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Research Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIINFLAMMATORY ACTIVITY OF *PERGULARIA DAEMIA* (FORSK.)

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ABSTRACT

Objectives: The *in vitro* antiinflammatory activity of acetone and ethyl acetate extracts of *Pergularia deamia* leaf and stem.

Methods: The different parts of extracts were subjected to preliminary phytochemical screening as per the standard protocols. *In vitro* anti-inflammatory activities were evaluated by red blood cell (RBC) membrane stabilization, protein denaturation, and antiproteinase methods.

Results: Preliminary phytochemical screening revealed that the presence of carbohydrates, phenol, tannins, flavonoids, alkaloids, steroids, and quinines in acetone extracts of plant. *In vitro* anti-inflammatory activities were tested using different concentrations of the extracts along with standard drug diclofenac sodium. The maximum anti-inflammatory activities were observed in ethyl acetate extracts of *P. daemia*. As the concentration of the extracts increased antiinflammatory activity also higher.

Conclusion: The plant, therefore, might be considered as a natural source of RBC membrane stabilizers and prevention of protein denaturation, so it is substitute medicine for the management of inflammatory disorder.

Keywords: *Pergularia daemia*, Leaf, Stem, Acetone, Ethyl acetate.

INTRODUTION

Since plant and plant products are being used as a source of medicine for a long ago. According to the World Health Organization, more than 80% of the world's population, mostly in poor and less developed countries depends on traditional plant-based medicines for their primary health-care needs. The efficacy and safety of herbal medicine have turned the major pharmaceutical population toward medicinal plant's research. Due to the global trend toward improved "quality of life," there is considerable evidence of an increase in demand from medicinal plant [1]. India is richly endowed with a wide variety of plant shaving medicinal values. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine [2]. In recent times, focus on plant research has increased worldwide, and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [3], and also major source of biodynamic compounds of therapeutic values exploration of the chemical constituents of the plant and pharmacological screening may provide us the basis for developing the lead for the development of novel agents [4].

Inflammation is a complex biological response of vascular tissue to harmful stimuli; pathogenic irritants are characterized by redness, warmth, swelling, and pain [5]. Inflammation is either acute or chronic inflammation; acute inflammation may be an initial response of the body to harmful stimuli and chronic inflammation the inflammatory response is out of proportion resulting in damage to the body [6]. Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability and increase of protein denaturation and membrane alteration. Bacterial infections cause an increased number of neutrophils, which produce an oxidative burst at the site of microbial invasion. The uncontrolled release of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks, and rheumatoid arthritis [7].

Acute inflammation is usually of sudden onset marked by the classical signs in vascular and oxidative processes predominate. Acute inflammation may be an initial response of the body to harmful stimuli. An increased movement of plasma and leukocytes, especially granulocytes from the blood into the injured tissues, is observed. Chronic inflammation is prolonged and persistent inflammation marked chiefly by new connective tissue formation; it may be a continuation of an acute or prolonged form. Steroidal and nonsteroidal anti-inflammation drugs (SAIDs and NASIDs) are currently the most widely used drugs in the treatment of acute inflammation disorders, despite their renal and gastric negative secondary effects [8]. NSAIDs and SAID are being used till now, as a result long-term uses of these drugs cause adverse side effect and damage human biological system such as liver and gastrointestinal tract as a result of adverse side effects such as gastric lesions, cardiovascular, and renal failure [9].

Pergularia daemia is belonging to the family Asclepiadaceae. It is commonly known as "Veliparuthi" widely distributed in the world tropics and subtropics from southern and tropical Africa through Arabia to Afghanistan, India, and Sri Lanka. It consists of coroglaucigenin, calactin, oleanolic acid, putranjivadione, calacin, calotropin, β-sitosterol, etc. The plant is pungent, cooling, anthelmintic, laxative, antipyretic, cures biliousness, asthma, ulcers, and useful in eye troubles [10]. Hence, there is no detail study on *in vitro* anti-inflammatory activities of acetone and ethyl acetate extracts of leaf and stem of *P. daemia.*

METHODS

Collection of plant materials

P. daemia was collected from Neivasal, Thanjavur (District), Tamil Nadu, India, during November 2016. The plant was identified by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The leaf and stem of the plant were collected and washed thoroughly in tap water followed by distilled water. It was shade dried at the room temperature for 7 days. After dried leaf and

stem were uniformly grinded well using mechanical grinder. The powder materials were stored separately in air-tight container.

Preparation of extracts

The powdered leaf and stem (250 g) of *P. daemia* were soaked in 250 ml acetone and ethyl acetate in separated conical flask, for 3 days and plug with cotton. On the 4th day, extracts were flittered through muscle cloth and followed by Whatman filter paper 1. The filtrates were concentrated by boil water bath and then crude form of extracts was stored at 4–8°C in air-tight container.

Qualitative method of phytochemical analysis

The leaf and stem of *P. daem*ia extracts contain alkaloids, carbohydrates, glycosides, protein, steroids, tannins, phenols, flavonoids, coumarins, saponins, quinines, terpenoids, phlobatannins, and anthraquinones were analyzed by standard procedure [11].

***In vitro* anti-inflammatory activity**

Heat-induced hemolysis

The heat-induced hemolysis was performed by Shinde *et al.* [12].

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuged tube. The tubes were centrifuged at 3000 rpm for 10 min and were washed 3 times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

The reaction mixture (2 ml) consists of 1 ml test sample of different concentrations (200–1000 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min, and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The percentage inhibition of hemolysis was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

***In vitro* protein denaturation**

In vitro anti-inflammatory activity was denaturation by inhibition of albumin denaturation techniques, which was carried out by Sakat *et al.* [13]. The reaction mixture (2 ml) was containing test extracts of different concentrations (200–1000 µg/ml), 1000 mg/ml diclofenac sodium (standard anti-inflammatory drug), and 1% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660 nm (UV-visible spectrophotometer). The experiment was performed in triplicate and calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

***In vitro* antiproteinase activity**

Antiproteinase activity was performed according to Oyedepo and Femurewa [14] with minor modifications. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 20 mµ Tris-HCl buffer (pH 7.4), and 1 ml test sample of different concentrations (100–500 µg/ml). The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicates. The percentage inhibition of proteinase inhibitory activity was calculated.

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

RESULTS

Phytochemical analysis of *P. daemia*

In the present study showed that the presence of phytochemical consistent of acetone and ethyl acetate extracts of leaf and stem of *P. daemia*. The maximum amount of bioactive phytocompounds such as carbohydrates, proteins, phenols, tannins, alkaloids, flavonoids, terpenoids, steroids, and quinines was observed in acetone extracts of leaf and stem of *P. daemia*, when as compared of ethyl acetate extracts of *P. daemia*. Whereas, saponins, glycosides, phlobatannins, and anthraquinones were absent in acetone and ethyl acetate extracts of *P. daemia* (Table 1).

In vitro* anti-inflammatory activity of acetone and ethyl acetate extracts of *P. daemia

Heat-induced analysis

Acetone and ethyl acetate extracts of *P. daemia* were analyzed using the heat-induced hemolysis method, and the activity was measured by the stabilization of human red blood cells (HRBC) at various concentrations (200–1000 µg/ml) were shown in Table 2. The maximum protection of membrane lysis was observed in ethyl acetate extracts of *P. daemia* at all the concentrations when compared to acetone extracts of leaf and stem of *P. daemia*. The increasing concentration of plant extracts RBC membrane protection was increased. Hence, *P. daemia* has anti-inflammatory activity against RBC.

***In vitro* protein denaturation**

Protein denaturation is a well-documented cause of inflammation. Table 3 summarizes that the maximum inhibition of protein denaturation was observed in ethyl acetate extracts of *P. daemia*. The highest inhibition of protein denaturation (58±3.77) was showed in ethyl acetate extracts of leaf at the concentration of 1000 µg when compared to acetone extracts of leaf and stem. The protection of protein denaturation was increased at increasing concentration of *P. daemia* was analyzed for anti-inflammatory activity, and the results were compared with standard drug diclofenac sodium (1 mg/ml).

***In vitro* antiproteinase activity**

The result showed that acetone and ethyl acetate extracts of *P. daemia* exhibited significant antiproteinase activity at different concentration, and values are representing in Table 4. The maximum antiproteinase activity was showed in the ethyl acetate extracts of *P. daemia*, which was

Table 1: Phytochemical analysis of different extracts of *P. daemia*

Name of phytochemical compounds	Acetone extracts		Ethyl acetate extracts	
	Leaf	Stem	Leaf	Stem
Alkaloids	++	+	-	-
Carbohydrates	+++	+++	+++	+++
Glycosides	-	-	-	-
Proteins	-	-	+	+
Steroids	++	++	++	++
Tannins	+	-	+	-
Phenols	+++	+++	++	++
Flavonoids	++	++	++	++
Coumarins	+	+	-	-
Saponins	-	-	-	-
Quinines	+++	+++	+++	+++
Terpenoids	+++	+++	+++	+++
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-

+++ (highly); ++ (moderate); + (mild); -(absent); + (present).
P. daemia: Pergularia daemia

Table 2: *In vitro* heat-induced analysis of different extracts of *P. daemia*

Concentration of plant extracts (µg/ml)	% of membrane stabilization				Diclofenac sodium (mg/ml)
	Acetone leaf	Acetone stem	Ethyl acetate leaf	Ethyl acetate stem	
200	10±0.94	20±0.94	18±1.10	35±3.69	-
400	20±1.05	29±4.4	29±2.13	45±2.13	-
600	29±1.15	40±1.41	39±2.86	48±2.86	-
800	30±0.98	50±3.07	55±2.60	52±3.07	-
1000	35±1.20	51±1.94	61±3.14	56±3.33	70±0.94

Values are expressed as mean±SE (*n*=3). SE: Standard error, *P. daemia: Pergularia daemia*

Table 3: *In vitro* protein denaturation of different extracts of *P. daemia*

Concentration of plant extracts (µg/ml)	% of inhibition of protein denaturation				Diclofenac sodium (1 mg/ml)
	Acetone leaf	Acetone stem	Ethyl acetate leaf	Ethyl acetate stem	
200	18±3.54	29±1.66	29±2.23	45±3.33	-
400	20±4.48	32±1.88	32±2.82	46±4.48	-
600	23±4.26	34±2.13	43±2.86	48±2.13	-
800	40±3.90	37±1.94	50±3.14	50±4.38	-
1000	45±3.29	46±4.44	58±3.77	53±4.53	63±2.34

Values are expressed as mean±SE (*n*=3). SE: Standard error, *P. daemia: Pergularia daemia*

Table 4: *In vitro* antiproteinase activity of different extracts of *P. daemia*

Concentration of plant extracts (µg/ml)	Antiproteinase activity				Diclofenac sodium (1 mg/ml)
	Acetone leaf	Acetone stem	Ethyl acetate leaf	Ethyl acetate stem	
200	8±4.02	11±4.26	12±5.21	9±3.90	-
400	13±4.53	15±4.64	23±4.71	14±4.12	-
600	19±4.34	24±4.12	33±5.54	26±5.00	-
800	33±4.33	28±4.74	43±6.47	36±5.08	-
1000	38±4.01	32±4.64	51±5.00	37±5.42	70±0.94

Values are expressed as mean±SE (n=3). SE: Standard error, *P. daemia: Pergularia daemia*

compared to acetone extracts and standard drug diclofenac sodium at the concentration of 1000 µg/ml.

DISCUSSION

Natural products have contributed significantly toward the development of modern medicine. Recently, traditional medicine worldwide is being reevaluated by extensive research on different plant species and their activity therapeutic principle. The phytochemical screening and quantitative estimation of the chemical constituents of the plant studies showed that the leaf and stem, etc. [11]. The medicinal plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, and steroids are active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of medicinal plants, particularly on the leaves and stem [15].

The plant extracts were also revealed to contain steroid, which are known to produce an inhibitory effect on inflammation [16] and alkaloids have been reported to exert analgesic, antispasmodic, and antibacterial activities [17]. Alghazeer and EI-Saltani [18] were mentioned the presence of tannins, alkaloids, saponin, and terpenoids in screened medicinal plants. Phenol acids are the most commonly occurring natural products noted for allelopathic activities.

Anthocyanins, glycosides, coumarins, saponins, steroids, and terpenoids were observed in a majority of species whose present may attribute to the medicinal properties of plants [19]. Secondary metabolites are such as saponins, terpenoids flavonoids, and alkaloids which have anti-inflammatory effects. Flavonoids, tannins, and alkaloids have hypoglycemia activities [20]. From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood sugar levels in animal studies [21]. The *P. daemia* contains alkaloids, tannins, and flavonoids, cardiac glycoside and terpenoids present in aqueous and methanolic extracts of *P. daemia* [22]. Similarly, in the present study showed that the various bioactive phytocompounds such as carbohydrates, phenols, tannins, flavonoids, alkaloids, and quinines in acetone and ethyl acetate extracts of *P. daemia*.

Inflammation is a body response to injury, infection, or destruction characterized by heat, redness, pain, swelling, and disturbed physiological function. The inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators form injured tissue and migrating cell. NSAIDs are one of the best classes of drug to prevent and treat the post-operative pain; the side effects with currently used drug are gastrointestinal ulceration and bleeding, renal damage, hyperglycemia, and hypertension [23]. The rich wealth of plant kingdom can represent a novel source of newer compound with significant anti-inflammatory activities [24].

The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that the extract may also well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bacterial enzymes and proteases which causes further tissue inflammation and damage. The membrane lysis was carried out by heat-induced hemolysis, and its stabilization was determined against the plant extracts and compared anti-inflammatory drug diclofenac sodium. The plant extracts may be possible to inhibit the release of neutrophils; lysosomal constituents include bactericidal enzymes

and proteinases, which on extracellular release cause further tissue inflammation and damage. The prevention of heat-induced RBC lysis is taken as a measure of anti-inflammatory activity of drugs. During inflammation, lysosomal hydrolytic enzymes are released which cause damaged of the surrounding organelles and tissue with attendance variety of disorders [25,26]. The acetone and ethyl acetate of *P. daemia* was effective in inhibiting the heat-induced hemolysis of erythrocyte membrane and its effectiveness was dose dependent. The plant extracts showed anti-inflammatory activity in the chronic inflammation. The anti-inflammatory activity of the plant extracts may be due to their content of flavonoids, tannins, and terpenoids and which inhibited the cyclooxygenase activity [27].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentration of inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain inflammatory disease is due to denaturation of protein. The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding [28]. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was studied. In this study, inhibition of protein denaturation was observed in ethyl acetate extracts of *P. daemia* leaf. The anti-inflammatory activity of ethyl acetate extract of *P. daemia* may be inhibited the release of active pain substance such as histamine, serotonin, polypeptide, or prostaglandins [29].

Proteinase has been implicated inflammatory disease of arthritic reactions. Neutrophils are known to be a rich source of proteinase. They contain many neutral serine proteinases in their lysosomal granules. Leukocyte proteinase plays an important role in the development of tissue damage during inflammatory reactions, and significant level of protection was provided by proteinase inhibitors [30]. Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the anti-inflammatory activities of many plants [31]. Due to the presence of bioactive compounds such as flavonoids, saponins, phenols, tannins, and cardiac glycosides in the extract may be contribute in its anti-inflammatory activity. *P. daemia* extract inhibits the development of proteinase inhibition and neutrophil infiltration in the topical inflammation.

CONCLUSION

In this study, the determination of phytocompounds in acetone and ethyl acetate extracts of leaf and stem of *P. daemia*. The highest amount of pharmacological active phytocompounds was observed in acetone extracts when compared to ethyl acetate extracts of *P. daemia. In vitr*o anti inflammatory activity of different extracts of leaf and stem of *P. deamia.* The human RBC membrane lysis was evaluated by heat-induced analysis. The maximum protection of HRBC membrane destructions was seen in ethyl acetate extracts of *P. daemia. In vitro* protein denaturation of different extracts of *P. daemia.* The highest degree of inhibition of protein denaturation was showed in ethyl acetate extracts of *P. daemia.* The antiproteinase activity was evaluated against acetone and ethyl acetate extracts of *P. daemia.* The maximum antiproteinase activity exhibited in ethyl acetate extracts when compared to acetone extracts of *P. daemia*. The potential use of *P. daemia* as an alternative natural antiinflammatory agent in acute and chronic inflammation. It is believed that polyphenolic components are responsible for this activity.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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