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Research Article

Phytochemical Analysis, Antioxidant and Antiarthritic Activity of Aqueous Extracts of *Gmelina arborea* Flowers

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Abstract

The plant *Gmelina arborea* (*G. arborea*) has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, antibacterial, antioxidant and antidiabetic. The aim of the present study was to evaluate qualitative phytochemical analysis, antioxidant and antiarthritic activities of aqueous extracts of *G. arborea* flowers. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity was assessed against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide scavenging assay using standard protocols. The *in vitro* antiarthritic potential of extracts was evaluated in terms of its inhibition of protein denaturation and membrane stabilization method. Freund's adjuvant arthritis model in Wistar rats was used to study the *in vivo* antiarthritic activity. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The inhibitory concentration 50% values are 85.43 and 107.59 µg/ml for DPPH radical scavenging assay and superoxide scavenging assay, respectively. The plant extracts exhibited dose dependent inhibition of protein denaturation and also protected the RBC cells by stabilizing the membranes. *G. arborea* flowers extracts (100 and 200 mg/kg) was given orally to arthritic rats induced with complete freund's adjuvant and changes in paw diameter, joint diameter, arthritic score and body weight were determined. Indomethacin was taken as standard. Rats treated with extracts displayed marked reduction in paw diameter, joint diameter and arthritic score along with substantial enhancement in body weight. The antiarthritic effects of these plant extracts may attribute either due to its inhibition of protein denaturation or stabilizing the membranes from the free radical attack which are generated due to the immunological and inflammatory reactions observed in most of the arthritic conditions.

Keywords: *Gmelina arborea;* Phytochemical Analysis; Antioxidant; Antiarthritic Activity

Introduction

Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defense mechanisms and among them, a key protective role is played by the antioxidant enzymes glutathione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase [1]. Rheumatoid Arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that causes pain, swelling, stiffness and loss of function in joints [2]. It occurs more frequently in women than in men and its prevalence depends upon age. In humans, RA is the most common inflammatory joint disease where skeletal complications start with focal erosion of cartilage initially followed by marginal and sub-chondral bone loss. Extended joint destruction with ankylosis and generalized bone loss are characteristic for late complications [3]. The steroidal and

non-steroidal anti-inflammatory drugs are used in the treatment of the disease, but they offer only temporary relief and produce severe side effects including gastrointestinal bleeding and cardiovascular toxicity. Consequently, there is a need to develop new long acting anti-inflammatory agents with minimum side effects. Herbal medicine is a form of alternative treatment for several ailments and plant derived drugs are gaining popularity both in developing and developed countries due to their natural origin and less side effects in the last few years. The World Health Organization (WHO) has listed 21,000 plants which are used for medicinal purposes around the world and India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones. Plant derived secondary metabolites in the plant extracts are the important source of drugs with desired pharmacological activity. Although herbal drugs are effective in treatment of various ailments, very often these drugs are unscientifically exploited and/or improperly used. Therefore, a detailed pharmacological evaluation and documentation of plants used in local health tradition is needed. *G. arborea* (Verbenaceae), popularly known as Gambhari, is an important medicinal plant in the Indian Ayurvedic system of medicine. The drupes, leaves, flowers, roots and bark are used in traditional medicine. The plant is used in snake-bite and scorpion sting. The juice of tender leaves added to cow's milk is used in gonorrhea. Leaves ground into paste with water are applied to the forehead for headache in fevers [4]. The plant is anthelmintic and is useful in treatment of piles, abdominal pains, burning sensations, fever [5] and diabetes [6]. A large number of phytoconstituents have been identified in different parts of *G. arborea* including flavonoids, steroids, alkaloids, glycosides, and lignans. Luteolin [7], indole alkaloids [8] and iridoid glycosides [9] have been isolated from the leaves. The occurrence of hentriacontanol [10] and lignans like arboreol, isoarboreol, methyl arboreol, arborone, gmelanone, gummadiol, gmelanone, and 7-oxodihydrogmelinol [11-13] in the heartwood has also been reported. Crude extracts of *G. arborea* have also been investigated for different pharmacological activities. The extract form of leaves is reported to have wound-healing properties [14]. The aqueous methanol extract (ME) of the bark showed an antidiarrheal activity in castor oil-induced diarrhea in mice [15]. The aqueous extract of the bark and fruit of *G. arborea* have been studied for a hepatoprotectant and antioxidant activity using liver slice culture [16]. The present study was designed to evaluate the antioxidant and antiarthritic activity of extract supporting its traditional use in arthritis.

Materials and Methods

Plant material

Flowers of *G. arborea* (Verbenaceae) were purchased from the local market of Indore Madhya Pradesh, India. The taxonomical recognition (Plant Authentication No. 178/Bot-DAVV/2018) was done from Department of Botany, DAVV University, Indore, Madhya Pradesh.

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). Clonidine (Unichem, Ltd.); Chlorpheniramine maleate (Alkem, Mumbai), All the chemicals used in this study were of analytical grade.

Preparation of extract

The collected yellow colored flowers (100 gm) were extracted (decoction) with water (500 ml) at a temperature not exceeding 110°C for 2hrs. The extract was allowed to cool, filtered and lyophilized. The yield of obtained dried extract was 9% w/w.

Qualitative phytochemical analysis of plant extract

The *G. arborea* extracts obtained was subjected to the preliminary phytochemical analysis [17,18]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

In vitro antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay of extract was determined using ultraviolet spectrophotometer [19,20], which involved the preparation of standard, test, and control.

Preparation of standard rutin solutions

Different solutions (20 - 100 µg/ml) of the rutin were prepared in methanol. 1.5 ml of each solution of rutin was mixed with 1.5 ml of 200 µM DPPH solution and fecundated for 30 minutes at room temperature in dark. Absorbance of each solution was taken after 30 minutes against methanol (as blank) at 517 nm.

Preparation of test solutions

Different solutions of the extract were prepared in methanol to give concentrations in the range of 20 - 100 µg/ml. 1.5 ml of each extract solution was blended with 200 µM DPPH solution and incubated for 30 minutes at room temperature. Optical density was measured after 30 minutes against methanol (as blank) at 517 nm.

Preparation of control solution

For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 minutes at room temperature in dark. Absorbance of the control was taken after 30 minutes against methanol (as blank) at 517 nm.

Superoxide scavenging assay

Different concentrations (20 - 100 µg/ml) of rutin and test sample were prepared. To the reaction mixture carrying 0.1 ml of NBT, 0.3 ml of extract and 1 ml of alkaline DMSO was added to give a final volume of 1.4 ml and the absorbance was fixed at 560 nm. Plain DMSO used as blank and reaction mixture without extract (water in place of extract) used as control. Depreciate absorbance of the reaction mixture designated the increasing of superoxide anion scavenging activity. 50% inhibition of extract was determined by plotting a graph between absorbance and concentration [21].

In vitro anti-arthritic activity

Protein denaturation inhibition study

Protein denaturation was executed as mentioned below. Different concentrations ranging from 100 - 500 µg/ml for both test sample and standard acetylsalicylic was composite with egg albumin solution (1 mM, 1 ml) and incubated at 27°C for around 15 minutes. Further reaction mixture was maintained at 70°C in a water bath for 10 minutes for denaturation. A sample was allowed to cool down and was spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control without sample and standard. All the experiment was performed in triplicate [22].

Membrane stabilization study

Extract sample subsisted of stock erythrocyte (RBCs) suspension 0.03 ml infused with hypotonic solution (5 ml) (154 mM NaCl in 10 mM Sodium Phosphate Buffer at pH 7.4) enclosing test sample ranging from concentration 100 - 500 µg/ml. The blank was performed. The standard drug acetylsalicylic was treated similarly. The experiment was done in triplicate. The mixtures were allowed for incubation at room temperature for around 10 minutes, followed by centrifugation (10 minutes at 3000 rpm). Absorbance of the supernatant was uniformed spectrophotometrically at 540 nm. The percentage inhibition of haemolysis was computed by following equation [23].

In vivo anti-arthritic activity

Complete Freund's adjuvant (CFA) induced arthritis

Complete Freund's adjuvant (CFA) model was performed to evaluate the antiarthritic activity. Albino Wistar male rats of weight 200 ± 25 gm were used for the experiment. Route of administration was P.O. Animals were placed in different cages in group under controlled conditions of temperature (22 ± 2°C). Golden feed diet and water were administered to all the animals regularly. The dose adopted 100 mg/kg and 200 mg/kg b.w. Paw diameter, joint diameter, arthritic score, body weight was used as a parameter for the activity. Institutional Animal Ethics Committee (IAEC) approval was taken (IAEC: SBRL/IAEC/PN-18040) for performing the experiments. Animals were divided into five groups. Vehicle/drug was administered orally and after 30 minutes of waiting period, 0.1 ml of CFA (0.05% Mycobacterium butyricum in mineral oil) was injected into left hind paw (subplantar surface) with a 26 gauge needle. Paw diameter, joint diameter, arthritic score, body weight measurements was carried out on days 3rd, 7th, 14th and 21st. All were compared to standard Indomethacin and evaluated [24].

Animal grouping

- Group I: Vehicle (Normal Saline, 10 ml/kg)
- Group II: CFA + Normal saline (10 ml/kg)
- Group III: CFA + Std (Indomethacin 3 mg/kg)
- Group IV: CFA + *G. arborea* extract treated (100 mg/kg)
- Group V: CFA + *G. arborea* extract treated (200 mg/kg).

Statistical analysis

Statistics were asserted as mean ± Standard Error Mean (SEM). Differences were considered significant at ***P < 0.001, or **P < 0.01 or * P < 0.05 when compared test group vs control (-ve) group. For numerical results, one-way analysis of variance (ANOVA) (compare all vs. control) was performed using Graph Pad InStat Version 3 (Graph Pad Software).

Results and Discussions

The results of qualitative phytochemical analysis of the crude powder of flower of *G. arborea* are shown in table 1. Aqueous ex-

tracts of flower sample of *G. arborea* showed the presence of, alkaloids, flavonoids, sterols, volatile oil, tannins and glycosides. *In vitro* anti-oxidant activity of the aqueous extract of *G. arborea* flowers was determined by evaluating the DPPH and superoxide scavenging assay. The experiment was done in triplicate for both the sample The IC_{50} values are 85.43 µg/ml and 107.59 µg/ml for DPPH radical scavenging and superoxide scavenging assay respectively, Rutin was used as reference standard. The *G. arborea* aqueous extract has significant antioxidant activity against DPPH scavenging assay and superoxide scavenging assay. Plant exhibits the antioxidant activity through the scavenging of free radicals (Table 2 and 3). The *G. arborea* aqueous extract was found to possess membrane stabilizing property which is one of the preliminary steps involved in the screening of anti-inflammatory property. Membrane of RBC structurally resembles the lysosomal membrane; the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane (Table 4). Anti-arthritic effect of *G. arborea* was studied significantly by using *in-vitro* inhibition of protein denaturation and it can be concluded that *G. arborea* extract can serve as an anti-arthritic agent as tabulated in table 5. Complete Freund's adjuvant (CFA) induced arthritis protocol was adopted. Vehicle/drug treatment was continued for the duration of 20 more days. Arthritic parameters like arthritic score, joint diameter and paw diameter were recorded. Arthritic parameters show that *G. arborea* possesses significant anti-arthritis activity. The aqueous extract of *G. arborea* possesses anti-inflammatory and anti-arthritic activity observed in parameters like paw edema, arthritic index and joint diameter. The presence of various phytoconstituents in *G. arborea* plant passes antiarthritic activity (Table 6-9).

S. No.	Secondary Metabolites	Result
1	Glycoside	Present
2	Alkaloid	Present
3	Sterols	Present
4	Volatile oil	Present
5	Tannins	Present
6	Triterpenoids	Present

Table 1: Phytochemical evaluation of *G. arborea* flowers.

S. No	Conc. (µg/ml)	Absorbance at 517 nm			Inhibition (%)		IC_{50} (µg/ml)	
		Extract	Control	Standard	Standard	Extract	Standard	Extract
1	20	0.383	0.562	0.304	52.86 ± 0.089	31.42 ± 0.399	10.10	85.43
2	40	0.332		0.265	55.50 ± 0.358	36.42 ± 0.224		
3	60	0.287		0.233	58.30 ± 0.016	40.97 ± 0.145		
4	80	0.254		0.209	61.68 ± 0.092	45.81 ± 0.083		
5	100	0.222		0.174	68.57 ± 0.342	56.53 ± 0.347		

Table 2: DPPH radical scavenging assay.

S. No	Conc. (µg/ml)	Absorbance of Standard	Inhibition (%)		IC_{50} (µg/ml)	
			Standard	Extract	Standard	Extract
1	20	0.364	51.95 ± 0.026	25.61 ± 0.377	11.18	107.59
2	40	0.339	57.30 ± 0.226	34.05 ± 0.038		
3	60	0.304	66.77 ± 0.234	38.06 ± 0.029		
4	80	0.263	72.42 ± 0.261	42.28 ± 0.290		
5	100	0.246	74.07 ± 0.062	47.73 ± 0.186		

Table 3: Superoxide scavenging assay.

S. No	Conc.(µg/ml)	% Inhibition		Absorbance at 540 nm	
		Standard	Extract	Standard	Extract
1	100	37.93 ± 0.247	8.67 ± 0.276	0.204	0.278
2	200	42.97 ± 0.310	12.40 ± 0.310	0.191	0.243
3	300	47.14 ± 0.117	16.95 ± 0.276	0.179	0.223
4	400	51.48 ± 0.117	20.76 ± 0.193	0.155	0.202
5	500	57.23 ± 0.089	22.90 ± 0.234	0.138	0.192

Table 4: Membrane stabilization activity of *G. arborea* extracts.

S. No	Conc.(µg/ml)	% Inhibition		Absorbance at 660 nm	
		Standard	Standard	Standard	Extract
1	100	67.73 ± 0.448	39.62 ± 0.448	0.179	0.339
2	200	73.49 ± 0.356	44.25 ± 0.625	0.147	0.313
3	300	79.89 ± 0.178	47.98 ± 0.448	0.112	0.292
4	400	82.80 ± 0.205	51.66 ± 0.719	0.098	0.271
5	500	85.23 ± 0.178	55.93 ± 0.544	0.083	0.247

Table 5: Effect of *G. arborea* extracts on inhibition of protein denaturation.

Groups	0 day	3 day	7day	14 day	21 day
G.1 (Vehicle)	3.44 ± 0.109	3.56 ± 0.113	3.50 ± 0.104	3.52 ± 0.131	3.52 ± 0.101
G.2 (Positive control)	3.58 ± 0.164	5.01 ± 0.164	5.23 ± 0134	5.89 ± 0.150	6.060.123
G.3 (Standard)	3.50 ± 0.118	4.08 ± 0.089	4.64 ± 0.105	4.89 ± 0.120	4.17 ± 0.188
G.4 (Extract-100 mg/kg)	3.51 ± 0.064	4.74 ± 0.125	5.06 ± 0.086	5.46 ± 0.063	4.89 ± 0.155
G.5 (Extract-200 mg/kg)	3.54 ± 0.097	4.42 ± 0.108	4.91 ± 0.093	5.17 ± 0.136	4.43 ± 0.162

Table 6: Anti-arthritic activity of *G. arborea* on rat paw diameter (in mm).

Groups	0 day	3 day	7 day	14 day	21 day
G.1(Vehicle)	6.10 ± 0.605	6.1 ± 40.593	6.10 ± 0.571	6.12 ± 0.557	6.12 ± 0.576
G.2 (Positive control)	6.17 ± 0.528	7.43 ± 0.522	7.86 ± 0.521	9.51 ± 0.523	9.67 ± 0.515
G.3 (Standard)	6.17 ± 0563	6.71 ± 0.572	7.18 ± 0.542	7.31 ± 0.504	6.65 ± 0.490
G.4 (Extract-100 mg/kg)	6.24 ± 0.464	7.55 ± 0.534	7.81 ± 0.500	8.21 ± 0.522	7.62 ± 0.461
G.5 (Extract-200 mg/kg)	6.24 ± 0.445	7.18 ± 0.487	7.57 ± 0.491	7.77 ± 0.484	7.10 ± 0.495

Table 7: Anti-arthritic activity of *G. arborea* on rat joint diameter (in mm).

Groups	Arthritic score
G.1(Vehicle)	0.00 ± 0.00
G.2 (Positive control)	3.50 ± 0.548
G.3 (Standard)	1.33 ± 0.516
G.4 (Extract-100 mg/kg)	2.33 ± 0.516
G.5 (Extract-200 mg/kg)	1.67 ± 0.516

Table 8: Anti-arthritic activity of *G. arborea* on rat Arthritic Index.

Groups	Initial	Final
G.1(Vehicle)	192.83 ± 6.555	217.17 ± 6.047
G.2 (Positive control)	198.67 ± 6.218	210.83 ± 6.555
G.3 (Standard)	203.17 ± 10.998	223.00 ± 7.772
G.4 (Extract-100 mg/kg)	196.17 ± 7.935	213.67 ± 8.756
G.5 (Extract-200 mg/kg)	194.00 ± 6.870	213.50 ± 10.232

Table 9: Effect of *G. arborea* and indomethacin drug on rat body weight (in gm).

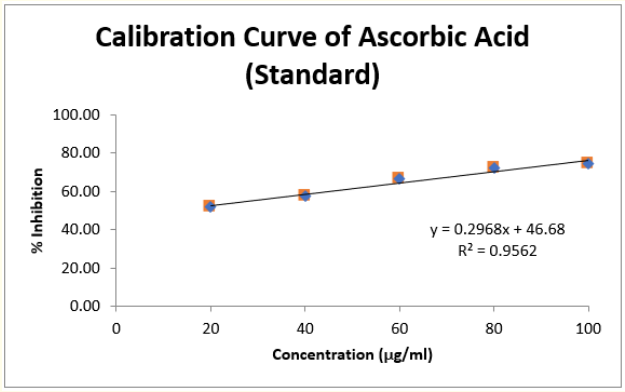

Figure 1: Calibration curve of ascorbic acid.

Conclusion

The current study established the scientific basis for the ethno medicinal use of *Gmelina arborea* for arthritis. The study also reveals the medicinal importance of the plant as an anti-inflammatory and free radical scavenger. The plant has the potential to be marketed in the future. The study also documents the use and importance of *Gmelina arborea* as an ethno medical plant and also suggests a possible alternative solution for arthritis to overcome the limitations and drawbacks of current drugs.

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