Original Article

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CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACTS OF *CAESALPINIA SAPPAN LINN* AND *ANAONA SQUAMOSA LINN.* IN A-549 CELL LINE

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Abstract

The present study was carried out to evaluate the in vitro cytotoxic activity of unexploited plants, heartwood of *Caesalpinia sappan* Linn and roots of *Annona squamosa* Linn. on A-549 lung cancer cell line, which are indigenous to India. Different concentrations of the methanolic extracts of heartwood and root parts of the plant (1000, 500, 250, 125, 50, 25, 12.5 µg/ml) were subjected to cytotoxic study against A-549 lung cancer cell lines by Trypan blue dye exclusion technique. In addition, a phytochemical screening of the ethanolic extracts was done. The phytochemical screening demonstrated the presence of different types of compounds like flavonoids, triterpenoids, alkaloids, acetogenins, phenols and sterols. The maximum reducing power of the *Caesalpinia sappan* and *Annona squamosa* extract at 680nm was found to be 0.976 ± 0.051 at 1000 µg/ml and 0.953 ± 0.037 at 1000 µg/ml respectively. The inhibition percentage with regard to cytotoxicity was found to be 87 % at 1000 µg/ml with IC_{50} value of 49 ± 0.03 µg/ml for *Caesalpinia sappan* and 85 % at 1000 µg/ml with IC_{50} value of 47 ± 0.02 µg/ml for *Annona squamosa* respectively. The ethanolic extracts of *Caesalpinia sappan* and *Annona squamosa* are showing potent cytotoxic activity against A-459 lung cancer cell line.

Key words: *Caesalpinia sappan* Linn., *Annona squamos* Linn., Ethanolic extracts.

Introduction

Caesalpina sappan Linn (Caesalpiniceae) is commonly known as "Sappan wood" or patang (Hindi). It is spreading tree or shrub upto 10 m in height found in India (West Bengal, Orissa, Kerala) Malaya, China and Sri Lanka.[1]. The seeds contain n-triactone, lupeol, β-amyrin, stigmosterol and diterpenoidal alcoholic compounds[2]. The chloroform extract of C. *sappan* on cell death in head and neck cancer cell lines. The results suggest that the chloroform extract of C. *sappan* may increased cell death in HNSCC4 and HNSCC31 cells, which are liked to increased cellular levels of p53 and p21WAF/CIPI[3].

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The methanol extract and two purified compounds, brazilin and hematoxylin, isolated from the wood showed a significant and dose dependant vasorelaxing effect[4]. Several triterpenoids, flavanoids, and steroids have been isolated from the heartwood of *Caesalpinia sappan*[5]. *Annona squamosa* Linn (Annonaceae) is commonly called custard apple in English and Sharifa in hindi[6]. 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. From the leaves of *Annona squamosa*, a tetrahydroisoquinoline alkaloid with cardio tonic activity[10] and a bioactive acetogenins like bulatacin and bullatacinone from its bark have been isolated[11]. Squamocin, another Annonaceous has been reported to exert antiproliferative effect on HL-60 cancer cells via

activation of caspase-3[12]. In the Ayurvedic system of medicine, herbal extracts but not purified compounds have been used from centuries because many constituents with more than one mechanism of action are considered to be beneficial. The present study aimed to evaluate the possible cytotoxic activity of the heartwood of *Caesalpinia sappan* and roots of *Annona squamosa* against lung cancer cell line.

Material and Methods

Plant Material

Heartwood of *Caesalpinia sappan* Linn and roots of *Annona squamosa* Linn were collected from Medicinal garden of SDM College of Ayurveda, Udupi and authenticated by Dr. T. Shridhar Bairy by comparison with the standard specimens deposited at the department of Drava Guna. SDM college of ayurveda. Udupi. Voucher specimens are kept at the NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore, Karnataka. India. The powdered shade dried plant materials were exhaustively extracted with ethanol using soxhlet apparatus. The extract was concentrated to dryness. A phytochemical screening of the ethanolic extracts was performed. Further more; the dried ethanolic extract was used for evaluation of cytotoxicity activities.

Preliminary Phytochemical Screening

Conventional standard protocols[13, 14] for detecting the presence of different chemical constituents in the plant extracts were employed. The tests for the secondary metabolites viz. alkaloids, tannins, sterols, saponins, amino acids, glycosides, proteins, sterols/terpenes, reducing sugars, non-reducing sugars, resins flavonoids and phenols were carried out with the ethanolic extracts of heartwood of *Caesalpinia sappan* and roots of *Annona squamosa* using preliminary phytochemical screening.

Assay of Cytotoxic Activity

The A-549 cell lines (lung carcinoma cells) used for the assay were obtained from Christian Medical College, Vellore. Tamil nadu. India. The stock cells were cultured in DMEM with 10% Fetal Bovine Serum (FBS), Penicillin (100 IU/ml) Streptomycin (100 µg/ml) and Amphotericin-B (5 µg/ml) in a humidified atmosphere of 5 % CO_2 at 37°C. The cells were dissociated with 0.2 % trypsin in phosphate buffer saline solution. The stock cultures were grown in 25cm^2 tissue culture flasks and all cytotoxicity experiments were carried out in 6 well plates.

Viability Staining by Trypan blue dye exclusion method

Cytotoxic activity of ethanolic extracts of *Caesalpinia sappan* and Annaona *squamosa* were analysed by Trypan Blue dye exclusion method[15]. Cell lines in exponential growth phase were washed with phosphate buffer saline (PBS) solution and trypsinized and re-suspended in complete culture media. Cells were plated at 30,000 cells/well in 6 well plates and incubated for 24 hours during which a partial monolayer forms. After incubation the cells were exposed to various concentrations of the drugs, which is the plant extract (1000µg/ml, 500µg/ml, 250µg/ml, 150µg/ml, 125µg/ml, 50µg/ml and 25µg/ml). The control well received only maintenance of medium. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 24 hours. Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control. At the end of 24 hours incubation, cell viability was determined.

Calculations and statistics

Experiments were performed in six replicates. Results were expressed as percentage growth inhibition of control. IC_{50} values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using Graphpad Prism version 3.00 were expressed as mean±S.E.M.

Table 01: Cytotoxic Activity of ethanolic extract of Caesalpinia sappan and Annona squamosa against A-549.

Concentration (µg/ml)	Cytotoxic activity (%)		IC_{50} (µg/ml)	
	C. Sappan	*A. squamosa*	*C. Sappan*	*A. squamosa*
1000	87	86		
500	71	67		
250	62	55		
125	58	52	49±0.03	47±0.02
50	56	47		
25	50	46		
12.5	40	36		

Each value represents mean ± S.E.M. of six replicates (n=6).

Results and Discussion

The results of the phytochemical screening of the investigated methanolic extracts showed the presence of different types of active constituents. Ethanolic extract of heartwood of C. *sappan* showed presence of flavonoids, triterpenes, polyphenols and sterols, ethanolic extract of roots of *A. squamosa* showed presence of alkaloids, acetogenins, and absence of flavonoids and terpenoids. The maximum reducing power of the C. *sappan* and *A. squamosa* extract at 680nm was found to be 0.9760±051 at 1000 µg/ml and 0.9530±037 at 1000 µg/ml respectively (Fig. 1a, Fig. 1b & Fig. 1c). The inhibition percentage with regard to cytotoxicity was found to be 87 % at 1000 µg/ml with IC_{50} value of 49±0.03 µg/ml for C. *sappan* (Table-1) and 85 % at 1000 µg/ml with IC_{50} value of 47±0.02µg/ml for *A.squamosa* (Table-01) respectively. The *in-vitro* screening of the ethanolic extracts of C. *sappan* and *A. squamosa* showed potential cytotoxic activity against the breast cancer cells. The results obtained are shown in table no-1. The results obtained from the present study showed that the C. *sappan* and *A. squamosa* are moderately cytotoxic activity. The cytotoxic activity may be due to the presence of flavonoids, alkaloids, acetogenins, sterols, polyphenols and terpenoids present in the heartwood of C. *sappan* and roots of *A. squamosa* respectively.

Conclusion

The results of the study revealed that the plant extracts have strong anticancer activity. Our phytochemical screening revealed the presence of terpenoid, flavonoids, alkaloids and acetogenins in the ethanolic extracts of C. *sappan* and *A. squamosa* respectively, which could be responsible for these noteworthy activities. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent against cancer and we therefore, suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *in-vivo* cytotoxic activity of the plants with a view to obtaining useful chemotherapeutic agent. In nutshell, extracts of C. *sappan* and *A. squamosa* have remarkable anticancer potentials against A-549 Lung cancer cell lines. Drug prepared from extracts of C.*sappan* and *A. squamosa* could be an excellent drug for treating lung cancer.

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