

**ANTIOXIDANT ACTIVITY AND IMMUNOMODULATORY ACTIVITY OF  
*Pedaliium murex* IN WISTAR ALBINO RATS****DHIVYA.M<sup>1</sup>, DHANALAKSHMI.J\*<sup>2</sup> AND S. SELVI<sup>3</sup>**

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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 world<sup>1</sup>. 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 industries<sup>2</sup>. 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 selectivity<sup>3</sup>. *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 proteins<sup>4</sup>. 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 versa<sup>5-6</sup>. The plant is also used by the local people as analgesic and antipyretic activities<sup>7</sup>. 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 world<sup>8</sup>. 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 model<sup>9</sup>.

## 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 ( $22 \pm 3$  °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 treatment<sup>10</sup>, 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 (Na<sub>2</sub>CO<sub>3</sub>). 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 the<sup>11</sup>. 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 with<sup>12</sup>. 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**

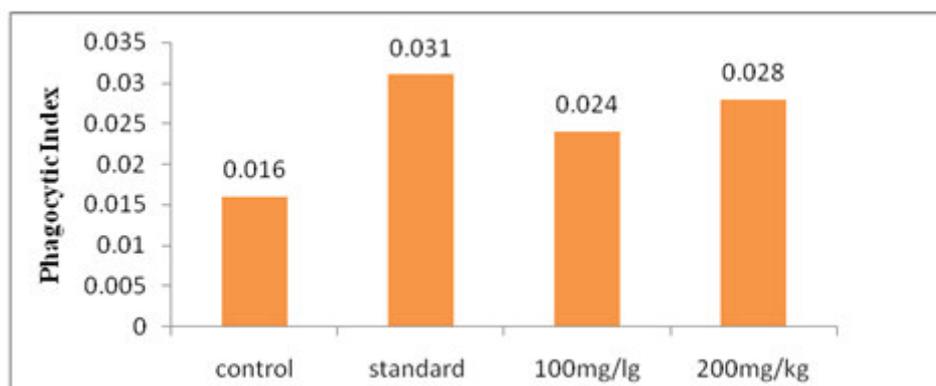
Values are expressed as (Mean  $\pm$  S.E.M), n = 6, \*\*P <0.01 ; When compared with control; (Statistically analyzed by one way ANOVA followed by Dunnett's test).

**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)

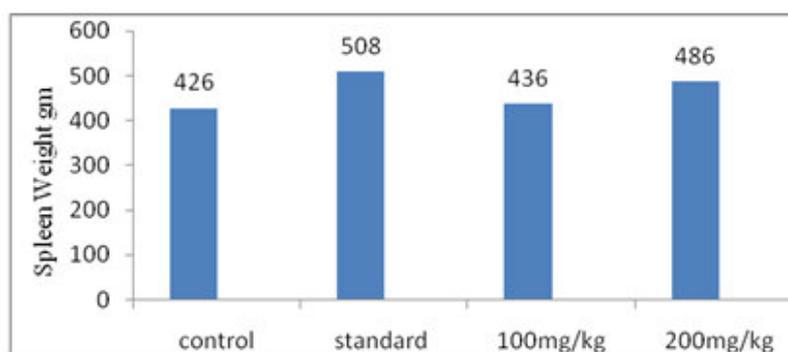
**Figure 1**  
**Phagocytic index in Ethanolic extract of *Pedalium murex***



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 with<sup>12</sup>.

**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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## REFERENCES

1. Duripandiyan V, Ayyanar M and Ignacimuthu S, Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamilnadu. India, BMC Camp, Alter med, 6: 35-41, (2006).
2. Adegoke A, Adebayo tayo and C Bukloa, Antibacterial activity and phytochemical analysis of leaf extract of *Lasientheraafricanum*. African journal of Biotechnology, 8(1): 77-80, (2009).
3. Vijyalakshmi R and Ravindhran R. Preliminary comparative phytochemical screening of root extracts of *Diospyrus ferra*(wild) and *Aera Lanata* (L.) Asian journal of Plant Sciences and Research, 2(5): 581-587, (2012).
4. Mukherjee PK, Quality Control of Herbal drugs. Edn 1, Business Horizons, New Delhi, 113-117, (2002).
5. Enomoto S, Okada Y, Güvenc A, Erdurak CS, Coşkun M and Okuyama T, Inhibitory effect of traditional Turkish folk medicines on aldose reductase (AR) and hematological activity, and on AR inhibitory activity of quercetin-3-O-methyl ether isolated from

- Cistus laurifolius*, Biol Pharm Bull, 27: 1140-1143, (2004).
6. Mathew S and Abraham TE, Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models, Food Chem, 94: 520-528, (2006).
  7. Shelke TT, Kothai R, Adkar PP, Bhaskar VH, Juvale KC, Kamble BB, *et al.* Nephroprotective activity of ethanolic extract of dried fruits of *Pedaliium murex* linn. J Cell Tissue Res, 9: 1687-1690, (2009).
  8. Kuttan G, Immunomodulatory effect of some naturally occurring sulphur-containing compounds. Journal of Ethnopharmacol, 72: 93-99, (2000).
  9. Bodhankar S, Makare N, Rangari V, Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. J Ethnopharmacol, 78: 133-137, (2001).
  10. Biozzi G, Benacerraf B, Halpern B, Quantitative study of the granulopetic activity of the reticuloendothelial system, Br. J. Exp. Pathol. 34: 426-457, (1953).
  11. Shareef H, S. Mahmud, U Farrukh, A Aqeel and HR Ghazala,. In vitro phytochemical analysis and antimicrobial activity of roots of *Operculinaturpethum* L. Inventi Impact: Ethnopharmacology, 1(1): 50-53, (2010).
  12. Liu Y, Jiao F, Qiu Y, Li W, Lao F, Zhou G, Sun B, Xing G, Dong J, Zhao Y, Chai Z and Chen C. The effect of Gd@C(82)(OH)(22) nanoparticles on the release of Th1/Th2 cytokines and induction of TNF-alpha mediated cellular immunity. Biomaterials, 68: 245-249, (2009).