**J. Bio-Sci. 31(1): 99-108, 2023** **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
**DOI:** https://doi.org/10.3329/jbs.v31i1.69538

# BIOACTIVE POTENTIALS OF *PETUNIA INTEGRIFOLIA* (HOOK.) SCHINZ & THELL. AGAINST *CULEX QUINQUEFASCIATUS* LARVAE, *ARTEMIA SALINA* NAUPLII AND *POECILIA RETICULATA* ADULTS

**Alimul Islam[1], Nelufa Yasmin[2] and Nurul Islam[2*]**

*[1]Institute of Environmental Science, University of Rajshahi, Bangladesh*
*[2]Department of Zoology, University of Rajshahi, Bangladesh*

**Abstract**

Petroleum ether (pet. ether), chloroform ($CHCl_3$) and methanol ($CH_3OH$) extracts of *Petunia integrifolia* (Hook.) Schinz & Thell. Whole plant was subjected to assess cytotoxicity against the brine shrimp *Artemia salina* L. *nauplii*, larvicidal activity against the mosquito *Culex quinquefasciatus* Say larvae and piscicidal activity against the *Poecilia reticulata* Peters adults. The *P. integrifolia* extract in pet. ether showed the highest cytotoxic activity against *A. salina* nauplii by giving the minimum $LC_{50}$ value of 49.25 parts per million (ppm) at 48 h, while in case of $CHCl_3$ and $CH_3OH$ extracts, the values were 63.37 and 342.55 ppm, respectively. Against *C. quinquefasciatus* larvae the three extracts gave $LC_{50}$ values of 4.92, 5.51 and171.26 ppm, respectively all after 48 h of exposure. Against *P. reticulata* the $LC_{50}$ values were 21.70 ppm for pet. ether extract at 48 h, and 31.91 ppm for $CHCl_3$ extract after 42 h of exposure but $CH_3OH$ extract offered no mortality. According to the intensity of activity, therefore, the extracts could be arranged in the descending order of: pet. ether > $CHCl_3$ > $CH_3OH$ extracts; and the sensitivity of the test organisms to the extract of *P. integrifolia* could be arranged in the following descending order: *C. quinquefasciatus* larvae (in Pet. ether; $LC_{50}$ = 4.92 ppm) > *P. reticulata* (in pet ether; $LC_{50}$ = 21.70 ppm) >*A. salina* nauplii (in pet. ether; $LC_{50}$ = 49.25 ppm).

**Key words:** *Artemia salina*, cytotoxic, *Culex quinquefasciatus*, larvicidal and piscicidal activities, *Petunia integrifolia*, *Poecilia reticulata*.

## Introduction

Plants produce a wide variety of bioactive chemicals, making them a valuable source of various medications. Medicinal plants have been discovered to be effective in treating and managing a variety of health issues over time. Natural products are used to produce over half of all current clinical medications (Stuffness and Douros 1982), and they play an essential part in the pharmaceutical industry's drug development programs (Baker et al. 1995). Synthetic insecticides have recently become the most widely used agrochemicals, and they have taken a significant market share. However, plant-based pesticides have been used as a potential toxic source in agriculture for over 150 years (Oberemok et al. 2015). The plant species *Petunia integrifolia* (Hook.) Schinz and Thell have been found with some remarkable bioactive properties such as medicinal, traditional and pharmaceutical potentials (Rahman et al. 2008). The genus *Petunia* has been reported as an antibacterial agent against *Streptococcus* sp., *Pseudomonas* sp., and *Salmonella* sp. (Kumar 2015). Floral

*Author for correspondence: n_islamm@yahoo.com

defensins of *Petunia* spp. inhibit the growth of the *Fusarium oxysporum* fungal strain (Lay et al. 2003). This plant can also be used as a mild anti-microbial and anti-oxidant medication (Rahman et al. 2008). Several steroidal chemicals such as petuniasterones found in *Petunia* species contribute to the plant's resistance to insect attack (Elliger and Waiss 1989) and directly repel aphids, tomato hornworm, asparagus beetles and leafhoppers in the crop field (Hummel et al. 2015). This herb is said to have been used as a hallucinogen in highland Ecuador that causes a sensation of flying, and a phenomenon sometimes attributed to the hallucinogenic tropane alkaloids, such as hyoscine (Butler et al. 1981, Schultes and Hofmann 1980). Even though *P. integrifolia* was described for its biological activities, however, detailed information of biological activities of the plant is still scanty. That is why this plant species has been subjected to evaluate the cytotoxicity, larvicidal and piscicidal potentials against three model organisms *viz*., *A. salina* nauplii, *C. quinquefasciatus* larvae and *P. reticulata* adults. The brine shrimp lethality bioassay is an innovation in the bioassay for phytochemicals demonstrating cytotoxic effects and a wide range of therapeutic potentials e.g., anticancer, antiviral, pesticidal and anti-AIDS activity (Wu 2014). The test on brine shrimp nauplii was adopted as a viable instrument for assessing innovative pharmacological natural compounds (Hosain et al. 2011). The mosquito species *C. quinquefasciatus* is a vector for pathogens that cause fatal diseases in humans and animals, such as Japanese encephalitis and lymphatic filariasis (Benelli and Beier 2017). The guppy, *P. reticulata*, commonly known as million fish or rainbow fish, is a widely distributed tropical and popular freshwater aquarium fish. Guppies in the wild eat a wide range of foods, including benthic algae and mosquito larvae that help limit the spread of malaria. In evolutionary ecology, and behavioral research, *P. reticulata* is utilized as a model organism (Magurran 2005). However, recently the fish species has been reported to have some detrimental influences on local fish populations (El-Sabaawi et al. 2016, Vallie 2022).

## Materials and Methods

### *Collection and preparation of plant materials*

The whole plant *P. integrifolia* was collected from the Rajshahi University Campus. The plant materials were cut into small pieces and spread out to dry under shade at room temperature in separate wooden trays. Well-dried materials were ground to powder with a blender, weighed, kept in conical flasks and then extracted with sufficient amounts of three solvents namely, petroleum ether (PE), chloroform (CF) and methanol (ME) at 50 g × 150 ml × 2 times for 48 h. Filtrations were done by Whatman filter paper Grade-40, and after evaporation, extracts left on the bottom of the round bottom flasks were collected in glass vials and kept in a cool place at 4ºC with appropriate labelling until used.

### *Hatching of brine shrimp cysts*

Brine shrimp cysts were purchased from the Pet and Aquarium Market of Nilkhet, Dhaka, Bangladesh. They were hatched in an aquarium using 1 liter of 1 M NaCl brine solution (pH 8.5), incubated for 48 h under fluorescent light, and the nauplii were hatched within 24-35h at 25-30°C. After hatching, the nauplii were transferred to test tubes. Ten nauplii were transferred to each tube containing a required amount of water and 2 ml NaCl solution was added to each test tube to fill up to the previously marked line.

***Hatching of mosquito larvae***

*C. quinquefasciatus* rafts were collected from the drains of Rajshahi University Campus and reared under laboratory conditions. The eggs were kept in 500 ml beakers with 250 ml pond water for 24 h in a dark place in the laboratory, and the larvae were hatched within 16-20 h at room temperature. After hatching, the larvae were transferred to test tubes. Ten larvae were transferred to each tube containing a required amount of water.

***Rearing of guppy fish***

Adults of *P. reticulata* were collected from an aquarium shop in Rajshahi City. The collected fishes were then transferred to the laboratory, kept in an aquarium, and reared with the continuous oxygen supply through an aerator. Five fishes were transferred to 500 ml beaker for experimentation.

***Preparation of test sample and cytotoxicity test***

Test samples at different concentrations were considered as doses prepared in test tubes by adding a calculated amount of DMSO (dimethylsulfoxide). Then water was added to fill the pre-marked (up to 10 ml) test tubes with the help of a pipette. The nauplii were counted by visual inspection and were released in test tubes containing 10 ml of water, and the test tubes were kept at room temperature along with a control batch. Observation of mortality was made after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure.

Plant extract solutions at different concentrations (for all three extracts of *P. integrifolia* in pet. ether 300, 250, 200, 150, 100 and 50 ppm; in $CHCl_3$ 500, 400,300, 200, 100 and 50 ppm and $CH_3OH$ were 550, 500, 400 and 300 ppm in artificial seawater containing 1% DMSO (v/v) used for this assay. Ten nauplii were used in each of the test tubes and three replicates were used for each of the concentrations. Later on, 1 ml of NaCl solution was added to each test tube. After 6, 12, 18, 24, 30, 36, 42 and 48 h incubation, the number of survivors and the dead nauplii were counted using a simple microscope and the percentage of the mortality for each of the doses was calculated as compared with the control. DMSO served as the negative control and the final concentration of DMSO in the assay volume was always kept below 1% to prevent possible false effects originating from DMSO toxicity. $LC_{50}$ value is the concentration of the sample required to kill 50% of the treated brine shrimp nauplii population. The $LC_{50}$ value of <1.0 mg/ml was considered toxic, while >1.0 mg/ml deemed non-toxic (Meyer et al. 1982).

***Preparation of test sample and larvicidal activity test***

The extract of *P. integrifolia* was tested against *C. quinquefasciatus* larvae after 24 h of emergence throughout the lethality test. Test samples at different concentrations considered as doses were prepared in test tubes by adding a calculated amount of DMSO to make the extract hydrophilic before adding water in each of the test tubes. Then water was added to fill the pre-marked (up to 10 ml) test tubes with the help of a pipette. Ten freshly emerged larvae were added to each of the test tubes with different concentrations of the selected doses along with a control batch. The counting of larvae was made after 6 h of application with 6 h intervals up to 48 h through visual inspections. The doses were selected for the extract of *P. integrifolia*

against the mosquito larvae in Pet. ether 50, 25, 15, 10 and 5 ppm; in $CHCl_3$ 25, 15, 10 and 5 ppm and in $CH_3OH$ 400, 300, 200 and 100 ppm for the final experimentation.

***Preparation of test sample and piscicidal activity test***

For the piscicidal activity test, *P. integrifolia* extract was placed in a 500 ml beaker and made hydrophilic by adding a small amount of DMSO. Then water was added to fill the pre-marked beakers up to 300 ml. In each of the beakers, five guppies were released and exposed for 48 h. The dead fish were counted after 6 h of application with 6 h interval up to 48 h through visual inspections and the findings were compared to the control. The final doses for the piscicidal activity test against guppies were obtained: *P. integrifolia* extracts in Pet. ether 66.67, 50, 41.67 and 33.33 ppm; in $CHCl_3$ 66.67, 50 and 33.33 ppm.

***Statistical analysis***

The lethality records of *P. integrifolia* on brine shrimp nauplii, mosquito larvae and guppy fishes were corrected by Abbott's formula: $P_r = (P_o - P_c / 100 - Pc) \times 100$, where $P_r$ = corrected mortality (%), $P_o$ = observed mortality (%) and $P_c$ = Per cent mortality in control (Abbot 1925). The data were then subjected to probit analysis (Finney 1947, Busvine 1971). The lethality relationship was expressed as a median lethal concentration ($LC_{50}$) for the test organisms.

**Results**

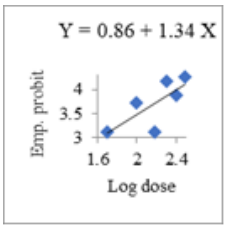
**Brine shrimp cytotoxicity**

The cytotoxic activity of the test plant extracts is shown in Table 1. The results showed promising cytotoxic potentiality of this plant against *A. salina* nauplii in three different solvents. *P. integrifolia* extracts in pet. ether gave $LC_{50}$ values 1253.92, 884.63, 289.79, 160.98, 119.23, 71.75, and 49.25 ppm; in $CHCl_3$ 1935.64, 845.16, 385.91, 157.99, 108.66, 91.05 and 63.37 ppm and in ME 840.31, 837.49, 587.75, 456.46, 418.05, 389.44 and 342.55 ppm all after 12, 18, 24, 30, 36, 42 and 48 h of exposure, respectively. According to the intensity of activity against *A. salina* nauplii, therefore, the extracts showed the following descending order: Pet. ether >$CHCl_3$ >$CH_3OH$.

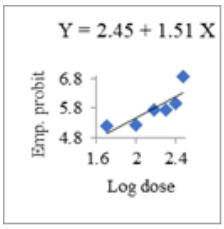
**Table 1.** $LC_{50}$ values of *P. integrifolia* extracts on *A. salina* nauplii, *C. quinquefasciatus* larvae and *P. reticulata* adults.

| Plant sp. | Test organisms | Solvents | $LC_{50}$ (ppm) at different exposure times points | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 12 h | 18 h | 24 h | 30 h | 36 h | 42 h | 48 h |
| *P. integrifolia* | *A. salina* nauplii | Pet. ether | 1253.92 | 884.63 | 289.79 | 160.98 | 119.23 | 71.75 | 49.25 |
| | | $CHCl_3$ | 1935.64 | 845.16 | 385.91 | 157.99 | 108.66 | 91.05 | 63.37 |
| | | $CH_3OH$ | 840.31 | 837.49 | 587.75 | 456.46 | 418.05 | 389.44 | 342.55 |
| | *C. quinquefasciatus* larvae | Pet. ether | 95.04 | 29.05 | 13.18 | 10.51 | 7.32 | 6.72 | 4.92 |
| | | $CHCl_3$ | 17.54 | 10.11 | 8.89 | 7.34 | 7.14 | 6.12 | 5.51 |
| | | $CH_3OH$ | 340.83 | 323.96 | 292.47 | 253.28 | 232.72 | 218.70 | 171.26 |
| | *P. reticulata* | Pet. ether | 58.71 | 49.86 | 44.73 | 33.25 | 31.40 | 22.99 | 21.70 |
| | | $CHCl_3$ | 67.01 | 55.15 | 51.65 | 50.11 | 43.75 | 31.91 | - |
| | | $CH_3OH$ | Not active | | | | | | |

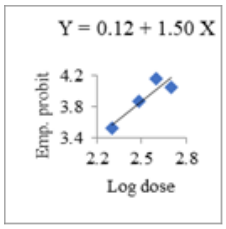
Regression lines of the $LC_{50}$ values for *P. integrifolia* extracts at the minimum (12 h) and maximum (48 h) exposures in three test organisms are shown in the figures below.



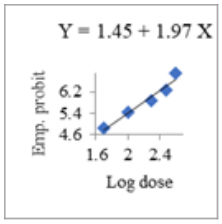

i) Pet. ether extract after 12 h



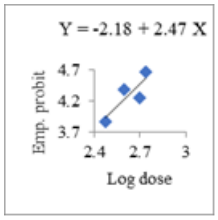

ii) Pet. ether extract after 48 h



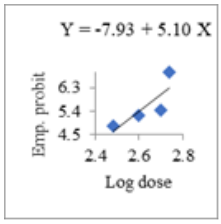

iii) $CHCl_3$ extract after 12 h




iv) $CHCl_3$ extract after 48 h

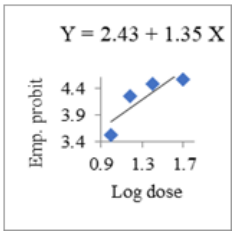



v) $CH_3OH$ extract after 12 h

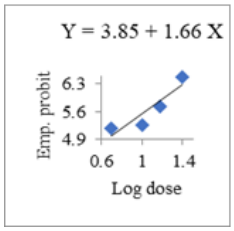



vi) $CH_3OH$ extract after 48 h

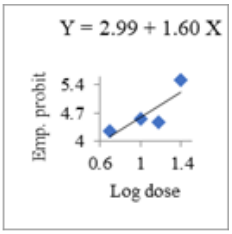
**Fig. 1(i-vi):** Regression lines of *P. integrifolia* extracts against *A. salina* nauplii at different hours of exposure.



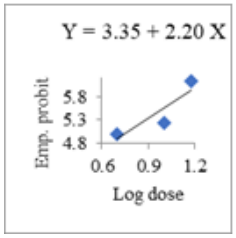

i) Pet. ether extract after 12 h



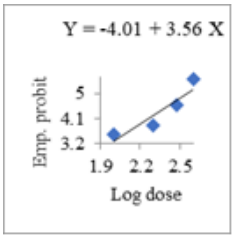

ii) Pet. ether extract after 48 h



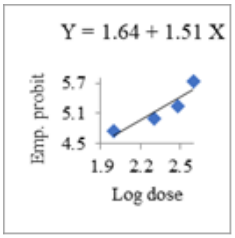

iii) $CHCl_3$ extract after 12 h




iv) $CHCl_3$ extract after 48 h

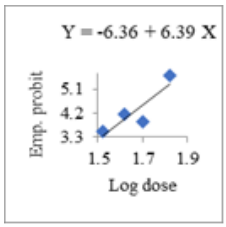



v) $CH_3OH$ extract after 12 h

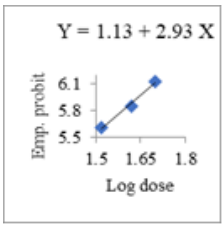



vi) $CH_3OH$ extract after 48 h

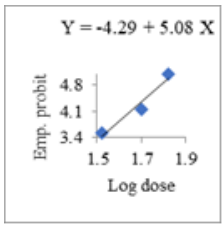
**Fig. 2(i-vi):** Regression lines of *P. integrifolia* extracts against *C. quinquefasciatus* larvae at different hours of exposure.



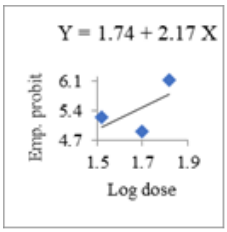

i) Pet. ether extract after 12 h




ii) Pet. ether extract after 48 h




iii) $CHCl_3$ extract after 12 h




iv) $CHCl_3$extract after 42 h

**Fig. 3 (i-iv):** Regression lines of *P. integrifolia* extracts against *P. reticulata* fish at different hours of exposure.

***Larvicidal activity***

The larvicidal activity of the *P. integrifolia* extracts is shown in Table 1. The plant extracts showed promising lethality against *C. quinquefasciatus* larvae in three different solvents. The extract in Pet. ether gave $LC_{50}$ values 95.04, 29.05, 13.18, 10.51, 7.32, 6.72 and 4.92 ppm; in $CHCl_3$ 17.54, 10.11, 8.89, 7.34, 7.14, 6.12 and 5.51 ppm; and in $CH_3OH$ 340.83, 323.96, 292.47, 253.28, 232.72, 218.70 and 171.26 ppm all after 12, 18, 24, 30, 36, 42 and 48 h of exposures, respectively. According to the intensity of activity the extracts could be arranged in the following descending order: pet. ether > $CHCl_3$ > $CH_3OH$.

***Piscicidal activity***

The piscicidal activity of the experimental plant extracts has also been presented in Table 1. The result showed promising toxic potential against *P. reticulata* in different solvents. The extracts in Pet. ether gave $LC_{50}$ values 58.71, 49.86, 44.73, 33.25, 31.40, 22.99 and 21.70 ppm after 12, 18, 24, 30, 36, 42 and 48 h; and in CF 67.01, 55.15, 51.65, 50.11, 43.75 and 31.91 ppm after 12, 18, 24, 30, 36 and 42 h of exposure, respectively. However, extracts in ME did not show any lethality against the tested fish, in which the following descending orders: pet. ether > $CHCl_3$.

**Discussion**

The investigation on the bioactive potentials of *P. integrifolia* against three test organisms was found promising and the findings got strong support from the previous works. Mishra et al. (2021) reported wound healing potential of *P. integrifolia* flowers. Their study provides a scientific evaluation for the wound healing

potential of the petroleum ether and water extracts of *Petunia integrifolia* flowers using excision and incision wound models in Swiss albino mice. Antioxidant activity was reported very recently by Petrova et al. (2020). The potential insecticidal activity of *P. integrifolia* was reported against stored product pests, e.g., *Callosobruchus chinensis*, *Sitophilus oryzae* and *Tribolium castaneum* (Islam et al. 2020). *Petunia* has been mentioned for the presence of acylsugars produced in the glandular trichomes of Solanaceae plants and linked to abiotic and biotic stress resistance (Nadakuduti et al. 2017). This plant species produces acylsugars to defend themselves against herbivores and various biological and environmental stresses (Nadakuduti et al. 2017).

Kumar (2015) previously reported that *P. axillaris* possesses antibacterial activity to cure various human diseases and ailments. According to his findings, bacteria are susceptible to *Petunia* spp. extracts. The bacteria *Staphylococcus aureus*, *Streptococcus* sp., *Pseudomonas aeroginosa*, and *Salmonella typhimurium* were strongly inhibited by the *P. axillaris* leaf extracts *in vitro*. Thenmozhi and Sivaraj (2011) described *Petunia* as a medicinal as well as aesthetic plant. They reported that the leaf and callus extracts were potential for callus induction and antibacterial activity against *Baccilus subtilis*, *Escherichia coli*, *Salmonella* spp. and *Streptococcus* spp. *P. hybrida* was described for producing a high level of plant defensins, similar to ornamental tobacco *Nicotiana alata* (Lay et al. 2003). Broekaert et al. (1995) revealed that defensins inhibit fungus, oomycetes and positive bacteria. *P. hybrida* floral defensins suppress the growth of *Botrytis cinerea* and *Fusarium oxysporum*, and they may play a role in pathogen defense in floral tissues (Lay et al. 2003). *Petunia* has been mentioned by Kays et al. (1994) for its poisonous potential and antibacterial and antifungal effects. They also revealed that the leaves of this plant produce an effective insecticide, which is widely employed as a natural insecticide and has all of the advantages of chemical compounds, such as quick action, broad spectrum insect activity, and rapid biodegradability. Sweet potato whiteflies are poisoned by a sticky secretion of sugar esters produced by the cultivar. Guerreiro (1984) reported some non-nitrogenous diterpene compounds as psychotropic substances rather than alkaloids in the related *P. patagonica*. Smoking *P. violacea* promotes similar effects of smoking *Coriaria thymifolia,* Butler et al. (1981). On the other hand, both plants are meant to make people feel like floating away from the ground or flying into the air. Tropane alkaloids are frequently blamed for this type of psychoactive experience. However, no alkaloids have been found in any section of *P. violacea* to date (Butler et al. 1981). Very recent investigation was done on this very well-known but less studied plant for the detection of nootrophic activity by Khan et al. (2022).

**Conclusion**

The results of this investigation depicted cytotoxic activity, coupled with larvicidal and piscicidal activities of *P. integrifolia,* which could be considered as further clarification of its potentials to be used in the pest control and pharmaceutical sectors. However, the genus *Petunia* has been reported as a source of antimicrobial, antibacterial, antifungal, and antioxidant agents. Several steroidal chemicals also found in *Petunia* species contribute to the plant's resistance to insect attack; and very recent finding broaden the horizon of *Petunia* potentials by adding nootropic activity; which triggers a hope for more potential leads in *P. integrifolia*. Thus, a thorough investigation on this plant is very much to be solicited.

**Acknowledgements**

The authors are grateful to the Ministry of Science and Technology, The People's Republic of Bangladesh, for a research grant; the Director of the Institute of Environmental Science, University of Rajshahi, and the Chairman, Department of Zoology, University of Rajshahi, Bangladesh, for providing laboratory facilities.

**Conflict of interest:** The authors declare that there is no competing interest.

**References**

Abbott WS (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD, Cragg GM, Gupta MP, Iwu MM, Madulid DR and Tyler VE (1995). Natural product drug discovery and development: new perspective on international collaboration. Journal of Natural Products 58: 1325-1357.

Benelli G and Beier JC (2017). Current vector control challenges in the fight against malaria. Acta Tropica 174: 91-96.

Broekaert WF, Terras FRG, Cammue BPA and Osborn RW (1995). Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiology 108: 1353-1358.

Busvine JR (1971). A critical review of the techniques for testing insecticides. Commonwealth Agricultural Bureau, London, pp. 345.

Butler EG, Robinson T and Schultes RE (1981). *Petunia violacea*: Hallucinogen or Not? Journal of Ethnopharmacology 4(1): 111-114.

Elliger CA and Waiss ACJ (1989). Insect growth inhibitors from *Petunia* and other solanaceous plants. Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, Chapter 14, pp. 188-205.

El-Sabaawi RW, Frauendorf TC, Marques PS, Mackenzie RA, Manna LR, Mazzoni R, Phillip DAT, Warbanski ML, Zandona` E. (2016). Biodiversity and ecosystem risks arising from using guppies to control mosquitoes. Biology Letters 12: 20160590. http://dx.doi.org/10.1098/rsbl.2016.0590.

Finney DJ (1947). Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, London, pp. 333.

Guerreiro E, de-Fernandez J and Giordano OS (1984). Beyerene derivatives and other constituents from *Petunia patagonica*. Phytochemistry 23(12): 2871-2873.

Hosain SB, Sultana S and Haque A (2011). Studies on antibacterial, cytotoxic and antioxidant properties of the seeds and leaves of *Ficus racemosa*. International Journal of Pharmaceutical Sciences and Research 2(4): 1040-1045.

Hummel HE, Langner SS and Breuer M (2015). Electrospunmesofibers: A novel biodegradable pheromone dispenser technology are combined with mechanical deployment for efficient IPM of *Lobesia botrana* in vineyards. Communications in Agricultural and Applied Biological Sciences 80: 331-341.

Islam A, Yasmin N, Hasan K and Islam N (2020). Pesticidal action of *Petunia integrifolia* (Hook.) Schinz & Thell. extracts against three stored product pests. Rajshahi University Journal of Environmental Science 9: 85-91.

Kays SJ, Severson RF, Nottingham SF, Chalfant RB and Chortyk O (1994). Possible biopesticide from *Petunia* for the control of the sweet potato whitefly on vegetable crops. Proceedings of the Florida State Horticultural Society 107: 163-167.

Khan I, Basedia DK and Dubey BK (2022). Investigation of Nootropic Activity of *Petnunia hybrida* Flower Extract. Psychology and Behavioral Science International Journal 18(3):555987. doi:10.19080/PBSIJ.2022.18.555987.

Kumar M (2015). *In vitro* evaluation of antibacterial activity of *Petunia axillaris* leaves extracts against human pathogenic bacteria. International Journal of Pharmaceutical and Life Sciences 6(3): 4363-4368.

Lay FT, Brugliera F and Anderson MA (2003). Isolation and properties of floral defensins from ornamental tobacco and petunia. Plant Physiology 131: 1283-1293.

Magurran AE (2005). Evolutionary Ecology: The Trinidadian Guppy. New York: Oxford University Press. Great Clarendon Street, Oxford OX2 6DP; pp. 192.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Nicols JL and McLaughlin (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica 45(5): 31-34.

Mishra A, Kirar BS, Jain P and Jain AP (2021). Assessment of Wound Healing Activity of Petunia integrifolia. International Journal of Pharmaceutical and Biological Science Archive, 9(5). https://www.ijpba.in/index.php/ijpba/article/view/246.

Nadakuduti SS, Uebler JB, Liu X, Jones AD and Barrya CS (2017). Characterization of trichome-expressed BAHD acyltransferases in *Petunia axillaris* reveals distinct acylsugar assembly mechanisms within the Solanaceae. Plant Physiology 175: 36-50.

Oberemok VV, Laikova KV, Gninenko YI, Zaitsev AS, Nyadar PM and Adeyemi TA (2015). A short history of insecticides. Journal of Plant Protection Research 55(3): 221-226.

Petrova I, Petkova N, Ivanov I and Todorova M (2020). Phytonutrients and Antioxidant Activity of Extracts from Five Edible Flowers Used in Culinary. Bulletin UASVM Food Science and Technology 77(2): 26-33.

Rahman MS, Rahman MZ, Wahab A, Chowdhury R and Rashid MA (2008). Antimicrobial activity of some indigenous plants of Bangladesh. Dhaka University Journal of Pharmaceutical Sciences 7(1): 23-26.

Schultes RE and Hofmann A (1980). The Botany and Chemistry of Hallucinogens. Revised ed. Charles C. Thomas, Illinois, USA, pp. 368.

Stuffness M and Douros J (1982). Current status of the NCI plant and animal product program. Journal of Natural Products 45: 1-14.

Thenmozhi M and Sivaraj R (2011). *In vitro* evaluation of the antibacterial activity of *Petunia* leaf and callus extracts. Journal of Agricultural Technology 7(2): 321-330. http://www.ijat-aatsea.com.

Vallie S (2022). What to know about guppies. https://www.webmd.com/pets/what-to-know-about-guppies.

Wu C (2014). An important player in brine shrimp lethality bioassay: The solvent. Journal of Advanced Pharmaceutical Technology and Research 5(1): 57-58.

*(Manuscript received on 25th May 2023 and revised on 22nd June 2023)*