

## PHYTOCHEMICAL INVESTIGATION AND CYTOTOXIC SCREENING OF *LAGENARIA SICERARIA* STANDLEY. FRUIT USING *BRINE SHRIMP* LETHALITY ASSAY MODELS

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### ABSTRACT

Cancer is one of the leading causes of mortality worldwide. Many of the cucurbitaceae plants possess antitumor activity on the traditional use. The present study was carried out to evaluate the anticancer activity of extracts *Lagenaria siceraria* Standley Fruit. This fruit has the antioxidant activity so the plant may have anticancer activity. The present research had carried out on laboratory level assay to avoid the use of different animal models. Preliminary phytochemical tests of successive extraction of *Lagenaria siceraria* Standley Fruit powder had performed to find out the different chemical moieties. Preliminary anticancer screening by exposure of different extracts Brine shrimp model was carried out to find out the lead extract which shows the promising cell growth inhibitory activity. The cytotoxic assay was selected because this is easy to done and give fastest promising results. Brine shrimp model were selected for the cytotoxic assay which shows the cytotoxicity that compared with standard antimetabolic drug (Colchicine 100PPM and 100 PPM). n-Butanol extract of *Lagenaria siceraria* Standley Fruit powder shows the promising anticancer activity or cytotoxicity that' so it is selected as a lead extract. Further isolation of active moiety from n-Butanol extract for anticancer activity by chromatographic techniques is almost completed.

**Keywords:** Cucurbitaceae, *Lagenaria siceraria*, anticancer activity, Brine shrimp model.

### INTRODUCTION

Cancer is one of the most fatal diseases in human population and one of the most frequent causes of death worldwide. To prevent the cancer, synthetic and natural sources are used in alone or combination. Today due to resistance of different allopathic

medicine natural source is preferred mainly to block the development of cancer in human. Plant shows different chemical moiety including flavonoids, terpenoids and steroids, Gangwal A (2010), S. L. Deore (2010), Rakesh P. Prajapati. (2010)<sup>[1-3]</sup> which have the pharmacological properties like Antiulcer

Vivek Shrivastav (2011), Antihyperlipidemic as well. Pankaj Nainwal (2011), Prerona Saha et al. (2011) S. L. Deore (2009), antioxidant, cytotoxic U. K. Mazumder (2011).

*Lagenaria siceraria* Standley, commonly known as bottle-gourd (in English), belongs to the Cucurbitaceae family. The plant is widely available throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial parts and fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardiotoxic, general tonic and diuretic properties.

*Lagenaria siceraria* Standley Fruit has different biological activities, as traditional medicinal plants, such as antihyperlipidemic, antidiabetic, antiulcer and prominently antioxidant activity. So the present communication deals with successive extraction of *Lagenaria siceraria* Standley Fruit. For anticancer activity. This activity was screened by different laboratory based models. The Brine shrimp model was selected because this is easy to do and give fastest promising results. The present research had carried out on laboratory level assays to avoid the use of different animal Models.

## MATERIALS AND METHODS

The dry fruit of the plant *Lagenaria siceraria* Standley was collected by cutting the fruit from climbing plant which was stay on other big plant trunk from the local area of Vaduj District of Satara, Maharashtra, India. The plant was identified by botanist, Dr. M. Y. Bachulkar, Taxonomist & Principal, B. Y. College Of Arts, Commerce and Science, Peth-Vadgaon Kolhapur, Maharashtra. After proper identification, voucher specimens (No.1 Sarang Sunil Mahamuni) was deposited in Dept. of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, Maharashtra - 416013.

## Reagents and Chemicals

n-Hexane, Chloroform, Dichloromethane, Ethyl acetate, n-Butanol, Methanol and Distilled water and preliminary

phytochemical reagents, Colchicine, Brine Shrimp eggs, rock salt, dried yeast, tap water.

## Equipment and Apparatus

Soxhlet, apparatus, Mettler analytical balance, Rotamentle (J-SIL), Rotary film evaporator (Evator). Haching, chamber, empty vials, capillary.

All experiment performed in year 2011-12 at Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, Maharashtra - 416013.

## Preparation of Extracts

Dry powder (250g) was used for carrying out soxhlet extraction with 2 liter of n-Hexane, Chloroform, Dichloromethane, Ethyl acetate, n-Butanol, Methanol and chloroform-water for 72h at room temperature. All the extracts were filtered and filtrates were evaporated using Rotary film evaporator and dried in vacuum drier. Extractive values are mention in table 1. Harborne, J. B (1998)

## Phytochemical screening

All the extracts obtained were subjected for phytochemical screening using standard procedure. Khandelwal K.R. (2006). The dried extracts (few mg) were dissolved in sufficient amount of respective solvents and tested for various constituents. The results of the tests are mentioned in table 2

## Brine shrimp lethality assay

The eggs were hatched in artificial sea water by dissolving 40 gm of rock salt 1000 ml of tap water (eggs were collected from local pet shop). Brine Shrimp eggs were incubated at 28°C for 48 hrs by using table lamp and Shrimps were feed by dried yeast. After hatching, ten naupilii were sucked out by capillary. These naupilii were added in already evaporated different concentrations of extract in vials containing 10 ml of sea water. Same procedure was followed for control and standard drug. After 24 hrs, live Brine Shrimps were counted and dead were taken into consideration for further calculation of percent lethality. Results are mentioned in table 3.

S. Kavitha Bagya *et al.* (2011), Muhammad Zia1 *et al.* (2009), Jerry L *et al.* (1998), Ahsan Hussain *et al.* (2007).

## RESULTS AND DISCUSSION

Fruit shows different chemical moiety mostly steroidal, triterpens, alkaloids, glycosides and For Brine Shrimp assay, n-Butanol extract shows 100% activity as compared to the

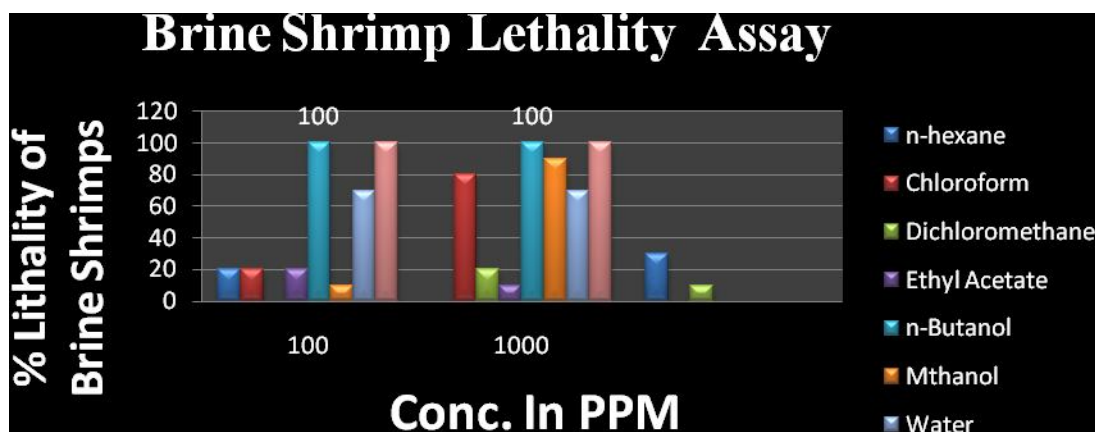
given antimitotic drug (Colchicine 100PPM and 100 PPM) respectively. n-Butanol extract of *Lagenaria siceraria* Standely Fruit powder showed the promising Antimitotic activity so it was selected as a lead extract. Further isolation of active moiety from n-Butanol extract for anticancer activity by chromatographic techniques is almost completed.

**Table 1: Percentage Yield of *Lagenaria Siceraria* Standely Fruit Extracts**

S. No.	Solvent	Colour	Consistency	% Yield
1	n-Hexane	Yellow	Sticky	0.21
2	Chloroform	Green	Non sticky	3.35
3	Dichloromethane	Brown	Sticky	0.21
4	Ethyl Acetate	Brown	Sticky	15.85
5	n-Butanol	Brown	Sticky	13.72
6	Methanol	Brown	Sticky	2.31
7	Aqueous(Water: Chloroform)	Black Brown	Non sticky	25.80

**Table 2: Phytochemical Investigation of *Lagenaria Siceraria* Standely Fruit**

S. No.	Constituents	Phytochemical Tests	Extracts(Fractions)						
			NH	CHL	DM	EA	NB	METH	WAT
1	Carbohydrates	Molisch's Test	-	+	+	-	+	+	-
2	Reducing sugar	Fehling's Test	-	+	-	-	-	-	+
3	Monosaccharide	Barfoed's	-	-	-	-	-	-	+
4	Pentose sugar	Bials orchinol	-	-	-	-	-	-	-
5	Hexose(fructose)	Selvinoff's	-	-	-	-	+	+	+
6	Non reducing Sugar	Tannic acid	-	-	-	-	-	-	-
7	Proteins	Ninhydrine	-	-	-	-	-	-	-
8	Steroids And	Liebermann Burchard Test	+	+	+	+	+	+	+
9	Tritrepinods	Liebermann Burchard Test	+	+	+	+	+	+	+
10	Anthraquinones	Borntrager's	-	-	-	+	-	-	+
11	Flavones	Shinoda	-	-	-	-	-	-	+
12	Alkaloids	Dragendorff Test	-	-	-	-	-	-	+
13	Tannins	Ferric chloride Test	-	-	-	+	-	-	+



**Fig. 1: Graphical presentation of Brine Shrimp lethality assay**

**Table 3: Result of Evaluation of Cytotoxic Potential of *Lagenaria Siceraria* Fruit**

S. No.	Drug/ Extracts	Conc. In PPM	No of Brine shrimps Dies	% Lethality
1	n-Hexane	100	2	20
		1000	0	0
		Control	3	30
2	Chloroform	100	2	20
		1000	8	80
		Control	0	0
3	Dichloromethane	100	0	0
		1000	2	20
		Control	0	0
4	Ethyl acetate	100	2	20
		1000	1	10
		Control	0	0
5	n-Butanol	100	10	100
		1000	10	100
		Control	6	60
6	Methanol	100	1	10
		1000	9	90
		Control	10	100
7	Water	100	7	70
		1000	7	70
		Control	0	0
8	Colchicine	100	10	100
		1000	10	100
		Control	0	0

**Fig. 2: Brine shimps****ACKNOWLEDGEMENT**

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