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Research Article

Comparative study of *Mimosa pudica* L. plant-derived extracts as new management strategies against *Spodoptera litura* (Fabricius)

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ABSTRACT: *Spodoptera litura*, a highly polyphagous insect, has been a consequential pest for many years. The widespread occurrence of *S. litura* has compelled farmers for the constant use chemical insecticides on crops. With the negative effects of insecticides, plant extracts have gained noteworthy importance because of their insecticidal and eco-friendly characteristics. In this study, Mimosa pudica seed and root crude extracts were used for insecticidal studies. The Total Flavonoid (TF) and Total Phenolic (TP) contents of the extracts were quantified. Third-instar larvae of *Spodoptera litura* were used to evaluate the larvicidal effects of methanolic extracts of *Mimosa pudica*. The total phenolic content of *M. pudica* was measured as 42.40±0.052 mg GAE/g in the seeds and 127.43±0.37 mg GAE/g in the roots. A total flavonoid content of 28.33±0.208 mg QE/g in the seeds and 30.40±0.529 mg QE/g in the roots of *M. pudica* was reported. Larval mortality recorded after 24 h of exposure to the crude extracts of *M. pudica* showed significant larvicidal effects for the methanolic extract at 5 mg/ml. GC-MS analysis revealed 20 phyto-compounds for the methanolic extract of Mimosa pudica seeds, while 12 phyto-compounds were identified in the root extract. These findings suggest that methanolic crude extracts from the roots and seeds of *M. pudica* can act as effective pesticide agents for managing lepidopteran pests.

KEYWORDS: Bio-pesticides, GC-MS, *Mimosa pudica, Spodoptera litura*

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INTRODUCTION

Spodoptera litura, a voracious eater and a polyphagous insect, feeds on major plants of about 40 different families, posing a significant threat to global food security. It can infest over 380 plant species, including crucial crops like cotton, rice, tobacco, soybeans, vegetables, and fruits (Wu *et al.*, 2019). Farmers sustain considerable losses due to larval feeding activities; the range of seriousness of the loss depends on parameters that include crop type, stage of plant growth, and also on population density. In instances where the pest infestation is severe, the total loss to the yield is reported up to a high of 50%, which leads to devastating consequences for farmers, such as huge financial setbacks (Natikar & Balikai, 2015; Sharma *et al.,* 2022).

Various strategies are applied to manage the destructive pest, though chemical control is widely effective and most commonly used (Ahmad *et al.,* 2022). Although effective, frequent and invariable application of chemicals has made the insect show resistance to synthetic insecticides as well as new insecticides (Saraswathi *et al.,* 2023; Ahmad *et al.,* 2022; Ahmad *et al.,* 2007; Armes *et al.,* 1997; Shad *et al.,*

2012; Kim *et al.,* 1998; Saleem *et al.,* 2008; Tong *et al.,* 2013; Shad *et al.,* 2012). With the overuse of chemicals for pest control and ill effects on the environment and human health, the need for alternative pest management strategies is crucial (Ahmad *et al.,* 2019; Memon *et al.,* 2019; Berenbaum, 1996).

The genetically modified BT crops provided an efficient method to protect the host plants against *S. litura,* which attracted significant attention, but soon resistance to transgenic crops was reported (Yinghua *et al.,* 2017; Wan *et al.,* 2008). Biological means of control are safe, sustainable solutions for pest management, such as predators (Li *et al.,* 2023), parasitoids (Nawaz *et al.*, 2020), nematodes (Tomar *et al.,* 2022), bacteria (Ali *et al.,* 2019), fungi (Kumar *et al.,* 2009; Islam *et al.,* 2023; Rasheed *et al.,* 2017), Nucleopolyhedrovirus (NPVs) (Sarwar *et al.,* 2021) are some of the widely used control agents. Plant extracts or botanical extracts are gradually gaining prominence and are in prime exploration as future and eco-friendly control strategies; in addition to their biodegradable nature, this strategy supports the requisite aspect of IPM programs (Kedia *et al.,* 2015). For example, a multitude of research has been conducted on neem, which reveals its positive efficacy against *S. litura* and other pests (Koul, 2004). Compounds extracted from neem have been proven to have insecticidal, anti-feedant, growth-inhibiting, growth-regulating, protein synthesis inhibition, reduced fecundity, and sterility activity against insects, alongside having toxic effects on a wide range of insect taxa, including Lepidoptera (Koul, 2004). Plants have novel insecticidal compounds in addition to medicinal properties alongside other applications. Active phytochemicals that include alkaloids, terpenes, terpenoids, steroids, phenols, and flavonoids can be found in certain specific segments in terms of plant leaves, stems, seeds, fruits, and roots (Senthil-Nathan, 2013).

The sensitive plant, *Mimosa pudica* L., also identified as Lajjalu in Ayurveda, is an annual and perennial herb (Ahmed *et al.,* 2012), known for its rapid leaf folding upon touch (Amador-Vargas *et al.*, 2014), and it has a rich history in traditional medicine (Yuan *et al.,* 2006b, 2007b). It contains various bioactive compounds, including flavonoids and phenolics (Yuan *et al.,* 2006c, 2007b), with demonstrated antioxidant, antibacterial, and anti-liver damage properties (Samuel *et al.,* 2008; Nazeema & Brindha, 2009; Haripyaree *et al.,* 2010; Sadia *et al.,* 2008). In pest control, utilising *M. pudica* extracts as a botanical insecticide offers a potential solution to manage *S. litura* infestations. As of now, no data are available on the larvicidal activity of *M. pudica* on *S. litura*. The current study reports the larvicidal activity of crude methanolic *M. pudica seed* and root extracts against *S. litura* larvae. Exploring *M. pudica* as a botanical larvicide represents a promising avenue for sustainable pest management, aligning with the growing demand for environmentally friendly and health-conscious agricultural practices.

MATERIALS AND METHODS

Plant sample collection

Mimosa pudica seeds and roots for the study were purchased from the Indian Institute of Horticulture Research (IIHR) located in Bengaluru, Karnataka, India. Following this, the samples underwent a thorough washing with deionised water and air dried. Dried materials were ground into fine powder. The powdered plant materials were then sifted through a strainer and stored for subsequent procedures.

Plant extracts preparation

Thirty grams of powdered *M. pudica* seeds and roots were placed in a thimble, which was then inserted into the Soxhlet apparatus. Approximately 300 ml of methanol were added to the Soxhlet extractor to start the distillation. After the extraction was completed, the solvent was evaporated by the process of using a hot water bath at 50-60°C.

Total Phenolic Content (TPC) assay

Standard Gallic acid solution of concentrations varying from 20-100 µg/ml was used to assess the TPC. The Folin-Ciocalteu method for total phenolic content was measured as per the protocol mentioned by Singleton and Rossi (1965) with slight modifications. Initial mixing was done with root and seed extracts and Folin-Ciocalteu reagent (1 ml each) and incubated for 5 min. To this mix, 10 ml of 7% Na_2CO_3(w/v) was added, vortexed for 40 s, and incubated at room temperature (90 min). The optical density of the total phenolics was recorded at 550 nm using a UV-Vis spectrophotometer. All experiments were conducted in triplicate. A blank using distilled water was run under similar conditions. The gallic acid equivalent using the calibration curve equation was used to express total phenolic content in mg/g. Results were reported as mean ± SD values.

Total flavonoid content (TFC) assay

The Total Flavonoid Content (TFC) of the methanolic root and seed extracts of *Mimosa pudica* was determined using the aluminium chloride ($AlCl_3$) colorimetric method described by Zhishen *et al.* (1999), with appropriate modifications. Quercetin was used as the reference standard, and a calibration curve was prepared using quercetin solutions at concentrations of 20–100 µg/ml. Briefly, 1 mL of appropriately diluted methanolic extract or quercetin standard solution was mixed with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% (w/v) $NaNO_2$. The mixture was allowed to react for 5 min at room temperature. Subsequently, 0.3 mL of 10% (w/v) $AlCl_3$ was added, and the mixture was incubated for a further 6 min. Thereafter, 2 mL of 1 M NaOH was added, and the final volume was made up to 10 mL with distilled water. The mixture was thoroughly homogenised, and the absorbance was measured at 510 nm against a reagent blank using a UV-Vis spectrophotometer. The assay was performed in triplicate. The total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

GC-MS ANALYSIS

GC-MS analysis was carried out for the methanolic extracts of *M. pudica* (seed and root) to detect various phytochemicals present in the extracts. Spectrometric data were obtained using a MassHunter Workstation Software and a GC-MS column, Agilent Technologies (model 19091S-433UI, HP5MS UI, 30 m x 0.250 mm, 0.25 µm film thickness, operational range: -60 to 325/350°C, serial number USE137316H). Data processing was performed with MassHunter Data Analysis and MassHunter GC-MS Acquisition B.07.01.1805 (Agilent Technologies) (Wang *et al.,* 2016).

Rearing of *Spodoptera litura*

Maintenance of larvae: *Spodoptera litura* disease-free third-instar larvae (Accession no: (NBAII-MP-NOC-02) were sourced from NBAIR (National Bureau of Agricultural Insect Resources), Hebbal, Bengaluru. Initially, castor leaves were used as a natural diet, and the insects were reared with the conditions of 24±2°C, 70±5% RH – relative humidity, and the cycle of 12 h:12 h (light: dark) in a BOD incubator.

Larvicidal activity

The leaf-dip method described by Park *et al.* (2002) was employed for the assessment of the activity of plant crude extracts. Castor leaf discs of 6.5 cm diameter were utilised to evaluate the larvicidal effectiveness. Castor leaves were rinsed with 70% double-distilled alcohol and air-dried for 15 min as prior preparation. Three discs per dose were immersed in crude extracts for 30 seconds and used for the study. Solvents were allowed to evaporate in a fume hood for one hour. Concentrations varying from 0.5, 1, 2, and 5 mg/L of *M. pudica* extracts (both root and seed) were used for the third-instar larvicidal study, and carbofuran insecticide (0.1%) was used as a positive control. The Negative control included castor leaves dipped in distilled water. The study had 20 larvae per treatment and was carried out in triplicate. Larval mortality was assessed 24 h later. Moribund larvae were considered dead.

Statistical analysis

The collected larval mortality data were analysed using statistical tests, including mean values and one-way ANOVA, with the GraphPad tool. A Holm-Sidak post hoc test was then conducted, and the results with $p<0.01$ were considered significant.

RESULTS AND DISCUSSION

Plant crude extracts have been used for their bioactive and therapeutic properties (Farzaei *et al.,* 2013). Their compounds have been examined for controlling pests, in the form of toxicity, repellence, anti-feedant effects, fumigation impacts, and influences on oviposition behaviours (Niroumand *et al.,* 2016). The phenolic content results measured as GAE were based on a calibration curve ($Y = 0.0025x$, $R^2 = 0.9934$, where x represents absorbance and Y indicates gallic acid equivalent in mg/g). Phenolic compounds in plant extracts contain hydroxyl groups that are crucial for their redox properties, contributing to antioxidant activity through free radical scavenging. The total phenolic content of *M. pudica* was found to be 42.40±0.052 mg/g in the seeds and 127.43±0.37 mg/g in the roots (Figures 1, 2). This high phenolic content suggests potential antioxidant activity. In a similar study, Zhang *et al.* (2011) stated that the TPC of *M. pudica* seeds was 2.31±0.04 mg GAE/g.

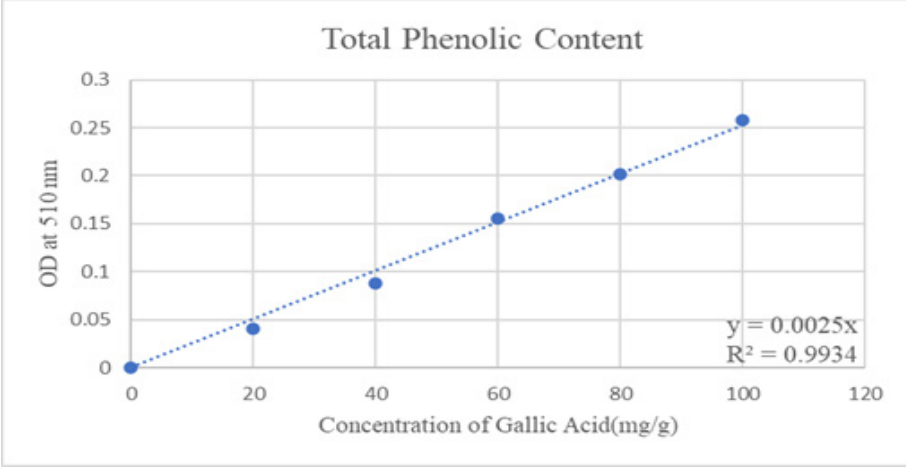


Figure 1. Standard calibration curve of gallic acid for determination of total phenolic content.

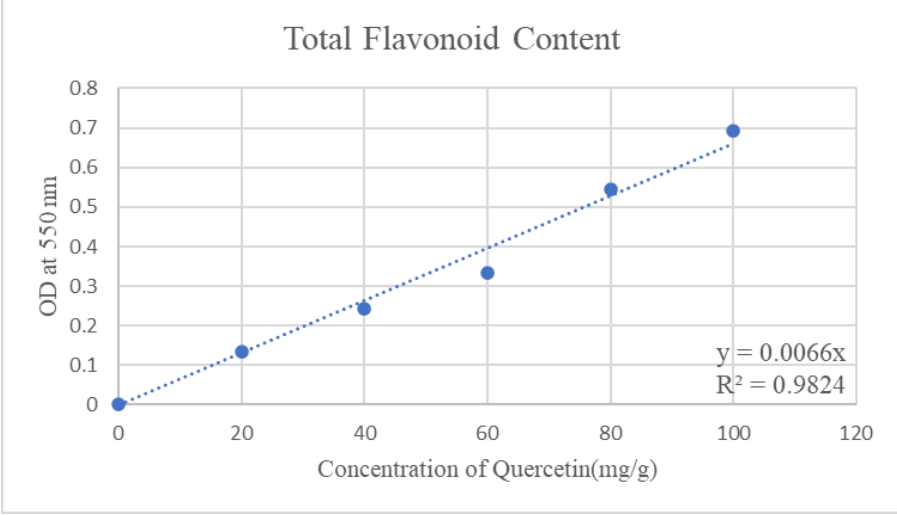


Figure 2. Standard calibration curve of quercetin for total flavonoid content estimation.

TFC of methanolic extracts of *Mimosa pudica* roots and seeds was measured in terms of Quercetin Equivalent (QE). The absorbance readings for the plant extracts and quercetin standards were plotted on a graph (Y = 0.0066x, R^2 = 0.9824, where x represents absorbance and Y denotes quercetin equivalent in mg/g). About 28.33±0.208 mg/g in

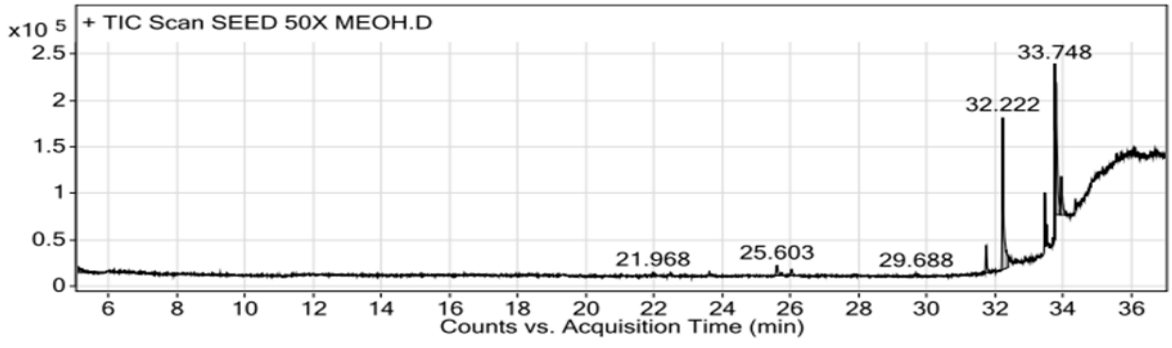


Figure 3. GC-MS graph analysis for methanolic extract of *M. pudica* seed extract.

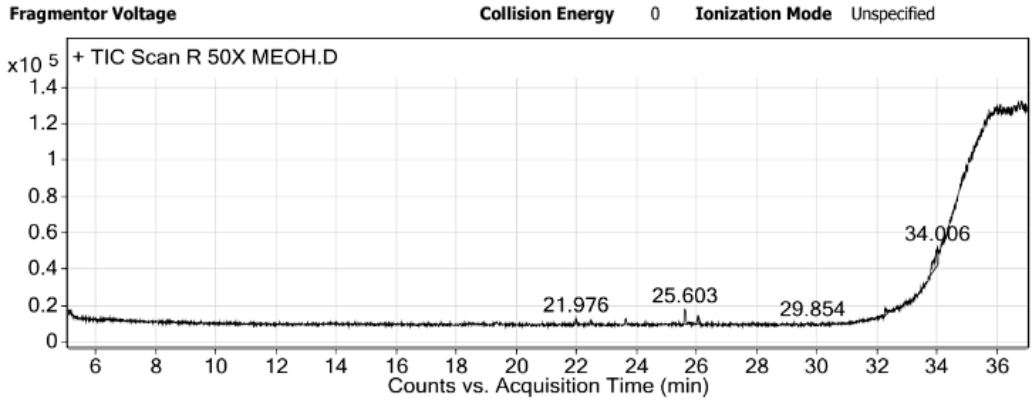


Figure 4. GC-MS graph analysis for methanolic extract of *M. pudica* root extract.

the seeds and 30.40±0.529 mg/g in the roots of *M. pudica* were reported for TFC (Figures 3, 4). Flavonoids, known for their antioxidant properties, are secondary metabolites whose effectiveness is influenced by the arrangement and number of free hydroxyl (OH) groups. According to studies by Zhang *et al.* (2011), the Total Flavonoid content (TF) of *M. pudica* seeds was found to be 0.43 ± 0.03. This group also determined the total flavonoid content in the whole of the plant stems and leaves. The result observed in our investigation was far higher than that of Zhang *et al.* (2011).

Larvicidal effect of *M. pudica* methanolic extracts on *S. litura* larvae

The results recorded during the study revealed that the methanolic root and seed extracts of *M. pudica* have a toxic effect on *S. litura* larvae. The crude extract of both the root and seed of *M. pudica* was larvicidal after 24 h of exposure. The mortality rate increases with the increased concentration of the crude seed extract of *M. pudica* (Table 1). The highest mortality rate of 100% was recorded at 5 mg/mL, with decreased larval mortality of 51.66% at 0.5 mg/ml. *M. pudica* crude root extract showed the highest mortality of 100% at 5mg/mL and the lowest mortality rate of 58.33% was recorded at 0.5mg/mL (Table 2).

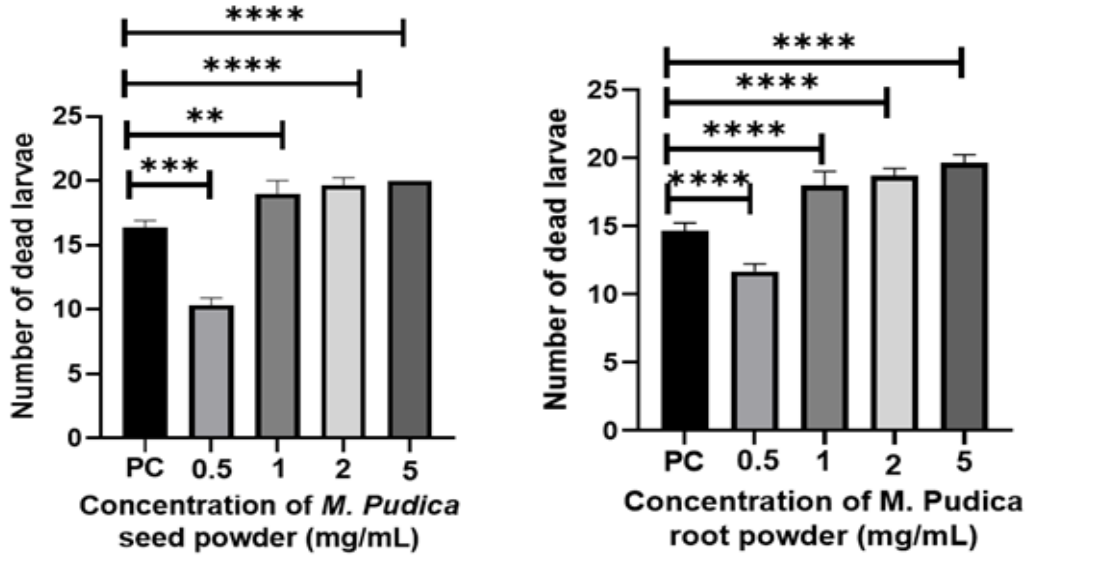
In 2016, Surendra and group investigated the larvicidal effects of an aqueous extract from *M. pudica* leaves on *Aedes aegypti* larvae at various concentrations and determined the larval mortality. They reported that the aqueous extract exhibited low larvicidal activity. Similarly, research on crude aqueous leaf extracts of *M. pudica* combined with titanium dioxide nanoparticles demonstrated significant larvicidal activity against mosquitoes (Montaser *et al.,* 2019; Marimuthu *et al.,* 2011). The observed larval mortality data were analysed using a one-way ANOVA, followed by a Holm-Sidak post hoc test by comparing experimental

Table 1. Larvicidal activity of *M. pudica* seed crude extract on *S. litura* at different concentrations

S. No.	Plant extract	Exposure (h)	Dose (mg/mL)	Mean±SD	Mortality rate of *S. litura* larvae (%)
1	*M.pudica* seed	24	0.5	10.333±0.5773	51.66%
2			1	19±0.5773	95%
3			2	19.666±1	98.3%
4			5	20±0.5773	100%
5	Negative control	24	-	0	0%
6	Positive control			16.333±0.5773	81.66%

Table 2. Larvicidal activity of *M. pudica* root crude extract on *S. litura* at different concentrations

Sl. No.	Plant extract	Exposure (h)	Dose (mg/mL)	Mean±SD	Mortality rate of S. litura larvae (%)
1	*M.pudica* root	24	0.5	11.66± 0.577	58.33%
2			1	18±1	90%
3			2	19±0	95%
4			5	20±0	100%
5	Negative control	24	-	0	%
6	Positive control			14.66± 0.5773	73.3%



Values are Mean ± SEM from three independent experiments. *P<0.0001 (one-way ANOVA followed by Holm-Sidak *post-hoc* test) compared to Positive Control treated group.
All the data represents average of n=3 independent experiments. Here, ****, p<0.0001; **, p< 0.001; **, p < 0.01.

Figure 3. illustrates the values for larval mortality against the application of *M. pudica* root and seed sample.

and control groups, with a significance level established at p<0.01 (Figure 5).

Phyto-compound characterisation

GC-MS results of the methanolic extract of *Mimosa pudica* revealed 20 phyto-compounds in the seeds and 12 in the roots, as detailed in Tables 3 and 4, respectively. n-Hexadecanoic acid (retention time: 32.222), which is known for its insecticidal, nematicidal, and pesticidal activities (Ravi *et al.,* 2018), and 9, 12-Octadecadienoic acid (Z,Z) (retention time: 33.781), which exhibits insectifuge and nematicidal properties (Selvan *et al.*, 2015),

Table 3. Compounds isolated through the GC-MS analysis of the methanolic extract of *Mimosa pudica* seed extract

Sl. No.	Peak	Start	RT	End	Height	Area	Area(%)	Compound name	Activity	References
1	1	5.129	5.137	5.393	4766.79	32504.04	6.52	1-Propanol, 2-amino-, (.+/-.)-	N/A	
2	2	21.893	21.968	22.06	4659.75	21100.57	4.23	7-Oxabicyclo [4.1.0] heptane, 3-oxiranyl	N/A	
3	3	22.395	22.477	22.535	3480.6	11750.25	2.36	Ethylamine, 2-(adamantan-1-yl)-1-methyl	Larvicidal	(Wiraswati *et al.*, 2023)
4	4	23.569	23.619	23.736	4808.3	17370.39	3.48	Bicyclo [5.1.0] octane, 8-(1-methy lethylidene)	Antibacterial, antifungal	(Khan *et al.*, 2015)
5	5	25.536	25.603	25.678	10952.94	41644.37	8.35	Cyclohexene, 2-ethenyl-1,3,3-trimethyl	Antioxidant, antibacterial, antiplasmodial and anticancer	(Wiraswati *et al.*, 2023)
6	6	25.678	25.728	25.786	2607.5	8984.44	1.8	R-(-)-Cyclo hexyl ethylamine	Larvicidal	(Luna-Hernández *et al.*,2021)
7	7	25.97	26.028	26.11	6767.25	25569.61	5.13	Bicyclo [5.1.0] octane, 8-(1-methyl ethylidene)-	Antibacterial, antifungal	Khan *et al.*, 2015)
8	8	29.622	29.688	29.705	3202.72	7115.88	1.43	1-Propanol, 2-amino-, (.+/-.)-	N/A	
9	9	31.699	31.739	31.836	29208.57	73568.56	14.76	Hexa decanoic acid, methyl ester	Antibacterial, antifungal	(Shaaban *et al.*, 2021)
10	10	32.178	32.222	32.381	163650.2	498577.18	100	n-Hexadecanoic acid	Insecticidal, nematicide, and pesticide	(Rajiv *et al.*, 2018)

Table 3. Continued

11	11	32.85	32.889	32.922	3897.93	8327.18	1.67	n-Nonadecanol-1	Antimicrobial and cytotoxic property	(Kuppuswamy *et al.*, 2013)
12	12	33.44	33.456	33.489	57577	74027.82	14.85	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anticancer, antioxidant, antitumor	(Ukwubile *et al.*, 2019)
13	13	33.489	33.506	33.539	21552.23	29287.54	5.87	cis-Vaccenic acid	Antibacterial	(Ali *et al.*, 2020)
14	14	33.714	33.748	33.764	190307.5	281446.95	56.45	9,12-Octadecadienoic acid (Z,Z)-	Anticancer, nematicide, insectifuge	(Selvan *et al.*, 2015)
15	15	33.906	33.781	33.906	142576.15	475712.38	95.41	9,12-Octadecadienoic acid (Z,Z)-	Anticancer, nematicide, insectifuge	(Selvan *et al.*, 2015)
16	16	34.33	33.939	34.096	41432.33	129691.63	26.01	Oleic Acid	Anticancer, antiinflammatory, insectifuge	(Selvan *et al.*, 2015)
17	17	35.54	34.356	34.39	10770.55	20922.15	4.2	1,4-Bis(trimethylsilyl) benzene	Larvicidal	(Sahayaraj *et al.*, 2022)
18	18	35.54	35.557	35.59	7850.82	10636.56	2.13	Cyclotetrasiloxane, octamethyl	Antibacterial	(Keskın *et al.*, 2012)
19	19	36.04	36.065	36.149	9090.88	32042.19	6.43	Cyclotetrasiloxane, octamethyl	Antibacterial	(Keskın *et al.*, 2012)
20	20	36.665	36.69	36.903	6591.52	40274.96	8.08	Cyclotetrasiloxane, octamethyl	Antibacterial	(Keskın *et al.*, 2012)

Table 4. Compounds isolated through the GC-MS analysis of the methanolic extract of *Mimosa pudica* root extract

Sl. No.	Peak	Start	RT	End	Height	Area	Area (%)	Compound name	Activity	Reference
1	1	5.112	5.179	5.208	2556.55	6068.54	6.47	R-(-)-Cyclo hexyl ethylamine	Larvicidal	(Luna-Hernández *et al.*, 2021)
2	2	21.935	21.976	22.046	3171.62	9100.79	9.7	(S)-(+)-1-Cyclohexylethylamine	Larvicidal	(Baskaran *et al.*, 2018)
3	3	22.416	22.477	22.543	2533.33	8960.35	9.55	Ethylamine, 2- (adamantan-1-yl)-1-methyl	Larvicidal	(Wiraswati *et al.*, 2023)
4	4	23.585	23.635	23.692	3295.02	10333.3	11.02	1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide	N/A	
5	5	25.547	25.603	25.686	8160	31039.43	33.1	Cyclo hexene, 2-ethenyl -1,3,3- trimethyl	Antioxidant, antibacterial, antiplasmodial and anticancer	(Wiraswati *et al.*, 2023)
6	6	25.686	25.72	25.792	1378.92	5006.64	5.34	R-(-)-Cyclo hexyl ethylamine	Larvicidal	(Luna-Hernández *et al.*, 2021)
7	7	25.986	26.053	26.11	4375.02	16532.32	17.63	Ethylamine, 2- (adamantan-1-yl) -1-methyl	Larvicidal	(Wiraswati *et al.*, 2023)
8	8	27.845	27.862	27.904	1344.68	2278.87	2.43	R-(-)-Cyclo hexylethy lamine	Larvicidal	(Luna-Hernández *et al.*, 2021)
9	9	29.813	29.854	29.879	1176.37	2264.56	2.41	D-Alanine	Larvicidal and insecticidal	(Zhou *et al.*, 2018)
10	10	32.205	32.297	32.364	3499.5	19298.03	20.58	(2S,3S)-(-)-3-Propyloxiranemethano	Antibacterial and antimicrobial	(Rakhmawatie *et al.*, 2023)
11	11	33.034	33.081	33.089	2949.32	3727.78	3.97	1,2-Bis(trimethylsilyl) benzene	N/A	
12	12	33.759	34.006	34.031	9911.65	93784.9	100	Cyclotetrasiloxane, octamethyl	Antimicrobial, antioxidant, antibacterial	(Rasyid *et al.*, 2023)

were reported to be major seed extract compounds. Minor compounds, such as Cyclohexylethylamine and 1-Propanol, 2-amino, also indicated insecticidal properties. The major compound identified from the analysis of the *M. pudica* root sample was Cyclotetrasiloxane, octamethyl (retention time: 34.006), which is a characteristic feature of antioxidant and antibacterial actions (Rasyid *et al.,* 2023). Additionally, another minor compound, R-(-)-Cyclohexylethylamine, found to possess larvicidal activity, was also reported (Luna-Hernández *et al.,* 2021).

In conclusion, this investigation reports that the crude extracts of *M. pudica* root and seed demonstrated larvicidal activity on *S. litura*. The mortality rate increases with the increased concentration of the crude extracts used. These results recommend that the extracts could be explored further as a potential natural product in pest management. However, their effects on non-target organisms need to be tested. Seeds methanolic extracts of *Mimosa pudica* accounted for 20 phyto-compounds, whereas 12 phyto-compounds were identified from root extract on characterisation by GC-MC analysis.

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AUTHOR CONTRIBUTION

Saraswathi Saraswathi, Nibedita Pradhan and Esther Shoba conceptualised and designed the study and contributed to the interpretation of the results. Debojyoti Chatterjee, L. Ignatius Raymond Peter, Froilanmark Chrishone and Saptak Kumar Basu conducted the study, analysed the data and prepared the initial manuscript under the supervision of Saraswathi Saraswathi and H. E. Krishnaiah. H. E. Krishnaiah contributed to the characterisation of the compounds. The manuscript was written by Saraswathi Saraswathi and critically reviewed by all the authors. All authors have read and agreed to the published version of the manuscript.