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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF *BOUGAINVILLEA VARIEGATA* AND *BOUGAINVILLEA SPECTABILLIS* ETHANOLIC AND METHNOLIC EXTRACT

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ABSTRACT

Present study was conducted to evaluate and compare the antimicrobial activity of *Bougainvillea variegata* leaves extract with *Bougainvillea spectabillis* leaves extract. Antibacterial activity of Methanolic and Ethanolic extract of these plant leaves were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. Antibacterial activity was done by disc diffusion method at a concentration of 500 µg/disc of the extract, using Ampicillin (Amp), Tetracycline (TE), Neomycin (N), Gentamycin (CN), Chloramphenicol (C), Ciprofloxacin (CIP), Erythromycin (E) as the standard. The bacterial strains used in the study were *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Enterococcus faecalis, Pseudomonas aeruginosa, Bacillus subtillis and Listeria monocytogenes* . Both Methanolic & Ethanolic *extracts* have shown activity against Gram positive and negative bacteria. Extracts were not effective against *Enterococcus faecalis*.

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INTRODUCTION

For primary health care medicinal plants are genuinely useful due to an effective source of both traditional and modern medicines. World Health Organization has advocated traditional remedy as safe medicine. Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical society that this would lead to the elimination of infectious diseases. However diseases and pathogenic organisms that were once thought to have been treated by antibiotics are attaining new forms which are resistant to antibiotic therapies (Levy and Marshall, 2004). Antibiotic resistance is a global issue now that effects the public health, which leads to epidemics of diseases (Iwu *et al*., 1999). Treatment failure and limited effectiveness of drugs is due to the global emergence of multi-drug resistant bacterial strains (Hancock, 2005). Emergence of multi-drug resistant strains has lead to the search of new antimicrobial sources which can be plants, so it is vital to study and screen the herbs for there antimicrobial properties. It will help to cure many diseases caused by microorganisms. Reservoirs of effective chemotherapeutics are present in many plants which can be a significant source of natural antibiotics (Gupta *et al*., 2009). Synthetic pharmaceutical products have limitations which opened avenues for 'Green Medicine' that is considered to be safe, more accessible and reasonable too.

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Research studies on herbal medicine is significantly required to identify and quantify the potential, to promote the use of herbal medicine (Kohli *et al*., 2011). Medicinal plants and herbal medicines provided a source of hope for novel drug compounds, as traditional medicine have made a significant contribution to human health (Iwu *et al*., 1999). Due to the major use of plants for remedies against infectious diseases, scientist are searching for active substances which shows antibacterial activity in plants (Betoni *et al*., 2006; Shibata *et al*., 2005). Secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, are present in Plants with extensive variety which have antimicrobial properties (Lewis and Ausubel, 2006; Cowan, 1999). However many active compounds have been isolated from medicinal plants and literature is present on those compounds. Regardless of this significant literature on the antimicrobial properties of plant extracts, no one has exploited those plant dervied chemical compounds for clinical use as successful antibiotics (Gibbons, 2004). Bougainvillea is a popular ornamental plant in warm climates including Ethiopia, Indonesia, Thailand, Pakistan, India, Sri Lanka, Australia, the Mediterranean region, the Caribbean, Central America, the United Arab Emirates and the southern mainland, United States and Hawaii. http://en.wikipedia.org/wiki/Bougainvillea. *Bougainvillea* has antibacterial activity in various extract. In developing countries 80% of the population still relies on the herbal medicines, for there health care estimated by the World Health Organization (Gupta *et al*., 2009).

MATERIALS AND METHODS

Collection of Plant Material

The plant Fresh leaves of *Bougainvillea variegata* and *Bougainvillea spectabillis* were collected during Feb.-March 2015 from the Garden of Qarshi Industries (Pvt.), Limited, Pakistan.

Extract Preparation of plant

The plant material was dried and crushed in grinder. The dried powder was obtained. From the dried powder of leaves 10g of sample was taken in 100ml of 70% Ethanol and 10 g of sample in 100ml of 70% Methanol. Flasks were placed in shaking water bath at 150 rpm for 24 hour. After 24 hr material was filtered through Whatman filter paper. And then solvent was allowed to be evaporate at room temperature. The extracts were stored in Screw caped bottles at 4ºC.

Test Organisms

Bacterial strains were selected from American type culture collection (ATCC).The strains used for the study were *Staphylococcus aureus* (ATCC-6538), *Salmonella typhimurium* (ATCC-14028), *Enterococcus faecalis* (ATCC-49452), *Pseudomonas aruginosa* (ATCC-27853, *Liateria monocytogenes* (ATCC-13932), *Bacillus subtillis* (ATCC-19659).Organisms were grown on there selective media, there purity was determined by morphological character and by performing Biochemical test and for *Salmonella typhimurium* serology test was performed.

Inoculum Preparation

Inoculum was prepared for each organism in sterile saline solution, by comparing the turbidity according to 0.5 McFarland standard, approximately having $1.0x10^8$ cells.

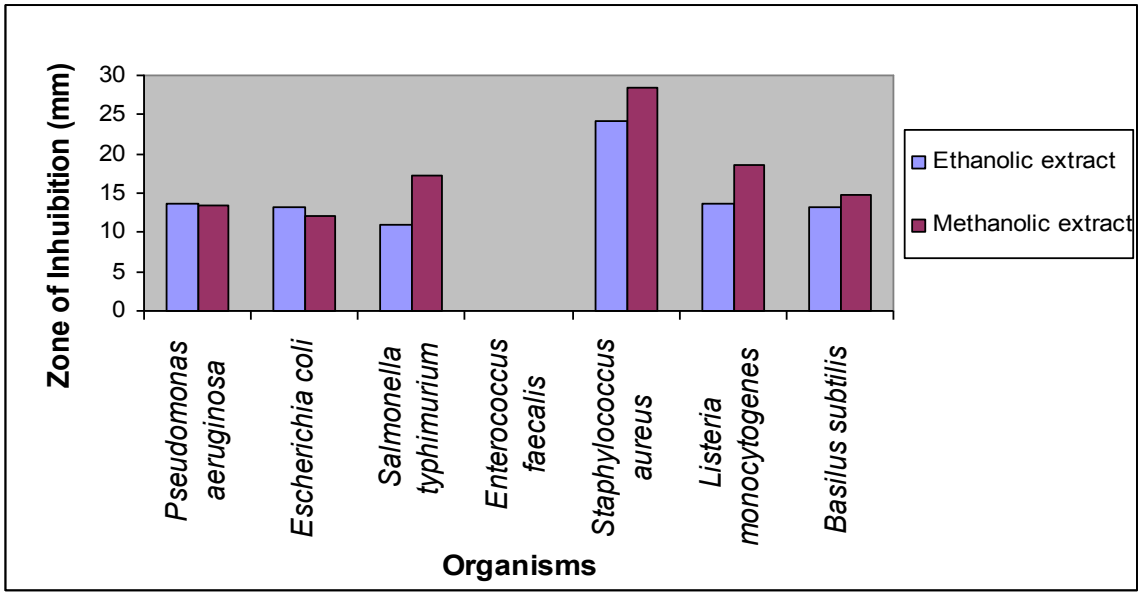


Fig. 1. Antibacterial activity of *Bougainville variegata* extract against Gram positive and Gram negative bacteria

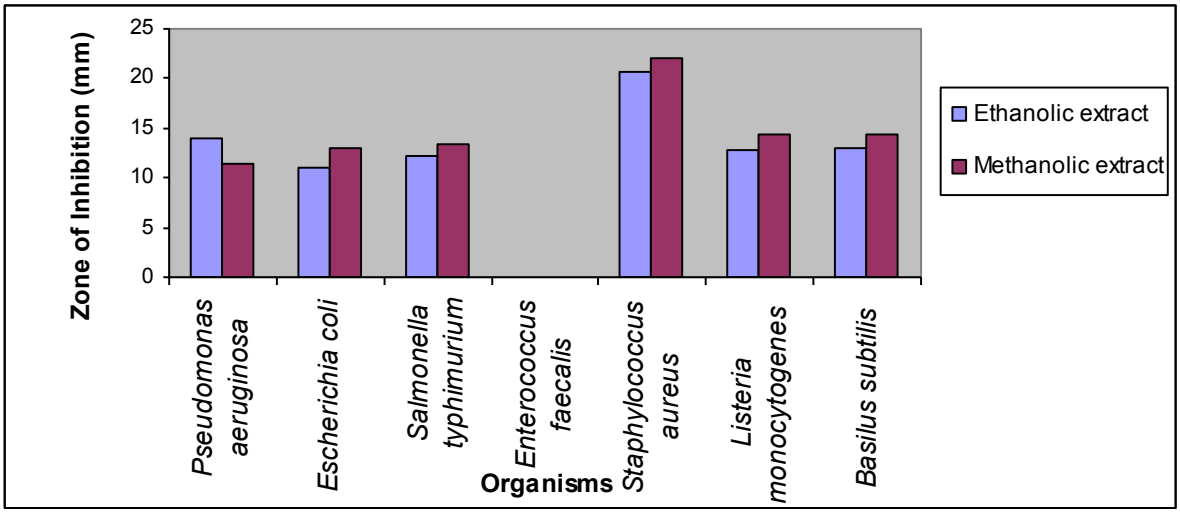


Fig. 2. Antibacterial activity of *Bougainville spectabilis* extract against Gram positive and Gram negative bacteria

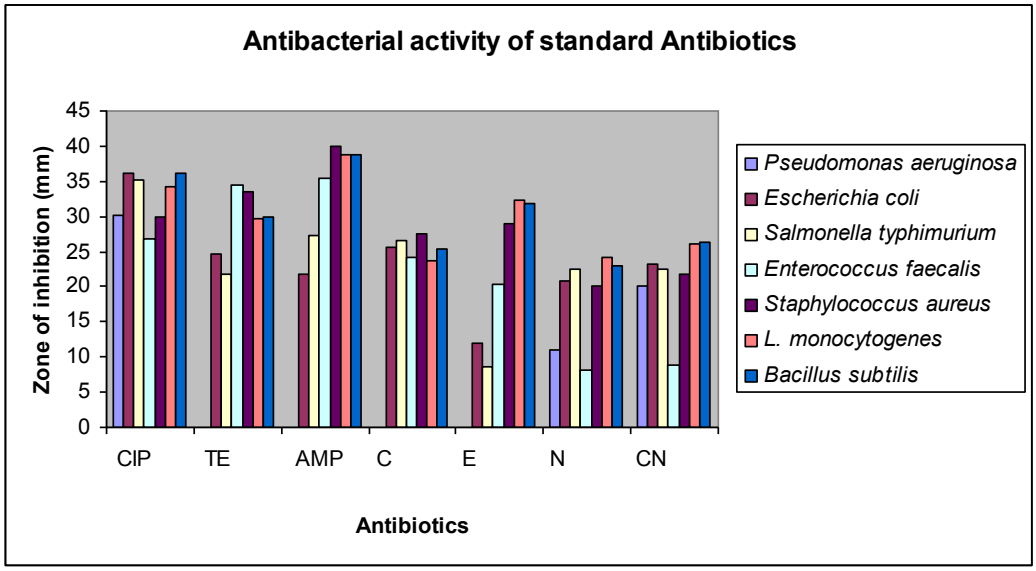
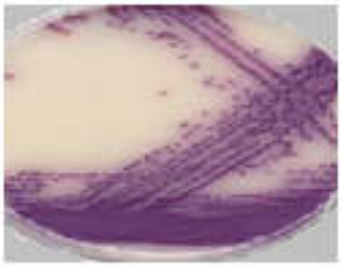


Fig. 3. Antibacterial activity of Standard Antibiotics against Gram positive and Gram negative bacteria

S. aureus (ATCC®6538) on Baird parker medium

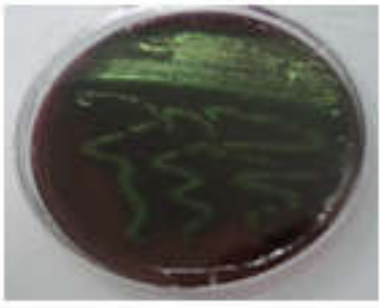
L.mononocytogens (ATCC®13932) on RAPID L.mono agar



S. typhimurium (ATCC®13076) on RAPID salmonella agar

E. faecalis (ATCC®49452) on S & B medium

P. aeruginosa on MSA (ATCC®27853)



E.coli on EMB (ATCC®25922

Fig. 4. Growth of Tested organisms on selective Media

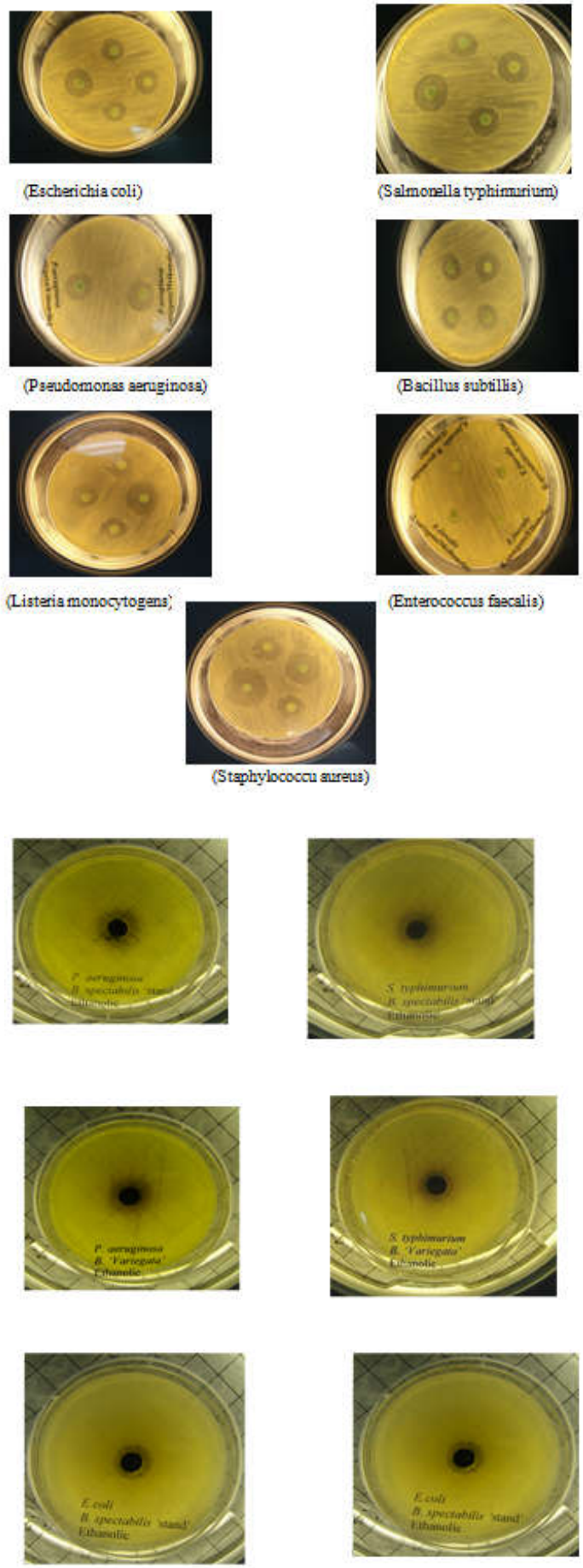


Fig. 5. Inhibition zone of *Bougainvillea Variegata* and *Bougenville Spectabillis* against tested Organisms by Disc diffusion Method

Antibacterial activity of the Extract

The antimicrobial activity of the extract was evaluated by disc diffusion method. Plates of Muller Hinton agar (OXOID) medium having media up to 4 mm were prepared. After the solidification of plates, Lawn of 24 hours fresh culture was prepared on the agar plates for each organism. Inoculum was taken by socking the sterile swab in prepared inoculum of test organisms, i.e. *Escherichia coli, Staphylococcus aureus, salmonella typhimurium, Enterococcus faecalis, Listeria monocytogenes, Bacillus subtillis* and *Pseudomonas aruginosa*, spread over the agar plates for respective organism. Ethanolic and Methanolic extract of *Bougainvillea* was dissolved in DMSO to make the concentration (500µg/disc).

Antimicrobial susceptibility test

As control antibiotics commonly used for the test organisms, there susceptibility was determined along with the extract of *Bougainvillea*. Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Baur *et al*. (1966) against a number of antibiotics.

The potency of antibiotics per disc are as follows. Ampicillin (Amp) (25 µg), Tetracycline (TE) (30 µg), Neomycin (N) (30 µg), Gentamycin (CN) (10 µg), Chloramphenicol (C) (30 µg), Ciprofloxacin (CIP) (05 µg), Erythromycin (E) (15 µg). All the antimicrobial susceptibility test discs were of OXOID.

Table 1. Inhibition zone of *Bougainvillea* extract against Gram negative bacteria

Gram negative Bacterial strains	Zone of inhibition (mm) by Bougainville variegata leaves extracts in different solvents		Zone of inhibition (mm) by Bougainville spectabilis leaves extracts in different solvents	
	Ethanol	Methanol	Ethanol	Methanol
Pseudomonas aeruginosa ATCC®27853	13.68	13.45	14.02	11.44
Escherichia coli ATCC®25922	13.23	12.01	10.93	12.91
Salmonella typhimurium ATCC®14028	10.93	17.26	12.20	13.45
Enterococcus faecalis ATCC®49452	00	00	00	00

Table 2. Inhibition zone of *Bougainvillea* extract against Gram positive bacteria

Gram positive Bacterial strains	Zone of inhibition (mm) by Bougainville variegata leaves extracts in different solvents		Zone of inhibition (mm) by Bougainville spectabilis leaves extracts in different solvents	
	Ethanol	Methanol	Ethanol	Methanol
Staphylococcus aureus ATCC®6538	24.19	28.54	20.67	21.97
Listeria monocytogenes ATCC®13932	13.75	18.53	12.85	14.35
Bacillus subtlilis ATCC®19659	13.14	14.78	12.92	14.44

Table 3. Inhibition zone of standard Antibiotics against Gram Negative bacteria

Gram Negative Bacterial strains	Zone of inhibition (mm) by Standard Antibiotics against different bacterial strains							
	CIP 5µg	TE 30µg	AMP 25µg	C30µg	E 15 µg	N 30 µg	CN 10 µg	DMSO
Pseudomonas aeruginosa ATCC®27853	30.10	00	00	00	00	10.99	20.03	00
Escherichia coli ATCC®25922	36.10	24.70	21.67	25.68	11.86	20.79	23.16	00
Salmonella typhimurium ATCC®14028	35.25	21.73	27.40	26.55	8.69	22.42	22.53	00
Enterococcus faecalis ATCC®49452	26.71	34.38	35.37	24.20	20.26	8.02	8.88	00

Table 4. Inhibition zone of standard Antibiotics against Gram positive bacteria

Gram positive Bacterial strains	Zone of inhibition (mm) by Standard Antibiotics against different bacterial strains							
	CIP 5µg	TE 30µg	AMP 25µg	C 30µg	E 15 µg	N 30 µg	CN 10 µg	DMSO
Staphylococcus aureus ATCC®6538	29.87	33.46	39.98	27.49	28.90	20.12	21.68	00
L. monocytogenes ATCC®13932	34.19	29.79	38.72	23.76	32.23	24.26	26.16	00
Bacillus subtilis ATCC®19659	36.17	29.93	38.66	25.40	31.83	23.06	26.35	00

With the help of micropippete10µl of extract was poured on the sterile filter paper disc (6mm), and placed on to the agar plate. Control of extracting solvents Ethanol and Methanol was also run. For half an hour plates were left at room temperature for proper diffusion of extract in disc and agar. Tests were performed on triplicate plates. Plates were incubated at 37ºC for 18-48 hrs. After 18-48 hrs inhibition zones of ethanolic & methanolic extracts and control were measured by using digital Vernier caliper. Mean of all the three results were taken for both extract and solvent.

RESULTS AND DISCUSSION

The antibacterial activity of ethanolic & methanolic extract of *Bougainvillea variegata* and *Bougainvillea spectabillis* was determined against the human pathogenic organisms both Gram negative & gram positive bacteria. Activity was determined by the 10µl of extract (500µg) results shows that Gram negative bacteria i.e *Pseudomonas aeruginosa, Escherichia coli and Salmonella typhimurium* are sensitive to

the *Bougainvillea* extract. While *Enterococcus faecalis* has shown no sensitivity against *Bougainvillea* extract. In case of Gram positive bacteria *Staphylococcus aureus, Listeria monocytogenes and Bacillus subtillis* are also sensitive to the *Bougainvillea* extract.

Antibacterial activity of Bougainvillea extract was also compared to other commonly used antibiotics against these Gram positive & negative bacteria. Comparative study showed that aginst *Pseudomonas aeruginosa* Tetracycline (TE) , Ampicillin (Amp), Chloramphenicol (C), Erythromycin (E) & Neomycin (N) , has shown no activity, while Bougainvillea extract has shown significant zone of inhibition but it was less than Ciprofloxacin (CIP), Gentamycin (CN). In case of *Escherichia coli*, *Salmonella, Listeria monocytogenes* and *Bacillus subtillis, Bougainvillea* extract has shown significant activity almost 50% of the compared antibiotics. All antibiotics have zone of inhibition against *Enterococcus faecalis* but *Bougainvillea* extract has shown no activity. Bougainvillea extract has shown excellent results against *Staphylococcus aureus* which are near to the standard antibiotics used.

Conclusion

The results of this study conclude that the antibacterial action of ethanolic and methanolic extract of *Bougainvillea variegate* and *Bougainvillea spectabillis* leaves may indicate their potential as antibacterial herbal remedies. The *Bougainvillea* has shown antibacterial activity against gram positive and negative pathogenic organisms in both ethanolic and methanolic extract, especially aginst *Staphylocoocus aureus.* Further phytochemical screening can be performed to analyze the active agents present in extract due to which *Bougainvillea* has shown antibacterial activity. Better treatment can be found in leaves extracts. Research on plants can increase the use of plants in therapy of diseases caused by pathogenic organisms.

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