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Phytochemical characterization, ovicidal and larvicidal potential of *Pentanema indicum* against *Spodoptera litura*

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Abstract
The different solvent extracts of *Pentanema indicum* (Asteraceae) are screened for ovicidal and larvicidal activity against *S. litura*. Qualitative and quantitative test were performed to know richness *P. indicum* for various bioactive metabolites. The investigation highlighted that methanol extract is more efficient for ovicidal as well as larvicidal activity. Whereas phytochemical analysis revealed the richness of *P. indicum* for phenolics, flavonoids, tannins and terpenoids and which may be responsible for efficient ovicidal and larvicidal potential of *P. indicum*. Hence further details study assists us to develop novel botanicals to manage *S. litura*.

Keywords: *Pentanema indicum*, *Spodoptera litura*, ovicidal, larvicidal, phytochemical

Introduction

Ever increasing human population and their increasing demand of daily needs like food, medicine, shelter and healthy environment are the serious issue in the today's fast growing world. For the sake of mankind we have to adapt effective strategies to increase agriculture production by preventing crop loss before and after harvest which keep equal pace between demand and production of food, shelters, medicine. Insect pest are serious problem in agriculture sector because its causes 30 to 40 % losses in major agricultural crops. (Ferry *et al*., 2004) [13]. Around 20,000 species of insects were destroyed agriculture production which leads lose of billion dollars revenue every year (Mariapackiam and Ignacimuthu, 2008) [18].

From last few decades insect pest have been controlled through indiscriminate application of chemical pesticides. Chemicals pesticides were efficiently control various agricultural pests due to their quick knock down effect and lead to increase agriculture production. The indiscriminate use of synthetic pesticides resulted to toxicity not only to non-target organism but also many other components of environment. It causes several problems like resistance to pesticides, resurgence of pests, elimination of natural enemies, toxic pesticides residues in food, water, air and soil which cumulatively affect human life's and disrupt the ecosystem, (Baskar *et al*., 2011, Balaraju *et al*., 2011) [4, 6]. So extensive screening of natural compound which having insecticidal properties are the urgent needs of today world. Therefore worldwide interest has been increased to develop alternative, environmental friendly strategies include search of novel plant based insecticidal compounds and botanical pest control agents. There are more than 2400 species of plant are said to rich source of bioactive compounds and many of such compounds are included in commercial biopesticides the potential source of bio-pesticides (Klocke, 1989, Rao *et al*., 2001) [16, 20]. These botanical pesticides are less toxic, biodegradable, environmental friendly and best alternative to chemical pesticides (Baskar *et al*., 2014) [5]. Therefore in the recent year screening of herbs, weeds and other natural products are increased to identify their insecticidal properties to develop effective alternative to hazardous chemical pesticides.

Asian armyworm *Spodoptera litura Fab* is a major polyphagous pest showed wide distribution in tropical countries such as Southeast Asia, India, China and Japan. It feed on wide range of agriculture crops numbering around 112 species from 44 families. (Baskar *et al*., 2011) [4]. It attacks several economically important crops and causes severe losses in agriculture production in all over the world (Ferry *et al*., 2004) [13]. Several botanical extracts have been tested against *Spodoptera litura* and some of them reported as a promising a biopesticides (Ningombam *et al*., 2017) [19]. However, the botanicals extracts and their complex mixture of compounds are still extensively investigated for their insecticidal, ovicidal, antifeedant properties against *Spodoptera litura* are still going throughout world.

Pentanema indicum well known medicinal plants belongs family Asteraceae (Compositae). The several ethanobotanical studies revealed the importance of *Pentanema indicum* to treat various ailments *viz*. cough, jaundice, contraceptive, anti-fertility and abortion (Tiwari, 2018) [25].

Besides, it is rich in various bioactive compounds germacranolide, vicoside A, vicodiol, vicolides, sesquiterpene lactones, vicogenin, vicosigenin, vicoside B, oleanane triperpenoids, n-alkanes and their derivatives (Srinivasan *et al*., 2007) [24]. Phytochemicals screening revealed the presence of various secondary metabolites groups like phenols, alkaloids, steroids, terpenoids and considerable pharmaceutical activities such as anti-inflammatory, analgesic, antiviral, anti-helmintic and antimicrobial (Srinivasan *et al*., 2007, Gondhali *et al*., 2019) [14, 24].

In addition members of family Asteraceae are well known for their insecticidal activity against various insect pests (Macedo *et al*., 1997, Amoabeng *et al*., 2018) [3, 17]. Therefore, the present study was focused on to examine the phytochemical characterization, Ovicidal, Insecticidal activity against the third instar larvae of *Spodoptera litura* under laboratory condition.

Materials and Methods

Plant collection

The fresh plant material of *E. pedunculatum* was collected from nearby Devchand College. The plant material was identified and authenticated based on its morphological characteristics.

Extract preparation

The plant material dried at room temperature then grind in electric mixer grinder to a fine powder. The fine powder (5 gm) extracted separately with 50ml acetone, ethanol, methanol and water on orbital shaker at 110 rpm for 6 hr at room temperature. All solvent extracts were filtered through the Whatman filter paper 1 to obtained supernatant. The supernatants were evaporated in hot air oven at 50ºC to get dry residue. The dry residue was dissolved in known amount of respective solvent and use for investigation.

Rearing of *Spodoptera litura*

Egg mass and larvae and adults of *Spodoptera litura* were collected from tobacco field located near Devchand College. The collected individuals were reared in rearing cage at laboratory. All larvae were fed regularly with castor leaf until the larvae become pupae. After adult emergence, cotton socked with 10% honey (sugar) solution mixed with a few drops of multi-vitamins was provided for adult feeding. Folded filter paper was provided from egg laying. After egg laying egg masses were collected from filter paper and allow for hatching larvae collected and fed with leaves. The entire process was repeated.

Ovicidal activity

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

Larvicidal activity

Larvicidal activity of crude extracts with different concentrations 5, 10, 25 and 50 mg/ml was carried out by topical application method on third instar larvae (Akhtar *et al*, 2012) [2]. A three micro litter extract of above mentioned concentrations were applied on the dorsum of the thorax and abdominal regions of third instar larva by using micro-pipette. Larvae were treated with azadirachtin and solvents were considered as positive and negative 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 treatment contained 20 larvae with three replicates. Larval mortality was observed and results were noted. Mortality data was corrected by using the Abbott's, (1925) [1] formula.

Qualitative test for phytochemicals analysis

All solvent crude extracts of *Pentanema indicum* were subjected to various phytochemical tests to identify phytoconstituents using standard protocols of Sofowara (1993) [23], Trease and Evans (1989) [26] and Harborne (1973) [15]. For qualitative tests of all solvent extract of *Pentanema indicum* diluted to obtained mg/ml concentration then use for the phytochemical analysis.

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 and adds few drops of 5% $FeCl_3$. The observation of Dark green/blue color confirmed the presence of phenolic compound.

Test for flavonoids

For flavonoids analysis, aliquot of extract (0.5 ml) was mixed with 0.5 ml of respective solvent. Add few drops of 1% of $AlCl_3$. Appearance of yellow colour confirmed the presence of flavonoids.

Test for Tannins

The tannin tests were performed by adding 0.5 ml of plant extract and 0.5 ml of respective solvent. Further few drops of 5% $FeCl_3$ added. Blackish color proved the presence of tannin compounds.

Test for Terpenoids

The presence of terpenoids conformed by mixing 0.5 ml of extract and 0.5 ml of solvent then 1 ml chloroform and 1 ml H_2SO_4 was added. Appearance of 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 appearance of orange color confirmed the presence of alkaloids.

Test for Anthraquinone

For anthraquinone test, few drops of magnesium acetate solution were mixed with 1 ml extract. The formation of pink color confirmed the anthraquinone.

Test for Glycosides

Glycosides test was performed by mixing 0.5 ml of extract, 0.5 ml chloroform, 0.5 ml glacial acetic acid. After add few drops of H_2SO_4. Appearance of violet to blue and then to green color indicates the presence of steroidal nucleus.

Test for Saponins

About 1 ml of plant extract and 1 ml of distilled water were mixed together then shaken vigorously. Appearance of a stable persistent froth indicates the presence of saponins.

Quantitative tests for phytochemical analysis

Total phenolics content (TPC)

TPC from all the extracts was evalauted by Folin-Ciocalteu specterophotometric method (Singleton and Rossi 1965) [22]. Aliquots of extracts (50 µl from mg/ml) and 1 ml pre-diluted Folin and Ciocalteu reagent (1:10) were mixed together. After 5 min, 800 µl of sodium carbonate was added. All the reaction mixtures were incubated for 30 min at room temperature and absorbance was read at 760 nm. Tannic acid was used to draw calibration curve and results were expressed as mg tannic acid equivalent (TAE)/g extract.

Total flavonoids content (TFC)

TFC was examined as method described by Sakanaka *et al*. (2009) [21]. 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 then kept for 5 min at room temperature Further 2 ml 1 M NaOH added then reaction mixture was mixed well and the absorbance was recorded immediately at 510 nm. Catechin was used to plot 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 (Bhat *et al*. 2007) [7]. Aliquot of extract (100 µl from mg/ml) was mixed with 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 recorded 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 methods of Chang and Lin (2011) [10]. Aliquot of extract (20 µl from mg/mL) was mixed with 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 then cooled immediately on ice bath. Further, 2 ml glacial acetic acid was added to the reaction mixture and absorbance was recorded at 548 nm. Ursolic acid was used to plot calibration curve 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) [12]. 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 represented 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 $P<0.05$.

Result and Discussion

Ovicidal activity

The different concentration of *P.indicum* extract was screened for ovicidal activity against *S.litura* and result were presented in Table 1. The ovicidal activity was assayed by accounting the no. of unhatched egg. The percentage of unhatched egg is directly proportional to percentage Ovicidal activity. The present investigation, the highest ovicidal activity (60%) was find with methanol extract at 50mg/ml concentration and least ovicidal activity (10%) noted with acetone and water extract at 5mg/ml. All tested concentration of methanol extract showed higher ovicidal activity than other tested solvent extract. At the same time as extract concentration increased, ovicidal activity was increased in all treatment. The positive control azadiractin (0.1%) presented 80% ovicidal potential and which was somewhat comparable with methanolic extract of *P.indicum*. The results were concurred with effective insecticidal ovidal activity shown by members of family Asteraceae due to presence of flavonoids, terpenoids, fatty acid, alkaloids (Carlos *et al*., 2019) [9].

Table 1: Percent ovicidal activity of *P. indicum* 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	20±0.5	25±0.5	30±1.0
Ethanol	20±0.5	35±1.0	40±1.0	45±0.5
Methanol	30±1.0	40±0.5	55±0.5	60±1.0
Water	10±0.5	10±0.5	15±1.0	25±1.0
Control	5±0.5			
Azadirachtin (0.1%)	70±1.0			

Values were the means of three replicates ± standard error.

Larvicidal activity

The larvicidal activity of different crude extracts of *P.indicum* was tested with 5, 10, 25 and 50mg/ml concentration against third instar larvae of an *S.litura* (Table 2). The perusal of the data clearly revaluated that at 50mg/ml concentration of methanolic extract of *P.indicum* showed potential larvicidal effect (60%) followed by acetone extract. Whereas, least larval mortaity was shown with ethanol at water extract at 5mg/ml concentration. In addition larval mortality was increased as concentration of extracts increased in all tested extract. The positive control azadiractin (0.1%) was shown notable larval mortality (70%) which was comparable with methanol extract of *P.indicum*. In present investigation potential larvicidal activity of *P.indicum* is strongly agreed with larvicidal activity of shown by members of Asteraceae family. Similarly, members of family Asteraceae showed the presence of various bioactive compounds from phenolics, flavonoids, terpenoids and alkaloids group which was responsible for efficient larvicidal activity against *Spodoptera* species (Macedo *et al*., 1997, Amoabeng *et al*., 2018) [3, 17]. Besides *Spodoptera* species, Asteraceae members also revealed effective insecticidal potential against several insect pest like stored grain pest, tarnished plant bug, whitefly and mosquito (Macedo *et al*., 1997, Boussaada *et al*., 2008, Fabrick *et al*., 2020) [8, 11, 17].

Table 2: Percent larvicidal activity of *P. indicum* extract against *S. litura*

Crude extract	Concentration mg/ml			
	5 mg/ml	10 mg/ml	25 mg/ml	50 mg/ml
Acetone	15±0.5	40±0.5	50±0.5	55±1.0
Ethanol	10±0.5	25±0.6	30±1.0	40±0.5
Methanol	20±1.0	35±0.4	45±0.5	60±1.5
Water	10±0.1	15±0.2	25±1.0	30±0.5
Control	5±0.9			
Azadirachtin (0.1%)	80±1.0			

Values were the means of three replicates ± standard error.

Phytochemical analysis

In the qualitative phytochemical analysis, a variety of secondary metabolites were recorded in all tested extract of *P.indicum* (Table. 3). The phenolics, flavonoids and tannins were commonly detected in all extract except for terpenoids, alkaloids, saponins, anthraquinones and glycosides. The

terpenoids and glycosides were found more intensively in acetone extract. However, saponins was only noted in ethanol extract and anthraquinones is completely absent in all extracts of *P.indicum*. Srinivasan *et al*., (2007) [24] and Gondhali *et al*., (2019) [14] recorded somewhat similar kind of observation in *P.indicum* for qualitative phytochemical analysis. The TPC, TFC, TTC, TTEC and TAC were quantitatively estimated from different extract of *P.indicum* and results were presented in Table 4. The maximum TPC (10.69±0.04 mg TAE/g DW) were found in water extract followed by acetone extract.

However, flavonoids (3.32±0.11 mg CE/g DW) were recorded in higher amount in methanolic extract then ethanolic extract.

Whereas acetone extract found elite for maximum recovery of TTC (5.53±0.09 mg CE/g DW), TTEC (2.80±0.06 mg UAE/g DW) and TAC (1.45±0.09 mg GE/g DW). The results concurred with previous studies for successive exploitation of insecticidal compounds from various phytochemical groups like phenolics, flavonoids, terpenoids, alkaloids and fatty acids (Carlos *et al*., 2019) [9].

Table 3: Qualitative phytochemicals test for different solvent extract of *P. indicum*

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

(+= present, - = absent).

Table 4: Quantitative phytochemicals test for different solvent extracts of *P. indicum*

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	7.92±0.01^{b}	0.68±0.09^{c}	5.53±0.09^{a}	2.80±0.06^{a}	1.45±0.09^{a}
	Ethanol	7.04±0.00^{c}	1.79±0.19^{b}	4.24±0.09^{b}	0.15±0.^{03cb}	0.38±0.03^{b}
	Methanol	6.50±0.00^{d}	3.32±0.11^{a}	3.74±0.09^{c}	0.24±0.08^{b}	0.47±0.03^{b}
	Water	10.69±0.04^{a}	0.72±0.03^{c}	3.23±0.06^{d}	0.00±0.00	0.02±0.01^{c}

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

Conclusion

The methanol extract of *P.indicum* at 50 mg/ml concentration exhibited significant ovicidal as well as larvicidal activity against *S. litura*. The qualitative phytochemical testing confirmed the presence bioactive metabolites *viz.* phenolics, flavonoids, tannins, terpenoids and alkaloids in *P.indicum*. Quantitative phytochemicals analysis revealed richness of *P.indicum* for phenolics, flavonoids, tannins and terpenoids which may be responsible for efficient insecticidal properties. Hence extensive investigation on *P.indicum* is needed to develop new botanicals for the management of *S. litura*.

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