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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC LEAVES EXTRACT OF *GARDENIA LATIFOLIA* USING FORMALIN-INDUCED PAW EDEMA MODEL

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ABSTRACT

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes has serious side effects. So, number of herbal medicines is recommended for the treatment of inflammation that has no side effects. Hence our study focused to investigate the phytochemical analysis, quantification of bioactive compounds, *in vitro* free radical scavenging activity (DPPH radical method) and anti-inflammatory activity (formalin-induced paw edema) of methanolic leaves extract of *Gardenia latifolia* (*G. latifolia*) which has boundless medicinal properties. Qualitative analysis of various phytochemical constituents, quantitative analysis of total phenolics (Folins ciocalteau reagent method) and flavonoids (Aluminium chloride method), and *in vitro* free radical scavenging activity (DPPH radical method) were determined by the well-known test protocol available in the literature. For anti-inflammatory activity, wistar albino rats were used and divided into four groups of six animals each group and Group 1 was treated as manage (formalin (0.2 ml of 2% v/v freshly prepared formalin resolution in distilled water), group 2 was received diclofenac sodium 30mg/kg, p.o. group 3 were dealt with methanolic leaves extract of *G. latifolia* (MEGL) (100mg/kg, p.o.). group 4 were handled with methanolic leaves extract of *G. latifolia* (MEGL) (200mg/kg, p.o.). The thickness was measured earlier and after injecting the formalin everyday at a fixed time for seven consecutive days utilizing a vernier caliper. Phytochemical analysis revealed the presence of phenols, flavonoids, proteins and amino acids, carbohydrates, saponins, diterpines. The total phenolics content of methanolic leaves extract was (0.77mg/100mg), followed by flavonoids (0.60mg/100mg). The activities of methanolic leaves extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 35.44 and 65.80µg/ml respectively. This plant also exhibit better anti-inflammatory activity. From the present observation, it is evidenced that *G. latifolia* would be an effective plant for the treatment of inflammatory reactions.

K**eywords:** Inflammation, *Gardenia latifolia*, Antioxidant, Formalin-induced paw edema, Phytochemical analysis

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infections, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as etiological factor for many chronic illnesses [1]. Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used in the treatment of pain and inflammation. Currently available NSAIDS are associated with unwanted side effects and have their own limitations. About 34-

46% of the users of NSAIDS usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa [2]. Hence there is a need for anti-inflammatory drugs with fewer side effects. Plants have been an important source of medicine for 1000's of years. Herbal medicine is still the mainstay of therapy for about 75-80% of the whole population in developing countries for primary health care [3]. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries [4]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine [5]. World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases[6]. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain [7]. However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. One such plant from Indian flora *G. latifolia*. *G. 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 varius ailments such as snake bite, skin diseases, stomach pais, caries in humans and ephemeral fever in live stocks [8,9]. Fruits are used for making perfumes [10]. 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]. Hence, in the present study, the antioxidant and anti-inflammatory activities of methanolic extracts of *G. latifolia* were evaluated using DPPH radical method and formalin-induced paw edema methods. Total phenolic and flavonoids contents of the crude extracts were also determined.

MATERIALS AND METHODS

Plant material

The leaves of *Gardenia latifolia* were collected from Bhimbetka mandideep (M.P.) in the month of January, 2019. Plant material (Leaves) 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 Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction

Dried powdered leaves (50 gm) of *G. latifolia* has been extracted with methanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C 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 *G. latifolia* leaves 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, flavonoids, glycosides, saponins, alkaloids, protein, amino acid and tannins.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso et al [16]. A volume of 2 ml of *G. latifolia* leaves extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

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 vivo* Anti-inflammatory activity**

Animals:-

Wistar rats (150-200 gm) had been group housed (n= 6) under a typical 12 h mild/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats got typical rodent chow and water advert libitum. Rats had been acclimatized to laboratory conditions for 7 days earlier than engaging in the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate crew (n=6) of rats was once used for every set of experiments. The animal reviews were approved through the Institutional Animal Ethics Committee (IAEC), constituted for the cause of manipulate and supervision of experimental animals through Ministry of atmosphere and Forests, government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared leave extracts of *G. latifolia* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [17] 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 [18].

Experimental designs

Group –1: Control

Group –2: Diclofenac sodium (Standard)

Group –3: Methanolic leaves extract of *Gardenia latifolia* (MEGL) (100mg/kg, p.o.)

Group –4: Methanolic leaves of *Gardenia latifolia* (MEGL) (200mg/kg, p.o.)

Formalin-induced paw edema model

The animals have been divided into four groups of six animals each group and were fasted for a interval of 24 h prior to the be taught. Group 1 was treated as manage (formalin (0.2 ml of 2% v/v freshly prepared formalin resolution prepared in distilled water), group 2 was received diclofenac sodium 30mg/kg, p.o. group 3 were dealt with methanolic leaves extract of *G. latifolia* (MEGL) (100mg/kg, p.o.). group 4 were handled with methanolic leaves extract of *G. latifolia* (MEGL) (200mg/kg, p.o.). The thickness was measured earlier than injecting the formalin and after injecting the formalin everyday at a fixed time for seven consecutive days utilizing a vernier caliper (precision) [19].

Statistical Analysis

All analysis used to be carried out utilizing graph pad prism for home windows. All statistical evaluation is expressed as imply ± commonplace error of the mean (SEM). Knowledge had been analyzed through one way ANOVA, the place relevant $p<0.05$ used to be considered statistically tremendous, compared with automobile followed by using Dunnett's scan.

RESULTS AND DISCUSSIONS

The yield of *G. latifolia* methanolic leave extracts was 8.908 % w/w. Preliminary phytochemical screening of *G. latifolia* methanolic leave extracts 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. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC and TFC of methanolic extract of *G. latifolia* leaves were found to be 0.77and 0.60 respectively. Results are provided in Table 2. 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 10µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 3 and Figure 1. The percentage inhibition of edema values of formalin induced rat paw edema is given in Table 4 and Figure 2. The inhibition was higher at a dose of MEGL 200 mg i.e., 87.00%. However the standard drug has exhibited the percentage inhibition of edema was 96.00%.

Table 1 Result of phytochemical screening of *G. latifolia*

S. No.	Constituents	Methanolic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenolics**	
	$Fecl_3$	+ve
5.	**Proteins and amino acids**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 2 Total phenolic and total flavonoid content of *G. latifolia*

S. No.	Extracts	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	**Methanolic**	0.77	0.60

Table 3 % Inhibition of ascorbic acid and extract using DPPH method

S. No.	Concentration (µg/ml)	Ascorbic acid % Inhibition	Extract % Inhibition
1.	10	36.77	27.74
2.	20	58.06	36.77
3.	40	64.51	45.16
4.	60	76.77	58.70
5.	80	86.45	67.09
6.	100	36.77	27.74
	IC 50	35.44	65.80

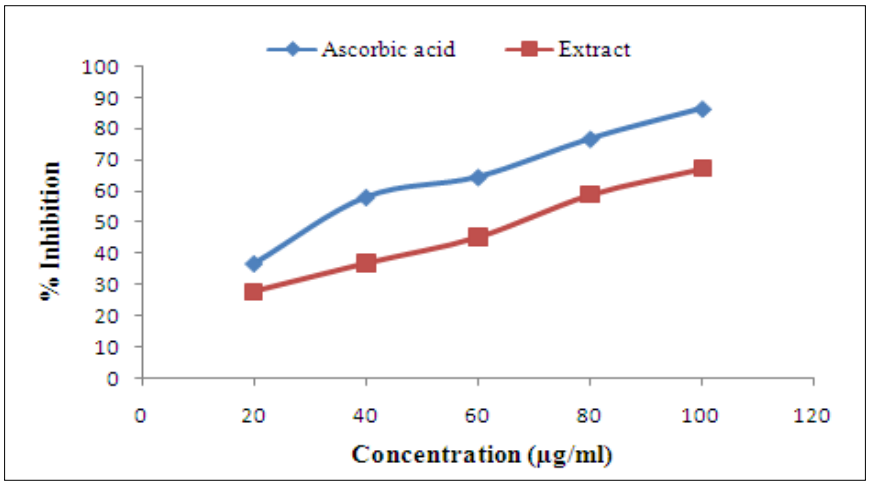

Figure 1 % Inhibition of ascorbic acid and extract using DPPH method

Table 4 Effect of different extracts on paw oedema induced by formalin in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.2 ml of 2% v/v	4.80±0.20	--
Diclofenac	30	3.50±0.19*	96.00
MEGL	100	4.10±0.21	84.00
MEGL	200	3.82±0.22	87.00

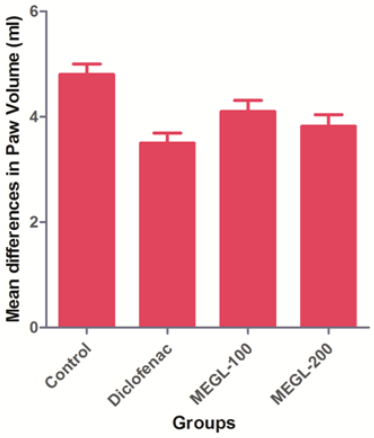

Figure 2 Effect of different extracts on paw oedema induced by formalin in rats

CONCLUSION

The methanolic extract of *G. latifolia* leaves exhibited significant anti-oxidant and anti-inflammatory activity. The anti-inflammatory effect could be attributed to antioxidant potential of *G. latifolia* which may be due to presence of flavonoid and phenolic compounds present in the leaves extract. The findings of the study validate the uses of leaves of *G. latifolia* as folklore medicine in treatment of inflammatory

conditions. However, the isolation of compounds responsible for anti-inflammatory effect is subject of further investigation.

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