Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research, 3(4),79-85

ISSN: 0976-822X

Research Article

Anti-Cancer and Anti-Microbial Activity of Hydro Alcoholic Extract of *Bougainvillea glabra*

Joshny J, Ramya Devi D, *Vedha Hari B.N

Department of Pharmaceutical Technology, School of Chemical and Bio-Technology, SASTRA University, Thanjavur, 613401, (T.N.). India.

ABSTRACT

Bougainvillea glabra, a well known and spread decorative plant in India and many parts of the world, was reported to have various medicinal properties such as anthelmentic, anti-diabetic, antiviral and insecticidal activity. We have performed the present study to focus and evaluate the antimicrobial and anticancer activity of the plant extract. We used the hydro-alcoholic extract of *Bougainvillea glabra* leaves for the antimicrobial studies, to test against Yeast, Gram positive bacteria and Gram negative bacteria strains by disc diffusion method. We have also estimated its maximum bactericidal activity by the same technique. The yeast used in the study was *Candida albicans*, while the bacteria used were *Salmonella typhi* as Gram negative and *Staphylococcus aureus* as Gram positive. And we have used Chloramphenicol as the standard drug for antibacterial test and Fluconazole for antifungal activity. The *in vitro* anticancer study was tested using human cancer cell line HeLa in which the cell growth inhibition was determined with the help of MTT assay. The hydroalcoholic extract of *B.glabra* showed significant anticancer activity with an IC_{50} value of 47.11µg/ml.

Key words: Gram positive bacteria, *S.typhi*, *S.aureus*, *C.albicans*, antimicrobial, anticancer, MTT assay.

INTRODUCTION

Plants have always been a part of medicinal science from the beginning of human civilisation to the present modern world of synthetic medicines. Even in the presence of variety of effective synthetic drugs, use of medicinal plants for maintaining human health has acquired a lot of importance in the present era. Though the modern antibiotics brought about a revolutionary change in eradicating the diseases, emergence of new antibiotic resistant pathogens brings up the need for a new antimicrobial active component search. Various phytochemical screening on plants revealed that the secondary metabolites synthesised by plants have many active components which have various properties like antimicrobial, antifungal, antidiabetic, anticancer, antioxidant etc. Hence from various studies the use of medicinal plant compounds in developing a new drug against different diseases has become a new field of research. Apart from anti microbial active components the need of anticancer active component is also increasing day by day. This is because the rate at which cancer is

Author for correspondence: Email: vedahari@scbt.sastra.edu

invading the humankind is very fast and its treatment is effective in slow pace. Due to these reasons search for an anticancer active component has turned to be a necessary factor.

Bougainvillea glabra, also called as paper flower is a climbing evergreen woody ornamental[1] shrub which inhabited to warmer climates is a native to Brazil and now also seen in areas like Middle East, Indian Sub-continent, and North America etc.[2] *B.glabra* from the family of *Nyctaginaceae* belongs to the genus *Bougainvillea* and this genus has 18 species of plants of which three of them *B. spectabilis*, *B. glabra* and *B.peruviana* have gained a lot of importance in the horticulture field. [2,3] *B. glabra* is reported to have a wide range of medicinal properties like anti viral[4], antioxidant, antibacterial [2,5], anti inflammatory[3], anti diabetic[2,6 ,3,7], anti fertility[1] and also considered to be larvicidal[6].

Considering all these facts, we planned for an investigation on *B. glabra* leaves to evaluate its antibacterial and anti cancer activities. Thus the present study mainly aims at the evaluation of antimicrobial anti cancer activity, using extraction of dried leaves.

MATERIALS AND METHODS

Plant materials: *B.glabra* fresh leaves were collected from Trichy, Tamil Nadu, India in the month of December and this was authenticated by Dr. N. Ravichandran, CARISM, SASTRA University, Thanjavur- 613 401.

Drying and Milling: The fresh leaves were dried under shade at room temperature for 2 weeks which helps to prevent the loss of active compounds from plants and mixer was used to ground the dried leaves to coarse powder for better and complete extraction of medicinal compounds from the plant leaves.

Preparation of extract: The powder (45g) obtained by grinding was gently packed and extracted using soxhlet extraction method where petroleum ether (40-60^0 C) was first used to remove the fats and pigmants, and the process was performed for 24 hrs. Further, hydroalcoholic solution in the ratio of 25:75 was introduced to treat the powder using same soxhlet extraction process for 24hrs and the extract obtained was evaporated to get a crude extract devoid of solvents at ambient conditions using water bath.

In vitro Anticancer Activity Evaluation by MTT assay :

Cell culture: The National Centre for Cell Science (NCCS), Pune provided the HeLa cell lines (human cervical adenocarcinoma cell) and this was grown in Eagles Minimum Essential Medium (EMEM) which contains 10% fetal bovine serum (FBS). All cells were maintained at 100% relative humidity at 37 ^{0}C with 5% CO_2 and 95% air.

Cell treatment: To make single cell suspensions, the monolayer cells were treated with trypsin-ethylenediaminetetraacetic acid (EDTA) to detach the cells. The viability of cells were counted using hemocytometer and to make final density of 1x10^5 cells/ml, the cell suspension was diluted using a medium containing 5% FBS. Each well in 96-well plate were seeded with 100µl of cell suspensions at plating density of 10,000cells/well and incubation for cell attachment was performed at same conditions at which cells were maintained. After 24 h the cells were treated with serial concentrations of the test samples. The test samples of different concentrations were prepared by serial dilution method. For this first the test samples were dissolved in pure dimethylsulfoxide (DMSO) then dilutions were carried out using serum free medium. Aliquots of 100 µl of these different drug dilutions were added to to 100 µl of medium present in the wells, gave the required final drug concentrations of 6.25, 12.5,25, 50, 100 µg/ml respectively. Followed by the addition of drugs, the plates

TABLE 1. % Growth Inhibition On HeLa Cell Line Of *B.glabra* Extract By MTT Assay

Sl. No	Concentration Of Extract (µg/ml)	Absorbance (nm)	Growth Inhibition (%)
1.	6.25	0.463 ± 0.009	7.197
2.	12.50	0.455 ± 0.008	8.728
3.	25	0.392 ± 0.003	21.277
4.	50	0.216 ± 0.002	56.528
5.	100	0.11 ± 0.011	76.474

were incubated for an additional 48 h at 37 ^{0}C with 5% CO_2, 95% air and relative humidity of 100%. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay: After 48h of incubation, each well was added with 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) and incubated at 37^{0}C for 4h. The medium with MTT was then flipped off and the formazan crystals formed were then solubilized in 100µl of DMSO. The absoebance was measured at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula[8]:

$$\% \text{ cell Inhibition} = 100 - \left\{ \frac{\text{sample absorbance}}{\text{Control absorbance}} \times 100. \right\} \text{flick}$$

STATISTICAL ANALYSIS

The IC_{50} is half the maximal inhibitory concentration of the toxic compound which results in the reduction of biogical activity by 50%. Nonlinear regression graph was plotted between % Cell inhibition and Log10 of concentration and IC50 was determined using GraphPad Prism software. The differences are considered to be statistically significant when $p < 0.05$.

Antimicrobial Activity Evaluation: Sub culturing of Test Organisms- For the antimicrobial activity, the bacterial strains used were *Salmonella typhi* (Gram negative bacteria), *Staphylococcus aureus* (Gram positive) which were suspended in Nutrient broth and kept for incubation at 37 ^{0}C for 24hrs. Potato dextrose broth was used to suspend the yeast strain *C.albicans* and was incubated for 3days at room temperature.

Sample Preparation: The 0.1ml of *B.glabra* hydro alcoholic extract was dissolved in 2% DMSO and used for the antimicrobial screening.

Agar well diffusion method: The 24h broth cultures of different bacteria are seeded on to the Muller Hinton Agar plates and then bored with 5 wells each of 10mm diameter using sterile cork borer. Each well was added with various concentrations (25, 50, 75, 100mg/ml) of the hydroalcoholic extract along with a control (0.1 ml of 2% DMSO). The standard antibiotic discs were also simultaneously placed into the wells and plants were allowed to diffuse at room temperature for 2hrs. Then plates were incubated at 37 ^{0}C for 18-24hrs and at room temperature for 3 days for bacteria and fungi respectively. The plates were removed at specified time interval and measured for zone of inhibition.[9] By measuring the diameter of zone of inhibition, the antimicrobial activity was determined and compared with both standard and control.

Minimum bactericidal/fungicidal (MBC/MFC) concentration: The MBCs were determined by colony count method. For this a 10µl from each of the culture was sub-cultured onto Muller Hinton agar plates which were then incubated for 24 h at 37 ^{0}C for bacteria and 3-4days at room temperature for fungi. The lowest concentration at which no growth was observed was noted by counting the number of colonies [9].

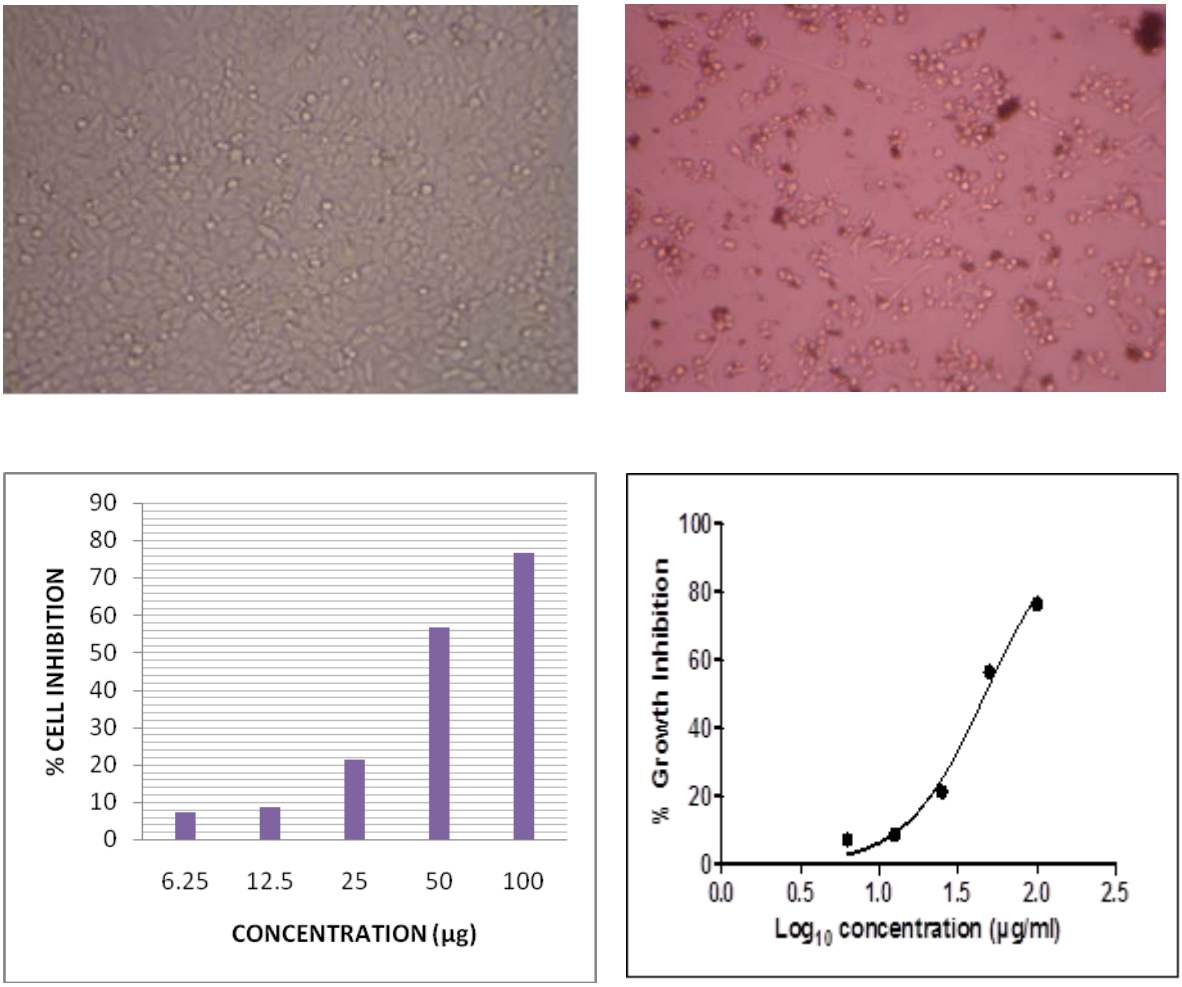

Fig 1. Anticancer effect of B.glabra extract against HeLa cells; a) control cells, b) 100 µg extract treated cells, c) concentration vs % cell inhibition, d) Log_{10}concentration vs % cell inhibition.

RESULTS

In vitro Anticancer Activity: In our present study the impact of hydroalcoholic extract of *B.glabra* on the growth of HeLa cell line was examined by performing MTT assay. After the treatment of cell line with various concentrations of extract, the results from MTT assay shows that there is an exponential increase in the growth inhibition as the concentration is increased. The results of the growth inhibition with increase in concentration against Hela cell line is shown in Table 1 and the fig 1.

At lowest concentration of 6.25µg/ml it showed an inhibition of 7.2% and at highest concentration of 100µg/ml it showed an inhibition of 76.36%. The hydroalcoholic extract of *B.glabra* was showing a promising inhibitory activity against the HeLa cells showing an IC50 value of 47.11µg/ml with a regression of 0.9861.

Antimicrobial Activity: The antimicrobial activity of hydroalcoholic extract of *B.glabra* was determined by the presence or absence of zone of inhibition and its diameter (mm), also minimum bacteriacidal concentration also determined. Results are as shown in Table 2:

From the data we can see that *B.glabra* showed no zone of inhibition for *Salmonella typhi* for 25mg/ml and 50mg/ml concentration of extract but showed good zone of inhibition for 75mg/ml and 100mg/ml. While in the

TABLE 2. Antimicrobial activity of hydroalcoholic extract of *B.glabra* by Agar disc diffusion assay

Extract Conc.(mg/ml)	Microorganism / zone of inhibition (mm)		
	Salmonella typhi (gram negative bacteria)	*Staphylococcus aureus* (gram positive bacteria)	*Candida albicans*
25	R	3	R
50	R	3	4
75	5	4	5
100	7	6	5
Chloramphenicol (10µg /disc)	14	16	_
Fluconazole (10µg /disc)	_	_	10

R: Resistance

case of gram positive bacteria *Staphylococcus aureus* though it started showing zone of inhibition from 25mg/ml, the diameter of inhibition zone was less compared to the gram negative bacteria *Salmonella typhi* at 75mg/ml and 100mg/ml, showing that later is much effective than the former. The results were compared with standard antibiotic Chloramphenicol (10µg /disc) for bacterias. The antifungal activity was also proved by *Candida albicans*, which showed god zone of inhibition for all concentrations except for 25mg/ml, which was compared with the drug Fluconazole (10µg /disc).

Apart from zone of inhibition the antibacterial and antifungal activities were proven with MBC/MFC (minimum bactericidal/ fungicidal concentration) too. The results are as given below:

From the above results of MBC/ MFC confirms that gram negative bacteria *S.typhi* shows better activity than *S.aureus* as former showed MBC from 800mg/ml while later started showing only at 1000mg/ml of extract. In the case of *C.albicans*, it showed MFC at a concentration of 400mg/ml from which we can infer that the hydroacohoic extract is effective against *C.albicans*.

DISCUSSION

There are various plant extracts which shows anticancer effects on various cell lines. Some of them includes, *Sansevieria roxburghiana*'s methanolic leaf extract which was evaluated against HepG2 liver cell line[10], the ethanolic extract of the matured root of *S. baicalensis* against glioma cell lines showed good activity [11], the diethyl ether extract Sukun (*Artocarpus altilis*) wood against Human Breast Cancer (T47D) Cells, the different solvent extract of whole plant of *Acanthus ilicifolius* (Acanthaceae) which was fractioned with different solvents were evaluated against HeLa and KB Cell lines where Ethyl acetate extract showed a good anticancer activity[12].

Our study showed significant anti cancer activity for hydro alcoholic extract of *B.glabra* with an IC_{50} of 47.11 µg/ml and % cell growth inhibition of 76.36 5 at 100 µg/ml. There are various other studies that support the results of the present study. Some of these studies include the ethanolic extacts of leaves of *C.parviflorum* which was examined for cytotoxic activity against two cell lines DLA and Hela cell where the results showed significant growth inhibition with IC50 61.24µg/ml for the former cells and 43.15µg/ml for the latter [13]. Similar results were also detailed in Raval P. Bhuvan et al. where the anticancer activity of bark extracts of *Symplocos*

TABLE 3. Minimum Bactericidal/Fungicidal Concentration of *B.glabra* hydroalcoholic extract diffusion

Sl No	Strains Used	Minimum Bactericidal/ Fungicidal Concentration (mg/ml)				
		200	400	600	800	1000
1.	*Salmonella typhi*	_	_	_	+	++
2.	*Staphylococcus aureus*	_	_	_	_	+
3.	*Candida albicans*	_	+	+ +	+ +	++

growth of fungi/bacteria + = lowest concentration with bactericidal/ fungicidal activity ++ = Concentrations showing increase in bactericidal activity

racemosa Roxb. (Symplococaceae) in different solvents which was assessed against HL60 (Human leukemia cell line) and HeLa (Human cervix cancer cell). In the above study they found that Butanolic extract showed good cytotoxic activity against HL60 (Human leukemia cell line) with IC_{50} 27183 ng/ml and HeLa (Human cervix cancer cell Line) with IC_{50} = 22861 ng/ml whereas Ethyl acetate extract showed less cytotoxic against HL 60 and HeLa with IC_{50} 117084 ng/ml and 137151 ng/ml respectively [14]. In another study on anticancer and cytotoxic activity against HeLa cells using saponins isolated from leaf extracts of *Gymnema sylvestre and Eclipta prostrata* also showed supportive results. The gymnemagenol from *Gymnema sylvestre* showed IC_{50} of 37 µg/ml and dayscyphin C from *Eclipta prostrata* showed IC_{50} 50 µg/ml and maximum cell death shown was 73% for the former and 53% [15]. Hence from above discussions it can be reported that *B.glabra* can be a potent anticancer agent for further studies.

The anti microbial activity showed by the extract of *B.glabra* gives a strong evidence for its use for various infectious diseases in folk medicine. The MBC/MFC performed helped to confirm the results of anti microbial activity shown by agar disc diffusion method. This plant seems to have very good anti fungal activity; hence apart from being an ornamental plant it also plays a role of medicinal plant.

CONCLUSION

Thus the present study on anticancer and antimicrobial activity of hydroalcoholic extract of *B.glabra* shows strong evidence to become a potent natural remedy against cancer and infectious diseases. The strong activities witnessed in this plant may be due to the presence of certain phytochemical compounds present in it. Hence from present study we can conclude that an ornamental plant like *B.glabra* can also play an important role as medicinal plant. The isolation and structural elucidation of individual compounds can be performed to quantify the activity; also *in vivo* animal studies can be performed to confirm the activity.

ACKNOWLEDGEMENT

The authours express their sincere gratitude towards the authorities of SASTRA University, Thanjavur, India, for their extensive support and help for the successful completion of this work. Authors would also wish to acknowledge KMCH college of Pharmacy, Coimbatore, India, for the facilities availed to perform the anti cancer study.

REFERENCES

1. Mishra N, Joshi S, Tandon VL, Munjal A. Evaluation of Antifertility Potential of Aqueous Extract Of *Bougainvillea spectabilis* Leaves in Swiss Albino Mice. International Journal Of Pharmaceutical Sciences and Drug Research. 2009; 1(1): 19-23.
2. Bhat M, Kothiwale SK, Tirmale AR, Bhargava SY, Joshi BN. Antidiabetic Properties of *Azardiracta indica* and *Bougainvillea spectabilis* : *In Vivo* Studies in Murine Diabetes Model. Evidence-Based Complementary and Alternative Medicine 2011; Article ID 561625, 9 pages. doi:10.1093/ecam/nep033.
3. Adebayo JO , Adesokan AA, Olatunji LA, Daniel O. Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biokemistri 2005; 17(1):45-50.
4. Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battelli MG *et.al.*, New ribosome-inactivating proteins with polynucleotide:adenosine glycosidase and antiviral activities from Basella rubra L. and Bougainvillea spectabilis Willd. Planta 1997; 203: 422-429.
5. Adebayo GI, Alabi OT, Owoyele BV, Soladoye AO. Anti-diabetic Properties of the Aqueous Leaf Extract of Bougainvillea glabra (Glory of the Garden) on Alloxan-Induced Diabetic Rats. Rec. Nat. Prod. 2009; 3(4): 187-192.
6. Saikia H, Lama A. Effect of Bougainvillea spectabilis Leaves on Serum Lipids in Albino Rats Fed with High Fat Diet. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(2): 141-145.
7. Malomo SO, Adebayo JO, Arise RO, Olorunniji FJ, Egwim EC. Effects of Ethanolic Extract of *Bougainvillea spectabilis* Leaves on Some Liver and Kidney Function Indices in Rats. Phytochemistry & Pharmacology-III. 2006; 17: 261-272.
8. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65, 55-63.
9. Anwer N, Salik S, Ahmed D. Antibacterial Activity of Otostegia limbata Int. J. Agric. Biol. 2009;11(5): 647-650.
10. Philip D, Kaleena PK, Valivittan K. Invitro Cytotoxicity And Anticancer Activity Of *Sansevieria Roxburghiana*. International Journal of Current Pharmaceutical Research.2011; 3(3): 71-73.
11. Scheck AC, Perry K, Hank NC, Clark WD. Anticancer activity of extracts derived from the mature roots of *Scutellaria baicalensis* on human malignant brain tumor cells. BMC Complementary and Alternative Medicine 2006, 6:27.
12. Khajure PV, Rathod JL. Potential Anticancer Activity Of *Acanthus ilicifolius* Extracted From The Mangroves Forest Of Karwar, West Coast Of India. World Journal of Science and Technology 2011, 1(1): 01-06.
13. Purushoth Prabhu T, Panneerselvam.P, Selvakumari.S, Sivaraman.D, Invitro and Invivo anticancer activity of Ethanolic extract of *Canthium parviflorum* Lam on DLA and Hela cell lines. Int. J. Drug Dev. & Res., Oct-Dec 2011, 3 (4): 280-285.
14. Raval PB, Patel DJ, Patel AB, Ganure LA. Potent In Vitro Anticancer Activity Of *Symplocos Racemosa* Bark. Rom. J. Biol. – Plant Biol., Volume 54, No 2, P. 135–140, Bucharest, 2009.
15. Khanna VG, Kannabiran K. Anticancer-cytotoxic activity of saponins isolated from the leaves of *Gymnema sylvestre* and *Eclipta prostrata* on HeLa cells. Int J Green Pharm 2009;3:2