International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 738-745
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IMMUNOMODULATOR ACTIVITY OF *SELAGINELLA BRYOPTERIS* (L.) BAKER

G. K. Mallaiah[1*] and G. Krishna Mohan[2]
[1]Vaagdevi Pharmacy College, Warangal, India
[2] Center for pharmaceutical sciences, JNTU Hyderabad, India

*Corresponding Author Email: gaddak70@gmail.com

ABSTRACT

Selaginella bryopteris (L.) (family: Selaginaceae), commonly known as Sanjeevni, is a magical herb that has the power to cure any malady. The immunomodulatory activity studies like various haematological and serological tests were used to determine phagocytic activity, humoral antibody response, and cell mediated immune response using the carbon clearance test, sheep erythrocyte agglutination method, and delayed type of hypersensitivity reaction in mixed Swiss albino mice and Wister rats. In this study, different doses (100, 200, and 400 mg/kg body weight/day) of the methanolic extract and its bioactive fractions possessed promising immunostimulant properties.

KEY WORDS

Selaginella Bryopteris Immunomodulatyory, Phagocytic Response, Delay Type of Hyper Sensitivity

INTRODUCTION

Selaginella bryopteris (L.) (Family: Selaginaceae), also known as "Sanjeevni," is a lithophytic pteridophytic plant with exceptional recovery abilities[1]. *Selaginella bryopteris* is the first known life-giving herb in India as the name of a wonder herb identified as Sanjeevni is mentioned in the well-known epic by the Hindi poet Tulsi das[2].

The species of the genus *Selaginella* (Family: *Selaginellaceae)* are referred to as "spike mosses". These are included in "fern allies". The spike mosses are creeping or ascendant plants with simple, scale-like leaves on branching stems from which roots also arise.

The plants are growing on barren rocks and soil. Some of the xerophytic species (observed in the desert) remain curled up in a tight brown or reddish ball (folded) under dry conditions. As soon as they come in contact with water, they uncurl, becoming green and normal (unfolded). Such plants are called "resurrection plants." Some of the species prefer moist environments and can be found in shady areas of the hills. A few species are found in tropical forests; other species are found as epiphytes[3].

In India, it is used as a major ingredient in local pills for the treatment of patients with spermatorrhoea, venereal diseases, constipation, colitis, indigestion, and urinary problems (diuretic). It is also used to treat patients who are unconscious and to lower the body temperature in patients with fever 4-6.

MATERIALS AND METHODS

(i) *Selaginell bryopteris* whole plants were collected from Budidagutta (Mandal: Bheemadevarapally), Warangal Urban district, Telangana. The plant was

authenticated by Prof. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal and the voucher specimens are preserved in the herbarium of the University College of Pharmaceutical Sciences, Kakatiya University, Warangal. The plant material was shade dried and crushed to a course powder. The crude powder drug was stored in airtight labelled containers.

The powdered drug (1 kg) was macerated with methanol in a round-bottomed flask for seven days. The flask was shaken intermittently to ensure the efficiency of extraction. After a while, it was filtered and concentrated under reduced pressure. The so obtained methnolic extract of the plant was kept in a desiccator to remove moisture and properly stored until used.

(ii) FRACTIONATION:

Methanol extract (15.49%) obtained by drug was dispersed in water and fractionated separately and sequentially with toluene (3.50%), ethyl acetate (2.50%), and n-butyl alcohol (1.50%).The solvent fractions were combined and concentrated under reduced pressure to afford the corresponding extracts.

Thin layer chromatographic and phytochemical studies were performed with metabolic extract and its corresponding fractions of *S. brysopteris,* using various solvent systems and tests.

REAGENTS:

India ink: It is a 1% w/v suspension of carbon black (Company. Veto.Co.Ltd., India).

Normal Saline: Nacl Claris Life Science Ltd., India, 5% w/v

Alsever's Solution[7]: Dextrose 2.05 gm, sodium citrate 0.80 gm, and sodium chloride 0.42 gm were dissoluted and volume was made with distilled water up to 100 ml. It is routinely used as anticoagulant blood preserver and permits the storage of whole blood for approximately two weeks in a refrigerator at 2-80°C.

Levamsole[8]: Levamsole (Khandelwal Laboratories, Mumbai, India) was used as a standard immune stimulating agent.

ANIMALS:

Swiss albino mice weighing between 18 and 25g (for the carbon clearance test) and Wister albino rats weighing between 180 and 250g (for immunomodulatory activity) were used. All the animals were procured from Mahaveer Agency (Regd.No.146/1999/CPCSEA), Hyderabad and maintained in the high-genic environmental conditions (25±2ºC) 12h/12h light/dark cycles in the animal house of the University College of Pharmaceutical Sciences, Warangal and provided a balanced diet with free access to mineral water accordingly. All the animal experimental protocols were duly approved by the institutional animal ethical committee (Regd. no. 166/1999/CPCSEA).

Acute Toxicity Study9 (Preparation of Doses and Determination of LD_{50}):

Acute toxicity studies were carried out according to the method described in the literature. Separately weighed in doses of 100, 300, 500, 1000, and 2000 mg/kg b.w. of methanolic extract of each drug, it was triturated with 2% w/v slurry of gum acacia with distilled water and administered orally to albino mice of either sex. The animals were observed continuously for any change in behavioral, neurological, autonomic profile, and mortality for the first few hours and later at 24 h and 48 h intervals for a period of 7 days. Based on the results obtained from this study, the doses were prepared (100, 200, and 400 mg/kg b.w. per day) for the present pharmacological studies. The LD_{50} for each extract was calculated and was found to be more than 500 mg/kg body weight.

Administration of Doses:

Doses of various extracts and its corresponding fractions were administrated orally to each animal using oral cannula fitted with a syringe of 5ml.

METHODOLOGY OF IMMUNOMODULATORY STUDIES

Immunological studies to evaluate the immunomodulatory activity of crude extracts (methanolic) and their fractions of *Selaginella bryopteris,* Experiments were carried out to determine phagocytic activity and cell-mediated immune response using carbon clearance tests and a delayed type of hypersensitivity reaction.

DETERMINATION OF PHAGOCYTIC ACTIVITY[10-11]:

Phagocytosis is a process by which certain body cells, collectively known as phagocytes, ingest and remove microorganisms, including malignant cells, inorganic particles, and tissue debris. Phagocytic activity of the Reticulo Endothelial System (RES) was assayed by a carbon clearance test.

(i)Carbon Clearance Test [12-22]. The phagocytic index was calculated as the rate of carbon elimination by the reticulo-endothelial system by the carbon clearance test. Twelve groups of animals were divided into I to XII (six in each) Group I was kept as a control (1ml gum acacia 2%), Group II was given the standard drug (Levamisole 50 mg/kg bwt/day), Group III, IV, and V were given methanolic extract at 100, 200, and 400 mg/kg bwt/day, Group VI and VII were fed toluene soluble fraction (50, 100 mg/kg bwt/day), and Group VIII and IX were fed ethy Groups X and XI received n-butanol soluble fraction (50 and 100 mg/kg bwt/day, respectively), while Group XII received aqueous residue (100 mg/kg bwt/day orally for 7 days).

At the end of seven days, the mice were injected with 0.1ml/10g of carbon ink suspension (1.6 w/v in 1% Gelatin in Saline) intravenously through the tail vein. Blood samples were collected from the retro orbital plexus immediately at 0 minute after 5-, 10-, and 15-minute intervals and transferred (centrifuge tube containing 0.15% w/v disodium edudate), a 50µl sample was with 0.1% sodium carbonate solution (2ml) and the absorbance was measured at 660 nm, taking 0.1% sodium carbonate solution as a blank. The carbon clearance was calculated using the following equation. (Where OD_1 and OD_2 are the optical densities at t_1 and t_2, respectively. t_1 and t_2 represent time intervals.

(ii)Delayed Type Hypersensitivity (DTH) Response[23-25]**:**

SRBCs suspended in normal saline, which sensitizes them for elicitation of DTH and also induces to study humarol antibody response against antigens (Sheep erythrocyte agglutination test) and DTH response to SRBC's was induced by injecting albino rates IP with antibody formation. Therefore, this system has major advantages, i.e., it enables two components of immune response to be measured in the same species under ideal conditions and is relatively simple and inexpensive to perform.

EXPERIMENTAL WORK

(i) TLC Studies:

The studies were performed for methanolic extract and its fractions. Methanolic extract and its ethyl acetate soluble fraction have shown the best separation *Selaginell bryopteris* in chloroform: methanol (90:10) to give five spots (Rf – 0.88, 0.73, 0.52, 0.44, and 0.32), and also n-butanol soluble fraction has shown two spots (Rf. 0.55, 0.38) in the same solvent system. Another solvent system is benzene: acetone (70:30) to give five visible spots of methanolic extract and its ethyl acetate soluble fraction (Rf – 0.80, 0.62, 0.57, 0.42,0.32 and 0.25) and n-butanol soluble fraction has shown two spots (Rf. 0.95, 0.77) in the same solvent system benzene: acetone (70: 30). The results were shown in Table number 1**.**

(ii) CHEMICAL REACTIONS

The preliminary microchemical investigation of the whole plant of ***Selaginella bryopteris L.,*** methanolic extract and its fractions was done by test tube reactions. By considering the above results, the plant may contain phenolic compounds (flavonoids), steroidal compounds, and their glycosides. The results were shown in Table number 2.

Table 1 TLC profile for *Selaginell bryopteris.*

Solvent System	No.of Spots	Rf Value	Remark
Methanolic Extract			
Ethyl acetate: Benzene: (50:50)	5	-	Tailing
Methanol: ethyl acetate: (90:10)	3	0.70, 0.50 0.29	Good Separation
Chloroform: Methanol (90:10)	5	0.88, 0.73,0.52,0.44,0.32	Best Separation
Benzene: acetone (70:30)	5	0.87, 0.57,0.42 0.32, 0.25	Good Separation
Ethyl acetate Soluble fraction			
Chloroform: Methanol (90:10)	5	0.85, 0.67,0.50,0.44,0.32	Best Separation
Benzene: acetone (70:30)	5	0.80, 0.62,0.57	Good Separation

		0.42, 0.25	
n-butanol Soluble fraction			
Chloroform: Methanol (90:10)	2	0.55, 0.38	Best Separation
Benzene: Acetone (70:30)	5	0.77, 0.37	Good Separation

Adsorbent:Silica gel G;Detection: Vanillin (1% w/v) in Sulphuric acid.

Table 2 Results of Chemical Tests:

Sl.No.	Name of the Test	Methonolic extract	Toluene fraction	Ethyl acetate	N-butanol	Aqueous residue
1	Alkaloids					
	a) Dragendorffs test	-	-	-	-	-
	b) Mayers test	-	-	-	-	-
	c) Wagner's test	-	-	-	-	-
	d) Hager's test	-	-	-	-	-
2	Carbohydrates/ Glycoasides					
	Molisch's test	+	-	-	+	+
3.	Steroids/Triterpenoids					
	Liebermann- Burchard Reaction:	+	+	+	-	+
4.	Saponins (Form test)	-	-	-	-	-
		+	-	+	+	-
	Lead Acetate					
5.	Flavonoids Shinoda's	+	+	+	+	-
	Ferric Chloride	+	+	+	+	-

'+': Present, '-': Absent; * Violet ppt.

(iii) Carbon Clearance Test:

Time dependent rate of carbon clearance was calculated as phagocytic index between the treated groups of animals (albino mice), compared with control and standard drug (Levamisole) groups. The mean phagocytic index of control and standard (Groups I and II) were found to be 0.0068 ± 0.0005 (P>0.05) and 0.0124±0.009 (P<0.001). The methanolic extract of *Selaginell bryopteris* treated Group III, IV and V have shown phagocytic index as 0.0075 ± 0.0009(P>0.05), 0.0091 ± 0.0007 (P <0.01) and 0.0130 ± 0.0010 (P<0.001) when animals treated with 100mg, 200mg, 400mg/kg body weight orally for 7 days. Toluene soluble fraction, Ethyl acetate soluble fraction and n-butanol soluble fraction of methanolic extract have given the phagocytic index as 0.0083 ± 0016(P>0.05), 0.0093 ± 0.0022 (P<0.01), 0.0110 ± 0.007, 0.0134 ± 0.0011 (P<0.001), and 0.0122 ± 0.0010, 0.0134 ± 0.0005 (P<0.001) respectively with 50mg and 100mg/kg body weight orally for 7 days. Aqueous residue of methanolic extract has shown phagocytic index as 0.0070 ± 0.0003 (P>0.05) with 100mg/kg body weight body weight orally for 7 days. Table 3, Figure 1.

(iv) Delayed type of hypersensitivity response:

Delayed type of hypersensitivity response to sheep red blood erythrocyte were calculated as a measure of paw volume (in ml) for each animal which compared with control (1ml 2%gum acacia) and standard drug (Levamisole, 50mg) Group I and II which were fed orally for 7 days. Paw volume was measured 0 hour and +24 hours and calculated percent increase of paw volume

after 24 hours. The DTH response of standard drug was found to be 20.2
±0.331 (P<0.001). Animals of Group III, IV and V were treated with methanolic extract (100mg, 200mg, 400 mg/kg/body weight) the percent activity for these groups were calculated at 24 hours. It was found to be 13.156 ±0.363, 20.072 ±0.206 (P>0.05) and 37.105 ±0.204 (P<0.001). Animals treated with Toluene, ethyl acetate and n-butanol soluble fractions of methanolic extract with 50mg and 100mg/kg body weight orally for 7 days. They were found to be DTH response 11.512 ±0.401, 17.69±0.3483 (P>0.05); 26.921±0.534 (P<0.01), 35.57±0.374 (P<0.001) and 21.15 ± 7.2(P>0.05), 34.28 ± 10.5(P<0.001), respectively. Aqueous residue of methanolic extract has
shown DTH response as 11.7 ± 4.0 with 100mg/kg body weight body weight orally for 7 days, Table 4 Figure 2.

(V) STATISTICAL ANALYSIS[26-27]:

Results of immunomodulatory activities in various animal models have been presented as Mean ± SD (Standard deviation) or Mean ± SEM (Standard Error of Mean). The significant difference was analyzed using student't' test. The variation present in a set of data was analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keul Multiple Comparison test p values < 0.05 were considered significant.

Table 3 carbon clearance test of *Sellaginella bryopteries*

Groups	Treatment doses mg/kg body wt/day	Mean of Absorbance ± SD				% Phagocytic Resp± SD
		0 min	5th min	10th min	15th min	
I	Control (2% GA)	0.0194±0.0002	0.0298±0.0008	0.0238±0.0011	0.0201±0.0008	0.0068±0.0005
II	Std. Lvl 50	0.0175±0.0004	0.0352±0.0004	0.0280±0.0002	0.0176±0.0003	0.0124±0.0005[a]
III	Me1 100	0.0177±0.0012	0.0278±0.0018	0.0220±0.0020	0.0187±0.0015	0.0075±0.0009
1V	Me2 200	0.0175±0.0014	0.0303±0.0006	0.0237±0.0022	0. 0179±0.0014	0.0091±0.0007[b]
V	Me3 400	0.0170±0.0009	0.0357±0.0015	0.0265±0.0014	0.0177±0.0008	0.0130±0.0010[a]
VI	TSF 50	0.0183±0.0013	0.0294±0.0009	0.0249±0.0009	0.0189±0.0010	0.0083±0.0016
VII	TSF 100	0.0178±0.0010	0.0301±0.0009	0.0245±0.0011	0.0189±0.0007	0.0093±0.0022[b]
VIII	ESF 50	0.0176±0.0010	0.0340±0.0008	0.0254±0.0009	0.0178±0.0010	0.0110±0.0007
IX	ESF 100	0.0177±0.0008	0.0377±0.0010	0.0279±0.0009	0.0187±0.0007	0.0134±0.001[a]
X	n-BSF 50	0.0166±0.0007	0.0349±0.0006	0.0228±0.0006	0.0179±0.0007	0.0122±0.0010[a]
XI	n-BSF 100	0.0168±0.0003	0.0371±0.0002	0.0254±0.0006	0.0177±0.0006	0.0134±0.0005[a]
XII	AR 100	0.0181±0.0002	0.0293±0.0003	0.0217±0.0004	0.0182±0.0003	0.0070±0.0003

n= six animals in each group.
Comparison with Control a=***p<0.001 very very significant; b=**p <0.01 very significant; c= *p <0.05 significant.
Comparison with standard x=***p<0.001 very very significant; y=**p <0.01 very significant; z= *p <0.05 significant.

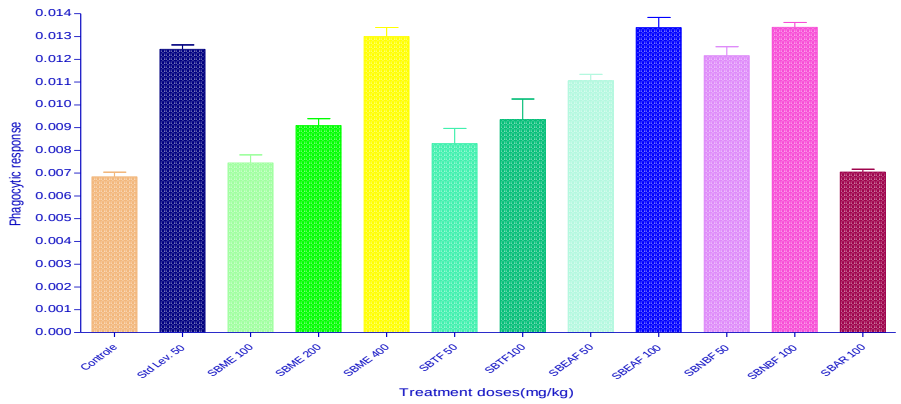

Figure 1: Carbon clearance tests of *Selaginella bryopteris*

Table 4 Delayed type hypersensitivity tests of *Selaginella bryopteris*.

Groups	Treatment doses mg/kg body wt/day	Paw Volume (Mean ±SEM)		Difference (B-A) (Mean ±SEM)	DTH Response (Mean ±SEM)
		0 hr	24th hr		
I	Control (2% GA)	1.47± 0.181	1.62 ± 0.14	0.15 ±0.365	10.89±0.463
II	Std. Lvl 50	1.67 ±0.140	1.98 ± 0.151	0.333±0.310	20.2 ±0.331[b]
III	Me1 100	1.67 ±0.140	1.83 ± 0.137	0.217±0.347	13.156 ±0.363
1V	Me2 200	1.77 ±0.137	2.12 ±0.113	0.35±0.156	20.072 ±0.206[b]
V	Me3 400	1.88 ±0.103	2.58 ±0.108	0.700 ±0.202	37.105 ±0.204[a, x]
VI	TSF 50	1.80±0.136	1.98 ±0.108	0.200 ± 0.316	11.512 ±0.401
VII	TSF 100	1.43 ±0.0845	1.68 ±0.0694	0.25 ±0.3347	17.69±0.3483[c]
VIII	ESF 50	1.55±0.254	1.92 ±0.182	0.383 ±0.384	26.921±0.534[b]
IX	ESF 100	1.43 ± 0.157	2.00 ± 0.130	0.500 ±0.358	35.57±0.374[a, y]
X	n-BSF 50	1.73 ± 0.27	2.10 ± 0.35	0.367 ± 0.14	21.15 ± 7.2[b]
XI	n-BSF 100	1.48 ± 0.12	1.98 ± 0.10	0.500 ± 0.13	34.28 ± 10.5[a, y]
XII	AR 100	1.98 ± 0.24	2.12 ± 0.35	0.233 ± 0.08	11.7 ± 4.0

n = six animals in each group.
Comparison with Control a=***p<0.001 very very significant; b=**p <0.01 very significant; c= *p <0.05 signific
Comparison with standard x=***p<0.001 very very significant; y=**p <0.01 very significant; z= *p <0.05 significant.

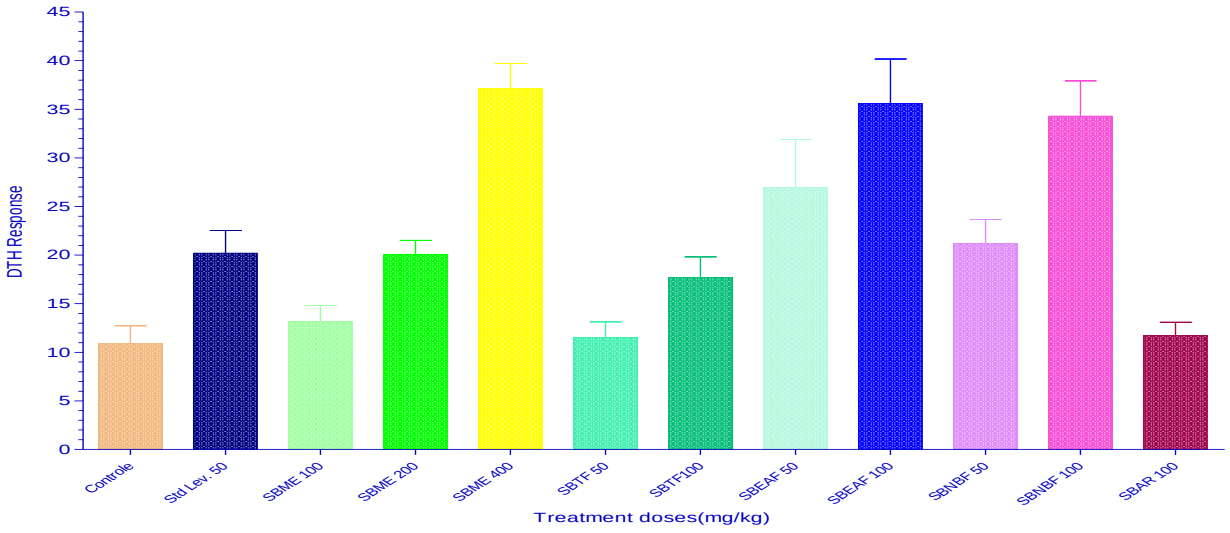

Figure 2: Delayed type hypersensitivity tests of *Selaginella bryopteris.*

DISCUSSION:

Selaginella bryopteris has shown significant activity in carbon clearance tests in experimental animals. It was observed that the phagocytic index was increased by the treatment with a methanolic extract of *Selaginella bryopteris.* The drug has been shown to increase the phagocytic index by 0.0075 ± 0.0009, 0.0091± 0.0007 ($P<0.05$) and 0.0130 ± 0.0010 ($P<0.001$), in dose dependent manner. Results of the study clearly indicate that methanolic extract and its fractions (ethyl acetate and n-butanol soluble fractions) activated the process of phagocyotosis. It is possible that the extract may influence the role of neutrophils, digestive enzymes in phagocytic vesicles, the role of monocytes, and fixed tissue macrophages in the spleen and lymph nodes. It can be stated that the *Selaginella bryopteris* influences the mechanism of phagocytosis, widespread monocyte-macrophage or reticulo-endothelial system, which resulted in a significant increase in the phagocytic index with carbon clearance test.

In the same experiment, the toluene soluble fraction of methanolic extract of *Selaginella bryopteris* showed moderate activity, but the ethyl acetate fraction and n-butanol soluble fraction (100mg/kg body weight) showed a potent increase in phagocytic index of 0.0093 ± 0.0022, ($P<0.05$) 0.0134 ± 0.001 and 0.0134 ± 0.001 and 0.0134 ± 0.0005 ($P<0.001$); i.e., the rate of carbon elimination in a dose dependent manner].

The effect of *Selaginella bryopteris* on cell-mediated immune response was studied by a delayed type of hypersensitivity (DTH) to sheep red blood erythrocytes. The methanolic extract showed a significant increase in DTH when compared with control and similar to standard. The percent increase was found to be 20.072 ± 0.206 and 37.105 ± 0.204% in the doses of 100 mg/kg body weight and 58.18 ± 0.13, 74.54 ± 0.21 % ($P<0.001$) in the doses of 200 and 400 mg/kg body weight. The drug influenced the cell-mediated immune response in a dose-dependent manner. The study also showed that the toluene soluble fraction had no significant effect while the ethyl acetate fraction and n-butanol soluble fraction (100mg/kg body weight increased the activity in a dose dependent manner as 26.921 ± 0.534, 35.57 ± 0.374 and 21.15 ± 0.72 and 34.28 ± 0.105 % ($P<0.001$) with 50 mg and 100 mg/kg body weight.

CONCLUSION

Studies on the immunomodulatory activity of *Selaginella bryopteris reveal* that the drug can influence the phagocytic activity, activating cell-mediated immune and humoral immune responses in a dose-dependent manner. These findings justify its use in various ailments resulting from diverse physiological conditions.

REFERENCES:

1. Ganeshaiah KN, Vasudeva R, Uma Shaanker R. In search of Sanjeevani. *Curr Sci.* 2009;97(4):484–9
2. Srimad Valmiki Ramayana. *Yuddakanda. Slokas.* 74th chapter:29–34
3. Pandey,B.P., *Ptendophyta* Chand's and company Ltd, New Delhi,*Pteridophyta*,1994, pp.46-50.
4. Singh S, Singh R. Ethnomedicinal use of pteridophytes in reproductive health of tribal women of pachmarhi biosphere reserve, madhya pradesh, India. *IJPSR.* 2012;3(12):4780–90.
5. Singh S, Singh R. Utilization of pteridophytes of achanakmar-amarkantak biosphere reserve, central India in women's health and beauty care practices. *IRJP.* 2013;4(1):235–40.
6. Shweta S, Singh R, Sahu TR. *Floral Diversity and their conservation.* Publisher Biotech Book; 2013. Ethnomedicinal uses of Pachmarhi Hills, Madhya Pradesh, India; pp. 267–90.
7. Shweta Singh, Singh Rita. *Ethnobotany of India.* Deep Publication Published; 2014. Ethnobotany of Pteridophytes in Bastar region of Chhathisgarh State.
8. Alsever, J.B. and Ainslie,R.W (new York), in: Journal of Medicine, Westbury, New York, 1941, 41,p- 126. http/www.usca.edu/biogeo/jacksonlab/reagents.html; http/www.biocompare.com/productdetails.
9. Mansure Kayatas, M., Levamisole treatment enhances protective antibody response to hepatitis B vaccination in hemodialysis patients. Artificial Organs. 2002, 26 (6), pp. 492-496.
10. Millars. Immunomodulators from higher plants. *Indian. J. Nat. Products* 2004. 20(1) pp. 3315-3318.
11. Sigurd,J., Normann, M.D.,Earl,P. and Benditt.M.D., Function of the Reticuloendothelial System-I. A study on the Phenomenon of Carbon Clearance inhibition. *J. Exp. Med*. 1965, 122, pp.693-707.
12. Barar. F.S.K. Essentials of Pharmacotherapeutics, II edition, S.Chand & Co. Ltd., 1987, P-775.
13. Dash, S., Nath, L.K., Bhise, S., Kar, P. And Bhattacharya.S., Stimulation of Immune Function Activity by the Alcoholic root extract of *Heracleum napalense* D.Don. *Indian of Pharmacol.*, 38 (5), 2006, pp. 336-348.
14. Gonda R, Tomoda M, Shimizu N and Kanari M. Characterization of an acidic polysaccharide from the

seeds of *Malva verticillata* stimulating the phagocytic activity of cells of the RES, *Planta medica,* 1990, 56, pp.73-76.

15. Hudson, L. and Hay, F.C., Practical Immunology, Harper and Row, New York, 1980, 2nd edn. P-73.
16. Biozzi, G., Benaceraf, B. and Halpern, B.N., Quantitative study of the granulopectic activity of the reticulo-endothelial system. II. A study of the kinetics of the R. E. S. in relation to the dose of carbon injected; relationship between the weight of the organs and their activity. *Brit. J. Expl.Pathol*. 1953, 34(4), pp.441-457.
17. Lempeerle, G. and Reichelt, M. The evaluation of phagocytic activity in, *Med. Klin. (Munich),* 1973, 54, pp.241-248.
18. Mungantiwar, A.A., Nair, A.M., Kamai, K.K. and Saraf, M.N. Adaptogenic activity of aqueous extract of the roots of *Boerhaavia diffusalinn*, *Indian Drugs,* 1997, 34 (4), pp.184-189.
19. Tuwain, A.A. and Saad, A.H., *British. J. Expl.Pathol.* 1986, 67, 251.
20. Senthamil, S.K., Suthanthirrajan, N. and Namasivayam, A., Reticuloendothelial function in acute noise stress. *Indian J. Physiol. Pharmacol.* 1992, 36 (4), 279.
21. Atal, C.K., Sharma, M.L., Kaul, A. and Khajuria, A. Immunomodulating agents of plant origin. I: preliminary screening. *J. Ethnopharmacol.* 1986, 18, pp.133-141.
22. Dash S, Nath LK, Bhise S, Bhuyan N. Antioxidant and antimicrobials activities of *Heracleum nepalense* D Don root. Trop, *J Pharm Res* 2005, 4, pp.341-347.
23. Neelam M., Subhash Bodhankar and Vinod Rangari. Immunomodulatory activity ofalcoholic extract of *Mangifera indica* (L) in mice. *J. Ethnopharmacol*, 2001, 78(2),pp.133-137
24. Ghule, B.V., Muruganantlian, G., Nakhat, P.D. and Yeole,P.G., Immunostimulant effect of *Capparis zeylanica* Linn. leaves, *J. Ethnopharmacol*, 2006,108, pp.311-315.
25. Ghosh, M.N., Fundamentals of Experimental Pharmacology., Scientific Book Agency, New Delhi, 1984, II edition, P-177.
26. Graph Pad, Instat St. Software.
27. Alafonso, R.G. (edn.), Remington's Pharamceutical Sciences, Mack Publishing Co. Pennsylvania, 1996, 18th edn. P-113.

***Corresponding Author:**
G. K. Mallaiah*
Email: **gaddak70@gmail.com**