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In vitro comparative study of *Bougainvillea spectabilis "stand"* leaves and *Bougainvillea variegata* leaves in terms of phytochemicals and antimicrobial activity

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[ABSTRACT] AIM: To study the qualitative analysis of phytochemicals and antibacterial activity of the ethanolic and methanolic extracts of *Bougainvillea spectabilis* and *Bougainvillea variegata* leaves. **METHODS:** Phytochemical constituents were determined qualitatively by the Harborne method, while antimicrobial activities were determined by measuring the zone of inhibition on Mueller Hinton Agar. **RESULTS:** The maximum inhibitory effects were obtained against the Gram positive microbe *Staphylococcus aureus* for the methanolic extracts of both *B. spectabilis* [(28.54 ± 0.18) mm] and *B. variegata* [(21.97 ± 0.06) mm]. The Gram negative microbes *Proteus vulgaris* [(16.00 ± 0.15) mm] and *Serratia marcescens* [(16.00 ± 0.06) mm] gave maximum inhibitory effects for the ethanolic extracts of *B. variegata,* while *Salmonella typhimurium* [(17.26 ± 0.12) mm] gave a maximum zone of inhibition for the methanolic extract of *B. spectabilis*.No inhibitory effects were observed for the extracts of *B. spectabilis* or *B. variegate* against *Enterococcus faecalis, Vibro cholera* or *Klebsiella pneumoniae.* **CONCLUSION:** Both *B. spectabilis* and *B. variegata* possess significant antimicrobial activity that, following additional studies, could replace commercially known antibiotics.

[KEY WORDS] *Bougainvillea spectabilis* "stand" leaves; *Bougainvillea variegata* leaves; Phytochemicals; Antimicrobial activity; Antibiotics

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1 Introduction

Plant-based medicines are important therapeutic weapons to cure human diseases, and are of relevance to pharmacology. The pharmacological properties of medicinal plants may be used as leads in developing novel therapeutic agents. For thousands of years, traditional plant-derived medicines have been used in most parts of the world, and their use in fighting microbial disease is becoming the focus of intense study [1-2]. Much of the research into traditional medicinal plant use has focused on Asian [3] and South American [4] plants.

Antimicrobial resistance has become a serious concern both in developed and developing nations. The rapid increase in the rate of infections, antibiotic resistance in microorganisms and the side effects of chemically modified synthetic antibiotics offers an opportunity to medicinal plants in terms of gaining popularity over synthetic drugs. Medicinal plants have been found useful in the cure of a number of diseases, including a wide range of bacterial diseases. Medicinal plants are a rich source of antimicrobial agents, phytochemicals and antioxidants [5-6]. Although medicinal plants produce slow recovery, the therapeutic use of medicinal plants is becoming popular because of their lower side effects and reduced resistance from microorganisms [7]. According to the World Health Organization, medicinal plants are the best source to

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obtain a variety of newer herbal drugs. About 80% of individuals from developing countries use traditional medicine, which contains materials derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [8-9]. The antimicrobial and chemotherapeutic activities of many plants have been reported [7, 10-11]. The antimicrobial activities of medicinal plants are due to the production of secondary metabolites, such as alkaloids, flavonoids, tannins, terpenoids, etc. that are naturally present in the plant for self-defense against different forms of attack [12]. Plant extracts have been used for thousands of years to cure many diseases, and scientific evidence about the antimicrobial activity of plants was first documented in the late 19th century [13-14].

Bougainvillea Comm. Ex. Juss. is a genus native to South America, and its name comes from Louis Antoine de Bougainville, a French navigator and military commander who was the first person who take note of this plant in 1768 [15]. The genus *Bougainvillea* belongs to the family Nyctaginaceae, also called (4 o'clock) family of plants. This genus has about 27 species, three of which are horticulturally important. *Bougainvillea spectabilis* Willd., *Bougainvillea glabra* Choisy and *Bougainvillea peruviana* (Humb. & Bonpl.) Modern technology has produced a large number of different hybrid species and important cultivars of this genus [15]. *B. spectabilis* is a large climber with typical cured thorns and hair on stems and leaves. *B. spectabilis* leaf extract repressed tomato spotted wilt tospovirus on *Capsicum annum* L. and ground water in laboratory trail tests [16]. *B. spectabilis* were highly effective in reducing okra yellow vein mosaic virus infection of okra [17]. Antiviral protein was characterized by Balasaraswathi *et al* [16] and anti-inflammatory activities were also observed by Joshi *et al* [18] for *B. spectabilis*. The alcoholic extract of *B. spectabilis* had a significant hypoglycemic effect in alloxan-induced diabetic albino mice, and was free from acute toxicity [19-20]. It was also found that the leaf extract of *B. spectabilis* contains D-pinitol (3-*O*-methylchiroinositol) which has insulin-like effects [21-22].

Literature reports indicate that the leaves of *B. glabra* have insecticidal [23], anti-inflammatory [20], anti-diarrheal [24], anti-microbial , anti-ulcer, and anti hyperglycemic activities [25].

The main objectives of the present study were to evaluate qualitatively the phytochemistry of *B. spectabilis* "stand" leaves and *B. variegata* leaves, and to determine the antimicrobial activity in relation to standard antibiotics.

2 Materials and Methods

2.1 *Preparation of samples*

B. spectabilis "stand" and *B. variegata* leaves were collected from surrounding areas of Hazara University Pakistan, and their identity was verified from Department of Botany, Hazara University, Pakistan. The leaves were washed with water, then with distilled water, and finally dried under shade at room temperature. The leaves were ground to a powder and stored in sterile containers for further use. One hundred grams of dried powder were dissolved in 150 mL of ethanol and methanol (Wako Chemicals, Japan). The wet powder was incubated at 50 °C for 24 h. After this, the dried powder was treated with 5 mL of dimethyl sulfoxide (DMSO) (Merck) and the filtrate was dried at room temperature according to the method described by Umamaheswari *et al* [26].

The treated powders were then subjected to different qualitative tests of screening for carbohydrates, proteins, amino acids, flavonoids, phytosterols, alkaloids, saponins, triterpenoids, tannins, anthraquinones, furanoids and phenolic compounds. All of these compounds were identified by the methods described by Harborne [27].

2.2 *Test microorganisms*

The *in vitro* activity of the treated powder was assayed against the bacterial strains which were obtained from American Type Culture Collection (AATC). All the ATCC strains were maintained on Nutrient agar slants (Oxoid) at 4 °C. The bacterial strains on which the antibiotic efficacy of the plant extracts were evaluated were *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 19433NA, *Micrococcus luteus* ATCC 4698, *Enterococcus faecalis* ATCC 49452, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 29905, *Serratia marcescens* ATCC 27137, *Shigella flexneri* ATCC 700930, *Vibrocholerae* ATCC 39315, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus spizizenii* ATCC 6633, *Bacillus subtilis* ATCC 19659 and *Klebsiella pneumoniae* ATCC BAA1705.

2.3 *Purity testing of each organism*

Each organism was inoculated from the working culture of Nutrient Broth (Merck) on their respective selective media for control, as well as for purity testing, i.e *Pseudomonas aeruginosa* on (PCA) Pseudomonas cetrimide agar (Oxoid, CM0579), *Salmonella typhimurium* on (XLD) xylose lysine deoxycholate agar (Oxoid, CM0469), *Staphylococcus aureus* on (MSA) mannitol salt agar (Oxoid, CM0085), *Enterococcus faecalis* on (S&B) Slanetz & Bartley (Oxoid, CM0377), *Escherichia coli* on (EMB) eosin methylene agar (Oxoid, CM0069), *Streptococcus faecalis* on (SAB) streptococcus agar base (Oxoid, CM 0701), *Enterococcus faecalis* on (BVA) brilliance VRE agar (Oxoid, PO11755), *Proteus vulgaris* on (TSA) triple sugar iron agar (Oxoid, CM0035), *Serratia marcescens* on (CLA) China blue lactose agar (Oxoid, CM0209), *Shigella flexneri* on (DCA) desoxycholate citrate agar (Oxoid, CM0227), *Vibrocholerae* (TCBS) cholera medium TCBS (Oxoid, CM0333), *Bacillus spizizenii* on (CBA) chromogenic *Bacillus cereus* agar *(Oxoid,* SR0230), *Bacillus subtilis* on **(**MYP) mannitol egg yolk polymyxin agar (Oxoid, CM0929) and *Klebsiella pneumonia* on (SCA) simmons citrate agar (Oxoid, CM0153) incubated at 37 °C for 24 h.

2.4 *Evaluation of antimicrobial activity*

After the incubation time, one colony of each bacterium

from the respective selective agar medium was inoculated into 5 mL nutrient broth and incubated for 4−6 h at 37 °C. The inoculums were standardized by matching their turbidity with McFarland No.1 standard. The test culture was spread evenly on the surface of pre-sterilized plastic petri dish containing solidified (MHA) Mueller Hinton agar (Oxoid CM 0337) with a sterile cotton swab. Wells were made in the MHA agar plate using a sterile cork borer of 6 mm. With the help of a sterile micropipette tip 0.1 mL of the ethanolic and methanolic treated extracts of both *B. spectabilis* and *B. variegata* were poured into the wells. The plates were incubated at 37 °C for 24 h. After 24 h, the diameter of the resulting zone of inhibition was measured, and the average values were recorded. Each antimicrobial assay was performed in at least triplicate.

2.5 *Comparison with standard antibiotics*

Standard discs (7 mm diameter) of ampicillin 'AMP' (25 μg), chloramphenicol 'C'.(30 μg), ciprafloxicin 'CIP' (5 μg) tetracycline 'TE' (30 μg), erythromycin 'E' (15 μg), neomycin 'N' (30 μg), and gentamicin 'CN' (10 μg) obtained from Oxoid Ltd., were used as positive controls for the antimicrobial activity testing against different Gram positive and Gram negative bacteria.

3 Results and Discussion

Since the discovery of antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases. However, diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapies [28]. Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns [29]. Keeping in view of these facts plant based medicines are gaining in popularity among citizens of highly developed world.

The results of the qualitative analysis of different phytochemicals presented in *B. spectabilis* and *B. variegata* are presented in Table 1. It is interesting to note that all important phytochemicals were present in *B. spectabilis,* on the other hand *B. variegata* contains fewer phytochemicals. The presence and absence of these phytochemicals indicates the effectiveness of antimicrobial activity. Proteins, carbohydrates and amino acids were present in both ethanolic and methanolic extracts in accordance with results obtained by Umamaheswari *et al* [26] and Gupta *et al* [30]. Umamaheswari *et al* [26] carried out phytochemical analysis of *B. spectabilis* leaves in ethanolic, methanolic, ethyl acetate, diethyl ether, chloroform and aqueous extract. They demonstrated the presence of amino acids and proteins in all extracts, except the ethyl acetate extract. Flavonoids, phytosterols, alkaloids, saponins, triterpenoids, tannins, anthraquinones, furanoids and phenols were present in both the ethanolic and methanolic extract of *B. spectabilis* (Table 1), while our results are in line with those of Umamaheswari *et al* [26] our results, except furanoids that are absent in their finding. All these phytochemicals proves the antimicrobial activity of *B. spectabilis*; while in the case of *B. variegata* only furanoids and phenols were present in the methanol extract, while the ethanolic extract contained all of the phytochemicals, except for triterpenoids and tannins. No previous screening of phytochemicals in *B. variegata* has reported previously in the literature. Despite the reduced level of phytochemical diversity in *B. variegata* it showed appreciable antimicrobial activity. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [31-32]. The literature reports numerous compounds that have been isolated from a variety of medicinal plants. Despite this abundant literature on the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been exploited for clinical use as antibiotics [33].

Table 1 Qualitative analysis of different phytochemicals in *B. spectabilis* "stand" and *B. variegata* "leaf extract"

Phytochemicals	*B. spectabilis*		*B. variegata*	
	Methanol	Ethanol	Methanol	Ethanol
Carbohydrates	++	++	++	++
Proteins	++	++	++	++
Amino acids	++	++	++	++
Flavonoids	++	++	--	++
Phytosterols	++	++	--	++
Alkaloids	++	++	--	++
Saponins	++	++	--	++
Triterpenoids	++	++	--	--
Tannins	++	++	--	--
Anthroquinones	++	++	--	++
Furanoids	++	++	++	++
Phenols	++	++	++	++

++ indicate present; -- indicate absent

3.1 *Antimicrobial activity of B. spectabilis stand against Gram positive and Gram negative bacteria*

Tables 2 and 3 show the antimicrobial activities of ethanolic and methanolic extracts of *B. spectabilis* against different Gram negative and Gram positive bacterial strains. The antimicrobial activity against these strains were measured by measuring their zone of inhibition (mm) in triplicate. Both extracts gave antimicrobial activities in the majority of strains.

Gram positive strains (*Staphylococcus aureus, Bacillus spizizenii, Bacillus subtilis, Streptococcus faecalis and Mi-*

Table 2 Zones of inhibitions (mm) produced by *B. spectabilis* leaf extract and *B. variegata* leaf extract against Gram positive strains

Bacterial Strains	*B. spectabilis*		*B. variegata*	
Gram positive strains	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
Staphylococcus aureus	24.19 ± 0.03	28.54 ± 0.18*	20.67 ± 0.04	21.97 ± 0.06*
Bacillus spizizenii	13.75 ± 0.05	18.53 ± 0.04	12.85 ± 0.03	14.35 ± 0.03
Bacillus subtilis	13.14 ± 0.02	14.78 ± 0.06	12.92 ± 0.03	14.44 ± 0.05
Enterococcus faecalis	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Streptococcus faecalis	15.00 ± 0.25	12.00 ± 0.06	15.50 ± 0.20	13.00 ± 0.15
Micrococcus luteus	08.00 ± 0.10	09.00 ± 0.15	09.50 ± 0.20	08.50 ± 0.10

*=Highest zone of inhibition

crococcus luteus) showed antimicrobial activity from both types of extracts. Maximum antimicrobial activity was seen against *Staphylococcus aureus* by the on methanolic extract where the zone of inhibition reached up to (28.54 ± 0.18) mm (Fig. 1). Umamaheswari *et al* [26] used *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis* and *Micrococcus luteus* as test microorganisms against various solvent extracts of *B. spectabilis*. They reported more antimicrobial activity for the ethanolic extract in comparison with the methanolic extract. No antimicrobial activity against *Enterococcus faecalis* was observed by *B. spectabilis*, and the results are in line with those of Umamaheswari *et al* [26].

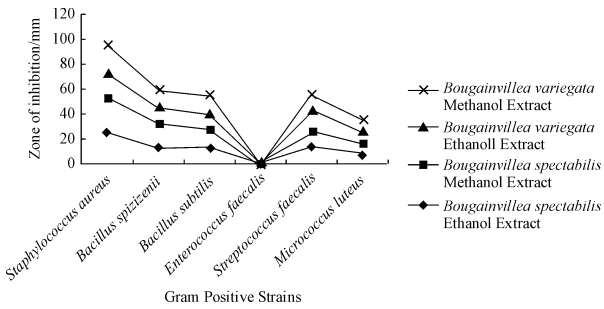

Fig. 1 Zone of inhibitions of Gram positive strains

The ethanolic and methanolic extracts showed antimicrobial activity against the Gram negative strains (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Serratia marcescens, Shigella flexneri*) on, except for *Vibro cholera* and *Klebsilla pneumoniae*. The highest antimicrobial activity was demonstated against *Salmonella typhimurium* where the zone of inhibition reached up to (17.26 ± 0.12) mm followed by *Pseudomonas aeruginosa* (15.00 ± 0.12) mm by the methanolic extract (Fig. 2). The ethanolic extract gave mm gave a larger zone of inhibition against *Proteus vulgaris* (14.55 ± 0.03) mm and *Serratia marcescens* (14.55 ± 0.05) mm. The potential reasons for higher antimicrobial activity was in accordance with that of Umamaheswari *et al* [26]. The range of effectiveness of plant extracts was different for each microbe because every medicine has its own activity spectrum against micro-organisms. The observations of no antimicrobial on *Vibro cholera* and *Klebsilla pneumoniae* were in accordance with the results of Umamaheswari *et al* [26]. Gupta *et al* [30, 34] used hydroalcoholic extracts of *B. glabra* 'snow white' and *B. glabra* 'choicy' against various Gram positive and Gram negative bacteria. They found that *B. glabra* 'choicy' inhibited all Gram negative and Gram positive, except *Proteus vulgaris*. While *B. glabra* 'snow white' showed no antimicrobial activity against *Bacillus subtilis* and *Micrococcus leuteus*. Ali *et al* [35] screened the methanolic extracts of *B. spectabilis* flowers with five different colors) for antibacterial and antifungal activity, brine shrimp lethality and phytotoxicity. They observed that the methanolic extract of the white flowers was the most biologically active among all of tested extracts. Similarly, the extract of the white flowers also exhibited toxicity against shrimp larvae with a LD_{50} value of 33.56 $mg \cdot mL^{-1}$.

3.2 *Antimicrobial activity of B. variegata against Gram positive and Gram negative bacteria*

Gram positive strains (*Staphylococcus aureus, Bacillus spizizenii, Bacillus subtilis, Streptococcus faecalis and Micrococcus luteus)* showed antimicrobial activity from both the ethanolic and methanolic extracts (Table 2). Maximum antimicrobial activities were observed from the methanolic extracts. Maximum zone of inhibition of (21.97 ± 0.06) mm was obtained against *Staphylococcus aureus* (Fig. 1), followed by *Bacillus subtilis* which showed (14.44 ± 0.05) mm. In the case of the ethanolic extract, *Staphylococcus aureus* showed a zone of inhibition of (20.67 ± 0.04) mm followed by *Streptococcus faecalis* at (15.50 ± 0.20) mm. No antimicrobial activity against *Enterococcus faecalis* was observed by either extract of *B. variegata.* Umamaheswari *et al* [26] found no zone of inhibition against *Enterococcus faecalis* with *B. spectabilis.* No previous study on the antimicrobial activity of *B. variegata* has been recorded previously.

Gram negative strains (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Serratia marcescens, Shigella flexneri*) showed antimicrobial susceptibility from the ethanolic and methanolic extracts, except from *Vibro cholera* and *Klebsilla pneumoniae*. The mMaximum zones of inhibition (Fig. 2) were observed for *Proteus vulgari* and *Serratia marcescens.* In both cases, the zone of inhibition reached up to 16.00 mm in ethanolic extract. Similarly, it was observed that ethanolic extract performed effectively against all microbes in comparison with methanolic extract. The possible reason for this difference is because of different spectrum of natural medicines against microbes [34].

3.3 *Comparison of antimicrobial activity of Bougainvillea spectabilis stand and Bougainvillea variegata leave extract with standard antibiotics*

Plant products which have been tested appear to be

Table 3 Zones of Inhibitions (mm) produced by *B. spectabilis* leaf extract *and B. variegata* leaf extract against Gram negative strains

Bacterial Strains	*B. spectabilis*		*B. variegata*	
Gram Negative Strains	Ethanol Extract	Methanol Extract	Ethanol Extract	Methanol Extract
Pseudomonas aeruginosa	13.68 ± 0.04	15.00 ± 0.12	14.02 ± 0.02	11.44 ± 0.01
Escherichia coli	13.23 ± 0.04	12.01 ± 0.02	10.93 ± 0.01	12.91 ± 0.01
Salmonella typhimurium	10.93 ± 0.03	17.26 ± 0.12*	12.20 ± 0.25	13.45 ± 0.05
Proteus vulgaris	14.55 ± 0.03	07.00 ± 0.10	16.00 ± 0.15*	08.00 ± 0.12
Serratia marcescens	14.55 ± 0.05	12.00 ± 0.15	16.00 ± 0.06*	18.00 ± 0.10
Shigella flexneri	11.00 ± 0.25	07.00 ± 0.10	13.00 ± 0.10	08.00 ± 0.10
Vibro cholerae	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Klebsilla pneumoniae	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00

*=Highest zone of inhibition

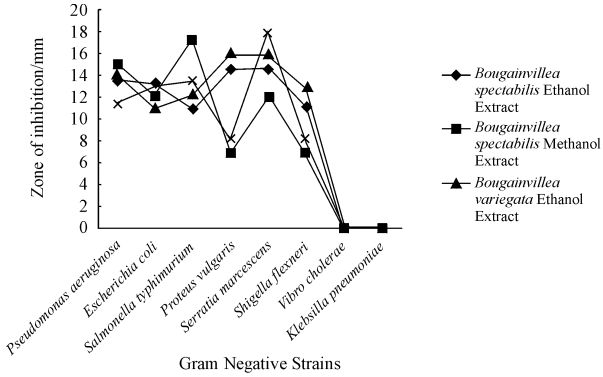

Fig. 2 Zone of inhibitions of Gram positive strains

effective against a wide spectrum of microorganisms, both pathogenic and non-pathogenic. Administered orally, these compounds may be able to control a wide range of microbes, but there is also the possibility that they may cause an imbalance in the gut micro flora, allowing opportunist pathogenic bacteria, such as coliforms, to become established in the gastrointestinal tract with resultant deleterious effects.

Table 4 shows the comparison of established antibiotics with *B. spectabilis* leaves and *B. variegata* leaves. The tandard discs (7 mm diameter) of ampicillin (AMP 25 μg), chloramphenicol (C 30 μg), ciprafloxicin (CIP 5 μg) and tetracycline (TE 30 μg), erythromycin 'E' (15 μg), neomycin 'N' (30 μg) and gentamicin 'CN' (10 μg) were used as positive controls, as well for antimicrobial activity bacterial strains. The results gave the clear idea that the antimicrobial activities for the extracts are comparable to those of the antibiotics. The antibiotics lose resistant after sometime but these extracts are natural extracts and lack the ability of losing resistant and without any side effects (Fig. 3).

A number of *in vitro* studies have reported the use of plant extracts in combination with antibiotics, with significant reduction in the minimum inhibitory concentrations (MICs) of the antibiotics against some resistant strains [36-38]. The curative effect of plant extracts in this combination study has been variably referred to as resistance modifying/modulating activity [33]. This ability of plant extracts to potentiate antibiotics has not been well explained. It is speculated that inhibition of drug efflux and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics [31, 39].

Table 4 Zones of inhibitions (mm) produced by standard antibiotic discs against Gram negative and Gram positive bacterial strains

Bacterial strains	Antimicrobial activity of standard antibiotics against different bacterial strains							
	CIP 5 μg	TE 30 μg	AMP 25 μg	C 30 μg	E 15 μg	N 30 μg	CN 10 μg	DMSO
Pseudomonas aeruginosa	30.10 ± 0.05	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	10.99 ± 0.03	20.03 ± 0.50	00.00 ± 0.00
Escherichia coli	36.10 ± 0.25	24.70 ± 0.10	21.67 ± 0.20	25.68 ± 0.05	11.86 ± 0.02	20.79 ± 0.04	23.16 ± 0.25	00.00 ± 0.00
Salmonella typhimurium	35.25 ± 0.22	21.73 ± 0.15	27.40 ± 0.30	26.55 ± 0.21	8.69 ± 0.05	22.42 ± 0.26	22.53 ± 0.30	00.00 ± 0.00
Enterococcus faecalis	26.71 ± 0.10	34.38 ± 0.24	35.37 ± 0.32	24.20 ± 0.05	20.26 ± 0.10	8.02 ± 0.23	8.88 ± 0.20	00.00 ± 0.00
Staphylococcus aureus	29.87 ± 0.05	33.46 ± 0.27	39.98 ± 0.32	27.49 ± 0.15	28.90 ± 0.30	20.12 ± 0.21	21.68 ± 0.15	00.00 ± 0.00
Bacillus spizizenii	34.19 ± 0.01	29.79 ± 0.32	38.72 ± 0.60	23.76 ± 0.20	32.23 ± 0.25	24.26 ± 0.05	26.16 ± 0.17	00.00 ± 0.00
Bacillus subtilis	36.17 ± 0.22	29.93 ± 0.23	38.66 ± 0.10	25.40 ± 0.10	31.83 ± 0.21	23.06 ± 0.07	26.35 ± 0.21	00.00 ± 0.00

CIP: ciprafloxicin; TE: tetracycline; AMP: ampicillin; C: chloramphenicol; E: erythromycin; N: neomycin; CN: gentamicin

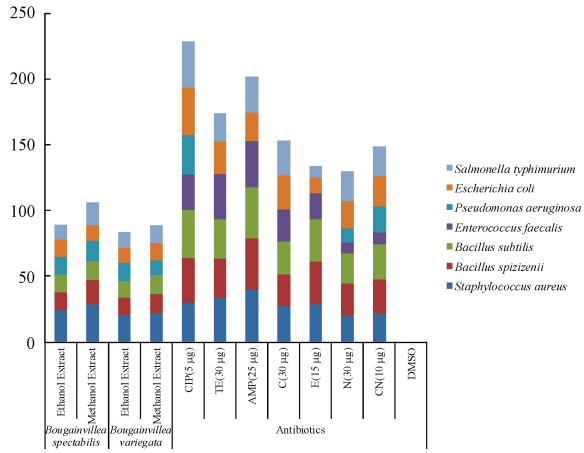

Fig. 3 Comparison of antimicrobial activity of *Bougainvillea* species and standard antibiotics

Chemotherapeutic agents, used topically or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious diseases.

4 Conclusion

The result proves efficiency of *Bougainvillea spectabilis* and *Bougainvillea variegata* against broad spectrum of pathogenic microorganisms. *Bougainvillea* species in near futures provides good source of natural medicines, if a comprehensive research is carried out in this field. *In vivo* studies are needed to assess their efficacy under clinical conditions. With an increasing public awareness of “green issues”, plant volatile oils and extracts, offer a more ecofriendly alternative to conventional formulations in a number of sectors where antimicrobial action is desirable.

References

[1] Chiariandy CM, Seaforth CE, Phelps RH, *et al.* Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties [J]. *J Ethnopharmacol*, 1999, **64**: 265-270.

[2] Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. [J]. *Curr Opin Microbiol*, 2000, **3**: 528-534.

[3] Patwardhan B, Warude D, Pushpangadan P, *et al.* Ayurveda and traditional Chinese medicine: a comparative overview [J]. *Evid Based Complement Alternat Med*, 2005, **2**: 465-473.

[4] Paz EA, Cerdeiras MP, Fernandez J, *et al.* Screening of Uruguayan medicinal plants for antimicrobial activity [J]. *J Ethnopharmacol*, 1995, **45**(1): 67-70.

[5] Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens [J]. *World J Agric Sci*, 2008, **4**(s): 839-843.

[6] Babu PD, Subhasree RS. Antimicrobial activities of *Lawsonia inermis*- a review [J]. *Acad J Plant Sci*, 2009, **2**(4): 231-232.

[7] Solanki R. Some medicinal plants with antibacterial activity. [J]. *Int J Compr Pharm*, 2010, **4**(10): 1-4.

[8] Ikegami F, Fujii Y, Ishihara Y, *et al.* Toxicological aspects of Kampo medicines in clinical use [J]. *Chem Biol Interact*, 2003, **145**: 235-250.

[9] Izzo AA. Drug interactions with St. John’s Wort (*Hypericum perforatum*): a review of the clinical evidence [J]. *Int J Clin Pharmcol Ther*, 2004, **42**: 139-148.

[10] Balandrin MF, Klocke JA, Wutule ES, *et al.* Natural plant chemicals: Sources of industrial and medicinal materials [J]. *Science*, 1985, **228**: 1154-1160.

[11] Satish S, Raveesha KA, Janardhana JR. Antibacterial activity of plant extracts of phytopathogenic *Xanthomonas campestris* [J]. *Lett Appl Microbiol*, 1999, **28**: 145-147.

[12] Sher A. Antimicrobial activity of natural products from medicinal plants [J]. *Gomal J Med Sci*, 2009, **7**(1): 72-68.

[13] Jones FA. Herbs-useful plants. Their role in history and today [J]. *Eur J Gastroenterol Hepatol*, 1996, **8**: 1227-1231.

[14] Zaika LL. Spices and herbs: their antimicrobial activity and its determination [J]. *J Food Safety*, 1975, **9**: 97-118.

[15] Kobayashi KD, McConnell J, Griffis J. *Bougainvillea. Ornamentals and Flowers* [M]. 2007.

[16] Balasaraswathi R, Sadasivam S, Ward M, *et al.* An antiviral protein from *Bougainvillea spectabilis* roots; Purification and characterization. [J]. *Phytochemistry*, 1998, **47**(8): 1561-1565.

[17] Pun KB, Sabitha D, Jeyarajan R. Screening of plant species for the presence of antiviral principles against okra yellow vein mosaic virus [J]. *Indian J Phytopathol*, 1999, **52**(3): 221-223.

[18] Joshi DD, Mujumdarm AM, Narayanan CR. Anti-inflammatory activity of *Bougainvillea spectabilis* leaves [J]. *Indian J Pharm Sci*, 1984, **46**(5): 187-188.

[19] Narayanan C. Pinitol-A new anti-diabetic compound from the leaves of *Bougainvillea spectabilis* [J]. *Curr Sci*, 1987, **56**(3): 139.

[20] Senapati AK, Dash GK, Ghosh T, *et al.* A study on anti-inflammatory and hypoglycaemic activity of *Bougainvillea spectabilis* [J]. *Indian J Nat Prod*, 2006, **22**(2): 2-9.

[21] Narayanan CR, Joshi DD, Mujumdar AM. Hypoglycemic action of *Bougainvillea spectabilis* leaves [J]. *Curr Sci*, 1984, **53**: 579-581.

[22] Adebayo JO, Adesokan AA, Olatunji LA, *et al.* Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats [J]. *Biochem*, 2005, **17**: 25-50.

[23] Schlein Y, Jacobson RL, Muller GC. Sand fly feeding on noxious plants: a potential method for the control of leishmaniasis. [J]. *Am J Trop Med Hyg*, 2001, **65**: 300-303.

[24] Edwin E, Sheeja E, Toppo E, *et al.* Anti-diarrhoeal, anti–ulcer and anti microbial activities of leaves of *Bougainvillea glbra* choicy [J]. *Ars Pharm*, 2007, **48** (2): 135-144.

[25] Edwin E, Edwin S, Amalraj A, *et al.* Antihyperglycemic activity of *Bougainvillea glabra* Choisy [J]. *Planta Indica*, 2006, **2** (3): 25-26.

[26] Umamaheswari A, Shreevidya R, Aparna N. *In vitro* antibacterial activity of *Bougainvillea spectabilis* leaves extracts [J]. *Adv Biol Res*, 2008, **2** (1-2): 1-5.

[27] Harborne JB. *Phytochemical methods* [M]. 1973: 149-188.

[28] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses [J]. *Nat Med*, 2004, **10**: S122-S129.

[29] Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin [J]. *Perspect New Crops New Uses*, 1999: 457-462.

[30] Gupta V, George M, Joseph L*, et al*. Evaluation of antibacterial activity of *Bougainvillea glabra* "snow white" and *Bougainvillea glabra* "choicy" [J]. *J Chem Pharm Res*, 2009, **1** (1): 233-237.

[31] Lewis K, Ausubel FM. Prospects for plant-derived antibacterials [J]. *Nat Biotechnol*, 2006, **24** (12): 1504-1507.

[32] Cowan MM. Plant products as antimicrobial agents [J]. *Clin Microbiol Rev*, 1999, **12** (4): 564-582

[33] Gibbons S. Anti-staphylococcal plant natural products [J]. *Nat Prod Rep*, 2004, **21**: 263-277.

[34] Gupta V, George M, Singhal M*, et al.* Pharmacognostical evaluation of the leaf of *Bougainvillea glabra* "snow white" [J]. *J Pharm Res*, 2009, **2** (11): 1775-1779.

[35] Ali MS, Ibrahim SA, Ahmed F*, et al*. Color versus bioactivity in the flowers of *Bougainvillea spectabilis* (Nyctaginaceae) [J]. *Nat Prod Res*, 2005, **19** (1): 1-5.

[36] Al-hebshi N, Al-haroni M, Skaug N. *In vitro* antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms [J]. *Arch Oral Biol*, 2006, **51**: 183-188.

[37] Darwish RM, Aburjai T, Al-Khalil S*, et al.* Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus* [J]. *J Ethnopharmcol*, 2002, **79**: 359-364.

[38] Betoni JEC, Mantovani RP, Barbosa LN*, et al.* Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases [J]. *Mem Inst Oswaldo Cruz*, 2006, **101** (4): 387-390.

[39] Zhao WH, Hu ZQ, Okubo S*, et al.* Mechanism of synergy between epigallochatechin gallate and *β*-lactams against methicillin resistant *Staphylococcus aureus* [J]. *Antimicrob Agents Chemother*, 2001, **45** (6): 1731-1742.