International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2022; 10(6): 125-131

Received: 18-09-2022
Accepted: 22-10-2022

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Ajuga parviflora Benth: Phytochemical composition and *in-vitro* pesticidal evaluation of butanol extract

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Abstract
The objective of this study was to assess the nematicidal, insecticidal, antifungal, and antibacterial potential in *Ajuga parviflora* Benth butanol extract (APBE). The GC-MS analysis of the extract resulted in the identification of over 32 constituents, comprising 81.7% of the total composition of which glycerol (15.8%), 3,5-dimethylphenyl isocyanate (6.5%), 2-ethyl-hexanol (6.5%), lageracetal (6.2%), butyl isobutyrate (5.0%), octylpivalate (4.4%), pyrrolid-2-one-5-methanol (4.4%) were identified as the major components. The observed results exhibited that APBE was significantly active against a nematode *Meloidogyne incognita* with significant egg hatching inhibition of 27.33±0.47% and motility of 91.66±1.24% at 200 µg/mL in a dose dependent manner. A promising insecticidal activity (92.09±0.37%) against *Lipaphis erysimi* was also observed in APBE. Similarly, the extract was found to be moderately active for its antibacterial potential against *Staphylococcus aureus* with 23.17±0.15% of inhibition compared to standard, amikacin (31.66±0.33%). The extract was found to be less active against *Curvularia lunata,* the soil borne fungus. Based on these observations it can be inferred that *Ajuga parviflora* could be a good natural source to develop a potential botanical pesticide against different insect and pests.

Keywords: *Ajuga parviflora*, nematicidal, insecticidal, antifungal, antibacterial, biopesticides

Introduction
According to previous studies, the popularity of medicinal and aromatic herbs as alternatives to contemporary allopathic treatments increased as a result of their low cost, little side effects, and holistic approach. There are approximately 18,440 plant species in the Indian Himalayan region (8000 angiosperms, 44 gymnosperms, 600 pteridophytes, 1159 lichens, 1736 bryophytes, and 6900 fungi), of which 45% have been documented to have medicinal characteristics [1]. Uttarakhand Himalaya has been described as a repository of many types of fragrant and medicinal plants with a remarkable variety. It has been claimed that because to its unique geographic location and various climatic conditions with a significant potential for domesticating plant species, which will be crucial for the sustainable lifestyle of hill people in the future [2,3]. In India, 72 genera and 435 species of plants belonging to Lamiaceae family have been documented, while in Uttarakhand, 49 genera and 143 species have been documented to occur [4,5]. There are approximately 64–70 species of the genus *Ajuga* L., also known as "bugleweed," which are found naturally and are particularly prolific in temperate and subtropical regions [6-9]. Previous research has demonstrated that a variety of *Ajuga* species have significant pharmacological properties. There have been reports of the widely dispersed tiny, clump-forming rhizomatous annual and perennial herbs in the western Himalaya and upper gangetic plains [10]. The whole plant of the *Ajuga* species, including the leaves, stems, and seeds, have been long used for treating stomach aches, diarrhoea, burns, cuts, and wound healing [11]. Numerous *Ajuga* species have been used for their ethnomedical purposes, including the treatment of rheumatism, skin conditions, fever, dysentery, toothaches, tuberculosis, gastrointestinal disorders, diabetes, malaria, liver protection, beneficial effects on muscle strength, and its prevention of heart conditions, stomach aches, pimples, constipation, and acidity [10, 12-14]. In recent years, studies have been focused on plant materials and their bioactive chemical constituents as a rich source of natural substances which can be used to develop ecologically safer methods for pest control. The main advantage of plant materials is that they are potentially less expensive, locally available, biodegradable, and sustainable; they can propagate easily, have no negative effects on the ecosystem [15].

It has been said that the entire plant belonging to *A. parviflora* can be utilized to treat a variety of illnesses and is also a potent medication. The biological activities of this herb include antidiabetic, antimicrobial, dermatological, antiviral, antibacterial, antifungal, antioxidant, insecticidal, brine shrimp toxicity, and synthesis of nanoparticles utilizing leaf extract of *A. parviflora* Benth. Taking into account its availability, collection, growth, and the portions used, the calculated conservation status of this herb has been classified as fragile [15, 16, 17]. Plant extracts from *A. parviflora* were tested against stored product insects, including the red flour beetle *Tribolium castaneum* (Herbst) and the lesser grain borer *Rhyzopertha dominica* (F.) [15]. Additionally, methanolic extract of this plant has been demonstrated to have insecticidal activity, brine shrimp lethality, and antibacterial activities [17].

Genus *Ajuga* is widely reported to have the clerodane and *neo*-clerodane diterpenoids with various biological activities [18]. Our research group has previously reported the essential oil composition from *A. parviflora* which includes *β*-caryophyllene (22.4%), *ϒ*-muurolene (12.7%), *ϒ*-terpinene (6.3%) and caryophyllene oxide (6.2%) as the major compounds. Moreover, the sterol compositions of *A. bracteosa*, *A. macrosperma* and *A. parviflora* have been reported and the *neo*-clerodane diterpenoids have been isolated from the DCM extract of *A. parviflora* [18, 19, 20]. Modern scientific study has supported the uses of this herb, but further investigation into the mechanisms underlying its secondary metabolites and varied biological activities is necessary to gain a deeper understanding of the crucial role of this species in the development of new and effective medications. The aim of the present study was to investigate the phytochemical analysis of butanolic extract of *A. parviflora* collected from Uttarakhand, India for pesticidal evaluation *viz.* nematicidal, insecticidal, antifungal and antibacterial activity. To the best of our knowledge, this is the first report on the phytochemical composition and pesticidal evaluation from the butanol extract of *A. parviflora*.

Material and Method

Plant material

The aerial parts of the plant were collected from district headquarter of Bageshwar, Uttarakhand, India (29°84′04″ N, 79°76′94″E, ~935 m a.s.l.) in the month of September 2018. The plant material was taxonomically identified by one of the author (Dr. D.S. Rawat) and voucher specimen (GBPUH-1020/09-10-2020) has been submitted in the herbarium of Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar (Uttarakhand).

Preparation of extract

Shade dried powder of the aerial part of *A. parviflora* was subjected for extraction in methanol by Soxhlet apparatus. The resultant extract was fractionated by hexane followed by DCM, ethyl acetate and butanol using separating funnel. The butanol extract was concentrated to dryness under reduced pressure using a rotary vacuum evaporator to yield concentrated crude extract. The crude extract collected and preserved until used for further studies.

Chemical composition

The GCMS-QP 2010 Ultra instrument was used to conduct the GC-MS analysis of the plant extract under the following circumstances: column: DB-5 silica capillary column (30 m × 0.25 mm × 0.25 m), carrier gas: helium, flow rate: 1.21 mL/min, injection temperature: 260 °C, injection mode: split, pressure: 73.3 kPa, split ratio: 20.0, and ion source temperature: 210 °C The oven's temperature was first set to 60 °C for 3 minutes, then increased to 250 °C at a rate of 7 °C/minute, held for 5 minutes, and then increased once more to 280 °C at a rate of 20 °C/min, held for 29 minutes. By comparing the mass spectra of the extract's contents to those in the NIST-MS, FFNSC Wiley Library, and literature publications, the constituents of the extract were identified [21].

Nematode population rearing

M. incognita population was collected from susceptible tomato plants (*Solanum lycopersicum* L.) growing in infected fields of Vegetable Research Centre (V.R.C.), Haldi, G.B.P.U.A&T, Pantnagar, Uttarakhand. Infested plant roots carrying large galls and egg masses were washed with tap water to remove soil and debris. Egg masses were then handpicked from the infected roots. Nematode second-stage juveniles (J_2) were obtained by extracting the egg masses in batches of similar size, averaging 20,000 eggs, with 2 mL tap water using 2 cm diameter sieve with a 215 mm aperture placed in a 3.5 cm diameter Petri dish. Incubating room was set at 25±2 °C [22] and all J_2 hatching in the first 3 days were discarded. The following J_2 generations were collected and used in the experiments.

In vitro mortality assay on second stage larvae (J_2) of *M. incognita*

The *in-vitro* tests were performed to determine whether APBE affected the second stage larvae's motility against *M. incognita*. The galled roots were sliced into small pieces of 2 cm and thoroughly cleaned under running water to remove the 2nd stage larvae (J_2) from the egg masses and dirt attached to them. The small pieces of roots placed in sodium hypochlorite (2%) solution were shaken for two minutes to separate the organic matter from the eggs. The eggs were sieved on a 38µm-pore, collected and cleansed after the solution was put through a series of sieves. To collect 2nd stage juveniles from hatched eggs after 48 h, the egg suspension was first incubated at 28±1 °C. A total of 100 juveniles were taken on petri dishes containing different concentrations (25-200 µg/mL) of oleoresins. Observations of three replicates were taken at the time intervals of 24 h, 48 h and 72 h using a stereo-binocular microscope. The juveniles placed in tween 20 (1%) were taken as control. All the treatments were arranged in CRD (completely randomized design) manner. To check the aliveness (mortality) of larvae, they were transferred to water. None of the immobile larvae recuperated their mobility, which confirmed the larval death. Percent nematode mortality was calculated by Abbott's formula [23, 24].

$$\%\ nematode\ mortality = 100 \times \left(1 - \frac{Nt}{Nc}\right)$$

Where,

Nt = number of viable nematodes after the treatment; Nc = number of viable nematodes in water control.

In vitro egg hatchability test of *M. incognita*

The *in vitro* experiment was carried out to assess the effectiveness of APBE on *M. incognita* egg hatching. To examine the impact of various oleoresin concentrations on the number of eggs hatched from egg masses, root knot nematodes of diseased capsicum plant were utilized. Two *M.*

incognita egg masses were suspended in a 25–200 µg/mL mixture of APBE in gridded petri dishes. The control group consisted of egg masses dissolved in a Tween 20 solution (1.0%). The treatments were maintained at a constant temperature of 27±1 °C in a BOD incubator with all of the treatments set up in triplicate and in a completely random order. To record the findings on egg hatchability at intervals of 24 h, 48 h, and 72 h, respectively, the number of eggs that hatched was counted under a microscope with a magnification of 40X.

Insecticidal activity using toxicity bioassay method

L. erysimi were directly collected from mustard field in Norman E. Borlogue Research Centre, G.B.P.U.A&T, Pantnagar and acclimatized in the laboratory of Department of Entomology, College of Agriculture, G.B.P.U.A&T, Pantnagar, until the emergence of 3rd instar larvae. *L. erysimi* from mustard plant were reared in a muslin-walled box in the growth chamber of glass house at 25±1 °C and relative humidity (RH) of 65±5%.

The experiment was evaluated in a petri plate utilizing the contact toxicity method in accordance with the methods being used [25, 26]. The aphids (*L. erysimi*) on the freshly picked and spotless mustard leaves were collected from the field. With a hole in the centre of the lid for ventilation, each leaf disc (44 sq. cm) from a fresh mustard plant was placed upside down on a 1 cm layer of agar solution (1% agar solution using 1 gm agar in 100 mL heated distilled water) in a Petri dish. After two hours, adult aphids were removed from leaves using a camel brush and gathered in a box. Before drying, mustard leaf discs were soaked for 30 seconds in solutions containing extracts ranging from 50 to 1000 µg/ml and their mixtures in tween 20. The control (1% tween 20) and three replications of each oleoresin concentration were used. 15 mature aphids treated with 3-4 hours of starvation were placed on agar layered petri dishes in each replicate. Observations were taken 24, 48, and 72 h after the nymphs were released. Abott's formula was used to count and determine the number of dead aphids. After performing a Probit analysis on the mortality data, the LC_{50} value of MBBEO was determined using the Statistical Package for the Social Sciences (SPSS) 16.0 software programme. The one-way ANOVA was used to statistically analyse the investigations' triplicate data as mean ± SD (significant at $p<0.05$).

Antifungal Activity

Curvularia lunata, a phytopathogenic fungus, was acquired from the Plant Pathology Department of the College of Agriculture at G.B.P.U.A&T. Pantnagar. The media was aseptically transferred onto petri plates containing the fungal colonies, and the fungi were then revived and grown on PDA (Potato dextrose agar) medium after one week of incubation at 25±2 °C. 1000 mL of boiling distilled water was added to 39g of PDA and carefully agitated. Each 500 mL conical flask received 200 mL of the medium after 500 mg of chloramphenicol had been added to the medium. The flasks were then sealed with non-absorbent cotton plugs. The medium was autoclaved for 30 minutes to sterilize it (15 lbs p.s.i. at 121.6 °C).

PDA plates were made by heating solidified PDA to 450 °C and then pouring 20 mL of each into sterile petri plates in a laminar flow to perform antifungal activity. After being inoculated with a test fungus disc (diameter=5 mm), PDA plates containing various concentrations of extract (25-500 µg/mL) of APBE were cultured for seven days at 26±2 °C and 68±2% RH until control plates reached full growth. After approximately 7 days, observations of three replicates for five different concentrations of APBE were recorded. Carbendazim was often used as standard fungicide. The percentage inhibition was computed using the formula [27].

$$\text{Percent inhibition}=\frac{X-Y}{X}\times 100$$

Where,

X= radial growth in control, Y= radial growth in treatment

Antibacterial Activity

The approach described was used to determine the antibacterial activity [28]. It was measured against typical gram positive, *Staphylococcus aureus*. Agar-agar, nutrient broth, and nutrient agar (AA, NB, and NA) were purchased from Hi media and kept at 4 °C until use. The media solution was produced by mixing 1000 mL of distilled water, 10 g of agar-agar powder, and 28 g of Muller Hinton Agar. For sterilization, the mixture was autoclaved at 120°C and 15-20 lbs. Similarly, mix 2.6 gm of nutrient broth in 200 mL of distilled water and autoclave at 128ºC and 15-20 lbs for bacterial culture inoculation. Nutritional agar media (20 mL) was added to sterile petri plates in laminar flow and allowed to solidify without being touched. The hardened petri plates should be kept at 37°C overnight for sterility testing.

After mixing the bacterial colonies, a pure culture of gram positive bacteria was created in a 5 mL test tube containing sterile nutrient broth. The bacterial tube should be kept at 37 °C overnight. Take a 5mm sterile paper disc and dip it into APBE solutions at various concentrations (ranging from 25 to 200 µg/mL). After some time, the paper disc was repositioned on the plate and left to establish bacterial colonies for 24 hours at 37ºC. Amikacin (standard) and tween 20 (1%) were employed as positive and negative controls, respectively. After 24 hours, the zone of inhibition (in mm) were measured and compared to a reference. To calculate zone of inhibition-

$$\text{Zone of inhibition}=\frac{\text{Diameter of inhibition zone- Diameter of disk}}{2}$$

Statistical analysis

The experimental data was expressed as mean±standard deviation and each treatment was duplicated three times. At a 1% level of significance ($p<0.01$), ANOVA was used to analyze the experimental data for nematicidal, insecticidal, antifungal, and antibacterial activities. A significant difference between the investigated data was found at each level of significance. Using the SPSS.16 software, three factor analysis was performed on the combined data.

Results and Discussion

Chemical Composition

Matching the mass fragmentation pattern of the target molecule with that of compounds existing in database of NIST-MS, FFNSC Wiley Library and Adams, 2007 [21], over 32 compounds were identified in APBE contributing 81.7% of the total chemical composition. Glycerol (15.8%) was identified as the major constituent followed by 3,5-dimethylphenyl isocyanate (6.5%), 2-ethylhexan-1-ol (6.5%), lageracetal (6.2%), butyl isobutyrate (5.0%), octylpivalate (4.4%), pyrrolid-2-one-5-methanol (4.4%), 4-methoxyindole (4.0%), diisobutyl succinate (3.9%), N-amino-1,2,3,4-

tetrahydroquinoline (3.5%), propyl pivalate (2.2%), (*E*)-cinnamaldehyde (1.8%), 2,3-dimethylphenyl isocyanate (1.8%), tetracontane (1.6%), 2,4-dimethylbenzoic acid (1.5%), phthalamide (1.4%), 2,5-ditert-butylphenol (1.2%) 1,1-diisobutoxy-butane (1.1%), isopropyl glycidyl ether (1.0%), allyl hydracrylate (0.9%), 1,3,4-eugenol (0.8%), 2-ethoxyethyl 2-ethylhexanoate (0.7%), 2-benzimidazolol (0.5%), widdrol (0.5%), 3-(4-isopropylphenyl)-*β*-alanine (0.5%), 3,4-diethoxybenzaldehyde (0.5%), bis-(2-ethylhexyl) maleate (0.5%), 2-methoxy-4-vinylphenol (0.4%), tris(2,4-di-tert-butylphenyl) phosphate (0.4%), hexatriacontane (0.3%) and heneicosane (0.3%). The GC chromatogram of APBE is given in Fig 1. The literature survey revealed no report on the chemical composition of butanolic extract of *A. parviflora*. So, it could be assumed as the first report on the chemical composition of APBE. However, the GC and GC/MS spectral analysis of the methanolic extract of *A. parviflora* have been reported with the identification of bioactive phytoconstituents such as 2-piperidinone, N-[4-bromo-n-butyl]-phthalic acid, squalene, phytol, mono-(2-ethylhexyl) ester, vitamin E, stigmasterol, 3-ethyl-3-hydroxyandrostan-17-one, 3,5-dehydro-6-methoxy- pivalate, cholest-22-ene-21-ol, and 2-methylenecholestan-3-ol [29]. Similarly, the chemical composition of *A. parviflora* essential oil has also been reported by our research group with the identification of *β*-caryophyllene (22.4%), *γ*-muurolene (12.7%), *γ*-terpinene (6.3%), caryophyllene oxide (6.2%), *α*-humulene (5.8%), *δ*-cadinene (4.3%), *α*-amorphene (3.8%) and *β*-selinene (2.5%) along with other minor constituents. The sterol compositions of *A. bracteosa*, *A. macrosperma* and *A. parviflora* have also been reported. Moreover, the compounds isolated by our group *viz*; *neo*-clerodane diterpenoids (deoxyajugarin-I, ajugarin-I chlorohydrin, and 3*β*-acetoxy-clerodinin C) have been reported to isolate from DCM extract of *A. parviflora* collected near Nainital, Uttarakhand, India [18, 19, 20].

Table 1: Chemical Composition of APBE

S. No	Compound Name	RT	% Composition	Molecular formula	Method of identification
1.	butyl isobutyrate	4.373	5.0	$C_8H_{16}O_2$	M^+ = 71, m/z= 116, 101, 89, 56 (100%)
2.	2-ethylhexan-1-ol	4.941	6.5	$C_8H_{18}O$	M^+ = 57, m/z= 112, 98, 84, 55 (100%)
3.	glycerol	5.709	15.8	$C_3H_8O_3$	M^+ = 61, m/z= 93, 73 (100%)
4.	allyl hydracrylate	6.219	0.9	$C_6H_{10}O_3$	M^+ = 73, m/z= 100, 85, 57 (100%)
5.	1,1-diisobutoxy-butane	6.852	1.1	$C_{12}H_{26}O_2$	M^+ = 57, m/z= 129, 103, 89, 73(100%)
6.	isopropyl glycidyl ether	6.983	1.0	$C_6H_{12}O_2$	M^+ = 57, m/z= 129, 103, 89, 73(100%)
7.	(*E*)-cinnamaldedhyde	7.302	1.8	C_9H_8O	M^+ = 131, m/z= 103, 77, 55(100%)
8.	lageracetal	8.191	6.2	$C_{12}H_{26}O_2$	M^+ = 57, m/z= 129, 103, 73, 55(100%)
9.	propyl pivalate	9.043	2.2	$C_8H_{16}O_2$	M^+ = 57, m/z= 103, 85, 71(100%)
10.	2-methoxy-4-vinylphenol	9.392	0.4	$C_9H_{10}O_2$	M^+ = 150, m/z= 135, 107, 77, 63 (100%)
11.	3-(4-isopropylphenyl)-*β*-alanine	9.519	0.5	$C_9H_{11}NO$	M^+ = 148, m/z= 132, 117, 91, 77 (100%)
12.	3,5-dimethylphenyl isocyanate	9.665	6.5	C_9H_9NO	M^+ = 147, m/z= 132, 118, 91, 77 (100%)
13.	2-benzimidazolol	9.968	0.5	$C_{10}H_{13}NO$	M^+ = 134, m/z= 117, 106, 65, 51 (100%)
14.	2,3-dimethylphenyl isocyanate	11.395	1.8	C_9H_9NO	M^+ = 147, m/z= 132, 118, 91, 77 (100%)
15.	octylpivalate	11.688	4.4	$C_{10}H_{16}D_2O$	M^+ = 57, m/z= 159, 103, 71, 55 (100%)
16.	2,5-ditert-butylphenol	11.742	1.2	$C_{14}H_{22}O$	M^+ = 191, m/z= 163, 74, 57, 55 (100%)
17.	1,3,4-eugenol	11.883	0.8	$C_{10}H_{12}O_2$	M^+ = 164, m/z= 91, 77, 65, 55 (100%)
18.	diisobutyl succinate	12.273	3.9	$C_{12}H_{22}O_4$	M^+ = 101, m/z= 130, 73, 57, 55 (100%)
19.	4-methoxyindole	12.648	4.0	C_9H_9NO	M^+ = 132, m/z= 147, 104, 50 (100%)
20.	2,4-dimethylbenzoic acid	12.870	1.5	$C_{10}H_{13}N$	M^+ = 132, m/z= 147, 104, 65 (100%)
21.	pyrrolid-2-one-5-methanol	13.448	4.4	$C_7H_{11}NO_3$	M^+ = 84, m/z= 157, 83, 55 (100%)
22.	widdrol	14.137	0.5	$C_{15}H_{26}O$	M^+ = 151, m/z= 123, 109, 55 (100%)
23.	phthalamide	14.272	1.4	$C_8H_8N_2O_2$	M^+ = 148, m/z= 164, 130, 91 (100%)
24.	N-amino-1,2,3,4-tetrahydroquinoline	14.527	3.5	$C_9H_{12}N_2$	M^+ = 148, m/z= 130, 119, 91 (100%)
25.	3,4-diethoxybenzaldehyde	17.473	0.5	$C_{11}H_{14}O_3$	M^+ = 137, m/z= 194, 149, 121,109 (100%)
26.	bis-(2-ethylhexyl) maleate	18.318	0.5	$C_{20}H_{36}O_4$	M^+ = 117, m/z= 100, 84, 71, 57 (100%)
27.	adipic acid dioctyl ester	20.491	0.6	$C_{22}H_{42}O_4$	M^+ = 129, m/z=147, 111, 84, 70, 57 (100%)
28.	2-ethoxyethyl 2-ethylhexanoate	21.207	0.7	$C_{22}H_{42}O_6$	M^+ = 57, m/z=171, 127, 99, 88, 70 (100%)
29.	heneicosane	21.598	0.3	$C_{21}H_{44}$	M^+ = 57, m/z=127, 113, 99, 85, 71(100%)
30.	bis-(2-ethylhexyl) phthalate	22.025	0.4	$C_{24}H_{38}O_4$	M^+ = 149, m/z=167, 113, 84, 71, 57 (100%)
31.	hexacosane	22.850	0.6	$C_{26}H_{54}$	M^+ = 57, m/z=127, 113, 99, 85, 71(100%)
32.	tetracontane	27.211	1.6	$C_{40}H_{82}$	M^+ = 57, m/z=127, 113, 99, 85, 71(100%)
33.	tris-(2,4-di-tert-butylphenyl) phosphate	31.767	0.4	$C_{42}H_{63}O_4P$	M^+ = 57, m/z=316, 291, 191,147, 91 (100%)
34.	hexatriacontane	35.645	0.3	$C_{36}H_{74}$	M^+ = 57, m/z=169, 141, 113, 99, 85, 71(100%)
Total (%)			81.7%		

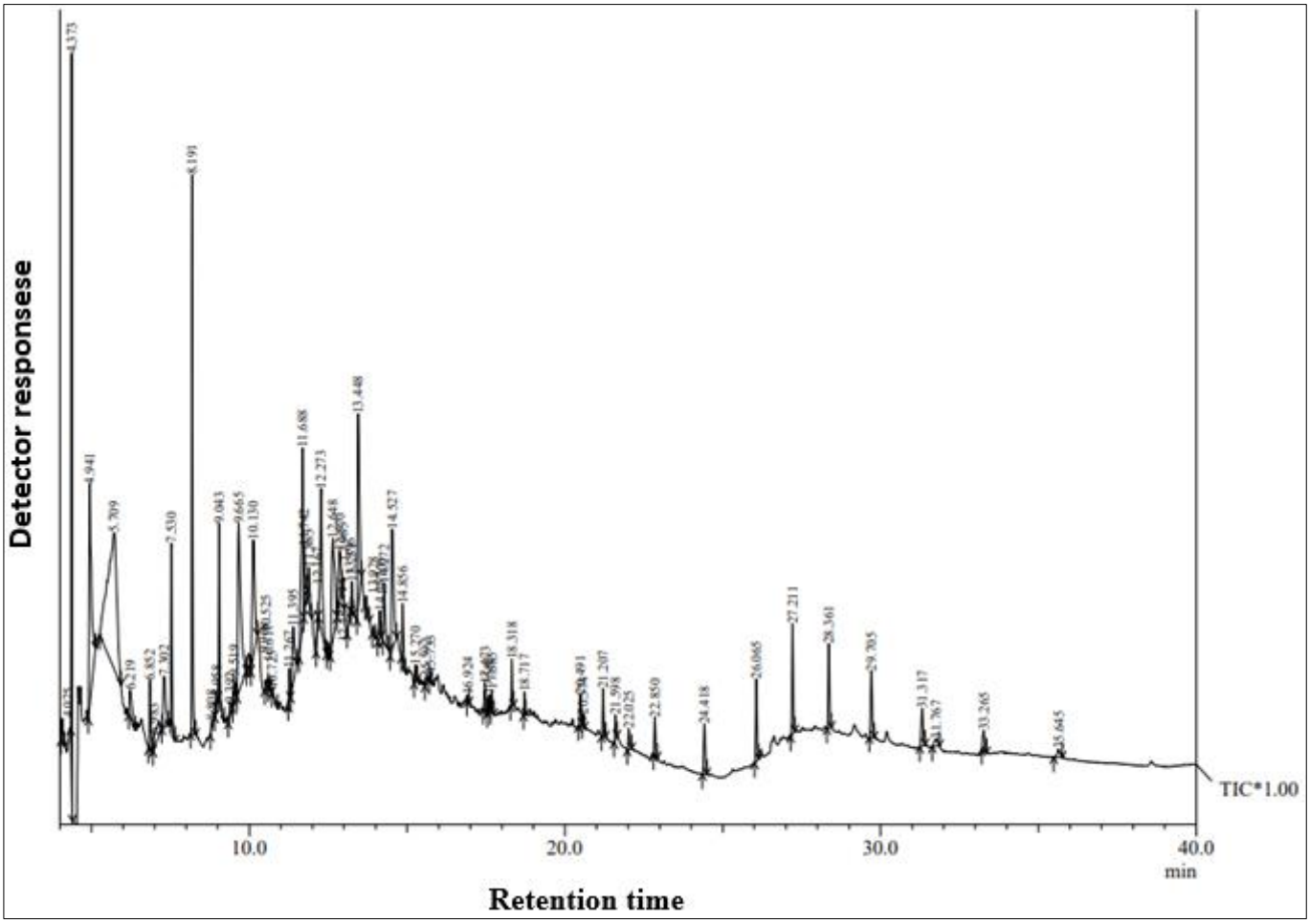

Fig 1: Chromatogram of APBE

Nematicidal activity

Table 2: Nematicidal activity of APBE against *M. incognita*

APBE (µg /mL)	Number of juvenile (J_2) mortality			Number of eggs hatching		
	24 h	48 h	72 h	24 h	48 h	72 h
25	15.66±1.24^{b}	19.33±0.47^{b}	27.33±0.94^{e}	29.33±0.94^{c}	42.33±1.24^{b}	68.66±2.49^{a}
50	24.66±0.47^{c}	29.66±1.24^{c}	40.66±1.69^{d}	21.00±0.81^{bc}	34.66±0.88^{b}	53.33±1.24^{a}
100	46.33±1.24^{d}	53.00±0.81^{d}	59.33±1.24^{c}	13.00±0.81^{abc}	20.33±1.24^{ab}	47.33±0.94^{a}
150	64.66±1.24^{e}	69.33±1.24^{e}	75.66±1.24^{b}	9.00±0.81^{ab}	12.66±0.47^{a}	37.33±1.69^{a}
200	80.33±0.47^{f}	85.66±0.47^{f}	91.66±1.24^{b}	6.33±0.47^{a}	9.33±0.47^{a}	27.33±0.47^{a}
Control (1% tween 20)	0.00±0.00^{a}	0.66±0.00^{a}	2.33±0.33^{a}	56.0±3.21^{d}	80.0±1.15^{c}	107.33±3.71^{b}
IC_{50} (µg/ml)	52.22±1.02			28.8±2.00		

APBE= *Ajuga parviflora* butanol extract. IC_{50}= Half maximal inhibitory concentration
Mean values followed by same letter are not significantly different according to Tukey's and Duncan's test (p<0.05).

The nematicidal activity of APBE was investigated by monitoring egg hatching and juvenile mortality of 2nd stage larvae of *M. incognita*. As the dose increased juvenile mortality increased whereas egg hatching decreased. Butanol is poisonous solvent towards nematodes. Therefore, all the butanolic samples were evaporated till dryness to remove the traces of butanol. The powdered extract solubilized in water and tween 20 was tested against nematodes with water as the negative control. The % mortality of APBE (80.33±0.47%, 85.66±0.47% and 91.66±1.24% respectively) and egg hatching inhibition of APBE (6.33±0.47%, 9.33±0.47% and 27.33±0.47%) was shown to be efficacious at highest dose level (200 µg/mL) when exposed for 24, 48, and 72 hours as shown in Table 2. The IC_{50} value of APBE for % mortality and egg hatching inhibition were found to be 52.22±1.02 and 28.8±2.00. The significant results might be due to the major compounds or synergistic effect of major/minor constituents present in APBE. Previous studies have revealed that the extracts of *Ajuga parviflora*, *Trichilia connaroides*, *Ajuga bracteosa*, and *Ajuga macrosperma* in different solvents *viz*. methanol, chloroform, acetone and aqueous exhibited significant anthelmintic activity on the isometric contractions of the poultry worm, *Ascaridia galli*. The methanol extracts of the roots of *A. parviflora* (IC_{50}= 16.79±2.93) inhibited the contractile activity in a dose-dependent manner, reducing the amplitude and frequency as compared to control which indicated the paralytic effect of the extracts on *A. galli* [30].

Insecticidal activity

Table 3: Insecticidal activity of APBE against nymph of *Lipaphis erysimi*

Conc (µg/mL)	Mortality % (mean±sd)		
	APBE		
	24 h	48 h	72 h
50	18.37±4.12^{e}	28.84±3.33^{e}	44.65±2.59^{e}
100	36.96±3.91^{d}	50.00±3.84^{d}	52.56±2.22^{d}
250	52.56±2.22^{c}	57.90±3.86^{c}	68.37±1.48^{c}
500	65.81±3.91^{b}	73.71±4.00^{b}	84.18±0.74^{b}
1000	81.62±4.12^{a}	89.52±4.26^{a}	92.09±0.37^{a}
Control	00.00±0.00^{f}	00.00±0.00^{f}	00.00±0.00^{f}
LC_{50}	59.62±2.99		

APBE= *Ajuga parviflora* butanol extract, LC_{50}=Half maximal lethal concentration

Mean values followed by same letter are not significantly different according to Tukey's and Duncan's test (p<0.05).

One of the most destructive pests in preventing Indian mustard (*Brassica juncea* L.) from producing to its full capacity is the mustard aphid (*Lipaphis erysimi*). The results reported in Table 3 showed that there was significant mortality of *L. erysimi* adults when exposed to different concentrations (50-1000 µg/mL) of APBE. The results showed varying mortality percentages at different dose levels where percent mortality increased with increase in dose level. At higher dose level (1000 µg/mL), APBE exhibited 81.62±4.12%, 89.52±4.26% and 92.09±0.37% mortality after 24, 48 and 72 h. The significant results might be due to the major compounds or synergistic effect of major/minor constituents present in APBE. The minimum median lethal concentration (LC_{50}) shown by APBE was observed to be 59.62±2.99 µg/mL. 0.080 µg/cm^2 dose of methanolic extract from *A. parviflora* have been reported to show significant insect repellent activity of 70.6% - 81.5% against the insects *Rhyzopertha Dominica and Trichilia castaneum*, respectively [31].

Antifungal activity

Table 4: Antifungal activity of APBE against *Curvularia lunata*

Conc (µg/mL)	25 µg/mL	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL
APBE	0.16±0.03^{b}	1.32±0.49^{b}	1.54±0.18^{ab}	2.31±0.07^{ab}	3.13±0.77^{a}
Carbendazim*	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

APBE= *Ajuga parviflora* butanol extract, *=Standard fungicide

Mean values followed by same letter are not significantly different according to Tukey's and Duncan's test (p<0.05).

APBE exhibited moderate antifungal activity against phytopathogenic fungi, *Curvularia lunata* at various dosages (25-500 µg/mL) (Table 5) revealing percent suppression of the tested fungus mycelial growth. The antifungal potential of APBE against *C. lunata* was 3.13±0.77 µg/mL, compared to carbendazim (100.00±0.00), the positive control when tested at highest dose of 500 µg/mL. The crude methanolic extract of *A. parviflora* has been reported to show moderate antifungal activity against nine pathogenic strains *viz*; *Aspergillus parasiticus*, *Aspergillus Niger*, *Yersinia aldovae*, *Candida albicans*, *Aspergillus effuses*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisia*, and *Trichophyton rubrum*, using itraconazole and amphoteracin B as positive control. It has also been reported that after 72 hours no antifungal activity was observed in the extract. These results are in agreement with the present study in a manner that the *A. parviflora* is slightly active against the tested fungi [17].

Antibacterial activity

Table 5: Antibacterial activity of APBE against *Staphylococcus aureus*

Conc (µg/mL)	25 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL
APBE	5.93±0.25^{a}	10.95±0.27^{a}	16.35±0.63^{a}	19.13±0.38^{a}	23.17±0.15^{a}
Amikacin	15.33±0.33^{a}	18.66±0.66^{a}	20.66±0.33^{a}	27.00±0.57^{a}	31.66±0.33^{a}

APBE= *Ajuga parviflora* butanol extract. *= Standard bactericide

Mean values followed by same letter are not significantly different according to Tukey's and Duncan's test (p<0.05).

Antibacterial potential against pathogenic bacterial strain *S. aureus* have been studied and expressed in terms of zone of inhibition (ZOI) in millimeter. At 200 µg/mL, APBE exhibited good antibacterial activity in comparison to standard: Amikacin (ZOI= 31.66±0.33) > APBE (23.17±0.15) (Table 5). The crude methanolic extract of *A. parviflora* exhibited antibacterial activity against *S. aureus* with 11 mm zone of inhibition at 100 and 1000µg/disc. The silver nanoparticles made from *A. parviflora* leaf extract also exhibited antibacterial activity against *Staphylococcus aureus* and other three different bacterial strains, including *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [17]. *Staphylococcus aureus* have been reported to have a maximum zone of inhibition of 14.58±0.41 mm at a concentration of 100 µl, respectively [17]. This is the first report on the antibacterial activity of butanol extract of *A. parviflora* against *S. aureus*.

Conclusion

The findings of this study highlighted the phytochemical makeup and biological properties of the butanol extract from the plant *A. parviflora*, which was collected from Uttarakhand, India. Our findings imply that *A. parviflora* extract has nematicidal and insecticidal activity. The present study showed that the mortality of *L. erysimi* was influenced by a number of factors, including the plant species employed, the components of the extracts, the application rate, and the exposure duration. The aforementioned findings demonstrated that although limited antifungal activity was noted, the plant extract had promising active ingredients against antibacterial activity. According to reported data, the activity shown in the extracts of the genus *Ajuga* might be caused possibly by the presence of the diterpenoids clerodane and *neo*-clerodane. Clerodane diterpenoids have also been reported to be possessing antibiotic, insecticidal, antiviral, anticancer, and

anti-microbial properties. The observations of present study revealed that the components of *A. parviflora* have a variety of bioactivities. The active components of the plant need to be isolated and identified in order to develop the plant based nematicides, insecticides, and antibacterial agents or to get a lead molecule for the development of noble pesticides.

References

1. Singh DK, Hajra PK. Floristic diversity. Changing perspectives of biodiversity status in the Himalayas; Gujral, G.S.; Sharma, V., Eds.; British Council Division: New Delhi; c1996. p. 23-38.
2. Prakash R. Traditional Uses of Medicinal Plants in Uttarakhand Himalayan Region. Sch. Acad. J Bio sci. 2014;2(5):345-353.
3. Joshi BC, Joshi RK. The Role of Medicinal Plants in Livelihood Improvement in Uttarakhand. Int. J Herb. Med. 2014;1(6):55-58.
4. Karthikeyan S. A statistical analysis of flowering plants of India.Flora of India, Introductory Volume (Part II); Singh, N.P.; Singh, D.K.; Hajra, P.K.; Sharma, B.D., Eds.; Botanical Survey of India: Calcutta; c2000. p. 201-217.
5. Uniyal BP, Sharma JR, Choudhery U, Singh DK. Flowering plants of Uttarakhand (A Checklist); Bishen Singh Mahendra Pal Singh: Dehradun; c2007, 404.
6. Plants of the World Online. Facilitated by the Royal Botanic Gardens Kew. http://www.plantsoftheworldonline.org/
7. WCVP; World Checklist of Vascular Plants. Facilitated by the Royal Botanic Gardens Kew; c2020. http://wcvp.science.kew.org/
8. Roskov Y, Ower G, Orell T, Nicolson D, Bailly N, Kirk PM, *et al*. Species 2000 ITIS Catalogue of life, Annual checklist; c2019. www.catalogueoflife.org/annual-checklist/2019
9. The Plant List, 2013.http://www.theplantlist.org/
10. Israili ZH, Lyoussi B. Ethno pharmacology of the plants of genus Ajuga. Pak. J Pharm. Sci. 2009;22(4):425-462. PMID: 19783524
11. Upadhyay SU, Patel VB, Patel AA, Upadhyay UM, Patel NM. *Ajuga bracteosa*: A Promising herb. Int. J. Pharma Sci. 2012;3(3):2085-2104.
12. Bouyahya A, El Omari N, Elmenyiy N, Guaouguaou FE, Balahbib A, El-Shazly M, *et al*. Ethnomedicinal use, phytochemistry, pharmacology, and toxicology of *Ajuga iva* (L.,) schreb. J Ethnopharmacol. 2020, 258.
13. Mamarasulov B, Davranov K, Jabborova D. Phytochemical, pharmacological, and biological properties of *Ajuga turkestanica* (Rgl.) Brig (Lamiaceae). Ann. Phytomed. 2020;9(1):44-57.
14. Hassan N, Din MU, Shuaib M, Hassan FU, Zhu Y, Chen Y, *et al*. Quantitative analysis of medicinal plants consumption in the highest mountainous region of Bahrain Valley, Northern Pakistan. Ukr. J Ecol. 2019;9(1):35-49.
15. Guruprasad BR, Akmal P. Assessment of repellency and insecticidal activity of *Ajuga parviflora* (Benth) and *Trichilia connaroides* (W&A) leaf extracts against stored product insects. J Entomol. Zool. Stud. 2014;2(4):221-226.
16. Chandra K, Nautiyal BP, Nautiyal MC. Herbal based traditional medicinal knowledge of local inhabitants in Rudraprayag district of Uttarakhand, India. Ethno bot. Res. Appl. 2013;11:99-313.
17. Rahman N, Ahmad M, Riaz M, Mehjabeen JN, Ahmad R. Phytochemical, antimicrobial, insecticidal and brine shrimp lethality bioassay of the crude methanolic extract of *Ajuga parviflora* Benth. Pak. J Pharm. Sci. 2013;26(4):751-756.
18. Singh P, Prakash O, Pant AK. Essential oil composition of *Ajuga parviflora* Benth. Growing in western Himalayan region of Uttarakhand (India). J Essent. Oil-Bear. Plants. 2015;18(3):697-701.
19. Singh P, Prakash O, Pant AK. Sterol composition of *Ajuga bracteosa* Wall Benth., *Ajuga macrosperma* Wall ex Benth. and *Ajuga parviflora* Benth. J Indian Chem. Soc. 2014;91(5):951-952.
20. Beauchamp PS, Bottini AT, Caselles MC, Dev V, Hope H, Larter M, *et al*. *Neo*-clerodane diterpenoids from *Ajuga parviflora*. Phytochem. 1996;43(4):827-834.
21. Adams RP. Identification of essential oil components by *gas chromatography/mass spectrometry*. Carol Stream: Allured Publishing Corporation. 2007;456:544-545.
22. Hussey RS, Barker RK. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 1973;57:1025-1028.
23. Kalaiselvi D, Mohankumar A, Shanmugam G, Nivitha S, Sundararaj P. Green synthesis of silver nanoparticles using latex extract of *Euphorbia tirucalli*: a novel approach for the management of root knot nematode *Meloidogyne incognita*. Crop Prot; c2019, 117.
24. Finney DJ. Statistical method in biological assay (No. Ed. 3). Charles Griffin & Company; c1978.
25. Cole RA. 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*. Entomologia Experimentalis et Applicata. 1994;72(2):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 gossypii* Glover (Hemiptera: Aphididae). J Essent. Oil-Bear. Plants. 2018;21(2):420-429.
27. Mckinney HH. Biological control of nematode pests by natural enemies. Annu. Rev. Phytopathol. 1923;18:415-440.
28. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm. Anal. 2016;6(2):71-79.
29. Burki S, Burki ZG, Ahme I, Jahan N, Owais F, Tahir N, *et al*. GC/MS assisted phytochemical analysis of *Ajuga parviflora* leaves extract along with anti-hepatotoxic effect against anti-tubercular drug induced liver toxicity in rat. Pak. J Pharm. Sci; c2020, 33.
30. Agarwal G, Pant AK, Hore SK. *In vitro* evaluation of anthelmintic efficacy of *Trichilia* and *Ajuga* species on *Ascaridia galli*. Hygeia: J Drugs Med. 2010;2(2):43-53.
31. Guruprasad BR, Akmal P. Assessment of repellency and insecticidal activity of *Ajuga parviflora* (Benth) and *Trichilia connaroides* (W&A) leaf extracts against stored product insects. J Entomol. Zool. Stud. 2014;2(4):221-226, 756.