International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 12-09-2024, Accepted: 13-10-2024, Published: 28-10-2024
Volume 9, Issue 10, 2024, Page No. 184-188

Qualitative estimation of phytochemicals from selected plants and their effect on Lepidopteran pest

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Abstract
Three plants (*Gnidia glauca* (Fresen.) Gilg, *Lygodium japonicum* (Thunb.) Sw. and *Ocimum basilicum* L.) were selected in order to investigate presence of various phytoconstituents. Plant leaves from three plants were collected, dried and extracted in Acetone, chloroform, methanol and water. Preliminary phytochemical analysis (qualitative) and total phenol, tannin, total alkaloid contents were carried out using spectrophotometric method. Antifeedant activity was evaluated against lepidopteran pest (*Spodoptera litura* Fab.) using a no-choice disc method. Methanolic extract of *G. glauca* recorded the highest tannin content (0.634±0.046 mg TAE/g DW) followed by methanolic extracts of *O. basilicum* (0.576±0.002 mg TAE/g DW). Methanolic extract of *L. japonicum* recorded the highest phenolic contents (0.826±0.034 mg CE/g DW). The *G. glauca* leaf extract (5%) in methanol exhibited highest antifeedant activity (52.56±0.003%) followed by 5% acetone extract (38.76±0.57%).

Keywords: Phytoconstituents, antifeedant, plant extract, antifeedant activity

Introduction

A recent global survey on major crops showed that, over 100 pests and pathogen species accounted for 20%–30% of crop losses (Savary *et al.*, 2019) [24]. Chemical pesticides play an important role to control these factors and improve production (Obembe *et al.*, 2017; Sharma *et al.*, 2019) [21, 26]. Lepidopteran pests belonging to family Noctuidae are highly destructive in agriculture due to voracious feeding habits and rapid reproduction. Lepidopteran pests can survive on every continent except Antarctica and there is hardly any plant not attacked by lepidopteran pests. These polyphagous pests including *Spodoptera litura* (Fab.) cause extensive damage to crops such as soybean, tomato, chickpea and cotton, causing significant losses in yield. Due to this reason in Asia-pacific area *S. litura* is considered as one of the most destructive pest (Ahmad *et al.*, 2013) [2]. Pesticide plays a significant role in controlling pests, diseases and increase crop yield world-wide (Tudi *et al.*, 2021) [32].

But, pesticide pollutes water, soil and air with harmful effects on human health, food quality and ecosystems (Damalas and Eleftherohorinos, 2011) [6]. To avoid the utilization of chemical pesticides, new alternatives and techniques are required. The use of biopesticides should be uplifted over synthetic pesticides (Chandler *et al.*, 2011) [5]. Biopesticdes produce fewer hazards to environment and human health being less toxic, residue free, target specific and accepted for organic farming (Kumar, 2015; Julia *et al.*, 2016) [17, 19]. Many plant derived compounds affects insects through repellence, feeding and oviposition deterrence and no harm to vertebrates (Senthil-Nathan, 2013) [25].

Phytochemical screening plays a crucial role in identifying the bioactive constituents responsible for the antifeedant activity of plant extracts. Qualitative analysis detects secondary metabolites such as tannins, phenols, flavonoids, terpenoids and alkaloids and which play key role in plant defense against pests (Divekar *et al.*, 2022) [7].

The present investigation was undertaken to study the phytochemical screening and antifeedant activity of *Gnidia glauca* (Fresen.) Gilg, *Lygodium japonicum* (Thunb.) Sw. and *Ocimum basilicum* L. against the lepidopteran pest *Spodoptera litura*. By identifying effective plant-based compounds, this research aims to contribute in the development of natural pest control strategies that can be incorporated into sustainable agriculture practices.

Materials and methods

Collection and extraction of the plant material

Fresh plant leaves of *G. glauca*, *L. japonicum* and *O. basilicum* were collected from the college campus and washed under tap water, dried in shade and powdered by using electric grinder. The powder is sieved and sopping in methanol, acetone, chloroform and distilled water separately for 24 hours. Extraction was done using method given by Eneh *et al.* 2016 [10]. The extract was filtered through Buchner Funnel and collected in borosil vials and stored at 4^0 C for further use.

Qualitative phytochemical analysis

Preliminary phytochemical analysis was performed by following the standard methods given by Harborne (1973) [15] Trease and Evans (1989) [31], Sofowara (1993) [30] and Gantra *et al*. (2012). All solvents extracts of selected plant species were diluted to mg/ml concentration and used for the qualitative phytochemical analysis.

Compunds	Test	Inference
Phenolics	One milliliter of extract was added along with a few drops of a 5% $FeCl_3$ solution	Phenolics are indicated by occurance of dark blue, green or black colour.
Flavonoids	1 ml of extract and a few drops of 1% $AlCl_3$ solution mixed	Mixture appears yellow shade suggests the presence of flavonoids.
Tannins	1 ml of the plant extract with a few drops of 5% $FeCl_3$, the tannin	The presence of tannins was indicated by a dark colour appearance.

Terpenoids	1 ml of extract, 1 ml of chloroform, and 1 ml of concentrated H_2SO_4 were combined	a reddish brown colour change at the interface indicate presence of terpenoids.
Alkaloids	1 ml was mixed with a few drops of Dragondoff's reagent	The appearance of orange precipitate shows the presence of alkaloids.
Saponins	1ml of extract was mixed with1 ml of distilled water and shaken well	Presence was indicated by a stable persistent forth

Quantitative estimation

Total Tannin Content (TTC)

For estimation of total tannin content (TTC) in plant extract, add 100 µl samples in 7.5 ml distilled water. Add 500 µl of Folin Ciocaltue Phenol Reagent and 35% 1 ml sodium carbonate to the mixture and dilute to 10 ml with distilled water. Shake the mixture well and keep at room temperature for 30 minutes. Observe the absorbance at 700 nm. Tannic acid is used as standard. (Kavitha, 2016) [18].

Total Phenol Content (TPC)

Total phenol content (TPC) was estimated by method given by Yadao *et al.* (2015) [33] with slight changes. Take 200µl (mg/ml) sample in test-tube, add 1 ml Folin Ciocaltue Phenol Reagent, after 5 minutes add 0.8 ml 7.5 % sodium carbonate. Keep the mixture for incubation for 60 minutes at room temperature, measure the absorbance at 765 nm. Catachein is used as Standard.

Test insect

For the present study, *Spodoptera litura* belonging to order Lepidoptera and family Noctuide is selected. Rearing was performed by using method given by (Arivoli and Tennyson, 2013) [3] and the culture was maintained throughout the study period.

Antifeedant activity

Antifeedant activities of plant extracts in different solvents were tested by leaf disc no choice method Isman *et al*. (1990) [16] and Arivoli and Tennyson (2013) [3]. Disc of fresh castor leaf measuring 4 cm diameter was prepared and dipped in 1% concentration of plant extracts separately. Treated discs were air dried and placed individually in to petri dish (9 cm diameter). Third instar pre starved larva of *Spodoptera litura* were introduced in each petridish. The larvae were allowed to feed on the treated castor leaf discs for 24 hours. Leaf discs treated with methanol, chloroform, water and acetone were served as positive and negative control respectively. The experiment was designed in three replicates. Unconsumed leaf area was recorded for each replicate after 24hours and percentage of antifeedant activity was calculated using formula:

$$\text{Antifeedant activity \%} = [(C-T) \div (C+T)] \times 100$$

Where, C- Leaf disc consumed by the larvae in control, T- Treated leaf disc consumed by the larvae.

Results and discussion

The results of phytochemical screening of plant extracts extracted in solvents such as acetone, methanol, chloroform and distilled water are recorded in Table 1. It clearly indicates the presence of different secondary metabolites in all four different solvents.

In the present investigation, phytochemicals such as tannin, phenol, flavoinoids, terpenoids, alkaloids were present in methanol extract of *G. glauca, L. japonicum* and *O. basilicum*. Acetone extract of *O. basilicum* and *L. japonicum* showed the presence of all phytochemicals except saponins. However, chloroform extracts shows least number of tested metabolites. The presence of photochemicals in plants may be related to their insecticidal properties (Adeniyi *et al*., 2010) [1].

Table 1: Qualitative phytochemical screening of leaf extracts of *Gnidia glauca, Lygodium Japonicum* and *Ocimum basilicum*

Name of the plant	Solvents	Phenolics	Flavanoids	Tannins	Terpenoids	Alkaloids	Saponin
Gnidia glauca	Water	+	+	+	+	+	-
	Acetone	+	+	+	+	-	+
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	+	-	-	+
Lygodium japonicum	Water	+	-	+	+	+	-
	Acetone	+	+	+	+	+	-
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	+	-	-	-
Ocimum basilicum	Water	+	+	+	-	-	-
	Acetone	+	+	+	+	+	-
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	+	+	-	-

"+" indicates the presence of phytochemicals "-" indicates absence of phytochemicals

Estimations of secondary metabolites

Total phenol content and total tannin content were estimated by using UV- Doublebeam Spectrophotometer (Shimadzu UV-1900i). At DIST-FIST, Science Instrumentation Facility Centre, Devchand College, Arjunnagar.

Total tannin content

To estimate TTC in plant extracts, FCR method was used. Methanolic extract of each plant displayed the presence of highest total tannin content, followed by acetone, aqueous and chloroform extracts as shown in Figure 1. Chloroform extract of all three plants recorded the lowest content of TTC and TTP as compared to methanol, acetone and aqueous extracts. Methanolic extract of *G. glauca* recorded the highest tannin content (0.634±0.046 mgTAE/g DW) followed by methanolic extracts of *O. basilicum* (0.576±0.002mgTAE/g DW). Petchidurai *et al.* (2023) [22] and El-Aswad *et al.* (2023) [9] reported the antifeedant and insecticidal potential of tannins.

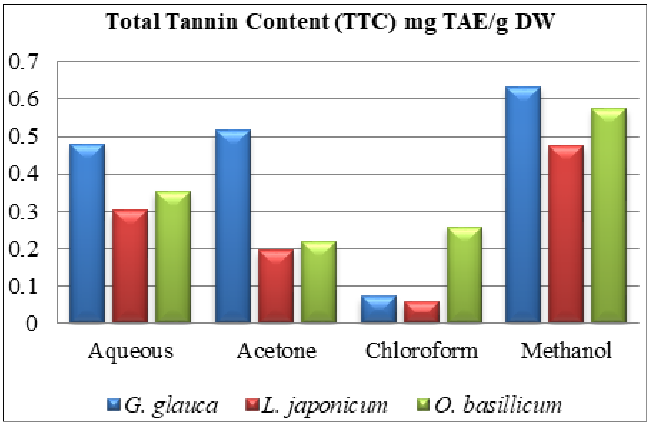


Fig 1: Total tannin content (TTC) mg TAE/g DW

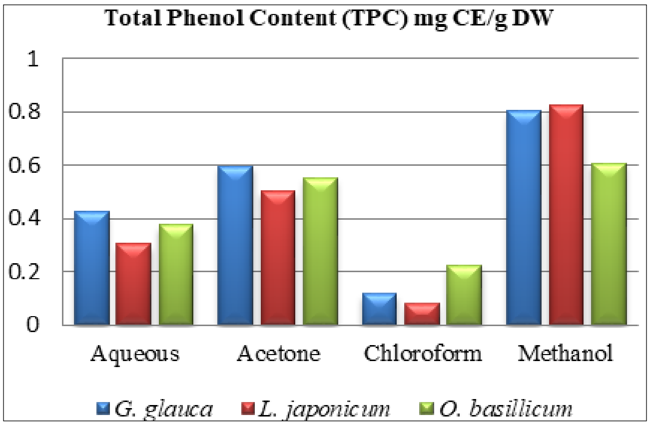


Fig 2: Total phenol content (TPC) mg CE/g DW

Total phenol content

Total phenol content of *G. glauca*, *L. japonicum* and *O. basilicum* are presented in Figure 2. Plant extracts in methanol exhibited the presence of highest total phenolic content, followed by acetone and aqueous extracts. Chloroform extract showed lowest phenolic content as compared to other solvents. Methanolic extract of *L. japonicum* recorded the highest phenolic contents (0.826±0.034 mg CE/g DW), followed by *G. glauca* (0.803±0.001 mg CE/g DW) and *O. basilicum* (0.609±0.062 mg CE/g DW) respectively. Presence of phenolics in plant extract is linked to insecticidal properties (Gorawade *et al*. 2021; Elsharkawy *et al*., 2018) [8].

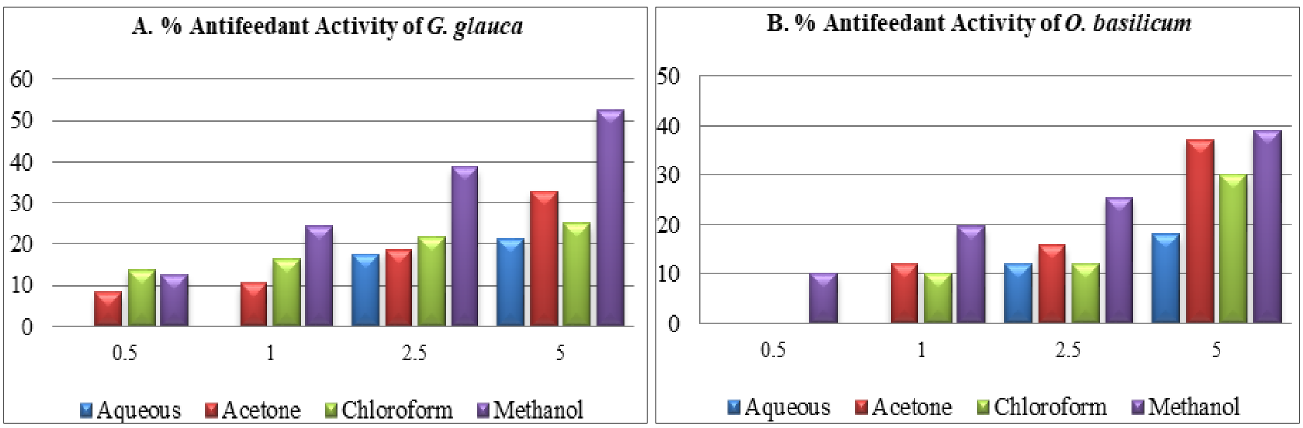
Antifeedant activity

Table 2: Percentage Antifeedant activity of *G. glauca, L. japonicum and O. basilicum* leaf extract against *Spodoptera litura*

Solvent	Treatment (%)	Antifeedant Activity (%)			
		Aqueous	**Acetone**	**Chloroform**	**Methanol**
G. glauca	0.5	-	8.23±0.023	13.66±0.177	12.36±0.003
	1.0	-	10.59±0.215	16.29±0.155	24.32±0.59
	2.5	17.62±0.006	18.66±0.177	21.68±0.1	38.76±0.57
	5.0	21.45±0.059	32.84±0.085	24.9±0.015	52.56±0.003
O. basilicum	0.5	-	-	-	10.15±0.026
	1.0	-	11.92±0.104	10.15±0.026	19.58±0.256
	2.5	11.92±0.103	15.82±0.123	11.92±0.104	25.37±0.006
	5.0	17.96±0.003	37.12±0.215	30.00±0.193	39.005±058
L. japonicum	0.5	-	8.23±0.056	-	11.96±0.03
	1.0	10.32±0.26	14.76±0.35	11.97±0.87	16.23±006
	2.5	13.92±0.104	19.67±0.56	16.67±0.016	27.12±0.25
	5.0	23.36±0.023	30.00±0.193	31.23±001	32.84±0.085
Azadiractin	1.0	58.99±0.066	-	-	-
Negative control	-	-	-	-	-

± =Standard Deviation

Antifeedant property of each of the plant extracts was evaluated by leaf area consumed by third instar larvae of *S. litura* in treated and in control (Table 2). The *G. glauca* leaf extract (5%) in methanol exhibited highest antifeedant activity (52.56±0.003%) followed by 2.5% concentration (38.76±0.57) and 5% concentration of Acetone extract (Figure 3.A.).. Figure 3.B. depicts highest antifeedant activity (39.005±058%) of *O. basilicum* (5%) in methanol extract. Methanolic extract (5%) of *L. japonicum* (Figure 3.C.) shows the highest antifeedant activity (32.84±0.085%). Aqueous extract of *O. basilicum 0.5* % concentration recorded the lowest antifeedant activity**.**



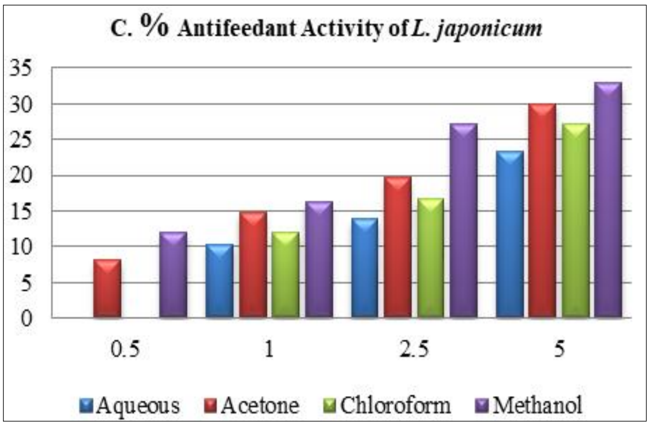


Fig 3: A. % Antifeedant Activity of *G. glauca*, B. % Antifeedant Activity of *O. basilicum* C. % Antifeedant Activity of *L. japonicum*

The present study proved the feeding deterrent effect against *S. litura*, methanolic extracts of all three plant extracts showed significant results than acetone, aqueous and chloroform extracts. These results corroborate with the findings of Gorawade *et al.* (2021) [13], who has reported the significant antifeeding activity in methanolic extracts (5%) of *Chromolaena odorata* and *Leonotis nepetifolia* (82.45 ± 0.16% and 71.77 ± 0.73%) respectively against *S. litura* than in acetone and aqueous extracts. Arumugam *et al*. (2015) [4] proved that, extracts of *Rivina humilis* in different solvents can be effectively used as antifeedant, ovicidal, larvicidal and oviposition deterrent against *Spodoptera litura*. Ningombam (2017) [20] has reported the effective antifeedant activity and phytochemical investigation of *Millettia apachycarpa* extracts against *Spodoptera litura* (Fabricius). Gorawade *et al.* (2019) [12] reported the effective antifeedant activity of different solvent extracts of *Eupatorium odoratum* L. on the third instar larva of *Spodoptera litura* (Fabricius), which results in high antifeedant activity in acetone extract followed by methanol and aqueous extracts. Ponsankar *et al.* (2019) [23] investigated a bioactive compound isolated from *Citrullus colocynthis* (L.) Schrad and screened bioassay against *S. litura* and earthworm species *Eisenia fetida*. Phytochemical profiling, antifeedant and larvicidal, insecticidal activity of Exacum *pendunculatum* (Shiragave, 2020 a) [29], *Gnidia glauca* (Fres.) Gil. Shiragave (2018**),** *Pentanema indica* (Shiragave, 2020 b) [29] against *Spodoptera litura* (Fabricius) has well documented.

Conclusion

The proposed study helps in finding out another way of controlling pests with locally available wild plant extract. This also helps in bio-controlling of pests without any adverse effects on ecosystem. The plants such as *G. glauca*, *O. basilicum* and *L. japonicum* are abundant in phytoconstituents including tannins, phenols, alkaloids, flavoinoids, terpenoids and saponins. Furthermore, methanolic extracts from all three plants demonstrated significant deterrent effects against tobacco cutworms. These plants could serve as valuable sources of phytopesticides for controlling tobacco cutworms.

Acknowledgement

We are thankful to DST-FIST, Science Instrumentation Facility Centre (SIFC), Devchand College, Arjunnagar for providing laboratory facility.

Conflict of interest

There is no conflict of interest involved in the study

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