Int J Pharm Bio Sci 2015 July; 6(3): (P) 230 - 237

Research Article Pharmacognosy

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

# CYTOTOXICITY OF *CIPADESSA BACCIFERA* (ROTH.) MIQ., ON HELA, JURKAT, MCF-7, AND KB CELL LINES.

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## ABSTRACT

*Cipadessa baccifera* (Roth.) Miq., belongs to Meliaceae family, is an ethnobotanically important plant. The aim of this study was to evaluate the cytotoxic activity of methanolic extracts of root, stem bark, leaf and fruit of *C. baccifera* against HeLa, MCF-7, Jurkat, and KB cell lines. Methanolic extracts of different plant parts were obtained and assessed for their cytotoxic activity by the MTT assay method. Leaf and root extracts showed potent cytotoxicity against MCF-7 and KB cell lines with significant $IC_{50}$ values. Fruit extract showed moderate cytotoxicity against HeLa and Jurkat cells. Cytotoxicity of bark extract was moderate on KB cells. Differential cytotoxicity was observed against the cell lines. Significant cytotoxic activity was reported in leaf and root extracts on MCF-7 and KB cell lines. Detailed cytotoxic evaluation of leaf and root extracts along with isolation and characterization of compounds is promising in the formulation of anticancer drugs against human breast cancer and human nasopharyngeal epidermoid cancer.

**KEYWORDS:** ***Cipadessa baccifera*, Cytotoxicity, HeLa, Jurkat, MCF-7, KB, MTT assay.**

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# INTRODUCTION

Cancer is a dreadful human disease and one of the leading causes of morbidity and mortality worldwide. It is responsible for one in eight deaths, more than AIDS, tuberculosis and malaria. In 2012, globally 8.2 million people died from cancer and this is expected to increase in the coming years[1] . One of the strategies of conventional cancer treatments is the use of chemotherapeutic drugs, which have shown adverse side effects[2] . Despite a whole variety of synthetic anticancer drugs available, phytochemicals, particularly secondary metabolites and their derivatives have played an important role in the development of several clinically useful anticancer formulations. Medicinal plants are a reservoir of phytochemicals of high medicinal properties. Currently, over 50% of the drugs used in clinical trials for cancer therapy are isolated from plants or their derivatives [3, 4] . The National Cancer Institute (NCI) USA has collected approximately 35,000 of the 27 million plant species and screened 114,000 plant extracts for potential anticancer activities [5] , of which 3000 species have been reported to possess anticancer properties [6] . Medicinal plants continue to be a subject of extensive screening in the search for safe bioactive compounds and attempts are continuously made to develop more effective anticancer drug formulations.

*Cipadessa baccifera* (Roth.) Miq., belonging to the family Meliaceae, is a shrub distributed in Northern Circars, Deccan, Western Ghats of India and Southwest of China. In India, it is a traditional medicinal plant used by folklore for treating a range of maladies, including diabetes, dysentery, malaria, rheumatism, piles, head ache, and psoriasis [7-10] .

Secondary metabolites like terpenoids, flavonoids, steroids, lignans, and coumarin known to exhibit biological activities, including antioxidant, antimicrobial, insecticidal, and cytotoxicity have been reported in *C. baccifera*. Systematic cytotoxic potential of *C. baccifera* has not been carried out despite its high traditional medicinal value and the presence of bioactive compounds. In view of this, the present study focuses on evaluating cytotoxic activity of plant extracts on various cancer cell lines.

# MATERIALS AND METHODS

### *i. Plant material*

The plant samples were collected from outskirts of Bangalore city and identified as *Cipadessa baccifera* (Roth.) Miq., using Flora of Bangalore [11] . Identification was authenticated by National Ayurvedic Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971. Separate voucher specimen BOT/Dec/11 is maintained in the herbarium of the Research Centre.

### *ii. Preparation of methanolic extracts*

Healthy and infection free samples viz., root, stem bark, leaf and fruit were collected, shade dried and pulverized. The powdered material was subjected to methanol extraction using soxhlet apparatus. The extracts were concentrated under reduced pressure using vacuum rotary evaporator. The concentrated methanolic extracts were stored at 4 ºC and used for cytotoxic studies.

### *iii. Chemicals*

Dulbecco's Modified Eagle's Medium (DMEM), 3-(4,5–dimethyl thiazol–2–yl)2,5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics were procured from Hi-Media Laboratories Ltd. Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol were obtained from Merck Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

### *iv. Cell lines and Culture medium*

HeLa (Human cervical adenocarcinoma cell line), Jurkat (Human T cell Leukemia cell line), MCF-7 (Human breast adenocarcinoma cell line) and KB (Human nasopharyngeal epidermoid carcinoma cell line) were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines were cultured in

DMEM supplemented with 10% inactivated 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 until confluent. The cells were dissociated with TPVG solution (0.2 % trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 $cm^3$ culture flasks and all experiments were carried out in 96 wells microtiter plate (Tarsons India Pvt. Ltd., Kolkata, India).

### *v. Preparation of Test and MTT solutions*

For cytotoxicity studies, the weighed test drug was separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/mL concentration and sterilized by filtration. Two fold serial dilutions were prepared from stock for carrying out cytotoxic studies.

MTT (Hi-media, Mumbai, India) was prepared by dissolving 5 mg powder in 1 ml phosphate buffered saline under hood. It was filtered through cellulose acetate filter with 0.2 µm pore size and 47 mm diameter (Sartorius AG, Germany) and stored at 8 °C.

### *vi. MTT Assay*

MTT assay used for determination of cell proliferation and cytotoxicity was done as per the modified method of Mosmann (1983) [12]. This colorimetric assay quantifies only living cells and has the advantage of being rapid and precise [13]. In this assay tetrazolium salt 3-(4, 5-dimethyl thiazol–2–yl)2,5-diphenyl tetrazolium bromide is reduced to a purple formazan product by mitochondrial dehydrogenase that are active in living cells. The intensity of the purple color developed is a measure of cell viability. The percentage of cells surviving was determined by comparing the absorbance of the treated cells with that of control [14].

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x $10^5$ cells/mL using DMEM containing 10% FBS. To each of the 96 wells microtiter plate, 0.1 ml of the diluted cell suspension was added. After 24 hours, a partial monolayer was formed and the supernatant was drained off. The monolayer culture was washed with medium and 100 µL of different concentrations of test drugs were added on to the partial monolayer in microtiter plate. Cultures were incubated at 37 °C for 3 days in 5% $CO_2$ atmosphere and at every 24 hour interval, cultures were examined microscopically and observations were recorded. After 72 hours of incubation, 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 4 hours at 37 °C in 5% $CO_2$ atmosphere avoiding exposure of light. After 4 hours, MTT was removed and the formazan formed with the MTT was solubilized by adding 100 µL of propanol. The absorbance was read at 570 nm using double beam UV-visible spectrophotometer [15].

The growth inhibition rate was calculated using the formula of Zheng et al. (2000) [16].

$$\text{Inhibition Rate } \% = \frac{OD_{\text{control well}} - OD_{\text{treated well}}}{OD_{\text{control well}}} \times 100$$

Concentration of the test drug needed to inhibit cell growth by 50% ($IC_{50}$) was generated from the dose-responsive curves for each cell.

### *vii. Statistical analysis*

Results of the experiments are expressed as mean±SD. The significance of difference among the various treated and control groups were analyzed by one-way ANOVA. The difference was found to be significant at $P \leq 0.05$. The $IC_{50}$ values were calculated using linear regression method of plots of the cell inhibition percentage against the concentration of tested compounds.

## RESULTS AND DISCUSSION

The cytotoxicity of methanolic extracts of *C. baccifera* were evaluated against HeLa, Jurkat, MCF-7 and KB cell lines by MTT assay. The $IC_{50}$ values of fruit, leaf, stem bark and root extracts are listed in table 1.

**Table 1**
***Cytotoxic activity ($IC_{50}$) of methanolic extracts of C. baccifera***

| Sample | MTT assay ( µg/mL) | | | |
|---|---|---|---|---|
| | HeLa | Jurkat | MCF-7 | KB |
| Fruit | 132.66±0.78* | 177.17±0.62* | 349.53±0.25* | 290.35±0.37* |
| Leaf | 417.10±1.24* | 803.60±0.91* | **072.88**±0.76* | **077.75**±0.65* |
| Stem Bark | 558.70±0.45* | 612.60±0.33* | 257.81±1.02* | 178.98±0.55* |
| Root | 855.75±0.86* | 549.72±0.67* | **097.40**±0.88* | **052.95**±0.93* |
| 6-Mercaptopurine[a] | 010.47±0.75* | 011.15±1.03* | 008.69±0.78* | 007.98±0.66* |

***n=18, mean ±SD*, P ≤ 0.05, a- positive control***

The methanolic extract from fruits of *C. baccifera* showed significant cytotoxicity at low concentration (100 µg/mL**)** against all the four cell lines. With increase in concentration, cytotoxic activity was moderate against HeLa and Jurkat and significant on MCF-7 and KB cell lines (Figure 1). The $IC_{50}$ values further substantiate significant toxicity of fruit extract against HeLa and Jurkat cells. Dose dependent increase in cytotoxic activity was noted against the cell lines for all the tested concentrations.
The cytotoxic activity of leaf extract was low against HeLa, while no cytotoxicity was noted against Jurkat cells. The percentage cytotoxicity against MCF-7 and KB cell lines at low concentration (100 µg/mL**)** was significant (68.92% and 69.58% respectively). With increase in concentration, progressive increase in percentage cytotoxicity was observed against MCF-7 and KB cell lines (Figure 2). Strong antiproliferative activity of leaf methanolic extract of *Azadirachta indica* on MCF-7 and HT 29 cell lines has been reported by Jafari et al. (2013) [17]. Similarly, high toxicity of leaf extract of *Dysoxylum gaudichaudianum* has been reported against MCF-7 and HT-29 cell lines [18]. Chhavi Sharma et al. (2014) reported inhibition of growth of MCF-7 and HeLa cells on treatment with ethanolic leaf extract of *Azadirachta indica* [19]. In the present study, $IC_{50}$ values of methanolic leaf extract demonstrate potent cytotoxicity against MCF-7 and KB cell lines.
At low concentration, bark extract exhibited moderate cytotoxic activity against MCF-7 and KB cell lines, while no cytotoxicity was observed against HeLa and Jurkat cells. Cytotoxicity was absent in the stem bark extract of *Soymida febrifuga* on MCF-7 cells with concentrations upto 100 µg/mL [20]. The present work showed significant increase in percentage cytotoxicity at higher concentrations (Figure 3). $IC_{50}$ values of stem bark extract was moderately significant against MCF-7 and KB cells when compared to HeLa and Jurkat.
Methanolic extract of root showed potential cytotoxicity against MCF-7 and KB cell lines at 100 µg/mL and dose dependent increase in cytotoxicity with increase in concentration was noted. However, no cytotoxicity was observed against HeLa and Jurkat cell lines (Figure 4). The $IC_{50}$ values of root extract were remarkably higher for MCF-7 and KB cell lines indicating significant cytotoxicity.
Reports on anticancer investigations on *Carissa opaca* and *Toona ciliata* plant extracts showed significant percentage cytotoxicity on MCF-7 cells (78.5% and 57%) at 500 µg/mL concentration [21]. In the present investigation, at 500 µg/mL concentration, fruit, leaf, bark, and root extracts showed percentage cytotoxicity of 69.93%, 93.4%, 88.64% and 90.71% respectively, against MCF-7 cells (Figure 1-4). It was observed that, with increase in extract concentration, there was significant increase in cell death and the plant extracts showed variable cytotoxicity against all the cell lines. Leaf and root extracts showed higher cytotoxic

activity when compared to stem bark and fruit extracts. This is the first report evaluating cytotoxicity of *C. baccifera* against Jurkat and KB cell lines. Cytotoxic studies of fruit and root extracts on cancer cell lines are reported for the first time in the present investigation.

**Figure 1**
***Cytotoxic activity of fruit extract***

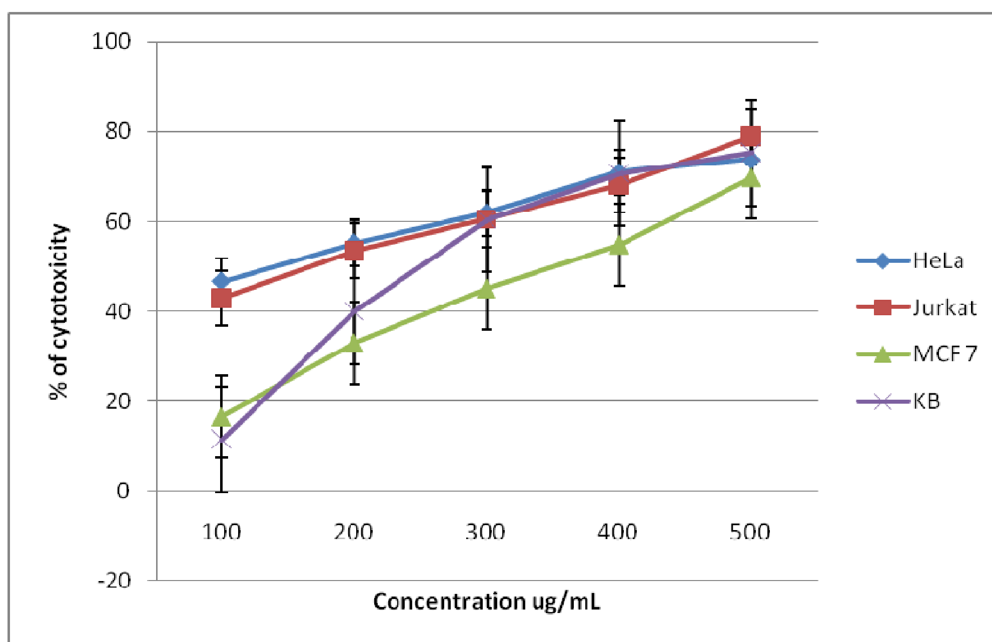



**Figure 2**
***Cytotoxic activity of leaf extract***

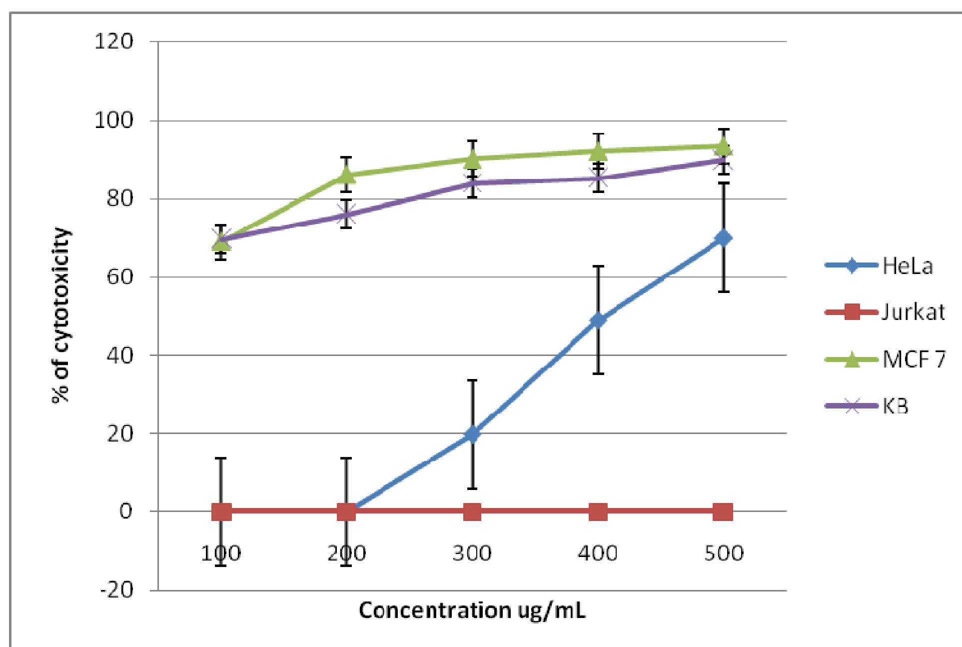


**Figure 3**
***Cytotoxic activity of bark extract***

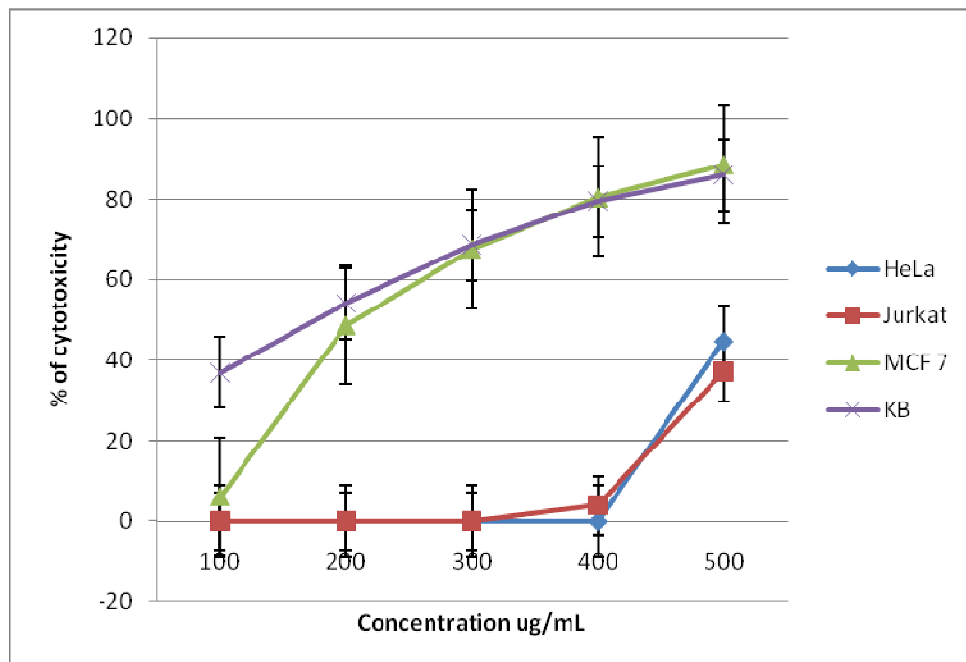
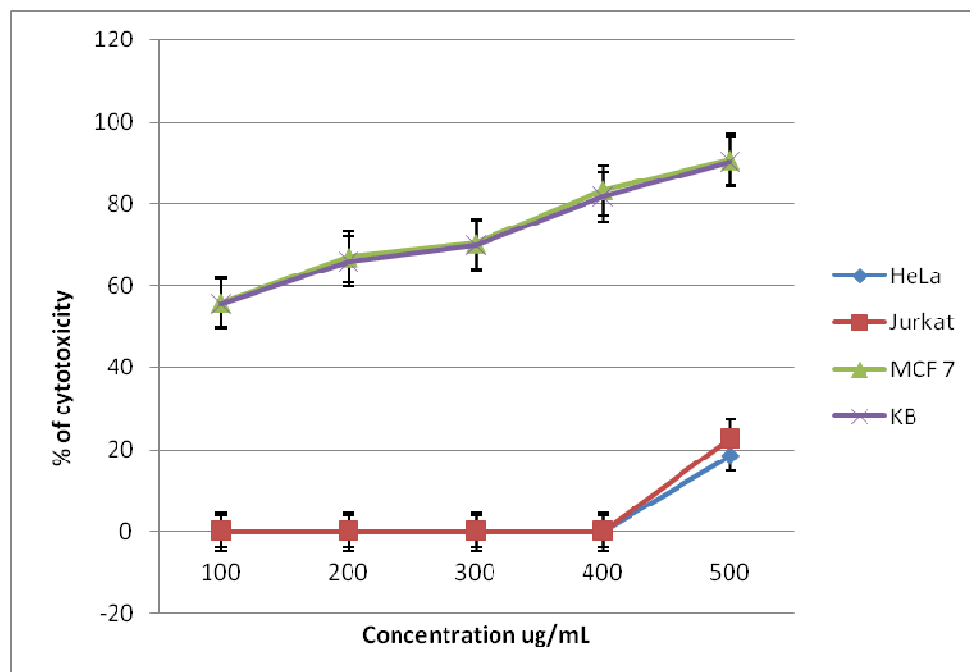



**Figure 4**
***Cytotoxic activity of root extract***



## CONCLUSION

*C. baccifera* is an ethno medicinally important plant used in the treatment of maladies like diabetes, dysentery, malaria and rheumatism. The cytotoxicity of methanolic extracts of leaf, stem bark, fruit and root on cancer cell lines HeLa, Jurkat, MCF-7 and KB cell lines varied significantly. The leaf and root extracts showed potent cytotoxic activity against MCF-7 and KB cell lines. The present study is the first report on cytotoxicity of root and fruit extracts on cancer cell lines. Isolation and characterization of compounds responsible for the cytotoxic activity and their evaluation as anticancer agents against human breast cancer and human nasopharyngeal epidermoid cancer is desirable.

### CONFLICT OF INTEREST

Conflict of interest declared none.

## REFERENCES


1. Jacques Ferlay., Isabelle soerjomataram., Rajesh Dikshit., Suktan Eser., Colin Mathers., et al. "Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012". International Agency for Research on Cancer WHO, Accessed on 29 March 2015. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
2. Richard R., Love MD., Howard MS., Leventhal., et al. Side effects and emotional distress during cancer chemotherapy. Cancer, 63: 604-612, (2006)
3. Newman DJ., Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal Natural Products, 70: 461-477, (2007)
4. Shashank K., Sanjay P., Abhay KP. *In Vitro* antibacterial, antioxidant, and cytotoxic activities of *Parthenium hysterophorus* and characterization of extracts by LC-MS analysis. Bio-Medical Research International, 1: 1-10, (2014)
5. Mohammad S. Anticancer agents from medicinal plant. Bangladesh J Pharmacol, 1: 35-41, (2006)
6. Hartwell., Jonathan L., Plants used against cancer: A survey, Lawrence, Mass, Quarterman publications, 8: (1982)
7. Malarvannan S., Giridharan R., Sekar S., Prabavathy VR., Sudha Nair. Ovicidal activity of crude extracts of few traditional plants against Helicoverpa armigera (Hubner) (noctuidae:Lepidoptera). Journal of Biopest, 2(1): 64-71, (2009)
8. Amit R., Shailendra S. Limonoids: Overview of significant bioactive triterpenes distributed in plant kingdom. Biol Pharm Bull, 29 (2): 191-201, (2006)
9. L.Liang., C-C Zhong., Xiao Z., -Y Xiao. Zhongcaoyao, 22: 6-8, (1991).
10. L.Liang., C-C Zhong., Xiao Z,-Y Xiao, *Zhongcaoyao,* 25: 236-237, (1994).
11. Ramaswamy SV., Razi BA., Flora of Bangalore District, Prasaranga, University of Mysore, Mysore, India, 316 (1973)
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65: 55-63, (1983)
13. Wasserman TH., Twentyman P., Use of a colorimetric microtiter (MTT) assay in determining the radio sensitivity of cells from murine solid tumors. Int J Radiat Oncol Biol Phys, 15(3): 699–702, (1988)
14. Guthrie N., et al. Palm oil tocotrienols and plant flavonoids act synergistically with each other and with Tamoxifen in inhibiting proliferation and growth of estrogen receptor- negative MDA-MB-435 and -positive MCF-7 human breast cancer cell culture. Asia Pacific J Clin Nutr, 6 (1): 41-45, (1997)
15. Francis D., Rita L. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and

reliability. Journal of Immunological Methods, 89 (2): 271-77 (1986)

16. Zheng Z., Zheng W., Huang Y., Yang Z., Li J., Cai H., Su W. Detection of antitumor and antimicrobial activities in marine organisms associated actinomycetes isolated from Taiwan strait, China. FEMS. Microbiology Lett, 88: 87-91, (2001).
17. Samineh Jafari., Soodabeh Saeidnia., et al. Cytotoxic evaluation of *Melia azedarach* in comparison with *Azadiracta indica* and its phytochemical investigation. DARU Journal of Pharmaceutical sciences, 21: 37, (2013)
18. Consolacion Y., Ragas., Vincent Antonio., et al. Chemical constituents and cytotoxicity of leaves of *Dysoxylum gaudichaudianum* (A. Juss.) Miq. Der Pharma Chemica, 6 (5): 182-187, (2014)
19. Chhavi Sharma., Andrea J Vas., Payal Goala., et al. Ethanolic Neem ( *Azadiracta indica)* leaf extract prevents growth of MCF-7 and HeLa cell and potentiates the therapeutic index of Cispatin. Journal of Oncology, Article ID 321754 (2014)
20. Varicola Karunasree., Ciddi Veeresham., Krothapalli KS., Sambasiva Rao., Kaleab Asres. Antioxidant, 5-Lipoxygenase inhibitory and anticancer activities of *Soymida febrifuga* A. Juss. Molecular & Clinical Pharmacology, 3 (2): 134-142, (2012)
21. Nisa S., Bibi Y., Zia M., Waheeda A., Chaudhary MF. Anticancer investigation on *Carissaa opaca* and *Toona Ciliata* extracts on human breast carcinoma cell line. Pak J Pharm Sci, 26 (5): 1009-12, (2013).