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Phytochemical analysis and inhibitory activity of Ornamental Plant (*Bougainvillea spectabilis*)

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ABSTRACT

Plants are the reservoir of effective chemotherapeutics and can provide valuable sources of natural antimicrobials. Bougainvillea is an ornamental plant that has also been used in traditional medicine as an anti-inflammatory, antidiabetic, antibacterial and antiviral agent. The purpose of this study was to make the phytochemical analysis and test the antimicrobial activity of Bougainvillea- spectabilis stem, leaves and flowers against Salmonella typhi. The phytochemical constituents of the dried powdered plant parts were extracted using aqueous and organic solvents (acetone, ethanol, methanol and chloroform) through the soxhlet and rotary evaporator. The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition by using the paper disc diffusion method. The results of the phytochemical analysis revealed the presence of alkaloid, flavonoids, glycosides, phlobotannins, saponins, steroids, tannins and terpenoids in distilled water, acetone chloroform, ethanol and methanol extracts of stem flowers and leaves extracts. Among different parts of Bougainvillea, leaves showed highest activity followed by flowers and stem. Stem extracts activity was higher in methanol. Leaves showed highest activity in ethanol while flowers extracts highest activity was observed in methanol and ethanol. Bougainvillea spectabilis has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

Key words: Antimicrobial susceptibility, *Bougainvillea spectabilis*, Disc diffusion method, *Salmonella typhi*

INTRODUCTION

Antimicrobial substances inhibits the growth of microorganisms such as bacteria, fungi, protozoans, etc. [1]. Synthetic chemicals have their side effects and the development of bacterial resistance [2] to the presently available antibiotics has necessitated the screening of several plant-based antimicrobials [3].

Plants have an infinite ability to produce aromatic substances, most of which are secondary metabolites and phenols or their oxygen substituted derivatives of which at least 12,000 have been isolated, a number likely to be less than 10% of the total. These substances act as plant defense mechanisms against predation by microorganisms, insects and herbivores. The pharmaceutical industry has come to consider them as a source of bioactiveagents [4, 5], which have gained considerable importance due to their potential as antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities[6].

Bougainvillea belongs to the *Nyctaginaceae* (4 O' clock) family of plants and has 14 species. Horticulturally important species are *Bougainvillea spectabilis* (*B. spectabilis) B. glabra* and *B. peruvina*. The leaves of *B. spectabilis* are reported to have antidiabetic, hepatoprotective, antiviral, antibacterial and insecticidal properties.

Reported constituents are flavonoids, betacyanine, alkaloids and tannins which are used as a medicine for variety of disorders [7, 8].

Salmonella is a gram-negative, bacillus belonging to the family *Enterobacteriaceae*. *Salmonella typhi* (*S. typhi*) is the most highly host adapted organism of concern in human medicine and is the cause of human typhoid fever. It is contracted from food or water that has been contaminated in some manner by another human being [9].

This study was designed to examine the antimicrobial effects of *B. spectabilis* against *S. typhi*. The antimicrobial potential justify the use of the plant by traditional medicine practitioners for the treatment of typhoid fever and other gastrointestinal infections.

MATERIALS AND METHODS

Flowers stem and leaves of *B. spectabilis* were collected, dried and blended in an electrical blender. Dry powder of the each plant material was weighed and extracted successively with different solvents (chloroform, methanol, ethanol, acetone and water) using soxhlet extractor and rotary evaporator. Antimicrobial activity was tested in four concentrations (25%, 50%, 75% and 90%) of each extract.

Phytochemical analysis

Phytochemical analysis of *B. spectabilis* leaves was carried out for the presence of chemical components such as, saponins, tannins, alkaloids, glycosides, flavonoids, terpenoids and phlobotannins [10].

Test for tannins: About 0.5 g of the dried powdered samples were boiled in 20 ml distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for blue-black or a brownish green coloration.

Test for phlobotannins: Aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate was taken as evidence for the presence of phlobatinins.

Test for saponins: Two g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion indicates the presence of saponins.

Test for flavonoids: Two ml aqueous filtrate of each plant extract was added to 5 ml of dilute ammonia solution. Few drops of concentrated H_2S0_4 were added before observation. A yellow coloration observed in each extract indicated the presence of flavonoids. But it was disappeared on standing.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The color change from violet to blue or green indicates the presence of steroids.

Test for terpenoids: Five ml of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for alkaloids: In 250 ml beaker, five gram of the sample and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. After filtration the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract and precipitation was observed. Precipitate formation was taken as alkaloids positive.

Antimicrobial susceptibility test

Inoculum preparation: Inoculum was prepared by taking colonies of *S. typhi* and transferring them to the tubes containing nutrient broth and incubated.

Measurement of zone of inhibition

The discs were saturated with different concentrations (25 %, 50 %, 75 % and 100 %) of each extract. Discs were placed onto the Nutrient and Mc Conkey agar plates, swabbed with the culture of *S. typhi*. Then the plates were inverted and incubated at 37 °C for 18, 24 and 48 hrs. The diameter of the zones of complete inhibition was measured.

MIC Determination

MIC was used to semi quantitatively measure the activity of *B. spectabilis*. Lowest concentrations of extracts from 5 % -15 % were tested and the concentration of the extracts that resulted in no visual growth was noted as the MIC.

RESULTS AND DISCUSSION

The study showed that the *B. spectabilis* extracts have the antimicrobial potential against *S. typhi*. The results also revealed that the ethanolic and methanolic extracts of leaves and flowers were more effective against the *S. typhi*. The results of the phytochemical analysis revealed the presence of alkaloid flavonoids, glycosides, phlobotannins, saponins, steroids, tannins and terpenoids in distilled water, acetone chloroform, ethanol and methanolextracts of stem flowers and leaves extracts of *B. spectabilis* (Table 1).

Table 1: Qualitative analysis of phytochemicals in *B. spectabilis* stem, leaves and flowers extracts

Sr. No	Chemical components	Distilled water			Acetone			Chloroform			Ethanol			Methanol		
		S	L	F	S	L	F	S	L	F	S	L	F	S	L	F
1.	Alkaloids	-	-	+	+	-	+	+	-	+	+	-	-	+	-	+
2.	Flavonoids	-	+	+	+	+	-	+	+	+	+	+	-	+	-	-
3.	Glycosides	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4.	Phlobotannins	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
5.	Saponins	-	-	+	-	-	+	-	+	-	-	-	+	+	+	-
6.	Steroids	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+
7.	Tannins	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+
8.	Terpenoids	-	-	+	-	+	-	+	+	-	+	_	+	-	+	-

S - stem, L - leaves, F - flowers

With an increase in concentration antimicrobial activity increases and highest activity was at 90% concentration (Fig. 1). Increase in incubation period has also a positive effect on antimicrobial activity (Fig. 2), and leaves showed highest activity followed by flowers and stem (Fig. 3).

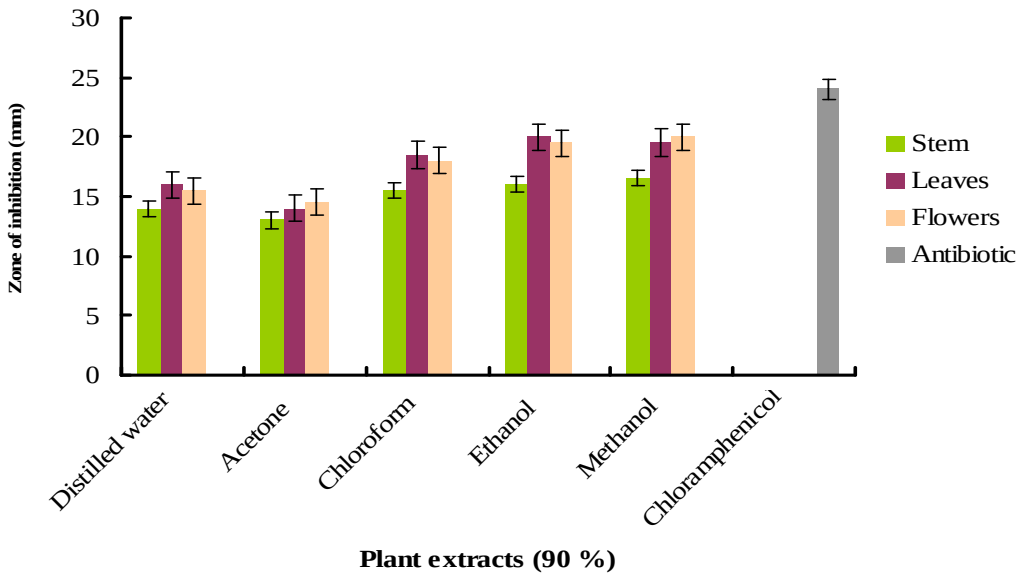

Fig. 1: Inhibitory activity of *B. spectabilis* and antibiotic against *S. typhi*

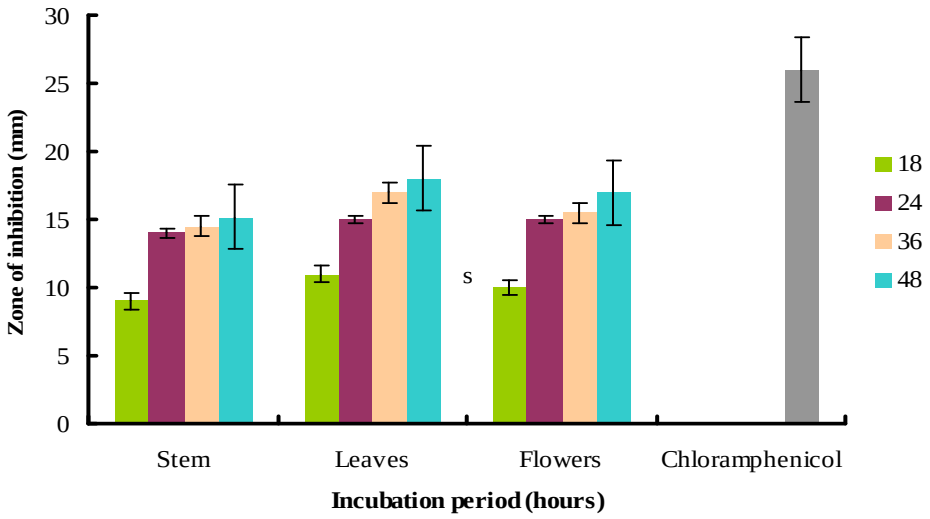

Fig. 2: Effect of incubation periods on activity of *B. spectabilis* and antibiotic against *S. typhi*

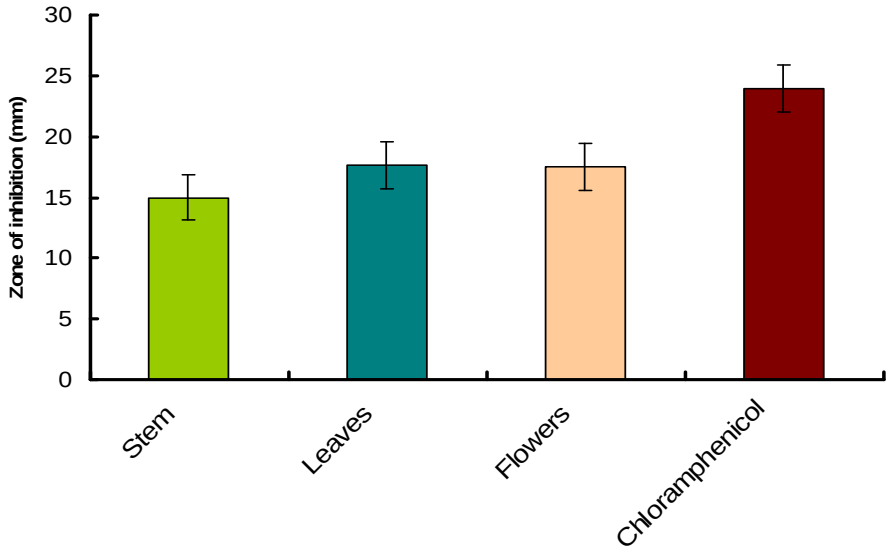

Fig. 3: Comparison of activity of *B. spectabilis* and antibiotic against *S. typhi*

Table 2: Minimum inhibitory concentration (MIC) of extracts against *S. typhi*

Sr. no.	Treatments	MIC (mg / ml)				
		Acetone	Aqueous	Chloroform	Ethanol	Methanol
1.	Stem	2.0	2.0	1.7	1.5	1.6
2.	Leaves	1.7	1.8	1.6	1.3	1.0
3.	Flowers	1.9	1.9	1.6	1.5	1.3

The results showed that the phytochemicals and antibacterial activity of different solvent extracts of *B. spectabilis* leaves extracts were effective against *S. typhi* but the chloroform extracts were more effective against *S. typhi* as compared to distilled water, acetone, ethanol and methanol extracts [7].

It is observed during the studies on different solvent extracts of many medicinal plants that ethanol and methanol extracts have higher activity as compared to other (distilled water, acetone) extracts against *S. typhi* [11].

Methanol extract as compared to aqueous and other extracts has a significant difference in inhibition activity against the *S. typhi* [12]. The organic solvents exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non polar and they were extracted only through the organic solvent medium [13].

CONCLUSION

The results suggested that the *B. spectabilis* stem, leaves and flowers have antimicrobial potential against *S. typhi*. These findings could be useful in searching new clinically important antimicrobials and helpful in the treatment of human and animal diseases and in replacing synthetic drugs as well.

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