https://ijrps.com

ISSN: 0975-7538
Research Article

Preliminary phytochemical screening, evaluation and comparison of anticancer activity of *Ficus gibbosa* blume by MTT assay method

Gini E.J*[1], Sivakumar T[2], Kuppuswamy S[3]

[1]Chemists College of Pharmaceutical Sciences and Research, Varikoli, Kerala, India
[2]Annamalai University, Chidambaram, Tamil Nadu, India
[3]Nirmala College of Pharmacy, Muvattupuzha, Kerala, India

ABSTRACT

The present study involves the preliminary phytochemical screening and evaluation of anticancer activity of the whole plant *Ficus gibbosa* blume. Extraction was carried out using the solvents petroleum ether, ethyl acetate, chloroform, ethanol and water. Among this, aqueous and ethanolic extract contains more active constituents, hence selected for the study. MTT assay method (invitro) was used for the evaluation of anti-cancer activity. Hacat and Mcf-7 cell lines were used for the evaluation. Preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponins, flavonoids, tannins, steroids, carbohydrates and proteins. Aqueous extract shows better activity against both Hacat and Mcf-7 cell lines and as the concentration increases there is an increase in the cell growth inhibition. For aqueous extract the IC50 value was found to be 46.54 µg/ml for Hacat and 92.4µg/ml for Mcf-7 cell lines. The present study concludes that both aqueous and ethanolic extract of entire plant of *Ficus gibbosa* blume possess anticancer activity against Hacat and Mcf-7 cell lines.

Keywords: Cytotoxic; *Ficus gibbosa* blume; linear regression analysis; MTT assay; Soxhlet extraction.

INTRODUCTION

Cancer, is one of the life threatening disease to human beings which is characterized by abnormal cell division (Akhila et al., 2012). Our life style, environment, and moreover genetic factors are the main causes of cancer (Abhishek et al., 2011). People are in search of an alternative method to chemotherapy for a cost effective treatment of cancer and also to overcome the side effects (Kiranmayi et al., 2011; Minu et al., 2014). From ancient times, various medicinal plants were used for treating different diseases by human beings and currently the demand for plant drugs are increased invariably (Mizanur et al., 2013; Trease et al., 2002).

Ficus gibbosa Blume belong to the family Moraceae, a large evergreen, climbing strangler with few aerial roots. Leaves alternately arranged with short petiolated, having smooth upper and rough lower surface, apex shortly and bluntly apiculate, flowers seen in clusters, hypanthodium, fruits small globose, yellowish when ripened (Wikipedia, Flowers of India).

The plant shows the presence of saponins, tannins, alkaloids, glycosides, flavonoids and carbohydrates (Rajagopal et al., 2013). Traditionally the bark powder is used to cure diabetic ulcers (Jayakumar et al., 2007). The juice of the bark and leaves of fig plants are used forgrinding the pills andmaking decoction in toxicology. Plant pacifies vitiated kapha, pitta, skin diseases, ulcers, hepatopathy, diabetes, ulcerative stomatitis, leucorrhoea and gynaecological problems (Wikipedia,Flowers of India)

The main method for the evaluation of anti -cancer activity by in-vitro method is MTT assay (McCauley et al., 2013). This method is more sensitive and reliable colorimetric assay which measures the reduction of yellow 3-(4,5 dimethyl thiazol 2-yl)-2,5 diphenyltetrazolium bromide by mitochondrial succinate dehydrogenase. The MTT enters the cell and pass into mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (eg: isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can occur in metabolically active cells, the level of activity is measure of the viability of cells (Ola Germaniuk et al) .The IC_{50} value is calculated to determine the concentration of an anticancer drug that kills 50% of the cells in a cancer cell line and the value was calculated by non-linear regression analysis (Minu et al., 2014).

* Corresponding Author
Email: ginibinu77@rediffmail.com
Contact: +91-9745234225
Received on: 08-09-2016
Revised on: 15-09-2016
Accepted on: 19-09-2016

Table 1: Phytochemical screening of Ficus gibbosa blume extracts

Sl no	Test	Pet.ether	Ethyl acetate	Chloroform	Ethanol	Aqeous
1	Carbohydrates	-	-	+	+	-
2	Glycosides	+	-	-	+	+
3	Saponins	-	-	+	-	+
4	Alkaloids	+	-	-	+	-
5	Flavonoids	-	-	-	+	+
6	Tannins	-	-	-	+	-
7	Proteins	-	-	-	-	+
8	Fixed oils	-	-	-	-	-
9	Steroids	+	-	+	+	-

+ :presence, **-** : absence

Table 2: % inhibition produced by aqueous extracts of Ficus gibbosa blume against Hacat cell lines

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage inhibition	IC 50 (µg/ml)
Sample-aqueous			
Control	**0.5427**		
6.25	0.4839±0.06	10.8347	
12.5	0.4112±0.05	24.2307	
25	0.3883±0.02	28.4503	
50	0.2547±0.04	53.0679	46.54
100	0.1857±0.02	65.7822	

Table 3: % inhibition produced by ethanolic extracts of Ficus gibbosa blume against Hacat cell lines.

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage inhibition	IC 50 (µg/ml)
Sample –alcoholic			
Control	**0.5427**		
6.25	0.4925±0.02	9.2501	
12.5	0.4865±0.03	10.3556	
25	0.3769±0.05	30.5509	
50	0.3626±0.01	33.1859	
100	0.2876±0.02	47.0057	
200	0.2504±0.02	53.8604	148
250	0.2073±0.05	61.8022	

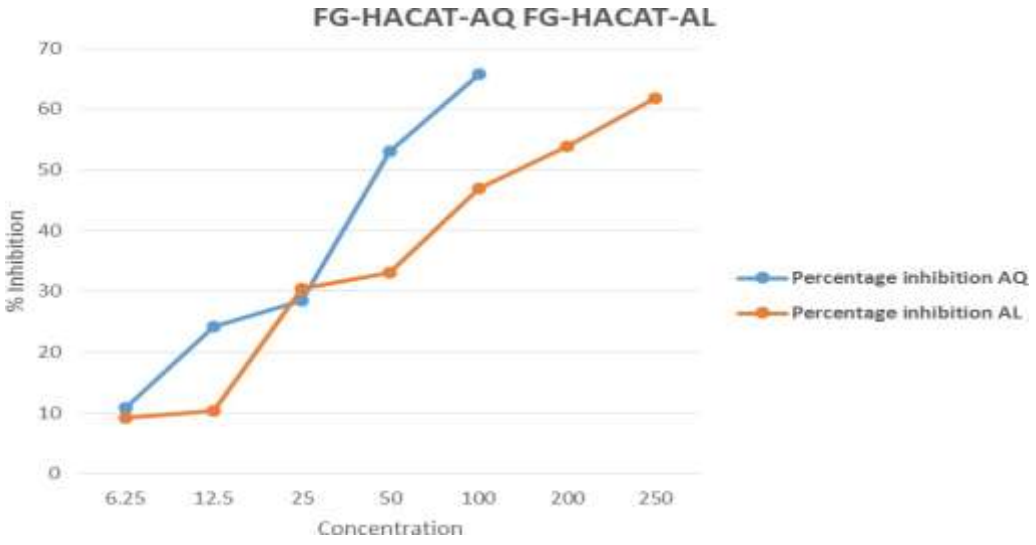

Figure 1: Comparison of % inhibition of extracts of Ficus gibbosa blume.

Table 4: % inhibition produced by aqueous extracts of Ficus gibbosa blume against Mcf-7 cell lines.

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage inhibition	IC 50 (µg/ml)
Sample –Aqueous			
Control	**0.691**		
6.25	0.5832±0.02	15.6006	
12.5	0.5655±0.05	18.1621	
25	0.5378±0.03	22.1706	
50	0.4596±0.02	33.4877	
100	0.3423±0.01	50.4631	92.4

Table 5: % inhibition produced by ethanolic extracts of FicusgibbosaBlume against Mcf-7 cell lines.

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage inhibition	IC 50 (µg/ml)
Sample –Alcoholic			
Control	**0.691**		
6.25	0.675±0.03	2.3155	
12.5	0.6572±0.01	4.8915	
25	0.6126±0.03	11.3459	
50	0.5593±0.04	19.0594	
100	0.4893±0.02	29.1896	
200	0.3493±0.05	49.4501	
250	0.3119±0.02	54.8625	224.5

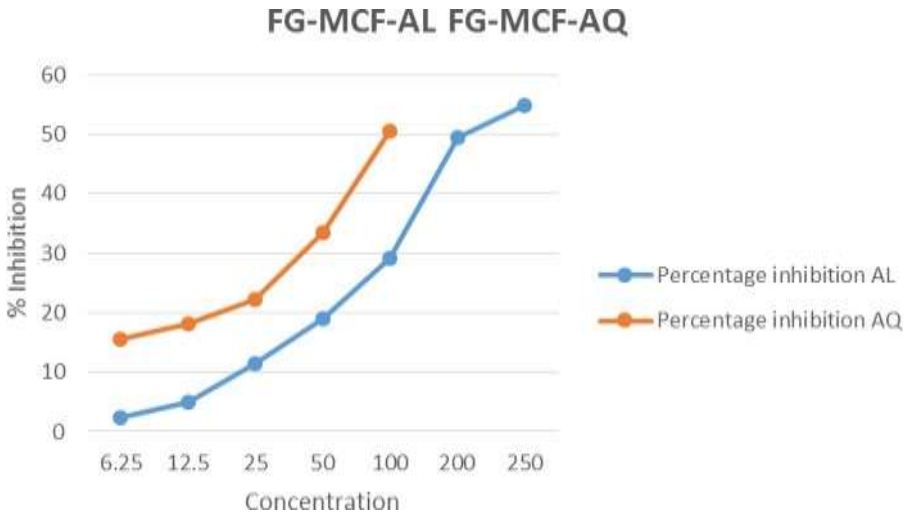

Figure 2: Comparison of % inhibition of the extracts of FicusgibbosaBlume by MTT Assay.

MATERIALS AND METHODS

Plant Material

The fresh plant material (entire lant) was collected from the northern Ghats of Kerala and was identified and authenticated by Mr.V.Chelladurai, Research Officer, central council for Research in Ayurveda & Siddha, Tamil Nadu.

Extraction and Phytochemical Analysis

The entire parts of the plant *Ficus gibbosa* blume was gabled of adhering impurities and then powdered. It was shade dried and 150gm of pulverised part was exhaustively and sequentially extracted/underwent successive solvent extraction using petroleum ether, chloroform, ethyl acetate, ethanol and distilled water respectively with Soxhlet extractor(gradient extraction). The extracts were concentrated in vacuum, weighed, labelled and stored in refrigerator until us (Trease et al., 2002).

Different qualitative tests were performed on the entire part of the plant extracts of *Ficusgibbosa*Blume for the identification of phytoconstituents (MukulTailang et al., 2008; Khandelwal et al., 2007).

In-Vitro cytotoxic activity of Ficus gibbosa blume entire plant by MTT Assay Method

The cells were trypsinized for 2 minutes and passed to T flasks in complete aseptic conditions. Extract was added to grown cells at the concentrations of 6.25 -

250µg/ml from a stock of 10mg/ml in 0.1% DMSO and incubated for 24 hours. Dilution of stock solutions was prepared in culture medium yielding final extract concentrations with a final DMSO concentration of 0.1%. This concentration of DMSO did not affect the cell viability. Control cells were incubated in culture medium only. Every concentrations of the plant extract were in triplicates on the same cell batch.

The % difference in viability was determined by standard MTT assay after 24 hours of incubation. The cell culture suspension was washed with 1x PBS and then 30 µl of MTT solution was added to the culture. Then incubated at 37°C for 3 hours. MTT was removed by washing with 1x PBS and 200µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lyses and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at 4000rpm for 2minutes to precipitate cell debris. Optical density was examined at 540nm using DMSO as blank in an ELISA reader.

$$\%\ viability = \left(\frac{OD\ of\ Test}{OD\ of\ Control}\right) \times 100$$

Experimental results were expressed as mean ± SD. All measurements were replicated three times. The IC_{50} values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

The plant used in this study was identified as *Ficus gibbosa* blume and preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponins, flavonoids, tannins, steroids, carbohydrates, proteins and the results were in Table 1. More active constituents were found to be present in aqueous and ethanolic extracts of *Ficus gibbosa* blume and therefore they were selected to perform the invitro cytotoxicity studies.

Determination of In-Vitro cytotoxic activity of aqueous and ethanolic extracts of entire plant *Ficus gibbosa* blume

The invitro cytotoxic activity by MTT assay on Hacat (skin cancer cells) and Mcf-7 (breastcancer cells) were performed. Control, aqueous, and ethanolic extracts of *Ficus gibbosa* blume were used.

MTT Assay using Hacat cell lines

The results of cell growth inhibition by different extracts against Hacat cell lines for various concentrations is shown in Table 2 & 3 respectively and graphically represented in Figure 1. MTT assay of aqueous and ethanolic extracts of *Ficus gibbosa* blume shows that the aqueous extract shows more anti proliferative activity against Hacat cell lines. As the concentration increases there is an increase in the cell growth inhibition with a maximum IC50 of 46.54µg/ml for aqueous extract.

MTT Assay using Mcf-7 cell lines

The % inhibition of various concentrations of aqueous and ethanolic extracts of

Ficus gibbosa blume were tabulated in Table 4&5 respectively and graphically represented in Figure 2. The aqueous extract of *Ficus gibbosa* blume shows better activity in MTT assay than ethanolic extract against Mcf-7 cell lines with an IC50 value of 92.4µg/ml and 224.5µg/ml respectively. The % inhibition increases with increase in concentration.

CONCLUSION

The present study reveals that the aqueous and ethanolic extracts of *Ficus gibbosa* Blume possess anticancer activity against Hacat (skin cancer) andmcf-7 (breast cancer) cell lines. Among the extracts, aqueous extract shows better anti-proliferative activity against both the cell lines. Hence further studies for the isolation and identification of active constituents from this extract may be carried out.

REFERENCES

Abhishek, B., Rohini, S. and Malleshappa, N.N.2011.Natural sources as potential anti -cancer agents: A review. International Journal of Phytomedicine, 3, 09-26.

Akhila, S.D., Shankaraguru, P., RamyaDevi, D. and VedhaHari, B.N.2012.Evaluation of invitro anti cancer activity of hydroalcoholic extract of *Tabernaemontanadivaricata*. Asian Journal of Pharmaceutical and Clinical research, 5, 59-61.

Jayakumar, G., Ajithbai, M.D., Sreedevi, S., Viswanathan, P.K. and Remeshkumar, B.2010.Ethnobotanical survey of the plants used in the treatment of diabetes. Indian Journal of Traditional Knowledge, 9, 100-104.

K.R.Khandelwal, Practical Pharmacognosy-Techniques and Experiments, Vol 16, NiraliPrakashan: 2007, 149 - 154.

Kiranmayi, G., CRamakrishnan, G., Kothai. R. andJaykar, B.In-vitro anti-cancer activity of methanolic extract of leaves of *Argemone Mexicana* Linn. International Journal of Pharm Tech Research, 3, 1329-1333.

McCauley, J., Zivanovic, A. and Skropeta, D.2013.Bioassays for anticancer activities. Methods Molecular Biology, 10, 191-205.

Minu, P.B., Sivakumar, T. and Giriraj, P.2014.Preliminary phytochemical screening and anticancer potential of ethanolic extract of *Madhucaneriifolia* (Moon) H.J.Lam. European Journal of Biomedical and Pharmaceutical Sciences, 1, 542-550.

Mizanur, R.M., Saifur, R., Sayed, K.A., SyedMasudur, R.D., Mahmodul, H., Nurul, A. and Naim, U.2013.Evaluation of cytotoxic and anthelmintic activities of the methanolic extract of *Thevetiaperuvi-*

ana. International Journal of Pharmacognosy and Phytochemical Research, 5, 92-95.

Mukul, T., Ashok, S.2008.Phytochemistry, Theory and Practical, Vol 1, Birla publications: 2008, 133 -135.

Rajagopal, P.L., Sreejith, K.R., Kiron, S.S., Robin, J. and Saritha, M.2013.Anti-inflammatory (invitro) activity of the leaves of *Ficus gibbosa* blume by HRBC membrane stabilisation. IOSR Journalof Pharmacy and Biological Sciences, 7, 55-57.

W.C. Evans and G.E. Trease. Pharmacognosy,Vol 14, Sanders: 2002, 409.

www.flowersofindia.net.

www.groups.molbiosci.northern.edu by Ola Germaniuk.

www.wikipedia.com.