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Research Article

# Evaluation of Anti-Microbial and Anti-Cancer Activity of Ethanolic Extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana*

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## Abstract

The present study aims to evaluate the antimicrobial and anticancer activity of ethanolic extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana.* The ethanolic extracts of both the plants showed antimicrobial activity against gram positive bacteria, gram negative bacteria and yeast. The antifungal activity of the extracts was measured as extent of mycelial growth and sporulation. By 96 h of incubation, the mycelial growth in control plates showed 3.8 fold increase and extensive sporulation whereas in plates pre-coated with ethanolic extracts of *B. shubhra* and *B. peruviana*, the fold increase in mycelial growth was 2.9 and 2.7 fold respectively with restricted mycelial sporulation. To study the anticancer activity of ethanolic extracts, Hela cells were treated with 20, 60 and 100 µg/ml of extracts. No inhibitory effects of the extracts were evident till 24 h incubation. After 48 h of treatment, 20 and 60 µg/ml of extracts showed no distinct changes in cells morphology, however at 100 µg/ml dose the cell number diminished, morphology was significantly altered and few floating (non-adherent) cells suggesting apoptotic cell population was observed. The cell viability measured by trypan blue exclusion method showed considerable decrease in viable cells at 100 µg/ml concentration of extracts. MTT assay showed a dose dependent inhibition of proliferation of the Hela cells. At 100 µg/ml concentration, the ethanolic extracts of *B. shubra* and *B. peruviana* showed 65% and 54% of growth inhibition respectively. These results suggest that both *B. shubra* and *B. peruviana* posses anticancer activity.

**Keywords:** Antibacterial; Antifungal; Anticancer; *Bougainvillea Shubhra; Bougainvillea peruviana*

## Introduction

Antibiotics (antimicrobial drugs) are widely used for the treatment and prevention of bacterial infections. These antimicrobial drugs may either have a bacteriostatic or bacteriocidal effect against different pathogenic bacteria. With the advent of discovery of new antibiotics the course of treating various pathogenic infections has revolutionized. However, the misuse of these antimicrobial drugs due to their easy accessibility, self medication, prescription of higher doses etc. have led to serious problems like emergence of drug resistance pathogens.

Multidrug resistance (MDR) specifically for methicillin-resistant *Staphylococcus aureus* (MRSA) is usually defined as resistance to three or more antibiotics [1] and the global emergence of MDR's is increasingly limiting the efficacy of the existing antibiotic drugs [2] e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), pneumococci resistant to penicillin and macrolides, and vancomycin-resistant *Enterococci* spp as well as multi-drug resistant Gram-negative organisms [3]. Infectious diseases caused by such antimicrobial-resistant microbes (ARM) have been frequently reported over last few years [4].

Various medicinal plants known to man-kind ought to be more promising for boosting immune system and maintaining human health. Phytochemical analysis has revealed presence of bioactive compounds viz flavonoids, alkaloids, tannins and polyphenols, which are known for their antioxidant and antimicrobial properties [5,6]. The efficacy of these plants in curing various ailments is well established and a large volume of work has been done in this field by researchers in India and abroad [7-9].

Several other studies report the ability of non-medicinal plants to synthesize various secondary metabolites that play an important role in plants defense mechanisms against predation by microorganisms, insects and herbivores [10]. Phytochemical screening of these plant secondary metabolites showed the presence of many active components which have antimicrobial, antifungal, anti-diabetic, anticancer, antioxidant potential etc. Hence, screening plants metabolites for developing a potential drug against various diseases has become a new field of research.

*Bougainvillea* belongs to *Nyctaginaceae* family and is a popular ornamental plant widely grown in tropical or subtropical regions.

Over 300 varieties of *bougainvillea* are grown around the world. *Bougainvillea. spectabilis, B. glabra* and *B. peruviana* are some of the horticulturally important species reported. Different hybrid species and cultivars of this genus have also been produced. Leaves, flowers, roots etc. of these plants have been explored for presence of active components.

Most of the phytochemical studies done on *B. spectabilis* and *B. glabra* leaves extract have shown good source of antioxidants with various biological activities like anticancer, antidiabetic, anti-inflammatory, antimicrobial etc. [11-13]. Islam MZ., *et al.* 2016 [14], reported *in-vitro* antioxidant and antimicrobial activity of B. glabra flower extracts. An antiviral protein identified in the root tissues of *Bougainvillea spectabilis* Wild was shown to be active against mechanical transmission of tomato spotted wilt virus [15].

In our previous study, phytochemical analysis of ethanolic leaves extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana* were shown to exhibit good antioxidant and anti-lipid peroxidation activity [16]. Our present study aims to evaluate the antimicrobial and anticancer activity of these extracts.

## Materials and Methods

### *Bougainvillea* extracts preparation

Dried powdered leaves of *Bougainvillea shubhra* and *Bougainvillea peruviana* plants were subjected to successive solvent extraction using soxhlet method [16]. The ethanolic extracts were concentrated by evaporation, reconstituted in DMSO and aliquots were stored at -20°C. These aliquots were suitably diluted using DMSO, filter sterilized and then used for evaluating antimicrobial and anticancer activity.

### Microbial cultures

All bacterial cultures viz *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella sonnie*, were grown on nutrient agar (HiMedia) and fungal and yeast cultures viz *Aspergillus niger, Saccharomyces cerevisiae* and *Candida albicans* were grown on Sabourauds agar (HiMedia). All microbial cultures were maintained at 4°C and sub-cultured at intervals.

### Evaluation of antimicrobial activity

The antimicrobial activity of the ethanolic extracts of *B. shubhra* and *B. peruviana* was determined using agar well diffusion method. Cell density of overnight grown microbial cultures was adjusted to $10^8$ cells/ ml. 0.1 ml of bacterial and yeast cultures were spread plated on Muller Hinton agar (HiMedia) and Sabourad agar plates respectively. Wells were bored and 40µl of extracts were added in respective wells. DMSO was used as vehicle control. After pre-diffusion at 4°C for 15 min, plates were incubated at 37°C for 24h. The antibacterial activity of the extracts was measured as zone of inhibition (mm).

### Evaluation of antifungal activity

Spore suspension of *Aspergillus niger* was spread plated on Sabourauds agar medium and incubated at room temperature for 48 h to obtain mycelial growth. Using a sterile cork borer, mycelial growth was scooped as discs (approx 10 mm) and placed on fresh Sabourauds agar medium pre-coated with 100µl of plant extract [17]. The extent of mycelial growth and sporulation was recorded in control and plates pre-treated with plant extract and fluconazole (positive control). The antifungal activity of extracts was a measure of extent of mycelial growth expressed in mm.

### Cell culture

HeLa cells were procured from NCCS, Pune. Cells were grown in Minimal essential medium (MEM) supplemented with 10% FBS (v/v) fetal bovine serum, in a humidified atmosphere at 37°C with 5% $CO_2$. When cells density reached near 70% confluency, cells were trypsinized and used for experiments.

Cell viability testing by Trypan Blue exclusion method $1x10^5$ cells/ ml were seeded per well in a six-well plate and were treated with 1% DMSO (vehicle control), 20, 60 and 100 µg/ml of ethanolic extracts of *B. shubhra* and *B. peruviana*. After treatment for 48 h, cells were harvested by trypsinization, washed with 1X PBS and final cell pellet was suspended in 1 ml PBS. 18µl of the cell suspension was mixed with 2µl of trypan blue and viable and non-viable cell count was done using a haemocytometer.

### MTT assay

The anti-proliferative activity of ethanolic extracts was studied by MTT assay [18] with slight modification. 3000 cells of Hela were seeded per well in 96 well plate and allowed to adhere. After 24 h of seeding, cells were treated with 20-100 µg/ml of ethanolic extracts of *B. shubra* and *B. peruviana*. A negative control (growth medium lacking extract) and vehicle control (medium containing 1% DMSO) were also maintained for each experiment. After 48 h of treatment, 20 µl of MTT solution (5 mg/ml in 1 X PBS) was added per well and incubated for 4 h to allow formation of formazon crystals. The spent media from each well was removed carefully and the crystals were dissolved by adding 100 µl of DMSO. The absorbance was measured on an ELISA plate reader at 540 nm with a reference wavelength of 690 nm.

## Results and Discussion

In the present study the ethanolic leaves extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana* were evaluated for their antimicrobial and anticancer activity.

The antimicrobial activity of the ethanolic extracts was tested by agar well diffusion method. Ethanolic extracts of both the plants showed inhibitory action against most of the gram positives, gram negatives and yeasts viz *Candida albicans* and *Saccharomyces*

cer*evesiae* (table 1) suggesting the presence of phytochemical compounds with broad spectrum inhibitory activity. There are reports suggesting antibacterial activity of leaves extract of different Bougainvillea species. The antibacterial activity of ethanolic extracts of *B. spectabilis* was reported by Umamaheshwari., *et al.* 2008 and Ban Abduljabbar Sidkey, 2018 [10,19]. Hydroalcoholic extract of Bougainvillea glabra 'Snow White' and *Bougainvillea glabra* 'Choicy' showed inhibitory effect against all gram positive and gram negative bacteria [20].

| Bacterial and Yeast Species | *B. shubra* | *B. peruviana* |
|---|---|---|
| | Zone of inhibition (mm) | |
| *E. coli* | 14 | 13 |
| *S. typhi* | 9 | 13 |
| *P. aeruginosa* | 15 | 14 |
| *S. sonnie* | 15 | 14 |
| *B. subtilis* | 15 | 14 |
| *S. aureus* | 16 | 15 |
| *Candida albicans* | 14 | 13 |
| *S. cerevisiae* | 16 | 15 |

**Table 1:** Antimicrobial activity of ethanolic extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana*.

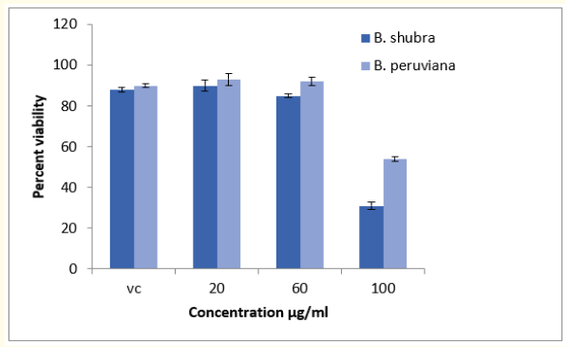
The ethanolic extracts of *B. shubhra* and *B. peruviana* were also evaluated for their antifungal activity wherein the extent of mycelial proliferation and sporulation in media coated with plant extracts and standard antifungal agent was compared with control media (Table 2). The extent of mycelial growth was same in control and ethanolic extract-treated plates after 24 h and no mycelial sporulation was seen. At 96 h, the mycelia in control plates showed extensive sporulation and about 3.8 fold increase in mycelial growth. The mycelial growth in fluconazole-treated plates was constrained showing only 2.6 fold increase and minimal sporulation. In plates coated with ethanolic extracts of *B. shubhra* and *B. peruviana* the fold increase in mycelial growth was 2.9 and 2.7 respectively suggesting the antifungal activity of both the extracts. Kumara SM., *et al.* 2012 [21] has reported antifungal activity of chloroform and ethanolic extracts of *B. spectabilis* flowers.

| Time period (h) | Control | *B. shubra* | *B. peruviana* | Fluconazole |
|---|---|---|---|---|
| | Extent of mycelial growth (mm) | | | |
| 24 | 19 ± 0.471 | 19 ± 1.09 | 19 ± 0.5 | 12 ± 0.5 |
| 48 | 48 ± 0.471 | 31 ± 0.816 | 30 ± 0.471 | 18 ± 0.5 |
| 96 | 73 ± 0.471 | 55 ± 1.414 | 51 ± 0.471 | 31 ± 0.471 |

**Table 2:** Antifungal activity of ethanolic extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana*. The extent of inhibition of mycelial growth is measured as mean ± SD.

To study the anticancer potential of ethanolic extracts of *B. shubhra* and *B. peruviana*, Hela cells were treated with 20, 60 and 100 µg/ml of extracts and observed up to 48h. After 24 h of treatment, the control and vehicle control (DMSO) treated cells appeared normal and showed no changes in the cells morphology. Even the morphology of cells treated with extracts did not show any significant changes. On further incubation till 48 h, the morphology of control and DMSO treated cells remained unaltered. Lower concentrations of ethanolic extracts also showed no distinct changes in cells morphology, however at 100 µg/ml concentration the cell number diminished, morphology was significantly altered and few apoptotic cell population was seen (Figure 1). The viable cell count by trypan blue exclusion method showed 90% percent cell viability in DMSO-treated cells. The cell viability at 20 and 60 µg/ml of extracts was equivalent to control, however at 100 µg/ml the cell viability decreased considerably viz 30.66% for *B. shubra* and 54.66% for *B. peruviana* (Graph 1).

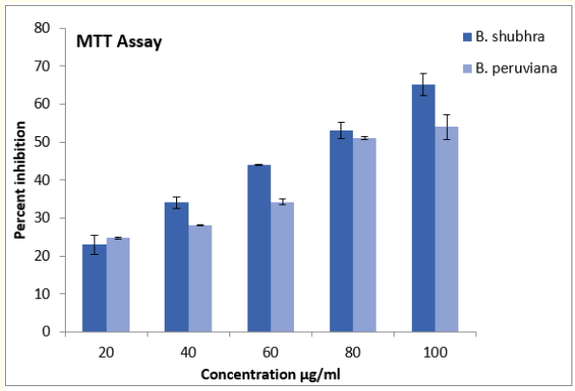
**Figure 1:** Morphology of Hela cells treated with ethanolic extracts of *Bougainvillea Shubhra* (WL) and *Bougainvillea peruviana* (PL).




**Graph 1:** Percent viability of Hela cells by Trypan Blue exclusion method.

The anti-proliferative effect of extracts was studied by MTT assay. Both the extracts exhibited inhibitory effect in a dose dependent manner. The percent growth inhibition of Hela cells at 100 µg/ml concentration was found to be 65% and 54% for *Bougainvillea*

*shubra* and *Bougainvillea peruviana* extracts respectively (graph 2), suggesting the anticancer potential of both the plants. Joshny., *et al.* 2012 [11] reported significant anti- cancer activity for hydro alcoholic extracts of *B. glabra* with an IC50 of 47.11 µg/ml and 76.365 % cell growth inhibition at 100 µg/ml. Liang., *et al.* 2017 [22] reported anticancer activity of *Bougainvillea spectabilis* Wild against different cancer cell lines.




**Graph 2:** Effect of ethanolic extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana* on Hela cells by MTT assay

## Conclusion

This study has evaluated the ethanolic extracts of *Bougainvillea shubhra* and *Bougainvillea peruviana* for its antibacterial, antifungal and anticancer activity. The inhibitory action against wide range of bacteria and fungi suggest that both these plants posses potential as antibacterial and antifungal agent. Both the plant extracts also possess a potent anticancer activity. The potent anticancer and antimicrobial activity of both the plants may be attributed to the presence of antioxidants [16]. Thus from the present study we can conclude that ornamental plants like *B. shubhra* and *B. peruviana* can also serve as important source of medicine. However further studies to isolate and characterize these phytochemical constituents need to be done.

## Conflicts of Interest

The authors have no conflicts of interest.

## Authors Contribution

MH: Literature search, concept and plan of study, maintenance of cell lines, standardization of assays for anticancer activity, supervision of study, interpretation and presentation of data, manuscript preparation, critical and final revision of manuscript. MV: Literature survey, concept and plan of study, cell line studies, standardization of assays for anticancer activity, supervision of study, interpretation of data. MP and DM: preparation of extracts, performing and standardizing antimicrobial assays, data collection and calculation, statistical analysis of data.

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## Bibliography

1. Styers D., *et al*. "Laboratory-based surveillance of current antimicrobial resistance patterns and trends among Staphylococcus aureus: 2005 status in the United States". *Annals of Clinical Microbiology and Antimicrobials* 5 (2006): 2.

2. Hancock RE. "Mechanisms of action of newer antibiotics for Gram positive pathogens". T*HE LANCET Infectious Disease* 5.4 (2005): 209-218.

3. Norrby RS., *et al*. "Lack of development of new antimicrobial drugs: A potential serious threat to public health". *The Lancet Infectious Diseases* 5.2 (2005): 115-119.

4. Vila J and Pal T. "Update on Antibacterial Resistance in Low-Income Countries: Factors Favoring the Emergence of Resistance". *The Open Infectious Diseases Journal* 4 (2010): 38-54.

5. Fawole FJ., *et al*. "Evaluation of antioxidant and antimicrobial properties of selected Indian medicinal plants". *International Journal of Medicinal and Aromatic Plants* 3.1 (2013): 69-77.

6. Edeoga HO., *et al.* "Phytochemical constituents of some Nigerian medicinal plants". *African Journal of Biotechnology* 4.7 (2005): 685-688.

7. AK and Malik A. "Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms". *Food Chemistry* 126 (2011): 228-235.

8. Daniele C., *et al*. "Vitex agnus castus: A Systematic Review of Adverse Events". *Drug Safety* 28 (2005): 319-332.

9. Chanda S., *et al*. "Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four Cassia species". *African Journal of Biotechnology* 11 (2012): 2490-2503.

10. Umamaheswari A *et al*., "In vitro Antibacterial Activity of Bougainvillea spectabilis Leaves Extracts". *Advances in Biological Research* 2.1-2 (2008): 1-5.

11. Joshny J., *et al*. "Anti-Cancer and Anti-Microbial Activity of Hydro Alcoholic Extract of Bougainvillea glabra". *International Journal of Current Pharmaceutical Research* 3.4 (2013): 79-85.

12. Venkatachalam RN., *et al*. "Bougainvillea spectabilis, a good source of antioxidant phytochemicals". *RJPBCS* 3.3 (2012): 605-613.

13. Joshi DD., *et al*. "Anti-inflammatory activity of Bougainvillea spectabilis leaves". *Indian Journal of Pharmaceutical Sciences* 46.5 (1984): 187-188.

14. Islam MZ., *et al*. "In-vitro Antioxidant and Antimicrobial Activity of Bougainvillea glabra Flower". *Research Journal of Medicinal Plant* 103 (2016): 228-236.

15. Balasaraswathi R., *et al*. "An antiviral protein from Bougainvillea spectabilis roots; Purification and characterization". *Phytochemistry* 47.8 (1998): 1561-1565.

16. Medpilwar M., *et al*. "In-vitro Antioxidant and Anti-lipid Peroxidation Activity of Ethanolic Extracts of Bougainvillea shubhra, Bougainvillea peruviana and Bougainvillea bhuttiana Golden Glow: A Comparative Study". *Journal of Natural Remedies* 15.1 (2015): 43-48.

17. Abdurrahman O., *et al.* "Antifungal Activity of Some Plant Extracts against Different Plant Pathogenic Fungi". *International Journal of Advances in Agricultural and Environmental Engineering* 3.2 (2016): 284-287.

18. Mosmann T. "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays". *Journal of Immunology Methods* 65.1-2 (1983): 55-63.

19. Ban Abduljabbar S. "Antibacterial and Antioxidant properties of Bougainvillea spectabilis L. and Myrtus communis L. Leaves extracts". *Global Journal of Bioscience and Biotechnology* 7.3 (2018): 336-342.

20. Gupta V., *et al*. "Evaluation of antibacterial activity of Bougainvillea glabra 'snow white' and Bougainvillea glabra 'choicy'". *Journal of Chemical and Pharmaceutical Research* 1.1 (2009):233-237.

21. Kumara Swamy M., *et al*. "Phytochemical screening and in vitro antimicrobial activity of Bougainvillea spectabilis flower extracts". *International Journal of Phytomedicine* 4 (2012): 3.

22. Liang C., *et al*. "In vitro anticancer activity and cytotoxicity screening of phytochemical extracts from selected traditional Chinese medicinal plants". *JBUON* 22.2 (2017): 543-551.

**Volume 4 Issue 1 January 2020**