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Research Article

CYTOTOXICITY STUDY OF *FICUS RELIGIOSA* LEAF EXTRACT AGAINST JURKAT CELL LINE

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ABSTRACT: Different parts of *Ficus religiosa* tree have traditional usage against many ailments and diseases. In the present study, 85% ethanol extract of *F. religiosa* leaves were partially purified by PLC Silica gel 60 F_{254}, 2 mm, 20X20 cm preparative thin layer chromatography using toluene and ethyl acetate (8:2, v/v) as solvent. Fraction 7 (FR7) showed highest brine shrimp larvicidal activity as regard to the leaf extract of *F. religiosa*. *In vitro* anticancer potential of same fraction against Jurkat cell line was investigated by means of flow cytometry using annexin V/7AAD apoptosis kit. *F. religiosa* leaf extract was found to markedly inhibit the proliferation of Jurkat cells in a dose-dependent manner.

Key words: *Ficus religiosa*, Cytotoxicity, Apoptosis, Jurkat cell line.

INTRODUCTION

Plant-derived polyphenolic compounds such as flavonoids, tannins, curcumin and the stilbene resveratrol are recognized as naturally occurring antioxidants and have been implicated as anticancer compounds (Mukhtar *et al*. 1988). In recent years, several reports have documented that plant polyphenolics, including curcumin, resveratrol and gallo-catechins such as gallic acid, epigallocatechin, epicatechin-3-gallate, and epigallocatechin- 3-gallate (EGCG), induce apoptosis in various cancer cell lines. Most of the current anticancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity. Several plant products have been tested for antitumor activity and some of these, such as vincristine (from Madagascar periwinkle, *Catharanthus roseus*) and taxol (from the Pacific yew tree, *Taxus brevifolia*) are now available as drugs of choice (Kviecinski *et al*. 2008).

Ficus religiosa or peepal tree or Sacred Fig or *Asvatha* is a large deciduous tree with few or no aerial roots. The tree is distributed throughout India. Recently Hyo *et al*. (2008) reported antioxidant property of methanolic leaf extract of *F. religiosa*. Patil *et al*. (2010) reported promising immunostimulant properties of ethanolic extract of leaves of *Ficus carica* (Fig tree) in mice. There are meager studies on anti-cancer activity of the tree parts. Lately, Uddin *et al*. (2011) has reported cytotoxic activity of *F. religiosa* leaves.

The present study aimed at investigating the cytotoxicity property of aqueous leaf extract of *Ficus religiosa* against Jurkat cell line (human T-cell leukemia) *in vitro*.

MATERIALS AND METHODS

Plant samples (*Ficus religiosa L*, Family: Moraceae) were collected in and around Bhubaneswar during winter season (November-December). The plants were authenticated by the botanists, OUAT, Bhubaneswar. After collection, leaves were cleaned, shade-dried and

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then powdered. A voucher specimen (Accession number 20104) representing this collection has been deposited in the department for further reference.

Preparation of plant sample

Five grams of the powder was extracted with 100 ml solvent by keeping on a rotary shaker overnight. An 85% ethanol solution was used as solvent of extraction. Extracts were filtered through Whatman No. 1 filter paper and filtrates were dried using a rotary evaporator at 40°C.

Purification of extract by HPTLC

HPTLC - UV analysis was performed on a computerized densitometer scanner 3, controlled by *winCATS* planar chromatography manager *version 1.4.2.* (CAMAG, Switzerland), having the facility of multiwavelength Scanning. The precoated TLC plate of silica gel 60 F_{254} of dimension 20 x10 cm and 0.2 mm thickness (EMerck, Germany) was used for fingerprinting study. Toluene and ethyl acetate were used as mobile phase (developer) in the ratio of 8:2. Chromatography procedure described by Kowalska *et al*. (2002) was followed.

For isolation of different fractions from the extracts, preparative thin layer plates (PLC Silica gel 60 F_{254}, 2 mm, 20 x 20 cm, EMerck, Germany) were used. Samples were applied across the entire width of the plate and different bands were scraped from the plate after UV detection. For elution of compounds, 85% ethanol was mixed with the scrapings separately and filtered with Whatman filter paper. After evaporation of ethanol, the fractions were lyophilized and the residue was weighed.

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to screen the cytotoxic activity of the fractions collected after preparative thin-layer chromatography (Meyer *et al*. 1982). For the experiment, lyophilized fractions were dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 µg/ml using simulated seawater. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 ml simulated seawater. After 24 h, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted. The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 s of observation. Vincristine sulphate was used as positive control.

Apoptosis study

The assay methodology followed is based on utilizing annexin V to detect the cell surface exposure of phosphatidylserine (PS) that occurs during apoptosis (Koopman *et al*. 1994, Vermes *et al*. 1995).

Jurkat cells were collected from ATCC (American Type Culture Collection). Cells were cultured in IMDM (Iscove's Modified Dulbecco's Media) with 10% FBS in presence of antibiotics, L-Glutamine and were maintained in a CO_2 incubator at 37°C with 5% CO_2. Cells were plated in a 24-well culture (Nunc) plates at 0.5 X 10^6 cells per ml. Fractions having the highest brine shrimp larvicidal activity were dissolved in water to obtain a concentration of 100 µ/200 µ. The aqueous samples were diluted in media and were added at indicated different doses (0.01, 0.1, 1, 10 and 100 µ/ml). The cells were incubated for 24 hours at 37°C in presence of 5% CO_2. Equivalent amount of media alone was added to the control wells. The efficacy of the samples to induce cell death was determined by annexin V/7AAD apoptosis kit (BD Biosciences). The samples were subjected to flow analysis, data were acquired using FACS Calibur (BD Biosciences) and were analyzed using CellQuestPro software (BD Biosciences).

RESULTS AND DISCUSSION

HPTLC, like HPLC and GC, is suitable for development of chromatographic fingerprint to determine and identify complex herbal extracts (Chen *et al.* 2006). Extracts of *F. religiosa* leaves has given eight (Rf value 0.14, 0.17, 0.22, 0.26, 0.38, 0.47, 0.56 and 0.72) visible bands after derivatization with p-anisaldehyde. Same numbers of peaks were also obtained after scanning with UV light at 254 nm (Fig. 1). Recent study by Rathee *et al*. (2015) has validated TLC densitometric quantification of the two marker compounds (stigmasterol and lupeol having Rf value 0.37 and 0.60 respectively) from methanolic extract of *F. religiosa* fruits using the solvent system of toluene: methanol (9:1, v/v). But, this could not be compared with the present study where

Table 1. Brine shrimp lethality bioassay of different purified fractions of *F. religiosa* leaf extracts (10 µg/ml concentration) with vincristine sulphate as positive control.

F. religiosa leaf extract		Vincristine sulphate	
Fractions	**% mortality**	**Concentration**	**% mortality**
FR1	43.19 ± 1.18	40 µg/ml	100
FR2	30.52 ± 2.27	20 µg/ml	100
FR3	37.95 ± 1.64	10 µg/ml	100
FR4	42.03 ± 2.08	5 µg/ml	93 ± 4.03
FR5	36.97 ± 1.88	2.5 µg/ml	75 ± 3.77
FR6	29.81 ± 2.02	1.25 µg/ml	62 ± 3.71
FR7	100	0.63 µg/ml	49 ± 2.08
FR8	35.47 ± 2.47	0.31 µg/ml	41 ± 2.83

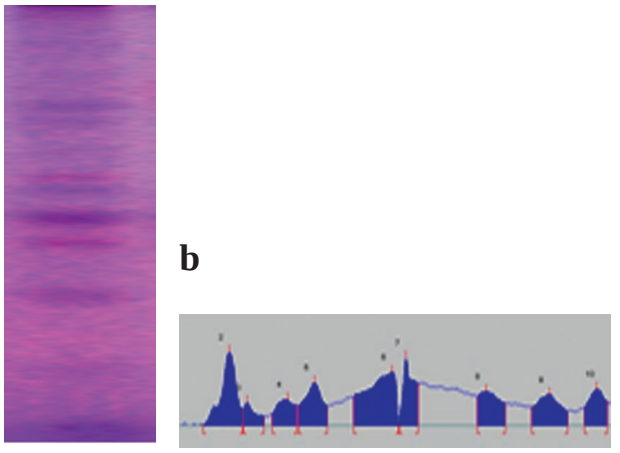

Fig. 1. HPTLC analysis of *Ficus religiosa* leaf extract showing characteristic bands after derivatization with p-anisaldehyde (a) and peaks after scanning at 254 nm (b).

toluene: ethyl acetate (8:2) was used as mobile phase for HPTLC fingerprinting of 85% ethanolic extract of *F. religiosa* leaves.

Out of all the fractions tested for cytotoxicity, fraction 7 (FR7, Rf value 0.56) showed highest brine shrimp larvicidal activity as regard to the leaf extract of *F. religiosa* (Table 1). Therefore, this particular fraction having the highest cytotoxic activity was selected in the next step for further investigation.

The results of flow cytometry assay using Jurkat cell line showed that FR7 of *F. religiosa* extract has apoptotic activity against cancer cells. At lower doses, it did not induce any cell death, but when used at higher doses, Jurkat cells had undergone apoptosis, as it showed that a significant number of cells have become positive both for annexin V and 7AAD. At a dose of 200 ul/ml (*i.e.*100 µ/ml), all cells died and this suggests that at higher dose, fraction 7 of *F. religiosa* extract was able to induce apoptosis in Jurkat cells (Fig. 2).

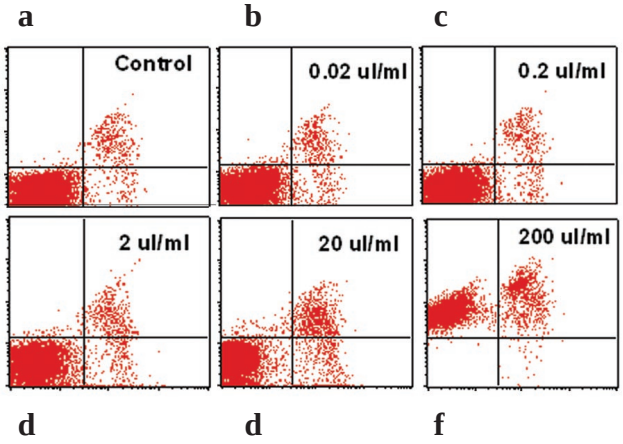

Fig. 2. Cell viability and apoptosis study on Jurkat cells after treatment with fraction 7 of *F. religiosa* leaf extract in different doses.
(a. control; b. 0.01 µg/ml; c. 0.1 µg/ml; d. 1 µg/ml; e. 10 µg/ml and f. 100 µg/ml).

Leaves of *F. religiosa* contain lupeol which is reported to have anticancer activity (Chaturvedi *et al.* 2008; Nigam *et al.* 2009). Uddin *et al.* (2011) reported cytotoxic activity (50% inhibition of cell growth, mg/ml) of *F. religiosa* leaves against normal mouse fibroblasts (NIH3T3), gastric cancer (AGS), colon cancer (HT29) and breast cancer (MDA-MB- 435S) cells to be 1.01, 2.16, >2.50, >2.50 for methanolic extracts; whereas >2.50, no cytotoxicity, >2.50, >2.50 for aqueous extracts respectively. The present study supported the above observation. However, the cytotoxicity of *F. religiosa* leaf extract is less, as compared to that of Vincristine.

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