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Cytotoxic and Anticancer Activity of *F. Racemosa* Fruit Extract on MCF7 Human Breast Cancer Cell Line by SRB Method

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ABSTRACT

Present study was aimed to investigate the *in vitro* cytotoxicity and anticancer activity of *F. racemosa* on MCF7 human breast cancer cell line. Effect of ethanolic extracts of tender fruits of *F. racemosa* on MCF7 human breast cancer cell lines by Sulphorodamine B (SRB) assay was carried out. Three observations *viz.* LC50, TGI, GI 50 were recorded. The absorbance was recorded on an Elisa plate reader at a wavelength of 540 nm with 690 nm. *F. racemosa* showed LC50, TGI and GI50 activity at ≥ 80 µg/ ml concentration. Thus, it can be concluded that *F. racemosa* fruit extract hassome cytotoxic and anticancer activity (*in vitro*)at ≥ 80 µg/ ml concentration of plant extract on MCF7 human breast cancer cell line.

Keywords: *F. racemosa*, anticancer, cytotoxic effect, MCF7, Sulphorodamine B method

Tumors of mammary glands are one of the most dreadful diseases in both human as well as canines. A canine mammary tumor (CMT) is the second most common tumor in bitches. One third to half of surgically removed tumors are considered as malignant (Misdorp, 2002). In humans, breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 i.e. 25% of all cancers (Levaggi *et al.* 2014). It is estimated that worldwide over 5,22,000 women died in 2012 due to breast cancer. As per GLOBOCAN 2012, in India, in the year 2012, 1,45,000 cases were diagnosed as breast cancer and among them 70,000 have been died (IARC, 2012). Thus, this is a matter of concern for both human as well as veterinary clinicians.

Most anticancer drugs used today have various side effects. Therefore, current interest has been diverted towards investigation into plants to identify the active principles that can have medicinal value. These herbal or naturally occurring chemicals with low side effects are occupying many synthetic chemicals. More than quarter of medicines in use today, comes from plants. Many medicinal plants contains the variable amounts of different phytochemicals such as saponins, triterpenoids, anthracyanins, alkaloids, phenols, flavanoids, resins, fatty acids and tannins. Many of these phytochemical agents have been found to possessanticancer activity. One of such plants is *Ficusracemosa*. It is known for various medicinal properties such as hepatoprotective, antioxidant, radioprotective, hypoglycemic, antidiuretic and antimitotic effects (Mandal, 1999; Veerapur *et al.* 2009; Ahmed, 2010; Joseph, 2010; Patil, 2012; Shivasharanappa and Londonkar, 2014).

Some of *in vitro* studies have revealed anticancer activity of *F. racemosa* on some cancer cell lines. These studies have indicated this plant as possible anticancer agent. Rana *et al.* (2004) observed 76% growth inhibition of Ehrlich ascites carcinoma cells when treated with *F. racemosa* root extract. Anti permeability effects of *F. racemosa* have been showed by Sarpate *et al.* (2009). Bark extracts of *F. racemosa* have also been found to have anticancer and cytotoxic activity against Lung anaplastic carcinoma Calu 6 cell line (Kambli *et al.* 2014). A study by Shivasharappa and Londankar, (2014) indicated that this plant also has some antimitotic effects. These previous studies have indicated that this plant could be a possible anticancer agent.

Therefore, in present investigation we attempted to study anticancer and cytotoxicity activity of hydroalcoholic extract from tender fruits of *F. racemosa* on human breast cancer cell line MCF7, the most commonly used cell line for breast cancer, by SRB assay.

MATERIALS AND METHODS

Procurement and processing of plant material

Before harvesting fruits, the plant was identified and authenticated by independent botanist at Department of Botany, St. Xaviors College, Mumbai. Tender fruits of *F. racemosa* plants were collected from forest area near Roha, Panvel, Maharashtra. After collection, fruits were washed thrice with clean water, mobbed with clean cloth. Fruits were then chopped in 4 pieces and shed dried. Dried plant material was then grinded to fine powder and stored till use.

Preparation of hydro-alcoholic extract

The extraction procedure was carried out at Department of Pharmacognosy, Institute of Chemical Technology, Mumbai. The mixture of plant powder and 70% ethanol at 1:5 proportions was made. The mixture was then added to the bulk extractor and extraction was made at 40-50^{o}C for 4 hours. The mixture was then filtered through thick cotton filter bag. The filtrate was then evaporated on water bath till filtrate acquires semisolid consistency. The final concentrated crude extract was then stored in clean glass bottle at refrigerator till use.

Cytotoxic and anticancer activity

Cytotoxic and anticancer activity of ethanolic extract of *F. racemosa* fruit was tested on MCF7 human breast cancer cell line. The SRB assay was carried out using facilities available at the Department of Anticancer Screening, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Mumbai. Three tests viz. LC50, GI50 and TGI were performed by SRB assay (Skehn *et al.* 1990; Shaban *et al.* 2012; Rajini *et al.* 2013). Each test was done in triplicate and graphs were then plotted using observed values. The drug adriamycin was taken as positive control.

Sulphorodamine B (SRB) assay protocol

RPMI 1640 growth medium containing fetal bovine serum (10%) and L-glutamine (2 mM) was selected for MCF7 human breast cancer cell line. The MCF7 cells (0.5- 1.0 $\times$ 10^5 cells/ml) were inoculated in 96 welled micro-titer plates. To Each well 90 µl of diluted cell suspension was then added. Prior to drug treatment inoculated micro-titer plate was then kept in incubator with 37^{0}C temperature, 5% CO_2 concentration, 95% air and 100% relative humidity. This incubation was done for 24 hours. After completion of 24 hours of incubation, when the partial monolayer was formed the supernatant was washed. Test compounds were solubilized in 70% ethanol and this aliquot wasthen frozen prior to use. At the time of addition, the test compound was thawed, and added (10 µl) to respective well containing 90 µl medium at 10, 20, 40 and 80 µg/ml of final concentrations. After the test drugs were added, micro-titer plates were incubated for 48 hours. The assay was then terminated by addition of cold TCA to wells. After completion of 72 hours, 50% cold TCA was added in wells over the test compound gently to in such a way to form overall concentration of TCA which becomes 10% and then the plate was incubated for an hour at 4^{o}C.

The supernatant was flicked off; the wells were washed 5 times with water to remove any traces of medium and air dried. SRB solution (0.4% (w/v) in 1% Acetic acid) was added to each of the wells (50-100 µl), and plates were incubated for 30 minutes at room temperature. After incubation, plates were washed four times with 1% Acetic acid to remove unbound dye. The plates were then air dried. To solubilize the bound dye, 10 mM tris base was added, the absorbance was read on an Elisa plate reader at a wavelength of 540 nm.

RESULTS AND DISCUSSION

In the present investigation the cytotoxicity and anticancer activity (*in vitro*) of hydroalcoholic extract of *F. racemosa* was carried out on MCF7 human breast cancer cell line by SRB assay. After completion of protocol the absorbance was read on an Elisa plate reader at a wavelength of 540 nm. Photography of cell cultures were taken (Fig. 1-3) and values were plotted on graph and LC50, TGI and GI50 were then calculated from the graph (Fig. 4). Along with adrinamycin and plant extract treated cells also showed kariolysis, apoptosis, roundening of cell (Fig. 2, 3). GI50

Table 1: Drug concentrations (µg/ml) and percentage of growth inhibition

	MCF7 Human Breast Cancer Cell Line															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
F. Racemosa	93.7	89.5	97.3	71.7	104.3	95.1	98.6	80.5	86.1	95.9	94.3	73.2	94.7	93.5	96.7	75.1
Adriamycin	-42.7	-57.2	-63.7	-74.6	-64.9	-65.8	-77.3	-78.4	-53.4	-49.4	-65.8	-80.4	-53.7	-57.5	-68.9	-77.8

means the drug concentration resulting in a 50% reduction in the net protein increase as compared to control cells. TGI is the drug concentration resulting in total growth inhibition. The LC50 is the drug concentration resulting in a 50% reduction in the measured protein at the end as compared to the beginning. *F. racemosa* showed LC50, TGI and GI50 activity at ≥ 80 µg/ ml concentration of plant extract on MCF7 cell line (Table 1 and 2).

Table 2: Drug concentrations (µg/ ml) required for LC50, TGI, and GI50 calculated from graph

MCF7	LC50	TGI	GI50
F. racemosa	>80	>80	>80
Adriamycin	<10	<10	<10

Results from present investigation indicate that *F. racemosa* has cytotoxicity as well as anticancer activity (*in vitro*) on MCF7 human breast cancer cell line. The effective concentration of crude extract was observed to be ≥ 80 µg/ ml of crude extract. This means that one or few phytochemical constituents of this crude plant extracts had possible anticancer activity. Our results are in concordance with some of the previous studies on this plant. Rana *et al*. (2009) had observed 76% growth inhibition of Ehrlich ascites carcinoma cells, when treated with *F. racemosa* root extract. Sarpate *et al.* (2009) stated that *F. racemosa* has anti permeability effects. Bark extracts of *F. racemosa* have also been found to have anticancer and cytotoxic activity against Lung anaplastic carcinoma Calu6 cell line (Kambli *et al.* 2014). A study by Shivasharappa and Londankar, (2014) indicated that this plant also has antimitotic effects. Another study by Manian *et al.* (2008) displayed strong antioxidant and free radical scavenger activity of *F. racemosa*. *F. racemosa* has been reported to be radioprotective againts radiation induced DNA damage (Barangi *et al.* 2012; Vinutha *et al.* 2015). These previous studies indicate that this plant has some phytochemicals which can have possible anticancer activity, either singly or in combination. The present results, together with previous studies, suggest that *F. racemosa* extract possess anti-oxidant, radio protective, anti-mitotic and anti-permiability activity. Mutation in genes is one of the major causes of cancer and these mutations can be due to radiation, oxidative stress, carcinogen or it may be hereditary. Therefore, overall observations from present and previous studies supports theory of anti-cancer activity of *F. racemosa*.

Legends of images

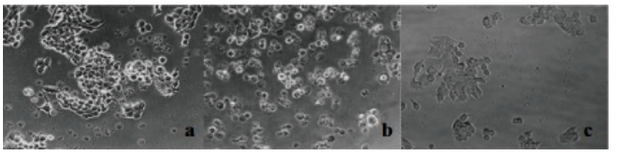

Fig. 1: Untreated MCF7 cell line with normal cellular morphology (a), Effect of Adriamycin on MCF7 cell line showing roundening of cells and apoptosis (b), Effect of ethanolic extract of *F. racemosa* on MCF7 cell line showing roundening of cells and apoptosis (c).

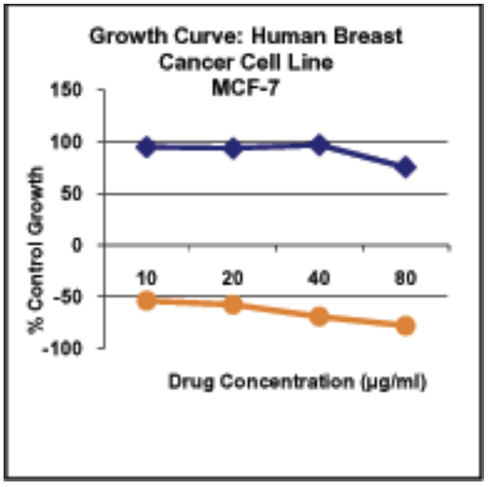

Fig. 2: MCF7 cell line Growth curve (Blue line: *F. racemosa*, Orange line: Adriamycin)

Furthermore, the major constraints in phytochemical studies are types of extracts and methodologies adopted. As we have used crude extract, the specific phytochemical agent might be in small concentration in this extract. Different methodologies used for extraction can have effect on final concentration of these compounds in crude extract. Constant heating, evaporation procedures may be responsible for the reduction in concentration. Also, one aspect that needs to be considered is the geographical area. The data from several recent studies suggested that geographical distributions affect the levels of phytochemicals and conversely their biological activities (Borokini and Ayodele, 2012; Jayanthy *et al.* 2013; Aslam *et al.* 2015; Khattak and Rahman, 2015). Therefore, these geographical variations must be taken into consideration while utilizing raw plant materials for industrial applications or traditional therapies.

Currently, natural products, especially plant secondary metabolites such as isoprenoids, phenolics and alkaloids, have been demonstrated to be the leading providers of novel anticancer agents (Shukla and Mehta, 2015). In general, the beneficial effect of plant products such as *F. racemosa* may be attributable to one or more phytochemicals including antioxidants, alkaloids, phenolic compounds and other substances. Therefore, additional studies with different individual fractions of the same extract are warranted to identify specific phytochemical agent which can have better anticancer activity. Understanding the modes of action of these active principals could provide useful information for their possible applications in therapy.

CONCLUSION

Crude extract of *F. racemosa* has some cytotoxic and anticancer activity (*in vitro*) on MCF7 human breast cancer cell line.

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