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## Research Article

# *In vitro* Cytotoxic Activity of *Luffa acutangula* on Human Neuronal Glioblastoma and Human Lung Adenocarcinoma Cell Lines

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**Abstract:** *In vitro* studies were carried out to evaluate the cytotoxic potential of the ethanolic and aqueous extracts of *Luffa acutangula* against human neuronal glioblastoma cells (U343) and human lung cancer cells (A549). Screening of these extracts was done using the brine shrimp lethality bioassay, MTT assay and SRB assay. The results showed the significant decrease of the viability of the cells in a concentration-dependent manner. The ethanolic and aqueous extracts of *Luffa acutangula* showed significant cytotoxic activity in both MTT and SRB assay. In brine shrimp lethality bioassay also aqueous extract showed more potent cytotoxicity as compared to ethanolic extract.
**Keywords:** *In vitro* cytotoxic, *Luffa acutangula*, U343, A549, MTT Assay, SRB assay

## INTRODUCTION

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of deaths after cardiovascular diseases [1]. For many years, the cytotoxic actions of the chemotherapeutic drugs were ascribed solely to their ability to induce genotoxic death [2]. Therefore, there is an urgent need to develop alternative therapeutic measures such as use of biological and natural products against this deadly disease. Cancer chemoprevention with strategies using foods and medicinal herbs containing antioxidant micronutrients has been regarded as one of the most visible fields for cancer control [3, 4].

One such plant, *Luffa acutangula*, (Family: Cucurbitaceae), commonly known as Ridge gourd and tiroi, is a large monoecious, annual climber, found wild and also cultivated throughout the greater parts of India. It contains crystalline bitter principle similar to cucurbitacin B, luffin, and colocynthin [5]. Seeds show presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid while fruits contain cucurbitacin B, E and oleanalic acid. The ancient literature also revealed that the plant is significantly used as abortifacient and antifungal agent [6]. Antioxidant activity of *Luffa acutangula* has been reported [7] and leaf extracts of *Luffa acutangula* exhibits high antiproliferative activity against various cell line as determined with MTT assay [8].

In context with the important phytochemical and therapeutic findings, it was considered worthwhile to assess the anticancer properties of fruit extract of *Luffa acutangula* against human neuronal glioblastoma cells (U343) and human lung cancer cells (A549) by using the *in-vitro* screening models such as brine shrimp lethality bioassay, MTT assay and SRB assay.

## MATERIAL AND METHODS

*Collection of plant and preparation of extracts*:

Fruit of *Luffa acutangula* was purchased from market of Udaipur, authenticated and a voucher specimen (PP 370) has been deposited in the Department of Pharmacognosy, Geetanjali Institute of Pharmacy (Udaipur, India).

*Ethanolic extract:*

The fruits were shade dried, powdered and about 100 g of powder was extracted with ethanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo*.

*Aqueous extract (Chloroform: water-1:99):*

The fruits were shade dried, powdered and macerated with chloroform water for seven days.

*Cell culture and treatment:*

U343 and A549 cells was procured from NCCS, Pune, grown in 75 $cm^2$ tissue culture flasks containing Dulbecco's minimum essential medium

(DMEM) supplemented with 10% Fetal Bovine Serum, Trypsin Phosphate Versene Glucose solution at 37 °C in $CO_2$ incubator in an atmosphere of humidified 5 % $CO_2$ and 95 % air. The cells were maintained by routine sub culturing in 75 $cm^2$ tissue culture flasks. The culture medium was changed every 48 h and the cells were usually split 1:3 when they reached confluence. Plates were changed to FBS-free medium before the beginning of the assay. For the cytotoxicity assay a range of concentrations of extract (62.5, 125, 250, 500 µg/ml) was used for 48 h treatment for the determination of $IC_{50}$.

*Test of cytotoxicity:*

Cytotoxic activity was determined by brine shrimp lethality bioassay, MTT dye-reduction assay and SRB assay.

**Brine shrimp lethality bioassay**

The brine shrimp (Artemia salina) eggs were brown in colour and very small in size. 100 milligram of eggs roughly represents 2.5 to 3.0 thousand of eggs. Hatching chamber was fabricated as per the design [9]. The chamber was made of glass, with aluminum lid on top. The chamber was divided into two equal parts with the help of a laminated plywood divider having a number of holes of 2 mm size. One of the compartments was illuminated with a lamp (60 watts) while the other was darkened. Both the chambers were aerated.

Samples of the extracts were prepared by dissolving 5 mg of both ethanolic and aqueous extracts in 5 mL of DMSO to get stock solution. From this stock 50, 250, 500 and 750 µL were taken and volume was made up to 5 mL with solution which contain specific volume of brine and yeast suspension to get the final drug concentration 10, 50, 100 and 150 ppm. Three replicates were prepared for each dose level. Control vials were prepared by adding equal volumes of distilled water. Nauplli were drawn in a pipette along with water and ten of such shrimps were transferred to each sample vial after they were counted in the stem of pipette against lighted background. The artificial sea water was added to each vial to make upto 5 mL, a drop of dry yeast suspension (3 mg in 5 mL sea water) was added to each vial as food for shrimps. The vials were maintained under illumination. After 24 h survivors were counted, by using 3X magnifying glass and the percent deaths and LC50 values were calculated by using Finney Computer program [9].

**MTT assay**

The tetrazolium 3- (4, 5 dimethylthiazoly-2)-2, 5-diphenyltetrazolium bromide) (MTT) is reduced to a colored product by the activity of NAD (P) H-dependent deshydrogenases and this indicates the level of energy metabolism in cells. Briefly cells were seeded in 96-well microplates with $1X10^4$ cells in 0.1 mL of DMEM medium supplemented with 10% FBS and routinely cultured in a humidified incubator (37 °C in 5% $CO_2$) for 24 h. Herbal extracts were added in serial concentrations (62.5, 125, 250, 500 µg $mL^{-1}$) and incubated for 24 h. Then the medium was discarded and 100 µL of tetrazolium dye (MTT) solution (1 mg $mL^{-1}$ in PBS) was added to every well and re-incubated for an additional 4 h. 100 µL of DMSO was added to dissolve the formazan crystals formed. The plate was then read on a microplate reader at 540 nm. MTT solution with DMSO (without cells and medium) acted as a blank control in microplate reading while the PBS-treated cells served as a control of 100% survival [10, 11].

**SRB assay**

The term cytotoxicity covers both cytostatic and cytocidal effects. The SRB assay can be used to determine which of these takes place for a particular test substance. 100 µL of cell suspension of optimum density was introduced into each well of 96 well plates. A range of concentrations (62.5, 125, 250, 500 µg $mL^{-1}$) of extracts to be tested was made in the culture medium.100 µL of each concentration of test sample was added in culture medium to the wells containing the cells and 100 µL medium only to the control wells. The cells were incubated with the samples for 48 h and fixed with ice-cold TCA for 1 h at 4°C. The plates were washed five times in distilled water and allowed to dry in the air. Then 50 µL sulphorhodamine (SRB) solution was added to each well of the dry 96-well plates and allow staining at room temperature for 30 min. The sulphorhodamine (SRB) solution was removed by washing the plates quickly with 1% v/v acetic acid five times to remove unbound dye. The bound SRB was solubilized by adding 100 µL of 10 mM unbuffered Tris Base (pH 10.5) to each well and shaking for 5 min on a shaker platform. Plates were read in a 96-well plate reader with the working wavelength 492 nm [12].

**Statistical analysis**

Data represent the mean ± standard error (SEM) of the indicated number of experiments. Graphs were prepared by Prism software. Statistical analysis of the data was carried out by one way ANOVA (Graph Pad Prism 5.02 Software) followed by Dunnet post hoc test. A value of $p< .05$, $p<0.01$, $p<0.0001$ were considered to be significant, very significant and highly significant, respectively. Linear regression analysis was used to calculate $IC_{50}$.

**RESULTS AND DISCUSSION**

**Brine shrimp lethality bioassay**:

Both ethanolic and aqueous extracts screened for BSL bioassay were found effective. The $LD_{50}$ of ethanolic and aqueous extracts was found to be 125 and 95 µg $mL^{-1}$, respectively. Since the BSL assay is very preliminary method to assess cytotoxic activity, the extracts were continued to explore in different *in vitro* cytotoxic models (Figure 1).

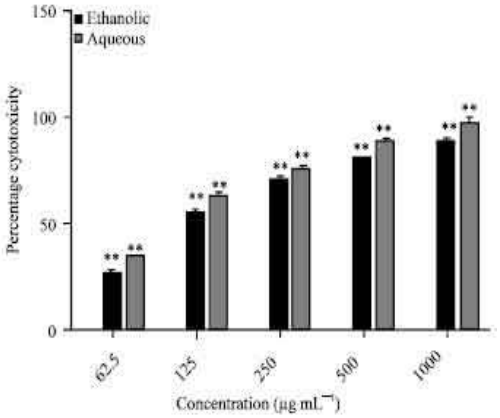


**Fig. 1 Effect of various extracts on brine shrimp lethality. All the values are Mean ± SEM of three samples, **p<0.01 compared to control. (***All the values are mean ± SEM of three samples, **P < 0.01 compared to control.)

***In vitro* cytotoxicity in U343 and A549 by MTT assay**:

Percentage cell death was determined after 48 h. Both the extracts showed significant cytotoxicity in U343 and A549 cells in the range of 62.5 to 250 µg $mL^{-1}$. (Fig. 2 and 3). The ethanolic extract of *Luffa acutangula* showed $IC_{50}$ 123 µg $mL^{-}$ against U343 cells and 127 µg $mL^{-1}$ against A 549 cells in MTT assay.

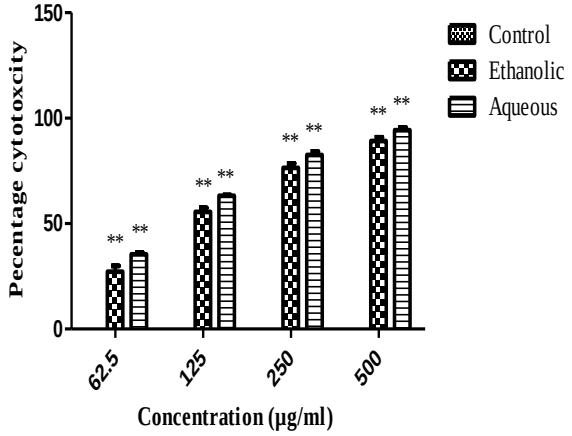


**Fig. 2 *In vitro* cytotoxic activity of various extracts in U343 cells (Human neuronal glioblastoma cells) by MTT assay at 48 hours of exposure. (***All the values are mean ± SEM of three samples, **P < 0.01 compared to control.)

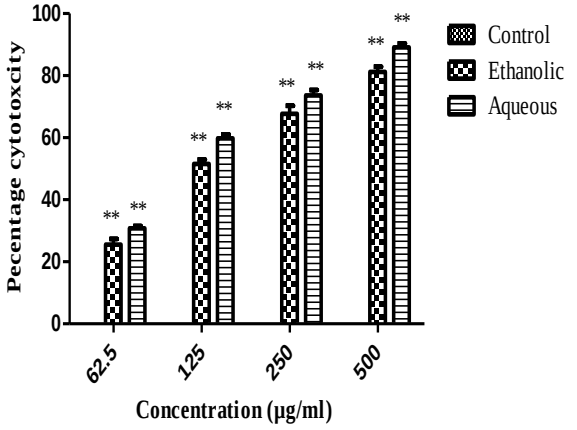


**Fig. 3 *In vitro* cytotoxic activity of various extracts in A549 cells (Human lung adrenocarcinoma cells) by MTT assay at 48 hours of exposure. (***All the values are mean ± SEM of three samples, **P < 0.01 compared to control.)

***In vitro* cytotoxicity in U343 and A549 by SRB assay**:

In SRB assay also both the extracts showed significant cytotoxicity in U343 and A549 cells. After 48 hours, both extracts showed potent activity with IC50 value in range of 62.5-125 µg mL-1 but the cytotoxicity of extracts was less in SRB assay as compared to MTT assay (Fig. 4 and 5).

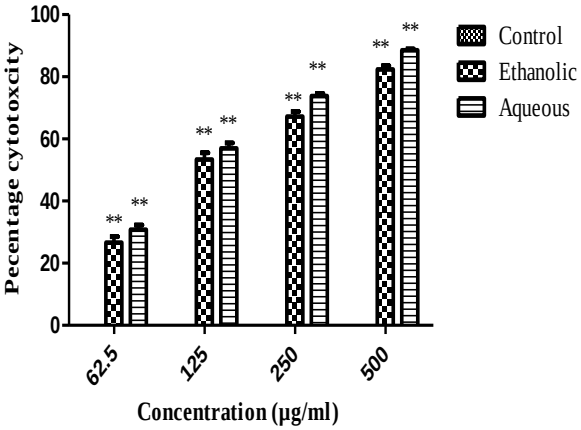


**Fig. 4 *In vitro* cytotoxic activity of various extracts in U343 cells (Human neuronal glioblastoma cells) by SRB assay at 48 hours of exposure. ***All the values are mean ± SEM of three samples, **P < 0.01 compared to control.

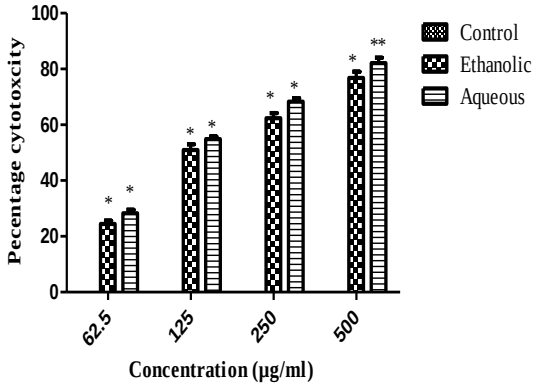


**Fig. 5 *In vitro* cytotoxic activity of various extracts in A549 cells (Human lung adrenocarcinoma cells) by SRB assay at 48 hours of exposure. (*****All the values are mean ± SEM of three samples, **P < 0.01 compared to control.)

The ethanolic extract of *Luffa acutangula* showed $IC_{50}$ 128 µg $mL^{-1}$ for U343 cells and 131 µg $mL^{-1}$ for A 549 cells in SRB assay, thus showed significant cytotoxic activity. The aqueous extract of *Luffa acutangula* demonstrated higher activity with $IC_{50}$ 108 µg $mL^{-1}$ in MTT and 114 µg $mL^{-1}$ in SRB assay for U343 cells and 112 µg $mL^{-1}$ in MTT and 119 µg $mL^{-1}$ in SRB assay for A549 cells, respectively after 48 h of exposure. In brine shrimp lethality bioassay also aqueous extract showed more potent cytotoxicity with $LC_{50}$ 95 µg $mL^{-1}$ as compared to ethanolic extract with $LC_{50}$ 125 µg $mL^{-1}$.

## CONCLUSION

The results obtained in the present study indicate that *Luffa acutangula* extracts exhibit potent cytotoxic activity in various *in vitro* models. The activities might be attributed to its polyphenolic content and other phytochemical constituents.

Our study revealed that aqueous extract significantly reduces cancer cell growth *in vitro*. In preliminary cytotoxic screening, considerable cell death was observed in the brine shrimp lethality bioassay. Particularly aqueous and alcoholic extracts at higher dose showed promising cytotoxicity in brine shrimp which gives preliminary information about toxic nature of compounds in rapidly multiplying cells, and supports their cytotoxic nature. The cytotoxic potency of the extracts is further confirmed by MTT and SRB assay using two cancer cell lines. U343 and A549 were found significantly sensitivity to *Luffa acutangula* and its studied extracts.

From results obtained it can be concluded that both extracts of *Luffa acutangula* may be a promising alternative to synthetic substances as natural compound with high antiproliferative activities and to be useful in cancer treatment and prevention and based on encouraging data obtained in *in vitro* study, we can further proceed with screening in tumor bearing mice. Findings of this study can be proved useful in accessing the *in vivo* anticancer efficacy of *Luffa acutangula*.

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