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Screening for secondary metabolite profile in *Momordica cymbalaria* Hook (Fenzl.) and their possible anti insect properties against *Spodoptera litura* Fab.

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Abstract
Momordica cymbalaria Hook (Fenzl.) is a non-cultivated, season-bound, tuberous vegetable plant harvested from wild sources. A little amount of research has been carried out on its phytochemistry. Further, none of the research articles focussed on its insecticidal value. Hence, the present research focussed on *M. cymbalaria* secondary metabolite profiling and screening its anti-insect properties. Saponins impacting feeding, growth, reproduction, and imparting mortality, and quinines causing cytotoxicity and immunotoxicity were found in acetone, methanol, and hexane extract of leaf, peel, seed, and tuber. Terpenes, one of the largest groups of secondary metabolites was found in acetone, hexane, and methanol extract of seed; acetone and methanol extract of leaf and tuber, and acetone extract of peel. Alkaloids resulting in pupal malformation, weight reduction, and extended development duration were seen in the hexane extract of peel; acetone extract of seed and tuber, and methanol extract of leaf and tuber. Coumarins, potent ovicidal and larvicidal compounds were found in acetone, hexane, and methanol extract of leaf; acetone and hexane extract of peel and tuber. Flavonoids were found in acetone, methanol, and hexane extract of seed and acetone and methanol extract of peel and acetone extract of leaf and tuber. Tannins were found predominately present in hexane extract of leaf, peel, seed and tuber. Phenols affecting larval growth, survival, adult emergence, pupal weight, and nutritional indices were present in hexane and acetone extract of leaf, peel, seed, and tuber. Further, the extracts tested on *Spodoptera litura* third instar larvae also revealed the presence of antifeedant, insecticidal, insect growth regulatory properties in the extracts.

Keywords: anti-insect properties; *M. cymbalaria*; screening; secondary metabolites

Introduction
Plants utilized in pest management for a long time are an excellent source of secondary metabolites. These naturally occurring bioactive secondary metabolites are the defense agents against herbivory. Among many, secondary metabolites *viz.*, terpenoid, alkaloid, flavonoid, phenol, saponin, coumarin, etc. are a few (Krishnaiah *et al*, 2007) [8]. Although a plethora of secondary metabolites has been identified from plant sources, continued search is worth doing as it adds to the repertoire of bioactive compounds. *Momordica cymbalaria* Hook (Fenzl.) is a non-cultivated, season-bound, tuberous vegetable plant harvested from wild sources. A little amount of research has been carried out on its phytochemistry. Further, none of the research articles focussed on its insecticidal value. Hence, the present research focussed on secondary metabolite profiling of *M. cymbalaria* leaf, peel, seed, and tuber and screening of its anti-insect properties against *Spodoptera litura* Fab.

Materials and Methods
Collection of plant sample
Leaf, peel (Fruit skin), seed, and tuber of *M. cymbalaria* were collected from Sangarankoil of Tenkasi district, Virudhunagar, Sattur, and Aruppukottai of Virudhunagar district, and T. Kallupatti, Saptur, and Tirumangalam of Madurai district. The vine along with the entire plant was collected during the rainy and winter season (October – January). The succulent tubers were collected during the pre-monsoon season once they started germinating (September – October).

Sample preparation
The collected plant parts were washed with distilled water and shade dried until the moisture content was completely removed. The dried samples were ground into a fine powder with a traditional stone grinder, sieved to uniform particle size using a 30 mesh sieve, and stored in air-tight plastic containers (500 ml capacity) separately.

Extraction of bioactive compounds
The powdered plant samples were extracted using three different analytical grade solvents *viz.*, hexane (Non-polar, BP – 69°C), acetone (Polar, BP – 56°C), and methanol (Most polar, BP – 65°C) sequentially at room temperature. 30 g of each powdered plant sample was soaked in 300 ml of the respective solvent sequentially (from non-polar to most polar) for seven days each in a 500 ml conical flask, covered with aluminum foil and shaken intermittently in a magnetic stirrer (Remi 2-MLH) (Patil, 2009). Later, each solvent extract was poured out into a desiccator for evaporating the solvent, the extractive was collected separately, and stored in glass vials (30 ml Capacity) kept in a refrigerator until further use.

Qualitative phytochemical screening of *M.* cymbalaria extracts
Phytochemical tests were carried out for all the extractives as per the standard methods described by Roopashree *et al*., (2008) [20] and Obasi *et al*., (2010). These experiments were replicated four times.

Test for saponins
To two ml of the plant extractive, two ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. Formation of one cm layer of foam indicated the presence of saponins.

Test for quinones
To one ml of the extractive, one ml of concentrated sulfuric acid was added. Formation of red colour indicated the presence of quinones.

Test for terpenoids
To 500 µl of the extractive, two ml of chloroform and concentrated sulfuric acid were added carefully. Formation of red brown color at the interface indicated the presence of terpenoids.

Test for triterpenoids
To 1500 µl of the extractive, one ml of Liebermann–Burchard Reagent (acetic anhydride + concentrated sulfuric acid) was added. Formation of blue green colour indicated presence of the triterpenoids.

Test for alkaloids
To two ml of the plant extractive, two ml of concentrated hydrochloric acid and a few drops of Mayer's reagent (Solution A: 1.358gm mercuric chloride + 60mL distilled water; Solution B: 5gm potassium iodide + 10mL distilled water; Working solution: solution A + solution B + distilled water to make final volume 100mL) were added. Presence of green colour or white precipitate indicated the presence of alkaloids.

Test for coumarins
To one ml of the extractive, one ml of 10% NaOH was added. Formation of yellow colour indicated the presence of coumarins.

Test for flavonoids
To two ml of the plant extractive, one ml of 2N sodium hydroxide was added. Presence of yellow colour indicated the presence of flavonoids.

Test for tannins
To one ml of the plant extractive, two ml of per cent ferric chloride was added. Formation of dark blue or greenish black colour indicated the presence of tannins.

Test for phenols
To one ml of the extractive, two ml of distilled water followed by a few drops of 10% ferric chloride were added. Formation of blue or green color indicated the presence of phenols.

Test for glycosides
To two ml of the plant extractive, three ml of chloroform and 10% ammonia solution were added. Formation of pink colour indicated the presence of glycosides.

Test for cardiac glycosides
To 500 µl of the extractive, one ml concentrated sulfuric acid, two ml glacial acetic acid and a few drops of five per cent ferric chloride were added. This was under layered with 1 ml of concentrated sulfuric acid. Formation of brown ring at the interface indicated the presence of cardiac glycosides.

Evaluation of anti–insect properties of *M. cymbalaria* extractives against *S. litura*
Oral toxicity of all the extractives was evaluated through poison food bioassay. The extractive was diluted using 0.1 percent emulsified water (1 ml of triton X-100 in 100 ml of water) to prepare desired concentrations (Amusan *et al.*, 2005; Murugan *et al.*, 2012) [1, 12]. The concentrations tested were 1, 10, 20, 30, 50, 70, 90 and 100 percent. 0.3ml of each concentration of each extractive was smeared on three cm dia. castor leaf discs (both abaxial and adaxial surface) using a camel hair brush, air dried for 10 minutes, and placed inside Petri dishes (15 cm dia.) lined with moist filter paper. 10 four-hour pre-starved third instar *S. litura* larvae were released into each Petri dish containing three treated leaf discs. 0.5 percent neem oil-treated leaf discs acted as the positive control. Solvent control and absolute control were also maintained. The experiment was replicated three times and carried out as a Completely Randomized Design. All the bioassays were performed at 25 ± 3° C and 80 % RH. After six hours, the leaf area fed was measured using a leaf area meter (Systronics India Ltd., Model. No: 211), and percent leaf area protection over absolute control was computed and feeding deterrent activity was calculated. The larvae were then fed with untreated castor leaves till pupation and insecticidal and insect growth regulatory effects were recorded.

Results and Discussion
Phytochemical constituents
The results of phytochemical evaluation revealed presence of major secondary metabolites such as tannins, saponins, flavonoids, terpenes, quinones, steroids, coumarins, phenols and alkaloids (Table 1).

Saponins were found in acetone, methanol and hexane extract of leaf, peel, seed, and tuber. It is reported that saponins play a major role in plant defence against herbivores in general and insect pests in particular. Their anti-insect properties include reduced feeding, impact on growth and reproduction and mortality. Saha *et al.* (2010) [21] reported that as the extract concentration increased, deterrency indices increased, in a dose-dependent manner against *S. litura*. Further, they reported reduced growth at reduced concentrations of saponin treatment.

Quinones were found in acetone, hexane as well as methanol extract of leaf, peel, seed, and tuber. Quinones cause cytotoxicity and immunotoxicity (Bolton *et al*. 2000). Krishnakumari *et al.* (2001) reported effective antifeedant activity of the Ventiloquinone A isolated from *Ventilago madaraspatana* against *Henosepilachna vigintioctopunctata* and *S. litura*. Similarly, strong antifeedant activity of 6-(4,7-hydroxy-heptyl) quinone from *Pergularia daemia* leaves against *S. litura* was reported (Pavunraj *et al.* 2011) [17].

Terpenoids and triterpenoids were found in acetone, hexane and methanol extract extract of seed; acetone and methanol extract of leaf and tuber, and acetone extract of peel. Terpenes are one of the largest groups of secondary metabolites (Ashour *et al* 2010; Georgian at al 2019) [2, 5]. Phytoterpenes' role to counter biotic and abiotic stresses is widely studied (Silva *et al* 2016; Li *et al* 2015; Thimmappa *et al* 2014; Mahdavi *et al* 2020) [22, 10, 24, 11]. Terpenes as active ingredients in pesticide development has seen a global resurgence due to its low risk to the environment and human health (Ninku *et al* 2021) [13].

Table 1: Preliminary phyto chemical screening for the presence of anti-insect secondary metabolites in *M. cymbalaria*

S. no	Screening for	Samples											
		Seeds			Peels			Biomass			Tubers		
		Hexane	Acetone	Methanol	Hexane	Acetone	Methanol	Hexane	Acetone	Methanol	Hexane	Acetone	Methanol
Phytochemical screening													
1.	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
2.	Quinines	+	++	+	+	++	+	+	+	+	+	+	+
3.	Terpenoids	+	+	+	-	+	+	-	+	+	-	+	+
4.	Triterpenoids	+	+	+	-	+	-	-	+	+	-	+	+
5.	Alkaloids	-	+	-	++	+	-	-	+	-	-	++	-
6.	Coumarins	-	+		-	+	-	-	+	-	-	+	
7.	Flavonoids	+	+	+	-	+	-	-	+	-	-	+	-
8.	Hydrolysable tannins	+	+	-	+	-	-	+	-	-	+	-	-
9.	Tannins	+			++			+++			+		
10.	Phenolics	+	-	-	+	-	-	+	-	-	+	-	-
11.	Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
12.	Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Anti – insect properties of *M. cymbalaria* against *S. litura* at higher concentrations

S.No	Extract	Anti – insect properties			
		Repellent	Antifeedant	Insect growth regulatory property	Insecticidal
Seed	Hexane	+	++	++	++
	Acetone	+	+++	+	+++
	Methanol	+	+	+	+
Peel	Hexane	-	+	+	-
	Acetone	+	+++	+	-
	Methanol	+	++	+	-
Leaf	Hexane	-	+	+++	+
	Acetone	+	+++	++	+++
	Methanol	+	++	+	+
Tuber	Hexane	-	+	+++	++
	Acetone	+	+++	++	+++
	Methanol	+	+	+	+

- Absent; + - Moderate; ++ - High; +++ - Very high

Alkaloids were present in hexane extract of peel; acetone extract of seed and tuber, and methanol extract of leaf and tuber. Alkaloids role as growth regulators is most effective since structures of some of them resemble structures of known growth regulators. Growth inhibition of *S. litura* larvae was caused by the alkaloids derived from *C. komarovii.* Pupal malformation, larval weight reduction, and extended development duration were observed (Sun *et al* 2012) [23].

Similarly, coumarins were found in acetone, hexane and methanol extract of leaf; acetone and hexane extract of peel and tuber, and they were found totally absent in any of the three solvent extract of seed. Coumarins are potent ovicidal and larvicidal compounds. They showed antifeedant responses in invertebrates and vertebrates (Hussain *et. al.* 2018) [6]. Prasad *et al.* (1998) [18] evaluated the effect of distyryl coumarins against *S. litura* larvae and found antifeedant response.

Flavonoids were found in acetone, methanol and hexane extract of seed and acetone and methanol extract of peel and acetone extract of leaf and tuber. Flavonoids are getting developed as effective alternatives to synthetic pesticides. They can inhibit enzymatic activity and prevent the growth of larvae of different insect species (Kim *et al* 2000) [7]. Some flavonoids interfere in the process of moulting and reproduction of several insects by inhibiting juvenile hormone and transcription of ecdysone receptor-dependent genes (EcR) (Oberdorster *et al* 2001) [16].

Tannins were found predominately present in hexane extract of leaf, peel, seed and tuber. Acetone extract also found to possess tannins. Tannins were totally absent in methanol extract of all the test samples. Tannins provide defense against insect herbivores by deterrence and/or toxicity. Tannins are astringent polyphenols and are effective feeding deterrents to many insect pests. They affect insect growth and development by binding to the proteins, reducing the efficiency to absorb nutrients and by causing midgut lesions (Rao et. al. 2017) [19]. Tannins inhibited the growth of the cutworm larvae and that the inhibitory effect was proportional to the amount of tannin ingested (Nomura and Itioka, 2002) [14].

Phenols were present in hexane and acetone extract of leaf, peel, seed, and tuber. Glycosides and cardiac glycosides were totally absent in *M. cymbalaria*. Wiwattanawanichakun *et al.* (2022) [25] tested the effect of *A. calamus* isolated phenolic compound, Chrysin (5,7-dihydroxy favone) against second instar *S. litura* larvae which showed toxicity 24 hours after topical application and reduction in carboxylesterase activities. Purified phenolic compounds from the bark of *Acacia nilotica* showed insecticidal potential against *S. litura.* The larval growth, survival, adult emergence, pupal weight and nutritional indices were adversely affected (Gautam *et al.* 2021) [4].

Presence of such diverse array of plant secondary metabolites in *M. cymbalaria* brightens the scope of developing it into a potent phyto insecticide. Further testing the extracts on *S. litura* revealed the presence of feeding deterrent, insecticidal and insect growth regulatory effects in *M. cymbalaria.*

Insect repellent activity was found in acetone and methanol extract of seed, peel, leaf and tuber and hexane extract of *M.*

cymbalaria seed. Feeding deterrent activity was noticed in all the extracts of *M. cymbalaria* seed, peel, leaf and tuber. However, it was found significantly higher in acetone extract followed by methanol and hexane extract. Insect growth regulatory (IGR) activities were also noticed in all the extracts and it was moderate in peel and higher in leaf and tuber extract. The *M. cymbalaria* peel did not present any insecticidal activity while the tuber and leaf acetone extract showed higher insecticidal effect against the cut worm, *S. litura*

References

1. Amusan AAS, Idowu AB, Arowolo FS. Comparative toxicity effect of bush tea leaves *(Hyptis suaveolens)* and orange peel *(Citrus sinensis)* oil extract on larvae of the yellow fever mosquito *Aedes aegypti*. *Tanzania* Journal of Health Research,2005:7(3):174-178.
2. Ashour M, Wink M Gershenzon J, Wink M. Biochemistry of plant secondary metabolism. Ann. Plant Rev,2010:40:258–303.
3. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. Chem Res Toxicol,2000:13(3):135-60. doi: 10.1021/tx9902082.
4. Gautam S, Samiksha, Chimni S S, Arora, S, Sohal S K. Toxic effects of purified phenolic compounds from *Acacia nilotica* against common cutworm. Toxicon,2021:203:22-29.
5. Georgian D C, Ramadoss N, Dona C, Basu C. Therapeutic and medicinal uses of terpenes. In Medicinal Plants; Springer: Berlin/Heidelberg, Germany, 2019, 333–359.
6. Hussain M I, Abbas S Q, Reigosa M J. Activities and Novel Applications of Secondary Metabolite Coumarins. *Planta Daninha*, 2018, 36(0). doi:10.1590/s0100-83582018360100016.
7. Kim J S, Kwon C S, Son K H. Inhibition of ά-glucosidase and ά-amylase by luteolin, a flavonoid. Bioscience, Biotechnology, and Biochemistry,2000:64:2458-2461.
8. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev,2007:1:97-104.
9. Krishnakumari G, Bhuvaneswari B, Swapna I R. *Antifeedant activity of quinones from Ventilago madaraspatana*. *Fitoterapia*,2001:72(6):671–675. doi:10.1016/s0367-326x (01)00280-5.
10. Li R, Tee C S, Jiang Y, Jiang X Y, Venkatesh P N, Sarojam R, Ye J. A terpenoid phytoalexin plays a role in basal defense of *Nicotiana benthamiana* against potato virus, X. Sci. Rep.,2015:5:9682.
11. Mahdavi A, Moradi P, Mastinu A. Variation in Terpene Profiles of Thymus vulgaris in Water Deficit Stress Response. Molecules,2020:25:1091.
12. Murugan K, Kumar P M, Kovendan K, Amerasan D, Subrmaniam J and Hwang J S 2012. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)." Parasitology research 111(4):1757-1769.
13. Ninkuu V, Zhang L, Yan J, Fu Z, Yang T, Zeng H. Biochemistry of Terpenes and Recent Advances in Plant Protection. International Journal of Molecular Sciences, 2021:22:5710. https:// doi.org/10.3390/ijms22115710
14. Nomura M, Itioka T. Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, Spodoptera litura Fabricius (Lepidoptera: Noctuidae). Applied Entomology and Zoology,2002:37(2):285-289. DOI:10.1303/aez.2002.285.
15. Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. African Journal of Pure and Applied Chemistry,2010:4(9):206-212.
16. Oberdorster E, Clay M A, Cottam D M, Wilmot F A, McLachlan J A, Milner M J. Common phytochemicals are ecdysteroid agonists and antagonists: A possible evolutionary link between vertebrate and invertebrate steroid hormones. Journal of Steroid Biochemistry and Molecular Biology,2001:77:229-238
17. Pavunraj M, Chellaiah M, Ignacimuthu S, Janarthanan S, Duraipandiyan V, Raja N, Vimalraj S. Antifeedant activity of a novel 6-(4,7-hydroxy-heptyl quinine from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helicoverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab.). Phytoparasitica,2011:39:145-150.
18. Prasad C V S S, Saraswat D, Ram G M, Sreenivasulu B. Effect of certain distyryl coumarins against the larvae of Spodoptera litura (Fabricius). Indian Journal of Plant Protection,1998:26(1):75-77.
19. Rao D E, Divya K, Prathyusha I V S N, Krishna C R, Chaitanya K V. *Insect-Resistant Plants.* Current Developments in Biotechnology and Bioengineering, 2017, 47–74. doi:10.1016/b978-0-444-63661-4.00003-7
20. Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. International Journal of Applied Research in Natural Products,2008:1(3):20-28.
21. Saha S, Walia S, Kumar J, Dhingra S, Parmar B S. Screening for Feeding Deterrent and Insect Growth Regulatory Activity of Triterpenic Saponins from *Diploknema butyracea* and *Sapindus mukorossi*. Journal of Agricultural and Food Chemistry,2010:58(1):434–440. doi:10.1021/jf902439m.
22. Silva L N, Zimmer K R, Macedo A J, Trentin D S. Plant Natural products targeting bacterial virulence factors. Chemistry Reviews,2016:116:9162–9236.
23. Sun H, Blanford S, Guo Y, Fu X, Shi W. Toxicity and influences of the alkaloids from *Cynanchum komarovii* AL. Iljinski (Asclepiadaceae) on growth and cuticle components of Spodoptera litura Fabricius (Noctuidae) larvae, Natural Product Research: Formerly Natural Product Letters,2012:26:10:903-912.
24. Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn A. Triterpene Biosynthesis in Plants. Annual Review of Plant Biology,2014:65:225–257.
25. Wiwattanawanichakun P, Saehlee S, Yooboon T, Kumrungsee N, Nobsathian S, Bullangpoti V. Toxicity of isolated phenolic compounds from *Acorus calamus* L. to control *Spodoptera litura* (Lepidoptera: Noctuidae) under laboratory conditions. Chemical and Biological Technologies in Agriculture 9, Article number: 10, 2022.