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SCREENING OF PHYTOCHEMICAL AND ANTIPROLIFARATION OF CELL GROWTH *LAGENARIA SICERARIA* STAND. FRUIT BY PHYTOTOXIC BIOASSAY 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. Preliminary phytochemical tests of successive extraction of *Lagenaria siceraria* Standely Fruit powder had performed to find out the different chemical moieties. Preliminary anticancer screening by exposure of different extracts Phytotoxic Bioassay model was carried out to find out the lead extract which shows the promising cell growth inhibitory activity. Cereals Moth seeds were selected for the Phytotoxic Bioassay which shows the phytotoxicity that compared with standard antimitotic drug (colchicine). n-Butanol extract of *Lagenaria siceraria* Standley Fruit powder shows the promising anticancer activity or cytotoxicity, so that it is selected as a lead extract. Further isolation of active moiety from n-Butanol extract for anticancer activity by chromatographic techniques is completed.

Keywords: Cucurbitaceae, *Lagenaria siceraria*, anticancer activity, Phytotoxic Bioassay.

INTRODUCTION

Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. It is a group of diseases characterized by the deregulate proliferation of abnormal cells that invade and disrupt surrounding tissues. To prevent the cancer, synthetic and natural sources are used alone or in 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 [1-3], trepinods [1-3] and steroids [1-3] which have the pharmacological properties like Antiulcer [4], Antihyperlipidemic [5-6], antioxidant [7-8], cytotoxic [9] as well. *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 cardiotonic, 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 Phytotoxic Bioassay was selected because this is easy to done 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) were prepared and deposited in the herbarium in Dept. of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur, Maharashtra – 416013

Reagents and Chemicals **:** n-Hexane, Methanol (SDFCL), Chloroform, Dichloromethane, Ethyl acetate (LOBA Chemicals), n-Butanol (FINAR), Distilled water and preliminary Phytochemical reagents, Colchicine (INDO GERMAN ALKALOIDS) Moth seeds (Kapiltirth market, Kolhapur) Mercuric chloride (FINAR),Tap Water, autoclaved distilled water, blotting paper.

Equipment and Apparatus: Soxhlet, apparatus, Mettler analytical balance, Rotamentle (J-SIL), Rotary film evaporator (Evator). Petri plats, Watman filter # 1 paper. 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, [10] Chloroform, Dichloromethane, ethyl acetate, n-Butanol, methanol and chloroform-water for72h 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.

Phytochemical Screening: All the extracts obtained were subjected for Phytochemical screening using standard procedure. [11-12] The dried extracts were dissolved in sufficient amount of respective solvents and tested for various constituents. The results of the tests are mentioned in table No. 2.

Surface sterilization: 0.1% $HgCl_2$ (mercuric chloride) solution was prepared in a beaker. [13] Moth seeds were put in it for 2 to 3 min rinsed with autoclaved distilled water and finally dried them with sterilized blotting paper.

Phytotoxicity Assay: Experiment consisted of two concentrations (100 and 1000 PPM) of the plant extracts were prepared in different solvent. [14-19] Filter papers (Whatman # 1) were placed in Petri plates and 10 ml of each concentration was added. Solvents were evaporated and 10 ml of tap water was added. To each Petri plate 10 Moth seeds surface sterilized with 0.1% mercuric chloride were placed. In control plates 10 ml of different solvents was added and evaporated. After evaporation of solvents tap water was added to each Petri plate. Positive controls made by tap water only. Standard control made by using Colchicine. Germinated Moth seeds were counted everyday from 1st to 5th day. The plates were sealed with cello tape to avoid moisture loss and placed at RT. In control plates 10ml of different solvents were added and evaporated. Root length was measured on 3rd and 5th day of incubation. The experiment was repeated in triplicate. Results are mentioned in table No. 3.

$$\% \text{ inhibition of the root length} = \frac{\text{Root length in test sample}}{\text{Root length in control}} \times 100$$

RESULTS AND DISCUSSION

The present study explores the potent antiproliferetive activity which may be either because of a direct cytotoxic effect of the extract on normal phytocells or restriction of cell division in normal cell cycle.

Fruit shows different chemical moieties mostly steroids, triterpens, alkaloids and glycosides. For Phytotoxic Bioassay, n-Butanol extract shows 26.08 % and 22.35%. Percent root growth as compared to the given antimitotic drug (Colchicine 100PPM and 100 PPM) respectively.

CONCLUSION

n-Butanol extract of *Lagenaria siceraria* Fruit powder showed the promising Antiproliferative 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.

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

Solvent	Colour	Consistency	% Yield
n-Hexane	Yellow	Sticky	0.21
Chloroform	Green	Non sticky	3.35
Dichloromethane	Brown	Sticky	0.21
Ethyl Acetate	Brown	Sticky	15.85
n-Butanol	Brown	Sticky	13.72
Methanol	Brown	Sticky	2.31
Aqueous(Water: Chloroform)	Black Brown	Non sticky	25.80

Table 2: Preliminary Phytochemical Screening of *Lagenaria Siceraria* Standely Fruit

Constituents	Phytochemical Tests	Extracts(Fractions)						
		NH	CHL	DM	EA	NB	METH	WAT
Carbohydrates	Molisch's Test	-	+	+	-	+	+	-
Reducing sugar	Fehling's Test	-	+	-	-	-	-	+
Monosaccharide	Barfoed's	-	-	-	-	-		+
Pentose sugar	Bials orchinol	-	-	-	-	-	-	-
Hexose (fructose)	Selvinoff's	-	-	-	-	+	+	+
Non reducing Sugar	Tannic acid	-	-	-	-	-	-	-
Proteins	Ninhydrin	-	'+	+	-	-	-	-
Steroids And	Liebermann	+	+	+	+	+	+	+
Tritrepinods	Burchard Test	+	+	+	+	+	+	+
Anthraqinones	Borntrager's	-	-	-	+	-	-	+
Flavones	Shinoda	-	-	-	-	-	-	+
Alkaloids	Dragendorff Test	-	-	-	-	-	-	+
Tannins	Ferric chloride Test	-	-	-	+	-	-	+

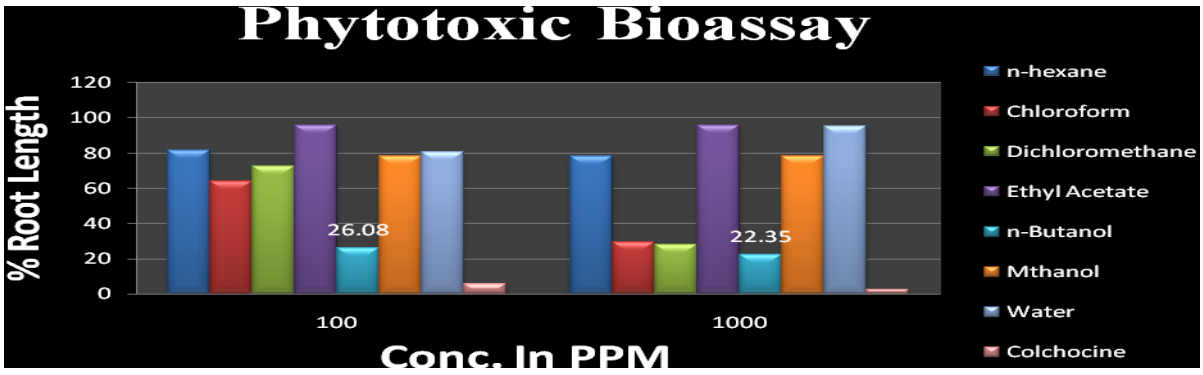
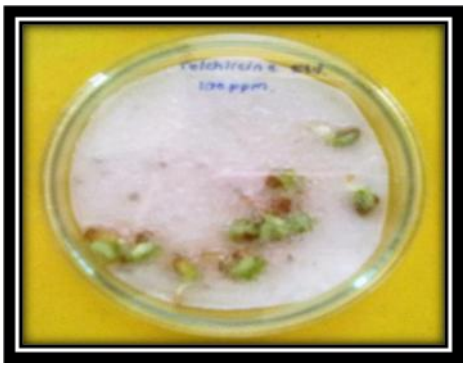


Figure 1: Graphical presentation of Phytotoxic Bioassay

Figure -2



a) **Colchicine 100ppm**

b) Colchicine 1000ppm

c) n-Butanol 100ppm

d) n-Butanol 100ppm

e) Water Control

Table 3: Result of Preliminary anticancer screening of *Lagenaria Siceraria* Standely Fruit

Sr. No.	Drug/ Extracts	Conc. In PPM	No of Moth seeds and Root Length of each										Average No of Root Length	% Root Growth
			1	2	3	4	5	6	7	8	9	10		
1	N H	100	5.7	6	5.5	3.7	5.7	2	6	3	0.5	6.5	4.46	81.83
		1000	4.9	3	4	3	4.5	2.4	6.4	4	3.8	6.7	4.27	78.34
	Control	-	7	3.5	8.5	4	7.5	8	5	4.5	5.5	1	5.45	-
2	CHL	100	4	5	4	7.5	11	4.5	1	1.5	0.6	4.5	4.36	64.02
		1000	3	2.5	4	3.5	2	1.3	0.9	0.8	0.5	1.5	2	29.36
	Control	-	7.5	6	2.5	11.5	9.5	10.2	6.5	7	1.5	5.9	6.81	-
3	DM	100	5.5	4.5	7	5.3	6	3.5	3.5	5.2	3.9	1.5	4.59	72.62
		1000	2.2	2.5	2.4	2.3	1.9	2.5	3	0	0.5	0.4	1.77	28.00
	Control	-	9.5	4.5	4	12	6.7	10	8.5	0	0.5	7.5	6.32	-
4	EA	100	4.5	4.4	4	3.5	5.5	4.9	5.5	2.5	5.4	4.5	4.3	95.98
		1000	2.5	7.5	5.4	4.4	5.5	4	2	5.4	2.3	4	4.3	95.98
	Control	-	3.7	2.5	6	7.8	0	6.7	4.5	4.8	6.5	2.3	4.48	-
5	NB	100	0	3.5	1.9	2.4	1.5	3	1.2	1.5	2.5	0	1.75	26.08
		1000	3.2	2.3	1.9	0	1.5	0	0	1.7	2.9	1.5	1.5	22.35
	Control	-	3	6.4	12.2	6	8.5	14.3	3.7	8.5	0.5	4	6.71	-
6	MTH	100	5.7	5.8	3.5	7.2	2.4	5.5	2.7	4.5	5	0	4.23	78.33
		1000	5.5	2.7	4.5	5	0	5.7	5.8	3.5	7.2	2.4	4.23	78.33
	Control	-	7.5	7	7	7.5	6	6	4.6	6.7	1.7	0	5.4	-
7	WAT	100	0	5.5	4.4	3.3	4.5	3.2	5.7	4.7	4	3.4	3.87	80.47
		1000	16.5	10	8.5	5	4.4	3	2.7	0.5	3.5	10	6.41	57.67
	Control	-	3	6.4	12.2	6	8.5	14.3	3.7	8.5	0.5	4	6.71	-
8	Colchicine	100	0.5	0.5	0.5	0.2	0.4	0.5	0.4	0.5	0.4	0	0.39	5.81
		1000	0.3	0.4	0.2	0	0	0.2	0.2	0.5	0	0	0.18	2.60
	Control	-	3	6.4	12.2	6	8.5	14.3	3.7	8.5	0.5	4	6.71	57.67

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