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Antibacterial and antifeedant activities of *Spilanthes acmella* leaf extract against Gram-negative and Gram-positive bacteria and brinjal fruit borer, *Leucinodes orbonalis* larvae

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PEER REVIEW

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Comments

The authors have evaluated different solvent extracts of the leaves of *S. acumella* against five diseases causing bacteria and an economically important pest *L. orbonalis*. The study provided excellent results against the tested microorganisms and an agricultural pest. This result could motivate the researchers in the related field (pharma, agricultural and pesticidal people).
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ABSTRACT

Objective: To evaluate the antibacterial and antifeedant activities of dichloromethane, acetone and aqueous extracts of *Spilanthes acmella* (L.) (*S. acmella*) Murr against selected bacterial strains and larvae of *Leucinodes orbonalis* Guen.

Methods: Solvent extracts were tested against pathogenic microbes using disc diffusion method and fruit disc no-choice method for antifeedant activity.

Results: The study revealed that dichloromethane extract of *S. acmella* leaf showed broad spectrum antibacterial activity against all the tested bacteria. Maximum zone of inhibition was observed in dichloromethane leaf extract of *S. acmella* against *Escherichia coli* [(18.9±0.34) mm] followed by *Staphylococcus aureus* [(18.6±1.31) mm], *Proteus vulgaris* [(17.2±0.68) mm], *Bacillus subtilis* [(17.0±0.76) mm] and *Klebsiella pneumonia* [(16.4±1.55) mm] at 5 mg/disc concentration. The aqueous extract was moderately inhibited the tested bacteria at all the concentrations. Dichloromethane extract showed good antifeedant activity against *Leucinodes orbonalis* (68.88%) when compared to acetone (60.80%) and aqueous (45.48%) extracts at 5% concentration. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, phytosterols, saponins, steroid, tannins and phenolic compounds.

Conclusions: The study suggests that the dichloromethane leaf extract of *S. acmella* could be used to develop a novel herbal formulation to control pathogenic bacteria and agricultural pests.

KEYWORDS

Antibacterrial, Antifeedant, *Spilanthes acmella*, *Leucinodes orbonalis*

1. Introduction

The increasing awareness of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards the studies on the potential antibacterial activity of plant-derived substances, an untapped source of antibacterial activity, which are used in traditional medicine in different countries. Medicinal plants contain physiologically active principles that have been exploited for many years in traditional medicine for the treatment of various ailments[1] and they contain antimicrobial properties[2]. Antimicrobials of plant origin

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have enormous therapeutic potential as they are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[3]. Antifeedant is described as substances that reduce the feeding of an insect and is found among all of the major classes of secondary metabolites: alkaloids, phenolics and terpenoids[4-6]. Terpenoids comprising the most potent and diverse forms of antifeedants[7,8]. Botanical insecticides have broad spectrum of activity, which is alternative to synthetic chemical insecticides for pest management.

Brinjal shoot and fruit borer (*Leucinodes orbonalis*) (*L. orbonalis*) is a monophagous pest. It is a very important pest on brinjal owing to its feeding habit. It is an internal borer that damages the tender shoots and fruits; it causes serious damages especially during the fruiting stage. The percent fruit infestation caused by this pest reached up to 90.86%[9] and larvae alone caused 12%-16% damage to the shoots and 20%-60% to the fruits[10].

Spilanthes acmella (*S. acmella*) is one of the important medicinal plants with rich source of therapeutic constituents[11]. It is native to the tropics of Brazil. *S. acmella* has been well documented for its uses as antimalarial[12], insecticidal[13], anti-inflammatory[14] and immunomodulating properties[15]. This study is aimed to assess different solvents extracts of *S. acmella* leaves on antibacterial and antifeedant properties.

2. Materials and methods

2.1. Plant collection and extraction

The fresh and healthy leaves of *S. acmella* were collected during the year 2012 from forest region of Wayanad district, Kerala, India. Plant specimen was identified by the authentic plant taxonomist. The leaves were shade-dried at room temperature and coarsely powdered in a powdering machine. A total of 500 g powder was taken in an aspirator bottle, soaked with dichloromethane (DCM) (w/v 1:3) and kept for 72 h with occasional shaking at room temperature. The content was filtered through Whatman No. 1 filter paper and the solvent was removed by using rotary vacuum evaporator at 40 °C. The crude extract was obtained and stored in refrigerator at 4 °C for further use. Remains were sequentially extracted with acetone and water.

2.2. Phytochemical screening

Phytochemical analysis of *S. acmella* leaves extracts was done using the Harborne[16] methods.

2.3. Tested microorganisms

A total of five bacterial strains were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. The Gram-negative bacteria are *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*(*K. pneumonia*) and *Proteus vulgaris* (*P. vulgaris*) and Gram-positive bacteria are *Bacillus subtilis* (*B. subtilis*)and *Staphylococcus aureus* (*S. aureus*). Strains were maintained on nutrient agar medium.

2.4. Preparation of inoculums

The mother culture was streaked on sterile nutrient agar plate to obtain pure colonies. After the incubation at 37 °C for 24 h, pure colonies were selected with sterile inoculating loop and transferred into a test tube with sterile Mueller-Hinton broth and vortex thoroughly. The bacterial suspension was equal to the 0.5 McFarland standards. These cell suspensions were diluted with sterile Mueller-Hinton broth to provide final cell counts of about 1×10^8 CFU/mL.

2.5. Rearing of L. orbonalis

The infested fruits were collected from the general crop of brinjal and kept in glass Petri dishes (10 cm×10 cm) with a layer of cut pieces of paper. Each Petri dish was covered with a piece of muslin cloth and tightened with rubber band. The diet was changed every alternate day. Proper hygienic conditions were maintained during the experimental period. The full grown larvae that came out of the fruits were in the form of pupae as spun cocoons at the periphery of muslin cloth covers and between the folds of the papers. These pupae were kept in separate glass jar. Soon after moth emergence, the black paper strips were kept inside the jar for egg laying. They were fed with 10% sugar solution soaked in cotton which was placed in watch glasses inside the glass jar. Eggs obtained from these moths were transferred from glass jar to glass Petri dishes (10 cm×10 cm) along with egg bearing paper strips. After hatching, the larvae were reared individually for two more generations on brinjal fruit. The larvae obtained from the later generation were utilized for the study.

2.6. Antibacterial susceptibility test

Antimicrobial activity was carried out using disc diffusion method[17]. The sterile Mueller Hinton agar obtained from

Himedia (Mumbai) were prepared by pouring 15 mL of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min and 0.1% inoculums (1×10^8 CFU/mL) suspension was swabbed uniformly and allowed to dry for 5 min. The different concentrations of extracts (1.25, 2.50 and 5.00 mg/disc) were loaded on 6 mm sterile paper disc. The loaded disc was placed on the surface of medium and the extracts were allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. DMSO was used as negative control and streptomycin (0.5, 1.5 and 5.0 mg/disc) was used as positive control. These studies were performed in triplicate.

2.7. Antifeedant activity

Antifeedant activity of the crude extracts of *S. acmella* was studied using fruit disc no-choice method. Fresh brinjal fruit discs of 10 mm thickness were used for this study. The brinjal fruit discs were dipped individually in 0.625%, 1.250%, 2.500% and 5.000% concentration of crude extracts. The fruit disc dipped in acetone + Tween 80 was used as negative control since it was used to dissolve the crude extracts. In each plastic Petri dish (1.5 cm×9.0 cm), wet filter paper was placed to avoid early dying of the test materials and three third instar larvae were introduced into each Petri dish that contains five discs of brinjal fruit. Five replicates were maintained for each treatment with 15 larvae per replicate (total n=75).

Progressive consumption of the fruit discs consumed by *L. orbonals* larvae was recorded after 24 h of treatment. The fruit discs were weighed using Mettler digital balance and the difference between initial and final weights were calculated. Real consumption was calculated as follows:

Weight loss due to desiccation (D)=initial weight-final weight

Real consumption=initial weight-(final weight+D)

The experiment was conducted at laboratory condition (27±2) °C with 14:10 light and dark photoperiod and (75±5)% relative humidity. Antifeedant activity was calculated according to the formula of Bentley *et al*[18]:

$$\text{Antifeedant activity}=\frac{\text{Consumption in control}-\text{Consumption in treated}}{\text{Consumption in control}}\times100$$

2.8. Statistical analysis

The data for zone of inhibition was analysed using One way ANOVA. Significant differences between treatments were determined using Tukey's multiple range test ($P\leq0.05$). The concentration dependent activity was analysed using linier regression for antifeedant activity.

3. Results

3.1. Preliminary phytochemical analysis of S. acmella leaf extracts

Phytochemical screening of *S. acmella* leaves revealed the presence of various bioactive compounds namely alkaloids, terpenoids, phytosterols, saponins, steroid, tannins and phenolic compounds (Table 1). DCM extract showed the presence of alkaloids, terpenoids, saponins, tannins and phenolic compounds. The acetone extract showed terpenoids, phytosterols, steroid, tannins and phenolic compounds. Alkaloids, terpenoids and steroid are detected in the aqueous extract.

Table 1.
Preliminary phytochemical analysis of different solvent leaf extracts of *S. acmella*.

S. No.	Test	Test applied	Extracts		
			DCM	Acetone	Aqueous
1.	Alkaloids	Mayer's test	+	−	+
2.	Terpenoids	Salkowski test	+	+	+
3.	Phytosterols	Liebermann Burchard test	−	+	−
4.	Saponins	Froth forming test	+	−	−
5.	Steroid	Salkowski test	−	+	+
6.	Tannins	Iron iii trichloride ($FeCl_3$)	+	+	−
7.	Phenolic compounds	Ferric chloride test	+	+	−

(Note): (−) Absents; (+) Present.

3.2. Antibacterial activity

In the present investigation, antibacterial activity of DCM, acetone and aqueous leaf extracts of *S. acmella* were studied against five bacterial strains using disc diffusion method. Among the tested extracts, DCM extract exhibited maximum zone of inhibition against *E. coli* (18.90±0.34) mm followed by *S. aureus* (18.60±1.31) mm, *P. vulgaris* (17.20±0.68) mm, *B. subtilis* (17.00±0.76) mm and *K. pneumonia* (16.40±1.55) mm at the concentration of 5 mg/disc. Acetone extract inhibited the growth of *S. aureus* (17.80±1.10) mm, *E. coli* (14.80±0.30) mm, *P. vulgaris* (14.60±0.30) mm, and *B. subtilis* (13.20±1.01) mm at the concentration of 5 mg/disc. While, the minimum zone of inhibition was observed against *K. pneumonia* (7.10±0.20) mm. The reference drug (streptomycin) showed inhibition zone ranged from (16.40±0.61) mm to (24.30±0.36) mm. The activity of DCM extract of *S. acmella* leaves found to be more pronounced than the acetone and aqueous extracts against all the organisms tested. In comparison, the aqueous extract showed less pronounced antibacterial activity. DMSO did not show any activity (Table 2).

Table 2

Antibacterial activity of DCM, acetone and aqueous leaf extracts of *S. acmella*.

Solvent extracts	Conc. (mg/disc)	Zone of Inhibition (mm)				
		B. subtilis	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*S. aureus*
DCM	1.0	12.50±0.57^{e}	14.70±0.65^{d}	8.30±0.25^{de}	10.30±0.40^{e}	13.50±1.73^{e}
	2.5	14.30±0.64^{ef}	16.80±0.17^{de}	10.80±0.17^{e}	13.20±0.17^{f}	16.00±0.28^{f}
	5.0	17.00±0.67^{f}	18.96±0.34^{e}	16.40±1.55^{f}	17.20±0.68^{g}	18.60±1.31^{f}
Acetone (Ac)	1.0	8.30±0.75^{c}	9.10±0.45^{c}	4.40±0.47^{c}	7.20±0.17^{d}	10.50±0.50^{d}
	2.5	10.50±0.50^{d}	12.90±0.70^{cd}	5.70±0.65^{c}	9.30±0.52^{de}	14.10±0.75^{e}
	5.0	13.20±1.01^{e}	14.80±0.30^{d}	7.10±0.20^{d}	14.60±0.30^{f}	17.80±1.10^{f}
Aqueous (AQ)	1.0	4.80±0.52^{b}	7.80±0.17^{b}	2.00±0.57^{b}	0.00±0.00^{a}	7.50±0.50^{b}
	2.5	5.30±0.25^{b}	9.30±0.36^{c}	4.60±0.41^{c}	2.40±0.52^{b}	9.30±0.52^{c}
	5.0	6.10±0.28^{bc}	11.90±0.55^{cd}	7.10±0.15^{d}	5.80±0.11^{c}	11.30±0.40^{de}
*Streptomycin	1.0	19.00±0.76^{fe}	20.40±0.91^{f}	16.40±0.61^{f}	17.60±1.51^{g}	20.10±1.45^{g}
	2.5	21.00±0.60^{g}	21.50±0.66^{fg}	18.80±1.17^{fg}	20.50±0.66^{h}	22.40±1.00^{g}
	5.0	22.80±1.01^{g}	23.80±0.51^{g}	21.80±0.68^{g}	23.50±1.04^{i}	24.30±0.36^{gh}
#DMSO		0.0±0.0^{a}				

The mean±SD followed by same letter do not differ significantly using Tukey's test $P \leq 0.05$. *Standard antibiotics for reference control; #Dimethyl sulfoxide 50%, for negative control.

3.3. Antifeedant activity

S. acmella leaves derived DCM, acetone and aqueous extracts showed antifeedant activity against larvae of *L. orbonalis* at different concentrations are illustrated in Figure 1. The results showed that the DCM extract of *S. acmella* was the most effective treatment that recorded the antifeedant activity of 68.88% against *L. orbonalis* followed by acetone (60.8%) and aqueous (45.48%) extracts of *S. acmella* at 5% concentration.

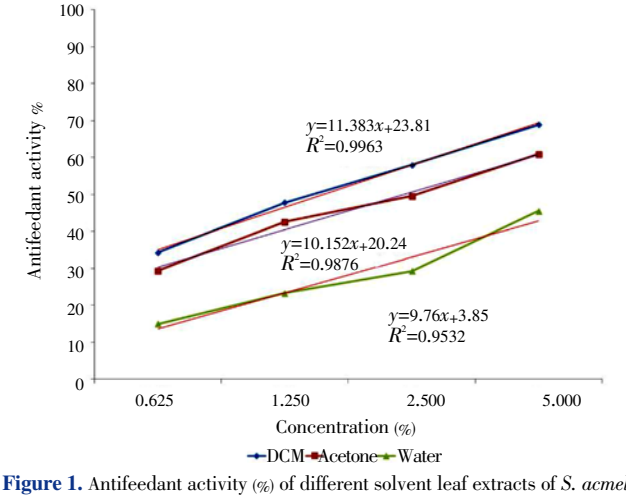

Figure 1. Antifeedant activity (%) of different solvent leaf extracts of *S. acmella* against *L. orbonalis*.

The linear regression indicates that the concentration dependent antifeedant activity against *L. orbonalis*. The DCM extracts exhibited the higher linear relationship between concentration and antifeedant activity [y=11.383x+23.81 (R^2=0.9963)] followed by acetone and aqueous extracts (Figure 1). All the treatments showed good R^2 value of more than 0.95 for concentration dependent activity.

4. Discussion

Plants are major source of potentially useful substances for the development of new chemotherapeutic agents. Various phytochemical compounds which are naturally occurring in plants as secondary metabolites have been implicated in the conferment of antimicrobial activity[19,20]. The increasing rate of antibiotic resistance of microorganisms necessitates the development and research of the new antibacterial agents or resistance modifiers. Medicinal plant-derived compounds have increased widespread interest in search of alternative antibacterial agents because the perception that they are safe and have a long history of use in folk medicine for the treatment of infectious diseases[21].

In the present study, solvent leaf extracts of *S. acmella* were tested for antibacterial activity against five microbial pathogens. Among them, *S. aureus*, a pyrogenic bacterium, was known to play a significant role in invasive skin diseases including superficial and *Salmonella typhi*, which causes typhoid fever to human beings[22]. The results of the present study pertaining to leaf extracts of *S. acmella* were active against all bacterial strains. DCM extract showed maximum zone of inhibition against *B. subtilis*e, *E. coli*, *K. pneumonia*, *P. vulgaris* and *S. aureus*, due to the presence of alkaloids, terpenoids, tannins and phenolics. The present findings coincide with the findings of Ngoci *et al*.[23] who reported that different phytochemicals in *Cissampelos pareira* L. showed antibacterial activity. Similarly, phenolics, flavonoids, tannins in *Limonium delicatulum* exhibited antimicrobial activity[24]. From this study it can be concluded that DCM and acetone extracts of the leaves of *S. acmella* showed wide range of antibacterial activity.

Today's awareness of bio-product quality and safety, research and development of plant-derived antifeedants have attracted increasing attention[25-27]. In this investigation, DCM extract of *S. acmella* exhibited the promising antifeedant activity. These findings coincide with the findings of Pavunraj *et al*.[28,29] who reported that hexane, chloroform and ethyl acetate extract of *Hyptis suaveolens* and *Melochia corchorifolia* exhibited antifeedant activity. These findings also against *L. orbonalis*. Muthu *et al*.[30] reported that hexane extract of *Flueggea leucopyrus* (Koen.) Willd. and chloroform extract of *Clerodendrum phlomidis* leaves showed maximum antifeedant activity against *Earias vittella*.

In the present study, maximum antifeedant activity was recorded due to the presence of different secondary substances like alkaloids and terpenoids in the tested extracts. The present findings coincide with the previous

reports of Baskar *et al*.[5,31] who reported that alkaloid and terpenoids containing extracts showed maximum antifeedant activity against *Helicoverpa armigera*. In conclusion, the extracts DCM of *S. acmella* leaf exhibited effective antibacterial and antifeedant activities against selected bacterial strains and brinjal fruit borer, *L. orbonalis* larvae. This study paves the way for further attention to identify the active compounds, which is responsible for the biological activities and to develop new formulations for safe health and environment.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Plant secondary substances are acting as defense against many pathogens and phytophagous insects. The activities of plants have been well documented in traditional practices. The authors have selected a medicinal plant for this study on the basis of traditional background and scientifically justified.

Research frontiers

Antibacterial and antifeedant activities of *S. acumella* against five pathogenic bacteria and brinjal fruit borer were evaluated. Different crude solvents extracts were prepared and evaluated. This plant has already been reported as medicinal plant used in traditional practices.

Related reports

Different solvent extracts of *S. acumella* showed the presence of secondary phytochemicals like alkaloids, phenolics and terpenoids in the present study. These chemicals from different plants were reported to possess antibacterial and antifeedant properties.

Innovations and breakthroughs

Maximum zone of inhibition of different bacteria were reported and the results are mostly comparable to the reference drug. Promising antifeedant activity against the larvae of *L. orbonalis* was recorded. Phytochemical analysis showed the presence of bioactive substance in the obtained solvent extracts.

Applications

The outcome from this study could be used by the pharmaceutical industries and pesticide industries to develop new drugs or pesticidal formulation.

Peer review

The authors have evaluated different solvent extracts of the leaves of *S. acumella* against five diseases causing bacteria and an economically important pest *L. orbonalis*. The study provided excellent results against the tested microorganisms and an agricultural pest. This result could motivate the researchers in the related field (pharma, agricultural, and pesticidal people).

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