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In vitro anti-inflammatory activity of different parts of *Pedalium murex* (L.)

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Abstract
The aim of the present study, phytochemical and anti-inflammatory activities of different parts of hydro alcoholic extracts of *Pedalium murex* (L.). The maximum phytocompounds were present in hydro alcoholic extracts of leaf of *P. murex* when compared to stem and pod. *In vitro* anti-inflammatory activity was screened against human red blood cell (HRBC) membrane stabilization method. The maximum percentage of stabilization (82.10±2.93%) was observed in hydro alcoholic extract of leaf at higher concentration. *In vitro* protein denaturation was screened by using egg albumin method. The maximum amount of inhibition of protein denaturation was showed in hydro alcoholic extract of stem of P. *murex.* The above result showed that the hydro alcoholic extract of *P. murex* possess anti- inflammatory activity. So future to find out the bioactive phytocompounds of plant extract of P. *murex*, which was used to design the drug for the treatment various type of inflammatory diseases.

Keywords: *In vitro,* Anti-inflammatory activity, Protein denaturation, *Pedalium murex*, Erythrocyte

1. Introduction
Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi herbivorous mammals. At least 12,000 phvto compounds have been isolated so far; a number estimated to be less than 10% of the total. The chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. Herbal medicines to have beneficial pharmacology activities, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects [1, 2].

Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells [3]. Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compounds, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well- documented cause of inflammation [4, 5].

Inflammation can be classified as either acute or chronic. Acute inflammation is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes. Chronic inflammation is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. Inflammation is a common clinical conditions and rheumatoid arthritis (RA) is a chronic debilitating autoimmune disorder [6]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs. Inflammation may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage [7]. NSAIDs are reported to possess prevention of the denaturation of proteins, which act as antigens and leads to auto-immune diseases [8].

Pedalium murex (L.) is a small herb distributed in tropical Africa, Ceylon, India and Mexico [9]. *P. murex* belongs to the family Pedaliaceae, is distributed in the coastal areas of southern India [10]. *P. murex* demulcent and diuretic also used for the treatment of disorders of urinary

Systems such as gonorrhea, dysuria, incontinence of urine, etc. [11, 12]. Pharmacognostical study of the leaves of the plant was reported [13]. It contains alkaloids, a greenish fatty oil, small amount of resin and ash. Fruit contains a mucilaginous alkaloid, fat, resin, and gum. Caffeic acid, cumaricacid daucosterol, acid, hepatatriacontonic acid [14], vanillic acid [15], ursolic acid and sitosterol were isolated from this plant. Flavonoids, triterpenoids, steroids, lipids, fatty acids, phenolic acids, amino acids and carbohydrates of *Pedalium murex* were reported [16]. Pharmacological activities of plant possess anti-bacterial [17], anti-microbial [18], anti-oxidant [19], aphrodisiac [20], anti hyperlipidemic [21], nephro protective [22], anti-inflammatory [23], anti-ulcer [24] and anti-diabetic [25] studies. Hence an attempt was made to evaluate the phytochemical properties and *in vitro* anti-inflammatory activities of *P. murex*.

2. Materials and methods

2.1 Collection of plant material

The different parts of *Pedalium murex* (L) were collected from in and around from ponsai, Nagapattinam (District), Tamilnadu, India during December 2015. The stem, leaf and pod of *Pedalium murex* (L.) were air dried at room temperature for 3 weeks. The dried parts were later ground well to powder.

2.2 Hydro alcoholic extract

20 g of plant powder materials was soaked with 200 ml of solvent in a sealed container for 3 days. Then the mixture was filtered through a Whatman no. 1 filter paper. Crude extract were obtained by evaporating the solvent in a water bath at low temperature (40-50 ºC) and stored in a refrigerator at 4 ºC – 8 ºC. Paste from of the extract obtained was subjected to screening test.

2.3 Qualitative phytochemical analysis (Sofowara, 1993)

The preliminary chemical tests were carried out for the extracts of *Pedalium murex* (stem, leaf and pod) to identify the presence of various phytoconstituents.

Detection of Carbohydrate

Fehling's test

One ml of extract was boiled on water bath with 1 ml each of Fehling solutions A and B. The color change was observed. A red precipitates indicated presence of sugar.

Barfoed's test

To 1 ml of extract, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. The color change was noted and recorded. A red precipitates indicated presence of sugar.

Benedict's test

To 0.5 ml of extract, 0.5 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2minutes and the result was observed. A red precipitates indicated presence of sugar.

Detection of Phenols

Lead acetate test

The extract (5 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

Detection of Tannins

Ferric chloride test

The extract (5 mg) was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicated the presence of tannins.

Detection of flavonoids

An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Detection of Saponins

Distilled water 2ml was added of each plant extracts and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1cm foam indicates the presence of saponins.

Detection of Glycosides

Legal's test

Chloroform (3ml) and ammonia solution (10%) was added to 2ml plant extract. Formation of pink color indicated the presence of glycosides.

Detection of Terpenoids

Chloroform (2ml) and concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red brown color at the interface indicated the presence of terpenoid.

Detection of Alkaloids

About 50 mg of solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

Mayer's test

To a 1 ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitate indicated test as positive.

Wagner's test

To a 1 ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. The color change was observed. A reddish-brown precipitates confirms the test as positive.

Dragendorff's test

To a 1 ml of filtrate, 2 ml of Dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive

Detection of steroids

To 0.5 ml of the plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids.

Detection of coumarins

10% NaOH (1ml) was added to 1 ml of the plant extracts formation of yellow color indicated presence of coumarins.

Detection of Quinone

Concentrated sulphuric acid (1ml) was added to 1ml of each of the plant extract. Formation of red color indicated the presence of Quinones.

Detection of Phlobatannins
Few drops of 10% ammonia solution were added to 0.5 ml of root extract. Appearance of pink color precipitates indicated the presence of phlobatannins.

Detection of Anthraquinones
Few drops of 2% HCL were added to 0.5 ml of seed extract. Appearance of red color precipitate indicated presence of anthraquinones.

2.4 *In vitro* anti-inflammatory activity
The activity was carried out by the method of Gandhisan *et al*., [26]**.** The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The 10% packed cells were washed with isosaline. Various concentrations of extracts were prepared (200,400,600, 800 and 1000µg/ml) using distilled water and 1 ml of plant extracts, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^{0}C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated by spectrophotometrically at 560 nm. Diclofenac (1 mg/ml) was used as reference standard drug and a control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$\% \text{ protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

2.5 Protein denaturation method
In vitro protein denaturation was adopted by Sakat *et al*. [27]. The anti-inflammatory activity of Pedalium *murex* (pod, seed and leaf) was studied by using inhibition of protein denaturation method. The reaction mixture (5ml) consist of 0.2 ml of egg albumin (from fresh hen's egg), 2.8ml phosphate buffered saline (pH: 6.4) and 2ml of varying concentration of plant extracts. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac at the final concentration of (1mg/ml) was used as reference drug and treated similarly for determination of absorbance.
The Percentage inhibition of protein denaturation was calculated as follows:

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

3. Results
3.1 Phytochemical analysis
The different parts of *pedalium murex* (L.) in hydro alcoholic extract was showed the presence of carbohydrates, glycosides, phenols, tannins, flavanoids, saponins, trepenoids, alkaloids, steroids, cumarins and quinone. Where as absence of phlabatannins and anthraquinone in all the parts of plant extracts.

Table 1: Phytochemical screening of *Pedalium murex* Linn. in different extracts

Name of the Phytocompounds	Hydro alcoholic extracts		
	Stem	Leaf	Pod
Carbohydrates	+++	+++	+++
Phenols	+++	++	++
Tannins	++	+++	+
Flavonoids	++	++	+++
Saponins	+++	+++	+
Glycosides	+	++	++
Trepenoids	+++	+++	++
Alkaloids	+++	+++	++
Steroids	++	+++	+++
Cumarins	++	+	+++
Quinone	+++	+++	+++
Phlabatannins	-	-	-
Anthraquinone	-	-	-

Highly (+++); Moderate (++); Mild (+); Absence (-); present (+)

3.2 Anti-inflammatory activity
In our study, absorbance of hemoglobin was determined in HRBC membrane stabilization method. The hemoglobin is released as a result of lyses of RBC membrane, due to less stabilization of membrane. The plant extracts exhibited membrane stabilization activity by hypotonic induced lyses of erythrocyte membrane. The research is based on to evaluate for newer anti-inflammatory agents from herbal medicine with potent activity and lesser side effect substitutes for drugs. The effect of hydro alcoholic extracts of different parts (leaf, stem and pod) of *Pedalium murex* on stabilization of RBC membrane is shown in table 2 and figure-1. The maximum percentage of stabilization was showed in hydro alcoholic extract of leaf 82.10±2.93% at 1000µg/ml as compared of other parts of *P. murex*. The minimum protection (19.16±3.04%) was observed in pod extract of *P. murex* at the 200µg/ml. It possess significant anti-inflammatory activity comparable with standard drug diclofenac sodium reference.

Table 2: *In vitro* anti-inflammatory activity of hydro alcoholic extracts of *P. murex*

Name of the Parts	Concentration of plant extracts / % anti-inflammatory activity				
	200 µg	400 µg	600 µg	800 µg	1000 µg
Pod	19.16±3.04	29.02±1.51	36.55±2.40	43.00±3.37	65.85±10.61
Stem	23.52±4.38	33.33±4.04	45.08±1.46	56.87±5.38	73.16±3.63
Leaf	32.25±2.40	42.15±3.97	51.95±5.72	65.03±10.61	82.10±2.93
Diclofenac Sodium (1mg/ ml)	42.14±2.00	53.13±4.08	62.52±5.01	73.17±7.43	77.40±2.69

Values are expressed in mean ± SE of 3 replicates

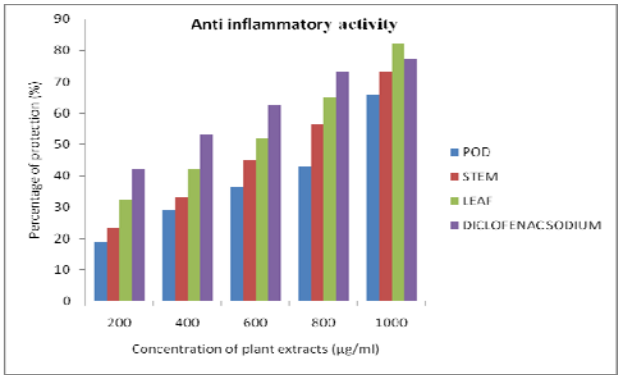

Fig 1: *In vitro* anti-inflammatory activity of hydro alcoholic extracts of *P. murex.*

3.3 Protein denaturation method

Protein denaturation means loses of biological properties of protein molecules. Denaturation of proteins is responsible for the cause of inflammation is conditions like rheumatoid arthritis, diabetes, cancer etc. Hence by, prevention of protein denaturation may also help in preventing inflammatory conditions. The present study showed the *in vitro* anti-Inflammatory activity of hydro alcoholic extract of different parts *P. murex* on inhibiting denaturation of proteins is shown in table 3 and figure-2. Maximum inhibition of 70.43±0.85% was observed in stem extract of *P. murex* at the concentration of 1000µg/ml. The minimum inhibition of 31.51±0.88% was showed in leaf extract at the concentration of 200µg/ml.

Table 3: *In vitro* protein denaturation of hydro alcoholic extracts of *P. murex*

Name of the parts	Concentration of plant extracts / % Protein denaturation				
	200µg	400 µg	600 µg	800 µg	1000 µg
Pod	36.58±0.90	46.87±5.95	55.24±4.35	61.45±2.54	68.83±5.17
Stem	37.75±5.00	40.81±5.00	54.08±0.83	62.22±0.83	70.43±0.85
Leaf	31.51±0.88	38.04±0.88	42.38±0.97	54.34±1.77	61.93±0.90
Diclofenac sodium (1mg/ ml)	27.23±2.56	36.10±2.46	45.55±1.99	60.57±18.97	73.38±2.31

Values are expressed in mean ± SE of 3 replicates

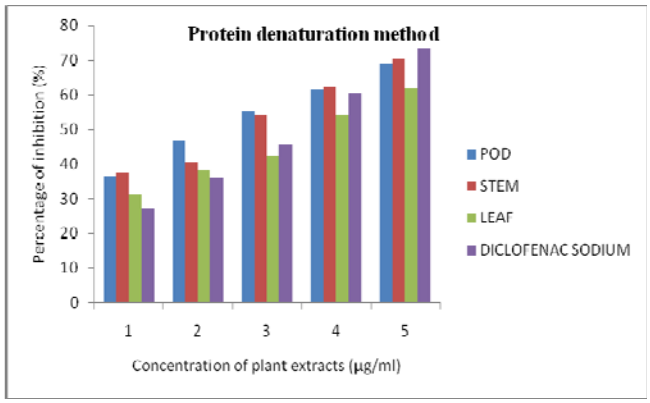

Fig 2: *In vitro* protein denaturation of hydro alcoholic extracts of *P. murex*.

4. Discussion

Herbal medicine is the use of plant extracts to treat various types of diseases. Medicinal plants exist in many local varieties depending on the regional flora [28, 29]. Many modern drugs were originally extracted from plant sources, they are now made synthetically, and many other drugs are descended from plant substances [30, 31].

The preliminary phytochemical screening tests may be useful in the detection of the bioactive phytocompounds may lead to the discovery and development drugs. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacological active phytochemical compounds [32]. Phytochemical investigations on the *P. murex* revealed the presence of various phytoconstituents such as triterpenoids, fatty acids, steroids, flavonoids, tannins, saponins, vitamins, proteins, sugars, vanillin, ursolic acid [33, 34, 35, 36]. These phytochemicals have various health benefits such as antioxidant, anti-microbial, anti-inflammatory, cancer preventive, anti-diabetic and anti-hypertensive effect [37, 38]. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardio-depressant properties [39]. Glycosides are naturally cardioactive drugs used in the treatment of coggestive heart failure and cardiac arrhythmia [40]. It has been reported that steroidal constituents found in the *P. murex* possess fertility potentiating properties, and they have been found to be useful in the treatment of impotence [41].

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [42]. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart diseases [43,44,45] and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vasculitis, celiac diseases, auto-immune diseases etc [46]. The erythrocyte membrane is analogous to the liposomal membrane [47] and its stabilization indicates that the extract may also inhibit the degradation. The stabilization of liposomal membrane is crucial point in limiting the inflammatory response via inhibiting the release of liposomal constituents of activated neutrophil.

Denaturation of proteins is a well-documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation [48]. The denaturation is used loosely to designate the change of proteins from a soluble to an insoluble form brought about by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals and dyes [49], and heat, light, and pressure [50]. Chick and Martin consider heat denaturation as a reaction between protein and water which implies in all probability a hydrolysis [51].

Some literature reported that denaturation of protein is one of the cause of rheumatoid arthritis [52, 53] due to the production of auto-antigens in certain rheumatic diseases. It may be cause to *in vitro* denaturation of proteins. Mechanism of denaturation is involved in alteration of electrostatic force, hydrogen, hydrophobic and disulphide bonds. Several author anti-inflammatory drugs have shown dose dependent ability to inhibit the thermally induced protein denaturation [54].

Similar results were observed from many reports from plant extract [55]. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release

cause further tissue inflammation and damage [47]. The precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the *P. murex* produce this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane protein [56].

The results of present study, phytochemical analysis of different parts of *Pedalium murex* revealed the presence of various bioactive phytochemical compounds was found in hydro alcoholic extracts. *In vitro* anti-inflammatory activity of hydroalcoholic extracts of *P. murex* (pod, leaf and stem) were screened against HRBC membrane and protein denaturation against egg albumin. The hydro alcoholic extract of different parts of *P. murex* showed significantly higher anti-inflammatory activity at increasing concentration. It may due to the presence of active principles of phytocompounds such as flavonoids and triterpenoids and related polyphenols may responsible for this anti-inflammatory activity. Hence, *P. murex* can be used as an anti-inflammatory agent. The investigation is based on the need for anti-inflammatory agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

5. Conclusions

The results of present study, phytochemical analysis of different parts of *Pedalium murex* revealed the presence of various bioactive phytochemical compounds was found in hydro alcoholic extracts. *In vitro* anti-inflammatory activity of hydroalcoholic extracts of *P. murex* (pod, leaf and stem) were screened against HRBC membrane and protein denaturation against egg albumin. The hydro alcoholic extract of different parts of *P. murex* showed significantly higher anti-inflammatory activity at increasing concentration. It may due to the presence of active principles of phytocompounds such as flavonoids and triterpenoids and related polyphenols may responsible for this anti-inflammatory activity. Hence, *P. murex* can be used as a anti-inflammatory agent. The investigation is based on the need for anti-inflammatory agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

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