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PHYTOCHEMICAL SCREENING AND IMMUNOMODULATORY ACTIVITY OF DIFFERENT EXTRACT OF *SPINACIA OLERACIA* LEAVES

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ABSTRACT: The extracts of *Spinacia oleracia* leaves studied for Phytochemical screening and different extract of *Spinacia oleracea* leaves was studied for immunomodulatory activity. The immunomodulatory effect was evaluated by delayed type hypersensitivity response using SRBCs. The n-hexane, dichloromethane, methanolic and aqueous extract of *Spinacia oleracea* leaves was studies for DTH response. The dichloromethane extract of *Spinacia oleracea* leaves extract showed significant increase in the hypersensitivity response, indicating its effect on cell mediated immunity. From the above result, it was concluded that the preliminary Phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds and the dichloromethane extract of *Spinacia oleracea* has a significant effect on cell mediated immunity.

INTRODUCTION: The leaves of *Spinacia oleracea* (Family: Chenopodiaceae) commonly known as Palak/Spinach is widely used in India for it various pharmacological effect. *Spinacia oleracea* useful in diseases of blood and brain, asthma, leprosy, biliousness; causes "kapha" (Ayurveda Leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestiblle, anthelmentic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting, biliousness, flatulence.). It has been used in the treatment of urinary calculi and has hypoglycemic properties.

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And have been used in the treatment of febrile conditions. Seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice [1, 2]. Since the plants is widely used for treatment of various ailments, the present study was investigated its effect on immunomodulatory activity in the experimental animal models. Spinach has a high nutritional value and is extremely rich in antioxidants, It is a rich source of Vitamin A (lutein), Vitamin C, Vitamin E, Vitamin K, magnesium, manganese, folate, and iron. It having the various biological activities like virus inhibitor [3], anthelmentic [4], Antioxidant [5], hepatoprotective [6] CNS Depressant Effect [7] and reducing risk of breast cancer [8].

MATERIALS AND METHODS:

Plant Material: The leaves of *Spinacia oleracea* were collected from outfield medicinal garden near to Gwalior, Madhya Pradesh. Plants were identified and voucher specimen deposited in Department of Pharmacognosy for future references.

A voucher specimen number is BU/Bot/10/05 for *Spinacia oleracea.* Different extract of *Spinacia oleracea* were used for Immunomodulatory activity.

Animals: Albino wistar rats (150–200 g) either sex, were used for pharmacological evaluation. The animals were housed in standard environmental conditions of temperature (21 ± 2°C), humidity (55 ± 10%) and a 12-h light–dark cycle. Rats were supplied with standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed with the approval of the Ethical committee and CPCSEA approved animal house and Institutional Animal Ethics Committee (CPCSEA Reg. N0. 1039/PO/Re/S/07/CPCSEA).

Drugs and Chemicals: Levamisole of Khandelwal labs, Mumbai was used as reference standard drug. All other reagents and chemicals used were of analytical grade.

Antigen: Fresh blood was collected from sheep sacrificed in the local slaughter house in a sterile bottle containing Alsever's solotion (2%dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride). Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free normal saline and adjusted to a concentration of 0.1ml of 0.5×10^9 cells/ml for immunization [9].

Qualitative Phytochemical Screening:
Preliminary phytochemical screening (Detection of Carbohydrate, Glycosides, Detection of Alkaloids, phytosterols and triterpenoids, Protein and Amino acid, Fixed oils and Fats, Phenolics and Tannins, Flavonoids, Detection of Saponin and Detection of Mucilage) was performed for all the extracts [10-11].

Acute toxicity study and Dose selection: Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guideline No. 423. Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of different extracts of plant *S.oleracea*. The treated animals were monitored for 14 days for mortality and general behavior.

Immunpmodulatory activity by Delayed-type hypersensitivity (DTH) response:
Dose and Treatment: Animals were divided into six groups each having 2 animals. Group 1-received Vehicle, Group-2 received n-hexane extract of *S.oleracea* (200mg/kg, p.o.), Group-3 received Dichloromethane extract of *S.oleracea* (200mg/kg, p.o.), Group-4 received Methanolic extract of *S.oleracea* (200mg/kg, p.o.), Group-5 received Water extract of *S.oleracea* (200mg/kg, p.o.), Group-6 received Levamisole (50mg/kg, p.o.). Levamisole (Khandelwal Pharmaceutical Ltd. Mumbai) was used as standard drug [12].

Preparation of antigen: Fresh blood was collected from sheep sacrificed in the local slaughter house in a sterile bottle containing Alsever's solotion (2%dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride). Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free normal saline and adjusted to a concentration of 0.1ml of 0.5×10^9 cells/ml for immunization [9].

Delayed-type hypersensitivity (DTH) response*:* Rats were divided in to ten groups of six animals. All the animals received drugs according to corresponding groups for 7 days, on 8^{th} day rats were immunized by injecting 0.5 ml of fresh sheep red blood cell suspension (SRBCs) (10^9 cells/ml suspended in normal saline) intraperitoneally. On 11^{th} day the thickness of right hind footpad was measured using vernier caliper. The rats were then challenged by injection of 20 µl of 1% SRBCs (suspended in normal saline) in right foot pad. Foot thickness was again measured after 24 hrs and 48 hrs of this challenge. The differences obtained for pre- and post challenge foot thicknesses were taken for the measurement of DTH and were expressed in mm [9, 13.]

RESULT AND DISCUSSION:
Qualitative Phytochemical Screening: The preliminary Phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds as shown in **Table 1.**

TABLE 1: QUALITATIVE CHEMICAL ANALYSIS OF *SPINACIA OLERACEA* BY CHEMICAL TESTS

S. No.	Test	n-hexane	Dichloromethane	Methanolic	Aqueous
1.	**Carbohydrate**				
	Molish test	-	+	+	+
	Felling test	-	+	+	+
2.	**Glycosides**				
	Borntrager test	-	+	+	+
	Killer killani test		+	+	+
3.	**Alkaloid**				
	Mayer test	-	+	+	-
	Dragendorff's test	-	+	+	-
	Hager test	-	+	+	-
4.	**Phytosterol and Triterpinoids**				
	Salkowaski test	-	+	+	-
5.	**Protein and Amino acid**				
	Millon's test	-	+	+	-
	Biuret test	-	+	+	-
	Ninhydrin test	-	+	+	-
6.	**Fixed oil and Fats**				
	Oily spot test	-	-	-	-
7.	**Phenolic and Tannins test**				
	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
8.	**Flavonoids**				
	Alkaline Reagent test	-	+	+	+
9.	**Saponin**				
	Foam test	-	-	-	-
10.	Gum and Mucilage	-	-	-	-

Acute toxicity study and Dose selection of different extracts: No mortality and behavioral changes were observed in the treated groups up to 2,000 mg/kg body weight. It was observed that none of the extracts were found to be lethal even at the dose of 2000 mg/kg. Hence, 1/ 10th dose was selected as effective dose or therapeutic dose. The cut off value of 200 mg/kg were selected for immunomodulatory activity. Doses equivalent to 200 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v Tween 80 solutions for experiment as shown in **Table 2.**

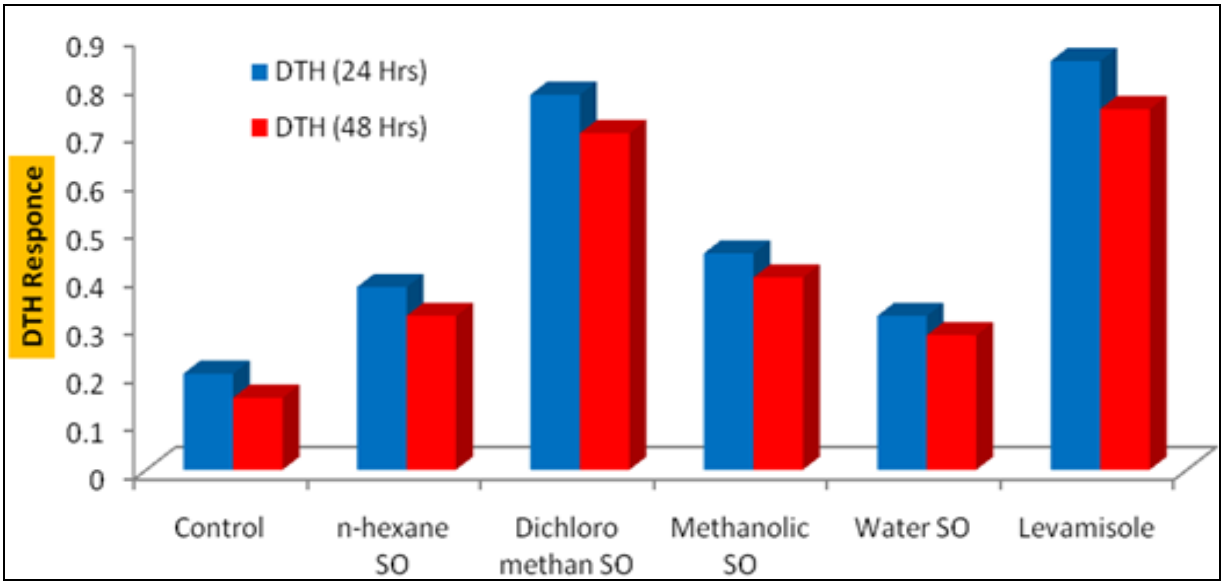
TABLE 2: ACUTE TOXICITY STUDIES OF PLANT EXTRACTS

S. No.	Treatment	Dose (mg/kg)	Number of animals	Mortality			Toxicity Profile
				After 24 hrs	After 7 days	After 14 days	
1	n- hexane extract of *Spinacia oleracea*	2000 mg/kg	3	0	0	0	Safe
2	Dichloromethane extract of *Spinacia oleracea*	2000 mg/kg	3	0	0	0	Safe
3	Methanolic extract of *Spinacia oleracea*	2000 mg/kg	3	0	0	0	Safe
4	Water extract of *Spinacia oleracea*	2000 mg/kg	3	0	0	0	Safe

Delayed-type hypersensitivity (DTH) response by SRBC: Cell mediate immunity involves effector mechanism carried out by T lymphocytes and their products (lymphokines). DTH require the specific recognition of a given antigen by T lymphocytes, which subsequently proliferate and release cytokines. The mean value of the DTH response to SRBC was 0.15±0.07 for group 1 (control), 0.32±0.04 for group 2, 0.70±0.06 for group 3, 0.40±0.03 for group 4, 0.28±0.04 for group 3, 0.75±0.05 for group 6 (standard group levamisole) as shown in table no 3. Group 2 Dichloromethane extract of *Spinacia oleracea* having maximum DTH value then compared with control. Shown in **Graph 1.**

TABLE 3: EFFECT OF DIFFERENT EXTRACTS ON DTH RESPONSE USING SRBCs

S no.	Groups	Treatments	Dose	DTH Response (mm) 24 Hrs	DTH Response (mm) 48 Hrs
1	Group I	Control	10ml/kg vehicle	0.20±0.04	0.15±0.07
2	Group II	n- hexane extract of *Spinacia oleracea*	200 mg/kg	0.38±0.03*	0.32±0.04*
3	Group III	Dichloromethane extract of *Spinacia oleracea*	200 mg/kg	0.78±0.08***	0.70±0.06***
4	Group IV	Methanolic extract of *Spinacia oleracea*	200 mg/kg	0.45±0.08**	0.40±0.03**
5	Group V	Water extract of *Spinacia oleracea*	200 mg/kg	0.32±0.02*	0.28±0.04*
6	Group X	Levamisole	50 mg/kg	0.85±0.02***	0.75±0.05***



GRAPH 1: EFFECT OF DIFFERENT EXTRACTS OF *SPINACIA OLERACEA* (SO) ON DTH

CONCLUSSION: Delayed type Hypersensitivity required the specific recognition of given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. DTH is a part of the process of graft rejection, tumor immunity and most important, immunity to many intracellular microorganisms. It can also be due to activation of complement, release of reactive oxygen or nitrogen species by activated phagocytes and pro-inflammatory cytokines (Smith and Kroes, 2000). Delayed type hypersensitivity (DTH) is antigen specific and cause erythema induction at the site of antigen infection in immunized animals. The cell-mediated immune response of different extracts of *Spinacia oleracea* were assessed by DTH reaction, i.e. foot pad reaction. The dichloromethane extract of *Spinacia oleracea* produced a significant, increase in DTH reactivity in rats. Increase in DTH reaction in rats in response to cell dependent antigen revealed the stimulatory effect of dichloromethane extract on T cells. The results of present study suggest that the dichloromethane extract of *Spinacia oleracea* leaves having maximum significant Immunomodulatory activity.

REFERENCES:

1. Kirtikar KR, Basu BD.: Indian Medicinal plants. Vol. 8. Deharadun: International Book Distributors; 2005; 2078-2079.
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement).New Delhi: Council of Scientific and Industrial Research; 1956.
3. Adam G, Mundry KW and Straub P. 2008, Isolation and Characterization of a Virus Inhibitor from Spinach (*Spinacia oleracea* L.) Journal of Phytopathology, 115: 357 – 367
4. Patil UK, Dave S, Bhaiji A, Baghel US, Yadav SK and Sharma VK. In-vitro Anthelmintic Activity of Leaves of *Spinacia oleracae* Linn. International Journal of Toxicological and Pharmacological Research; 2009, 1, 1, 21-23
5. Verma RK, Sisodia R. and Bhatia AL.: Role of Spinacia oleracea as Antioxidant: A biochemical study on mice brain after exposure of gamma radiation. Asian Journal of Experimental Sciences, 2003; 17, 51-57.
6. Gupta RS and Singh D. Amelioration of CCl4- induced hepatosuppression by *Spinacia oleracea* L. leaves in wistar albino rats. Pharmacologyonline, 2006; 3: 267-278.
7. Guha D, Das S.: CNS depressive role of aqueous extract of *Spinacia oleracea* L. leaves in adult male mice albino rats. Indian J Exp Biol; 2008; 46: 185-190.
8. Longnecker MP, Newcomb PA, Mittendorf R, Greenberg R and Willet W. Intake of Carrots, Spinach, and Supplements Containing Vitamin A in Relation to Risk of Breast Cancer. Cancer Epidemiology, Biomarkers & Prevention, 10997; 6: 887-892.

9. Yadav Y, Mohanty P K and Kasture S B. Evaluation of immunomodulatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in wistar rats. Int. J. of Pharm. & Life Sci 2011; 2(4):687-694.
10. Khandelwal K.R. Practical Pharmacognosy Techniques and Experiments. 15th ed., Pune, Nirali Prakashan; 2006, pp. 15–163.
11. Kokate CK. Practical Pharmacognosy. Delhi, Vallabh Prakashan, 4th ed., Delhi, Vallabh Prakashan; 1997,107 -111
12. Dashputre NL, Naikwade NS. International Journal of Pharma Sciences and Research (IJPSR) 2010; 1(3): 178-184.
13. Gokani RH, Lagiri SK, Santani DD, and Shah MB. Evaluation of Immunomodulatory activity of *Clerodendrum phlomidis* and *Premna integrifolia* Root. International Journal of Pharmacology 2007; 3(4): 352-356.

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