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Der Pharmacia Lettre, 2017, 9[12]: 16-22
[http://scholarsresearchlibrary.com/archive.html]

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ISSN 0975-5071
USA CODEN: DPLEB4

Novel Efficacy of *In vitro* Anti- Haemolytic and Anti-Cancer Activities of *Ficus krishnae*

Amarvani P Kanjikar, Aruna LH, Ramesh L Londonkar*

Department of Biotechnology, Biopharmaceutical and Nanobiotechnology, Gulbarga University, Kalaburagi, India

***Corresponding author:** Ramesh LL, Professor and Chairman,Department Of Biotechnology, Gulbarga University,Kalaburagi-585106, Karnataka,* India, *Fax. No. 08472-245632, Tel: 08472 245446; E-mail:londonkarramesh53@gmail.com*

ABSTRACT

In breast cancer cell cycle invasion, metastasis of aggressive are final and fetal stages during cancer progression. Therapeutically, there are few methods for the treatment of aggressive and metastatic breast cancer. Phyto-medicines are increasingly being used against cancer because of their availabiity; less adverse effects, targeted, non-toxic and low cost therapy when compared with chemotherapy. The present investigation aims to examine the anti-proliferative potentiality of different extracts derived from the Ficus krishnae stem bark (medicinal plant) on the growth of breast cancer cells. The F. krishnae extract directly suppress the proliferation and colony formation in breast cancer cell lines (MCF7). Meanwhile, the F. krishnae extracts did not significantly affect viability of non-tumorigenic normal blood cells instead of that, it protect the blood cell from lyses due to oxygen stress. MTT assay on breast cancer cell lines i.e., MCF7 shows the IC50 value of 74.46 ± 3.1 □g/ml, 103.38 ± 3.3 µg/ml, >1000µg/ml and >1000µg/ml of PE, CH, ME and AQ extracts respectively. Whereas the methanol extract has shown maximum protection of the blood cell from lyses was 94.59% and minimum protection at 65.18% by petroleum ether extract. The overall results concluded that the Ficus krishnae has the promising treatment for breast cancer due to presence of bioactive compounds.

Keywords: Ficus Krishnae, MTT, Cytotoxicity, Anti-heamolysis and MCF7.

INTRODUCTION

Breast cancer is the second leading cause of cancer death in women, it accounts for approx 30% of all cancers were diagnosed in the United States. Although the research has been done on breast cancer, the causes and mechanism of this cancer still remain not known [1,2]. The breast cancer development process has stepwise 4 stages begins in mammary ducts and progresses in 4 stages. At the stage 0, the tumor cells growth does not affect the functions of neighboring cell, tissue and noninvasive. In stage Ist, the tumor cell undergoes invasive and start to affect neighboring cells and tissue but it not reaches the lymph node. In stage IInd the cells of lymph node individual begin clumping and formation of inflammation. In stage IIIrd, the cancer spreads throughout the body organs such as lung, liver and barin [3,4]. There are many mechanisms related to breast cancer are genetic modifications, includes specific gene amplification, point mutation, deletion, chromosome rearrangements and aneuplody, epigenetic mechanism associates the 12 other types of cancer [5,6]. There are many methods to treat breast cancer patients by chemotherapy, radiotherapy and surgery. But these methods are not satisfactory due to its adverse effects and cost effective [7] New novel agents acting on targets in breast cancer are currently under investigation.

According to world health organization, 80% of rural people depend on the medicinal plants as primary drugs used against many healths related problems. The medicinal plant extract formulations helps in direct attack the cancer cells without harming the normal cells of the body organs [8-10]. The genus *Ficus* is the member of moreacae family growing in tropical and subtropical regions of worldwide; it is considered as one of the largest genera of medicinal plants with about 750 species of woody plants, herbs and shrubs [11]. One hundred and fifteen species of medicinal plants are distributed throughout the India and approximately 43 species are found in Meghalaya alone [12].

Ficus krishnae is also known as Makkhann Katori in Hindi and Krishna fig or Krishna's butter cup in English. It is mainly found in India, tropical Africa and Sri Lanka. The plant has 10 m in height, fast growing tree with spreading branches and aerial roots [13]. Various parts of the plant are used to treat ulcers, vomiting, fever, inflammations, leprosy and also used as aphrodisiac, as a tonic, in piles and gonorrhoea. Stem bark and leaves are useful in treatment of diabetes. The aerial roots are styptic; useful in syphilis, biliousness, dysentery and inflammation of liver [14,15]. The *Ficus krishnae* stem bark extracts of plant has not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s). Hence, the present study was intended to screen *in-vitro* MTT and anti-heamolysis assay methods has been used to measure the efficiency of the plant extracts under investigation against breast cancer cells and erythrocytes as explained in the materials and methods for their possible further lead in drug design.

MATERIALS AND METHODS

Hyposaline induced haemolysis

The hyposaline induced haemolysis was evaluated for *Ficus krishnae* stem bark *in vitro* by the previously described method [16]. Collected the Blood sample from healthy adult volunteers in steril Alsever's solutions and used within 5 hours after blood collection. The blood samples were centrifuged at 3000 rpm for 15 min, and the RBC cells were washed 3 times with phosphate saline buffer (PBS, pH 7.4) and 10% (v/v) RBC suspension was made witn PBS. The assay mixture with different concentration of petroleum ether, chloroform, methanol and aqueous extracts, 1 ml of PBS, 2 ml (0.36%) of hyposaline and 0.5 ml of 10% RBC suspension in different test tubes. Hyposaline used as positive control. The tubes were incubated for 30 min at 370˚C and

centrifuged. After incubation the supernatant were measured at 540 nm in UV spectrophotometer and percentage of prevention of heamolysis of four extract was calculated using the relation.

$$Percentage\ prevention\ of\ \ haemolysis = \frac{Absorbance\ of\ \ treated\ \ sample}{Absorbance\ of\ \ control} \times 100$$

Anti-cancer activity

Determination of cell viability by MTT Assay

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), MEM and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium

MCF 7 (Human Breast cancer) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells are cultured in MEM (Minimum Essential Media) supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures are grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, 10mg of test drug was separately dissolved in distilled DMSO and volume was made up with MEM and supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Cell viability by MTT assay

Cell viability assay with MCF-7 cells was done as per the protocol described method [17], the monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates are then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line and calculated with fallowing formula.

$$\%\,Growth\ Inhibition = \frac{Mean\ OD\ of\ \ individual\ \ test\ group}{Mean\ OD\ of\ \ control\ \ group} \times 100$$

Statistical analysis

Data was expressed as mean ± SD. Analyses of variance (ANOVA) were done for the comparison of results using Fischer's test. Statistical significance was set at $p<0.05$.

RESULT AND DISCUSSION

Anti-haemolysis activity

Several plants possess the bioactive substances that having a haemolytic and anti-haemolytic effect on human erythrocytes. The mean corpuscular fragility determined that the membranes of human erythrocytes from blood types have varying stability [18]. Plants extract may create serious adverse effects, which may include induction of hemolytic anemia. Therefore, many of the commonly used plants need to be evaluated for anti-haemolytic activity [19].

The heamolysis activity of petroleum ether, chloroform, methanol and aqueous extract of *Ficus krishnae* stem bark was screened at different concentration ranging from 62.5 to 1000 µg/mL. (Table 1). It was observed that there is an increase in concentration of extract with increases the prevention of lyses in RBC cells, when compared with standard. This can be concluded that the methanol extract of the plant has a potential anti-hemolytic activity followed by chloroform, aqueous and petroleum ether.

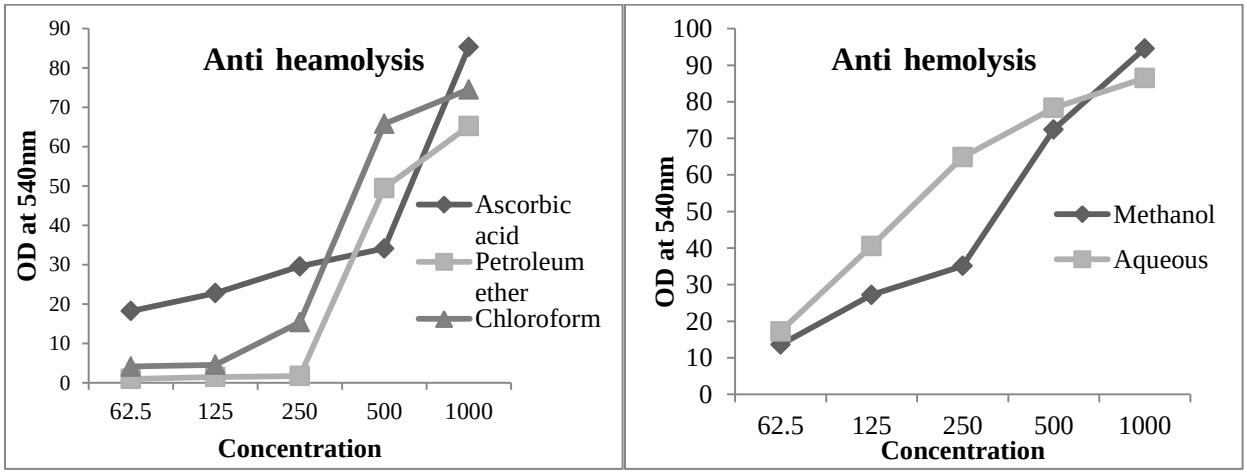

Figure 1. Anti-heamolysis activity of different extract of stem bark of *Ficus krishnae.*

Cell viability test by MTT assay

In the present investigation the cytotoxicity effect of *F. krihnae* on breast cancer cell line of petroleum ether, chloroform, methanol and aqueous extracts was carried out by SRB assay, after incubation absorbance were read at wavelength of 540 nm. Photo of the cells were taken and percentage of inhibition value were plotted on graph in Figures 1 and 2. From the graph IC_{50} values were calculated and were predicted in Table 1. Petroleum ether extract of *F. krishnae* has showed IC50 value 74.46 ± 3.1

µg/ml means the drug concentration has 50% reduction in cell viability on breast cancer cell lines, followed by chloroform extract which has showed 103.38 ± 3.21 µg/ml, whereas methanol and aqueous extract revealed IC50 values >1000 µg/ml (Figure 3).

Ficus krishnae stem bark extract has potential activity on breast cancer cell lines when compared with our results are in concordance with some of the previous studies on this genus, *F. racemosa* has anti permeability effects [20]. *F. racemosa* bark extracts have also exhibited anticancer and cytotoxic activity against lung cancer [21], *F racemosa* of bark extract has the anti-breast cancer activity [22]. *Ficus* glomerata the plant also has shown antimitotic effects [23], another study displayed a strong antioxidant and free radical scavenging activity of *F. racemosa* [24].

Table 1: Cytotoxic IC_{50} values of different extract of *Ficus*, against MCF 7.

Sl. No	Name of test sample	IC_{50} (µg/ml)
1	Petroleum ether extract 160094 (1)	74.46 ± 3.1
2	Chloroform extract 160094 (2)	103.38 ± 3.2
3	Methanol extract 160094 (3)	>1000
4	Aqueous extract 160094 (4)	>1000

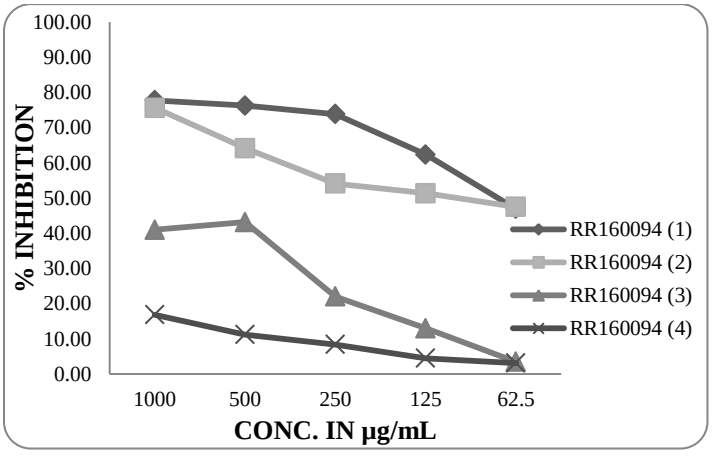

Figure 2: Cytotoxic effect of the sample 160094 (1, 2, 3, 4) on MCF 7 cell.

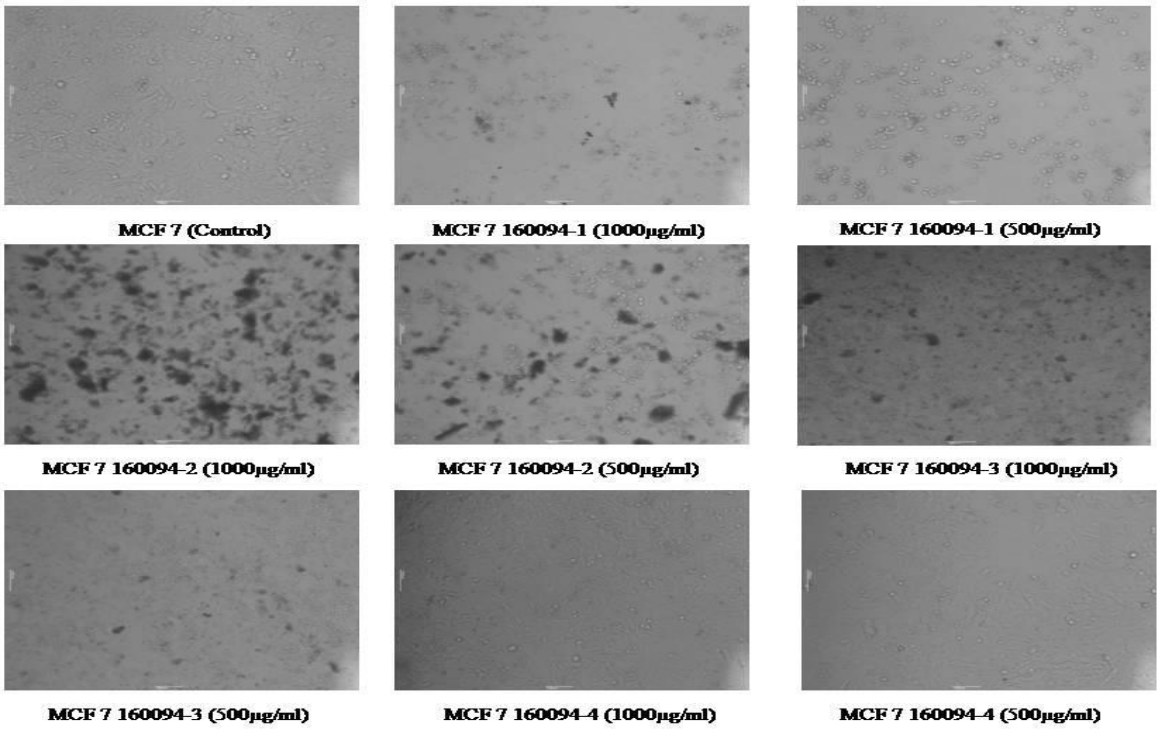

Figure 3. MTT assay of *F. krishnae* stem bark extract against breast cancer cell MCF 7.

CONCLUSION

Cytotoxicity activity of *F. krishnae* stem bark extract revealed that the effect of extracts is toxic only to breast cancer cells. Cytotoxicity activity of hemolytic assay on human erythrocytes has revealed a nontoxic nature of *Ficus krishnae* for all 4 extracts. The results of present study denouement that all the 4 extracts of *Ficus krishnae* have the encouraging effects as potential drug for cancer treatment in future because the plant has the good sources of bioactive compounds. Further, investigation on *in vivo* research has to be carry out to known the mechanism of inhibition in molecular level. Investigation has to extend for isolation of pure compounds responsible for anti-breast cancer activity.

Acknowledgment

The authors are thankful to the University Grant Commission (UGC), New Delhi for providing financial support to carry out this research work under Rajiv Gandhi national fellowship (RGNF) scheme.

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