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**PHYTOCHEMICAL SCREENING OF *LAGENARIA SICERARIA* STANDLEY FRUIT
AND IT'S ANTICANCER ACTIVITY USING POTATO DISCS ASSAY MODEL**

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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 on Potato disc assay was carried out to find out the lead extract which shows the promising cell growth inhibitory activity. The potato discs assay was selected because this is easy to done and give fastest promising results. Potato was selected for this assay in which the *Agrobacterium tumefaciens* produces tumor on potato discs. The tumor formation was compared in between different extracts of *Lagenaria siceraria* Standley Fruit and standard Antimitotic drug (colchicine).

n-Butanol extract of *Lagenaria siceraria* Standley Fruit powder shows the promising anticancer activity that' so it is selected as a lead extract. Further isolation of chemical moieties of same extract will be possible.

Keywords: Cucurbitaceae, *Lagenaria siceraria*, Anticancer Activity, Potato Discs Assay

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 [1]. 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, 2, 3], terpenoids [1, 2, 3] and steroids [1, 2, 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 cardiogenic, 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 potato discs assay 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. S. R. Yadav Shivaji University 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 [10], Methanol (SDFCL), Chloroform, Dichloromethane, Ethyl acetate (LOBA Chemicals), n-Butanol (FINAR), Distilled water and preliminary phytochemical reagents, Colchicine (INDO GERMAN ALKALOIDS), Russet Potatoes 1 medium sized potato (Kapiltirth market, Kolhapur), Bleach, Sterile Water, DMSO, Knives, Micropipettes (100/1000) with disposable tips, Disposable Culture Tubes Eppendorf tubes (Shri Laxmi Diagnostics), LB Broth agar medium (HiMedia).

Agrobacterium Strains

Commercially available stock culture of *Agrobacterium tumefaciens* [available from NCIM (No. 2145), Pune, Maharashtra] was used for experiment. The *A. tumefaciens* strains for Antitumor Potato Disc Assay {isolated from crown gall sample and identified in the Microbiology Laboratory, Department of Microbiology, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur – 416013} were used during antitumor assay. Subculture was prepared by using YEB medium (composition: Beef extract 0.05g, sucrose 0.05g, yeast extract 0.1g, peptone 0.5g, MgSO₄.7H₂O 30mg, distilled water 100ml, Agar 2g) according to NCIM.

Equipment and Apparatus

Soxhlet apparatus, Mettler analytical balance, Rotamentle (J-SIL), Rotary film evaporator (Evator), Colony counter magnifying glass. 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 (IP) 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**.

Phytochemical Screening

All the extracts obtained were subjected for phytochemical screening using standard procedure [11-12]. 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**.

Bacterial Culture Preparation

Using commercially available stock culture of *Agrobacterium tumefaciens* a small amount of subculture (1 loop) of bacteria aseptically

transferred to 5 ml autoclaved LB broth and incubated at 28°C overnight. Entire starter culture was then transferred 250 ml autoclaved LB broth and incubated at 28°C for 48 hrs. A suspension of the 48 hrs bacterial culture in 1X phosphate Buffered Saline (PBS) was prepared.

Potato Discs Assay

Potatoes (*Solanum tuberosum* L., Solanaceae) were sterilized using 20% bleach solution and cut into 5mm×8mm or 1cm×1cm in size from the center of potato tissue by sterilize cork borer. Five discs were placed on 1.5% agar media. The experiment was performed in three groups; Test group (7 different Extracts), Control group (DMSO and water), Standard (colchicine) and each had two concentrations 100 PPM and 1000 PPM. All extracts were dissolved in DMSO as stock solution and further inoculums were prepared as follows:

a) 1.5 ml water, 2.5 ml 48 hrs bacterial culture and 5 ml extracts were added in DMSO.

b) Controls were prepared by replacing extract with only DMSO and water. Same procedure was follow for standard antimitotic drug colchicine. From different groups, 0.05 ml sample were added on five potato discs in respective labeled Petri plates. Plates were incubated at 30°C for 7-21 days [13-17]. Tumors of each plate were counted and experiment was repeated in triplicate. Results are mentioned in **Table 3**.

RESULTS AND DISCUSSION

The present study thus explores the potent anticancer activity which may be either because of a direct cytotoxic effect of the extract on *Agrobacterium tumefaciens* or restriction of tumor formation. Fruit shows different chemical moieties mostly steroids, triterpens, alkaloids and glycosides. For Potato Disc Assay, n-Butanol extract shows maximum activity (Average no. of Tumors for 100 PPM and 1000 PPM are 4.8 and 1 respectively) as compared to the given Antimitotic drug (Colchicine Average no. of Tumors for 100 PPM and 1000 PPM are 5 and 3.2 respectively).

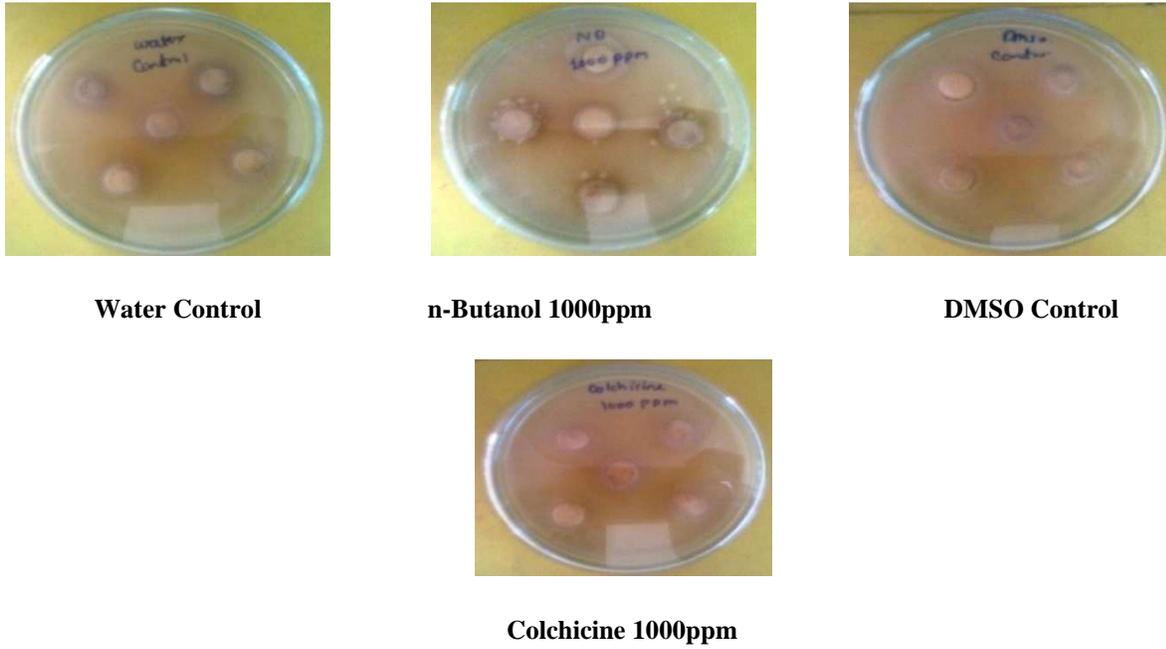


Figure 1: Potato Discs Assay

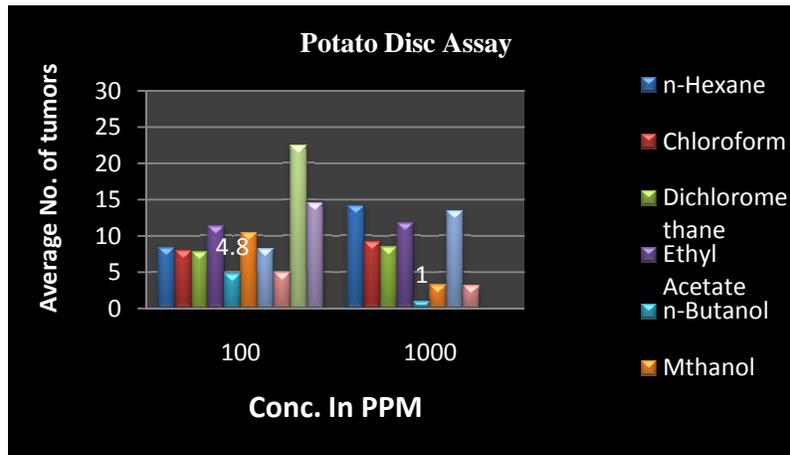


Figure 2: Graphical Presentation of Anticancer Activity

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: Preliminary Phytochemical Screening of *Lagenaria Siceraria* Standely Fruit Using Potato Disc Assay Model

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	+	+	+	+	+	+	+
9	Tritrepinods	Burchard Test	+	+	+	+	+	+	+
10	Anthraquinones	Borntreger's	-	-	-	+	-	-	+
11	Flavones	Shinoda	-	-	-	-	-	-	+
12	Alkaloids	Dragendorff Test	-	-	-	-	-	-	+
13	Tannins	Ferric chloride Test	-	-	-	+	-	-	+

Table 3: Result of Evaluation of Anticancer Activity of *Lagenaria Siceraria* Standely Fruit Using Potato Disc Assay Model

S. No.	Drug/ Extracts	Conc. In PPM	No. of Tumors					Average No. of Tumors
			1	2	3	4	5	
1	n-Hexane	100	20	10	3	4	5	8.4
		1000	15	12	13	15	16	14.2
2	Chloroform	100	10	15	2	-	5	8
		1000	1	7	-	20	9	9.25
3	Dichloromethane	100	12	13	6	3	5	7.8
		1000	-	2	10	10	12	8.5
4	Ethyl acetate	100	8	8	10	11	20	11.4
		1000	15	15	7	17	5	11.8
5	n-Butanol	100	6	5	6	1	6	4.8
		1000	-	-	-	1	1	1
6	Methanol	100	9	14	15	4	-	10.5
		1000	-	2	6	3	2	3.25
7	Water	100	-	3	8	20	2	8.25
		1000	2	5	9	25	15	13.5
8	Colchicine	100	6	3	5	-	7	5
		1000	6	6	1	1	2	3.2
9	Water control	-	2	20	15	20	16	14.6
10	DMSO	-	-	-	-	25	20	22.05

CONCLUSION

n-Butanol extract of *Lagenaria siceraria* Standely Fruit powder showed the promising antitumor activity so it was selected as a lead extract. Further isolation of active moiety from n-Butanol extract for anticancer or

antitumor activity by chromatographic techniques is almost completed.

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