

MICROSCOPY, ANTI-DIARRHOEAL AND REDUCING POWER OF PLANT EXTRACTS OF *BOUGAINVILLEA GLABRA* “SNOW WHITE”

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Summary

The aim of this paper was to evaluate microscopy, anti-diarrhoeal and reducing anti-oxidant power of *Bougainvillea glabra* “Snow White”. An animal study (antidiarrhoeal activity) was carried on experimental albino rats. Extract of leaf was extracted by soxhlet apparatus by using hydro alcoholic solvent (50:50). hydroalcoholic extract in 200 mg kg⁻¹ and 400 mg kg⁻¹ doses were administered in two group and loperamide was administered in dose 3mg kg⁻¹ in separate group. After 5 h fecal matter was collected and test groups were compared with those in the control animals and analyzed statistically. In the antioxidant activity reducing power of plant extracts were evaluated. Different concentration of plant extract (100- 1000 micro litre) were dissolved in 1 ml of water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe (CN)₆] (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. The extracts were shown anti diarrhoeal activity and reducing antioxidant power. These activities were statistically significant (P< 0.05) when compared with control. These results suggest that *Bougainvillea glabra* “Snow White” is able to reduce free radical and effective in bowl imbalance.

Key Words: Anti-diarrhoeal activity, Anti-oxidant, *Bougainvillea*

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Introduction

Diarrhoea has been recognized as one of the most important health problems in developing countries. Worldwide distribution of diarrhoea accounts for more than 5-8 million death each year in infants and small children less than 5 years according to who estimation for the year 1998, there were about 7.1 million deaths due to diarrhea [1]. Microbial infections are a real cause of diarrhoea.

In recent years much attention has been devoted to natural antioxidant and their association with health benefits [2]. Plants are potential sources of natural antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or promotion of their decomposition and suppression of such disorders [3, 4].

The present study relates to one such plant, which has these activities. *Bougainvillea glabra* "Snow White" is a cultivar of *Bougainvillea glabra*, family: Nyctaginaceae [5]. All superficial feature of this cultivar is same as the *Bougainvillea glabra* but the bracts of this cultivar are white with greenish veins [6]. *Bougainvillea glabra* choicy have been used by the traditional practitioner of Mandsaur in variety of disorders like diarrhoea, 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 [7]. The reported constituents in leaf are alkaloids, flavanoids, tannins, sapononins and proteins [8]. The leaves of *Bougainvillea glabra* choicy are reported to have insecticidal activity [9], anti-inflammatory [5], anti-diarrhoeal activity [10], anti hyperglycemic activity [11], anti-ulcer [10] and anti-microbial activity [10].

In spite the numerous uses and pharmacological activity attributed of *Bougainvillea glabra* choicy but no microscopical and 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 microscopical evaluation, anti diarrhoeal activity and reducing antioxidant power of extracts.

Material and Method

Plant material

Bougainvillea glabra leaves was collected from the Balaji Nursery, Jagatpura, Jaipur (Rajasthan), India. The botanical identity of this plant was confirmed by the Dr. N.S. Shekhawat, Head of the Department of Botany, Jai Narayan Vyas University, Jodhpur, (Raj.), India. The specimen was deposited in the museum of the Department of Pharmacognosy, Jaipur National University, Jaipur-302025, (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 °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 successively extracted with acetone and hydro-alcohol (50:50) each for 72 h. The extracts were filtered while hot and the solvent was removed by distillation under reduced pressure and percentage yield of the extracts were determined.

Chemicals and instruments

Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 Camera. Solvents viz. petroleum ether, chloroform, acetone, ethanol (95%) and reagents viz. phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. ferrous chloride were purchased from Merck (Merck KGaA, Darmstadt, Germany). Methanol, ferric chloride, potassium ferricyanide and trichloroacetic acid (TCA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Microscopy

Microscopical studies for the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940) [12]

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b) [13] and Harborne (1998) [14].

Animals

Adult Albino rats of either sex weighing 150–200 g bred in the animal house in the School of Pharmaceutical Sciences, Jaipur National University, Jaipur were housed in a controlled room with a 12 h light-dark cycle, at room temperature of 22 ± 02 °C, humidity 30-60%, and kept on standard pellet diet (altromin pellets) and water *ad libitum*. Animal maintenance and handling were in accordance to internationally accepted standard guidelines for use of laboratory animals. Animals kept under fasting for overnight, but allowed for free access of water before commencement of experiments. The experiment were conducted according to the guidelines and ethical norms, approved by Ministry of Social Justice and Empowerment, Government of India and the study was got approved from the Institutional Animal Ethical Committee (IAEC), (Approval no.1054/ac/07/CPCSEA) of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Acute toxicity studies

The acute toxicity test (LD_{50}) of extracts were determined according to the OECD guidelines [15]. The extracts were safe up to the dose of 2000 mg kg^{-1} and from results suitable dose was chosen for each activity in each extract for further experimentation.

Extract and drug administration

Each extract and standard drugs were suspended in tween 80 (5%) and administered orally through intragastric tube at different doses in mg kg^{-1} Body weight.

Castor oil induced Diarrhoea

Rats were divided in four groups (n=6). The rats were fasted for 18 h and water was provided *ad libitum* and were grouped as follows. Control group was given 10 ml / kg of vehicle (5% Tween 80 in water) orally. Standard group were administered with standard drug of Loperamide (20 mg/kg) orally. Next the remaining groups III, to IV were treated with 200 mg/kg and 400 mg/kg

respectively (P.O.) body weight of extracts. After 1 hour of the above treatment all the rats were orally given 1 ml/100gm wt. castor oil. Each group of rats were then housed separately and observed for 5 h and weight of diarrhoeal faeces was taken. After 5 h in preweighed transparent plastic dishes placed beneath the individual rat cases [10].

Reducing antioxidant power

The reducing antioxidant power of plant methanolic extracts was determined. Different concentrations of plant extracts (200 – 1200 ppm) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Double beam -SHIMATZU). Increased absorbance of the reaction mixture indicates increase in reducing power [16].

Statistical Analysis:

Values are expressed as mean±SEM. Statistical Difference in mean were analyzed using one way ANOVA followed by Dunnett's test $p < 0.05$ was considered significant.

Result and Discussion

Microscopic studies

Epidermis layer is continuous and can be distinguish into upper and lower epidermis, without intercellular space and compact in nature. Upper epidermis is straight walled, single layer containing trichomes (uniseriate, multicellular, bulbulous) and stomata actinomyces type). Lower epidermis is similar to upper epidermis but it contains trichomes and stomata. Cuticle present on above epidermis and lower to lower epidermis but it is wavier at lower side. Just below the epidermis collenchyma layers present this can be characterized by thick cellulosic deposition. It present in whole length of midrib but not in middle lamina. Cells of upper layers are small and 4 to 5 layers but cells of lower are comparatively big and 2 to 3 layers. Mesophyll can be differentiate in spongy and palisade parenchyma cells. Palisade is single layered readily elongated covering 1/ 10 of the lamina part. Spongy parenchyma is Thin layered loosely arranged containing intercellular spaces. Cells also contain starch (in large amount) and Ca. oxalate crystals (in small amount). It covers remaining part of lamina. Vascular bundles present in spongy tissues, usually 5 in bundles arc shaped, more prominent towards lower side. Each is surrounded by pericycle single layer. Vascular bundles are surrounded by endodermal layer. Phloem present towards dorsal side while xylem toward ventral side. (Fig. 1)

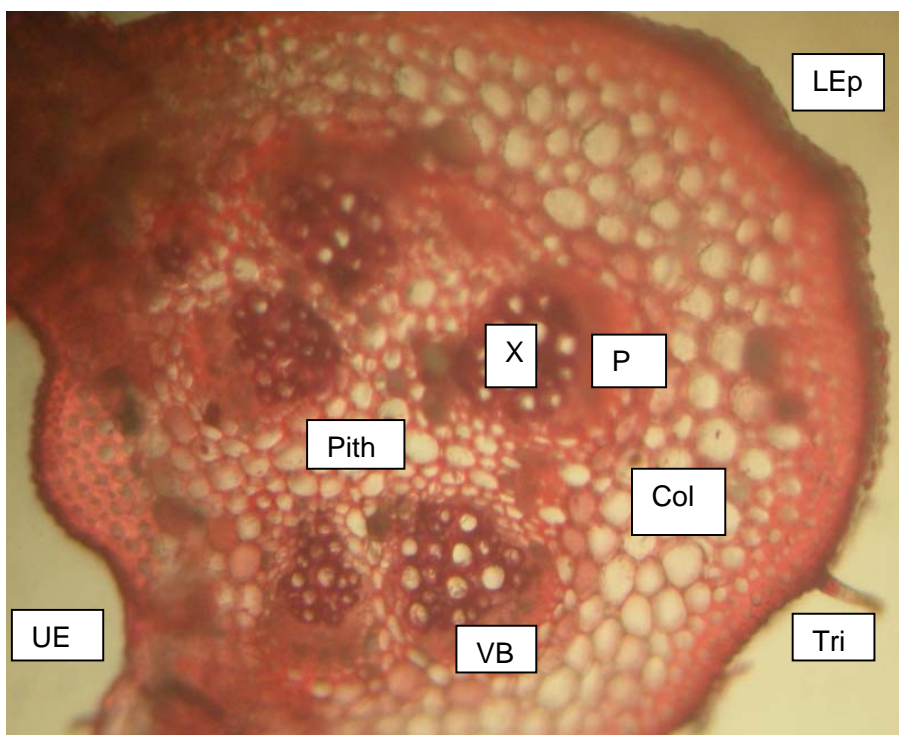


Fig.1 T.S. of leaf of *Bougainvillea glabra* “Snow White” through midrib & lamina.

Where LEp- lower epidermis, col- collenchymas, VB- vascular bundles, GT- ground tissue, UEp- upper epidermis, X- xylem, Ph- phloem, Pith, Tri- trichome.(Fig. 1)

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of alkaloid, glycosides (minute amount), flavanoids, tannins, steroid, protein and saponins (Table 1).

Table 1. Preliminary phytochemical screening of extracts of *Bougainvillea glabra* “Snow White”

Tests	Pet. ether	Acetone	Ethanol + water(50:50)
Alkaloid	-	+	+
Carbohydrate	-	-	-
Flavanoids	-	+	+
Phenolics compound & Tannins	-	+	+
Protein	-	+	+

+ Denotes presence of respective class of compounds

Antidiarrhoeal activity

Hydro alcoholic extract in different doses for anti diarrhoeal activity was shown significant ($p < 0.05$) and comparatively 400mg/kg was shown to more effective than the 200mg/kg dose (Fig. 2) (Table 2). Many plants easily available in india are used in traditional folklore medicine for the treatment of diarrhoea and dysentery. Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract. In the present study, the plant extracts that have not been studied so far, was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea based on traditional use. The aqueous and acetone extracts showed marked dose dependent anti-diarrhoeal activity comparable to standard drug loperamide. Previous studies reports have been demonstrated the anti-diarrhoeal activity of tannin, flavanoids, alkaloids, saponins, reducing sugars and sterols and/or terpenes containing plant extracts. The high activity of acetone and hydro alcoholic extracts suggests that tannins and reducing sugars present in these extracts would be the main constituents to exhibit this activity along with flavanoids.

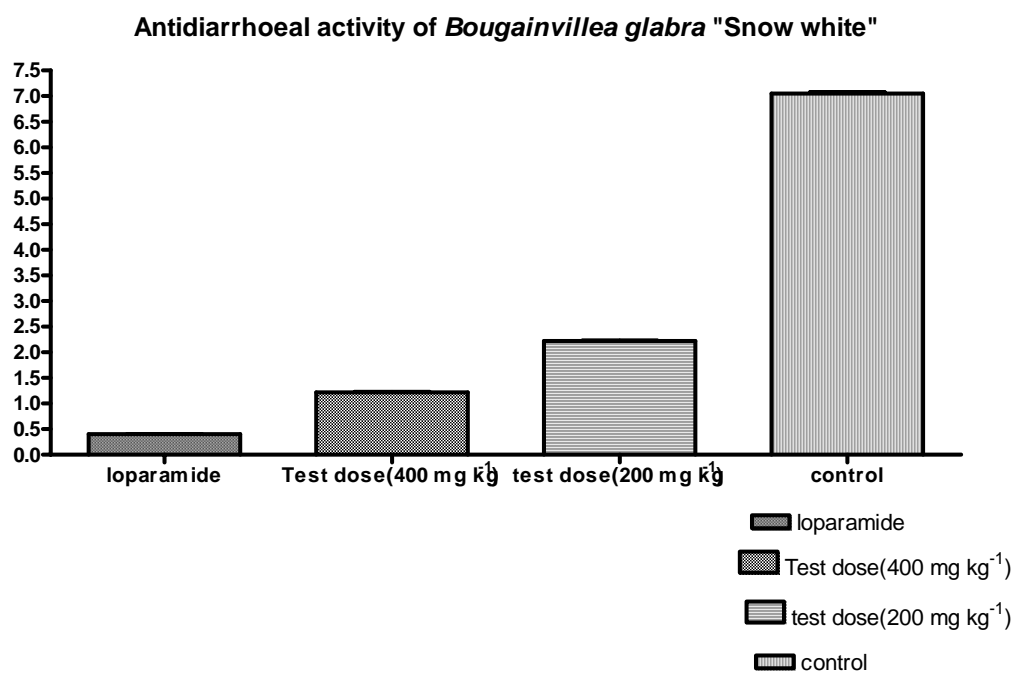


Fig. 2 . Anti diarrhoeal activity of hydro alcoholic extract of *Bougainvillea glabra*

Table 2: Effect of various leaf extracts of *Bougainvillea glabra* "Snow white" as Anti diarrhoeal agent

Treatment/ Dose(mg kg ⁻¹)	wt. of faeces after 5 h (gm)	% protection
Control (I) (10 ml kg ⁻¹)	7.050 ± 0.034	-----
Loparamide (II) (3 mg kg ⁻¹)	0.4017± 0.010*	94.30
Hydro alcoholic ext.(III) (200 mg kg ⁻¹)	1.217± 0.020*	82.74
Hydro alcoholic ext.(IV) (400 mg kg ⁻¹)	2.222 ± 0.019*	68.48

Results are expressed as Mean±SEM. p<0.05 (Dunnett's test),* p<0.0001, compared to control ,n=6

Reducing anti-oxidant power:

Reducing power means the reductive ability of antioxidant, and it is evaluated by the change of Fe (III) to Fe (II) in the presence of the sample extracts [17]. The reducing power of methanolic plant extracts are demonstrated in (Fig. 3) (Table 3). From the figure we can say that reducing power increased with an increase in extracts concentration. The all data show that all the samples increased their reducing ability when the concentration of extracts was increased. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds (Shimada et al., 1992) which is also related to presence of reductant agent [18]. In addition, the number and position of hydroxyl group of phenolic compounds also rule their antioxidant activity [19].

Table 3: Effect of various leaf extracts of *Bougainvillea glabra* "Snow white" as reducing oxidant power

Extracts	Reducing power
Acetone extract	0.05073± 0.01353*
Hydro alcoholic extract	0.06102 ± 0.01513*

Results are expressed as Mean±SEM. *p<0.05 (Dunnett's test)

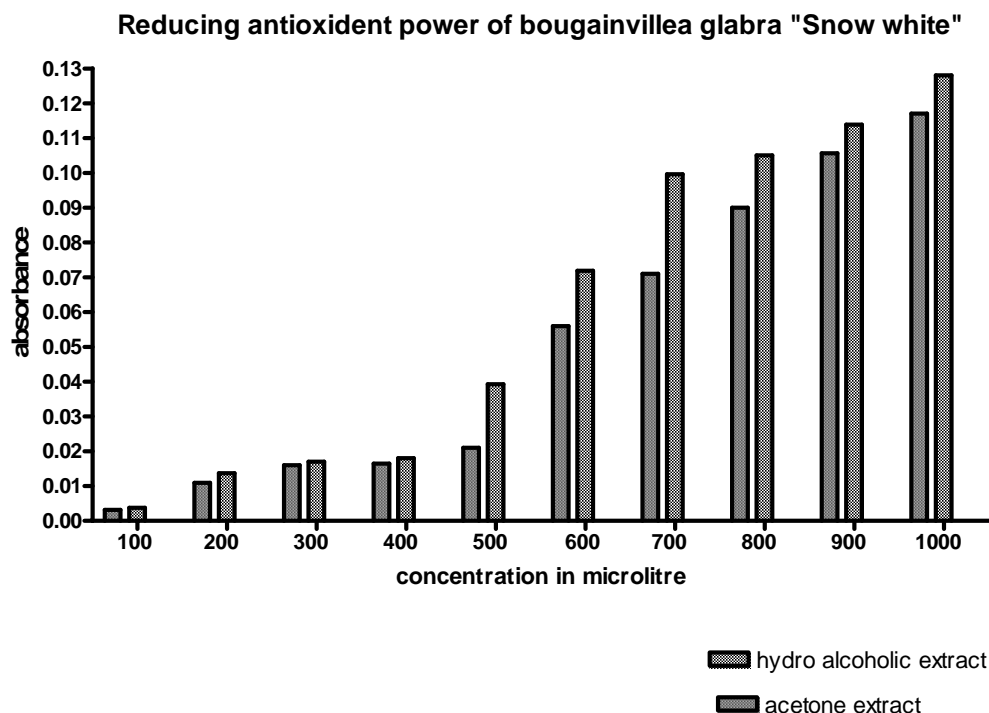


Fig. 3 Reducing antioxidant power of various extracts of *Bougainvillea glabra* "Snow White"

Conclusion

The result obtained in the study shows that leaf of *Bougainvillea glabra* "Snow white" extracts of different solvents (hydro alcoholic and acetone) possess reducing anti-oxidant power which are probably mediated via inhibition of various oxidant formation formation and release in the body. And anti-diarrhoeal study of this plant shows that hydro alcoholic extract of this plant have anti-diarrhoeal activity which is may be presence of tannins and flavanoids. So further studies are needed to elucidate the exact mechanism by which *Bougainvillea glabra* "Snow white" inhibits diarrhoea.

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