

## Studies on *in vitro* cytotoxicity of *Ficus hispida* leaves

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*Ficus hispida* Linn. is explored for its *in vitro* cytotoxicity against oral (KB) and colon (COLO 205) human cancer cell lines. Three extracts (alcoholic, hydro-alcoholic and aqueous) and four fractions (n-hexane, chloroform, n-butanol and aqueous) from the leaves were prepared and were evaluated using sulforhodamine B assay at 10, 30 and 100 µg/ml. The growth inhibition demonstrated by all extracts and fractions were in dose dependent manner. The alcoholic extract was most active followed by aqueous and 50% aqueous-alcoholic extract. Further on the evaluation of fractions, n-butanol fraction was highly significant among the four fractions of alcoholic extract against both oral and colon cancer cell lines.

Key words : *Ficus hispida*, Cytotoxicity, SRB assay, KB human cancer cell lines

### INTRODUCTION

Plants have been used as folkloric sources of medicinal agents since the beginning of mankind. As the age of modern medicine, single pure drugs emerged, and plant-derived active principles, their semi-synthetic and synthetic analogs have served as a major route to new pharmaceuticals. It is already estimated that 122 drugs from 94 plant species have been discovered through ethnobotanical leads (Fabricant and Farnsworth, 2001). Plants commonly used in traditional medicine are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries.

*Ficus hispida* Linn. (Family-Moraceae) is well known medicinal plant, growing in damp and shady areas and found throughout India. Almost all parts of the plant are used in Indian folklore medicine for the treatment of various ailments like leucoderma, skin diseases, jaundice and as anti-poisonous. The methanolic extract of leaves had showed anti-diarrhoeal (Mandal *et al.*, 2002), anti-inflammatory (Vishnoi and Jha, 2004) activity. The plant is reported for its hepatoprotective activity in rats (Mandal *et al.*, 2000) and antioxidant potential against cyclophosphamide induced abnormalities in rat liver (Shanmugarajan and Devki, 2008). This paper describes cytotoxic screening results of the leaves from *F. hispida* against oral human cancer cell lines.

### MATERIALS AND METHODS

#### **Plant collection:**

Leaves of *Ficus hispida*. Linn was collected from the campus of Indian Institute Intergrative medicine (IIIM) in the month of December and was authenticated at source by taxonomist of the institute. A voucher specimen has been deposited at the herbarium of the Institute vide IIIM collection No.50301, Acc. No.20049.

#### **Preparation of plant extracts and fractions:**

The authenticated and freshly collected leaves were chopped and dried under shade. Three extracts of the plant material were made with 95% alcohol, alcohol-water (1:1) and water using repeated solvent extraction procedure. For the alcoholic extract, dried powdered plant material was extracted in Soxhlet extractor with 95% alcohol and concentrated to dryness under reduced pressure. Hydro-alcoholic extract was prepared by percolating another lot of dried plant material with hydro-alcohol (1:1) and then concentrating it to dryness. For the preparation of aqueous extract, the dried powdered plant material was heated with distilled water on steam bath for 2 hours, the supernatant was decanted and filtered through celite powder and pooled extract was concentrated on rotavapour and dried by lyophilizer. The alcoholic extract was fractionated sequentially with n-hexane, chloroform, n-butanol and water. The dried alcoholic extract was macerated with n-hexane. The combined solvent portion was evaporated under reduced pressure to yield hexane fraction. The residue was further macerated with chloroform and combined organic layer

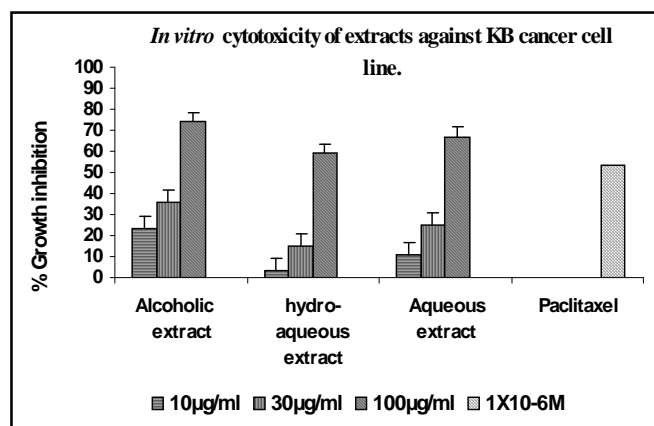
was evaporated under reduced pressure to yield chloroform fraction. The residue obtained was dissolved in distilled water and partitioned between n-butanol and water. The process was repeated four times, the organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield n-butanol fraction. The aqueous part was concentrated under reduced pressure to give aqueous fraction.

#### *In vitro* cytotoxic assay:

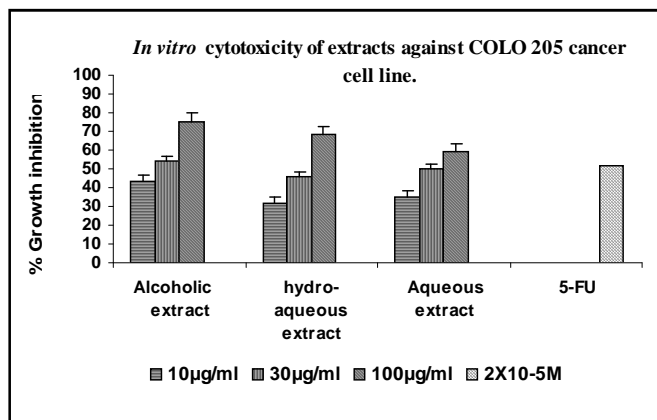
The human cancer cell lines were obtained from National Center for Cell Science, Pune, India. The media were supplemented with FCS (10%), penicillin (100 units/ml), streptomycin (100 µg/ml) and glutamine (2mM). The cells were grown in CO<sub>2</sub> incubator (Hera Cell: Heraeus; Germany) at 37°C with 90% relative humidity and 5% CO<sub>2</sub>. The *in vitro* cytotoxicity of extracts and fractions was determined using sulforhodamine-B (SRB) as described previously (Bhahwal *et al.*, 2007). In brief, the stock solution (20 mg/ml) of the alcoholic, hydro-alcoholic and aqueous extracts was prepared in dimethylsulfoxide (DMSO), dimethylsulfoxide – water (1:1) and hot water, respectively and were further diluted with growth medium (RPMI-1640/ DMEM with 2mM glutamine, pH 7.4, 10% fetal calf serum, 100 µg/ml streptomycin, and 100 U/ ml penicillin) to obtain desired concentrations. The stock solution of hexane, chloroform and butanol fractions was prepared in dimethylsulfoxide where as aqueous fraction was dissolved in distilled water. The cells were grown in tissue culture flasks and incubated at 37°C. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in the growth medium. Cells with more than 97% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cell suspension (10<sup>5</sup> to 2 x10<sup>5</sup> cells/ml depending upon mass doubling time of cells) was transferred culture plate and incubated for 24 h. The test materials (100 µl) were then added to the wells and cells were further allowed to grow for another 48h. The cell growth was stopped by layering 50µl of 50% trichloroacetic acid, incubated at 4°C for an hour, then plates were washed and air-dried. Sulforhadamine B (100 µl, 0.4% in 1% acetic acid) was added to plates and incubated at room temperature for 30 min. The unbound SRB dye was quickly removed by washing with 1% acetic acid and plates were air dried. Further tris-HCL buffer (100 µl, 0.01 M, pH 10.4) was added to the plates and optical density was recorded on ELISA reader at 540 nm. Suitable blanks and positive controls were also included. Each test was done in triplicate.

## RESULTS AND DISCUSSION

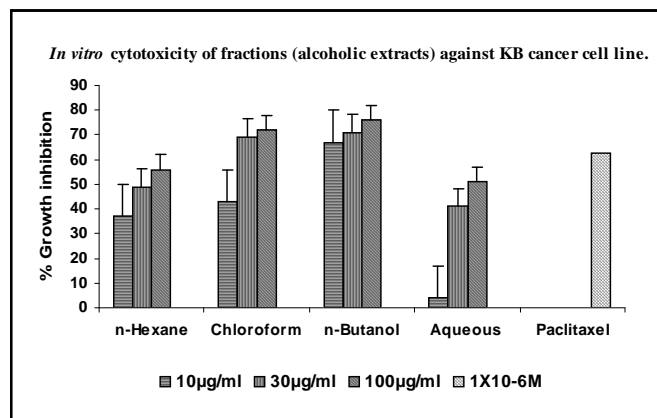
The search for new biologically active compounds from natural sources has always been of great interest (Taylor *et al.*, 1996). Bioassays offer special advantages for identification of medicinal plant extracts. Most often, a desired biological response is not due to one component but rather to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity and then any “active” extract should be fractionated directed with bioassays to exploit the bioactive compounds (Jerry and Lingling, 1998). In the present investigation the *in vitro* cytotoxic effect of *Ficus hispida* leaves extracts and fractions was evaluated against human cancer cell lines. Among all the three extracts prepared (alcoholic, hydro-alcoholic and aqueous) the alcoholic extract had showed significant per cent growth inhibition against both oral and colon cancer cell lines in dose dependent manner at 10, 30 and 100 µg/ml (Fig.1 and Fig.2). Activity profile of the extracts (decreasing order) was alcoholic extract > aqueous extract > hydro-alcoholic extract for oral (KB) cancer cell line (Fig.1) and alcoholic extract > hydro-alcoholic extract > aqueous extract for colon (COLO 205) cancer cell line (Fig. 2). Further four fractions (n-hexane, chloroform, n-butanol and aqueous) from the active alcoholic extract were prepared and evaluated. The result demonstrated that n-butanol fraction was most cytotoxic against both the cancer cell lines (Fig.3 and Fig.4). The activity profile for the fraction was in following order (decreasing) n-butanol > chloroform > n-hexane > aqueous for oral cancer cell line (Fig.3) and for colon cancer cell line it was n-butanol > aqueous > chloroform > n-hexane (Fig. 4). The result indicates that the plant *F. hispida* possess significant cytotoxicity against both oral



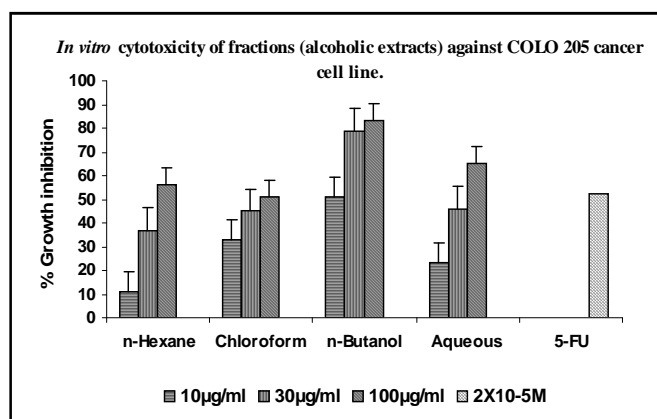
**Fig. 1 :** *In vitro* cytotoxicity of the extracts of *Ficus hispida* (leaves) against oral (KB) human cancer cell lines



**Fig. 2 :** *In vitro* cytotoxicity of the extracts from *Ficus hispida* (leaves) against colon (COLO 205) human cancer cell lines



**Fig. 3 :** *In vitro* cytotoxicity of the fractions (alcoholic extract) from *Ficus hispida* (leaves) against oral (KB) human cancer cell lines



**Fig. 4 :** *In vitro* cytotoxicity of the fractions (alcoholic extract) from *Ficus hispida* (leaves) against colon (COLO 205) human cancer cell lines

and colon human cancer cell lines and the active chemical constituents may be present in the alcoholic extract and in its n-butanol fraction and are in non-polar in nature. Further studies are required to isolate active compounds responsible for the activity.

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