IJPSR (2016), Vol. 7, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 November, 2015; received in revised form, 10 March, 2016; accepted, 23 March, 2016; published 01 May, 2016

IN VITRO ANTICANCER ACTIVITY OF *GMELINA ASIATICA* L. LEAF AGAINST HUMAN BREAST CANCER CELL LINE (MCF-7)

A. R. Florence [1] and S. Jeeva [*2]

Department of Botany [1], Department of Botany and Research centre [2], Scott Christian College (Autonomous), Nagercoil, Tamilnadu, India.

Key words:

MTT assay, MCF-7 cell line, *Gmelina asiatica*, Cytotoxicity, Growth inhibition

Correspondence to Author:
S. Jeeva

Assistant Professor,
Department of Botany and Research centre, Scott Christian College (Autonomous), Nagercoil -629 003, Tamilnadu, India.

E-mail: solomonjeeva@gmail.com

ABSTRACT: The present study was taken to prove the cytotoxic effect of ethanolic extract of *Gmelina asiatica* leaf on human breast cancer cell line MCF-7. The *in vitro* cytotoxicity was evaluated by performing MTT assay. Cell morphological characteristics were observed by using phase contrast microscope. The results demonstrated in MCF-7 cancer cell lines treated with ethanolic extract of *G. asiatica* leaf showed the cell death which is dose dependent. Only 8.2 % of cell viability was observed at 1000µg/ml concentration of *G. asiatica* leaf extract. It is concluded that the ethanolic extract of *Gmelina asiatica* leaf can be used for the treatment of breast cancer.

INTRODUCTION: Cancer is a scourge afflicting disease to mankind from the time immemorial. Cancer diseases are characterized by a rapid and uncontrolled cellular growth, local tissue invasion and distant metastases [1] and the free radicals have been implicated in carcinogenesis [2]. Modern technology of present century, the treatment of cancer remains an enigma. It is a major public health burden in both developed and developing countries and the incidence of cancer is increasing annually [3, 4]. Medicinal plants have played an important role in the last half century in the treatment of cancer and secondary metabolites and their derivatives have been applied towards cancer. The antitumor agents are able to kill or inactivate tumor cells without damaging normal tissues.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(5).2116-21

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (5).2116-21

Currently, plants derived anticancer drugs in regular clinical use for the cancer treatment. They are vinblastine and vincristine was isolated from *Catharanthus roseus* (Apocyanaceae) which is used for the treatment of a variety of cancer, including testicular, breast, lung cancers and Kaposi's sarcoma [5, 6]. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity [4]. The screening of compounds with ten cancer cell lines (A-549, BEL-7402, BGC-823, SGC-7901, DU-145, HT-29, MCF-7, MDA-MB-231,U-251, B-16) where 324 compounds showed cytotoxicity against cancer cell lines [7].

Breast cancer is the second most prevalent cancer and leading death in women [8]. Approximately one-third of the women with breast cancer developed metastases and ultimately died of the disease. MCF-7 is an estrogen receptor-positive human cancer cell line, which derived from a patient with metastatic breast cancer [9]. Growth of MCF-7 cells is inhibited by tumor necrosis factor (TNF alpha).

Many plants are claimed to induce apotosis in MCF-7 cells such as *Antrodia camphorate* [10] and *Gmelina asiatica* [11]. The goal of screening medicinal plant is to search for excellent anticancer agent avertable to human malignancies.

Gmelina asiatica L. (Syn: *Gmelina parvifolia* Roxb.), is a deciduous large sized bush or shrub, commonly growing to about 4 m to 6 m tall and much branched. The whole plant of *G. asiatica* is medicinally important and many reports claim to cure diseases according to the Indian traditional system of medicines [12-19]. The previous literature reviews showed that *G. asiatica* root possess potent antiproliferative activity against MCF-7 and MDA-MB-231 human breast cancer cell lines [20]. Experiments conducted by Merlin *et al*. (2010) in the petroleum ether, chloroform, ethyl acetate and ethanol extracts of *G. asiatica* aerial parts showed potent cytotoxicity activity [11].

The chloroform extract of aerial parts of *G. asiatica* is effective against Dalton's Ascitic Lymphoma in Swiss Albino Mice was studied by Merlin and Parthasarathy (2010) [21]. The current study was undertaken with the objective to rationalize the cytotoxicity effect of ethanol extract of *G. asiatica* leaf on MCF-7 cell lines. The main aim of this research is to produce anticancer agent as well as the ability to prevent the growth of the breast cancer MCF-7 cell line.

MATERIALS AND METHODS:

Collection and sample preparation:

Leaves of *G. asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamilnadu, India and identified using Gamble and Fisher [22]. The healthy mature leaves were freshly collected and rinsed thoroughly with tap water to remove extraneous contaminants and kept in shade at room temperature for about two weeks to dry. They were made into powder with the help of a mechanical grinder and sieved. Dried and powdered samples were subjected to soxhlet with ethanol until the solvent was colorless. The extracted solvent was collected and the extracts were evaporated under reduced pressure by rotary evaporator. The dried extracts were kept in the refrigerator at 4^{o}C until use.

Cell lines:

Cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune. The cells were maintained in minimal essential medium (MEM) supplemented with 10% foetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL). The cells were cultured at 37˚C in a humidified atmosphere of 5% CO_2 incubator.

Reagents:

Minimal Essential Media (MEM), foetal bovine serum (FBS), trypsin, Methyl thiazolyldiphenyl-tetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO) and MCF-7 cells were purchased from Hi-Media & Sigma Aldrich, Mumbai.

In vitro assay for cytotoxicity activity (MTT assay):

The anticancer activity of samples on MCF-7 was determined by the MTT assay [23]. MCF-7 Cells (1×10^5/well) were plated in 0.2 mL of medium/well in 96-well plates. Then the plates were incubated 5% CO_2 incubator for 72 h. Then, various concentrations of the samples were added in 0.1% DMSO for 24 h 5% CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20 µL/well (5 mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) in phosphate-buffered saline solution was added. After 4 h of incubation, 1 mL of DMSO was added. Viable cells were determined by the absorbance at 540 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The effect of the samples on the proliferation of MCF-7 cells was expressed as the % cell viability was calculated manually using the formula:

$$\%\,\text{Cell viability} = \frac{\text{A540 of treated cells}}{\text{A540 of control cells}} \times 100\%$$

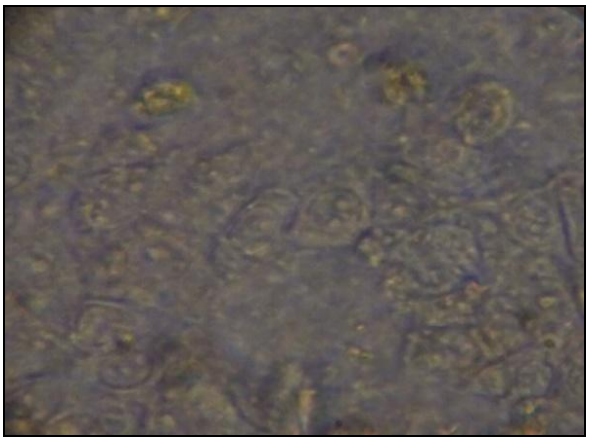
RESULTS: Cytotoxicity of *G. asiatica* leaf extract was assessed in the growth of MCF-7 cells (Human breast cancer cells) by MTT (3,4.5-dimethyl thiazole-2-yl)-5-5diphenyltetrazolium bromide) assay, which is based on the reduction of MTT. The cytotoxic activity was investigated using MTT assay, and the human breast cancer cell line MCF-7 cells are treated at different concentrations (7.8,

15.6, 31.2, 62.5, 125, 250, 500 and 1000µg/ml^{-1}). The viability of the control was designated as 100% and the others were expressed as percent compared to the control. The results demonstrated a strong dose-dependent growth inhibition in treated cell lines. As concentration increases percentage of inhibition also increased (**Fig. 2**). Greater than 80% cell death was observed at 1000µg/ml concentration. However, a complete cell death was not observed even at higher concentration of the sample. But the ethanolic extract of *G. asiatica* showed only 8.2% of viable cells was observed at 1000µg/ml concentration against the MCF-7 cancer cell line (**Table 1** and **Fig. 1**). From these findings it is observed that the reduction noticed in the viable cells with the treatment of *G. asiatica* extract is due to cell death.

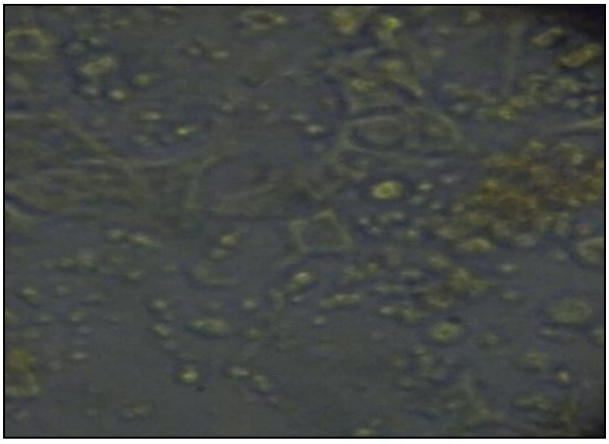
TABLE 1: SURVIVAL RATE OF MCF-7 CELLS TREATED WITH ETHANOLIC EXTRACTS OF *GMELINA ASIATICA* AT THE CONCENTRATION OF 0-1000 µG/ML

S.No	Concentration µg/ml	Dilution	Absorbance 540nm	% cell Viability
1	1000	Neat	0.07	8.2
2	500	1:1	0.12	14.1
3	250	1:2	0.18	21.1
4	125	1:4	0.23	27.0
5	62.5	1:8	0.29	34.1
6	31.2	1:16	0.35	41.1
7	15.6	1:32	0.44	51.7
8	7.8	1:64	0.49	57.6
9	control	-	0.85	100

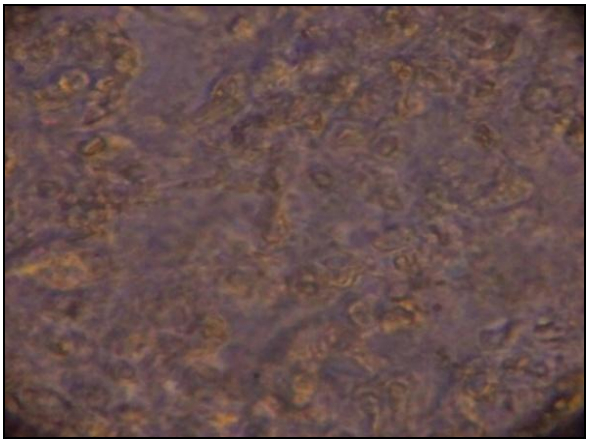
(a)1000µg



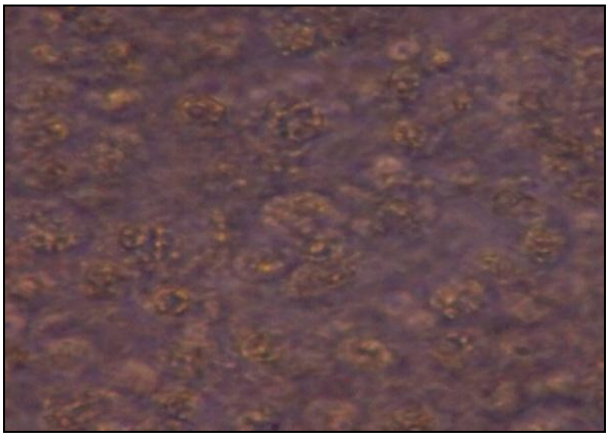
(b) 500µg



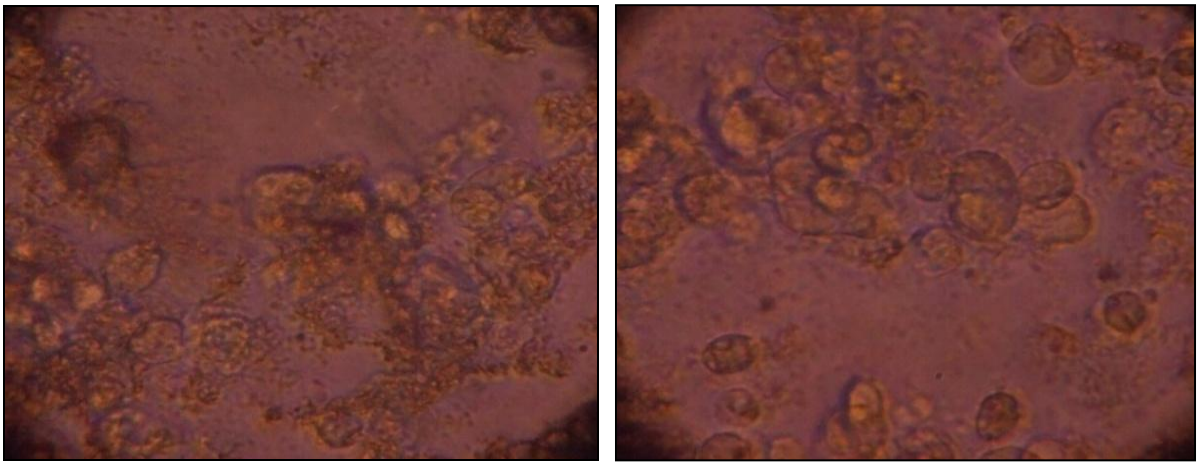
(c) 250 µg



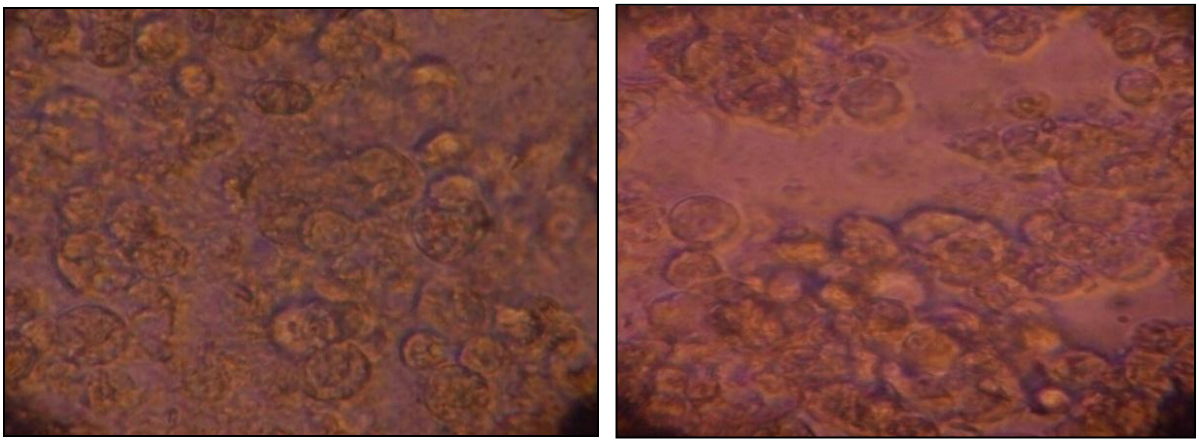
(d) 125 µg



(e) 62.5µg **(f) 31.2µg**



(g) 15.6µg **(h) 7.8µg**



(i) MCF7 Cancer cell line (control)

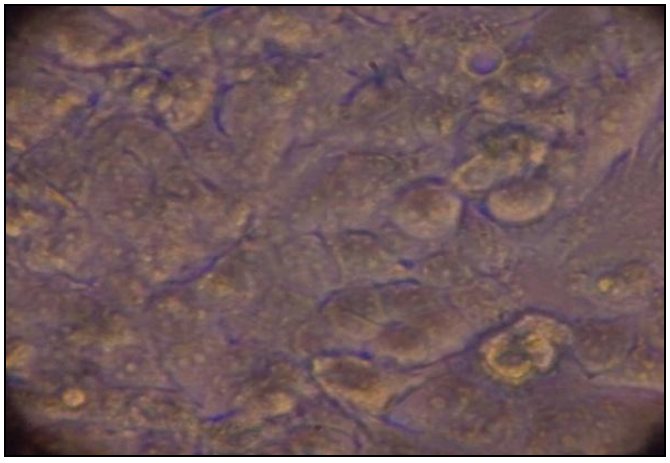


FIG. 1: MORPHOLOGICAL CHARACTERISTICS OF MCF-7 CELLS VISUALIZED WITH A PHASE CONTRAST MICROSCOPE. MCF-7 CELLS WERE TREATED WITH ETHANOLIC EXTRACTS OF *GMELINA ASIATICA* LEAF AT DIFFERENT CONCENTRATIONS OF 0-1000µg/ml (a-h) AND CONTROL CELLS (i)

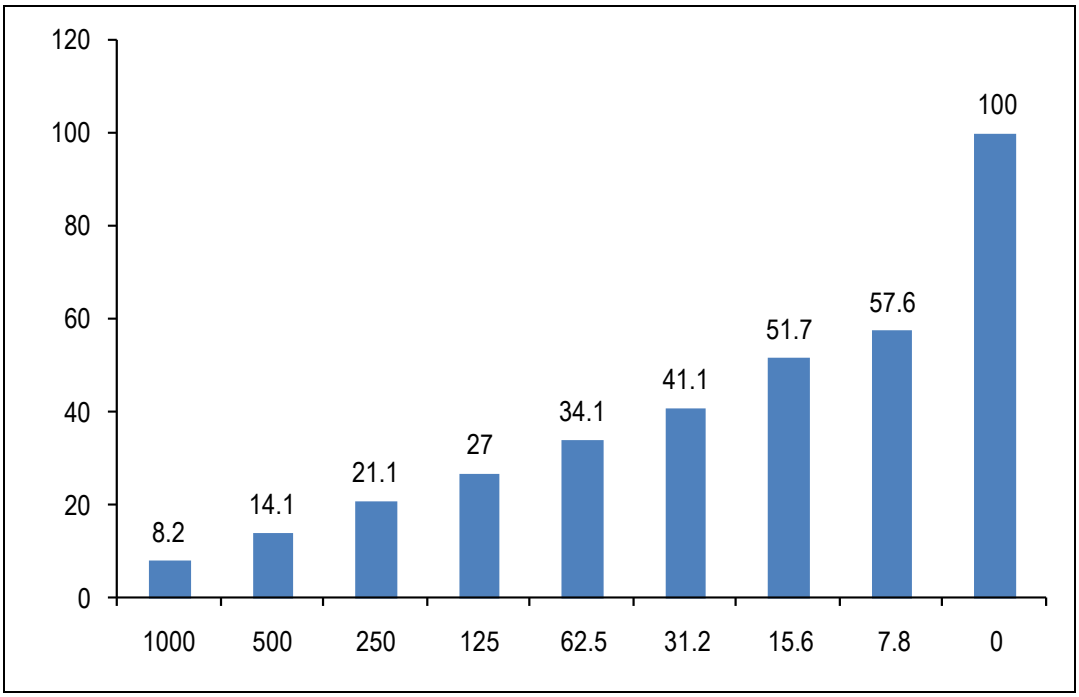


FIG. 2: EFFECT OF *G*MELINA *ASIATICA* LEAF EXTRACT ON CELL VIABILITY. MCF-7 CELLS WERE TREATED WITH 0 - 1000µG/ML OF *G*MELINA *ASIATICA*.

DISCUSSION: Agents capable of inhibiting cell proliferation, inducing apotosis or modulating signal transduction are currently used for the treatment of cancer [24]. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment [25]. Breast cancer is the most common malignancy among women.

MCF-7 cell has become a prominent model system for the study of breast cancer as it relates to the susceptibility of the cells to apoptosis. Despite the fact that many tumors initially respond to chemotherapy, breast cancer cells can subsequently survive and gain resistance to the treatment [26].

In the present study, the effects of ethanol extracts of *G. asiatica* leaf had high anticancer activity as evidenced from the MTT assay in a concentration dependent manner. The ethanol extract of *G. asiatica* showed notable cell death against the MCF-7 cancer cell line (**Table. 1).** Previous studies have highlighted the importance of anticancer effects plant in the bioactive compounds like flavonoids, phenols, phytosterols and also fatty esters such as n-dodecanoic acid, 9,12,15-octadecatrienoic acid, stigmesterol, (breast cancer preventive) and vitamin E from plant derivatives [27-29] which has been confirmed in the present study.

Balijapalli *et al*. (2010) investigated the ethyl acetate extract from *G. asiatica* roots as antiproliferative agents on human breast cancer cells (MCF-7) which was due to the presence of lignins and flavonoids [20]. Merlin and Parthasarathy (2010) and Merlin *et al*. (2010) have investigated that the chloroform extract of aerial parts of *G. asiatica* possess potential anticancer activity in caspase 3 deficient breast cancer cell line MCF-7 [21, 11]. The chloroform extract of 50µg/ml significantly increased the percentage of cells with condensed nuclei when compared to other solvents [30].

CONCLUSION: The present investigations provide important information that ethanolic extract of *G. asiatica* leaf is considered to have potent cytotoxic activity against MCF-7 cells. The outcome of the present study encourages to carrying out further studies to be extended for other cell lines and *in vivo* cytotoxicity investigations are required to identify anticancer activity.

ACKNOWLEDGEMENTS: The authors appreciate the National Centre for Cell Sciences (NCCS), Pune, for providing MCF-7 cancer cell line and thank the Department of Botany and Research Centre, Scott Christian College (Autonomous), Nagercoil, Kanyakumari,

Tamilnadu, India for the infrastructure provided to carry out this study.

REFERENCES:

1. Chabner BA and Collins JM: Cancer Chemotherapy: Principles and Practice, Philadelphia: Lippincott JB. 1990: 276–313.
2. Player T: Free Radicals, Lipid Peroxidation and Cancer, Mc Brien DCH, Slater TF, (eds). Academic Press, London, 1982: 173.
3. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics (2002) CA. Cancer Journal of Clinician 2005; 55:74-108.
4. Shoeb M: Anticancer agents from medicinal plants. Bangladesh Journal of Pharmacology 2006; 1:35-41.
5. Heijden RV, Jacobs DI, Snoeijer W, Hallard D and Verpoorte R: The *Catharanthus* alkaloids: Pharmacognosy and Biotechnology. Current Medicinal Chemistry 2004; 11:607-628.
6. Cragg GM and Newman DJ: Plants as source of anticancer agents. Journal of Ethnopharmacology 2005; 100:72-79.
7. Han HJ, Tan NH, Zeng GZ, Fan JT, Huang HQ, Ji CJ, Jia RR, Qin-Shi Zhao YJ, Zhang XJ and Wang LQ: Natural Inhibitors of DNA Topoisomerase I with Cytotoxicities. Chemistry and Biodiversity 2008; 5:1364-1368.
8. Parkin DM, Bray F, Ferlay J and Pisani P: Estimating the world cancer burden: Globocan, 2000. International Journal of Cancer 2001; 94:153-156
9. Levenson AS and Jordan CV: MCF-7: the first hormone-responsive breast cancer cell line. Cancer Research 1997; 57:3071-3078.
10. Yang HL, Chen CS, Chang WH, Lu FJ, Lai YC, Chen CC, Hseu TH, Kuo CT and Hseu YC: Growth inhibition and induction of apotosis in MCF-7 breast cancer cells by *Antrodia camphorate*. Cancer Letters 2006; 231:215-227.
11. Merlin NJ, Parthasarathy V and Santhoshkumar TR: Induction of apoptosis in human breast cancer cell line MCF-7 by phytochemicals from *Gmelina asiatica*. African Journal of Biotechnology 2010; 9(28):4451-4456.
12. Kiritikar KR and Basu BD: Indian Medicinal plants, International Book Distributors, Dehradun, Edition 2, Vol.3, 1975: 2327-2328.
13. Kiritikar KR and Basu BD: Indian Medicinal plants, Periodical Expert Book agency; New Delhi, Edition 2, 1984: 838
14. Apparanantham T, Chelladurai V and Subramaniam V: Some tribal folk medicines of point calimere (Kodikkarai) in TamilNadu. Bulletin of Medico-Ethno-Botanical Research 1982; 3:173-177.
15. Parekh J and Chanda SV: In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology 2007; 31:53-58
16. Parekh J, Jadeja D and Chanda S: Efficacy of aqueous and methanol extracts of some medicinal for potential antibacterial activity. Turkish Journal of Biology 2005; 29:203-210.
17. Vikneshwaran D, Viji M and Lakshmi KR: Ethnomedicinal plants survey and documentation related to Palaiyar community. Ethnobotanical Leaflets 2008; 12:1108-1115.
18. Kusuma G and Joshi VK: Nomenclature of Anukta Dravya. Ancient Science of Life 2010; 29(4):17-23.
19. Bakkiyaraj S and Pandiyaraj S: Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. International Journal of Pharma and Biosciences 2011; 2(2):B484-491.
20. Balijepalli MK, Tandra S and Pichika MR: Antiproliferative activity and induction of apoptosis in estrogen receptor-positive and negative human breast carcinoma cell lines by *Gmelina asiatica* roots. Pharmocognosy Research 2010; 2(2):113-119.
21. Merlin NJ and Parthasarathy V: Potential Antitumour Activity of *Gmelina asiatica* Aerial Parts Against Dalton Ascites Lymphoma in Mice. Asian Journal of Chemistry 2010; 22(4):3193-3199.
22. Gamble JS and Fischer CEC: Flora of Presidency of Madras. Adlard and Son Ltd., London, V. 1-3, 1935: 1-2017.
23. Mosmann T: Rapid calorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological methods 1983; 65(1):55-63.
24. De Flora S and Ferguson LR: Overview of mechanisms of cancer chemopreventive agents. Mutation Research 2005; 591: 8-15.
25. Howells LM and Manson MM: Prospects for plant-derived chemopreventive agents exhibiting multiple mechanisms of action. Current Medicinal Chemistry-Anticancer agents 2005; 5:201-213.
26. Campbell RA, Bhat-Nakshatri NM, Constantinidou DP, Ali S and Nakshatri H: Phosphatidyl-inositol/3-kinase/Akt-mediated activation of estrogen receptor α: a new model for anti-estrogen receptor resistance. Journal of Biological Chemistry 2001; 276:9817-9824.
27. Kametani T and Furuyama H: Synthesis of Vitamin D3 and related compounds. Medicinal Research Reviews 1987; 7(2):147-171.
28. Turkey JM, Fu T, Ruscetti FW, Mikovits JA, Bertolette FC and Birchenall-Roberts MC: Vitamin E succinate induces Fas-mediated apotosis in estrogen receptor-negative human breast cancer cells. Cancer Research 1997; 57:881-890.
29. Awad AB, Fink CS, Williams H and Kim U: In vivo (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. European Journal of Cancer Prevention 2001; 10(6):507-513.
30. Kerr JF, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer 1972; 26:239-257.

How to cite this article:
Florence AR and Jeeva S: *In vitro* Anticancer Activity of *Gmelina Asiatica* L. Leaf against Human Breast Cancer Cell Line (Mcf-7). Int J Pharm Sci Res 2016; 7(5): 2116-21.doi: 10.13040/IJPSR.0975-8232.7(5).2116-21.