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In vitro Antibacterial Activity of *Bougainvillea spectabilis* Leaves Extracts

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Abstract: The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antibacterial activity of various solvent extracts of *Bougainvillea spectabilis* leaves. Antimicrobial activity of different solvent extracts of *Bougainvillea spectabilis* leaves were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. The bacteria used in the study were *Staphycococcus aureus, Bacillus subtilis, Streptococcus faecalis, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii, Klebsiella pneumoniae, Proteus vulgaris, Serratia marcescens, Shigella flexneri and Vibrio cholerae.* It was observed that ethanolic, methanolic, chloroform and ethylacetate extracts showed maximum inhibitory effect on all tested bacteria except *Vibrio cholerae* compared to other solvent extracts. The alcoholic extracts were more active against all Gram positive and Gram negative bacteria.

Key words: Antibacterial activity · bacteria · *Bougainvillea spectabilis* · herbal medicine and pytochemicals

INTRODUCTION

Human infections particularly those involving microorganisms i.e., bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. Over the last centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs [1-3].

The World Health Organisation estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs for their primary health care needs. Herbs are supposed to be safe but many unsafe and fatal side effects have recently been reported [4,5]. Hence, there is an urgent need to study the screening of antimicrobial properties of herbs, which will be helpful in the treatment of several diseases caused by microorganisms.

Many plant families represent reservoir of effective chemotheraupeutics and can provide valuable sources of natural antimicrobials [6,7]. Thus for many thousands of years, plant extracts have been used for a wide variety of purposes [8]. Plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [9].

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which atleast 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defence mechanisms against predation by microorganisms, insects and herbivores.

The genus Bougainvillea, in the Nyctaginaceae (4 O' clock) family of plants, has 14 species, with three that are horticulturally important *Bougainvillea spectabilis*, *B. glabra* and *B. peruvina*. *Bougainvillea spectabilis* is a large climber with distinctive cured thorns and hair on stems and leaves. *Bougainvillea spectabilis* leaves extract inhibited tomato spotted wilt tospovirus on *capsicum annum* and ground water in laboratory tests [10]. *Bougainvillea spectabilis* were highly effective in reducing okra yellow vein mosaic virus infection of okra [11]. Antiviral protein was characterized by Balasaraswathi *et al.,* [10] and anti-inflammatory activities were also observed by Joshi *et al.,* [12] by *Bougainvillea spectabilis*.

The aim of the present study was to evaluate the antibacterial activity of *Bougainvillea spectabilis* against various bacterial strains.

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MATERIALS AND METHODS

Plant material: *Bougainvillea spectabilis* leaves were collected, washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored in sterile containers for further use. 50 g of dried powdered leaves sample was taken and treated with petroleum ether. The treated sample was dissolved in 150 ml of ethanol, methanol, diethyl ether, chloroform, ethyl acetate and water respectively. All the preparations were kept in shaker for 3 days. Then the solvents were filtered through filter paper to remove free extractable substances. The filtrate was concentrated by drying at room temperature for several days till dried leaves sample were obtained.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, anthroquinones, saponins, triterpenoids, flavonoids, carbhohydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and furanoids using the method of Harborne [13].

Test organisms: The strains used for the present study were Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii, Klebsiella Pneumoniae, Proteus vulgaris, Serratia marcescens, Shigella flexneri, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Micrococcus luteus.

Agar disc diffusion method: This method was adopted to determine the antibacterial activity of leaves extract against the test organisms.

The Muller Hinton Agar (MAA) was (3.1 g/100 ml) weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile Petri plate. The disc was saturated with 50 micro liter of the extract and was allowed to dry. The disc was placed on MAA plate swabbed with the culture of microorganisms.

The plate was incubated at 37°C for overnight. The microbial growth was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of leaves (*Bougainvillea spectabilis*) extracts.

RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis of ethanolic, ethylacetate, diethylether, methanolic, Chloroform and aqueous extracts of *Bougainvillea spectabilis* leaves were presented in Table 1. Proteins and aminoacids were found in all solvent extracts except ethylacetate extract. Quinones were found in all solvent extracts. Saponins were absent in methanolic and aqueous extracts. Triterpenoids were found in ethanolic, diethylether and ethylacetate extracts. Flavonoids were absent in ethanolic and methanolic extracts. Alkaloids were absent in ethanolic and diethylether extracts. Sterols were absent in aqueous extract. Glycosides were absent in aqueous extract. Tannins were extracted in all solvents except ethylacetate. Furanoids were absent in ethanolic and methanolic extracts. Phenols were absent in ethanolic and aqueous extracts. Carbohydrates were found in ethanolic, diethylether and aqueous extracts.

In the present study the phytochemicals occurring in the various solvent extracts of the plant leaves (ethanolic, methanolic, ethylacetate, Diethylether, chloroform and aqueous extracts) were analyzed qualitatively by phytochemical screening. These pytochemicals present in extracts may be responsible for the antibacterial activity of the plant leaves extract.

Table 1: Qualitative analysis of phytochemicals in *Bougainvillea spectabilis* leaves extracts

Extracts	Ethanolic extract	Methanolic extract	Diethyl ether extract	Ethyl acetate extract	Chloroform extract	Aqueous extract
Proteins and amino acids	+	+	+	-	+	+
Quinones	+	+	+	+	+	+
Saponins	+	-	+	+	+	-
Triterpenoids	+	-	+	+	-	-
Flavonoids	-	-	+	+	+	+
Alkaloids	-	+	-	+	+	+
Sterols	+	+	+	+	+	-
Glycosides	+	+	+	+	+	-
Tannins	+	+	+	-	+	+
Furanoids	-	-	+	+	+	+
Phenols	-	+	+	+	+	-
Carbohydrates	+	-	+	-	-	+

+ Present - Absent

Table 2: Antimicrobial activity of *Bougainvillea spectabilis* leaves against various Gram positive bacteria

S. No	Solvent extracts	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus faecalis*	*Micrococcus Luteus*
1	Ethanolic Extract	++	++	++	+
2	Methanolic Extract	+	+	++	+
3	Chloroform Extract	-	+	++	+
4	Diethylether Extract	-	-	+	+
5	Ethylacetate Extract	+	+	+	+
6	Aqueous extract	-	+	+	-
7	Positive control	Tetracycline ++	Gentamycin ++	Ampicillin ++	Tetracyclin ++

+ denotes zone diameter less than 10 mm

++ denotes zone diameter greater than 10 mm

- denotes the absence of inhibition zone.

Table 3: Antimicrobial activity of *Bougainvillea spectabilis* leaves against Gram negative bacteria

S. No	Solvent Extracts	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhii*	*Klebsiella Pneumoniae*
1	Ethanolic extract	+	+	+	++
2	Methanolic extract	+	++	+	-
3	Chloroform extract	+	+	+	++
4	Diethylether extract	-	-	+	++
5	Ethylacetate extract	+	+	+	++
6	Aqueous extract	-	-	+	+
7	Positive control	Ampicillin ++	Gentamycin ++	Ampicillin ++	Ampicillin ++

+ denotes zone diameter less than 10 mm

++ denotes zone diameter greater than 10 mm

- denotes the absence of inhibition zone.

Tables 2 and 5 showed the antimicrobial activities of different solvent extracts of *Bougainvillea spectabilis* leaves against different Gram positive and Gram negative bacterial strains. Their antibacterial potency was assessed by the presence or absence of inhibition zones and zone diameters (mm). As can be seen from Tables 2 and 5 the aqueous extract of *Bougainvillea spectabilis* showed the antimicrobial activity against Gram positive bacteria like *Bacillus subtilis and streptococcus faecalis* and Gram

Table 4: Antimicrobial activity of *Bougainvillea spectabilis* leaves against Gram negative bacteria

S. No	Solvent Extracts	*Proteus vulgaris*	*Serratia marcescens*	*Shigella flexneri*	*Vibrio cholerae*
1	Ethanolic extract	++	++	++	-
2	Methanolic extract	+	++	+	-
3	Chloroform extract	+	++	+	-
4	Diethylether extract	-	+	++	+
5	Ethylacetate extract	+	+	+	-
6	Aqueous extract	-	-	+	+
7	Positive control	Ampicillin ++	Gentamycin ++	Tetracyclin ++	Tetracyclin ++

+ denotes zone diameter less than 10 mm

++ denotes zone diameter greater than 10 mm

- denotes the absence of inhibition zone.

negative bacteria like *Shigella flexneri, Vibrio cholerae, Salmonella typhii, Serratia marcescens* and *Klebsillea pneumoniae.*

Ethanolic extract showed inhibitory effect on all Gram positive bacteria selected for the present study and all Gram negative bacteria except *Vibrio cholerae.* Methanolic extract showed inhibitory effect against all Gram positive bacteria selected for the present study and all Gram negative bacteria except *Klebsiella pneumoniae* and *Vibrio cholerae*.

Diethyl ether extract showed inhibitory effect on Gram positive bacteria selected for the present study and all Gram negative bacteria except *Vibrio cholerae.* Ethylacetate extract showed inhibitory effect on all Gram positive bacteria selected for the present study and all Gram negative bacteria except *Vibrio cholerae.* Chloroform extract showed inhibitory effect on all Gram positive bacteria except *Staphylococcus aureus* and all Gram negative bacteria selected for the present study except *Vibrio Cholerae.*

The ethnobotanical approach assumes that the popular uses of plants can offer strong clues to the biological activity of plants. The high percentage of positive results found in this and previous studies [14] shows that this approach is also promising for antimicrobial activity. The results of the present study reveals the fact that the organic solvent extracts (ethanolic and methanolic extracts) exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted

Table 5: Inhibition Zone Diameter of *Bougainvillea spectabilis* leaves extracts against Gram positive and Gram negative bacteria

S.No	Bacteria	Zone diameter (in mm)					
		Ethanolic extract	Methanolic extract	Chloroform extract	Diethyl Ether extract	Ethyl Acetate extract	Aqueous extract
	Gram Negative						
1	*Escherichia coli*	9	7	9	-	8	-
2	*Pseudomonas aeruginosa*	7	16	8	-	11	-
3	*Salmonella typhii*	8	7	9	7	7	8
4	*Klebsiella Pneumoniae*	12	-	15	11	12	9
5	*Proteus vulgaris*	15	8	10	-	7	-
6	*Serratia marcescens*	15	13	15	-	9	8
7	*Shigella flexneri*	14	8	9	11	7	7
8	*Vibrio cholerae*	-	-	-	1	-	7
	Gram Positive						
9	*Staphylococcus aureus*	13	10	-	-	8	-
10	*Bacillus subtilis*	13	7	10	-	7	-
11	*Streptococcus faecalis*	16	12	7	11	8	-
12	*Micrococcus luteus*	9	7	8	7	6	-

only through the organic solvent medium [15, 16]. The present study justifies the claimed uses of *Bougainvillea spectabilis* leaves in the traditional system of medicine to treat various infections diseases caused by the microorganisms. Comparison of controls with the solvent extracts of *Bougainvillea spectabilis* leaves revealed that the leaf extracts are more effective towards pathogenic organisms.

CONCLUSION

The results suggested that the different solvent extracts under study showed antibacterial activity. The antibacterial action of various extracts of *Bougainvillea spectabilis* leaves may indicate their potential as antibacterial herbal remedies. Further work is needed to locate the active principle from the various extracts and their phytopharmaceutical studies. Research into the effects of local medicinal plants is expected to boost the use of these plants in the therapy against disease caused by the test bacterial species and other microorganisms. It is possible that better therapy for many microbial diseases can be found in the leaves extracts. The Preliminary results of this investigation indicates that *Bougainvillea spectabilis* leaves have high potential of antimicrobial activity.

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