Evaluation of antibacterial activity of *Bougainvillea glabra 'snow white'* and *Bougainvillea glabra 'choicy'*

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Abstract

The aim of the present study was to evaluate and compare the antimicrobial activity of *Bougainvillea glabra* 'Snow White' leaves extract with *Bougainvillea glabra* 'Choicy' leaves extract. *Bougainvillea glabra* 'Snow White' is a cultivated variety of *Bougainvillea glabra* 'Choicy' which have white bracts with greenish veins. Antimicrobial activity of different solvent extracts of these plant leaves were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. Antimicrobial activity was done by disc diffusion method at a concentration of 500 µg/disc of the extract, using ofloxacin (5µg/disc) as the standard. The bacterial strains used in the study were *Staphycococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhii, Klebsiella pneumoniae, Proteus vulgaris, and Vibrio cholerae.* Hydroalcoholic extract was more active against all bacteria. *Bougainvillea glabra* 'snow white' was not effective against *Bacillus subtilis* and *Micrococcus leuteus* while *B. glabra* 'choicy' was not effective against *Proteus vulgaris*.

Key Words: Antibacterial activity, *Bougainvillea glabra*, Hydroalcoholic extract, Ofloxacin

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 drugs [1-3]. The World Health Organization 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 chemotherapeutics 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]. The genus *Bougainvillea* in the Nyctaginaceae (4 O' clock) family of plants, has 18 species, with three that are horticulturally important *Bougainvillea spectabilis*, *B. glabra* and *B. peruviana*. *Bougainvillea glabra* 'Snow White' is a cultivar of the *B. glabra* 'Choicy' which have white bracts with the greenish veins [9,10]. *Bougainvillea glabra* 'Choicy' have been used by the traditional practitioner of Mandsaur in variety of disorders like diarrohea, reduce stomach acidity, cough and sore throught, decoction of dried flowers for blood vessels and leucorrohea and decoction of the stem in hepatitis. The main part used is leaves. The reported constituents in leaf of *Bougainvillea glabra* 'Choicy' are alkaloids, flavanoids, tannins, sapononins and proteins [11]. The leaves of *Bougainvillea glabra* 'Choicy' are reported to have insecticidal activity [12], anti-inflammatory [9], anti-diarrhoeal activity [13], anti hyperglycemic activity [14], anti-ulcer and anti-microbial activity [13]. In spite the numerous uses and pharmacological activity attributed of *Bougainvillea glabra* choicy but no pharmacological information regarding the leaves of this plant cultivar *Bougainvillea glabra* 'Snow White'. Hence, the present investigation is an attempt in this direction and includes evaluation of anti-bacterial activity of hydroalcoholic extract.

Experimental Section

Plant Material

The plant materials (leaves) were collected during Feb.-March 2009 from the Balaji Nursery, Jagatpura, Jaipur (Rajasthan), India. The botanical identity of both plants was authenticated by the Dr. N.S. Shekhawat, Head of the Department of Botany, Jai Narayan Vyas University, Jodhpur, (Raj.), India.

Preparation of Plant Extract

The plant material was dried in shade and crush in the grinder. The dried powder was obtained. The dried powdered material was initially defatted with pet. ether (60-80 ^{0}C) in a soxhlet apparatus for 72 h according to successive solvent extraction. The pet. ether extract was dried and collected. The mark was dried and extracted with hydro-alcohol (50:50) each for 72 h. The extract was filtered while hot and the solvent was removed by distillation under reduced pressure and percentage yield of the extract was determined.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate[15] and Harborne[16].

Test Organisms

Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were *Staphycococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Micrococcus luteus* (ATCC 9341), *Escherichia coli* (NCIM 2931), *Salmonella typhii* (ATCC 13313), *Klebsiella pneumonia* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *and Vibrio cholera* (MTCC 1738).

Antimicrobial Activity

The antimicrobial activity of the extract was assessed by disc diffusion method [17]. Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into a petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *vibrio cholere*, *salmonella typhii*, *micrococcus leuteus* and *Proteus vulgaris* were spread over the media with the help of a sterile swab socked in bacterium and is used for antibacterial study. Acetone, ethanolic and aqueous extract residues were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 500 µg/disc and used for the study. Ofloxacin 5 µg/disc was used as the standard. Then the sterile filter paper discs (6mm) having a capacity to hold 10 µl of extracts were immersed in definite concentration of plant extracts and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37 °C for 24 hours. After the incubation periodis over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.

Results and Discussion

The result of preliminary phytochemical screening revealed the presence of alkaloid, glycosides (minute amount), flavanoids, tannins, steroid, protein and saponins in hydroalcoholic extract of leaves of *Bougainvillea glabra'*Snow White' but *Bougainvillea glabra* 'Choicy' had not the glycosides in the leaves extract [Table 1].

Table 1. Preliminary phytochemical screening of extract of *Bougainvillea glabra* "Snow White" and *Bougainvillea glabra* 'Choicy'

Tests	*B. glabra* 'Snow White'	*B. glabra* 'Choicy'
Alkaloid	+	+
Carbohydrate	-	-
Flavanoids	+	+
Phenolics compound & Tannins	+	+
Protein	+	+
Steroids	-	-
Glycosides	- (slightly)	+
Fat & oils	-	-

Hydroalcoholic extract of *Bougainvillea glabra* 'Snow White' showed inhibitory effect against all gram positive and gram negative bacteria except *Bacillus subtilis* and *Micrococcus leuteus* because every medicine have his own spectrum against micro-organisms so *Bougainvillea glabra* "Snow White " have his own spectrum against bacteria's so we can say that it is more effective against gram negative and positive bacteria except *Bacillus subtilis* and *Micrococcus leuteus* . And *Bougainvillea glabra* 'Choicy' showed inhibitory effect on all gram negative and gram positive bacteria selected for the present study except *Proteus vulgaris* respectively [Table 2].

Table 2. Comparative study of hydroalcoholic extract of leaves of *Bougainvillea glabra* 'Choicy' and *Bougainvillea glabra* 'Snow White'

Bacterial Strains	**Diameter of zone of inhibition**		
	Hydroalcoholic extract of *B. glabra* 'choicy' 500 µg/disc	**Hydroalcoholic extract of *B. glabra* 'Snow White' 500 µg/disc**	**Ofloxacin 5 µg/disc**
Gram Positive Bacteria			
Staphycococcus aureus	10	12	26
Bacillus subtilis	15	----	24
Micrococcus luteus	13	----	22
Gram Negative Bacteria			
Escherichia coli	12	13	23
Salmonella typhii,	14	13	24
Klebsiella pneumonia	16	14	25
Proteus vulgaris	----	12	22
Vibrio cholerae	17	18	27

Conclusion

The result suggested that different solvent extracts under study showed antibacterial activity. The anti-bacterial action of various extracts of *Bougainvillea glabra* 'Choicy' 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 meditional plants is expected to boost the use of these plants in the therapy against disease caused by the test bacterial species and other micro-organisms. It is possible that

better therapy for many microbial diseases can be found in the leaves extracts. The prilimnary results of this investigation indicates that *Bougainvillea glabra* 'Choicy' and *Bougainvillea glabra* 'Snow White' leaves have good potential of antimicrobial activity.

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