.0**
Pendimethalin	0.0	0.0	0.0	0.0	0.0	0.0	**100.0**	**100.0**	**100.0**
S.E.M	0.8	0.8	0.2	0.6	0.4				
C.D. 1%	0.3	0.3	0.7	0.3	0.4				
C.D. 5%	0.2	0.2	0.5	0.2	0.2				
C.V.	**13.4**								

HS-RCLE: *H. spicatum* rhizomes chloroform extract, S.E.M.: Standard error mean, C.D.: Critical Difference, C.V.: Coefficient of Variance.

Table VIII. Docking results of compounds in HS-RCLE with target 1YHZ by AutoDock 4.2.

Compounds/Ligand (PubChem SID)	ΔG_b	Ki	I.E.	B.E.	R.M.S.D.	Residual Interactions		
						Van der Waals	Pi-alkyl	Pi-sigma
curcumenone (153845)	-7.52	3.10	-8.41	-8.24	77.44	Phe330, Phe290, Asp72, Phe288, Arg289, Leu282, Ile287, Ser286	Tyr121, Trp279, Tyr70, Tyr334	
coronarin *E* (9971144)	-7.87	1.71	-8.47	-8.40	76.29	Asn525, His398, Trp524, Asp397, Cys231, Leu305, Asn230, Glu306	Pro232, His406, Pro403, Cys402	
α-selinene (10856614)	-7.57	2.82	-7.87	-7.87	90.16	Tyr121, Asp72, Ser81, Gly80, Tyr442, Ile439	Phe330, Leu333, Met436	Tyr334, Trp432, Trp84
germacrene-*D* (5317570)	-7.06	6.63	-7.36	-7.36	77.64	Ser81, Tyr334, Asp72, His440, Gly80	Ile439, Tyr442, Trp84	Trp432, Phe330
pendimethalin (38479)	-4.37	625.52	-6.16	-7.50	93.23	Phe290, Ala201, Phe288, Phe331, Gly119, Ser200, Gly118, Gly117, His440, Tyr130, Glu199, Gly441, Ser122, Phe330	Tyr121 (pi donor hydrogen bond)	Trp84

1YHZ: PDB ID for crystal structure of enzyme acetohydroxyacid synthase (AHAS) from the weed *Raphanus raphanistrum* sub *sativus*, ΔG_b: Free energy of binding (kcal/mol), Ki: Estimated inhibition constant (µM) at temperature 298.15 K, I.E.: Final intermolecular energy (kcal/mol), B.E.: Binding energy (kcal/mol), Residual Interactions: H-bonds + Hydrophobic, R.M.S.D.: Root mean square distance.

(6.0 %) as major phytoconstituents, while its ethyl acetate oleoresin has been reported to be dominated by curcumol (13.0 %), curzerene (10.4 %) and isovelleral (9.7 %) (Rawat et al. 2021). *H. coronarium* rhizome chloroform extract has been reported to be dominant with coronarin E (20.1 %) followed by 1,8-cineole (12.6 %), α-terpineol (9.5 %), isopulegol (8.2 %), dodecane

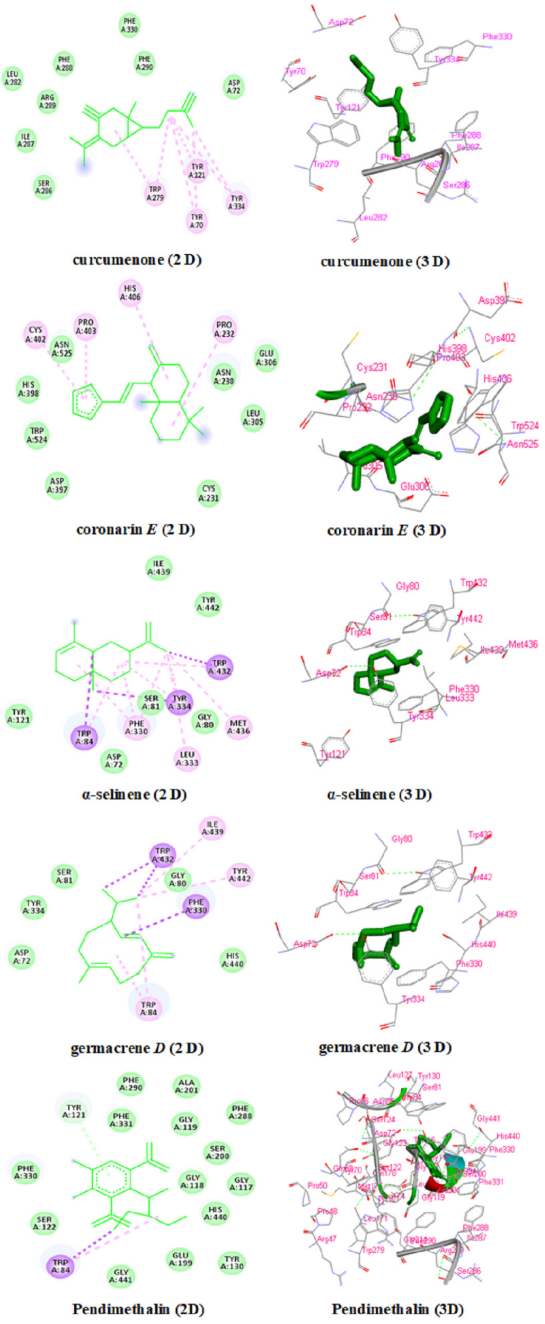

Figure 4. 2D and 3D interactions of major compounds in HS-RCLE with target 1YHZ. 1YHZ: PDB ID for crystal structure of enzyme acetohydroxyacid synthase (AHAS) from the weed *Raphanus raphanistrum* sub *sativus*, amino acid residues in green rings are showing van der Waals interactions, amino acid residues in pink rings are showing pi-alkyl interactions, amino acid residues in purple rings are showing pi-sigma interactions, amino acids in red rings are showing unfavorable bumps.

(7.3 %), α-pinene (6.2 %) and α-fenchene (5.9 %) (Arya et al. 2022).

Recently, chloroform extract of *H. coronarium* has been reported to show nematicidal activity against *M. incognita* (Arya et al. 2022). Plant extracts of several species *viz; Curcuma longa* (Rashid et al. 2021), *Kaempferia rotunda* (Krishnakumar & Varghese 2022), and *Ocimum tenuiflorum* (George et al. 2022) have shown significant nematicidal activity against *M. incognita*. Nematicidal activity of *Mentha longifolia* against *M. graminicola* (Gowda et al. 2022), *Moringa oleifera* against *Haemonchus contortus* (Páez-León et al. 2022), and *Chelidonium majus* against *Bursaphelenchus xylophilus* (Lee et al. 2022) have also been reported. These findings suggest that the extract has the potential for the development of novel nematicidal compounds for the control of the root-knot nematodes.

The chloroform extract of *H. coronarium* rhizomes has also been found to show 60 % mortality of *S. litura* at a 100-ppm dose level (Arya et al. 2022). Plant extracts of several species *viz; Moringa oleifera* (Kaur et al. 2022), *Piper retrofractum* (Ratwatthananon et al. 2020), and *Alpinia galanga* (Datta et al. 2019) have shown insecticidal activity against *S. litura*. Also, the insecticidal activity of *Dodonaea viscosa* against *Spodoptera exigua* (Ramírez-Zamora et al. 2020), *Origanum onites* against *Sitophilus oryzae* (Erenler et al. 2018), and *Thymus kotschyanus* against *Oryzaephilus surinamensis* (Ghasemi et al. 2020) have also been reported. Some selected major compounds of the extracts like germacrene-D (Ravi Kiran et al. 2006), curzerene (Govindarajan et al. 2018), and isovelleral (Daniewski et al. 1995).

Rawat et al. 2019 reported complete inhibition of *R. raphanistrum* seedling growth at the dose level of 200 ppm of *H. spicatum* rhizomes essential oil. Chloroform extract of *H.*

Table X. Docking results of compounds in HS-RCLE with target 3HNR by AutoDock 4.2.

Compounds/ Ligand (PubChem SID)	ΔG_b	Ki	I.E.	B.E.	R.M.S.D.	Residual Interactions		
						Van der Waals	Pi-alkyl	Pi-sigma
curcumenone (153845)	-7.45	3.46	-8.35	-8.32	92.17	Phe290, Phe330, Tyr121, Tyr70, Phe288, Ser286, Ile287, Gly335	Phe331, Tyr334	Trp279, Arg289 (C-H)
coronarin *E* (9971144)	-9.36	137.07	-9.96	-9.94	91.86	Trp279, Tyr70, Asn85, Phe330, Phe288, Gly335	Phe331, Tyr334	Asp72 (pi-anion), Tyr121 (pi-H donor)
α-selinene (10856614)	-7.49	3.21	-7.79	-7.80	89.47	Gly118, Ser122, Gly123, Leu127, Gly117, Tyr130, Ile444, Gly441, Glu199	His440, Phe330	Trp84
germacrene-*D* (5317570)	-7.34	4.20	-7.63	-7.64	76.49	Phe290, Tyr121, Gly119, Ser200, Phe331, Gly118, Gly117, Glu199, Tyr130, Ile444, Gly441	His440	Trp84, Phe330
fluconazole (3365)	-5.20	154.71	-6.99	-7.05	92.79	Tyr130, Leu122, Tyr70, Asp72, Asn85, Gly441, Gly118, Gly123	His440 Phe330, Gly117 (pi-pi T-shaped)	Trp84, Ser122 (pi donor H bond)

3HNR: PDB ID for crystal structure of enzyme melanin biosynthetic enzyme trihydroxy naphthalene reductase from fungus *Curvularia lunata*, ΔG_b: Free energy of binding (kcal/mol), Ki: Estimated inhibition constant (μM) at temperature 298.15 K, I.E.: Final intermolecular energy (kcal/mol), B.E.: Binding energy (kcal/mol), Residual Interactions: H-bonds + Hydrophobic, R.M.S.D.: Root mean square distance.

Table XI. *in silico* PASS prediction bioactivities of major compounds in HS-RCLE.

Major compounds	Predicted biological activities (Pa>Pi, Pa=Probable activity and Pi=Probable inactivity)							
	Antioxidant	Anti-amylase	Anti-helminthic (nematodes)	Anti-inflammatory	Insecticidal	Anti-fungal	Anti-bacterial	Anti-feedant
dodecane	0.170>0.079	0.512>0.005	0.628>0.005	-	0.378>0.007	0.377>0.055	0.287>0.065	-
δ-elemene	0.164>0.086	0168>0.165	0.552>0.010	0.745>0.011	0.392>0.006	0.655>0.013	0.424>0.025	-
trans-bergamotol	0.353>0.016	0.024>0.012	0.200>0.090	0.594>0.033	0.340>0.011	0.454>0.039	0.430>0.024	-
germacrene-*D*	0.193>0.049	0.195>0.057	0.457>0.009	0.457>0.070	0.447>0.005	0.570>0.022	0.427>0.025	-
curzerene	0.321>0.020	0.118>0.106	0.334>0.079	0.695>0.016	0.215>0.032	0.491>0.032	0.377>0.036	-
α-selinene	0.153>0.100	0.298>0.221	0.387>0.019	0.256>0.162	0.475>0.004	0.548>0.024	0.355>0.042	-
furanodiene	0.383>0.014	0.204>0.053	0.300>0.038	0.413>0.089	0.219>0.032	0.268>0.097	0.226>0.097	-
isovelleral	0.167>0.083	0.161>0.084	0.186>0.106	-	0.233>0.029	0.393>0.051	0.381>0.035	-
curcumenone	0.145>0.109	0.287>0.035	0.250>0.162	0.684>0.018	0.220>0.031	0.238>0.115	0.219>0.102	-
n-hexadecanoic acid	0.661>0.002	0.583>0.003	0.348>0.025	0.515>0.052	-	0.407>0.048	0.300>0.060	-
valerenic acid	0.184>0.062	0.123>0.121	0.202>0.088	0.638>0.025	0.200>0.037	0.468>0.036	0.281>0.067	-
linderazulene	0.288>0.025	0.215>0.047	0.254>0.056	0.256>0.113	0.275>0.021	0.264>0.099	0.199>0.118	-
coronarin *E*	0.166>0.084	0.206>0.156	-	0.737>0.012	0.112>0.105	0.471>0.036	0.212>0.107	-
trioxsalen	0.478>0.012	0.121>0.117	0.253>0.057	0.457>0.070	0.221>0.031	0.310>0.077	0.300>0.060	-

coronarium rhizomes causing 98.88% inhibition of *R. raphanistrum* seed germination at 1000 ppm dose level has been reported (Arya et al. 2022). Plants of the family Zingiberaceae have also been reported to show herbicidal activity *viz*; *Curcuma zedoaria* essential oil has been

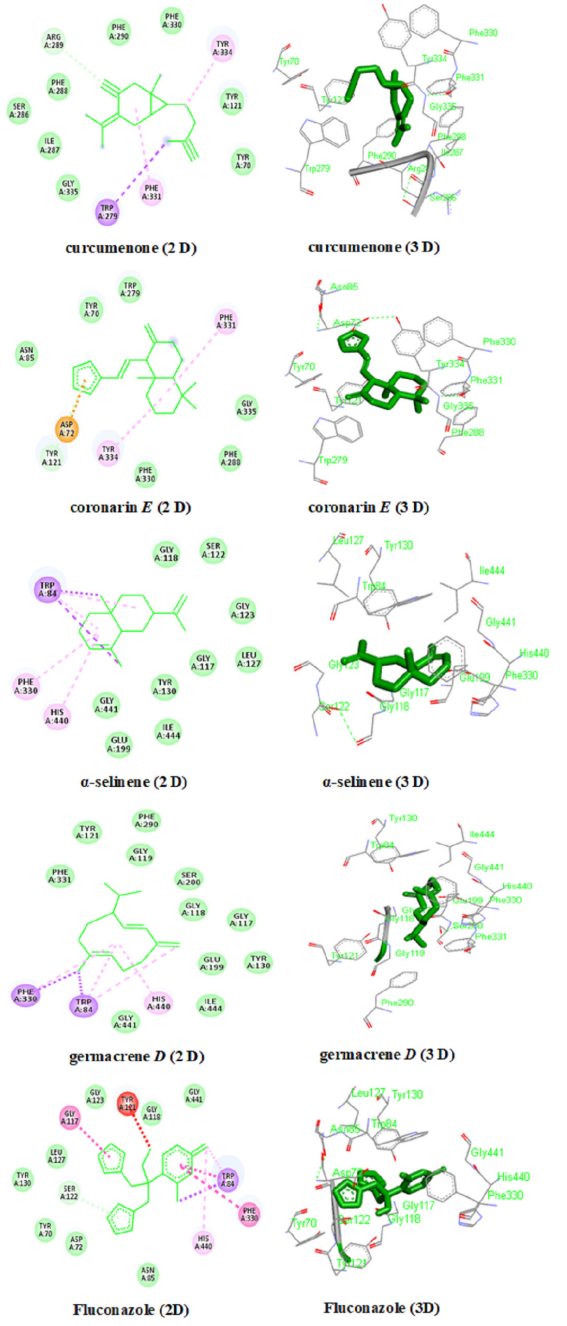

Figure 5. Isolated compounds docked into the binding pocket of 3HNR. 3HNR: PDB ID for crystal structure of enzyme melanin biosynthetic enzyme trihydroxy naphthalene reductase from fungus *Curvularia lunata*, amino acid residues in green rings are showing van der Waals interactions, amino acid residues in pink rings are showing pi-alkyl interactions, amino acid residues in purple rings are showing pi-sigma interactions, amino acids in red rings are showing unfavorable bumps.

reported to show allelopathic effects on the vigor and germination of lettuce achenes and tomato seeds. The root system was found to be more heavily damaged than the hypocotyl, especially in tomatoes compared to lettuce (de Melo et al. 2017). *Alpinia zerumbet* has also been investigated to exhibit herbicidal activities against *Lactuca sativa* seedlings (Xuan et al. 2019).

The Zingiberaceous plants have also been reported to show fungicidal activity. The methanol extract of *Curcuma longa* rhizomes has been reported to show antifungal activity against plant pathogenic fungus *viz; Fusarium oxysporum*, *Pythium debaryanum*, *Phytophthora infestans*, *Fusarium solani* and *Alternaria alternata* (Abdelgaleil et al. 2019). *Etlingera flexuosa* has been reported as an antifungal agent for *Candida albicans* (Pitopang et al. 2020). Jantan et al. 2003 investigated nine Zingiberaceae species namely *Zingiber officinale*, *Z. cassumunar*, *Z. zerumbet*, *Curcuma aeruginosa*, *C. manga*, *C. xanthorrhiza*, *Kaempferia galanga*, *Alpinia galanga* and *Boesenbergia pandurata* for their antifungal activities against five dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. nanum*, and *Epidermophyton floccosum*) and three filamentous fungi (*Aspergillus niger*, *A. fumigatus*, and *Mucor* sp.).

No studies on the antifungal activity of *H. spicatum* oils against *Curvularia lunata* have been reported. However, *Hedychium spicatum* essential oils and chloroform extract have been reported to show significant antifungal activity against *Rhizopus stolonifer*, *Trichoderma viride*, and *Trichoderma lignorum* (Bisht et al. 2006). Rawat et al. 2021 reported the methanol and ethyl acetate oleoresins of *Hedychium spicatum* rhizomes to exhibit moderate to strong antifungal activity against *Colletotrichum falcatum*,

Rhizoctonia solani, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*.

CONCLUSIONS

H. spicatum is rich in chemical constituents, diverse in pharmacological activities, and abundant in resources, which is widely used in clinics from the traditional to modern era. In addition, the existing clinical applications suggest that *H. spicatum* has a certain therapeutic potential in the treatment of rheumatic pain and cardiovascular diseases, but the gap in the research activities carried out on its pesticidal potential has to be fulfilled for the benefit of farmers and agricultural sectors in view of environmentally benign concepts. Taking together, the cumulative *in vitro* and *in silico* computational bio-efficacy analysis of the pesticidal activities provides useful leads on harnessing the potential of HS-RCLE as an environmentally safe bio-pesticide. The insight into biochemical ligand-target protein interactions and toxicity analysis described in the present study will be helpful in the logical selection of bioactive natural compounds for the development of practically viable bio-pesticidal products. To make a more comprehensive evaluation of the quality of *H. spicatum*, it is necessary to further strengthen the research on quality control, look for more specific indicative components and more stable and reliable analysis methods, and establish a scientific and reasonable quality evaluation system. It is worthwhile to further investigate *H. spicatum* in depth to make discoveries and breakthroughs. Chloroform extract of *H. spicatum* has been a source of labdane diterpenes known for significant anti-cancer and anti-diabetic activities, furthermore, these labdane diterpenes could be isolated for further look out into their pesticidal activities. The data generated in the present study will strengthen the database for judicious exploitation of the plant material as it is near to endangered besides the study has academic importance.

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Avneesh Rawat: Planning original draft, experimental execution, data interpretation, statistical tests, data analysis, molecular docking studies, and manuscript preparation. Om Prakash: Research Advisor of Avneesh Rawat, Provide some professional guidance and valuable advice during the writing process of this paper. Kirti Nagarkoti: Formal analysis, collected and sorted out the relevant literature and other information, Ravendra Kumar: co-advisor, analyzed, reviewed, and edited the manuscript, Ashok Kumar Verma: analyzed, reviewed, and edited the manuscript, Satya Kumar: Contributed as a member of the Advisory Committee and helped in nematicidal activity. Ravi Mohan Srivastava: Contributed as a member of the Advisory Committee and helped in insecticidal activity. Mamta Latwal and Ganesh Pandey: Formal analysis, helped in assisting with GC-MS analysis.