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IN-VITRO ANTI INFLAMMATORY ACTIVITY OF ULTRA SONIC BATH ASSISTED, METHANOL EXTRACT OF *LEPIDIUM SATIVUM* LINN. SEEDS

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ABSTRACT

Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. There are certain problems associated with use of animals in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of methanol extract of *Lepidium sativum* seeds. Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritis diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of tissue proteins in vivo. Anti-inflammatory agent that can prevent protein denaturation. Therefore, would be worthwhile for anti-inflammatory drug development.

Keywords: *In-vitro* anti-inflammatory activity, Ultra sonication, Protein denaturation. UV- Spectrophotometer.

INTRODUCTION

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis [1]. Plants have formed the basis of sophisticated traditional medicine practices that have been used for thousands of years by people in China, India, and many other countries. Nowadays plants are still important sources of medicines, especially in developing countries that still use plant-based for their healthcare. *Lepidium sativum* Linn. belongs to family cruciferaceae. It is a cool season annual plant, cultivated throughout India. It is considered as the one of the better medicinal plants in various African countries, where the seeds are chewed to cure throat disease, asthma and headache and are useful for diuresis and rapid bone fracture healing. *Lepidium sativum* is reported to exhibit antihypertensive [2], diuretic [3], Analgesic, anticoagulant [4], antirheumatic [5], hypoglycemic [6], laxative, prokinetic [7], antidiarrheal, and antispasmodic properties. It has been shown to possess antiasthmatic [8] and bronchodilatory activity [9]. Hence, the Present Study was aimed to investigate the invitro anti-inflammatory effect of methanol extract of *Lepidium*

sativum Linn. Seeds.

MATERIALS AND METHODS

Plant material

The Plant material was collected from the distributor SHRI-SHAIL MEDI-FARM, Nagpur, Maharashtra, India. It was identified by Dr. B. Prathibha Devi, Department of Botany, Osmania University, Hyderabad. Air-dried under the shed at room temperature. Dried seed material was pulverized and the powder kept in polyethylene bags.

Preparation of extract and Preliminary phytochemical screening

Accurately weighed plant material was soaked in the conical flask by using methanol solvent. Extraction was done by using ultra sonic bath sonication. Solvent recovery done by using simple distillation method. Extract was collected and stored in refrigerator. Preliminary phytochemical screening done to investigate different phytoconstituents present in the *Lepidium sativum* Linn. seeds.

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In vitro Anti-inflammatory bio assay

The reaction mixture (5ml) consisted of 0.2 ml of egg (from fresh hen's egg), 2.8 ml of phosphate buffer saline (PBS, pH -6.4) and 2ml of varying concentrations of the test extract methanol extract of *Lepidium sativum* Linn. seeds. So that the final concentrations become 25, 50, 150, 250, 500, 1000 µg/ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2°C in a BOD incubator (BIOTECH) for 15 mins and then heated at 70°C for 5 mins. After cooling, their absorbance was measured at 660nm (LAB INDIA, UV 3000) by using vehicle as blank. Diclofenac sodium at the final concentration of (50, 100, 300, 600, 1200, 2500, µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\% \text{ of inhibition} = 100 \times [V_t / V_c - 1]$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

The extract or drug concentration for 50% inhibition (IC_{50}) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS

Preliminary phytochemical screening of *Lepidium sativum* seed extract results revealed that Alkaloids, Carbohydrates, Tannins, Triterpenoids, Flavanoids, Inulin, Amino acids are present and Saponins, Glycosides are absent.

In vitro Anti-inflammatory bio assay

The in vitro anti-inflammatory effect of methanol extract of *Lepidium sativum* seeds was evaluated against the denaturation of egg albumin. The results are summarized in table 1.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the test extract throughout the concentration range of 25 to 1000 µg/ml Diclofenac sodium (at the concentration range of 50 to 2500 µg/ml) was used as the reference drug which also exhibited concentration dependent inhibition of protein denaturation. The results are summarized in table 2.

However, the effect of Diclofenac sodium was found to be less as compared with that of methanol extract of *Lepidium sativum* seeds. This was further confirmed by comparing their IC_{50} values. The results are summarized in table 3.

Table 1. Influence of methanol extract of *Lepidium sativum* Linn. seeds against protein denaturation.

Concentration(µg/ml)	% Inhibition
Control	-
25	3.15
50	6.92
150	12.32
250	22.32
500	30.61
1000	66.65

Table 2. Influence of Diclofenac sodium against protein denaturation

Concentration (µg/ml)	% Inhibition
Control	-
50	10.5
100	22.5
300	32.3
600	61.6
1200	119.4
2500	190.5

Table 3. IC_{50} values of methanol extract of *Lepidium sativum* Linn. seeds and Diclofenac sodium against protein denaturation.

Treatment	IC_{50} value (µg/ml)
Methanol extract of <i>Lepidium sativum</i> Linn. seeds	770
Diclofenac sodium	485

CONCLUSION

From the findings of the present preliminary experiment it can be concluded that the seeds of *Lepidium sativum* had marked anti-inflammatory effect against the

denaturation of protein in vitro. It is suggested that anti-inflammatory effect of this plant should be further evaluated in other experimental models in pursuit of newer phytotherapeutic against inflammatory diseases.

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