IJPSR (2016), Vol. 7, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 March, 2016; received in revised form, 24 May, 2016; accepted, 13 July, 2016; published 01 August, 2016

ANTICANCER ACTIVITY OF *CALOPHYLLUM INOPHYLLUM* L., ETHANOLIC LEAF EXTRACT IN MCF HUMAN BREAST CELL LINES

K. Jaikumar, Sheik Noor Mohamed M., Anand D and P. Saravanan *

P.G & Research Department of Botany, Ramakrishna Mission Vivekananda College, Mylapore, Chennai-600004, Tamilnadu, India.

Keywords:

Calophyllum inophyllum, MTT assay,MCF-7 breast cancer cell line

Correspondence to Author:
Dr. P. Saravanan

Assistant Professor,
Department of Botany, Ramakrishna Mission Vivekananda College, Mylapore, Chennai -600004, Tamilnadu, India.

Email: sarviveka@gmail.com

ABSTRACT: Cancer is a major health problem; almost thought - out the world. Herbal medicines have a vital role in the prevention and treatment of cancer. The present study, the in vitro anticancer activity of *Calophyllum inophyllum* ethanolic leaf extract was evaluated in MCF-7 Breast cancer cell lines. Anticancer activity was evaluated with MTT standard colorimetric assay against MCF-7 cells with IC50 value of 120µg/mL. The biologically active metabolites were identified and quantified by a GC-MS method shows presence of 11 different bioactive compounds. The cytotoxic effect was found to be concentration dependent. Increased concentration of the leaf extract showed increased cytotoxicity.

INTRODUCTION: Cancer is a major public health burden in both developing and developed countries. As estimated there would be 10.9 million new cases, 6.7 million deaths and 24.6 million persons living with cancer around the world in 2012 [1-3]. Natural products like medicinal plants have been used for the treatment of various diseases for thousands of years.

Calophyllum inophyllum L. (Clusiaceae) commonly known "Punnaga" in Sanskrit, is a tree that can grow 8-20 meter tall with a broad spreading crown of irregular branches which exudes white latex when bruised. The leaves have opposite arrangements, and are petiolate, thick and shiny with numerous parallel secondary veins.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(8).3330-35

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (8).3330-35

The flower is 25 mm wide and occurs in racemose or paniculate inflorescences consisting of 4 - 15 flowers [4].

The fresh fruit and its oil extracts have been traditionally used externally against rheumatism, in topical infection and seborrhea in human adult. The dried leaf and its decoction used to cure rheumatism, skin infections, cuts and sores [5]. Five bioactive compounds isolated from *Calophyllum inophyllum L.*, leaves namely calophyllic acid and isocalophyllic acid mixture, 3-oxofriedelin-28-oic acid, canophyllic acid, amentoflavone and shikimic acid showed dose dependent lipid lowering activity in *in vivo* experiemtents [6].

Kashman and Patil reported that (+) - calanolide A and inophyllum B isolated from *Calophyllum lanigerum Miq.* and *Calophyllum inophyllum L.* showed strong activity against Human Immuno Deficiency virus type 1 [HIV-1] [7]. Tamanu oil demonstrates anti-inflammatory activity. This activity is due partly to the 4-phenyl coumarin calophylloide [8-9] and to a group of xanthones in the

oil, including dehydrocycloguanandin, calophyllin-B, and euxanthone. Al the xanthones in tamanu oil show anti-inflammatory activity[10]. Even though, the mechanism of action is not yet completely known in treatment of various infections, it may assume that the active ingredients could be play role in mode of action. The present study aimed to evaluate the possible cytotoxic activity of the ethanolic leaf extract of *Calophyllum inophyllum L* using MCF-7 human breast cancer cell line.

MATERIALS AND METHODS:

Collection of Plant Material:

The leaves of *Calophyllum inophyllum L.* were collected during month of September, from Rajiv Gandhi Salai (OMR), in Chennai, Tamilnadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, India. Herbarium of the plant was prepared and preserved in the Department.

Preparation of Leaf Extract:

The fresh leaves were collected and washed with running tap water, chopped into small pieces and shade dried for 10 days and grounded into powder. About 50 gm of powdered leaves were extracted with 300ml of ethanol in Soxhlet apparatus for 6 hours. Dry ethanolic extracts were obtained after removing the solvent by evaporation and stored in vials at 4°C until further analysis [11].

GC-MS Analysis:

The phytochemical investigation of methanolic leaf extract was performed on a GC Calrus 500 Perkin Elmer System comprising an AOC- 20 I auto sampler. Gas chromatography interfaced to a Mass spectrometer (GC-MS) instrument employing with column Elite-1 fused silica capillary column 30 X 0.25mm x 1μmdf composed of 100% Dimethyl poly silloxane, operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml per minute with injection volume of 0.2μl was employed (split ratio of 10:1) injector temperature maintained at 250°C, with ion-source temperature 280°C. The oven temperature was programmed from 110° C (Isothermal for 2 min) with an increase of 10°C per-minute to 200°C, then with 5°C increase per-minute upto 280°C ending with a 9min hold isothermal at 280°C. Mass spectral analysis was taken at 70eV: a scan interval of 0.5 seconds Mass scan fragments from 45 to 450 Da. Total GC running time is 36min and total MS running time is also 36min**.**The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2,chromatogram were compared again NIST database for compound identification[12].

Cell Line and Culture Medium:

MCF-7 cell line obtained from NCCS, Pune, India (National Centre for Cell Science), was used in this study. Cells were cultured in liquid medium (RPMI 1640) supplemented 10% Fetal Bovine Serum (FBS), penicillin/streptomycin (250 U/mL), gentamycin (100μg/mL) and amphotericin B (1mg/mL) and maintained under an atmosphere of 5% CO_2 and 95% air at 37°C.

MTT Assay:

The ethanolic leaf extract of *Calophyllum inophyllum* was tested for *in vitro* cytotoxicity, using MCF-7 cells by 3-(4,5- dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [13]. Cells were allowed to grow to confluence over 24 h before use. Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, MCF-7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (1 – 120μg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10μl, 5mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 570nm on a scanning multi-well spectrophotometer (Biotek, USA). Percent cell viabilities were calculated against selected concentrations.

Cell survival curves were then calculated for the fractions showing strongest cytotoxic activities and 50 percent inhibition concentration (IC50) values were calculated from the curve for each fraction. IC50 was defined as the concentration at which each cell line growth was inhibited by 50 percent.

Data represented the mean values for six independent experiments.

Cell viability (%) = A570 treated cells/A570 control cells x 100 [14]

Cytotoxicity(%) = 100-Cell viability

Statistical analysis

One way-ANOVA was used to analyze statistical differences between groups under different conditions.

RESULTS:

GC-MS analysis of Compounds:

To identify the anti-cancerous compounds present in the ethanolic leaf extract of *Calophyllum inophyllum* was subjected to GC-MS analysis. The spectrum reviled the presence of eleven bioactive compounds were shown in the **Table 1**and **Fig 1.** Of the eleven compounds identified, the most prevailing compounds were n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl) (51.20%), Phytol (35.45) based on the peak area.

TABLE 1: GC-MS PROFILE OF ETHANOLIC LEAF EXTRACT OF *CALOPHYLLUM INOPHYLLUM*.

S.no	Rt	Name of the Compound	Molecular Weight	Molecular Formulae	Peak Area %
1	11.18	Caryophyllene	204.35	$C_{15}H_{24}$	5.52
2	11.68	Z,Z,Z-1,4,6,9-nonadecatetraene	260.45	$C_{19}H_{32}$	2.97
3	12.53	1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl (15-(1a,3a,3aa,4a,8aa)	204.35	$C_{15}H_{24}$	4.02
4	15.95	Z,E-2-Methyl-3, 13,Octadecadein-1-ol	280.48	$C_{19}H_{36}O$	3.95
5	16.60	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	294.47	$C_{19}H_{34}O_2$	0.51
6	17.83	Hexadecanoic acid, ethyl ester	284.47	$C_{18}H_{36}O_2$	13.59
7	19.10	Phytol	296.53	$C_{20}H_{40}O$	35.45
8	19.52	Dasycarpidan-1-methanol,acetate(ester)	326.43	$C_{20}H_{26}N_2O_2$	1.41
9	25.48	Benzo(b,t)-1,2,4- triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	361.55	$C_{22}H_{11}N_5O$	11.48
10	27.28	2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1	376.35	$C_{22}H_{16}O_6$	15.95
11	27.63	n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)	376.53	$C_{23}H_{36}O_4$	51.20

*RT- Retention-Time

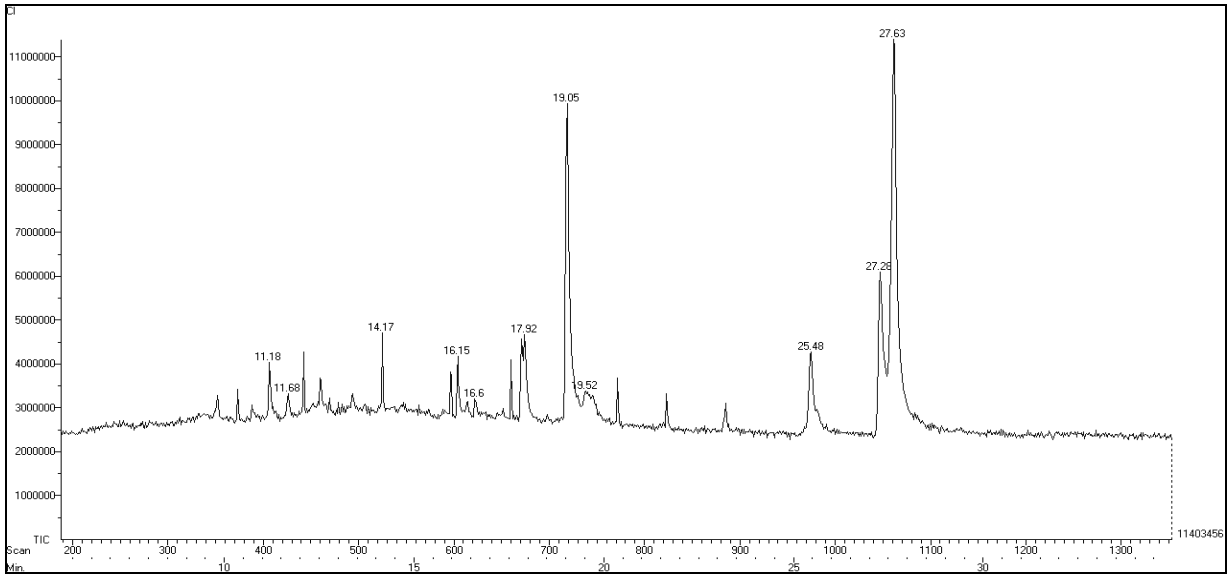


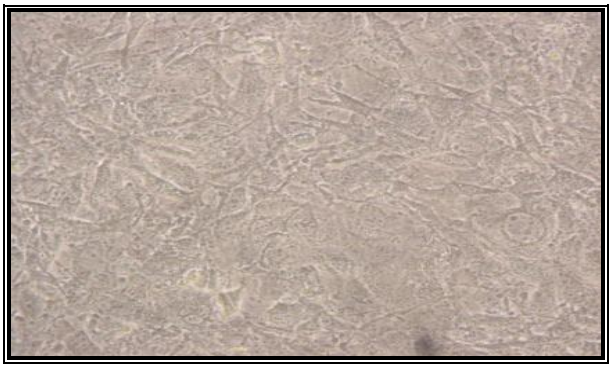
FIG.1: GC-MS CHROMATOGRAM OF ETHANOLIC LEAF EXTRACT OF *CALOPHYLLUM INOPHYLLUM*

Proliferative effects of MCF-7 cells:

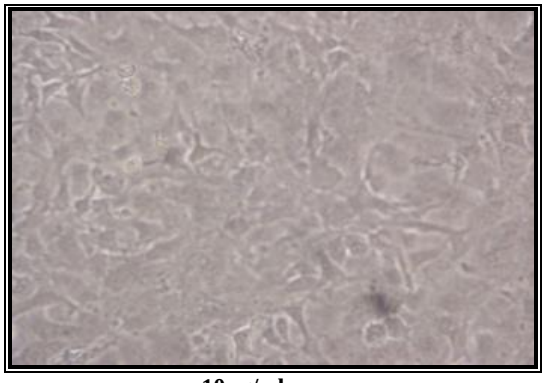
The anticancer effect of *Calophyllum inophyllum* on MCF-7 breast cancer cell line was evaluated through micro-culture MTT assay. The results obtained were presented in **Table 2** and **Fig. 2** and 3. It was observed that leaf extract showed 22.54%, 30.99%, 32.40%, 33.81%, 38.03% and 50.71% of cytotoxicity effects in 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml, 100µg/ml, 120µg/ml concentrations respectively. The median inhibitory concentration (IC_{50}) of *Calophyllum inophyllum L.* leaf extract against MCF-7 breast cancer cell line was found to be 120µg/ml. However, in all cases the percentage of growth inhibition increases with the increasing concentration of the plant extract.

TABLE 2: *IN- VITRO* CYTOTOXICITY ASSAY (MTT ASSAY):

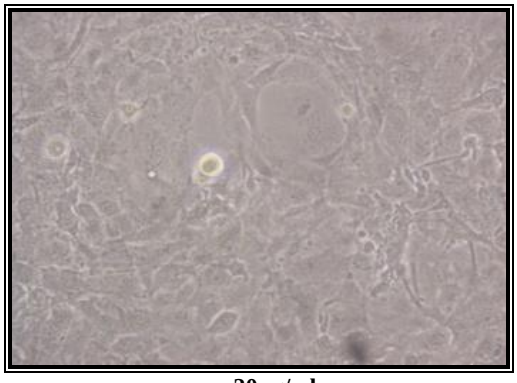
S.No	Concentartion [µg/ml]	Absorbance 570nm [O.D]	Cell Viability [%]	Cytotoxicity [%]	IC_{50} Value
1	120	0.35	49.29	50.71	120
2	100	0.44	61.97	38.03	µg/ml
3	80	0.47	66.19	33.81	
4	40	0.48	67.60	32.40	
5	20	0.49	69.01	30.99	
6	10	0.55	77.46	22.54	
7	Control	0.71	100	0	



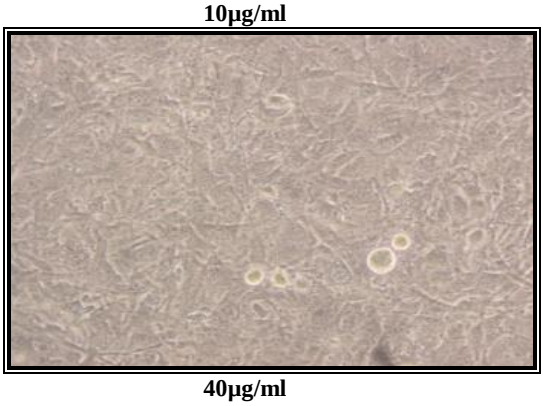
Control



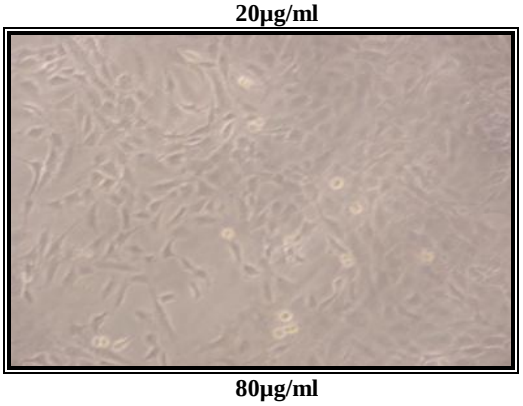
10µg/ml



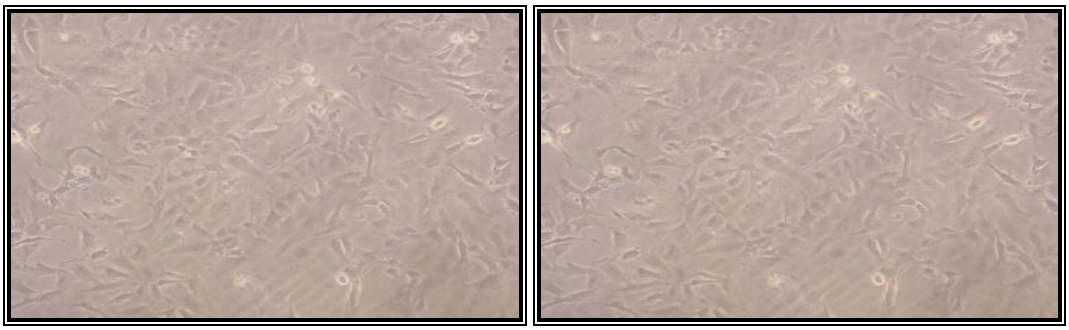
20µg/ml



40µg/ml



80µg/ml



100µg/ml **120µg/ml**

FIG. 2: CYTOTOXIC EFFECT OF ETHANOLIC LEAF EXTRACT OF *CALOPHYLLUM INOPHYLLUM*ON MCF-7 BREAST CANCER CELL LINES AFTER

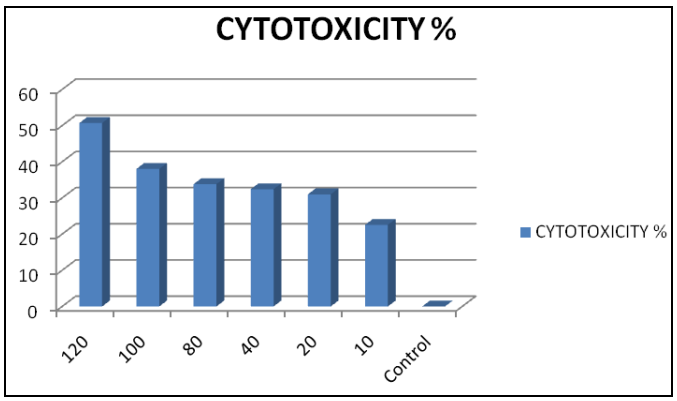


FIG.3: (MCF-BREAST CANCER CELL LINE)

DISCUSSION: Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Plants are rich sources in secondary metabolites with interesting biological activities [15].

Earlier reports of *Calophyllum inophyllum L.* leaf extract using alcoholic solvents by Malarvizhi 2011 yielded seventeen compounds [16]. By comparing the earlier reports, two compounds are similar where as other nine compounds were different.

The difference in plant components from previous study might arise from different extraction procedure whereas, the present study of ethanol leaf extract exhibited important bioactive compounds which posses various biological activities. Phytol is one among the nine compounds of the present study. Nanadagopalan 2015 reported the presence of Phytol in the leaves of *Kirganelia reticulata* aerial parts, which was found to be effective in different stages of arthritis[17].

Calophyllum inophyllum methanol leaf extract against MCF-7 and HT-29 cancer cell lines showed weak inhibition percentage of 31.25% and 22.56% [18] where as in the present study ethanol leaf extract showed anticancer effects of an IC_{50} value of 120µg/ml against MCF-7 cell line. The differences in anticancer activity from previous study, that ethanol leaf extract fraction possess the anticancer molecules. Further this bioactive molecule from this plant paves way for the discovery of new novel anticancer drug.

CONCLUSION: The GC-MS analysis of ethanol leaf extract of *Calophyllum inophyllum* revealed the presence of many important secondary metabolites having various pharmacological activities including anticancer activity. The IC_{50} value of ethanol leaf extract of *Calophyllum inophyllum L.* against MCF-7 breast cell lines showed potent anticancer activity, which are anti-proliferative, and apoptotic nature. In future studies this drug has to be characterized for clinical trials.

ACKNOWLGEMENT: The authors are thankful to The Secretary and Principal, Ramakrishna Mission Vivekananda College, Mylapore, Chennai, India for providing all facilities and we specially thank Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Chennai, India for GC-MS studies and validation of the results. The corresponding author acknowledge the financial support provided by University Grants Commission (UGC), New Delhi in the form of Minor Project Scheme.

REFERENCES:

1. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin2005; 55: 74-108.
2. Dervan PA: 1999, Understanding Cancer. Jefferson, NC: McFarland.
3. Hartwell JL Plants used against cancer: a survey. Lawrence, MA. Quarterman Publications1982;438-439.

4. Saravanan R, Dhachinamoorthi D, Senthilkumar K, Thamizhvanan K: Antimicrobial activity of various extracts from various parts of *Calophyllum inophyllum L*. Journal of Applied Pharmaceutical Science2011; 1(03):102-106.
5. Uma Shankar Mishra, Murthy PN, Sudhir Kumar Sahoo and Kanhu Charana Sahu: Formulation and evaluation of herbal tablet containing methanolic extract of *Calophyllum inophyllum*. International Journal of Pharmacy 2012; 2(1):181-186.
6. Janki Prasad, Atul Shrivastava, Khanna AK, Bhatia G, Awasthi SK, Narender T:Antidyslipidemic and antioxidant activity of the constituents isolated from the leaves of *Calophylluminophyllum.*Phytomedicine2012; 19(14):1245-1249.
7. Guang-Ying Chen, Guo-Zhu, Chang-Ri Han, Jun Zhao, Xiao-Ping Song, Wang-Fun Fong: A new pyranoxanthone from the stems of *Calophyllummembranaceum.* ARKIVOC 2008; 13:249-254.
8. Bhalla TN, Saxena RC, Nigam SK, Misra G, Bhargava KP: Calophylloide: a new nonsteroidal anti-inflammatory agent. Indian Journal of Medical Research 1980; 72: 762-765.
9. Saxena RC, Nath R, Nigam SK, Bhargava KP: Effect of calophyllolide, a non-steroidal anti-inflammatory agent, on capillary permeability. Journal of Medicinal Plant Research 1982; 30(1): 366-367.
10. Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, Viswanathan S, Kameswaran L: Anti-inflammatory and C.N.S. depresent activities of xanthones from *Calophyllum inophyllum* and *Mesuaferrea*. Indian Journal of Pharmacology1980; 12(3): 181-19
11. Anand D, John wyson W, Saravanan P, Rajarajan S: Phytochemical Analysis of Leaf Extract of *Eclipta alba* (L.) Hassk by GC-MS Method. International Journal of Pharmacognosy and Phytochemical Research2014; 6(3): 562-566.
12. Saravanan P, Jaikumar K, Sheik Noor Mohamed M, Anand D: Phytochemical analysis of bioactive compounds from *Calophyllum inophyllum* L. leaf extract using GC-MS analysis. International Journal of Pharmacognosy and Phytochemical Research2015; 7(5): 956-959.
13. Mossman. T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods1983; 65: 55-63.
14. Donatus EO, Ephraim C: Isolation, characterization and antibacterial activity of alkaloid from *Daturametel* Linn leaves. African Journal of Pharmacy and Pharmacology2009; 3(5): 277-281.
15. Mohamed Zaky Zayed, Fasihuddin Badruddin Ahmad, Wei-Seng Ho, Shek-Ling Pang: GC- MS analysis of Phytochemical constituents in leaf extracts of *Neolamarckiacadamba* (Rubiaceae) from Malaysia. International Journal of Pharmacy and Pharmaceutical Sciences2014; 6(9): 123-127.
16. Malarvizhi P, Ramakrishnan N: GC-MS analysis of biologically active compounds in Leaves of *Calophyllum inophyllum L*. International Journal of Chem Tech Research2011; 3(2):806-809.
17. Nanadagopalan V, Johnson Gritto M, Doss A: GC-MS analysis of biomolecules on the leaves extract of *Sterculiaurens* Roxb. Journal of Pharmacognosy and Phytochemistry2015; 3(6): 193-196.
18. Sankara Aditya J, Nareshkumar L, Animisha Mokkapati: In vitro anti-cancer activities of few plant extracts against MCF-7 and HT-29 cell lines. International Journal of Pharma Sciences 2013; 3(2): 185-188

How to cite this article:
Jaikumar K, Md. Sheik NM, Anand D and Saravanan P: Anticancer Activity of *Calophyllum Inophyllum* L., Ethanolic Leaf Extract In MCF Human Breast Cell Lines. Int J Pharm Sci Res 2016; 7(8): 3330-35.doi: 10.13040/IJPSR.0975-8232.7(8).3330-35.