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Research Article

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In vitro antioxidant and *in vivo* anti-tumor activity of *Luffa acutangula* against Dalton's Lymphoma Ascites (DLA) cells bearing mice

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ABSTRACT

The objective of the present study is to explore the anti-oxidant activity of the methanolic and aqueous extracts and anticancer activity of the ethanolic and aqueous extracts of the Luffa acutangula in Swiss albino mice against Dalton's Lymphoma Ascites cells. Both methanolic and aqueous extracts exhibited antioxidant activity determined by in vitro models. Anticancer activity of ethanolic and aqueous extracts of Luffa acutangula was evaluated in Swiss albino mice at the doses of 200 and 400 mg/kg body weight orally. Both extracts at both doses were administered for 13 consecutive days. After 24 h of the last dose and then eighteen hours of fasting, the mice were sacrificed and antitumor effect of ethanolic and aqueous extracts was assessed by evaluating tumor weight and tumor volume. Both the extracts showed significant decrease in ($p<0.0001$) tumor volume and weight. The results demonstrated that the extract has potent dose dependent anticancer activity comparable to that of cisplatin. Aqueous extract at both doses (200 and 400 mg/kg) and ethanolic extract at 400 mg/kg dose showed potent anticancer activity.

Keywords: *Luffa acutangula*, DLA, anticancer activity, cisplatin.

INTRODUCTION

Cancer is considered as one of the most dreaded diseases and it's a class of diseases characterized by uncontrolled cell proliferation or metastasis of abnormal cell in the body [1]. At present, chemotherapeutic agents, surgery and radiation are the commonly used treatment strategies in cancer; however they are not fully effective against the high prevalence or low survival rate. Hence, there is a great interest in the development of safe, low-cost anti-cancer agents from natural sources [2].

Many plants and plant derived agents have been used for cancer treatment since 1950 [3]. The phytoconstituents present in the plants are mainly responsible for its cytotoxic activity. The isolation of vincristine and vinblastine from vinca and podophyllotoxins from Podophyllumhexandrum are considered as milestones in the development of anti-cancer agents [4], [5]. This led to the discovery of other compounds in cancer treatment such as taxanes, camptothecins, and combretastatins [6], [7].

Plant-derived agents act by modulating various signaling pathways in cancer cells. Plant-products are known to modulate multiple signaling pathways simultaneously; hence, they can be very effective in inhibiting uncontrolled cell proliferation of cancer cells, which have multiple survival strategies. Naturally herbs are curative, whereas chemotherapy and cytotoxic drugs are inherently destructive [8].

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 [9]. 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 [10]. Leaf extracts of *Luffa acutangula* exhibits high antiproliferative activity against various cell line as determined with MTT assay [11]. 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 Dalton's Lymphoma Ascites in Swiss albino mice.

EXPERIMENTAL SECTION

Collection of plant and preparation of extracts

Fruit of *Luffa acutangula* was purchased from market of Udaipur, authenticated and a voucher specimen has been deposited in the Department of Pharmacognosy, B. N. Institute of Pharmaceutical Sciences (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*.

Methanolic extract

The fruits were shade dried, powdered and about 100 g of powder was extracted with methanol 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.

Chemical and Reagents

1, 1-Diphenyl-2-picryl hydrazine (DPPH) and 2, 2-azino bis (3-ethyl benzo thiazoline-6-sulphonic acid (ABTS) were purchased from Sigma Chemicals, USA. Gallic acid was obtained from Nice Chemicals, Mumbai. Ascorbic acid (Ranbaxy Fine Chemicals Ltd.) was used as standard for the study.

Animals

Swiss albino mice weighing 22-28 g were used in the experiment. They were obtained from animal house of B. N. Institute of Pharmaceutical Sciences, Udaipur and were acclimatized to the experimental room having temperature 23±2 °C, controlled humidity conditions and 12-h light - dark cycle. Animals were caged in poly acrylic cages (38 x 23 x 10 cm) with maximum of four animals per cage. The mice were fed with standard food pellets and water ad libitum. Before commencement of the experiment the mice were acclimatized to laboratory conditions for 7 days. All procedures described were reviewed and approved by the Animal Ethical Committee and study was conducted after obtaining ethical committee clearance (Clearance certificate No. 100/LSC/BNCP-12/IAEC).

Antioxidant Activity Assays

1. 1-Diphenyl-2-Picryl Hydrazine (DPPH) Radical Scavenging Activity

The antiradical activity for the plant extracts was assessed on the basis of the radical-scavenging effect of the stable DPPH free radical. The concentration of DPPH was kept at 300 μM in MeOH. 1 mg of the extracts dissolved separately in 1 mL of MeOH, from which different concentrations (5-200 μg/mL) were prepared. 10 μL of each extract solution was allowed to react with 200 μL DPPH at 37°C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as reference [12].

2. 2-Azino Bis (3-Ethyl Benzo Thiazoline-6-Sulphonic Acid (ABTS) Radical Scavenging Activity

For ABTS assay, the stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared and the working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in a dark place. The solution was then diluted by mixing ABTS•+ solution with methanol to obtain an absorbance of 1.00 ± 0.02 units at 734 nm using the spectrophotometer. Fresh ABTS•+ solution was prepared for each assay. Different concentrations of standard solution of ascorbic acid were prepared. The 1 mg of the extracts dissolved separately in 1mL of MeOH, from which different concentrations (5-200 μg/mL) were prepared. 200 μL of each concentration was allowed to react with 4 μL of the ABTS•+ for 2 h in a dark place. Then, the absorbance was read at 734 nm [13].

Nitric Oxide (NO) Scavenging Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions. The nitrite ions can be determined by Griess Illosvoy reaction [14]. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

% Inhibition = $[(A_0 – A_1) / A_0 X100]$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract/Standard [15].

Total Phenolics

Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent using gallic acid as a standard phenolic compound. 1.0 mL of extract solution containing 1.0 mg extract was diluted with 46 mL of distilled water in a volumetric flask. 1.0 mL of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as gallic acid equivalents in mg/g of dry extract [16].

Total Flavonoids

Aluminum chloride colourimetric method was used for determination of flavonoids. To the 10 mL volumetric flask 2 mL of water and 1 mL of plant extract (1 mg/mL) were added. After 5 min 3 mL of 5 % sodium nitrite and 0.3 mL of 10 % aluminum chloride were added. After 6 min, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 510 nm. The percentage of total flavonoids were calculated from calibration curve of quercetin (10-250 µg) plotted by using the same procedure and total flavonoids was expressed as quercetin equivalents in milligrams per gram sample [17].

Acute Toxicity Study

Acute toxicity study was carried out for ethanolic and aqueous extracts of *Luffa acutangula* according to the method described by Litchfield and Wilcoxon, 1949 using male Swiss albino mice orally [18]. The LD_{50} values were found to be 4 g/kg body weight respectively.

Dalton's Lymphoma Ascites (DLA) cell induced solid tumor model

DLA cells were aspirated from peritoneal cavity of the tumor bearing mice. Tumor viability was determined by Tryphan blue exclusion test and cells were counted using Haemocytometer. The Ascitic fluid was suitably diluted in saline to get a concentration of 10^6 cells/ml of tumor cell suspension and 0.1 ml of this solution was injected into the right hind limb of Swiss albino mice to obtain a solid tumor. Treatment was started on 3rd, 5th, 7th, 10th, 12th and 14th day of tumor inoculation [19]. Cisplatin was injected on first day as standard.

Treatment schedule

70 Swiss albino mice were used in the experiment, which were divided into seven groups (n=10), they were fed with food and water *ad libitum*. All the animals in each groups received DLA Cells except Group-I. This was taken as day '0'. Group-I animals served as normal saline control (5 mL/kg i.p.) and group-II animals served as DLA control without any drugs. 24 h after DLA transplantation, groups-III and IV animals received ethanolic extract of *Luffa acutangula* at a dose of 200 and 400 mg/kg orally, groups-V and VI animals received aqueous extract at a dose of 200 and 400 mg/kg orally for 13 alternative days, respectively. Group-VII animals received reference drug cisplatin (3.5 mg/kg i. p) on the first day[11]. After 24 h of the last dose and then 18 h of fasting, animals of each group were sacrificed by cervical dislocation to measure tumor volume and tumor weight.

Tumor volume = $4/3\ \pi\ ab^2$, where a and b represent the major and minor radii respectively [20].

Tumor weight: At the end of the fourth week, animals were sacrificed under anesthesia using diethyl ether; tumor was extirpated and weighed. The percentage inhibition was calculated by the formula:

% Inhibition = (1-B/A) × 100; where, A is the average tumor weight of control group, B is that of treated group. [21]

RESULTS AND DISCUSSION

Methanolic and aqueous extracts of *Luffa acutangula* exhibited antioxidant activity in different *in vitro* models (Table **1)**. IC_{50} values of methanolic and aqueous extracts were, 110 µg/mL and 102 µg/mL for DPPH (Fig. **1**) and 125 µg/mL and 115 µg/mL for ABTS (Fig. **2**), and 190 µg/mL and 198 µg/mL for NO scavenging activity (Fig. **3**), respectively. Total Phenolic content of methanolic and aqueous extracts of *Luffa acutangula* fruit was found to be 1.25 and 0.68 mg/g respectively compared with gallic acid. Total flavonoid content of methanolic and aqueous extracts of *Luffa acutangula* was found to be 0.52 mg/g and 0.78 mg/g respectively compared with quercetin. Phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activities [22]. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent [23]. The results obtained in the present study indicate that *Luffa acutangula* extracts exhibit potent free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that plant could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

Table 1: *In Vitro* Antioxidant Activities of *Luffa acutangula* Extracts

Sample	Anti-oxidant activity (IC_{50} µg/ml)		
	DPPH	ABTS	Nitric Oxide Scavenging Activity
Methanolic extract	110	125	190
Aqueous extract	102	115	198
Ascorbic acid	8.2	12	26

Table 2: Effect of various extracts against DLA induced solid tumor volume (in cm^3) in mice

Treatment	Volume of tumor on 4th week	% Reduction in Tumor volume
Control	5.59±1.07	0.00
Standard	0.22±0.14***	96.06±0.34***
Ethanolic (200 mg/kg)	3.85±0.78	31.12±0.83***
Ethanolic (400 mg/kg)	3.06±0.60*	45.25±1.10***
Aqueous (200 mg/kg)	2.76±0.24*	50.80±0.69***
Aqueous (400 mg/kg)	2.00±0.42**	64.22±0.90***

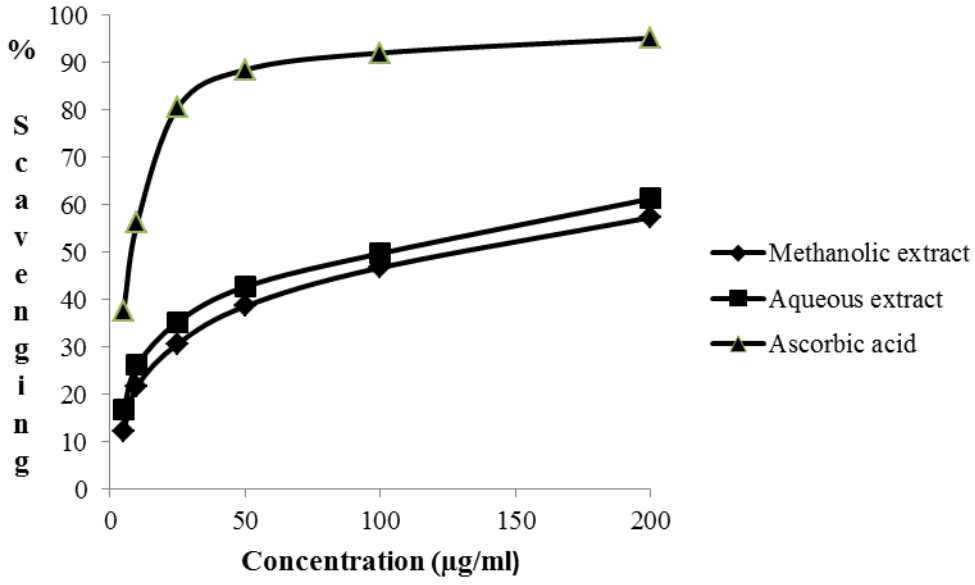

Fig 1: Scavenging of DPPH free radical by various extracts

Effect of extracts against DLA induced Solid tumor volume in mice:

Both the ethanolic and aqueous extracts of *Luffa acutangula* significantly reduced development of solid tumor in mice and maximum reduction in tumor volume was observed in cisplatin treated mice. Administration of ethanolic extract at 400 mg/kg body weight and aqueous extract at 200 and 400 mg/kg body weight were found most effective in reducing tumor growth (Table 2).

Table 3: Effect of various extracts against DLA induced Solid tumor weight in mice

Treatment	Weight of tumor on 4th week (in g)	% Tumor weight reduction
Control	8.35±1.27	0.00
Standard	1.20±0.22***	85.62±0.64***
Ethanolic (200 mg/kg)	5.58±0.72*	33.17±0.18***
Ethanolic (400 mg/kg)	4.92±0.14**	41.07±1.08***
Aqueous (200 mg/kg)	4.30±0.56***	51.41±0.69***
Aqueous (400 mg/kg)	3.70±1.12***	58.19±0.87***

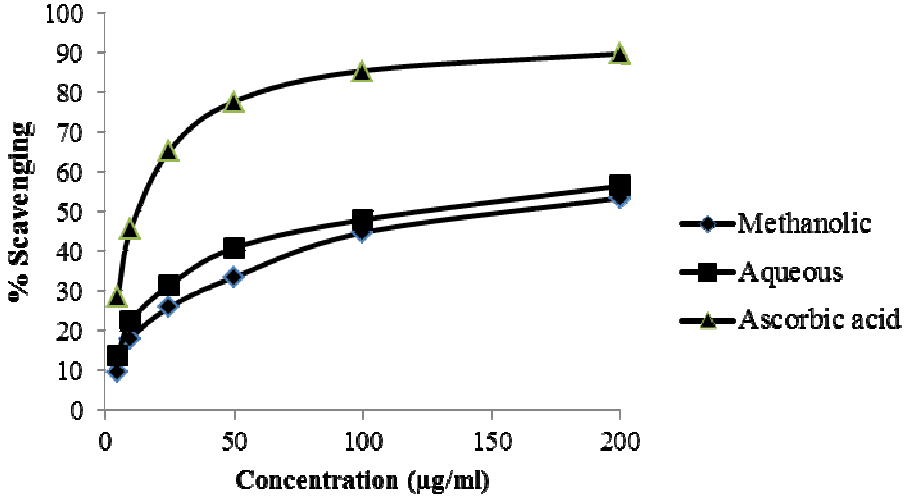

Fig 2: Interaction of different extracts with ABTS radical

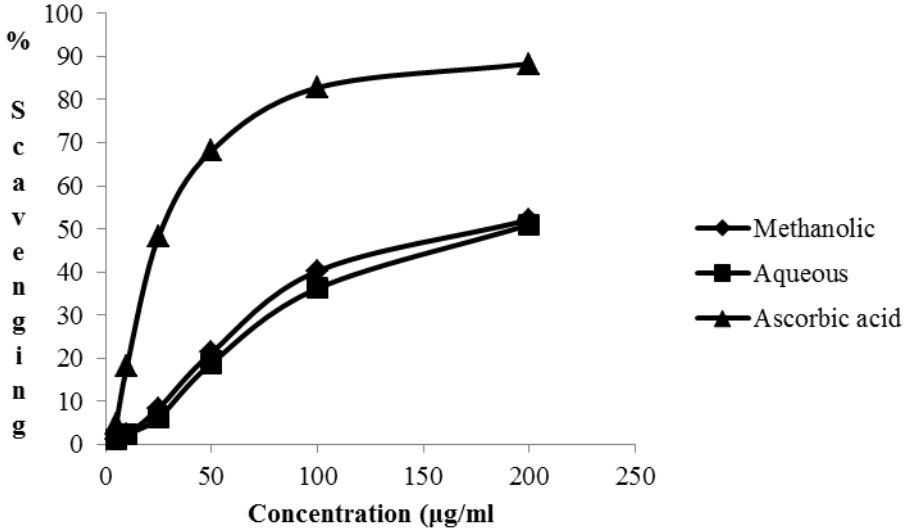

Fig 3: Nitric oxide scavenging activity of various extracts

Effect of different extracts against DLA induced solid tumor weight in mice:

The DLA inoculation significantly increased the tumor weight in vehicle treated mice on day 30th. In drug treated mice significant reduction in tumor weight was observed at every monitored day. Among the treatments, aqueous extract of *Luffa acutangula* at 400 mg/kg showed 58.19 % reduction. (Table 3) The animals treated with standard (cisplatin) were able to prevent solid tumor weight by 85.62%.

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