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Phytochemical profiling, ovicidal, larvicidal potential of *Exacum pendunculatum* L. against *Spodoptera litura*

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Abstract
The present study was focused on to evaluate ovicidal, larvicidal activity and phytochemical profile of different solvent extracts of *Exacum pendunculatum* L. The efficacy of acetone, methanol, ethanol and water extracts of *E. pendunculatum* was assayed against third instar larvae of *Spodoptera litura* for their insecticidal activity. The highest ovicidal (80%) and larvicidal (90%) activity was recorded in methanol extract at 50 mg/ml concentration. Qualitative and quantitative phytochemicals tests were revealed the presence of considerable amounts of phenolics flavonoids, tannins, terpenoid and alkaloids in *E. pendunculatum*. The recorded phytochemicals might be responsible for effective bio-pesticidale potential of *E. pendunculatum*. Hence further details phytochemical investigation will assist us to develop a new botanical formulation for the management of *S. litura*.

Keywords: *Exacum pendunculatum*, *Spodoptera litura*, larvicidal, ovicidal

Introduction
Insect pest are the major issue in the agricultural sector, which leads to approximately 50% losses in the total agricultural production (Ferry *et al*., 2004) [14]. Regular application of chemical pesticides to maintain crop yield causes several environmental and health issues. Chemical pesticides affect the non-target organisms and human beings, directly or indirectly. Many researcher were confirmed that repeated use of pesticides to control pests has created so many serious environmental problems like genetic resistance in the pest, loss of natural bio-control agent, pollinators, toxic residues in food chain, water bodies, air and soil, which cumulatively affects human life and leading to disrupt ecosystem. (Baskar *et al*., 2009) [6]. More than 650 species of insects and mites have developed resistance to one or more insecticides (Baskar and Ignacimuthu, 2012, Kannaiyan, 2002) [6, 22]. Under such alarming situation plant based bio pesticides are effective and alternative strategy to control insect pest. Plant synthesizes variety of secondary metabolites like polyphenols, alkaloids, steroids, terpenoids, essential oils, lignans, fatty acids and sugars to protect them from insect attack (Isman, 2006) [19]. Due to rich in such bioactive chemicals, it can be utilized in the development of environmentally safe bio-pesticides for insect control. (Arivoli and Tennyson, 2013) [4]. Along with effectiveness against variety of agricultural insect pests; they are easily degradable, beneficial to environment, agriculture product consumers and potentially suitable to use in integrated pest management programs. (Baskar *et al*., 2009, Elangovan *et al*., 2012) [6, 12].

Spodoptera litura Fab is a major pest which having cosmopolitan distribution all over the world. It feeds on around 112 cultivated crops which causes severe losses in agriculture production (Sharma and Seth, 2005) [29]. It attack several economically important crops *viz*., cotton, groundnut, chilly, tobacco, castor, mungbean, soya bean, cabbage, cauliflower, tomato and pulses in India, China and Japan (Ferry *et al*., 2004, Mallikarjuna *et al*., 2004) [14, 23]. Several studies have been reported the promising plants based product as a biopesticides against *Spodoptera litura* (Baskar *et al*., 2011, Ningombam *et al*., 2017) [8, 24]. However, the search of plant derived chemicals that have potential to use as insecticides, antifeedants and growth inhibitors against *Spodoptera litura* are still continuing throughout world.

Exacum is the genus belongs to family Gentianaceae. It comprises 20 species like *E. affine*, *E. axillare*, *E. bicolor*, *E. pedunculatum*, *E. sessile* etc. It is mainly distributed in India and only few in Malayan, China and Africa. However, 19 species, 5 varieties are distributed in India (Biswal *et al*., 2011, Patil *et al*., 2014) [9, 25]. *Exacum pedunculatum* is one of the common species in south India and generally occurs at eastern and western Ghats. It is traditional medicinal herb and due to bitter test, local people used it as a remedy for diabetes and skin disease. (Shahina and Namp, 2016) [28]. the phytochemicals screening of *E. pedunculatum* exhibited the presence of secondary metabolites like alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and triterpines. These bio-metabolites are well familiar to protect the plants from the insect attack as well as various discese (Vinayaka *et al*., 2016, Jarag *et al*., 2019) [21, 33]. Therefore, the present study was undertaken to evaluate the bio- efficacy of *E. pedunculatum* against the pest *Spodoptera litura* under laboratory condition.

Materials and Methods
Plant collection and extract preparation
The fresh plant material of *E. pedunculatum* was collected from Shivaji University campus, Kolhapur, Maharashtra, India during monsoon season. The plant material was identified and authenticated based on its morphological characteristics. The entire plant material was air dried at room temperature then grind in mixture grinder to make fine powder. The dried powder of entire plant (5 g) was taken into conical flask then 50 ml of acetone, methanol, ethanol

and water were added in each flask respectively. The conical flasks were kept on the orbital shaker for 2 hrs at 100 rpm at room temperature. All extracts were filtered through a Buchner funnel using Whatman number 1 filter paper. The filtered crude extract was evaporated under reduced pressure using rotary evaporator. The yield of dried residue for each extract was noted and further dissolved in known amount (5 ml) of respective solvent and used for in investigation.

Rearing of *Spodoptera litura*

Egg mass and larvae of *S. litura* were collected from tobacco field located around Devchand Collage, Kolhapur, and Maharashtra. The collected larvae were reared in plastic containers and fed them regularly with fresh leaves of castor (*Ricinus communis*) until the larvae became pupae in the laboratory conditions. After pupation, the pupa was collected and placed inside the rearing cage. After adult emergence, cotton soaked with 10% honey (sugar) solution mixed with a few drops of multi-vitamins was provided for adult feeding. Folded filter papers were provided for egg laying. After egg lying, egg masses were collected from the filter paper and allowed for hatching. After hatching the larvae were collected from the cage and fed with standard artificial diet. The entire rearing process was repeated and the insect culture was maintained throughout the study period.

Ovicidal activity

The ovicidal activity was carried out by spraying (0.5 ml) different concentration of crude extracts (5, 10, 25 and 50 mg/ml) on fresh laid eggs of *S. litura*. The eggs sprayed with solvent and azadirachtin is considered as negative and positive control. For each concentration five replicate of 20 eggs were maintained. The number of hatched and unhatched eggs was recorded up to 96 h and percentage of egg mortality was calculated by Abotts formula (Abotts, 1925) [1]. The experiment was carried out in the control laboratory condition with temperature 27 ± 2° C, 14:10 light:dark photoperiod and 75 ± 5% relative humidity.

Larvicidal activity

Larvicidal activity of crude extracts with different concentrations 5, 10, 25 and 50 mg/ml was determined by topical application method on third instar larvae (Akhtar *et al*, 2012) [2]. A three micro litter extract of above mentioned concentrations were applied separately on the dorsum of the thorax and abdominal regions of third instar larva by using micro-pipette. Larvae were treated with solvents and azadirachtin were considered as negative and positive control respectively. Further larvae were transferred to rearing tubs (8cm ×18cm) lined with wet paper towels and tubs closed with muslin cloth. The treated and control larvae were feed on normal castor leaves. Each concentration treatment contained 20 larvae with three replicates. Larval mortality was observed and results were recorded. Mortality data was corrected by using the Abbott's, (1925) [1] formula and then used for statistical analysis.

Qualitative test for phytochemicals analysis

All solvent extracts of *E. pedunculatum* were subjected to various chemical tests to identify phytoconstituents using standard methods of Sofowara, (1993) [31], Trease and Evans, (1989) and Harborne (1973) [32]. For qualitative tests of all solvent extract of *E. pedunculatum* diluted to obtained mg/ml concentration then use for the phytochemical tests.

Test for Phenolics

The presence of phenolics was confirmed by mixing of 0.5 ml of plant extract and 0.5 ml of respective solvent. Then add few drops of 5% $FeCl_3$. Dark green/blue color appears confirmed the presence of phenolic compound.

Test for flavonoids

For flavonoids test, aliquots of extracts 0.5 ml mixed with 0.5 ml of respective solvent. Add few drops of 1% of $AlCl_3$. Appearance of yellow colour proved the presence of flavonoids.

Test for Tannins

The tannin tests was carried out by adding 0.5 ml of plant extract and 0.5 ml of respective solvent then add few drops of 5% $FeCl_3$. Blackish color shows presence of tannin compounds.

Test for Terpenoids

The presence of terpenoids conformed by mixing 0.5 ml of plant extract and 0.5 ml of solvent. Further add 1 ml chloroform and then add 1 ml H_2SO_4. Reddish brown color confirmed the presence of terpenoids.

Test for Alkaloids

For alkaloid test add 0.5 ml of plant extract and 0.5 ml of respective solvent. After adding of few drops of dragondroff reagent. The presence of orange color confirmed the alkaloids.

Test for Anthraquinone

For anthraquinone test, few drops of magnesium acetate solution were mixed with 1 ml extract. The formation of pink color signified that anthraquinone was present.

Test for Glycosides

About 0.5 ml of plant extract and 0.5 ml chloroform mixed then add 0.5 ml glacial acetic acid. After add few drops of H_2SO_4. Violet to blue and then to green indicates the presence of steroidal nucleus.

Test for Saponins

About 1 ml of plant extract and 1 ml of distilled water were mixed and shaken vigorously. A stable persistent froth indicated the presence of saponins.

Quantitative tests for phytochemical analysis

Total phenolics content (TPC)

TPC from all the extracts was estiamted by Folin-Ciocalteu specterophotometric method (Singleton and Rossi, 1965) [30]. Aliquots of solvent extracts (50 µl from mg/ml working stock) were mixed with 1 ml pre-diluted Folin and Ciocalteu reagent (1:10). After 5 min, 800µl of sodium carbonate was added to the reaction mixture. All the reaction mixtures were incubated at room temperature for 30 min and absorbance was read at 760 nm. Tannic acid was used to plot calibration curve and results were expressed as mg tannic acid equivalent (TAE)/g extract.

Total flavonoids content (TFC)

TFC was estimated as method described by Sakanaka *et al*.

(2009) [27]. Aliquots of extracts 20 µL (mg/mL) were mixed with 150 µL of 5% $NaNO_2$ solution. After 5 min, 300 µL of 10% $AlCl_3$ was added and kept for 5 min at room temperature and then 2 ml 1 M NaOH was added. The reaction mixture was mixed well and the absorbance was recorded immediately at 510 nm. Catechin was used to obtain calibration curve and results were expressed as mg catechin equivalents (CE)/g extract.

Total tannins content (TTC)

TTC was estimated by using vanillin-HCl method described by Bhat *et al*. (2007) [10]. A known volume of extract (100 µL from mg/ml stock) was added to 2 ml reagent (4% (w/v) vanillin in methanol and 8% (v/v) HCl in methanol (1:1 ratio)). After 20 min incubation, absorbance was measured at 500 nm. Catechin was used as standard and results were reported as mg catechin equivalents (CE)/g extract.

Total terpenoid content (TTEC)

TTEC was determined according to Chang and Lin (2011) [11] with few modifications. Aliquot of extract (20 µl from mg/mL working stock) was added to the 150 µl freshly prepared 5% (w/v) vanillin in glacial acetic acid and 500 µl perchloric acid. The reaction mixture was heated for 45 min at 60 °C and cooled immediately on ice bath. Further, 2 ml glacial acetic acid was added to the reaction mixture and absorbance was measured at 548 nm. Ursolic acid was used as standard and results were reported as mg ursolic acid equivalents (UAE)/g extract.

Total Alkaloids content (TAC)

TAC was estimated by adapting protocol of Fadhil *et al*. (2007) [13]. Appropriate aliquot of plant extract (50 µl from mg/mL working stock) was added to 1 ml buffer solution and 1 ml BCG reagent. Further, reaction mixture was extracted with 2 ml chloroform and absorbance was measured at 470 nm. Galanthamine was used as standard and results were reported as mg galanthamine equivalents (GE)/g extract.

Statistical analysis

The results are presented as mean ± standard error (SE). The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 16.0 and the significant differences between the means were compared by using Duncan's multiple range test (DMRT) at $P<0.05$.

Results and Discussion

Ovicidal activity

The solvent extracts of *E. pedunculatum* with different concentration were screened for ovicidal activity against *S. litura* and results were presented in Table 1. The ovicidal activity of all extracts against *S. litura* was assayed by accounting the number of unhatched eggs. The percentage of unhatched eggs is directly proportional to percentage ovicidal activity. In present investigation, the highest ovicidal activity (70%) (Table 1) was found with methanol extract at 50 mg/ml concentration and least ovicidal activity (10%) noted with water extract. As compared to other all tested concentration of methanol extract showed higher ovicidal activity. However, tested positive control azadiractin at (0.1%) concentration exhibited 80% ovicidal activity which found comparable with methanol extract of *E. pedunculatum*. At the same time as the extract concentration increased, ovicidal activity was increased in all treatments. The results are good agreement with effective ovicidal activity of *E. pedunculatum* solvent extract against eggs of mosquito (Elangovan *et al*., 2012) [12].

Table 1: Percent ovicidal activity of *E. pedunculatum* extract against *S. litura*

Crude extract	Concentration mg/ml			
	5 mg/ml	10 mg/ml	25 mg/ml	50 mg/ml
Acetone	20±0.5	20±0.5	25±0.5	40±1.0
Ethanol	35±0.3	40±0.6	55±1.0	60±0.5
Methanol	40±1.0	55±0.5	65±1.5	70±1.0
Water	10±0.1	15±0.5	25±1.0	30±1.0
Control	5±0.5			
Azadirachtin (0.1%)	80±1.0			

Values were the means of three replicates ± standard error.

Larvicidal activity

The larvicidal activity of different crude extracts of *E. pedunculatum* was tested against third instar larvae of an *S. litura*. The perusal of the data clearly revealed that methanol extract at 50 mg/ml concentration showed potential larvicidal effect (80%) (Table 2) followed by ethanol extract (70%). Whereas, least larvicidal effect (5%) was noted with water extract at 5 mg/ml concentration. The positive control azadirachtin showed 90% mortality which was comparable to methanolic extract of *E. pedunculatum*. In addition all tested methanolic extract concentration showed significant mortality rate as compared to other. As seen in ovicidal activity, as extract concentration increased larvicidal activity was increased. The results are concurred with efficient larvicidal potential shown by various solvent extract of *E. pedunculatum* at various concentrations. Similarly insecticidal potentiality of *Exacum* sp is revealed by various Studies (Grainge *et al*., 1984, Gupta and Thorsteinson, 1960, Jacobson, 1975, Amoabeng *et al*., 2019) [3, 15, 16, 20].

Table 2: Percent larvicidal activity of *E. pedunculatum* extract against *S. litura*

Crude extract	Concentration mg/ml			
	5 mg/ml	10 mg/ml	25 mg/ml	50 mg/ml
Acetone	10±0.5	30±1.0	40±1.0	60±0.5
Ethanol	25±1.0	40±1.0	50±1.5	70±1.5
Methanol	30±0.5	45±0.5	60±1.0	80±2.0
Water	5±1.0	15±1.5	20±1.0	25±0.5
Azadirachtin (0.1%)	90±1.5			

Values were the means of three replicates ± standard error.

Phytochemical analysis

The phytochemical analysis was revealed the presence of variety of secondary metabolites in all tested extracts of *E. pedunculatum*. Qualitative tests were revealed the presence of secondary metabolites such as flavonoids, phenolic, terpenoid, tannin, anthraquinone, glycosides, alkaloids in methanol, Ethanol and acetone extracts of *E. pedunculatum* (Table 3). However water extract shown least number of tested metabolites.

Quantitative estimation of TPC, TFC, TTC, TAC and TTEC were studied from different solvent extracts of *E. pedunculatum*. TPC (9.18±0.06 mg TAE/g DW), TFC (2.03±0.20 mg CE/g DW) and TTC (2.87±0.04 mg CE/g DW) were recorded highest in methanolic extract followed by ethanol extracts. However, TAC (0.88±0.05 mg GE/g DW) and TTEC (0.21±0.01 mg UAE/g DW) (Table 4) were

estimated maximum in the acetone extract. The result was good agreement with Vinayaka *et al*. (2016) [33] and Jarag *et al*., (2018) [21] who recorded alkaloids, phenolics, flavonoids, tannins, terpenoids and saponins from acetone, methanolic and water extract of *E. pedunculatum*. Similarly, Huttada and Hiremath, (2016) [18] highlighted the richness of *E. pedunculatum* for the variety of secondary metabolites. The recorded values of alkaloids, phenolics, tannins are strongly agreed with value reported for *Exacum bicolor* (Paulsamy and Jeeshna, 2011) [26]. These secondary metabolites play vital role in chemical defense mechanisms of plant against insects (Amoabeng *et al*., 2019) [3]. In the present investigation recorded secondary metabolites might be responsible for the higher ovicidal and larvicidal activity of *E. pedunculatum* against *S. litura*.

Table 3: Qualitative phytochemicals test for different solvent extract of *E. pedunculatum*

Phytochemical constituents	Acetone	Methanol	Ethanol	Water
Phenolics	+	+++	++	+
Flavonoids	++	+++	+	+
Tannins	-	++	+	-
Terpenoids	+++	+	+	-
Alkaloids	+	-	-	-
Anthraquinones	-	-	-	-
Glycosides	+	+	+	-
Saponins	-	-	-	-

(+= present, - = Absent).

Table 4: Quantitative phytochemicals test for different solvent extracts of *E. pendunculatum*

Plant part	Solvents	TPC (mg TAE/g DW)	TFC (mg CE/g DW)	TTC (mg CE/ g DW)	TTEC (mg UAE/g DW)	TAC (mg GE/ g DW)
Whole plant	Acetone	7.64±0.02^{c}	0.95±0.06^{c}	0.00±0.00	0.21±0.01^{a}	0.88±0.05^{a}
	Ethanol	9.02±0.05^{ab}	1.37±0.09^{b}	0.00±0.00	0.03±0.00^{b}	0.45±0.04^{b}
	Methanol	9.18±0.06^{a}	2.03±0.20^{a}	2.87±0.04^{a}	0.03±0.01^{b}	0.49±0.02^{b}
	Water	8.83±0.11^{b}	0.43±0.03^{d}	0.71±0.16^{b}	0.00±0.00	0.30±0.03^{c}

Values were the means of three replicates ± standard error. Mean values with different alphabets in the same row showed statistically significant differences ($p<0.05$) according to DMRT.

Conclusion

The methanol extract of *E. pedunculatum* at 50 mg/ml concentration demonstrated highest ovicidal as well as larvicidal activity against *S. litura* followed by ethanol, acetone, and water. The phytochemical analysis revealed the presence secondary metabolites *viz*. alkaloids, flavonoids, terpenoids, phenolics and tannins in *E. pedunculatum*. Hence it is inferred that *E. pedunculatum* can be used further for details phytochemical investigation to develop a new botanical formulation for the management of *S. litura*.

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