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Phytochemical analysis, antioxidant, antidiabetic and anti-inflammatory activity of bark of *Gardenia latifolia*

Farheen Ansari, Salaj Khare*, B. K. Dubey, Amit Joshi, Amit Jain, Suresh Dhakad

Technocrats Institute of Technology- Pharmacy, Bhopal (M. P.), India

ABSTRACT

Gardenia latifolia (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of various ailments such as snake bite, skin diseases, stomach pains, caries in humans and ephemeral fever in live stocks. Many beneficial medicinal products are used to treat various serious diseases and disorders like diabetes, cancer and cardiovascular diseases without side effects. Hence our study focused to investigate the phytochemical analysis, quantification of bioactive compounds, in vitro free radical scavenging activity (DPPH radical method), anti-inflammatory activity (Carrageenan induced hind paw oedema), in vitro anti-diabetic activity by enzyme inhibition activity of bark of *Gardenia latifolia* (hydroalcoholic extract) which has boundless medicinal properties. The results of this study showed the evidence that the extracts when tested for their phytochemicals and free radical scavenging activity were found to have considerable antioxidant potential. This plant also exhibit better in vitro enzyme inhibitory activity and anti-inflammatory activity. The results of this study indicate that the hydroalcoholic extract of *Gardenia latifolia* bark has significant pharmacological properties.

Keywords: *Gardenia latifolia,* Phytochemical analysis, DPPH radical method, Anti-inflammatory activity, Anti-diabetic activity

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***Address for Correspondence:**

Salaj Khare, Technocrats Institute of Technology- Pharmacy, Bhopal (M. P.), India

INTRODUCTION

Medicinal plants are the bio resources given by natural world used to heal a group of human diseases to evaluate their probable sources for new drugs[1]. Plant parts have been a typical source of medicine from an ancient time and have been the primary source of drugs in Indian systems of medicine and other ancient systems in the world[2]. Phytochemicals are the natural bioactive compounds present in plants and the most substantial bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds[1,3]. Inflammation is the reaction of the living tissues to injury; it comprises systemic response (involving nervous and hormonal adjustments and proliferation of the lymphoreticular system); and local response (pain, redness, warmth and swelling). The three important aspects of inflammation that render themselves readily to measurement are erythema (local vasodilation), edema (increased capillary permeability) and formation of granulation tissue. Compounds claimed to possess antiinflammatory activity can be evaluate either by their ability to reduce one or more of these phenomena in experimentally induced inflammation or by testing their anti-inflammatory activity in experimental arthritis produced in animals[4]. The commonly used drug for managing of inflammatory conditions is non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers[5]. Diabetes mellitus is a complex disease characterized by gross derangement in carbohydrate, fat and protein metabolism due to deficiency in insulin secretion and/or action[6]. Mammalian α-amylase is a prominent enzyme in the pancreatic juice which breaks down large and insoluble starch molecules into absorbable molecules ultimately maltose[7]. α-glucosidase, on the other hand, anchored in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet[8]. Inhibitors of α-amylase and α-glucosidase delay the breakdown of carbohydrate in the small intestine and decrease the postprandial blood glucose excursion levels in diabetic patients[9]. The inhibition of these two prominent enzymes has been found as a useful and effective strategy to lower the levels of postprandial hyperglycemia[10]. The incidence of diabetes mellitus in the world is increasing at an alarming rate, affecting close to 5 % of its population. Crude extract acquired from the G. latifolia was evaluated for its radical scavenging properties and assessed that it could be a rich

source of natural oxidants with potential applications[11]. *G. latifolia* is a small deciduous tree or large shrub. Root used as a remedy for indigestion in children. Fruits used in affections of the mammary glands. Pounded pulp is applied to forehead in fever. Stem and fruit used for stomach pain. Fruit extract is used in treating snake bite, sores of hand and feet, stomach ache and wounds. To treat caries, stem bark crushed and boiled in water is applied to affected areas. Bark is used in skin diseases. The bark and wood gave beta sestorol, hederegenin, Me-esters of oleanic and gypsogenic acids. Root gave gardenins. Saponins from bark decreased formation of histamine and may find use in asthma (market drug is expectorant and weak spasmolytic, but was not found effective in asthma). The stem bark contains hederagenin, D-mannitol, sitosterol and siaresinolic, episiaresinolic, oleanolic andspinosic acid[11,12]. The present study was focused to evaluate the phytochemical analysis, anti-inflammatory activity, in vitro antidiabetic activity of *G. latifolia* bark.

MATERIALS AND METHODS

Plant material

The plant material (Barks) for the proposed study was collected from rural area of Bhopal (M.P.)

India. Plant material (Barks) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for Phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Gardenia latifolia bark was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material has been taken place.

Extraction

Dried powdered of bark of *Gardenia latifolia* has been extracted with hydro alcoholic solvent (70:30) using hot continuous percolation process for 48 hrs and dried using vacuum evaporator at 40^{0}C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[13].

Qualitative phytochemical analysis of plant extract

The *Gardenia latifolia* barks extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [14, 15]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TFC are determined. Hydroalcoholic extract obtained from bark of *Gardenia latifolia* plant material of subjected to estimate the presence of TFC by standard procedure.

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [16]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method [16]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

In vitro anti -diabetic assays

α-Amylase inhibitory activity

The α-amylase inhibitory activity was determined according to the method described by Jyothi et al[17]. A total of 500 µl of test samples and standard drug (10-50µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

In vivo Anti-inflammatory activity

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared bark extracts of *Gardenia latifolia* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423[18] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 5, 50, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method[19].

Experimental designs

Group –1: Control

Group –2: Diclofenac Sodium (Standard)

Group –3: Hydroalcoholic extract of *Gardenia latifolia* (HAGL) (100mg/kg, p.o.)

Group –4: Hydroalcoholic extract of *Gardenia latifolia* (HAGL) (200mg/kg, p.o.)

Carrageenan induced hind paw oedema

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control (0.1 ml of 1% (w/v) of Carrageenin subcutaneously), Group 2 was received Diclofenac Sodium 30mg/kg, p.o. Group 3 were treated with hydroalcoholic extract of *Gardenia latifolia* (HAGL) (100mg/kg, p.o.). Group 4 were treated with hydroalcoholic extract of *Gardenia latifolia* (HAGL) (100mg/kg, p.o.). Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contralateral paws were measured at time interval after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition[20, 21].

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group

Vt- Edema volume of test group

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSIONS

The yield of *Gardenia latifolia* hydroalcoholic barks extracts was 3.9 % w/w. Preliminary phytochemical screening of *Gardenia latifolia* hydroalcoholic barks revealed the presence of various components such as phenolic compounds, carbohydrates, flavonoids, saponins and diterpins among which flavones were the most prominent ones and the results are summarized in table 1.

Table 1: Result of phytochemical screening of *Gardenia latifolia*

S. No.	Constituents	*G. Latifolia*
1.	**Alkaloids** Hager's test	 -ve
2.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
3.	**Phenolics** $FeCl_3$	 -ve
4.	**Proteins and Amino acids** Xanthoproteic test	 -ve
5.	**Carbohydrates** Fehling's test	 +ve
6.	**Saponins** Foam test	 +ve
7.	**Diterpins** Copper acetate test	 +ve

The content of total flavanoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance Table 2& Fig 1. Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 3 and Fig. 2.

Table 2: Total flavonoid content of hydroalcoholic extract

S. No.	Plant	Total flavonoid (QE) (mg/100mg)
1.	***Gardenia latifolia***	**0.501**

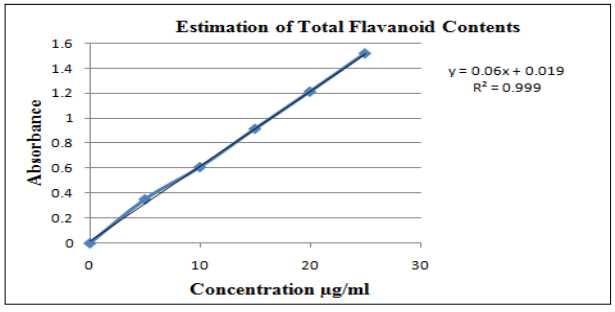

Figure1: Graph of estimation of total flavonoid content

Table 3: DPPH assay of ascorbic acid and hydroalcoholic extract

S. No.	Concentration	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	39.89	28.98
2	20	46.56	30.14
3	40	61.25	36.45
4	60	62.25	42.25
5	80	74.45	55.56
6	100	83.32	60.21
IC 50		27.18	72.73

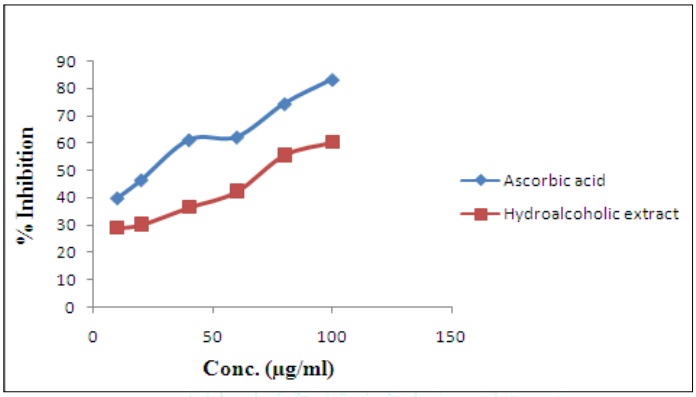

Figure 2: DPPH Assay

The inhibitory action of Hydroalcoholic bark extract of *Gardenia latifolia* were increased as the concentration increases in both the extract and the standard acarbose and results were shown in table 4 and fig. 3. The percentage inhibition of edema values of Carrageenan induced rat paw edema is given in table 5. The inhibition was higher at a dose of HAGL 200 mg i.e., 87.25 %. However the standard drug has exhibited the percentage inhibition of edema was 96.93%.

Table 4: Results of *In vitro* antidiabetic studies

S. No	Acarbose		Hydroalcoholic extract	
	Conc.	% Inhibition	Conc.	% Inhibition
1.	100	45.65	100	35.12
2.	200	61.56	200	55.56
3.	300	68.78	300	58.98
4.	400	72.15	400	72.23
5.	500	78.89	500	82.12
IC_{50}	100.25		IC_{50}	203.63

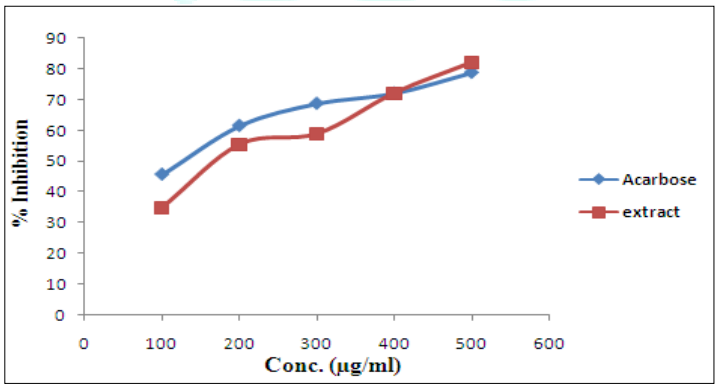

Figure 3: α-amylase inhibitory activity of *Gardenia latifolia* bark extract.

Table 5: Anti-inflammatory effect of HAGL on carrageenan induced rat paw edema

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	% of Inhibition
Control	0.1 ml of 1% (w/v)	1.55 ± 0.02	--
Diclofenac	30	0.90 ± 0.04*	96.93
HAGL	100	1.10 ± 0.03	84.03
HAGL	200	1.05 ± 0.05*	87.25

CONCLUSION

By considering the above data it can be concluded that the bark of *Gardenia latifolia* has medicinal values since it contains more secondary metabolites and its free radical scavenging activity were found to have considerable antioxidant potential. This plant also reveals better in vitro enzyme inhibitory activity (alpha amylase) which is involved in regulation and absorption of carbohydrate and also exhibits good antiinflammatory activity. The present data, illustrate that the hydroalcoholic extract of *Gardenia latifolia* bark has good medicinal properties and it will be useful in treating various diseases including diabetes.

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