Antibacterial Activity of the Leaves of Bougainvillea spectabilis against E. coli NCIM 2832 and M. aureus NCIM 5021

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ABSTRACT
The significance of phytochemicals in chemotherapy is well known. However, most of these are used for systemic applications. There is an equally important need to search affordable source of such phytochemicals which can be used topically as disinfectants primarily as first aid dressing, prior to being treated by registered medical practitioners. This study aims to find one such disinfectant from the leaf extracts of a very common plant like Bougainvillea spectabilis which can be used as an ethanolic extract (tincture). It has been observed to retard the growth of a common gram negative organism like E.coli and a gram positive organism like M. aureus, giving enough time for the patients to be shifted for treatment by appropriate medicines.

Keywords: Phytochemicals, antibacterial activity, Bougainvillea spectabilis.

INTRODUCTION
Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years1. Many medicinal plants are used daily in Ayurvedic practices. In India more than 7,000 medicinal plant species are known. These plants contain many biological active compounds having antimicrobial property and were used as a antimicrobial drugs in traditional medicines. According to a report of World Health Organization, more than 80% of world’s populations depend on traditional medicine for their primary health care needs2. Recently, medical practitioners are switching over to such plant based medicines, as there is a high rate of developing of resistant pathogens due to excessive selection pressures created by misuse and rampant use of classical antimicrobials like antibiotics.

The genus Bougainvillea is a native of South America and derived its name from Louis Antione de Bougainville (1729–1811), who encountered the plant in Brazil in 1768 and first introduced it to the rest of the world3. The genus Bougainvillea in the Nyctaginaceae (4 O’ clock) family of plants has 18 species, with three that are horticulturally important B. spectabilis, B. glabra and B. peruviana4.

The Bougainvillea spectabilis reported to have medicinal properties viz. anti-diabetic5, due to the presence of D-pinitol (3-O-methylchiroinositol)6, antiviral7, anti-inflammatory8, antioxidant9 and anti-fertility potential10. Similarly, B. glabra is reported to have antiulcer, anti-diarrhoeal, and anti-microbial properties11. Leaves and inflorescence of Bougainvillea glabra, have been used in Mexican traditional medicine as a remedy for minor upper and lower respiratory tract illnesses, such as cough, cold, bronchitis12,13. Its antimicrobial effect can be associated with the presence of betalains pigments14, as well as steroidal compounds with anti-inflammatory activity15. Gupta (2009)16 found that a 500 g/disc hydro alcoholic extract from leaves of B. glabra was active against some pathogenic microorganisms.

The present investigation attempts to determine the possible antibacterial activity of aqueous and solvent extracts of Bougainvillea spectabilis leaves.

There are plenty of reports of its medicinal uses of the bark. However, there is little report about the pharmacological properties of the leaf extracts.

MATERIALS AND METHODS
Plant Material
The fresh green leaves of Bougainvillea spectabilis visibly free from disease and weighing 1gm or more were collected locally.

The botanical identity of the plant of Bougainvillea spectabilis was authenticated from botany departament of Smt. K. W. College, Sangli(India) by Dr. K. S. Patil.

Preparation of Extract
The leaves were washed thoroughly several times with potable water and then air dried. Dried leaves were pulverized to fine powder, which was soaked (20 g) separately in 100ml of ethanol, 100ml of acetone and also in similar quantity of distilled water for 24 to 48hrs.

This was then filtered and centrifuged at 7000 x g for 10mins to remove all insoluble materials.

The clear supernatant was stored at 4°C for further studies.

Test Microorganisms
The bacterial cultures used were Escherichia coli NCIM 2832, Micrococcus aureus NCIM 5021.
**Antibacterial Activity**

The growth pattern of the test organisms were checked by growing these in sterile liquid medium containing meat extract 0.5%, peptone 1% and NaCl 0.5% at pH 7.0. The organisms were added at 10% level (v/v), with a cell density of $6 \times 10^8$ cells/ml along with 10 ml of the respective extract.

Similarly second set of experiments were set, where instead of extracts, same quantity of the respective solvents were added. This was the solvent control set.

The third set of flasks was used where 10 ml of extracts were added but no organism was inoculated. This was essential for adjusting zero reading of the spectrophotometer.

The flasks were incubated for 150 minutes on a rotary shaker at 120 r.p.m.

The growth was monitored by measuring absorbance at 540 nm at 30 minutes interval.

The solvent extracts were also subjected for GC MS analysis to identify the compounds showing antibacterial properties.

**Statistical Analysis**

All the experiments were repeated 3 to 5 times till the data obtained were statistically valid by checking the variance with Tukey Kramer multiple comparison test.

**RESULTS AND DISCUSSION**

**Figure 1:** Growth pattern of *E. coli* in presence of ethanolic extracts (♦) and acetone extracts (■) of the leaves. A positive control (▲) was kept where in no leaf extracts were added.

The objective of this study was to find out exactly what was the response of the *Escherichia coli* NCIM 2832, *Micrococcus aureus* NCIM 5021 growing in presence of the aqueous and solvent extracts of the leaves of *Bougainvillea spectabilis*, by studying their growth patterns.

**Figure 2:** Growth pattern of *M. aureus* in presence of ethanolic extracts (♦) and acetone extracts (■) of the leaves. A positive control (▲) was kept where in no leaf extracts were added.

It can be noted from the results (Fig.1 and Fig.2) that there is a significant reduction in the growth rate of both the organisms in presence of ethanolic extract and acetone extract but no inhibition was observed. It was not possible to show such effects from agar-well diffusion method.

**Figure 3:** Growth pattern of *E. coli* in presence of aqueous extracts (♦) of the leaves. A positive control (▲) was kept where in no leaf extracts were added.

**Figure 4:** Growth pattern of *M. aureus* in presence of aqueous extracts (♦) of the leaves. A positive control (▲) was kept where in no leaf extracts were added.
However, aqueous extracts of the leaves did not show any significant reduction in growth rate of either of the organisms as shown in Fig.3 and Fig.4. Infact, there was an activation of growth in case of E. coli.

The GC MS analysis showed the presence of Myristic acid, Palmitic acid and Phytol in the ethanolic extract of plant. The reduced growth rate of E. coli and M. aureus was primarily due to the presence of palmitic acid and to some extent by palmitic acid. These observations concur with the ones reported by16. Phytol being a diterpene also has antibacterial properties57 and possibly this too had the effect of bringing down the growth rate. However, none of these compounds are soluble in water and hence, the absence of antimicrobial activity of the aqueous extract probably be due to this reason. The activated growth of E. coli is probably due to the readily available and easily metabolizable carbohydrates from the leaf.

CONCLUSION

It can be thus concluded that as per the observations, the solvent extracts (other than water) of the leaves of Bougainvillea spectabilis are good enough for use as a first aid disinfectant for minor wound dressings and the injured individual must be shifted to a medical center for proper medication. This is due to the fact that the extracts can only retard the growth rate of any infecting organisms. Such disinfectants could to some extent replace the normal formulations like carbolic acid and cetrimide, especially when these are not available immediately and handy.

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REFERENCES


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