

**ANTIOXIDANT ACTIVITY AND IMMUNOMODULATORY ACTIVITY OF
Pedaliium murex IN WISTAR ALBINO RATS****DHIVYA.M¹, DHANALAKSHMI.J*² AND S. SELVI³**

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ABSTRACT

Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. An important amount of therapeutic products are derived from medicinal plants (77% for cardiovascular diseases and 74% for digestive diseases). *Pedaliium murex* is a member of the sesame family, Pedaliaceae. It is found in different parts of the world such as tropical Africa, Srilanka, India, Mexico and Pakistan. The present study was carried out for the characterization, physical, chemical, microbial and immunological strength of the development of immune system for human era. The phytochemical compounds were analyzed. Twelve male Wistar albino rats (200-220g) two months of age were used as experimental animals and were divided into four groups. In this animal model studies assessment of immunomodulatory activity was carried out by various hematological and serological tests like determine phagocytic activity and spleen weight. In this study, different doses (100, 200 mg/kg body weight/day) of the ethanolic extract of the fruits showed significant activity and increased phagocytic response and spleen weight the result were compared with control and standard drug (septilin). The activity reported was dose dependent and set of data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test Multiple Comparison test values **P<0.01 were considered significant. It has been concluded that the test extract possesses promising immunostimulant or immune suppressive properties.

KEY WORDS: *Pedaliium murex*, Phytochemical, Immunomodulatory

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INTRODUCTION

Medicines in India are used by about 60% of the world's population. The traditional medicines are derived from medicinal plants, minerals and organic matter. The herbal drugs are prepared from medicinal plants only. There are about 45000 medicinal plant species in India with concentrated spots in the region of Eastern Himalayas, Western Ghats and Andaman Nicobar Island. India is the largest producer of medicinal herbs is called the botanical garden of the world¹. Nowadays, pharmaceutical and cosmetic industries are increasingly using plant resources from rural or unpolluted areas. It is a well known fact that large Western pharmaceutical and cosmetical companies buy various plant products, especially medicinal plants harvested from the wild flora in countries that has not yet applied intensive chemicalization in agriculture. Some of the species from the wild flora cannot provide sufficient material for the pharmaceutical, cosmetic and food industries². The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. These Bio-active chemicals may be concentrated in various parts of the plant, such as roots, stems, leaves, flower buds, flowers, fruits and seeds. Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity³. *Pedaliium murex* is a member of the sesame family, Pedaliaceae. It is found in different parts of the world such as tropical Africa, Srilanka, India, Mexico and Pakistan. In India, it occurs mainly in the Western and Corommandal coasts as a weed of waste places and is generally called under the Hindi name "Gokhru or gokhar". The fruits are rich in flavonoids, Saponins, sterol and soluble proteins⁴. An infusion or extract prepared from the different parts of *P. murex* in cold water is used as demulcent, diuretic and also found to be used in the treatment of disorders of urinary systems such as gonorrhoea, dysuria, incontinence of urine and vice versa⁵⁻⁶. The plant is also used by the local people as analgesic and antipyretic activities⁷. Immunomodulation is a procedure which can

alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reaction, it is named as an immunostimulative drug which primarily implies stimulation of non-specific system. Immunosuppressant implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be considered in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing, so search for better agents exerting these activities is becoming the field of major interest all over the world⁸. As we know, the human population is in direct exposure to different risk factors such as pathogenic agents and mycotoxins which impair immune function and this is controlled by the use of immunomodulators. In our present study, we have attempted to evaluate the immunomodulatory potency in Ethanolic extract of fruits of *Pedaliium murex* had specific and non-specific immune responses on animal model⁹.

MATERIALS AND METHODS

Collection of Plant material

The fruits of *Pedaliium murex* were collected from in and around Chittode, Erode, and Tamilnadu, India. The plant was identified and authenticated at Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore, and Tamilnadu, India. And species are preserved in the herbarium of BCAS, Erode.

Extraction of fruits of Pedaliium murex

The fruits of *Pedaliium murex* were dried in the shade, powdered and passed through a 40-mesh sieve. The dried fruits of *Pedaliium murex* (300 g) were transferred into a Soxhlet's apparatus containing 1200 ml of ethanol at 78.3° C for 72 hours. The extract was concentrated to dryness under vacuum desicator.

Qualitative analysis of phytochemicals of Ethanolic extracts of Pedaliium murex

Phytochemicals such as Alkaloids, Flavonoids, Tannins, Saponins, Phenols, Glycosides, Sterols and Terpenoids.

INVIVO STUDY

Experimental animals

The experiments were conducted after obtaining approval from the Animal Ethical Committee, Nandha College of Pharmacy, Erode, and Tamilnadu.

Groupings

Twelve Wister rats (200–220 g) of approximately two months of age were used as experimental animals and were divided into four groups of six rats. The animals were caged individually after wounding for treatment till completion of wound healing. The animals were housed in standard environmental conditions of temperature (22 ± 3 °C), humidity ($60 \pm 5\%$), and a 12 h light/dark cycle. During the course of the experiment the rats were administered a standard pellet diet and water.

Preparation of the Test sample

Suspension was made in 0.1% sodium carboxy methylcellulose and administered to animals to give a concentration of 1mg/ml. CMC was used as a vehicle.

Carbon ink suspension

Commercially available camel brand black ink suspension was purchased from the local market and diluted in a ratio 1:50 with normal saline and used for carbon clearance test in a dose of 1 ml/200 g body weight of rat.

$$K = (\ln OD_{15 \text{ min}}) - (\ln OD_{0 \text{ min}}) / (t_{15 \text{ min}} - t_{0 \text{ min}})$$

Where, OD 15 min and OD 0 are the optical densities at time t 15 and t 0, respectively.

Effect on spleen weight

The animals were sacrificed by cervical dislocation and their spleens were harvested for weighing. The results of this analysis were compared with that of vehicle control.

Statistical analysis

The mean + SEM was calculated. Statistically analyzed by one way ANOVA followed by

Experimental design

Animals were divided into 4 groups of 3 each.

Group I control

Received 4 ml of 0.1% Carboxyl methyl cellulose (i.p.) for 7 consecutive day.

Group II

Received 100 mg/kg (0.1 ml) b.w. Ethanolic extract of fruit of PM, i.p. for 7 consecutive day.

Group III

Received 200 mg /kg b.w. (0.2 ml) Ethanolic extract of PM, i.p. for 7 consecutive day.

Group-IV

Received 50mg /kg b.w. (0.05 ml) Standard drug of Septilin, i.p. for 7 consecutive day.

Macrophage phagocytosis by carbon clearance method

Phagocytic activity of the 'reticulo endothelial' system in vivo was determined by carbon clearance test. After completion of the extract treatment¹⁰, on the 8th day, immediately after the last dose was administered to all the animals of each group, the control as well as the treated groups received an intravenous injection of carbon suspension (1:50 dilution of Indian ink camel) in a dose of 1 ml/200 g body weight. Blood was withdrawn from the retro orbital venous plexus before injection (0 min) and 15 min after injection of the carbon suspension, 50 µl of blood was lysed with 4 ml of 0.1% sodium carbonate solution (Na₂CO₃). The optical density was measured spectrophotometrically at 660 nm wavelength. The results were expressed as phagocytic index:

Dunnett's test) to estimate the significance of difference between various individual group.

RESULTS

The results of the present study "Immunomodulatory activity of *Pedalium murex* in Wistar Albino rats" are discussed.

Pedalium murex fruits extract revealed the presence of alkaloids, flavonoids, tannins, Saponins, phenols, glycosides, sterols and Terpenoids (Table 1). The presence of phenolic compounds and saponins can stimulate or suppress the immune system due to the hydroxyl groups in the structure. These groups can affect the enzyme or electron transferring system regulating an immunomodulatory property especially phagocytic activity. The results of this study were consistent with the work of other investigators in determining the effectiveness of selected natural plant products against disease. This study is in accordance to the¹¹. The phagocytic index in all the groups treated with *Pedalium murex* showed a significant phagocytic index when compared with the control group. Maximum phagocytic index was observed in *Pedalium murex* when compared with the control and as well as other groups. The phagocytic index was increased in the dose dependent manner (table 2). Values are expressed as (Mean \pm S.E.M), n = 6, **P <0.01 ; When compared with control; (Statistically analyzed by one way ANOVA followed by Dunnett's test). The effect of ethanolic extract administration as such and that in combination with standard drug and

spleen weight is shown in Table 3. Significant positive effect was observed in Groups II & IV, compared to vehicle control (P<0.01). Values are expressed as (Mean \pm S.E.M), n = 6, **P <0.01 ; When compared with control; (Statistically analyzed by one way ANOVA followed by Dunnett's test) The phagocytic index in all the groups was treated with Ethanolic extract of *Pedalium murex* and standard drug. In this showed a significant phagocytic index when compared with the control group and as well as shown in the figure 1. Maximum phagocytic activity was observed in groups II and IV compared to vehicle control (p<0.01). This study is in accordance with¹². Initially, effects on spleen weight were determined to investigate immunomodulatory effect of ethanolic extract of *Pedalium murex* and that in combination with control group and as well as shown in the figure 2. Among different organs of the immune system, spleen represents a major secondary lymphoid organ involved in elicitation of immune response. Results from the present study revealed a significant increase in the weight of spleen (p<0.01) compared to vehicle control group, suggesting an enhancement of immune status in group II&IV.

Table 1
Preliminary phytochemicals screening of Ethanolic extract of Pedalium murex

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Glycosides	+
Sterols	+
Terpenoids	+

+ - indicates present

Table 2
In vivo phagocytosis (carbon clearance method)

Group	Treatment of 7 days	Phagocytic index
I	Control	0.016 \pm 0.001
II	Standard drug(50mg/kg)	0.031 \pm 0.0009**
III	Plant extract(100mg/kg)	0.024 \pm 0.0021
IV	Plant extract(200mg/kg)	0.028 \pm 0.0007**

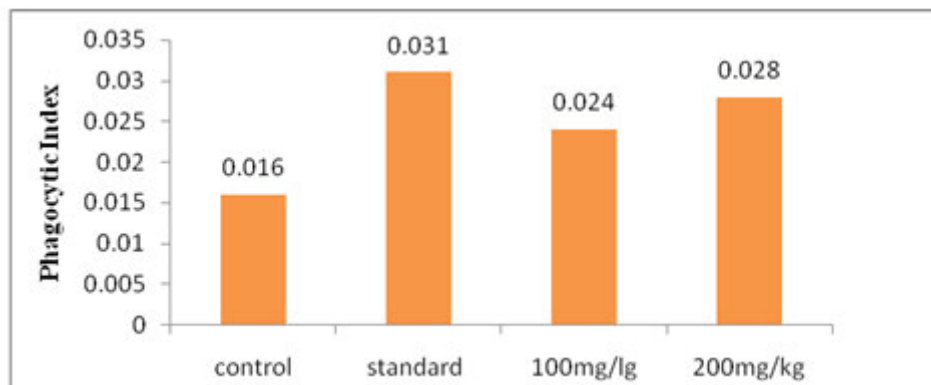
Values are expressed as (Mean \pm S.E.M), n = 6, **P <0.01 ; When compared with control; (Statistically analyzed by one way ANOVA followed by Dunnett's test).

Table3
Effect on spleen weight

Group	Treatment of 7 days	Spleen weight (mg)
I	Control	426± 24
II	Standard drug(50mg/kg)	508± 18**
III	Plant extract(100mg/kg)	436± 38
IV	Plant extract(200mg/kg)	486± 22**

Values are expressed as (Mean ± S.E.M), n = 6, **P < 0.01 ; When compared with control; (Statistically analyzed by one way ANOVA followed by Dunnett's test)

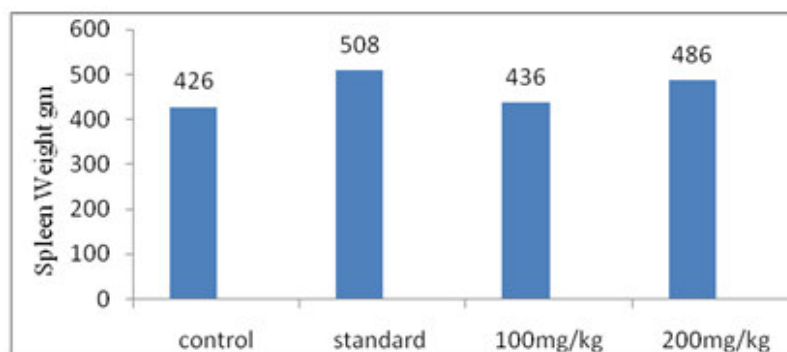
Figure 1
Phagocytic index in Ethanolic extract of *Pedalium murex*



The phagocytic index in all the groups was treated with Ethanolic extract of *Pedalium murex* and standard drug. In this showed a significant phagocytic index when compared with the control group and as well as shown in

the figure 1. Maximum phagocytic activity was observed in groups II and IV compared to vehicle control ($p < 0.01$). This study is in accordance with¹².

Figure 2
Spleen weight of ethanolic extract of *Pedalium murex*



Initially, effects on spleen weight were determined to investigate immunomodulatory effect of ethanolic extract of *Pedalium murex* and that in combination with standard drug. Among different organs of immune system, spleen represents a major secondary lymphoid organ involved in elicitation of immune response. Results from the present study revealed a significant increase in the weight of spleen ($p < 0.01$) compared to vehicle

control group, suggesting an enhancement of immune status in group II&IV.

CONCLUSION

The plant extractive studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no

side effects when compared to the commonly used synthetic chemotherapeutic agents. The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The present study verified the traditional use of *Pedaliium murex* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the saponins, phenols, flavonoids, terpenoids, alkaloids, and tannin. According to the results reported here, the ethanolic extract of *Pedaliium murex* was found to have dose dependant immunomodulatory activity in the experimental models compared to control. The presence of phenolic compounds and saponins can stimulate or suppress the immune system due to the hydroxyl groups in the structure. These groups can affect the enzyme or electron transferring system regulating an immunomodulatory property especially phagocytic activity. The results of this study were consistent with the work of other investigators in determining the effectiveness of selected natural plant products against disease. In the present investigation carbon clearance is an indicator of enhanced in vivo phagocytic activity and competency of the granuloplectic system in removal of foreign particles, therefore, an indicator of enhanced immunological response against foreign particles or antigens. This study demonstrates that *Pedaliium murex* treatment is potentiated more the phagocytosis of reticulo endothelial system. Maximum phagocytic index was observed in Ethanolic extract of *Pedaliium*

murex and standard drug when compared with the control and as well as shown in the figure 2. Maximum phagocytic activity was observed in groups II and IV compared to vehicle control ($p < 0.01$). Recent reports indicated that many plant products used in traditional medicine have been reported to have immunomodulating activities. Initially, effects on spleen weight were determined to investigate immunomodulatory effect of ethanolic extract of *Pedaliium murex* and that in combination with standard drug. Among different organs of immune system, spleen represents a major secondary lymphoid organ involved in elicitation of immune response. Results from the present study revealed a significant increase in the weight of spleen ($p < 0.01$) compared to vehicle control group, suggesting an enhancement of immune status in group II&IV. Results from the present study revealed among different organs of immune system present, spleen represents a major secondary lymphoid organ involved in elicitation of immune response. In our study clearly showed that the Ethanolic extract of *Pedaliium murex* had immunosuppressive or immunostimulatory activity.

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