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EVOLUTION OF ANTI MICROBIAL AND ANTI OXIDANT ACTIVITY OF ALCOHOLIC EXTRACT OF BOUGAINVILLEA GLABRA FLOWERS

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial, anti oxidant activity of *Bougainvillea glabra* leaves extract, plant belongs to family Nyctaginaceae. Antimicrobial activity of alcoholic 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, Proteus vulgaris,* the anti oxidant activity of extract was tested by using DPPH radical scavenging method at concentration of 5, 10, 15, 20, 25, 30 µg/ml, using Ascorbic acid as standard. The outcomes of the present study indicated that the alcoholic extract of the *Bougainvillea glabra* flowers shows the significance anti microbial and anti oxidant activity in a concentration of 500µg/ml and 30 µg/ml respectively.

KEYWORDS: *Bougainvillea glabra*, alcoholic extract Antibacterial activity, antioxidant activity, DPPH method.

INTRODUCTION

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] Hence, the present investigation is an attempt in this direction and includes evaluation of antibacterial, anti oxidant activity of alcoholic extract.

Experimental Section

Plant Material

The plant material was collected flower market in Tirupathi and authentified by Dr.B.Sitaram, Professor, Department of Dravyaguna, S. V. Ayurvedic Medical College, Tirupati. (Internet source)

Preparation of plant extract

The fresh petals of flower *Bougainvillea glabra* were shade dried. The dried petals were grinded to get coarse powder. 250 gm of coarse powder was subjected to cold maceration process using ethanol as solvent. The extraction was continued for 7days at room temperature with occasional shaking. Then the extract was filtered, collected and concentrated at 70^{o}C on a heating mantle until a softy mass obtained. It was then thoroughly air dried to remove all the traces of solvent and then was subjected to freeze drying. The obtained plant extract was preserved in cold condition i.e. below 0^{o}C till the end of treatment period.

Preliminary Phytochemical Screening

Standard qualitative screening test of the extract was carried out for various plant constituents. The crude

extract was screened for the presence or absence of secondary metabolites using standard procedures.[15,16]

Anti-microbial evolution

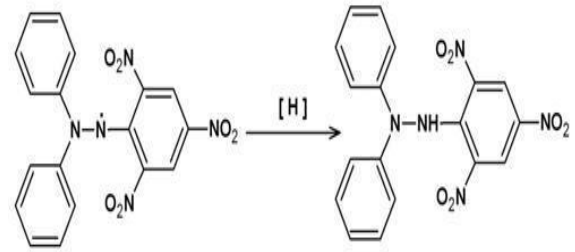
Test Organisms Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were Staphycococcus aureus (NCIM 2079), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 2931, Proteus vulgaris (NCIM 2027).

Procedure

The antimicrobial activity of the extract was assessed by disc diffusion method. 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, Staphylococcus aureus, and Proteus vulgaris were spread over the media with the help of a sterile swab socked in bacterium and is used for antibacterial study. The ethanolic extract residues were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 100, 250,500 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 period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.

4.8.2 Anti-oxidant activity

The antioxidant activity by DPPH radical scavenging method Free radical scavenging activity of different extracts of leaves and flowers of. plant were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Here, only those extracts are used which are Solubilise in ethanol and their various concentrations were prepared by dilution method.14 The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu).15 Reference standard compound being used was ascorbic acid and experiment was done in triplicate.16 The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.



Structural changes of DPPH during oxidation

The percent DPPH scavenging effect was calculated by using following equation

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction

A1 was the Absorbance in presence of test or standard sample.

RESULTS AND DISCUSSION

Table 1: Results of Preliminary Phytochemical.

S. No	Name of the Test	Result
1.	Flavonoids	++
2.	Phenols	++
3.	Alkaloids	++
4.	Saponins	++
5.	Carbohydrates	++
6.	Proteins & amino acids	++
7.	Tannins	++
8.	Cardiac glycosides	++

Screening of ERD

Table 2: Anti Microbial Evolution of Compounds.

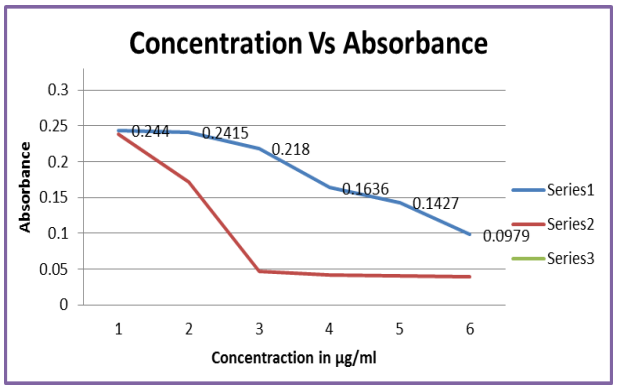
	Alcoholic extract of *Bougainvillea glabra*				Ofloxacin		
	Zone of inhibition in mm						
		100µg/ml	250mg/ml	500µg/ml	100µg/ml	250mg/ml	500µg/ml
Name of the organisms	*Staphylococcus aureus*	10	14	18	12	20	26
	Bacillus subtilis	8	16	20	14	18	24
	Escherichia coli	6	18	22	12	16	25
	Proteus vulgaris	12	14	20	12	14	22
Control	DMSO	-	-	-	-	-	-

Table 2: Anti oxidant activity of alcoholic extract of *Bougainvillea glabra.*

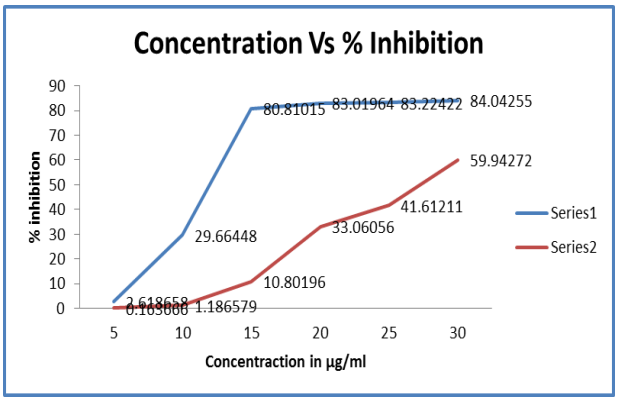
Concentration (µg/ml))	Ascorbic acid (Abs)	Alcoholic extract of Bougainvillea glabra (Abs)
5	0.2380	0.244
10	0.1719	0.2415
15	0.0469	0.218
20	0.0415	0.1636
25	0.0410	0.1427
30	0.0390	0.0979
Control	0.2444	

Table 3: % inhibition of alcoholic extract of *Bougainvillea glabra* with ascorbic acid.

Concentration (µg/ml))	Ascorbic acid (% Inhibition)	Alcoholic extract of Bougainvillea glabra (% Inhibition)
5	2.618658	0.163666
10	29.66448	1.186579
15	80.81015	10.80196
20	83.01964	33.06056
25	83.22422	41.61211
30	84.04255	59.94272



Graph-1: Concentration Vs Absorbance.



Graph-2: concentrations Vs % Inhibition.

DISCUSSION

The present results reveals that the alcoholic extract shows the activity less than the standard. The extract was diluted with concentration of 100 µg/ml, 250µg/ml, 500µg/ml. In that the extract with concentration of 500µg/ml shows the significance activity than the remaining concentrations. The Alcoholic extract of *Bougainvillea glabra* tested for antioxidant activity by using DPPH Assay method. Here the results were compared with the standard Ascorbic acid. The result reveals that the extract shows results less than the standard. The concentration of the extract was taken in to 5-30 µg/ml. The % of inhibition shows that the up to 30µg/ml .The % inhibition is therefore it shows more activity than compare with other concentrations.

CONCLUSION

The outcomes of the present study indicated that the alcoholic extract of the *Bougainvillea glabra* flowers shows the significance anti microbial and anti oxidant activity in a concentration of 500µg/ml and 30 µg/ml respectively. The results were compared with standards like ofloxacin and ascorbic acid respectively.

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