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In-vitro anticancer activity of Chlorophytum tuberosumand Lagenaria siceraria

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Abstract: *Both Chlorophytum tuberosum B. and Lagenaria siceraria are rich in antioxidant secondary metabolites like saponin, triterpenoids and flavonoids. Cytotoxicity and or anticancer activity of antioxidant phytochemicals like steroidal and triterpenoidal saponins, flavonoids is well elaborated in literature. Hence, aim of present study is to screen these plants for in-vitro anticancer activity against human cell lines MCF-7, HeLa, COLO-205, Hep-G2 and HL-60.*

Keywords: *Chlorophytum tuberosum, flavonoids, Lagenaria siceraria, SRB assay, saponin*

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I. Introduction

Natural world of chemistry has given world number of wonderful anticancer moieties and hence medicinal plants are major target of anticancer drug screening. [1]*Chlorophytum tuberosum* B. of family Liliaceae commonly known as "safedmusli" is a very well known plant for its aphrodisiac as well as immunomodulatory activity in India. *C. tuberosum* root contains mainly steroidal saponins. [2-3]

Lagenariasiceraria of family Cucurbitaceaecommonly known as "bottle gourd"is useful in different systems of traditional medication for the treatment of diseases and ailments of human beings. The plants contain triterepnoid saponins: cucurbitacins, flavones, C-glycosides beta glycosides.[4-5]

Both *Chlorophytum tuberosum* and *Lagenaria siceraria* are used since many years and found to play important role in immunomodulation and protection from major diseases due to antioxidant potential. [2-5] Anticancer role of antioxidant phytochemicalsis well elaborated in many literatures. Hence, it is decided to screen both selected plants for in-vitro anticancer activity against cell lines Human Breast Cancer Line (MCF-7), Human Cervical Cancer Line (HeLa), Human Colon Cancer Line (COLO-205), Human Hepatocellular Carcinoma Cell Line (Hep-G2) and Human Leukemia Cell Line (HL-60).

II. Materials and methods

Fruits of *Lagenaria siceraria* (LS) and tubers of *Chlorophytum tuberosum* (CT) collected from local area in Dhule district and Melghat region of Amravati district respectively. The plant materials were authenticated from authorized person ofbotanical institute/ lab.The plant materials dried in shade and converted to powder of coarse size by pulverization.The parts of plant material/s subjected for successive extraction by using different solvents according to polarity: Petroleum ether, chloroform, ethanol and water. Preliminary phytochemical screening of these four extracts performed to know maximum phytochemical containing extract. The rich phytochemical containing ethanol extract further subjected for fractionation and analyzed by TLC for presence of different phytochemicals.

2.1 In vitro anticancer activity by SRB assay[6-8]

Less number of anticancer drugs and their unpredictable cure enforces need of screening of medicinal plants to get more potent anticancer drugs. In vivo methods of anticancer screening require animal and thus ethical permissions. Again due to the high costs and time-consuming nature of animal cancer model studies, the initial screening of anticancer compounds from natural products is best accomplished using in vitro cancer cell methods. Cultured human cancer cell lines are preferred to screen chemicals and natural product extracts in vitro to speed the discovery of new anticancer drugs.

Present study has undertaken to carry out in vitro anticancer effects of extracts and fractions on selected human cancer cell lines. The *Chlorophytum tuberosum*chloroform extract (CTCA), *Chlorophytum tuberosum* ethanolic extract (CTEE), *Chlorophytum tuberosum* aqueous extract (CTAE), *Lagenaria siceraria* chloroform extract, *Lagenaria siceraria* ethanolic extract (LSEE), *Lagenaria siceraria*aqueous extract (LSAA)

prepared as above were used for the anticancer activity. Adriamycin (ADR)was used as a standard anticancer drug.

2.2 SRB Assay procedure

The anticancer activities of extracts were studied at Advanced Center for Treatment, Research and Education in Cancer (ACTREC), Mumbai where 14 cell lines were maintained in ideal laboratory conditions. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates 90 µL/well at appropriate plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, in 5% CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs.After 24 h, cells from one plate of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental extracts were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration of 100, 200, 400 and 800 µg/ml. Aliquots of 10 µl of these different dilutions were added to the appropriate micro-titer wells already containing 90 µl of cell suspension, resulting in the required final drug concentrations of 10, 20, 40 and 80 µg/ml.For each of the experiments a known anticancer drug was used as a positive control.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mMTrizma base, and the absorbance was read on an Elisa Plate Reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells 100.

The summary of parameters is as follows.

- GI50 Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, drug concentration resulting in a 50% reduc-tion in the net protein increase
- TGI Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
- LC50 Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] × 100 = 7minus;50.
- GI50 value of = 20 µg/ml is considered to demonstrate activity.

III. Results and discussion

The part of plants materials was collected from local area in Dhule district and Amravati district under Melghat region. The *Chlorophytum tuberosum* are authenticated by Department of Botany, Government Vidarbha Institute of Science & Humanities (GVISH), Amravati while *Lagenaria siceraria* authenticated by Department of Botany, S.S.V.P.S's Dr. P.R. Ghogrey Science College, Dhule. Preliminary phytochemical screening showed presence of different phytochemicals in extracts while fractions showed presence of following phytochemicals (Table 1)in chromatographic (TLC) studies.

Table 1: Phytochemicals present in extracts and fractions

Extract	*Chlorophytum tuberosum B.*	*Lagenaria siceraria*
Petroleum ether	Fatty acids	-
Chloroform	Alkaloids	Triterpenoids
Ethanol	Carbohydrate, flavonoids, saponin, glycoside	Saponins, tannins, flavonoids
Water	Carbohydrate, protein, flavonoids, Amino acid	Carbohydrate, Tannin, protein, flavonoids

Different extractscontaining saponin and flavonoids of *Lagenaria siceraria* and *Chlorophytum tuberosum* are selected for evaluation of in-vitro anticancer properties against cell lines MCF-7 (Table 2, figure

1), HeLa (Table 3, figure 2), COLO-205 (Table 4, figure 3), Hep-G2 (Table 5, figure 4) and HL-60 (Table 6, figure 5).

Ethanolic extract of Chlorophytum tuberosum and Lagenaria siceraria shows prominent result against HL-60 cancer cell line (Table 6, figure 5) could result significant lead molecule and none of extract of both plants found effective against MCF-7 (Table 2, figure 1), HeLa (Table 3, figure 2) and COLO-205 (Table 4, figure 3).

The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of diseases requiring use of anticancer agents. Further work on the chemical isolation and structure determination of the compounds from bioactive fractions in order to find the structure activity relationship is warranted. Since secondary metabolite content may vary as a function of multiple factors, such as environmental conditions and harvest period, reproduction of this analysis over a long period of time is needed before the effectiveness of our method is totally demonstrated.

Table 2: % Control Growth against Human Breast Cancer Line MCF-7

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
CTCE	101.3	98	96	94.3
CTEE	92.2	92.9	91.2	105.6
CTAE	91.17	83.12	89.27	100.22
LSCE	90.1	88.13	91.1	92.12
LSEE	91.6	92.8	93.7	101.5
LSAE	94.12	95.17	98.12	108.12
ADR	-14.5	-25.7	-44.9	-52.7

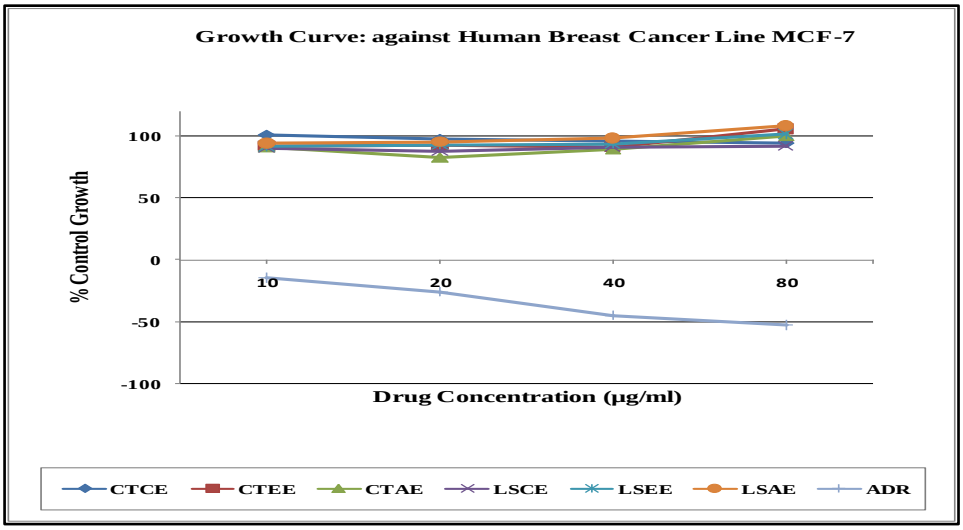

Figure 1: Growth Curve: Human Breast Cancer Line MCF-7

Table 3: % Control Growth against Human Cervical Cancer Line HeLa

Test Component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
CTCE	104.2	103.2	104	102.4
CTEE	106	104.4	106.7	107.3
CTAE	106	103	102	105
LSCE	98.2	103.4	102.4	102.6
LSEE	107.4	107.2	109.7	109.2
LSAE	95	98	100	90
ADR	-59.6	-62.2	-63.1	-62.3

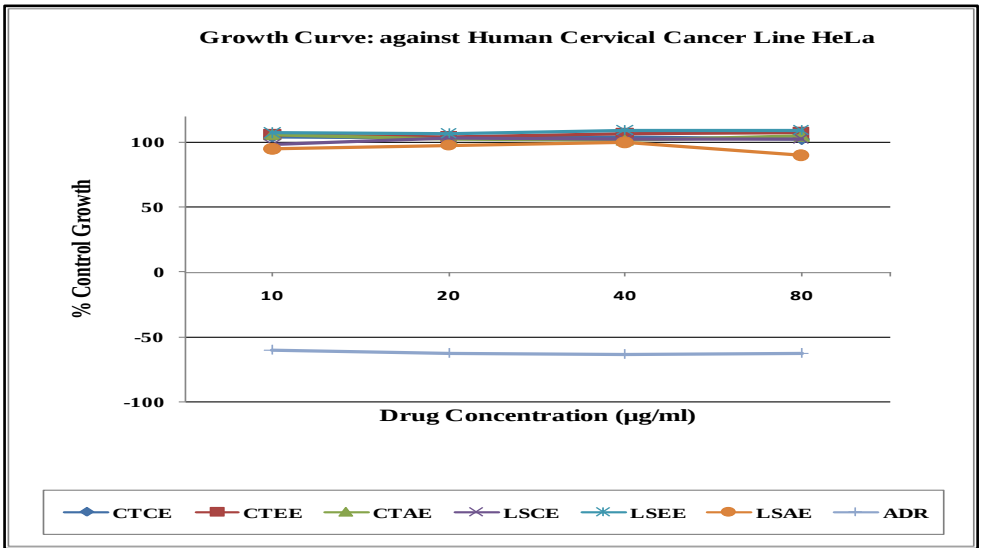

Figure 2: Growth Curve: Human Cervical Cancer Line HeLa

Table 4: % Control Growth against Human Colon Cancer Line COLO-205 in the form of Drug Concentrations (µg/ml)

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
CTCE	102.3	102.4	98.3	96.4
CTEE	97.6	100.1	100.2	96.8
CTAE	104.2	103.4	98.1	92.1
LSCE	99.3	94.2	92.3	91.2
LSEE	97.5	98.2	100.9	97.7
LSAE	98.5	92.2	91.2	94.6
ADR	-23.9	-25.3	-30.7	-46.2

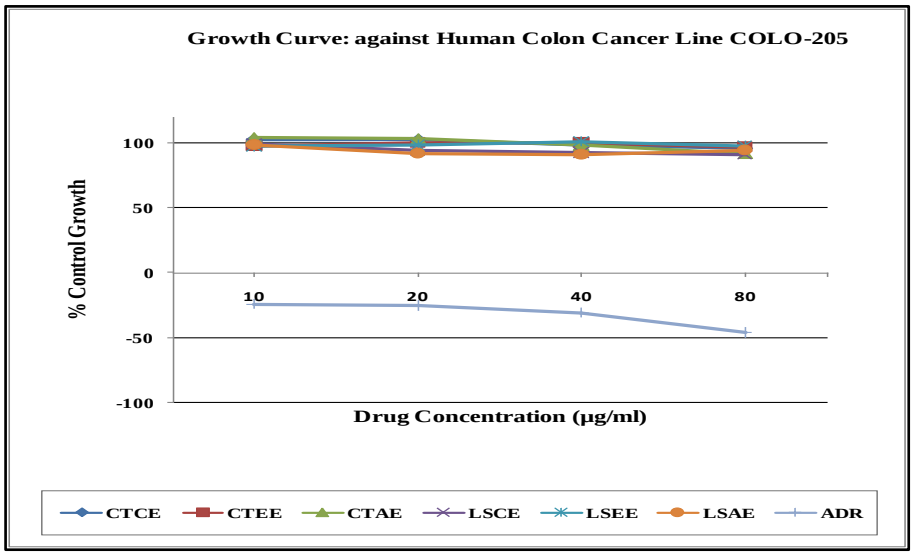

Figure 3: Growth Curve: Colon Cancer Line COLO-205

Table 5: % Control Growth against Human Hepatocellular Carcinoma Cell Line Hep-G2 in the form of Drug Concentrations (µg/ml)

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
CTCE	105.1	101.9	100.6	98.7
CTEE	103.7	105.1	103	102.3
CTAE	105.2	106.9	102.1	105.3

LSCE	106	105.4	102.3	102.7
LSEE	105.7	106.4	105.3	106.4
LSAE	101	103.2	102.1	98
ADR	-37.2	-44.7	-70.1	-71.5

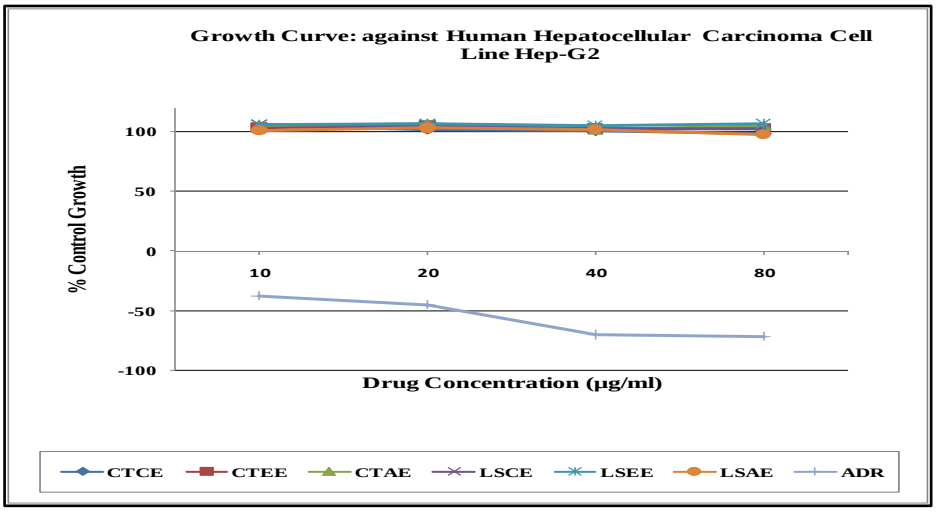

Figure 4: Growth Curve: Human Hepatocellular Carcinoma Cell Line Hep-G2

Table 6: % Control Growth against Human Leukemia Cell Line HL-60 in the form of Drug Concentrations (µg/ml)

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	20	40	80
CTCE	93	82	84	76
CTEE	-6.1	-6	-12.9	-22.2
CTAE	-2.3	2	-3.2	-11.17
LSCE	-2.3	3	2.3	-2.1
LSEE	-16.6	1	-12.1	-23.4
LSAE	91	86	85	72
ADR	-34.6	-37.3	-30.7	-18.1

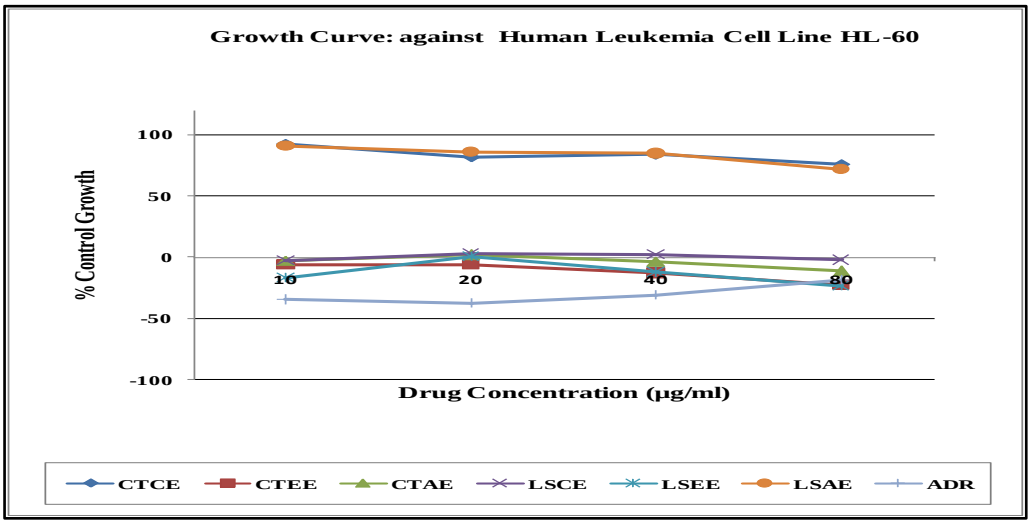

Figure 5: Growth Curve: Human Hepatocellular Carcinoma Cell Line HL-60

IV. Conclusion

Cancer is one of the leading causes of mortality worldwide. Currently available, in vitro screening techniques SRB and MTT assay are the most reliable techniques used to evaluate anticancer activity on the cancer cell lines. The SRB assay provides a better linearity with cell number and a higher sensitivity and its staining is not cell dependent. It is known that, in contrast to the MTT assay the SRB assay stains recently lysed cells. The present study was carried out to evaluate the anticancer activity for different extracts of *Lagenaria siceraria* fruit and *Chlorophytum tuberosum* tubers. Ethanolic extract of *Lagenaria siceraria* fruit and *Chlorophytum tuberosum* tubers found to be active against Human leukemia Cell Line (HL-60).So finally it can be concluded that both plant parts can be promising candidates in future anticancer therapy.

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