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In vitro Antioxidant and Cytotoxicity Activity of Aqueous and Alcoholic Extracts of *Annona Squamosa* Linn

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ABSTRACT

Aqueous and alcoholic extracts of the aerial part of *Annona squamosa* Linn were screened for their possible antioxidant activity by DPPH free radical scavenging and cytotoxicity on proliferation of HT29 colon cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) microculture tetrazolium viability assay. The cells were exposed to different concentrations (100, 50, 25, 12.5, 6.25and 3.125 µg/ml. In DPPH radical scavenging assay the % inhibition at100 (µg/ml) was 6.48 and 67.97 respectively and % cytotoxicity in MTT assay at100 (µg/ml) was 46.75 and 67.24 with IC50 of 38.04 and 36.80 respectively. From the above results it was observed that alcoholic extract of *Annona squamosa* aerial part was more significant than the aqueous extract of *Annona squamosa* aerial part.

Key Words: *Annona squamosa,* DPPH, MTT assay, cytotoxicity, IC50.

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INTRODUCTION

The incidence of colon cancer is rising in every country of the World. It is the fourth most common cause of cancer death (after lung cancer, stomach cancer and liver cancer). Thus, colon cancer is a worldwide disease and needs to be addressed seriously. Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various ailments including cancer. Almost 60% of drugs approved for cancer treatment are of natural origin [1]. There is always the hope that the search among the traditional medicinal plants may provide potent and safe medicines.

Annona squamosa Linn. is a small evergreen tree is cultivated throughout India for its fruits, different parts of *Annona squamosa* Linn. are used in folkloric medicine for the treatment of various disease [2]. This plant is commonly called custard apple in English & sharifa in Hindi & sitaphalam in telgu in India [3]. *Annona squamosa* Linn, belonging to family *Annonaceae* is commonly found in India It is considered beneficial for cardiac disease, diabetes hyperthyroidism & cancer. The root is considered as a drastic purgative3. An infusion of the leaves is considered efficacious in prolapsusani of children, the crushed leaves are sniffed to overcome hysteria & fainting spells, they are also applied on ulcer & wounds. The ripe fruits of this plant are applied to malignant tumors to hasten suppuration. A paste of seed powder has been applied to the head to kill lice. It is also used for destroying worm in the wound of cattle's [4].

The present study aimed to evaluate the possible Cytotoxic activity of the aerial part of *Annona squamosa* Linn used in the treatment of several diseases, but with no reports on its inhibitory effect on colon cancer potential. Therefore, the aim of the present study was to evaluate the anticancer activity on HT-29 Human colon cancer cell line.

MATERIALS AND METHODS

Plant material Collection: Arial part of the plant was collected from Savadatti nearby Belgaum. Plant is identified and authenticated in Ghatprapha Ayurvedic College and research center, Ghataprapha. By Soxhlet apparatus aqueous and ethanolic extracts were prepared.

Cell Culture: Human colon cancer cell line (HT29) obtained from the NCL PUNE India.

MTT assay

MTT solution preparation: 10 mg in 10 ml of Hank's balanced solution.

Cell culture : The cell line were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamycin, Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% Co2 at 37ºC for 3-4 days. After 3-4 days remove the supernatant and replace MEM media with Hank's

balanced solution supplemented with Gentamycin, Penicillin and Streptomycin. Incubate overnight.

Invitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and adds fresh Hank's balanced salt solution and treated with different concentration of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. After 24 hrs incubation at 37ºC in a humidified atmosphere of 5% Co2, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4 hr incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubalised by adding DMSO (200µl) and optical density was measured at wavelength of 570nm. The test denotes the survival cells after toxic exposure. Percentage inhibition of the extract against all cell line was calculated using the following formula.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of control}} \times 100$$

% cell inhibition = 100 - % cell survival

The effects of extracts were expressed by IC50 values calculated from dose response curves [5]

DPPH radical scavenging activity:

For assessment of DPPH radical scavenging activity DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid and extract. After that 5ml of sample solution was mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517 nm and calculated the % inhibition of DPPH radical [6].

Calculations and statistics

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Absorbance Control} - (\text{Sample with DPPH} - \text{sample without DPPH})}{\text{Absorbance of control}} \times 100$$

Results were expressed as percentage growth inhibition of control. IC50 values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using Graphpad Prism version 5.00. And results were shown in Table 01 and Table 02 represented in fig 1 and fig 2 respectively.

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RESULTS & DISCUSSION

The plant is reputed to possess varied medicinal properties like, cytotoxic [7] and antioxidant activities [8]. Numerous Annonaceous acetogenins have been shown antimalarial, cell growth inhibitory [9], antiparasitic and antimicrobial activities. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease [10]. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases ,cancer, neurodegenerative diseases inflammation and problems caused by cell and cutaneous aging [11]. In the present study the cytotoxic activity of aqueous and alcoholic extracts of *Annona squamosa* Linn *aerial part* on HT-29 human colon cancer cell lines were evaluated with MTT assay. The IC_{50} of the aqueous and alcoholic extracts was found to be38.04 and 36.8 µg/ml on HT 29, cell lines respectively. Among the tested extracts alcoholic extract was more selective cytotoxic against HT-29 cell line.

Table No 1: Cytotoxicity Activity of aqueous (A.aq) and alcoholic (A.al) extract of *Annona squamosa* Linn against HT-29 cell line

Sl.N0	Concentration(µg/ml)	Cytotoxic activity (%)		IC_{50} (µg/ml)		R2	
		A.aq	A.al	A.aq	A.al	A.aq	A.al
1	100	46.75	67.24	38.04	36.8	0.9981	0.9976
2	50	35.7	50.75				
3	25	23.69	32.69				
4	12.5	14.25	18.32				
5	6.25	7.12	6.6				
6	3.125	6.68	6.1				

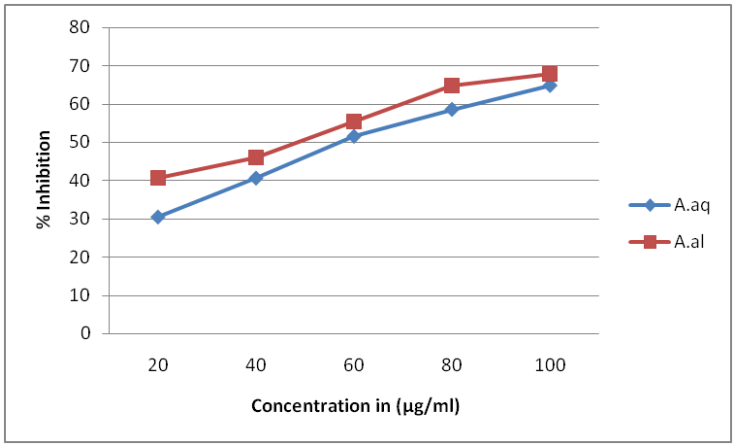

Figure No 1

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DPPH radical scavenging activity

Table No 2: Effect of aqueous (A.aq) and alcoholic (A.al) extract of Annona squamosa Linn on DPPH

Sl.N0	Concentration (µg/ml)	% Inhibition	
		A.aq	A.al
1	20	30.46	40.62
2	40	40.62	46.09
3	60	51.56	55.47
4	80	58.59	64.84
5	100	64.8	67.97

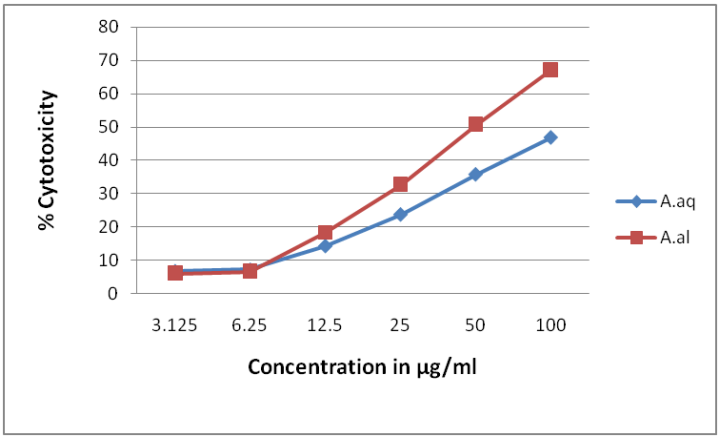

Figure 2

The results of this study, clearly indicate that aqueous and alcoholic extracts of *Annona squamosa* Linn *aerial part* had significant scavenging effect on the DPPH free radical which increased with increasing concentration from 20-100 µg/ml.The percentage inhibition activity of aqueous and alcoholic extracts was 64.8, 67.97 at 100 µg/ml concentration respectively. The scavenging effect of sample was lower than that of Ascorbic acid. The extracts possess statistically significance DPPH free radical scavenging activity.

CONCLUSION

In vitro cytotoxic activity against HT29 cell line at different concentrations were evaluated. Cytotoxic effect *against* HT29 colon cancer cell line is considered as a predictive anticancer activity indicator and IC50 value calculated for *both* extracts was below 50 µg/ml, which indicates that aqueous and alcoholic extracts of Annona squamosa Linn. potentially present an interesting cytotoxic activity and should be evaluated against primary cultures to determine the selectivity of their effects. We therefore, suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *invivo* and cytotoxic activity of these extracts to obtain useful chemotherapeutic agent. The antioxidant effect of these extracts may also contribute for the Cytotoxic activity.

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