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Phytochemical screening and *in vitro* antimicrobial activity of *Bougainvillea spectabilis* flower extracts

Kumara Swamy M*[1], Sudipta KM[1], Lokesh P[1], M Neeki A[1], Rashmi W[1], S Bhaumik H[1], S Darshil H[1], R Vijay[1] and SSN Kashyap[1]

*Corresponding author:

Kumara Swamy M

[1]Department of Biotechnology, Padmashree Institute of Management and Sciences, Kommagatta, Kengeri, Bangalore - 560060, India.

Abstract

Various flower extracts (Chloroform, ethyl acetate, ethanol and water) of *Bougainvillea spectabilis* were screened for their phytochemical constituents and also investigated for their antimicrobial activities. Phytochemical screening of flower extracts revealed the presence of alkaloids, flavonoides, phlobatannins and terpenoids. Steroids, phenol, tannins, cardinolides and volatile oils were absent in all the extracts. All flower extracts of *B. spectabilis* inhibited the growth of few of the bacterial and fungal strains tested with varied effectiveness. The maximum antibacterial activities were observed in ethanol and water extracts. The maximum antifungal activities were observed in chloroform and ethanol extracts. Thus the bioactive natural products in flower extracts of *Bougainvillea spectabilis* can be used in the development of new pharmaceuticals that address unmet therapeutic use.

Keywords: Antimicrobial, phytochemical screening, *Bougainvillea spectabilis* , medicinal plant.

Introduction

The medicinal plants are of great interest to human health. Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years [1, 2]. Many medicinal plants are used daily in Ayurvedic practices. In India more than 7,000 medicinal plant species are known. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Plants contain numerous biologically active compounds, many of these have been shown to exhibit antimicrobial properties and therefore they were in use as 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 needs [3].

Knowledge of the phytochemicals is desirable not only for the discovery of healthcare products, but also in disclosing new sources of economic materials like alkaloids, tannins, oils, gums etc., [4]. The systematic screening of plant extracts or plant derived substances still remains an interesting strategy to find new lead compounds in many plant species. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [5]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [6].

Bougainvillea spectabilis belonging to family Nyctaginaceae is an important horticultural plant. The Bougainvillea is an immensely showy, floriferous and hardy plant native to South America. Plants produce vibrant blooms nearly year-round and virtually it is pest-free and disease resistant. *B. spectabilis* leaves extract inhibited tomato spotted wilt to spovirus on *capsicum annum* and ground water in laboratory tests [7]. *B. spectabilis* was highly effective in reducing okra yellow vein mosaic virus infection of okra [8]. Antiviral protein was characterized by Balasaraswathi *et al.,* [7] and anti-inflammatory activities were also observed by Joshi *et al.,* [9] in *B. spectabilis*. *B. spectabilis* Wild. (Nyctaginaceae) have been identified to be of prime importance in controlling and preventing diabetes [10, 11]. In vitro antibacterial activity of *Bougainvillea spectabilis* leaves extracts is been reported by Umamaheswari et al [3]. The effect of the ethanolic extract of *B. spectabilis* leaves on some liver and kidney function indices in rats. Traditional practitioners in Mandsaur use the leaves for a variety of disorders, for diarrhea, and to reduce stomach acidity, used for cough and sore throat for blood vessels and leucorrhea for hepatitis. The aqueous extract of *B. spectabilis* leaves showed anti-fertility potential in Swiss Albino mice [12]. To the best of our knowledge, in *B. spectabilis* there is little or no scientific information concerning the antimicrobial activity of flower extracts.

Thus considering the vast potentiality of *Bougainvillea spectabilis* as sources for antimicrobial drugs, a systematic investigation was undertaken to screen for phytochemicals, antibacterial and antifungal activity from its flower extracts.

Materials and methods

Plant material and collection

The flowers of *Bougainvillea spectabilis* were collected from Padmashree campus, Kommagahatta village, Kengeri , Bangalore, Karnataka.

Extraction

Flowers of *Bougainvillea spectabilis* were dried in the micro-oven and ground into powder by using an electronic blender. 5g of blended material was transferred into a beaker and 100 ml of each chloroform, ethyl acetate, ethanol, and water was separately added and allowed to stand for 48h and then filtered. The mixture was filtered using Whatman No. 1 filter paper. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Phytochemical studies

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne [13].

Test for steroids

2ml of acetic acid was added in 0.5 ml of ethanolic extract and then 2ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for terpenoids

In 5ml of solvent extract, 2ml of chloroform was added and then 3ml of concentrated sulfuric acid was added carefully. A reddish brown coloration of the interface was regarded as positive for the presence of terpenoids.

Test for tannins

About 0.5 g of leaf powder was weighed into a beaker and 20 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl3 were then added. Production of greenish precipitate indicated the presence of tannins.

Test for flavonoids

2ml of sodium hydroxide was added in 2ml of solvent extract. Appearence of yellow color was regarded as the presence of flavonoids.

Test for alkaloids

A little amount of picric acid solution was added in 2ml of solvent extract. Formation of orange color showed the presence of alkaloids.

Test for saponins

About 1ml of solvent extract was introduced into a tube containing 1 ml of distilled water, the mixture was vigorously shaken for 2 min, and formation of froth indicated the presence of saponins.

Test for phenols

2ml of ferric chloride solution was added in 2ml of solvent extract. Formation of deep bluish green solution indicated the presence of phenols.

Test for anthraquinones

0.5g of crude powder was added in 10ml of benzene and filtered. Then 0.5ml of ammonia solution was added in the filtrate and shaken well. Violet color in the layer phase indicated the presence of antraquinones.

Test for cardiac glycoside

0.5g of extracts was dissolved in 2ml of glacial acetic acid containing 1 drop of ferric chloride. Then 2ml of conc.sulphuric acid was added under layered. Brown ring was formed at interphase indicated the presence of deoxy sugar which is the characteristic of cardiac glycoside.

Test for phlobatannins

Few drops of 1% hydrochloric acid was added in 1ml of solvent extract and boiled. Red precipitate was formed indicated the presence of phlobatannins.

Test for cardenolides

2ml of benzene was added to 1ml of solvent extract. Turbid brown color was observed indicated the presence of cardenolides.

Test for volatile oils

2ml of extract solution in which 0.1ml of sodium hydroxide and small quantity of dilute HCl was added and shaken well. White precipitate was formed with volatile oils.

Bacterial Cultures And Media

Bacterial isolates of *Bacillus, Klebsilla, Proteus vulgarius, Pseudomonas aeruginosa, Rhizobium* were obtained from the Department of Microbiology, Padmashree Institute of Management and Sciences, Bangalore. All bacteria were grown on nutrient agar (NA) at 37°C. For antibacterial assays, bacteria were inoculated into nutrient broth (Himedia, India) and incubated overnight at 37°C.

Antibacterial Assay

Antibacterial assay of the extracts was carried out by disc diffusion method [14]. Briefly, freshly grown liquid culture of the test pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs of eight mm diameter were soaked with 40µl of 50mg/ml the extracts and air dried to evaporate the solvent and the discs were applied over the seeded NA plates at equidistance. The plates were incubated at 37°C for 18 to 24 h. After the incubation period, the plates were observed for a clearance zone around the discs which indicates a positive antibacterial activity of the respective extracts. The clearance zones formed around each disc were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Fungal Cultures And Media

Fungal isolates of *Trichoderma sp., Penicillium sp., Aspergillus niger, Rhizopus* were obtained from the Department of Microbiology, Padmashree Institute of Management and Sciences, Bangalore. All fungi were grown on Potato dextrose agar at 25°C. For antifungal assays, fungi were inoculated into normal saline.

Antifungal Assay

Antifungal assay of the extracts was carried out by disc diffusion method [14]. Briefly, freshly inoculated liquid culture of the test pathogens were seeded over the Potato dextrose agar plates with a sterile swab. Sterile filter paper discs of eight mm diameter were soaked with 40µl of 50mg/ml the extracts and air dried to evaporate the solvent and the discs were applied over the seeded PDA plates at equidistance. The plates were incubated at 25°C for 18 to 24 h. After the incubation period, the plates were observed for a clearance zone around the discs which indicates a positive antifungal activity of the respective extracts. The clearance zones formed around each disc were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antifungal activity of the extracts.

Results and Discussion

In the present study the phytochemicals occurring in the various solvent extracts of *Bougainvillea spectabilis* flowers (ethanolic, ethyl acetate, chloroform and aqueous extracts) were analyzed qualitatively by phytochemical screening. The results revealed the presence of various secondary metabolites of therapeutical importance. The major phytochemicals found were phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids. However, all extracts tested showed the absence of sterols, anthraquinones, cardenolides and volatile oils. Ethyl acetate extract yielded maximum phytochemicals (Table1). Alkaloids were present only in ethyl acetate extract. Flavonoids were found in chloroform and ethanol extracts. Saponins were present in all the extracts except chloroform. Phlobatannins were present only in ethyl acetate extract. Terpenoids were present in chloroform and water extract. When compared to leaf extracts, the flower extract showed very less number of phytochemicals [3].

The present work reveals the antimicrobial activities of different solvent extracts of *Bougainvillea spectabilis* flower against different bacterial and fungal strains (Tables 2 and 3). Their antimicrobial potency was assessed by the presence or absence of inhibition zones and zone diameters (mm). It was observed that the antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains [15, 16]. Moreover, different studies found that the type of solvent has an important role in the process of extracting [17, 18, 19, 20]. Several authors have reported the antimicrobial activity of crude extracts of various plants [21, 22, 23]. The negative control discs were soaked with 40 µl DMSO and the positive control with 40 µl of 50 µg/ ml chloromphenicol. Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments.

The negative control discs were soaked with 40 µl DMSO and the positive control with 40 µl of 50 µg/ ml flucanazole. Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments.

In the present study, a considerable antimicrobial activity was observed in *B. spectabilis*. All flower extracts of *B. spectabilis* inhibited the growth of few of the bacterial and fungal strains tested with varied effectiveness. The ethanol and chloroform extracts have shown relatively greater activity than that of any other extracts at 40µl concentration. This may be due to the presence of flavonoids and saponins present in their extracts. Similarly Hegazi et al., [24] and Tsao et al., [25] have reported the antimicrobial activity of flavonoids. The observed antibacterial effects on the isolates are believed to be due to the presence of phlobatannins and flavonoids which have been shown to possess antibacterial properties [26, 27]. Soetan et al., reported the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench [28]. The mild antimicrobial activity was observed in water and ethyl acetate

Table 1. Preliminary phytochemical analysis of *B. spectabilis* leaf extracted with different solvents.

Phytochemicals	Chloroform	Ethyl acetate	Ethanol	Water
Alkaloids	-	+	-	-
Flavonoids	+	-	+	-
Saponins	-	+	+	+
Phlobatannins	-	+	-	-
Terpenoids	+	-	-	+
Steroids	-	-	-	-
Phenol	-	-	-	-
Tannins	-	-	-	-
cardinolides	-	-	-	-
Volatile oils	-	-	-	-

Note: + Present, -Absent.

Table 2. Antibacterial activity of leaf extracts of *B. spectabilis* at 40 µl 50 µg/ ml concentration.

Bacteria	Chloroform (mm	Ethanol (mm)	Ethyl acetate (mm)	Water (mm)
Klebsiella pneumoniae		-	-	7±0.4
Proteus vulgaris	3±0.2	6±0.4	-	-
Pseudomonas aeruginosa		-	-	-
Bacillus subtilis	4±0.8	7±0.2	3±1.0	7±0.7

Table 3. Antifungal activity of leaf extracts of *B. spectabilis* at 40 µl 50 µg/ ml concentration.

Fungi	Chloroform (mm)	Ethanol (mm)	Ethyl acetate (mm)	Water (mm)
Aspergillus niger	8±0.6	9±0.3	-	7±0.3
Penicillium notatum	-	8±0.7	-	-
Rhizopus oryzae	-	-	9±0.5	-
Trichoderma viride	5±0.4	13±0.3	-	-

extracts. This may be due to the presence of terpenoids and saponins. Also Tchesche and Wulff (1973) have confirmed the weak antibacterial and fungistatic effects of the majority of saponins [29]. The results obtained are encouraging as the ethanol and chloroform extracts have shown considerable antibacterial activity against the tested organisms.

In conclusion, the screening and scientific evaluation of plant extracts against microbes may provide new antimicrobial substances. Also, plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Hence, the present investigation clearly reveals the presence of several phytochemicals in flower extracts of *B. spectabilis* which could be used as a source of antimicrobial agents.

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