European Journal of Chemistry

View Journal Online
View Article Online

Comparative chemical composition and pesticidal evaluation of *Acorus calamus* accessions collected from different geographical locations

Tisha Joshi [1], Kirti Nagarkoti [1], Navadha Joshi [2], Avneesh Rawat [1], Om Prakash [1,*], Ravendra Kumar [1], Ravi Mohan Srivastava [3], Satya Kumar [4], Shilpi Rawat [4] and Dharmendra Singh Rawat [5]

[1] *Department of Chemistry, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, 263145, India*
[2] *Department of Chemistry, Graphic Era Hill University, Dehradun, Uttarakhand, 248002, India*
[3] *Department of Entomology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, 263145, India*
[4] *Department of Plant Pathology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, 263145, India*
[5] *Department of Biological Sciences, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, 263145, India*

* *Corresponding author at: Department of Chemistry, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, 263145, India.*
e-mail: oporgchem@gmail.com (O. Prakash).

RESEARCH ARTICLE

10.5155/eurjchem.14.1.129-143.2387

Received: 16 December 2022
Received in revised form: 21 January 2023
Accepted: 28 January 2023
Published online: 31 March 2023
Printed: 31 March 2023

KEYWORDS

Herbicidal
Insecticidal
Nematicidal
Biopesticides
Acorus calamus
Molecular docking

ABSTRACT

The objectives of the present study were to investigate the phytochemical composition of essential oils (EO) from rhizomes of *Acorus calamus* collected from Jorhat, Assam; Munsyari and Pantnagar, Uttarakhand, India. EOs were studied for different pesticidal activities *viz*; nematicidal, insecticidal, and herbicidal activity. To study the synergistic effect of EOs on pesticidal activity, four combinations of EOs were prepared. Phenylpropanoids with β-asarone as the main compound were identified in all collections with varying percentages. Its contribution was found to be 85.8% in Munsyari EOs followed by 74.3% in Pantnagar and 62.6% in Assam collections. All EOs exhibited dose-dependent *in vitro* nematicidal activity against *Meloidogyne incognita* in terms of immobility and inhibition of egg hatching. The activity was observed as maximum in the EO combination of all three collections (1:1:1) whereas minimum in the Assam collections. In insecticidal activity against *Lipaphis erysimi* and *Selepa celtis*, maximum mortality was observed in Munsyari collections. The oils were assessed for sprout inhibition activity in terms of seed germination inhibition, coleoptile growth of the shoot and root against *Raphanus raphanistrum*. Maximum seed germination inhibition, % shoot, and root growth inhibition were found in all collections EO combinations. To predict the possible mode of action and the structure-activity relationship between major compounds of EOs and biological activities, *in silico* molecular docking and ADME/Tox studies were performed. The docking results revealed the mode of action of proteins of insects, nematodes, and weeds and were found in support of *in vitro* experiments. The study may be helpful for the development of herbal-based pesticides after proper clinical trials.

Cite this: *Eur. J. Chem.* **2023**, *14(1)*, 129-143 **Journal website**: www.eurjchem.com

1. Introduction

In the present scenario, pest management faces significant economic and environmental problems due to the widespread overuse of pesticides. Biodegradability, non-target toxicity, and lingering effects of synthetic pesticides have become a severe concern. Herbicides, insecticides, nematicides, molluscicides, rodenticides, bactericides, piscicides, avicides, animal repellents, antimicrobials, fungicides, disinfectants, and sanitizers are examples of pesticides. Dichlorodiphenyltrichloroethane (DDT), the first synthetic organic pesticide, was considered extraordinary because of its wide range of activity, insolubility, low cost, and ease of application. However, a ban on the use of DDT in agriculture was proposed due to its long-lasting effects on soil health and aquatic biota [1]. The current pest control method involves the usage of synthetic insecticides such as carbamates and organophosphate compounds. However, these neurotoxins also increase insect pest resistance, and more than 500 types of insects and mites are no longer effective against these synthetic pesticides [2]. Modern agricultural production has dominated the world over synthetic pesticides with a market share of approximately 95%. The uncontrolled use of these pesticides has undesirable impacts and detrimental effects on biodiversity and human health [3]. To combat the adverse effects of synthetic pesticides, herbal pesticides such as EO, extracts, and pure compounds and their formulations are

https://dx.doi.org/10.5155/eurjchem.14.1.129-143.2387

being developed [4]. Botanical pesticides are widely available, easily biodegradable, and inexpensive and have been reported to be effective against a variety of agricultural pests [5]. The popularity of using medicinal and aromatic herbs as alternatives to modern allopathic therapies has increased due to their low cost, lack of side effects, and holistic approach [6]. Active ingredients in botanical pesticides, particularly the distinctive structural patterns of secondary metabolites such as alkaloids, terpenes, flavonoids, phenolics, phytosterols, and polyketides, are capable of conferring antibacterial, antifungal, herbicidal, and insecticidal actions, as recently seen in the case of various phytochemicals, including *Citrus limonum* and *Emblica officinalis* [7-12].

Acorus calamus (Sweet flag) is an angiosperm that belongs to the Acoraceae family of the order Acorales. It is native to China, Japan, South East Asia, Asia, and Sulawesi and is found in moderate and sub moderate rainfall regions around the world [13]. In India, *A. calamus* is found naturally in swamps and is also cultivated in the Himalayas, particularly in Karnataka, Assam, Tamil Nadu, Kerala, and Andhra Pradesh, up to an altitude of 2200 m [14]. The members are aromatic, perennial, marshy herbs with rhizomes, bearing EO [15]. It has traditionally been used as a medicine for a variety of diseases, including fever, asthma, bronchitis, cough, and, most notably, digestive problems such as gas, bloating, colic, and poor digestive function. EOs from rhizomes of *A. calamus* have been reported to treat a variety of diseases, including epilepsy, antidepressant, anxiolytic, antifungal, and antibacterial activities [16].

The objectives of the present study were to investigate the phytochemical analysis of the rhizomes of *A. calamus* EOs collected from Pantnagar and Munsyari, Uttarakhand and Jorhat, Assam in India (ACPREO, ACMREO, and ACAREO) for pesticidal evaluation viz. nematicidal, herbicidal, and insecticidal activity. EOs from three ecological niches and their different combinations of *A. calamus* Assam-Pantnagar rhizome EO (AC(A:P)REO), *A. calamus* Munsyari-Pantnagar rhizomes EO (AC(M:P)REO), *A. calamus* Assam-Munsyari rhizomes EO (AC(A:M)REO) and *A. calamus* Assam-Munsyari-Pantnagar rhizomes EO (AC(A:M:P)REO) in the ratio of 1:1 and 1:1:1 were also studied for their synergistic/antagonistic effects against pesticidal activities. Structure-activity relationships (SARs) between major compounds of EOs with possible pesticidal activities have been studied by *in silico* molecular docking and ADME/Tox studies.

2. Experimental

2.1. Collection of plant material

The herb *Acorus calamus* was collected from Pantnagar and Munsyari, Uttarakhand, and Assam in India in July-September 2021. The plant was identified by Dr. Dharmendra Singh Rawat (taxonomist). The herbarium number for the Pantnagar (GBPUH-910) and Munsyari (GBPUH-912) collections was deposited in the Department of Biological Sciences, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India.

2.2. Phytochemical analysis

2.2.1. Isolation of EO from rhizomes of A. calamus

The EOs from *Acorus calamus* were isolated by the hydro distillation method in a Clevenger-type apparatus. The plant material was crushed prior to hydrodistillation for 3-4 h. To obtain reproducibility in EO yield, the hydro distillation was repeated 3 times [17]. The oils were collected as neat and desiccated over anhydrous sodium sulphate to remove traces of water if any. The EOs obtained were stored at a low temperature (4 °C in the refrigerator) for GC-MS analysis to identify the EO components and to determine the pesticidal activities.

2.2.2. GC-MS analysis of EO

The DB-5 silica capillary column (30 m × 0.25 mm; 0.25 m) was used to analyze the EOs using GCMS-QP 2010 Ultra equipment. The following parameters were programmed for the experiment: carrier gas He, column flow rate of 1.21 mL/min, injection temperature of 260 °C, split injection mode, pressure of 69.0 KPa, split ratio of 1:10 and interphase temperature of 270.0 °C. The oven temperature was first set at 50 ° C for 2 minutes, then increased to 210 ° C at a rate of 3 °C/min while isothermal for 2 minutes, and then increased to 240 ° C at a rate of 8 °C/min while isothermal for 11 minutes. MS was recorded using a 1:100 split mode and an injection volume of 0.1 L under EI conditions (70 eV). By comparing the fragmentation pattern and retention indices of the mass spectra with those of the MS library (NIST14.lib, FFNSC2.lib, WILEY8.LIB), as well as by comparing the spectra with the data from the literature, the constituents of the EOs were identified [18].

2.3. Nematicidal activity

2.3.1. Nematode population collection

The population of *Meloidogyne incognita* was reared on capsicum at the Vegetable Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, in a glasshouse maintained at 25±2 °C. Capsicum (*Capsicum annuum*) was taken as the infected root knot plant. The roots were dissected under a stereo binocular microscope (Magnus inverted microscope with 4× magnification) where perineal patterns were prepared to identify the species of root knot nematode (RKN) (*Meloidogyne* spp.) as described in the reference [19]. The mature egg masses were handpicked from the roots of infected capsicum and incubated in distilled water in a growth chamber at 25 °C. Emerged juveniles were collected and stored at 5 °C until use [20].

2.3.2. In vitro nematicidal evaluation of EOs on egg hatching of M. incognita

The eggs of *M. incognita* were obtained from pure nematode culture. Gridded Petri dishes containing three triplicates of each treatment with four different doses (50-200 ppm) of EOs (ACAREO, ACMREO, ACPREO) and their combinations in 1:1 viz. AC(A:P)REO, AC(A:M)REO, AC(P:M)REO, and 1:1:1 for AC(A:M:P)REO in tween 20 (1% solution), respectively. Distilled water in tween 20 (1% solution) was taken as a negative control. The egg masses of *M. incognita* were suspended in different doses of EOs taken in Petri dishes. All treatments were organized in a completely randomized design (CRD) and kept at an ambient temperature of 27±1 °C in a BOD incubator (Remi Cl-16 plus). Observations of the mean egg hatchability of eggs were recorded after 24, 48, and 72 h of exposure periods. The number of eggs hatched was counted under a microscope (Olympus CX3) at 40× magnification. All the above experiments were carried out following the standard protocols [21].

2.3.3. In vitro nematicidal evaluation of EOs on the immobility of M. incognita (J_2)

The study was carried out according to the protocols developed [22]. The larvae were isolated from galled roots with egg masses from a pure culture of nematodes. After the galled roots were removed from the pot, they were washed with tap

water to remove the soil adhered to it. The roots were cut into small pieces of approximately 2 cm and transferred to a 2% NaOCl solution and shaken for two minutes to separate the organic debris from the eggs. The suspension was poured through a series of sieves and the eggs sieved in 38 µm pores were collected and washed cautiously on tap water. The egg suspension was then poured and incubated at 27±1 °C so that the juveniles (J_2) of the hatched eggs were collected within 48 h. Approximately one hundred juveniles (J_2) were counted and placed on gridded Petri dishes containing different doses of EOs. Tween 20 in distilled water was taken as a negative control. The Completely Randomized Design (CRD) method was used for all of the experiments. The immobilized juveniles were counted after time periods of 24, 48, and 72 h using a stereo-binocular microscope (Olympus CX3) to observe the mortality of the nematode.

2.4. Insecticidal activity

The insecticidal activity of EOs individually and in combination was studied against *Selepa celtis* and *Lipaphis erysimi.* In the present study, the insecticidal activity of EOs was performed using the contact toxicity method developed earlier [23] with slight modifications.

2.4.1. Rearing of insects

S. celtis is a commonly found nocturnal, polyphagous, multivoltine lepidopterous pest. The eggs of this pest are laid in masses and the newly laid eggs are yellowish and translucent, with a round shape and reticulate markings on the chorion [24]. The insects were reared in a glass jar covered with muslin cloth under ideal laboratory conditions (Temperature 27 °C, relative humidity 75-80%). Fresh castor leaves were fed to the insect larvae of *S. celtis* on a daily basis for 7 days. Finally, the third instar larvae were starved for 24 h before being used to investigate insecticidal activity.

L. erysimi was directly collected from the mustard field at the Norman E. Borlogue Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, and acclimatized in the laboratory of Department of Entomology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, until the emergence of the third instar larvae. *L. erysimi* of the mustard plant was reared in a muslin-walled box in the growth chamber of a glass house at 25±1 °C and relative humidity (RH) of 65±5%.

2.4.2. Experimental procedure for Selepa celtis

The protocol practiced for the activity [23] was followed. The fresh leaf of soybean was cut in a square shape (4×4 sq. cm) and dipped in various concentrations (50-200 ppm) of EOs and their combinations in tween 20 (1% solution), for around 30 seconds before placing in Petri dishes for feeding trials. Moisture paper was placed at the bottom of the Petri dish to keep the treated leaves fresh and maintain the proper humidity level. In each Petri plate, five third-instar larvae were released to assess their feeding performance at different concentrations of EO and control (1% tween 20). Three triplicates of each treatment were used for analysis. Observations were made by counting the number of dead insect larvae after the time intervals of 24, 48, and 72 h.

2.4.3. Experimental procedure for Lipaphis erysimi

According to the protocols used [25,26], the experiment was evaluated on a Petri plate using the contact toxicity method. Fresh and clean mustard leaves, along with aphids (*L. erysimi*) on them, were obtained from the field. Each leaf disc (4×4 sq. cm) from a fresh mustard plant was placed upside down on a 1 cm layer of agar solution (1 % agar solution using 1 g of agar in 100 ml of boiled distilled water) in a Petri dish to maintain moisture in the leaf and a hole in the center of the lid for ventilation. The adult aphids were separated from the leaves with the help of a camel brush after 2 hours and collected in a box. The mustard leaf disc was soaked for 30 seconds in EOs (50-200 ppm) EOs, and their combination in Tween 20, respectively, for one minute before being dried. Three replications of each EO concentration were taken along with the control (1% Tween 20). In each replicate, treated leaves with 3-4 hours starved 15 adult aphids were placed on agar-layered Petri plates. Observations were noted at the time interval of 24, 48, and 72 h after the release of the nymph. The number of dead aphids was counted and calculated using Abbott's formula.

2.5. Herbicidal activity

The herbicidal activity was determined according to the earlier method described according to the standard protocols that are generally used [27]. Seeds of Radish (*Raphanus raphanistrum*) were obtained from the Vegetable Research Centre, Pantnagar, India. The seeds of radish (*R. raphanistrum*) were sterilized in 1% H_2O_2 for 3-4 hours. To evaluate the herbicidal activity of the sample, 2.0 mL of various concentrations (50-200 ppm) of EOs and their combinations in tween 20 (1% solution), respectively, were poured into Petri dishes lined with filter paper. Ten seeds of *R. raphanistrum* were placed in each Petri dish. A solution of 1.0 % tween 20 in distilled water was used as a negative control, while pendimethalin was used as a positive control. The experiments were conducted in CRD mode, each treatment was studied in replicates. The experiment was carried out at room temperature (25-28 °C) for five days. The germination rate, root length, and shoot lengths were observed after the time interval of 24, 48, 72, 96, and 120 h, respectively, to check the sprout inhibition activity. The percentage of seed germination was calculated using the following formula.

$$\text{Germination \%} = Nt \times 100/N \quad (1)$$

where, Nt = Proportion of germination seeds in each treatment for the final measurement, N = Number of seeds in the bioassay.

2.6. Molecular docking studies

Molecular docking is a method for predicting the activity and affinity of small molecules with macromolecules like proteins in different orientations in order to understand the host-guest relationship at the minimum energy level. Molecular docking is a virtually developed crucial approach to determining the binding energy and learning about the numerous ligand-protein interactions through software such as Autodock 4.2.6 (The Scripps Research Institute, USA). Gasteiger charges were calculated and registered as a pdbqt file using Autodock Tools (ADT ver.1.5.7) [28]. Different 3D complex structures for the target proteins for different pests were data mined from Protein Data Bank (PDB) *viz.* acetylcholinesterase protein (PDB ID: 1C2O) for *M. incognita* [29], anionic peroxidase (PDB ID: 1OG5) for *R. raphanistrum* [30] (PDB ID: 3QQQ) for *S. celtis,* and PDB ID: 5F3Y for *L. erysimi* have been taken directly from PDB (www.rscb.org/pdb) for validation of the biological activities tested. Thus, it is being reported for the first time. The main identified compounds like β-asarone and *Z*-methyl isoeugenol, which were common in the EOs of three different accessions of *A. calamus*, were used as ligands (guest molecule) obtained from PubChem (3D structures). To examine the chemical interactions of complicated structures, 3D structures of ligands and proteins were created using BIOVIA Discovery Studio 2019.

Table 1. Quantitative and qualitative composition of ACAREO, ACMREO, and ACPREO.

Compound	% Contribution			Kovatt index
	ACAREO	ACMREO	ACPREO	
α-Pinene (MH)	t	-	-	938
Camphene (MH)	t	-	-	954
β-Pinene (MH)	t	-	-	980
β-*trans*-Ocimene (MH)	1.0	0.2	0.1	1050
Linalool (OM)	1.0	0.1	t	1095
Camphor (OM)	0.1	-	-	1141
Shyobunone (OS)	-	-	5.4	1324
α-Copaene (SH)	t	-	-	1376
β-Elemene (SH)	0.3	-	-	1389
Aristolene (SH)	0.5	-	0.4	1416
(*E*)-Caryophyllene (SH)	-	0.2	-	1419
Calarene (SH)	0.9	0.2	1.1	1433
α-Guaiene (SH)	t	-	-	1439
α-Humulene (SH)	-	t	-	1452
(*Z*)-Methyl-isoeugenol (OS)	4.2	4.6	4.5	1453
γ-Curcumene (SH)	-	t	-	1480
Germacrene D (SH)	0.1	-	0.1	1485
6-Epishyobunone (OS)	12.5	2.2	-	1488
Dehydroxy-isocalamendiol (OS)	-	-	1.9	1497
Bicyclogermacrene (SH)	0.1	-	-	1500
Isoshyobunone (OS)	4.0	0.6	-	1510
δ-Cadinene (SH)	0.5	0.1	0.1	1524
α-Calacorene (SH)	0.3	0.1	-	1544
α-Elemol (OS)	0.1	-	-	1549
Elemicin (PP)	0.6	-	-	1554
(*E*)-Isoelemicin (OM)	4.9	4.5	-	1570
Y-Asarone (PP)	-	0.4	-	1574
β-Oplopenone (OS)	0.5	-	-	1607
(*Z*)-Asarone (PP)	4.0	-	-	1617
β-Asarone (PP)	62.6	85.8	74.3	1617
Z-Isoelemicin (OM)	-	-	4.2	1644
α-Cadinol (OS)	0.8	0.2	-	1654
Cedrenyl acetate (OS)	-	0.6	-	1668
α-Asarone (PP)	-	-	2.3	1676
Isocalamenediol (OS)	0.5	0.1	-	1690
Cyclohexasiloxane (OS)	-	t	-	1710
Khusinol acetate (OS)	0.3	-	-	1823
Total	96.0	93.4	94.4	

ACMREO: *A. calamus* Munsiyari rhizomes essential oil, ACPREO: *A. calamus* Pantnagar rhizomes essential oil, ACAREO: *A. calamus* Assam rhizomes essential oil, MH: Monoterpenoids hydrocarbons, SH: Sesquiterpenoids hydrocarbons, OM: Oxygenated monoterpenoid, OS: Oxygenated sesquiterpenoids, PP: Phenylpropanoid, t = Trace compounds (<0.1).

All of the water molecules were eliminated by introducing Kollmann charges and polar hydrogens in proteins. Using the graphical user interface program (AutoDock Tools), the pdbqt files were prepared for protein and ligands and a grid map was created using a grid box called AutoGrid (grid space of 0.375 Å and grid size set to 40×40×40 points). Cluster analysis was used to evaluate the docked results with an RMS (root mean square) tolerance of 2.0. The docking score used to express the binding affinity of the test ligands (compounds) with the receptors (protein) indicates the estimated binding free energy (B.E.) (kcal/mol) in negative terms, which is reported as the enzyme inhibition constant (Ki) value. Higher calculated binding free energy values in negative terms or lower enzyme inhibition constant values suggest higher ligand-receptor binding affinities [31].

2.7. In-silico ADME/Tox studies

A computer-based drug design method called ADME/Tox analysis is a virtual and preliminary stage of drug discovery. It is presumed that due to the limited knowledge about the characteristics of absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox), a promising potential for a future drug is possible. To predict the pharmacokinetic characteristics and the safety profile of the discovered drugs/phyto-constituents, ADME/Tox profiling using web-based software (SwissADME and pkCSM) was used to reduce the timeline and expensive experiments compared to traditional methods [32,33]. The main compounds identified compounds like β-asarone, *Z*-methyl isoeugenol, 6-epishyobunone, (*Z*)-asarone, (*E*)-isoelemicin, and shyobunone from different accessions of *A. calamus* were studied for their *in silico* potential.

2.8. Statistical analysis

Each treatment was repeated three times and the experimental data were expressed as mean±standard deviation. ANOVA was used to analyze the experimental data for nematicidal, insecticidal, and herbicidal activities at a 1% level of significance ($p < 0.01$). The data examined were significantly different at the respective level of significance.

3. Results and discussion

3.1. GC-MS analysis of EOs from A. calamus accessions

More than 27 constituents were identified in ACAREO that contributed 96.0% of total oil. Based on the class composition, ACAREO was found to be potentially dominant in phenylpropanoids (PP) (71.4 %) followed by oxygenated sesquiterpenoids (OS) (18.7 %), oxygenated monoterpenoids (OM) (5.9%), sesquiterpenoid hydrocarbons (SH) (2.7%) and monoterpenoid hydrocarbons (MH) (1.0%). β-Asarone (62.6%) was found to be the main component of the oil, followed by 6-epishyobunone (12.5%), (*E*)-isoelemicin (4.9%), (*Z*)-methyl-isoeugenol (4.2%), (*Z*)-asarone (4.0%) isoshyobunone (4.0%) and multiple other constituents in a low percentage, β-*trans*-ocimene (1.0%), linalool (1.0%), α-cadinol (0.8%), calarene (0.9%), elemicin (0.6%), δ-cadinene (0.5%), β-oplopenone (0.5%), isocalamenediol (0.5%), aristolene (0.5%), *etc*. mentioned in Table 1.

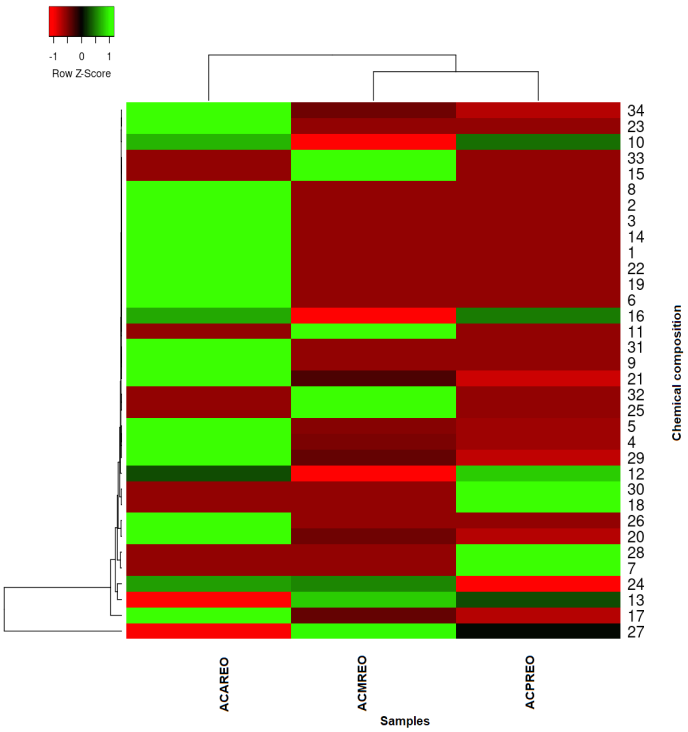


Figure 1. Heat map for the comparative study of ACAREO, ACMREO, and ACPREO.

The persual of Table 1 revealed that in ACMREO more than 18 components representing 93.4% of the total oil composition were identified with the class composition of potentially rich PP (90.8%) followed by OM (4.6 %), OS (1.5%), SH (0.6%) and MH (0.2%). *β*-asarone (85.8%) was found to be the main component of the oil, followed by (*Z*)-methyl isoeugenol (4.6%), (*E*)-isoelemicin (4.5%), 6-epishyobunone (2.2%) and the other minor components (< 1.0%) identified were isoshyobunone (0.6%), cedrenyl acetate (0.6%), γ-asarone (0.4%), calarene (0.2%), (*E*)-caryophyllene (0.2%), β-*trans*-ocimene (0.2%), and α-cadinol (0.2%).

Similarly, more than 12 constituents with 94.4% constituents were identified in ACPREO along with their class composition revealing PP (81.1%) followed by OS (7.3%), OM (4.2%), SH (1.7%), and MH (0.1%). Similarly, to ACAREO and ACMREO, β-asarone (74.3%) was found to be the main component in ACPREO followed by shyobunone (5.4%), (*Z*)-methyl isoeugenol (4.5%), isoelemicin (4.2%), α-asarone (2.3%), dehydroxy-isocalamendioil (1.9%) and calarene (1.1%). The minor constituents (< 1.0%) were aristolene (0.4%), β-*trans*-ocimene (0.1%), and germacrene-D (0.1%).

Comparing the phytochemical analysis of ACAREO, ACMREO, and ACPREO from different ecological regions, it was observed that the EOs were dominated by PP with 67.4% in ACAREO, 94.0% in ACMREO and 81.1% in ACPREO, respectively. Common dominating compounds identified were β-asarone, (*Z*)-methyl-isoeugenol, *Z*-asarone, γ-asarone, (*E*)-isoelemicin, and α-asarone, *etc*. The EOs were found to vary quantitatively; OS contributed 18.7% in ACAREO, 1.5% in ACMREO, and 7.3% in ACPREO, while the contribution of OM contributed 5.9% in ACAREO, 4.6% in ACMREO and 4.2% in ACPREO. SH (2.8, 0.7, and 1.7%) and MH (1.2, 0.3, and 0.1%) could be identified in ACAREO, ACMREO, and ACPREO, respectively. The main OS identified were 6-epishyobunone, isoshyobunone, shyobunone, and dehydroxy-isocalamendiol, while the OM was identified as (*E*)-isoelemicin and (*Z*)-isoelemicin. EOs varied in terms of quantity viz; β-asarone (62.6, 85.8, and 74.3%), (*Z*)-methyl-isoeugenol (4.2, 4.6, and 4.5%), β-*trans*-ocimene (1.0, 0.2, and 0.1%), linalool (1.0, 0.1, and 0.01%), calarene (0.9, 0.2, and 1.1%) and δ-cadinene (0.5, 0.1, and 0.1%), among ACAREO, ACMREO, and ACPREO, respectively. In terms of qualitative makeup, 6-epishyobunone, (*E*)-isoelemicin, and isoshyobunone identified in ACAREO and ACMREO, were absent in ACPREO. The isomeric compounds of β-asarone like (*Z*)-asarone were present only in ACAREO, whereas α-asarone, dehydroxy-isocalamendiol, shyobunone, and *Z*-isoelemicin were only identified in ACPREO. The detailed comparative qualitative and quantitative composition of the identified constituents is depicted in Table 1.

Two main bioactive aromatic constituents have been identified from the rhizomes of *A. calamus* as (α)-asarone and (β)-asarone beside calamenol, calameon and calamen [34]. β-asarone from the wild collection (85.34%) and cultivated collections (78.49%) have been reported from *A. calamus* rhizomes [35]. 50 populations of *A. calamus* species have been reported to be collected from the Indian Himalayan Region (IHR) to study natural variations in β-asarone concentration, ploidy level, and genetic divergence [36]. A significant relationship was observed between the concentration of β-asarone and its distribution along the latitudinal and longitudinal ranges, but not with ecological factors (mean annual temperature and mean annual precipitation), *etc*. Previously, the chemical composition was along with biological activities such as antioxidants, antibacterial, *etc*. in the EOs from *A. calamus* rhizomes and leaves have been reported from our laboratory [37-41] with PP as the main component, particularly β-asarone. The present study also reveals the presence of β-asarone along with other major phenyl propanoids and terpenoids. The reported results on the molecular and genetic levels were found to be in total agreement with our current and previous reports that the level of ploidy is primarily responsible for the biosynthesis of β-asarone in plants rather than climatic conditions [36]. However, the qualitative and quantitative variation in other constituents could possibly be due to environmental factors such as altitude, temperature, soil, *etc*.; α-asarone, and β-asarone have been reported to be the main bioactive component of the chemical composition of *A. calamus* EO [42].

Table 2. *In vitro* egg hatching inhibition and nematicidal mortality of EOs and their combinations against *M. incognita* *.

Doses of EOs	ACAREO	ACMREO	ACPREO	AC(A:M)REO (1:1)	AC(M:P)REO (1:1)	AC(A:P)REO (1:1)	AC(A:M:P)REO (1:1:1)
Egg hatching inhibition							
50 ppm	50.3±4.0^{a}	25.4±1.7^{a}	41.2±2.43^{a}	39.8±3.67^{a}	44.9±2.13^{b}	49.7±1.5^{a}	34.8±3.0^{b}
100 ppm	38.0±3.76^{ab}	16.5±1.67^{a}	30.6±2.7^{a}	33.1±4.03^{a}	35.7±3.06^{ab}	46.0±2.0^{a}	25.0±3.63^{ab}
150 ppm	24.4±3.23^{bc}	8.7±1.67^{a}	18.9±4.56^{ab}	24.1±3.76^{a}	29.5±3.67^{ab}	38.7±2.5^{a}	20.1±3.2^{ab}
200 ppm	18.1±3.36^{d}	8.6±1.73^{a}	14.9±1.36^{b}	21.6±2.5^{a}	20.5±2.5^{a}	33.7±3.5^{a}	6.9±2.53^{a}
Control	149.4±3.1^{e}	149.4±3.1^{b}	149.4±3.1^{c}	149.4±3.1^{b}	149.4±3.1^{c}	149.4±3.1^{b}	149.4±3.1^{c}
Nematicidal mortality							
50 ppm	7.6±1.73^{c}	19.4±2.73^{d}	8.4±2.03^{b}	38.6±2.06^{c}	46.6±2.2^{c}	32.1±2.83^{c}	50.0±1.23^{c}
100 ppm	24.7±2.76^{b}	30.9±2.9^{cd}	24.0±2.4^{b}	47.8±2.5^{c}	54.7±1.8^{c}	33.3±4.43^{bc}	54.8±1.9^{c}
150 ppm	37.4±2.36^{b}	47.0±3.33^{bc}	45.2±1.83^{a}	53.7±2.56^{b}	58.8±2.56^{b}	44.6±3.43^{b}	66.9±1.83^{b}
200 ppm	52.7±4.76^{a}	55.4±2.63^{ab}	54.1±3.3^{a}	55.6±2.23^{bc}	70.7±2.9^{a}	54.2±2.73^{b}	72.2±3.16^{b}
Control	6.22 ± 1.05^{a}	6.22 ± 1.05^{a}	6.22 ± 1.05^{a}	6.22 ± 1.05^{a}	6.22 ± 1.05^{d}	6.22 ± 1.05^{a}	6.22 ± 1.05^{a}

* Results obtained using three factor CRD Analysis ($p < 0.05$), ACAREO: *A. calamus* Assam rhizomes essential oil, ACMREO: *A. calamus* Munsyari rhizomes essential oil, ACPREO: *A. calamus* Pantnagar rhizomes essential oil, *A. calamus* Assam-Pantnagar rhizomes essential oil (AC(A:P)REO), *A. calamus* Munsyari-Pantnagar rhizomes essential oil (AC(M:P)REO), *A. calamus* Assam-Munsyari rhizomes essential oil, (AC(A:M)REO) and *A. calamus* Assam-Munsyari-Pantnagar rhizomes essential oil(AC(A:M:P)REO. Within a column, mean values (a, b, c, d, e) followed by same letter are not significantly different according to Tukey's and Duncan's test ($p < 0.05$).

The variability of each trait during treatment (ACAREO, ACMREO, and ACPREO) was depicted by a heat map analysis (Figure 1) and showed the variation in EO composition. The distribution of trait was identified by colour, where green colour showed the maximum value of the trait, while red represented the minimum value. All samples were clearly divided into two main clusters on the basis of their chemical classes. In the first cluster, only one ACAREO sample was present; in the second cluster there were two samples from Uttarakhand *viz.* ACMREO and ACPREO, and the numbering represented the chemical constituents.

3.2. Nematicidal activity

Meloidogyne incognita (Nemata: Meloidogynidae) is one of the most important species with a wide range of hosts and distribution. Plant EOs are complex mixtures of organic constituents with different stereochemical behaviour of their constituents, which have a lot of potential for nematode control and can be developed as nematicides by developing derivatives of EO constituents with improved nematicidal activity [43]. The nematicidal activity of EOs was observed in egg hatching and mortality against combinations of *M. incognita* and EO to check the synergistic / anti-agonistic effect of the constituents of EOs.

3.2.1. Effect of EO and their combinations on egg hatching of M. incognita

Compared to the negative control, concentration and time-dependent hatching of *M. incognita* egg masses were observed in EOs. The EOs were tested against juvenile (J_2) larvae of *M. incognita* (root knot nematode). Dose-dependent activity was observed in terms of egg hatching inhibition activity, the rate of egg hatching was inversely proportional to the concentration of EO (minimum egg hatching revealed good activity), while directly proportional to the exposure time period (Table 2). At the highest concentration (200 ppm), AC(A:M:P)REO exhibited the lowest (lesser the egg hatchability, higher the effectivity of EOs) mean egg hatching value of 6.9±2.53, whereas ACAREO exhibited a maximum mean egg hatching value of 18.1±3.36. At the lowest dose of 50 ppm, ACMREO exhibited a minimum mean egg hatching value of 25.4±1.7, whereas ACAREO exhibited the highest mean egg hatching of 50.3±4.0 (Table 2). The mean egg hatching value exhibited by the control was 149.4±3.1.

In terms of IC_{50} values, the overall mean egg hatching order was observed as AC(A:M:P)REO (59.8±1.9 ppm) < AC(M:P)REO (IC_{50} = 59.8±1.5 ppm) < AC(A:M)REO (IC_{50} = 70.5±1.6 ppm) < AC(A:P)REO (IC_{50} = 81.4±1.9 ppm) < ACMREO (IC_{50} = 86.7±2.0 ppm) < ACPREO (IC_{50} = 98.4±1.9 ppm) < ACAREO (IC_{50} = 114.8±3.0 ppm) suggesting the major influence of AC(A:M:P)REO and AC(M:P)REO on egg hatching inhibition compared to other combinations and individual EOs, which could be due to the synergistic effect of major/minor or trace constituents with different quantitative and qualitative makeup of EOs (Figure 2a). Furthermore, the direct correlation of higher % of β-asarone with lower IC_{50} values was observed.

3.2.2. Effect of EO and their combinations on nematicidal mortality of M. incognita

The percentage of mortality of the nematodes was observed directly proportional to the concentration of the oil samples and also directly proportional to the exposure time period. At 200 ppm, AC(A:M:P)REO exhibited the highest mean mortality inhibition value of 72.2±3.16, whereas ACAREO exhibited the lowest mean mortality inhibition value of 52.7±4.76. At the lowest concentration of 50 ppm, similar results were observed where AC(A:M:P)REO exhibited a maximum inhibition value of 50.0±1.23 and ACAREO exhibited a minimum inhibition value of 7.6±1.73 whereas the mean mortality value for the control was found to be 6.22±1.05 as shown in Table 2. β-asarone (85.4±1.1%) and methyl isoeugenol (3.1±0.2%) have been reported to be the main constituents of *A. calamus* EO and exhibited significant nematicidal activity against *M. incognita,* which were found to support the experimental results of the present study [44]. This confirmed that the higher the percentage composition of *β*-asarone, higher the nematicidal potency of EO.

In terms of the overall order of mean mortality, variation in lethal concentration, LC_{50} values were observed as: AC(A:M:P)REO (IC_{50} = 60.7±0.9 ppm) < AC(M:P)REO (IC_{50} = 66.5±1.1 ppm) < AC(A:M)REO (IC_{50} = 77.5±1.1 ppm) < AC(A:P)REO (IC_{50} = 85.1±1.5 ppm) < ACMREO (IC_{50} = 90.2±1.3 ppm) < ACPREO (IC_{50} = 93.8±1.3 ppm) < ACAREO (IC_{50} = 102.2±1.5 ppm). The lowest IC_{50} values of AC(A:M:P)REO and AC(M:P)REO were observed to show higher nematodes mortality compared to other combinations that could be due to the major/minor or trace constituents with different quantitative and qualitative makeup of EOs (Figure 2b).

The nematicidal activities of EOs of 26 plant species and compounds (geranial, isoeugenol, methyl isoeugenol, eugenol, methyl eugenol, and neral) have been reported to exhibit nematicidal activity against the pinewood nematode, *Bursaphelenchus xylophilus* [45]. The nematicidal activity of different plant extracts (*Nicotiana tabacum* L, *Syzygium aromaticum* L, *Piper betle* L and *A. calamus* L) has been reported against *M. incognita* with EC_{50} values 5-10 times lower than synthetic pesticides viz; chlorpyrifos, carbosulfan, and deltamethrin [46,47]. Thus, it can be inferred that mortality was observed to be dose- and time-dependent. The mixture of EOs exhibited higher mean mortality compared to individual EOs due to the addition/synergistic effect of other constituents which were found in accordance with the previously reported data and could act as a potent nematicide.

Table 3. Insecticidal activity of EO and its combinations in mustard aphid (*L. erysimi*) and castor hairy caterpillar (*S. celtis*) *.

Doses of EOs	ACAREO	ACMREO	ACPREO	AC(A:M)REO (1:1)	AC(M:P)REO (1:1)	AC(A:P)REO (1:1)	AC(A:M:P)REO (1:1:1)
L. erysimi							
50 ppm	42.2±3.0^{ab}	51.1±2.9^{ab}	38.9±1.9^{ab}	43.3±2.1^{ab}	29.0±2.0^{a}	38.9±1.8^{a}	54.3±1.7^{b}
100 ppm	48.9±2.1^{ab}	54.3±2.4^{ab}	34.4±1.3^{a}	50.0±1.9^{ab}	42.2±1.3^{ab}	48.9±2.2^{a}	68.9±1.6^{bc}
150 ppm	68.9±1.7^{b}	71.1±1.5^{bc}	65.5±1.3^{bc}	68.0±1.4^{bc}	58.9±1.3^{bc}	54.4±2.4^{a}	83.3±1.3^{c}
200 ppm	76.7±1.3^{b}	95.6±5.0^{c}	80.0±1.7^{c}	81.1±1.1^{c}	81.1±3.4^{c}	57.8±2.3^{a}	84.4±1.1^{c}
Control	8.33±0.4^{a}	8.33±0.4^{a}	8.33±0.4^{a}	8.33±0.4^{a}	8.33±0.4^{a}	8.33±0.4^{a}	8.33±0.4^{a}
S. celtis							
50 ppm	24.3±1.7^{a}	38.2±2.6^{ab}	31.5±1.6^{ab}	20.0±1.3^{ab}	40.0±2.4^{ab}	22.2±1.4^{a}	31.5±1.9^{ab}
100 ppm	31.0±1.4^{ab}	46.7±1.3^{b}	46.7±1.3^{ab}	33.3±1.8^{abc}	55.6±2.0^{b}	31.1±2.0^{ab}	40.4±3.0^{ab}
150 ppm	55.6±2.3^{bc}	80.0±1.3^{c}	40.0±2.0^{b}	51.1±1.4^{bc}	66.6±2.0^{b}	44.4±1.7^{ab}	62.2±1.7^{bc}
200 ppm	68.9±1.4^{c}	84.5±1.1^{c}	75.5±1.0^{c}	67.8±1.5^{c}	68.8±1.0^{b}	58.4±2.6^{b}	80.0±1.3^{c}
Control	15.3±0.8^{a}	15.3±0.8^{a}	15.3±0.8^{a}	15.3±0.8^{a}	15.3±0.8^{a}	15.3±0.8^{a}	15.3±0.8^{a}

* Results obtained using three-factor CRD Anaysis ($p < 0.05$), ACAREO: *A. calamus* Assam rhizomes essential oil, ACMREO: A. calamus Munsyari rhizomes essential oil, ACPREO: *A. calamus* Pantnagar rhizomes essential oil, *A. calamus* Assam-Pantnagar rhizomes essential oil (AC(A:P)REO), *A. calamus* Munsyari-Pantnagar rhizomes essential oil (AC (M:P)REO), *A. calamus* Assam-Munsyari rhizomes essential oil, (AC(A:M)REO), *A. calamus* Assam-Munsyari-Pantnagar rhizomes essential oil (AC(A:M:P)REO. Within a column, mean values (a, b, c) followed by same letter are not significantly different according to Tukey's and Duncan's test ($p < 0.05$).

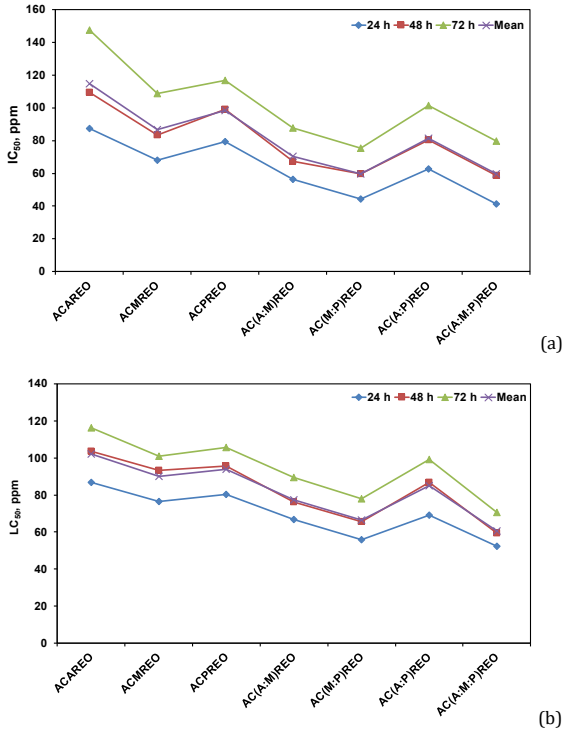


Figure 2. Comparison of *A. calamus* EO samples (a) egg hatching inhibition (IC_{50}) and (b) nematicidal mortality (LC_{50}).

3.3. Insecticidal activity

Insecticide resistance is a genetic phenomenon, and the most reported mutations affect insecticide target proteins and metabolism [48]. In the present study, the insecticidal potential of EOs and their combinations against the insect mustard aphid (*L. erysimi)* and the castor hairy caterpillar (*S. celtis*) to develop environmental friendly insecticides are examined.

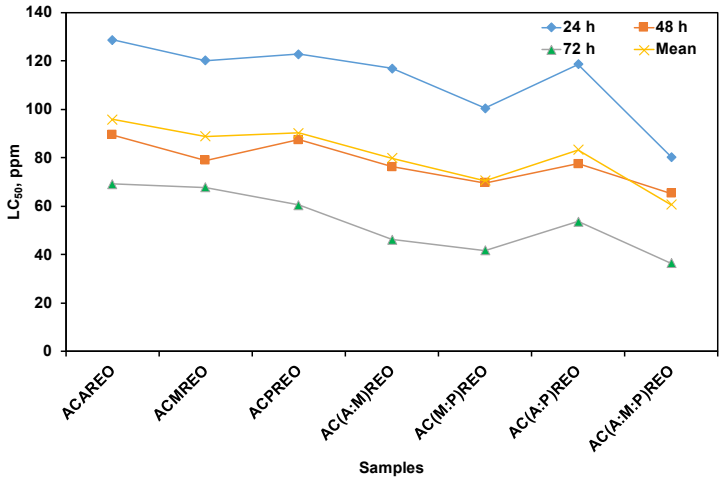
3.3.1. Effect of EO and its combinations on insecticidal activity against L. erysimi

The % mortality of the second instar nymphs of mustard aphid was found to increase with increasing concentration of EOs. The % mortality increased with increase in time and concentration and was found to be maximum after 72 h. The mean mortality of aphids in ACMREO showed a maximum% mortality of 95.6±5.0 whereas ACAREO showed a minimum% mortality of 76.7±1.3. At the dose level of 50 ppm, AC(A:M:P)REO showed a maximum % mortality of 54.3±1.7 and ACAREO showed a minimum % mortality of 42.2±3.0, whereas the % mortality exhibited by the control was 8.33±0.4. The detailed % mortality data against *L. erysimi* has been mentioned in Table 3.

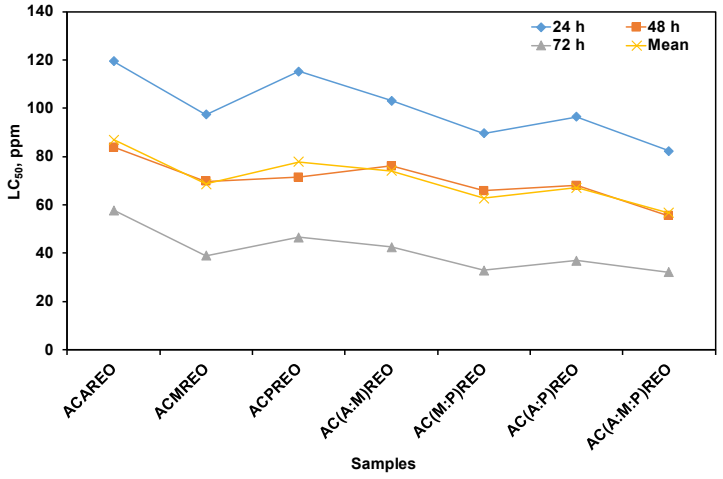
In terms of the overall order of insecticidal activity, variations in LC_{50} values were observed: AC(A:M:P)REO (LC_{50} = 60.7±2.2 ppm) < AC(M:P)REO (LC_{50} = 70.6±2.9 ppm) < AC(A:M)REO (LC_{50} = 79.8±3.5 ppm) < AC(A:P)REO (LC_{50} = 83.3±3.3 ppm) < ACMREO (LC_{50} = 88.9±2.8 ppm) < ACPREO (LC_{50} = 90.2±3.1 ppm) < ACAREO (LC_{50} = 95.8±3.0 ppm) (Figure 3a). The mixture of EOs was found to have lower IC_{50} values compared to individual EOs suggesting the synergistic effect of EOs with higher percent mortality against *L. erysimi.* Furthermore, a direct correlation of a higher percentage of β-asarone with lower IC_{50} values was observed.

3.3.2. Effect of EOs and their combinations on insecticidal activity against S. celtis

It was observed that as the concentration of EO increased, the percent mortality of the insect larvae increased.



(a)



(b)

Figure 3. LC_{50} of *A. calamus* essential oils (a) Mustard aphid (*Lipaphis erysimi*) and (b) castor hairy caterpillar (*Selepa celtis*).

The % mortality also increased over time and exhibited the highest mortality after 72 h. The mean mortality of insect larvae in ACMREO exhibited a maximum % mortality of 84.5±1.1 whereas AC(A:P)REO exhibited a minimum % mortality of 58.4±2.6. At the lowest dose value of 50 ppm, AC(A:M:P)REO exhibited maximum % mortality of 31.5±1.9 and AC(A:M)REO exhibited minimum % mortality of 20.0±1.3 whereas the % mortality exhibited by the control was 15.3±0.8. The minimum inhibition shown by the combination of EOs at 50 and 200 ppm could be due to the antagonistic effect of chemical constituents present in EOs. The ethanolic extract of *A. calamus* has been reported to be toxic against *P. xylostella* [49]. α- and β-asarone present in *A. calamus* were not completely responsible for the larvicidal activity of the EO, but other constituents also played a significant role in the rendering of toxicity. Similarly, in the present study, EOs with varying % of *β*-asarone was not solely responsible for the mortality of insect larvae, but other minor EO constituents could have shown a synergistic / antagonistic effect with each other. Detailed% mortality data against *S. celtis* have been mentioned in Table 3.

In the present study, the toxicity (LC_{50}) of individual and combination EO against *Selepa celtis* was studied at 24, 48, and 72 h. The LC_{50} values were observed: AC(A:M:P)REO (LC_{50} = 56.7±2.5 ppm) < AC(M:P)REO (LC_{50} = 62.8±2.8 ppm) < AC(A:P)REO (LC_{50} = 67.1±2.9 ppm) < ACMREO (LC_{50} = 68.7±2.9 ppm) < AC(A:M)REO (LC_{50} = 74.0±3.0 ppm) < ACPREO (LC_{50} = 77.8±3.5 ppm) < ACAREO (LC_{50}= 87.0±3.1 ppm) (Figure 3b). Although no particular pattern was observed in the mean mortality of insect larvae, the IC_{50} values of the mixture of EOs were found to be the lowest due to the addition/synergistic effect of EOs. Furthermore, a direct correlation of % of β-asarone with lower IC_{50} values was observed. EOs of *A. calamus* have been reported to have insecticidal activities against a different group of insects; α- and β-asarone have been reported to be potent growth inhibitors and antifeedants [50]. The EO of the *A. calamus* rhizomes and its constituents have been reported to develop natural fumigants/insecticides for insect pest control [51].

To our knowledge, no previous studies have been reported on the insecticidal activity of *A. calamus* against mustard aphid (*L. erysimi*) and castor hairy caterpillar (*S. celtis*). However, various insecticidal activities against different pests have been reported *in vitro*. Rhizome-derived (*Z*)-asarone has been reported to exhibit activity against adult *Sitophilus zeamais* [52]. *Cis*-asarone has been reported to cause 83% mortality at 1000 ppm, while *trans*-asarone resulted in 30% mortality at 1000 ppm against third instar larvae of *P. xylostella* [53] that showed that different isomers exhibit different results. Based on the above findings and reports from the literature, it can be inferred that EO (individually and in combination) from *A. calamus* accessions loaded with different secondary metabolites, such as terpenoids / phenylpropanoids, have different potentials for insecticidal activity.

Table 4. % Seed germination, root and shoot length inhibition of EOs and their combinations against *R. raphanistrum* *.

Samples (ppm)	Mean seed germination	Mean shoot length (cm)	Mean root length (cm)
ACAREO			
50	7.4±0.48^{bc}	6.3±0.8^{bc}	1.9±0.2^{a}
100	6.4±0.6^{ab}	4.8±0.6^{b}	1.8±0.3^{a}
150	5.9±0.68^{ab}	3.7±0.6^{a}	1.1±0.2^{a}
200	4.5±1.57^{a}	3.7±0.4^{a}	0.6±0.1^{a}
Control	9.06±0.6^{c}	8.9±0.3^{c}	3.9±0.4^{b}
ACMREO			
50	7.1±1.16^{bc}	6.1±0.2^{bc}	1.2±0.5^{a}
100	6.0±0.68^{ab}	4.3±0.4^{ab}	1.0±0.3^{a}
150	5.5±0.68^{ab}	3.0±0.3^{a}	0.9±0.4^{a}
200	4.4±0.78^{a}	2.4±0.2^{a}	0.4±0.2^{a}
Control	9.06±0.6^{c}	8.9±0.3^{c}	3.9±0.4^{b}
ACPREO			
50	7.3±0.68^{bc}	6.3±0.5^{b}	1.6±0.2^{a}
100	6.1±0.68^{ab}	4.9±0.3^{ab}	1.3±0.1^{a}
150	5.5±0.68^{ab}	4.1±0.2^{a}	0.7±0.3^{a}
200	4.5±0.94^{a}	3.1±0.2^{a}	0.3±0.1^{a}
Control	9.06±0.6^{c}	8.9±0.3^{c}	3.9±0.4^{b}
AC(A:M)REO			
50	6.3±0.36^{a}	5.1±0.2^{b}	1.4±0.05^{a}
100	5.4±0.24^{a}	3.6±0.2^{ab}	1.0±0.1^{a}
150	4.5±0.48^{a}	2.6±0.5^{a}	0.7±0.1^{a}
200	4.2±1.3^{a}	1.5±0.6^{a}	0.3±0.2^{a}
Control	9.06±0.6^{b}	8.9±0.3^{c}	3.9±0.4^{b}
AC(M:P)REO			
50	5.9±0.68^{a}	5.1±0.2^{b}	1.1±0.2^{a}
100	5.1±0.68^{a}	3.6±0.2^{ab}	0.8±0.05^{a}
150	4.3±0.68^{a}	2.6±0.5^{a}	0.6±0.1^{a}
200	4.0±0.68^{a}	1.5±0.6^{a}	0.3±0.05^{a}
Control	9.06±0.6^{b}	8.9±0.3^{c}	3.9±0.4^{b}
AC(A:P)REO			
50	6.6±0.92^{b}	5.5±0.5^{a}	1.5±0.1^{a}
100	5.9±0.68^{ab}	4.6±0.3^{a}	1.2±0.1^{a}
150	5.0±1.16^{ab}	4.1±0.2^{a}	0.9±0.1^{a}
200	4.3±0.94^{a}	3.2±0.3^{a}	0.6±0.1^{a}
Control	9.06±0.6^{c}	8.9±0.3^{b}	3.9±0.4^{b}
AC(A:M:P)REO			
50	5.9±0.68^{b}	4.3±0.5^{a}	1.0±0.2^{a}
100	4.8±0.76^{ab}	3.3±0.3^{a}	0.6±0.2^{a}
150	4.1±0.76^{ab}	2.4±0.2^{a}	0.4±0.1^{a}
200	3.6±0.36^{a}	1.5±0.4^{a}	0.2±0.05^{a}
Control	9.06±0.6^{c}	8.9±0.3^{b}	3.9±0.4^{b}
Pendimethalin			
50	0.8±0.24^{a}	0.2±0.05^{a}	0.7±0.05^{a}
100	0.7±0.24^{a}	0.1±0.09^{a}	0.4±0.01^{a}
150	1.0±0.48^{a}	0.0±0.08^{a}	0.3±0.005^{a}
200	0.4±0.36^{a}	0±0.005^{a}	0.0±0^{a}

* Results obtained using three-factor CRD Anaysis ($p < 0.05$), ACAREO: *A. calamus* Assam rhizomes essential oil, ACMREO: *A. calamus* Munsyari rhizomes essential oil, ACPREO: *A. calamus* Pantnagar rhizomes essential oil, *A. calamus* Assam-Pantnagar rhizomes essential oil (AC(A:P)REO), *A. calamus* Munsyari-Pantnagar rhizomes essential oil(AC (M:P)REO), *A. calamus* Assam-Munsyari rhizomes essential oil, (AC(A:M)REO) and *A. calamus* Assam-Munsyari-Pantnagar rhizomes essential oil(AC(A:M:P)REO. Within a column, mean values (a, b, c) followed by same letter are not significantly different according to Tukey's and Duncan's test ($p < 0.05$).

3.4. Herbicidal activity

Herbicides are chemicals that can harm or kill unwanted plants, primarily weeds. Natural compounds derived from allelopathic and medicinal plant residues may help reduce the use of synthetic herbicides for weed control, resulting in reduced pollution and safer agricultural products [54]. In the present study, the herbicidal activity of EOs and their combinations were tested against *R. raphanistrum,* major weed in summer crops.

3.4.1. Effect of EO and its combinations on herbicidal potential against R. raphanistrum for seed germination

It was observed that as the concentration of the EOs increased, the percentage of seed germination inhibition increased. The% inhibition of seed germination also increased over time and exhibited maximum inhibition after 72 h. Combinations of EOs in definite proportions were found to exhibit higher inhibition compared to individual EOs for herbicidal activity. Perusal of Table 4 revealed that at the highest dose level of 200 ppm, AC(A:M:P)REO exhibited maximum herbicidal activity of 3.6±0.36 whereas ACAREO exhibited minimum herbicidal activity of 4.5±1.57. Similarly, at the lowest dose level of 50 ppm, AC(A:M:P)REO exhibited maximum herbicidal activity of 5.9±0.68, whereas ACAREO exhibited minimum herbicidal activity of 7.4±0.48 against *R. raphanistrum.* Pendimethalin (standard) exhibited the lowest mean seed germination of 0.4±0.36 and 0.8±0.24 at a dose value of 200 and 50 ppm, while the negative control exhibited a mean value of 9.06±0.6. Detailed% seed germination inhibition against *R. raphanistrum* is mentioned in Table 4. The data mentioned above on the herbicidal activity of *A. calamus* were found to be consistent with the findings of a previous study in which the allelopathic potential of rhizome oil showed inhibition of seed germination of *Lactuca sativa* and *Lolium perenne* with IC_{50} values of 450 and 737 µg/mL, respectively [55].

The general order of individual EOs and their combinations of IC_{50} values were in the following order: Pendimethalin (LC_{50} = 16.8±0.8 ppm) < AC(A:M:P)REO (LC_{50} = 47.6±0.9 ppm) < AC (M:P) REO (LC_{50} = 61.0±1.4 ppm) < AC (A:M) REO (LC_{50} = 78.9±1.2 ppm) < AC (A:P) REO (LC_{50} = 94.2±1.6 ppm) < ACMREO (LC_{50} = 110.0±1.8 ppm) < ACPREO (LC_{50} = 118.9±1.7 ppm) <

Table 5. Molecular docking analysis of the main compounds of *A. calamus* EOs against target proteins from various pesticidal activities *.

Compounds	ΔGb	Ki	I. E.	B.E.	RMSD	Residual Interactions		
						Van der Waals	Pi-alkyl	Pi-sigma
PDB ID: IC2O								
β-Asarone	-6.51	16.77	-7.71	-7.66	96.34	GlY441, MET436, ASP72, SER81, GLY80, ASN85	ILE439, TYR442, HIS440, TRP84, PHE330, TYR334	TRP 432
(Z)-Methyl isoeugenol	-5.85	51.50	-6.75	-6.80	89.38	TYR442, GLY80, GLY441, HIS440, TYR442	PHE330, TRP84, MET436, ILE439	TRP432
PDB ID: 3QQQ								
β-Asarone	-5.14	171.50	-6.31	-6.26	86.45	GLN63, GLU62, ASN64, ALA30, GLY33	PRO65, LYS68, LEU34, PHE37, LEU67	-
(Z)-Methyl isoeugenol	-5.42	107.09	-6.33	-6.29	90.32	ALA47, HIS114, THR118, LEU50	PHE51, HIS105, HIS70, ILE40, PHE37, PHE41	VAL110
PDB ID: 5F3Y								
β-Asarone	-5.26	140.20	-6.31	-6.27	88.56	GLU1166, LYS1173, ALA1169, ASP1193, GLU1147, ARG1151, ALA1195, LEU1117, THR1152, SER1194, GLY1118, SER1114	VAL1170, ARG1148, LEU1115	PHE1144
(Z)-Methyl isoeugenol	-5.42	107.13	-6.45	-6.40	91.43	GLY1233, GLY1231, GLY1216, ASN1214, SER1218, PHE1217, GLU1267, ARG139	LEU1215, PHE1268, SER1232 (pi donor hydrogen bond)	-
PDB ID: 3HNR								
β-Asarone	-5.36	118.70	-6.55	-6.51	91.47	GLY119, SER122, GLY123, GLU199, SER200	PHE331, TYR121, TRP84, HIS440	GLY118
(Z)-Methyl isoeugenol	-5.35	120.14	-6.24	-6.20	91.31	ASN525, ASP397, HIS406, ASN230, PRO232, GLU306	CYS402, PRO403, TRP524, HIS398	
PDB ID: 3HNR								
Pendamethalin	-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

* Gibbs free energy, ΔG; Estimated inhibition constant, Ki; Intermolecular energy, I.E.; Binding energy, B.E.; Root mean square deviation, R.M.S.D.; Residual interactions represent amino acid interactions that form Vander Waals, pi-alkyl, pi-sigma interactions with the ligand.

ACAREO (LC_{50} = 138.6±2.7 ppm) which revealed that AC(A:M:P)REO was more active than other combinations due to the synergistic effect of the major/minor constituents as shown in Figure 4. Among the individuals and combinations of EOs, minimum egg hatching was exhibited by the combination of EOs.

3.4.2. Effect of EOs and their combinations on herbicidal potential against R. raphanistrum for root and shoot length

The concentration of EOs showed an inverse relationship with the growth of the roots and shoots of *R. raphanistrum*. % root growth and % shoot growth were observed as a function of dose levels for each treatment of EOs. The % shoot growth was observed to be maximum with 1.5±0.4 for AC(A:M:P)REO, while minimum with 3.7±0.4 for ACAREO, at 200 ppm. At the dose level of 50 ppm, AC(A:M:P)REO exhibited a maximum % shoot growth inhibition of 4.3±0.5, whereas ACAREO exhibited a minimum inhibition of 6.3±0.8. The % root growth was observed at maximum with 0.2±0.05 for AC(A:M:P)REO, while at minimum with 0.6±0.1 for ACAREO, at 200 ppm. At the dose level of 50 ppm, AC(A:M:P)REO showed a maximum% inhibition of root growth of 1.0±0.2, whereas ACAREO showed a minimum inhibition of 1.9±0.2. The % shoot growth (0.2±0.05 at 50 ppm and 0±0.005 at 200 ppm) and % root growth for pendimethalin (0.7±0.05 at 50 ppm and 0.0±0 at 200 ppm) were observed higher compared to individual EO and combination of EOs. The mean value of the negative control was found to be 8.9±0.3 and 3.9±0.4 for % shoot and root growth. The detailed results pertaining to the % root and shoot length for EOs are mentioned in Table 4.

To date, no herbicidal activity of *A. calamus* EOs against *R. raphanistrum* has been reported in the literature search. The isolated compounds from *A. calamus* have been reported to exhibit phytotoxicity against *Agrostis stolonifer* and *Lemna paucicostata*), but not *Lactuca sativa* [56]. β-asarone was reported to be primarily responsible for observed herbicidal activity. The results of the present study showed that phenylpropanoids and oxygenated monoterpenoids are abundantly present in EO and could be responsible for herbicidal activities that were found consistent with the results of previous studies [57,58]. It can be inferred that EOs obtained from *A. calamus* individually or in combination or by developing a formulation can be a good source of herbal-based eco-friendly weedicides after their proper clinical trials.

3.5. Molecular docking analysis

In medicinal chemistry and drug discovery, molecular docking is used to predict how two molecules will interact. When the optimum confirmation of the ligand-protein complexes and their relative orientation was explored, docking was found to be helpful for drug design. The enzyme's active site was docked with major bioactive compounds of *A. calamus* from three altitudinal locations. The Gibbs free energy (ΔG), the intermolecular energy (I.E.), the binding energy (B.E.) for each bioactive compound against different protein targets, the inhibition constant (Ki), the root mean square deviation (RMSD), and residual interactions were listed in Table 5. *Z*-methyl isoeugenol exhibited the lowest binding energy of -6.40 kcal/mol (PDB ID: 5F3Y) and -6.29 kcal/mol (PDB ID: 3QQQ) for insecticidal activity against *L. erysimi* and *S. celtis*. The lowest binding energy for the acetylcholinesterase protein present in *M. incognita* for nematicidal activity (PDB ID: 1ODC), was shown by β-asarone (B.E. = -7.66 kcal/mol). Similarly, for *Raphanus sativus* anionic peroxidase for herbicidal potential (PDB ID: 4A5G), the lowest binding energy was exhibited by β-asarone (B.E. = -6.51 kcal/mol), while the binding energy for Pendimethalin (B.E. = -7.50 kcal/mol) was found lower than β-asarone and *Z*-methyl isoeugenol with no major differences in B.E. referring to the effectivity of β-asarone as a potent insecticide. Although not only was one major compound identified that was alone in charge of pesticidal activity, but rather a bioactive mixture of EO, it is possible that the considerable findings observed were caused by the synergistic action of the main / minor constituents of EO. The computed binding energy of each ligand-protein interaction, free energy data, and the inhibition constant values were observed to show direct correlation.

Table 6. Drug likeness and ADME/Tox analysis of the major compounds of *A. calamus* EOs *.

Major compounds	β-Asarone	Shyobunone	(*Z*)-methyl-isoeugenol	6-Epishyobunone	(*E*)- isoelemicin	(*Z*)-asarone
Drug likeness						
Lipinski rule	Yes	Yes	Yes	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55
Absorption						
Water solubility (log mol/L)	-2.536	-4.724	-2.633	-4.724	-2.462	-2.536
$CaCO_2$ Permeability (log Papp in 10^{-6} cm/s)	1.914	1.339	1.794	1.339	1.92	1.914
Human intestinal absorption (% Absorbed)	95.49	97.176	95.485	97.176	95.49	95.49
Skin permeability (log Kp)	-1.885	-1.581	-1.489	-1.581	-1.966	-1.885
P-glycoprotein substrate	No	No	No	No	No	No
Distribution						
VDss (log L/kg)	0.087	0.355	0.213	0.355	0.073	0.087
Fraction unbound (human) (Fu)	0.263	0.265	0.26	0.265	0.261	0.263
BBB permeability (log BB)	0.229	0.629	0.323	0.629	0.229	0.229
CNS permeability (log PS)	-1.993	-2.009	-1.826	-2.009	-1.993	-1.993
Metabolism						
CYP2D6 substrate	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	No	Yes	No	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No
Excretion						
Total clearance	0.441	0.324	0.273	0.324	0.282	0.441
Renal OCT2 substrate	No	No	No	No	No	No
Toxicity						
Hepatotoxicity	0.63	0.69	0.69	0.69	0.69	0.69
Carcinogenicity	0.56	0.62	0.62	0.62	0.62	0.62
Immunotoxicity	0.67	0.96	0.96	0.96	0.96	0.96
Mutagenicity	0.92	0.97	0.97	0.97	0.97	0.97
Cytotoxicity	0.55	0.93	0.93	0.93	0.93	0.93

* Lipinski rule of five; molecular weight < 500 Da, number of H donor bonds ≤ 5, number of H acceptor bonds ≤ 10, number of rotatable bonds < 10, lipophilicity (octanol/water partition coefficient, log P) < 5 and total polar surface area < 140 Å^2; Yes represents not more than one violation; skin permeability is expressed as Log Kp; P-gpI = P-glycoprotein inhibitor; Human intestinal absorption (HIA); BBB (blood-brain barrier) permeability is expressed as log BB (log BB > -1.0 is moderately cross blood-brain barrier); VDss (Volume of distribution should be less than 0.45; Cytochromes (Cyp) of P450 family (CYP2D6 and CYP3A4 substrate, CYP1A2, CYP2C19, CYP2D6, CYP2C9 and CYP3A4 inhibitors); toxicity measured in probability values varying from 0.00 to 1.00.

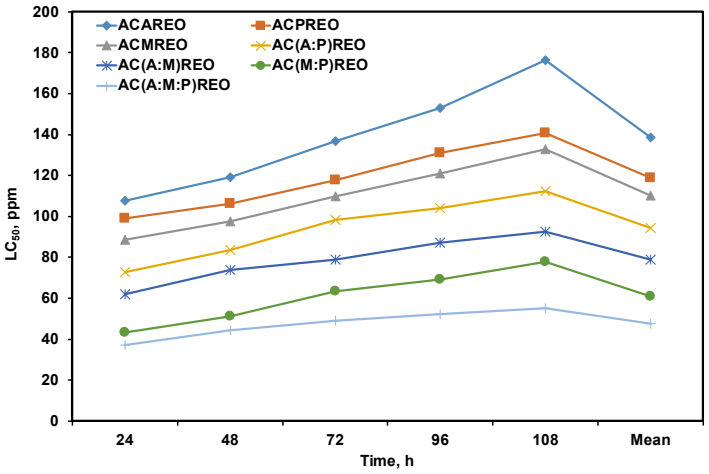


Figure 4. Comparison of seed germination (IC_{50} values) for *A. calamus* EO samples.

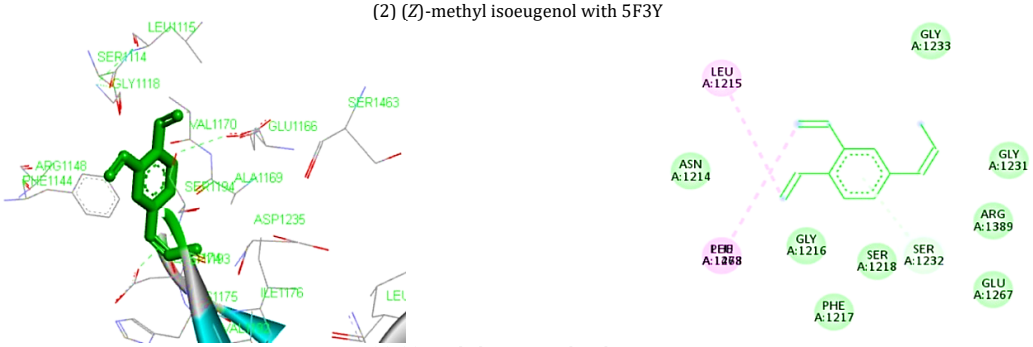
The principal chemical components contained in *A. calamus* EO, according to the aforementioned calculated results, ligand-protein interactions for a variety of pesticidal activities had the lowest binding energies for the main compounds, and these findings were consistent with previously reported *in vitro* results [44,45, 51,57]. In Figure 5, the residual interactions that form Van der Waals, pi-alkyl and pi-sigma interactions, and the strongest ligand-protein interactions, as indicated by the lowest binding energies, are depicted in 3D docked conformations. These interactions showed the strength and catalytic activity of the binding complex.

3.6. ADME/Tox studies analysis

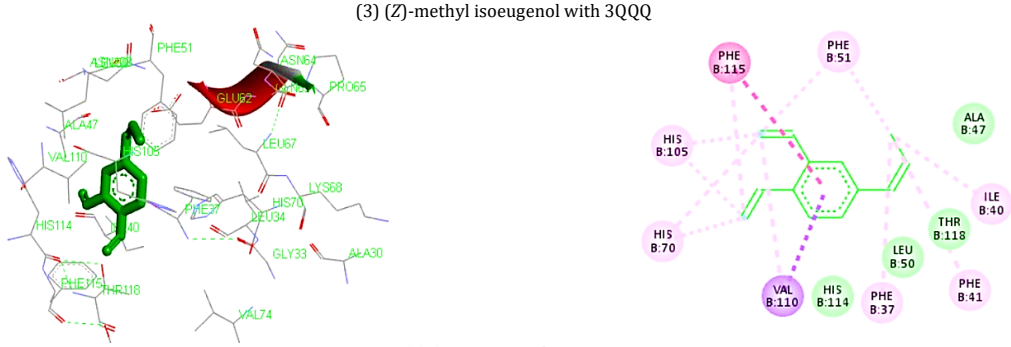
To evaluate a drug for safety and efficacy, which is essential for regulatory approval, drug developers can use ADME features. Absorption was predicted from % of human intestinal absorption (HIA), lipophilicity, and water solubility of the compound. The prediction of drug likeness was done using the Lipinski rule of five, in which the main compound of *A. calamus* EOs did not violate more than one rule to exhibit drug-like behavior [59], thus it would be considerable for absorption (Table 5).

(1) *β*-asarone with 1C2O

(2) (*Z*)-methyl isoeugenol with 5F3Y



(3) (*Z*)-methyl isoeugenol with 3QQQ



(4) β-asarone with 3HNR

(5) Pendimethalin with 3HNR

Figure 5. Schematic illustration of the three-dimensional interactions of the main components EO of *A. calamus* docked into the active site of selected proteins involved in various pesticidal activities. The interactions are based on the lowest binding energies and showed the strongest interactions with the amino acid residues at the binding site. (1) β-asarone with 1C2O (*M. incognita*), (2) (*Z*)-methyl isoeugenol with 5F3Y (*L. erysimi*), (3) (*Z*)-methyl isoeugenol with 3QQQ (*Selepa celtis*), (4) β–asarone with 3HNR (*Raphanus sativus*), (5) Pendimethalin with 3HNR (*Raphanus sativus*).

The logarithmic Kp values for all major compounds were found within in the range of -1.48 to -1.96 cm/s, inferring low skin permeability. The distribution was also predicted for β-asarone, shyobunone, (*Z*)-methyl-isoeugenol, 6-epishyobunone, (*E*)-isoelemicin and (*Z*)-asarone that showed high Gastro intestinal absorption and no permeability of the blood brain barrier. None of the tested constituents were observed to act as a substrate for permeability glycoprotein (P-gp). Metabolism was predicted based on the inhibitor interaction, that is, the cytochromes of the P450 family (CYP1A2, CYP2C19, CYP2D6, CYP2C9 and CYP3A4 inhibitors) involving the mechanism in which different drugs compete for the same enzyme binding site and inhibition of cytochromes could lead to drug efficacy or toxicity (Table 6). Excretion and toxicity were predicted using the Web-based online tool Protox II, where LD_{50} values and toxicological parameters (hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity) were predicted for the main compounds that did not show toxicity [60]. Based on the ADMET study, we can conclude that *A. calamus* EOs and their combinations can be considered effective and efficacious against a therapeutic target and show a good pharmacokinetic profile.

4. Conclusion

The results of the present experimental work using plant-based EOs individually and in combination compared to synthetic pesticides against insect pests, weeds, and nematodes responsible for crop damage were summarised and interpreted. The combination of EOs was found to exhibit a potent synergism in modulating crop-associated pesticides due to the presence of the main/minor constituents of EOs compared to individual EOs. β-Asarone was the chemical biomarker in all EOs of *A. calamus* from three different ecological niches. The current study demonstrates that *A. calamus* EO from three different ecological niches was found to be rich in phenyl propanoids (67.4% in ACAREO, 94.0% in ACMREO and 81.1% in ACPREO), and identified β-asarone, *Z*-methyl isoeugenol, 6-epishyobunone, (*E*)-isoelemicin and shyobunone as major components of EO. After evaluating the pesticidal evaluation of EOs, significant results were obtained in nematicidal and herbicidal activities, while excellent results were observed in insecticidal activity, which could be due to the presence of the aforementioned EO constituents and their addition or synergistic effects. These results could be attributed to the presence of the aforementioned EO constituents and their combination or synergistic effects. Additionally, computer-assisted PASS prediction confirmed *in vitro* pesticidal activity, and ADME/Tox tests distinctively showed that the main components of *A. calamus* have drug-like qualities without toxicity.

Acknowledgements

The authors acknowledge the support of lab facilities provided by Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, and are thankful to the Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, India, for providing the required GC-MS facility for the analysis of the essential oil samples.

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered to.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Om Prakash, Ravendra Kumar; Methodology: Tisha Joshi, Om Prakash, Ravendra Kumar, Ravi Mohan Srivastava, Satya Kumar, Shilpi Rawat; Software: Kirti Nagarkoti, Avneesh Rawat; Validation: Navadha Joshi, Om Prakash, Ravendra Kumar, Ravi Mohan Srivastava, Satya Kumar, Shilpi Rawat, Dharmendra Singh Rawat; Formal Analysis: Kirti Nagarkoti, Navadha Joshi, Avneesh Rawat; Investigation: Tisha Joshi; Resources: Tisha Joshi, Om Prakash, Ravendra Kumar, Ravi Mohan Srivastava, Satya Kumar, Shilpi Rawat; Data Curation: Tisha Joshi, Kirti Nagarkoti, Om Prakash; Writing - Original Draft: Tisha Joshi, Kirti Nagarkoti; Writing - Review and Editing: Tisha Joshi, Kirti Nagarkoti, Om Prakash; Visualization: Tisha Joshi, Kirti Nagarkoti, Om Prakash; Funding acquisition: Tisha Joshi; Supervision: Om Prakash, Ravendra Kumar; Project Administration: Om Prakash.

ORCID and Email

Tisha Joshi
tishajoshi1998@gmail.com
https://orcid.org/0000-0002-6951-3993
Kirti Nagarkoti
kirti.nagarkoti121@gmail.com
https://orcid.org/0000-0002-7332-1265
Navadha Joshi
joshinavadha@gmail.com
https://orcid.org/0000-0003-2343-1121
Avneesh Rawat
aveeneshrawat@gmail.com
https://orcid.org/0000-0003-1142-0994
Om Prakash
oporgchem@gmail.com
https://orcid.org/0000-0002-2767-6997
Ravendra Kumar
ravichemistry.kumar@gmail.com
https://orcid.org/0000-0001-7299-1761
Ravi Mohan Srivastava
ravimohanento@gmail.com
https://orcid.org/0000-0002-0216-117X
Satya Kumar
skumar8326@rediffmail.com
https://orcid.org/0000-0002-2555-4769
Shilpi Rawat
rawatshilpi19@gmail.com
https://orcid.org/0000-0002-8606-0885
Dharmendra Singh Rawat
drdsrawat.alpinist@gmail.com
https://orcid.org/0000-0003-2613-2709

References

[1]. Agboola, A. R.; Okonkwo, C. O.; Agwupuye, E. I.; Mbeh, G. Biopesticides and conventional pesticides: Comparative review of mechanism of action and future perspectives. *AROC Agric.* **2022**, *1*, 14–32.
[2]. Chawla, M.; Kaushik, R. D.; Malik, M. K.; Pundir, V.; Singh, J.; Rehmaan, H. Development and optimization of RofA-PAMAM dendrimer complex materials for sustained drug delivery. *Mater. Today Commun.* **2022**, *33*, 104881.
[3]. Ngegba, P. M.; Cui, G.; Khalid, M. Z.; Zhong, G. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture* **2022**, *12*, 600.
[4]. Kumar Malik, M.; Kumar, T.; Kumar, V.; Singh, J.; Kumar Singh, R.; Saini, K. Sustainable, highly foldable, eco-friendly films from Mandua starch derivative. *Sustain. Energy Technol. Assessments* **2022**, *53*, 102398.
[5]. Malik, M. K.; Bhatt, P.; Singh, J.; Kaushik, R. D.; Sharma, G.; Kumar, V. Preclinical safety assessment of chemically cross-linked modified mandua starch: Acute and sub-acute oral toxicity studies in Swiss albino mice. *ACS Omega* **2022**, *7*, 35506–35514.
[6]. Nagarkoti, K.; Kanyal, J.; Prakash, O.; Kumar, R.; Rawat, D. S.; Pant, A. K. *Ajuga*L.: A systematic review on chemical composition, phytopharmacological and biological potential. *Curr. Bioact. Compd.* **2021**, *17*, e010621189843.
[7]. Acheuk, F.; Basiouni, S.; Shehata, A. A.; Dick, K.; Hajri, H.; Lasram, S.; Yilmaz, M.; Emekci, M.; Tsiamis, G.; Spona-Friedl, M.; May-Simera, H.; Eisenreich, W.; Ntougias, S. Status and prospects of botanical biopesticides in Europe and Mediterranean countries. *Biomolecules* **2022**, *12*, 311.
[8]. Rana, S.; Dixit, S. Screening of phytochemicals in citrus limonum peel extract to evaluate its antimicrobial potential. *International Journal of Natural Products Research* **2017**, *7*, 7–16.

[9]. Chugh, C. A.; Bharti, D. Chemical characterization of antifungal constituents ofEmblica officinalis. *Allelopathy Journal* **2014**, *34*, 155–178.

[10]. Rana, S.; Dixit, S.; Mittal, A. In silico target identification and validation for antioxidant and anti-inflammatory activity of selective phytochemicals. *Braz. Arch. Biol. Technol.* **2019**, *62*, e19190048.

[11]. Malik, M. K.; Bhatt, P.; Kumar, T.; Singh, J.; Kumar, V.; Faruk, A.; Fuloria, S.; Fuloria, N. K.; Subrimanyan, V.; Kumar, S. Significance of chemically derivatized starch as drug carrier in developing novel drug delivery devices. *Nat. Prod. J.* **2022**, *12*, e190822207708.

[12]. Malik, M. K.; Kumar, V.; Sharma, P. P.; Singh, J.; Fuloria, S.; Subrimanyan, V.; Fuloria, N. K.; Kumar, P. Improvement in digestion resistibility of mandua starch (Eleusine coracana) after cross-linking with epichlorohydrin. *ACS Omega* **2022**, *7*, 27334–27346.

[13]. Khwairakpam, A. D.; Damayenti, Y. D.; Deka, A.; Monisha, J.; Roy, N. K.; Padmavathi, G.; Kunnumakkara, A. B. *Acorus calamus*: a bio-reserve of medicinal values. *J. Basic Clin. Physiol. Pharmacol.* **2018**, *29*, 107–122.

[14]. Abdul Kareem, V. K.; Rajasekharan, P. E.; Ravish, B. S.; Mini, S.; Sane, A.; Vasantha Kumar, T. Analysis of genetic diversity in Acorus calamus populations in South and North East India using ISSR markers. *Biochem. Syst. Ecol.* **2012**, *40*, 156–161.

[15]. Singh, B. K.; Pillai, K. K.; Kohli, K.; Haque, S. E. Isoproterenol-induced cardiomyopathy in rats: influence of Acorus calamus Linn.: A. calamus attenuates cardiomyopathy: A. calamus Attenuates Cardiomyopathy. *Cardiovasc. Toxicol.* **2011**, *11*, 263–271.

[16]. Raina, V. K.; Srivastava, S. K.; Syamasunder, K. V. Essential oil composition ofAcorus calamus L. from the lower region of the Himalayas. *Flavour Fragr. J.* **2003**, *18*, 18–20.

[17]. Polish Pharmacopoeia. https://www.urpl.gov.pl/en/polish-pharmacopoeia (accessed January 1, 2023).

[18]. Adams, R. P. *Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured publishing corporation*; Allured Publishing Corporation, 2007.

[19]. Hussey, R. S.; Barker, K. R. comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. *Plant disease reporter* **1973**, *57*, 1925–1928.

[20]. Eisenback, J. D. *Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (Meloidogyne spp.). In: Sasser JN, Carter CC (eds) An advanced treatise on Meloidogyne, vol I: biology and control*; North Carolina State University Graphics, Raleigh, N.C.: USA, 1985.

[21]. Babaali, D.; Roeb, J.; Hammache, M.; Hallmann, J. Nematicidal potential of aqueous and ethanol extracts gained from Datura stramonium, D. innoxia and D. tatula on Meloidogyne incognita. *J. Plant Dis. Prot. (2006)* **2017**, *124*, 339–348.

[22]. Zaidat, S. A. E.; Mouhouche, F.; Babaali, D.; Abdessemed, N.; De Cara, M.; Hammache, M. Nematicidal activity of aqueous and organic extracts of local plants against Meloidogyne incognita (Kofoid and White) Chitwood in Algeria under laboratory and greenhouse conditions. *Egypt. J. Biol. Pest Contr.* **2020**, *30*, 46.

[23]. Yang, Y.-C.; Lee, S.-H.; Lee, W.-J.; Choi, D.-H.; Ahn, Y.-J. Ovicidal and adulticidal effects of Eugenia caryophyllata bud and leaf oil compounds on Pediculus capitis. *J. Agric. Food Chem.* **2003**, *51*, 4884–4888.

[24]. Gadad, H.; Naqvi, A. H.; Mittal, V.; Singh, J.; Das, S. Biology and Damage Pattern of Hairy Caterpillar Selepa celtis Moore (Lepidoptera: Nolidae) on Terminelia arjuna. *Int. J. Curr. Microbiol. Appl. Sci.* **2021**, *10*, 25–31.

[25]. Cole, R. A. Isolation of a chitin-binding lectin, with insecticidal activity in chemically-defined synthetic diets, from two wild brassica species with resistance to cabbage aphid Brevicoryne brassicae. *Entomol. Exp. Appl.* **1994**, *72*, 181–187.

[26]. Koorki, Z.; Shahidi-Noghabi, S.; Mahdian, K.; Pirmaoradi, M. Chemical composition and insecticidal properties of several plant essential oils on the melon aphid,Aphis gossypiiGlover (Hemiptera: Aphididae). *J. Essent. Oil-Bear. Plants* **2018**, *21*, 420–429.

[27]. Sahu, A.; Devkota, A. Allelopathic Effects of Aqueous Extract of Leaves of Mikania Micrantha H.B.K. on Seed Germination and Seedling Growht of Oryza Sativa L. and Raphanus Sativus L. *Sci. World* **2013**, *11*, 91–93.

[28]. Abhishek Biswal, R.; Venkataraghavan, R.; Vivek, P.; Ivo Romauld, S. Molecular docking of various bioactive compounds from essential oil of Trachyaspermum ammi against the fungal enzyme Candidapepsin-1. *J. Appl. Pharm. Sci.* **2019**, *9*, 21–32.

[29]. Kabdal, T.; Himani; Kumar, R.; Prakash, O.; Nagarkoti, K.; Rawat, D. S.; Srivastava, R. M.; Kumar, S.; Dubey, S. K. Seasonal variation in the essential oil composition and biological activities of Thymus linearis Benth. Collected from the Kumaun region of Uttarakhand, India. *Biochem. Syst. Ecol.* **2022**, *103*, 104449.

[30]. Hu, Y.; Liu, X.; Wu, X.; Zhang, Z.; Wu, D.; Chen, C.; Su, W.; Zhang, L.; Li, J.; Wang, H.-M. D. Several natural phytochemicals from Chinese traditional fermented food-pickled Raphanus sativus L.: Purification and characterization. *Food Chem. X* **2022**, *15*, 100390.

[31]. Misra, A.; Kishore, D.; Verma, V. P.; Dubey, S.; Chander, S.; Gupta, N.; Bhagyawant, S.; Dwivedi, J.; Alothman, Z. A.; Wabaidur, S. M.; Sharma, S. Synthesis, biological evaluation and molecular docking of pyrimidine and quinazoline derivatives of 1,5-benzodiazepine as potential anticancer agents. *J. King Saud Univ. Sci.* **2020**, *32*, 1486–1495.

[32]. Domínguez-Villa, F. X.; Durán-Iturbide, N. A.; Ávila-Zárraga, J. G. Synthesis, molecular docking, and in silico ADME/Tox profiling studies of new 1-aryl-5-(3-azidopropyl)indol-4-ones: Potential inhibitors of SARS CoV-2 main protease. *Bioorg. Chem.* **2021**, *106*, 104497.

[33]. Han, Y.; Zhang, J.; Hu, C. Q.; Zhang, X.; Ma, B.; Zhang, P. In silico ADME and toxicity prediction of ceftazidime and its impurities. *Front. Pharmacol.* **2019**, *10*, 434.

[34]. Das, B. K.; Swamy, A. V.; Koti, B. C.; Gadad, P. C. Experimental evidence for use of Acorus calamus (asarone) for cancer chemoprevention. *Heliyon* **2019**, *5*, e01585.

[35]. Bhat, S.; Ashok, B. K.; Bhat, D. V.; Acharya, R.; Shukla, V. A Comparative Phytochemical Evaluation of Wild and Cultivated Acorus calamus Linn (Vacha) with Special Reference to Beta-Asarone Content. *Inventi Rapid Pharm Analysis and Quality Assurance* **2011**, *2*, 1–4.

[36]. Varshney VK, S. B. H.; Ginwal HS, M. N. High levels of diversity in the phytochemistry, ploidy and genetics of the medicinal plant Acorus calamus L. *Med. Aromat. Plants (Los Angel.)* **2015**, *s1*.

[37]. Kumar, R.; Prakash, O.; Pant, A. K.; Hore, S. K.; Chanotiya, C. S.; Mathela, C. S. Compositional variations and anthelmentic activity of essential oils from rhizomes of different wild populations of Acorus Calamus L. and its major component, β-asarone. *Nat. Prod. Commun.* **2009**, *4*, 1934578X0900400.

[38]. Joshi, N.; Prakash, O.; Pant, A. K. Essential oil composition andin vitroAntibacterial activity of rhizome essential oil and β-asarone fromAcorus calamusL. Collected from lower Himalayan region of utarakhand. *J. Essent. Oil-Bear. Plants* **2012**, *15*, 32–37.

[39]. Chaubey, P.; Archana; Prakash, O.; Rai, K.; Kumar, R.; Pant, A. K. in vitro Antioxidant Activity and Total Phenolic Content of Rhizome Extracts from Acorus calamus Linn. *Asian J. Chem.* **2017**, *29*, 2357–2360.

[40]. Chaubey, P.; Parki, A.; Prakash, O.; Kumar, R.; Pant, A. K. Comparative study of chemical composition and antioxidant activity of essential oil extracted from Acorus calamus L. leaves. *J. Herb. Drugs* **2018**, *8*, 203–211.

[41]. Parki, A.; Chaubey, P.; Prakash, O.; Kumar, R.; Pant, A. K. Seasonal variation in essential oil compositions and antioxidant properties of Acorus calamus L. accessions. *Medicines (Basel)* **2017**, *4*.

[42]. Hermes, L.; Römermann, J.; Cramer, B.; Esselen, M. Quantitative analysis of β-asarone derivatives in Acorus calamus and herbal food products by HPLC-MS/MS. *J. Agric. Food Chem.* **2021**, *69*, 776–782.

[43]. Ozdemir, E.; Gozel, U. Nematicidal activities of essential oilsagainst meloidogyne incognita on tomato plant. *Fresenius Environ. Bull* **2018**, *27*, 4511–4517.

[44]. Kundu, A.; Dutta, A.; Mandal, A.; Negi, L.; Malik, M.; Puramchatwad, R.; Antil, J.; Singh, A.; Rao, U.; Saha, S.; Kumar, R.; Patanjali, N.; Manna, S.; Kumar, A.; Dash, S.; Singh, P. K. A Comprehensive in vitro and in silico Analysis of Nematicidal Action of Essential Oils. *Front. Plant Sci.* **2020**, *11*, 614143.

[45]. Park, I.-K.; Kim, J.; Lee, S.-G.; Shin, S.-C. Nematicidal Activity of Plant Essential Oils and Components From Ajowan (Trachyspermum ammi), Allspice (Pimenta dioica) and Litsea (Litsea cubeba) Essential Oils Against Pine Wood Nematode (Bursaphelenchus Xylophilus). *J. Nematol.* **2007**, *39*, 275–279.

[46]. Wiratno; Taniwiryono, D.; Van den Berg, H.; Riksen, J. A. G.; Rietjens, I. M. C. M.; Djiwanti, S. R.; Kammenga, J. E.; Murk, A. J. Nematicidal activity of plant extracts against the root-knot nematode, Meloidogyne incognita. *Open Nat. Prod. J.* **2009**, *2*, 77–85.

[47]. Nicol, J. M.; Turner, S. J.; Coyne, D. L.; Nijs, L. den; Hockland, S.; Maafi, Z. T. Current nematode threats to world agriculture. In *Genomics and Molecular Genetics of Plant-Nematode Interactions*; Springer Netherlands: Dordrecht, 2011; pp. 21–43.

[48]. Li, J.; Zhang, G.; Guo, L.; Xu, W.; Li, X.; Lee, C. S. L.; Ding, A.; Wang, T. Organochlorine pesticides in the atmosphere of Guangzhou and Hong Kong: Regional sources and long-range atmospheric transport. *Atmos. Environ. (1994)* **2007**, *41*, 3889–3903.

[49]. Jiyavorranant, T.; Chanbang, Y.; Supyen, D.; Sonthichai, S.; Jatisatienr, A. The effects of Acorus Calamus Linn. And Stemona tuberose lour. Extracts on the insect pest, Plutella xylostella (Linnaeus). *Acta Hortic.* **2003**, 223–229.

[50]. Asha, D. S.; Bawankar, R.; Babu, S. Current status on biological activities of Acorus Calamus - a review. *Int. J. Pharm. Pharm. Sci.* **2014**, 66–71.

[51]. Liu, X. C.; Zhou, L. G.; Liu, Z. L.; Du, S. S. Identification of insecticidal constituents of the essential oil of Acorus calamus rhizomes against Liposcelis bostrychophila Badonnel. *Molecules* **2013**, *18*, 5684–5696.

[52]. Yao, Y.; Cai, W.; Yang, C.; Xue, D.; Huang, Y. Isolation and characterization of insecticidal activity of (Z)-asarone from Acorus calamus L. *Insect Sci.* **2008**, *15*, 229–236.

[53]. Balakumbahan, R.; Rajamani, K.; Kumanan, K. Acorus calamus: An overview. *J. Med. Plant Res.* **2010**, *4*, 2740–2745.
[54]. Khanh, T. D.; Chung, I. M.; Tawata, S.; Xuan, T. D. Weed suppression by Passiflora edulis and its potential allelochemicals. *Weed Res.* **2006**, *46*, 296–303.
[55]. Satyal, P.; Paudel, P.; Poudel, A.; Dosoky, N. S.; Moriarity, D. M.; Vogler, B.; Setzer, W. N. Chemical compositions, phytotoxicity, and biological activities of Acorus calamus essential oils from Nepal. *Nat. Prod. Commun.* **2013**, *8*, 1179–1181.
[56]. Chen, Z.; Zou, Y.; Wang, J.; Li, M.; Wen, Y. Phytotoxicity of chiral herbicide bromacil: Enantioselectivity of photosynthesis in Arabidopsis thaliana. *Sci. Total Environ.* **2016**, *548–549*, 139–147.
[57]. Feng, G.; Chen, M.; Ye, H.-C.; Zhang, Z.-K.; Li, H.; Chen, L.-L.; Chen, X.-L.; Yan, C.; Zhang, J. Herbicidal activities of compounds isolated from the medicinal plant Piper sarmentosum. *Ind. Crops Prod.* **2019**, *132*, 41–47.
[58]. Azirak, S.; Karaman, S. Allelopathic effect of some essential oils and components on germination of weed species. *Acta Agric. Scand. B Soil Plant Sci.* **2008**, *58*, 88–92.
[59]. Adnan, M.; Nazim Uddin Chy, M.; Mostafa Kamal, A. T. M.; Azad, M. O. K.; Paul, A.; Uddin, S. B.; Barlow, J. W.; Faruque, M. O.; Park, C. H.; Cho, D. H. Investigation of the biological activities and characterization of bioactive constituents of Ophiorrhiza rugosa var. Prostrata (D.don) & Mondal leaves through in vivo, in vitro, and in silico approaches. *Molecules* **2019**, *24*, 1367.
[60]. Banerjee, P.; Ulker, O. C. Combinative ex vivo studies and in silico models ProTox-II for investigating the toxicity of chemicals used mainly in cosmetic products. *Toxicol. Mech. Methods* **2022**, *32*, 542–548.