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PHYTOCHEMICAL ANALYSIS AND CYTOTOXICITY ASSESSMENT OF LEAF EXTRACTS FROM *BARLERIA LONGIFLORA* AGAINST *ARTEMIA SALINA*

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ABSTRACT

To investigate the phytochemical profile and cytotoxicity effect, *Barleria longiflora* leaf extracts obtained using the soxhlet extraction. The phytochemical constituents present in B. longiflora leaf extracts were confirmed by qualitative analysis shows the presence of alkaloids, flavonoids, tannin, steroids, glycosides and triterpenoids were present in various leaf extracts. Ethanol extract of leaf responded for good cytotoxicity properties against *Artemia salina*. The results revealed that the ethanolic extract of B. longiflora exhibited the highest mortality rate 93% (LC- 18.63) at 1000 ppm concentration. As the concentration decreases the mortality % also decreased. The present study on preliminary phytochemical analysis and cyto-toxicity properties of plant *Barleria longiflora* contributes to a greater knowledge of this under studied medicinal plant.

KEYWORDS: *Barleria longiflora*, Qualitative analysis, *Artemia salina* and cytotoxicity.

INTRODUCTION

Nature products from the bowls of the earth, a great number of plants having medicinal value, that grow abundantly in and around our lands and used by millions of people in India in their daily use from time immemorial (Apparanantham and Chelladurai, 2017). It's is an evident from the human history that medicinal plants have been the treatment regimen to cure a variety of diseases, including diseases caused by insects, fungi, bacteria, and virus. The

effects shown by the plants are due to the chemicals present in them and they work in the same manner as the conventional drugs (Sharma et al., 2017). India is favoured with a fine wealth of medicinal plants. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines (Alagesaboopathi, 2011).

Cancer had always been the most serious disease in humans around the world due to high morbidity and mortality. Herbal remedies used in traditional folk medicine provide an intriguing and largely unexplored source for the development of potentially new chemotherapy drugs that could help to overcome the growing issue of resistance and toxicity of currently available commercial antibiotics (Ali et al., 2001) on the market. Medicinal plants have been regarded as important sources that could produce potential chemotherapeutic agents for cancer treatment. Over 400 plant chemicals have been identified using cytotoxicity bioassays. Vinblastin, vincristine, etoposide and taxol are prominent examples of plant -derived compounds accredited as anti-cancer drugs (Amirghofran et al., 2009).

Artemia salina shrimp larvae test is a preliminary method frequently used in testing the cytotoxic properties of compounds (Riskianto et al., 2022). It is predicated on the test substances' capacity to kill brine shrimp (*Artemia salina*), a basic zoological organism. Brine shrimp lethality test (BSLT) is a simple, cost effective, requires small amount of test material (Quazi et al., 2017).

The genus Barleria belongs to the family Acanthaceae (Makholelaet al., 2003). Africa has the highest concentration of Barleria, with two centers of diversity: one in tropical east Africa (home to over 80 species) and another in southern Africa (approximately 70 species) (Balkwill et al., 1998). “White long flowered nail dye” is the Common name of B. longiflora. Ithasbeen used traditionally to cure inflammation, kidney stone issues, dropsy and cough. It is made up of four anthraquinones β-sitosterol pentacyclic, titerpene arnidiol, stigmasterol and campesterol. The Leaves, stems and roots of *B. lupulina* and flower of B. prionitis holds potential antibacterial and anti-inflammatory activities (Amoo et al., 2009; Chavan et al., 2010). However, cytotoxicity of *B. strigosa* extract has been characterized by (Choudhury et al., 2015). *B. lupulina* is the source of many phytochemicals with antibacterial and anticancer qualities, such as the barlerin alkaloid. Hence the Acanthaceae plant having significant uses

that has to be scientifically proven the plant *Barleria longiflora* a very less explored plant tested against *Artemia salina* to discover its cytotoxicity properties.

MATERIALS AND METHODS

Collection of Plant Material: The fresh plant materials were collected from the Theerthamalai hills in Dharmapuri District. The plant materials were washed under running tap water to remove the surface pollutants. The separated leaves were dried under shade. The dried leaf samples were powdered and used for further studies.

Figure 1: *Barleria longiflora* L. f.

Extraction of leaf

The shade dried powdered leaves were extracted using petroleum ether, ethyl acetate, ethanol, and water in the increasing order of polarity using soxhlet extraction method. The solvent was removed using vacuum rotary evaporator. Crude extracts were collected until thick and viscous paste or powdered of extract is visible. The residues were then used to prepare one percent stock solution with above solvents. From the stock solution, different dilutions were prepared with distilled water includes 1 ppm, 10 ppm, 100 ppm and 1000 ppm.

Tests for secondary metabolites screening

Qualitative phytochemical examinations for secondary metabolites were carried out for all the centrifuged filtrates (successive extracts) as per the following standard procedures (Shaikh, 2020). This is mainly performed to identify the presence of secondary metabolites in the plant extracts.

Cytotoxic Study

Brine shrimp lethality assay

Hatching of eggs

The method of Meyer et al., (1982) was followed with slighter modification. The eggs of brine shrimp were collected from Beena aquarium, Coimbatore. Artificial sea water is prepared with distilled water and NaCl. The pH was adjusted to 8- 8.5 which is optimum for brine shrimp egg hatching. The setup was allowed for 48 hours for hatching. The hatched larvae were collected and separated. The larvae were kept undisturbed for 24 hours. The bioassay was carried out in 24-hour old nauplii of brine shrimp.

Bioassay

1% stock solution was prepared using 0.2 g plant extract powder and 2 ml of corresponding solvent. Varoius concentrations were prepared by serial dilution methods in 1 ppm, 10 ppm, 100 ppm, and 1000 ppm dilutions. 2 ml of the dilutions were made up to 5 ml using artificial sea water. 10 nauplii were injected using filler in each tube. Triplicated were maintained to get better results. Artificial sea water with DMSO was used as a positive control. Results were obtained after 24 hours.

Statistical analysis

Data was analyzed using Microsoft Excel 2010. Lethal concentration (LC 50) represents the concentration of the test material that caused 50% mortality of all test organisms within the specified period. Based on the mortality of the test organisms reported in these bioassays, the LC 50 and fiducial limits were computed at a 95% confidence level using probit analysis using SPSS software package 16.0 (Statistical Package of Social Sciences). Results with p & lt; 0.05 were marked as statistically significant.

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \text{X100}$$

RESULT AND DISCUSSION

Qualitative phytochemical screening

The results of preliminary phytochemical screening of crude leaf extract of *Barleria longiflora* for major secondary phytochemicals was carried out and the results summarized in

Table 1. The results revealed that the petroleum ether extract of leaf contains flavonoids, steroids and glycosides. The ethyl acetate extract contained alkaloid, steroid, glycoside and triterpenoids. The ethanol extract contained alkaloids, flavonoids, tannin, steroids and triterpenoids. The aqueous extract contained alkaloids, triterpenoids. The highest secondary metabolites result was obtained in ethanol leaf extract of B. longiflora.

Table 1: Preliminary phytochemical analysis of solvent extracts of *Barleria longiflora*.

S. No.	Chemical Constituent	Tests	Organic solvents			WA
			PE	EA	ET	
1	Alkaloids	a) Dragendorff's test	_	+	+	+
		b) Mayer's test	_	_	+	_
		c) Picric acid test	_	_	+	+
2	Flavonoids	10% HCl& 5% NaOH test	+	_	+	_
3	Tannins	5% $FeCl_3$ test	_	_	+	_
4	Steroids	Liebermann-Burchard's test	+	+	+	_
5	Triterpenoids	a) Liebermann-Burchard's test	_	+	+	_
		b) Salkowski's test	_	_	_	+
6	Saponins	Foam test	_	_	_	_
7	Glycosides	Keller - Kiliani test	+	+	_	_
8	Gum & Mucilages	Whistler & Be Miller test	_	_	_	_
9	Fixed oils	Spot test	_	_	_	_
10	Anthraquinones	Sanker and Nahar test	_	_	_	_

+ PRESENCE, - ABSENCE, PE- Petroleum ether; EA-Ethyl acetate; ET- Ethanol; WA-Water

Cytotoxicity study

A simple and cheap bioassay to evaluate the potency of phytochemicals in plant extracts is the brine shrimp lethality assay (BSLA). The results of this investigation showed a direct relationship between the extract's concentration and the degree of lethality. Following a 24-hour observation period, every shrimp in the control group survived. Nevertheless, the most mortality was noted at 1000 ppm and the lowest at 1 ppm doses.

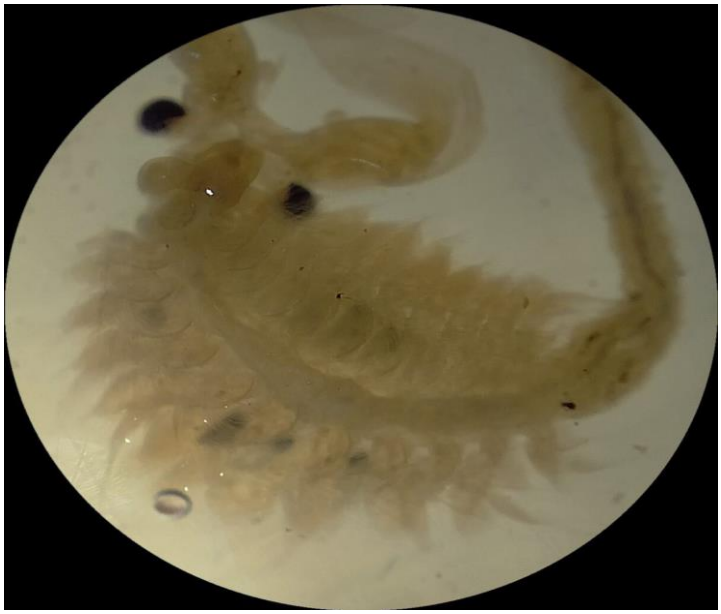


Figure 2: Microscopic view of Brine shrimp.

The cytotoxicity study was carried out in all the four-leaf extracts given in table 2. There were three dilutions which are considered as replicants. The % mortality was based on counting the number of motile nauplii and subtracting it from total nauplii to get the number of dead organism and the percentage were calculated.

The LC50 value of ethanolic extract shows good results comparing to all other extracts (LC 50- 18.63) followed by Ethyl acetate, Aqueous and Petroleum ether extract (LC 50- 28.84, 214.40 and 955.36). But in 1000 ppm concentration the aqueous leaf extract shows sudden increase in mortality rate compared to ethyl acetate extract.

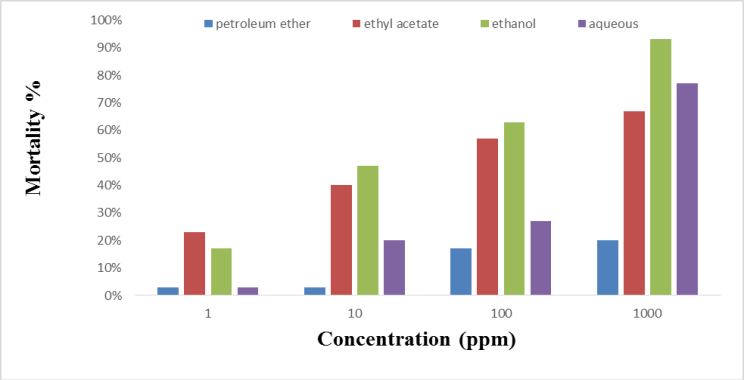
Table 2: Cytotoxicity study of various leaf extract of *Barleria longiflora* on Brine shrimp.

S. no	Treatment	Concentration (ppm)	No. of surviving organism			No. of live nauplii	No. of death nauplii	Mortality %	LC50
			R1	R2	R3				
1	Control distilled water	1 10 100 1000	10 10 10 10	10 10 10 10	10 10 10 10	29 29 28 30	0 0 0 0	0 0 0 0	0
2	Standard Vincristine sulphate	1 10 100 1000	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	30 30 30 30	100 100 100 100	0
3	Petroleum ether extract of B.	1 10	9 9	10 9	10 10	29 29	1 2	3 3	955.36

	longiflora	100 1000	8 8	7 9	10 7	25 24	5 6	17 20	
4	Ethyl acetate extract of B.longiflora	1 10 100 1000	8 7 5 3	8 6 4 4	7 5 4 3	23 18 13 10	7 12 17 20	23 40 57 67	28.84
5	Ethanolic extract of B. longiflora	1 10 100 1000	8 5 3 1	8 4 4 0	9 7 4 1	25 16 11 2	5 14 19 28	17 47 63 93	18.63
6	Aqueous extract of B. longiflora	1 10 100 1000	9 6 4 4	10 9 8 0	10 9 10 3	29 24 22 7	1 6 8 23	3 20 27 77	214.40

LC50= Lethal concentration when 50% of organisms dead; % mortality= Percentage of dead organisms with reference to the total number of organisms used for assay.

Graphical representation of Cytotoxicity study of various leaf extract of *Barleria longiflora* against Brine shrimp



The highest larval mortality was recorded in 1000 ppm concentration of ethanol extract ie., 93% and as concentration decreases (1 ppm) the mortality % also decreased. As it shows better result in preliminary phyto-chemical screening. The presence of secondary metabolites (alkaloids, flavonoids, tannins and steroids) could be accounted for its cytotoxicity properties. Some of the evidence for the brine shrimp lethality test showed that acetone and methanol extracts of *Acanthus polystachyus*, as well as all extracts of *Rhynchosia elegans*, were hazardous. The presence of alkaloids and flavonoids in the acetone and methanol extracts of *Acanthus polystachyus* could account for the reported toxicity. A study on the brine shrimp cytotoxicity of endemic Papuan plants, such as *Piper methysticum* and *Evodia suaveolens*, found that they were not only cytotoxic to brine shrimp, but also rich in alkaloids and flavonoids (Lestari, 2015). Seremet et al., (2018) found that pyrrolizidine alkaloids isolated

from *Senecio vernalis*, *Symphytum officinale*, *Petasites hybrid*, and *Tussilago farfara* were cytotoxic to brine shrimp.

As the ethanol leaf extract of *Barleria longiflora* subjected to major secondary metabolites it shows positive lethality rate. These chemicals work by causing gastrointestinal sickness. As a result, when these substances enter the larva's body, it disrupts its digestive system. This causes the larvae to fail to receive a taste signal, thus they are unable to recognize the food, and the larvae starve to death (Muaja, 2013).

CONCLUSION

Barleria longiflora leaf ethanol extracts were shown to be cytotoxic to brine shrimp, indicating the presence of active components. Although BSLA is insufficient for establishing the mechanism of action of bioactive compounds in plants, it provides a preliminary screen that may be followed by a more specialized bioassay after the active chemical is identified. Research may lead to the development of valuable medicinal medications.

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