WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 01, 172-179. Research Article ISSN 2277– 7105

EVALUATION OF CYTOTOXIC POTENTIALITY OF DIFFERENT EXTRACTS OF *GLOCHIDION VELUTINUM* WIGHT'S LEAVES THROUGH BRINE SHRIMP LETHALITY BIOASSAY

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Article Received on
11 Nov. 2015,

Revised on 02 Dec. 2015,
Accepted on 23 Dec. 2015,

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ABSTRACT

The aim of the work was to investigate the cytotoxic potentiality of leaf of *G. velutinum* through Brine shrimp lethality bioassay (BSLA). Methanol, Ethyl Acetate and n – hexane extracts of *G. velutinum* leaf was investigated on eggs of *Artemia salina*. Various phytochemical constituents like carbohydrates, glycosides, glucosides, alkaloids, glycosides, steroids, tannins, flavonoids and saponin were found in preliminary phytochemical screening. In Brine shrimp lethality bioassay (BSLA), methanol, ethyl acetate and n-hexane extract has cytotoxic activity with LC50 of 428.47µg/ml, 651.92 µg/ml and 598.54µg/ml respectively. So extensive research work is necessary to find out the active constituent responsible for this activity.

KEYWORDS: Brine shrimp lethality bioassay (BSLA), Dimethylsulfoxide (DMSO), cytotoxicity.

INTRODUCTION

From the beginning of human civilization, medicinal plants have a great importance on ailments of various human diseases.[1] Day by day, herbal medicine is becoming much more attractive as alternative medicines useful for treating or preventing various disorders in human and their mode of action.[2] About 25% of drugs prescribed originated from plant source and more than 3000 species have anticancer property.[3,4] The plant *Glochidion velutinum* Wight belongs to Euphorbiaceae family is a small tree or large shrub having coraceous, pinnate venation leaves, yellow flowers and depressed fruits. The chemical

constitutes includes tannins, flavonoids, alkaloids and steroidal saponins. Traditionally the plant is claimed to have antidiabetic, anticancer and antidiarrheal activities.[5]

The in vivo lethality bioassay in a simple zoological organism (eggs of *Artemia salina)* which is known as the brine shrimp lethality bioassay (BSLA), developed by Meyer et al.[6] This bioassay is a rapid (24 h), simple (e.g., no aseptic techniques are required), easily mastered, inexpensive, that requires small amounts of test material (2-20 mg or less).[7] The result found from the bioassay has a ppositive correlation with cytotoxic activity in some human solid tumors.[7] Since the introduction of this brine shrimp lethality bioassay (BSLA), this *in vivo* test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated. The aim of the work was to assess the cytotoxic potentiality of different extracts of *Glochidion velutinum* wight's leaves extract.

MATERIALS AND METHODS

CHEMICALS

Methanol, Dimethylsulfoxide (DMSO) and Folin-Ciocalteu reagent were purchased from Merck, Germany. Vincristine sulphate was collected from Techno Drugs Ltd., Bangladesh. All chemicals and reagents used were of analytical grade.

COLLECTION OF PLANT MATERIAL

The fruit of the plant was collected from Gazipur, Bangladesh and identified by the taxonomist of the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

PREPARATION OF PLANT MATERIAL & EXTRACTION PROCEDURE

Leaf of the plant were first washed with water to remove adhering dirt and then cut into small pieces and sun-dried for few days and then dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C). Dried leaf was grinded into coarse powder using high capacity grinding mill. The powdered leaf was used for serial extraction by Soxhlet apparatus at elevated temperature (65°C) using n-Hexane, Ethyl Acetate and Methanol consecutively (500 mL of each solvent). After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be completed when the leaf materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. The filtrates obtained were dried at

temperature of 40±2°C to have gummy concentrate of the crude extract. The extract was kept in a suitable container with proper labeling and then stored in cold and dry place for further use.[8]

PHYTOCHEMICAL SCREENING

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents i.e. carbohydrates through molisch's test and fehling's test, flavonoids, glucosides through general test for glycoside and glucoside, steroids through liebermann-burchard's test, saponins through frothing test, tannins through Ferric chloride and Potassium dichromate test, alkaloids through mayer's test, hager's test, wagner test and dragendorff's test. These phytochemicals were identified from their respective characteristic color changes as stated in the standard procedures.[9]

BRINE SHRIMP LETHALITY BIOASSAY

Lethal activity of the leaf extracts of *Glochidion velutinum* was determined by Brine shrimp lethality bioassay described by Meyer *et al*.[6] Brine shrimp eggs (*Artemia salina* leach) were hatched in simulated seawater (3.8% NaCl) with continuous oxygen supply for two days and got the nauplii. Stock solution of the sample was prepared by dissolving 20 mg of extract in 400 µL of pure dimethylsulfoxide (DMSO) and adding sea water to make the total volume 20 mL. Thus the stock solution gained concentration of the extract as 1µg/µL. Then specific volumes of stock solution was transferred into different test tubes so that the final concentration of the extract becomes 6.25, 12.5, 25, 50, 100, 200, 400 and 800µg/mL in the respective test tubes after volume adjustment to 5 mL with sea water. In the control tubes 75µL and 150µL DMSO were taken and volume was adjusted to 5mL with sea water (as in the sample tubes). Vincristine sulfate was used as positive control and evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06µg/mL). Using a Pasteur pipette 10 living nauplii were put to each of the test tubes. After 24 hours the test tubes were observed and the number of nauplii survived in each test tube were counted.The mortality was corrected using Abott's formula.[10]

$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$

Where, P_t = Corrected mortality, P_o= Observed mortality and P_c= Control mortality.

LC_{50} values of the test samples after 24 hours are obtained by regression analysis.

RESULTS

PHYTOCHEMICAL SCREENING

In the present study, various qualitative tests were done to detect the presence of different phytochemical compounds in the methanolic, ethyl acetate and n-hexane extracts of the leaf of *Glochidion velutinum*. The results of the phytochemical testing are given in Table 1.

Table 1: Results of Phytochemical Screening of the extracts.

Test		Extracts		
		Methanol Extract	Ethyl Acetate Extract	n-Hexane Extract
Cabrohydrate	Molisch's Test	++	++	++
	Barfoed's Test	+	-	-
	Fehling's test	++	++	++
	Benedict's Test	++	+	-
Glycoside		+	+	+
Glucoside		+	+	+
Alkaloids	Mayer's Test	++	-	+
	Hager's Test	++	-	+
	Dragendorff's Test	++	+	+
	Wagner's Test	++	+	+
Steroids		+	-	-
Tannin	$FeCl_3$ (Ferric Chloride) Test	+	-	-
	Lead Aceatate	+	+	+
Flavonoids		+	+	-
Saponin		+	+	-
Triterpenoid		-	-	-

['++' sign indicates strongly presence & '+' sign indicates presence of phytochemical group of compounds while the '-' sign indicates absence of phytochemical group of compounds tested for]

BRINE SHRIMP LETHALITY BIOASSAY (BSLA)

All the extracts of leaf were subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol extract of leaf was found to be the most toxic to Brine Shrimp nauplii, with LC50 of 428.47µg/ml, whereas anticancer drug vincristine sulphate showed LC50 value 0.699µg/ml. On the other hand, ethyl acetate extracts showed good and n-hexane extract showed 651.92 µg/ml and 598.54µg/ml respectively (table 2).

Table 2: LC_{50} values of the different extracts in brine shrimp lethality bioassay.

Test Sample	Concentration (µg/mL)	% Mortality	LC_{50} (µg/mL)
GHL	6.25	10	598.54
	12.5	20	
	25	20	
	50	30	
	100	50	
	200	60	
	400	50	
	800	60	
GEAL	6.25	10	651.92
	12.5	10	
	25	10	
	50	30	
	100	50	
	200	40	
	400	40	
	800	60	
GML	6.25	20	428.47
	12.5	20	
	25	20	
	50	30	
	100	50	
	200	60	
	400	70	
	800	70	
VS	0.06	10	0.699
	0.125	20	
	0.25	30	
	0.5	40	
	1	50	
	5	90	
	10	100	

DISCUSSION

Different crude extracts of leaf of *G. velutinum* have been shown to possess phytoconstituents including carbohydrates (monosaccharides, reducing sugars), glycosides, glucosides, alkaloids, glycosides, steroids, tannins, flavonoids and saponin. No triterpenoid was detected. These phytoconstituents present in the extracts may account for their various pharmacological activities shown in other investigations.[9]

It is a very useful tool to screen a wide range of chemical compounds for their various bioactivities. It has been well utilized to screen and fractionation of physiologically active

plant extracts as well. Table 2 shows the lethality of different extracts of leaf of *G. velutinum* to the Brine Shrimp nauplii. The degree of lethality shown by the extracts was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (6.25 µg/mL) to the highest concentration (800 µg/mL). This concentration dependent increment in percent mortality of Brine Shrimp nauplii produced by the leaf extracts of *G. velutinum* indicates the presence of cytotoxic principles in these extractives.

The significant correlation between the Brine shrimp assay and in vitro growth inhibition of human solid tumor cell lines demonstrated by the national Cancer Institute (NCI, USA) is significant because it shows the value of this bioassay as a pre-screening tool for antitumor drug research.[11] In toxicity evaluation of plant extracts by Brine shrimp lethality bioassay LC_{50} values lower than 1000 µg/mL are considered bioactive.[6] Principle of brine shrimp toxicity for plant extracts or compounds above 1000µg/ml is non-toxic, between 500 & 1000µg/ml is weakly toxic, and below 500µg/ml is toxic which were established as LC_{50} values.[12] So, methanol extract of leaf is said to be toxic to Brine Shrimp nauplii with LC50 of 428.47µg/ml and ethyl acetate and n-hexane extracts can be said to weakly toxic with 651.92 µg/ml and 598.54µg/ml respectively (table 2). Preliminary phytochemical screening revealed the presence of alkaloids. So the observed cytotoxic action may be due to the presence of such compounds. Again, reports exist on the role of alkaloids and steroids in cytotoxic activity of plant extract.[13,14,15] In addition, phenolics and flavonoids are also known to show cytotoxicity in Hoechst 33258 fluorescence assay by inhibiting cellular DNA in a concentration-dependent manner.[16] Sandhaya S et al. found that three parts (leaf, stem and root) *G. velutinum* are highly lethal to *Artemia salina* due to presence of high phenolic content.[5]

CONCLUSION

Results reveal that, the presence of various phytochemical constituents among all the extracts. They are also cytotoxic due to presence of various phytochemical constituents. Further research is required to investigate the extract of leaf of *G. velutinum* for various pharmacological activities for the benefit of human beings.

ACKNOWLEDGEMENT

First of all, I would like to express my heartfelt gratitude to the Almighty Allah for being enabled me to complete the task of this research. My parents also deserve special appreciation for their huge support and prayers. For that, a great thank for my family. I am

privileged to express my heartfelt gratitude to my honorable teacher and supervisor, Dr. Md. Sohel Rana, Professor, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, for this expert planning, sincere direction, supervision, advices, and continuous follow up from the very beginning of this work. I would also like to acknowledge the guidance of my senior brothers Atunu Raz and helping hands from my friends and juniors (Aumit Roy, Kakon Shutra Dhar, Ariful Islam, Md. Nasir Uddin and Md. Nuruzzaman Neon).

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