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## PHYTOCHEMICAL INVESTIGATION AND STUDY OF ANTI-NFLAMMATORY ACTIVITY OF *MORINGA OLEIFERA* LAM.

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### ABSTRACT

*Moringa oleifera* lam. is a highly valued plant as well member of family Moringaceae, is a fast growing plant widely available in tropics and subtropics with much economic importance for industrial and medicinal uses. Though a number of medicinal plants are being used to bring about traditional cure of inflammatory conditions, our present investigation includes preliminary phytochemical investigation of extracts and further screening them for possible anti-inflammatory potential by using carrageenan induced paw edema model in rats. Ethanolic extract has shown significant ( $p < 0.001$ ) reduction in rat paw edema to the extent of 52.7% at a dose of 300 mg/kg from 3<sup>rd</sup> hour as compared control group. However, petroleum ether (60-80<sup>o</sup>c) extract, chloroform extract and distilled Water extract has reduced the paw edema to the extent of 23%, 24% and 22% respectively at 300 g/kg concentration when compared with control group. Where as Ibuprofen also significantly ( $P < 0.001$ ) reduced paw edema from 3<sup>rd</sup> hours when compared to control group.

Hence, the present study was under taken to investigate the anti-inflammatory activity of Ethanolic extract at some medicinal plant in rats by using carrageenan induced paw edema model. The extract was compared with the standard drug ibuprofen (100mg/kg). The % yield of petroleum ether (40-60<sup>o</sup>C), chloroform, ethanol and water extract was 5%, 2.45%, 11% and 1.5% respectively.

Ethanol extract has shown good significant ( $p < 0.001$ ) reduction in paw edema to the extent of 52.7% at 300 mg/kg concentration, respectively from 3<sup>rd</sup> hours when compared to control group. However, petroleum ether (60-80<sup>o</sup>c) extract, chloroform extract and distilled Water extract has reduced the paw edema to the extent of 23%, 24% and 22% respectively at 300 mg/kg

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concentration when compared with control group. Where as Ibuprofen also significantly ( $P < 0.001$ ) reduced paw edema form 3<sup>rd</sup> hours when compared to control group.

**Key Wards:** *Moringa oleifera* lam., Anti-inflammatory, Phytochemical Investigation.

## INTRODUCTION

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans. Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 B.C. Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles.<sup>1</sup> The use of medicinal parts is accepted as the most common form of traditional medicine. Among the entire flora, it is estimated that 35,000 to 70,000 species have been used for medicinal purpose. Some 5000 of these have been studied in biomedical research. In developing countries, herbal medicines continue to play important role in primary health care, especially where coverage of health service is limited.<sup>2</sup>

The seeds of *Moringa oleifera* lam (MOL), (Moringaceae), commonly known as drumsticks, are recommended in disease at liver<sup>3</sup>, spleen, tetanus and paralysis. The seeds are rich in mineral, vitamins and mucilages<sup>4</sup>, a pharmaceutical adjuvant<sup>5</sup>, in addition to ascorbic acid oxidase. They are also used in the treatment at ascites and rheumatism<sup>6</sup>. A small or medium sized tree up 10m tall, with thick, soft, corky, deeply fissured bark and tomentose twigs.<sup>7</sup> inflammatory disease are among the commonest and oldest disease afflicting man and animals. Despite significant advances in knowledge about inflammation, the ideal anti-inflammatory drug is still elusive. Plant

based medicaments have already provided uncompromising remedies for some disease condition. With drugs. Such as morphine, digitalis, atropine etc. Hence, there is a good reason to search for an ideal anti-inflammatory drug from the plant kindom.<sup>8</sup>

## MATERIAL AND METHODS

### Collection of Plant Material

The Seeds of *Moringa oleifera* lam were collected from local areas of Chopda Maharashtra and authenticated by Department of Botany, Agharkar Research Institute, pune, The Voucher no. is F-164. The seeds were dried in shade and reduced to coarse power using mechanical grinder and passed through a sieve No.40 to obtain about powder of desired particle size.

### Preparation of Crude Extraction

The dried powder (1 Kg) was extracted with petroleum ether, chloroform, ethanol in soxhlet extractor and another batch of powder (1 Kg) was macerated with distilled water for 24 hours. The extracts were concentrated in rotary flash evaporator under vacuum.

### Successive Solvent Extraction

The dried powder (1 Kg) was extracted with various solvents having different polarity like petroleum ether (40-60<sup>o</sup>C), chloroform and ethanol in succession. Each extract was concentrated in rotary flash evaporator under vacuum. The percentage yield of petroleum ether (40-60<sup>o</sup>C), chloroform, ethanol and distilled water extract was recorded in Table No. 1.

**Table No. 1:** Showing the percentage yield of *Moringa oleifera* lam. Seeds extracts.

Sr. No.	Extract	Weight of residue	% Yield
1.	Petroleum-ether	35 gm	5%
2.	Chloroform	15 gm	2.45 %
3.	Ethanol	66 gm	11 %
4.	Distilled Water	10 gm	1.5 %

**Preliminary Phytochemical Investigation****Extract of Cruds Drug**

The percentage yield of petroleum ether (40-60°C), chloroform, ethanol and distilled water extract was recorded in Table 1.

**Qualitative Chemical Analysis<sup>9</sup>**

The extracts were subjected to preliminary qualitative chemical analysis in Table No.2.

**Table No. 2:** Showing the qualitative chemical investigation of *Moringa oleifera* Lam. Seeds extracts

Tests / Extracts	Ethanol Extract	Pet. Ether (60-80°C)	Chloroform	Distilled Water
<b>Test for Sterols</b>				
1. Salkowski's test				
2. Sulphur test	+	+	-	-
3. Liebermann Burchards's test	+	+	-	-
	+	+	-	-
<b>Test for Glycoside</b>				
1. Baljet's test	+	-	-	-
2. Keller- Kellani test	+	-	-	-
3. Raymond's test	+	-	-	-
4. Bromine water test	+	-	-	-
5. Legal's test	+	-	-	-
<b>Test for Saponins</b>				
1. Foam Test	-	-	-	-
<b>Test for Carbohydrates</b>				
1. Molish's Test	+	+	+	+
2. Barfoed's test	-	+	-	-
3. Benedict's test	+	-	-	-
<b>Test for Alkaloids</b>				
1. Mayer's test	+	-	-	+
2. Wagner's test	+	-	-	-
3. Dragendorff's test	+	-	-	-
4. Hager's test	-	-	-	-
<b>Test for Flavonoids</b>				
1. Ferric Chloride test	+	-	-	-
2. Shinoda test	+	-	-	+
3. Zn-Hcl reduction test	-	-	-	-
4. Lead Acetate test	+	+	-	-

<b>Test for Tannins</b>				
1. Ferric Chloride test	-	-	-	+
2. Gelatin test	-	-	-	-
<b>Test for Proteins</b>				
1. Million's test	+	-	-	-
2. Xanthoprotein test	+	-	+	-
3. Ninhydrin test	+	-	-	-
4. Biuret test	+	-	+	-

(+) Present (-) Absent

### Identification of Active Principle by Thin Layer Chromatography<sup>10</sup>

The Ethanolic extracts were subjected to thin layer chromatography.

The spot were visualized as violet and pink at  $R_f$  values 0.87 and 0.96 for amino acid  
The spot were visualized as pink and blue at  $R_f$  values 0.96 and 0.54 for sterols.

### Pharmacological Screening

Male albino rats were utilized for studying carrageenan induced hind paw edema. all the suspensions were administered orally 30 min. before the injection of carrageenan, paw volume was measure by plethysmographic method and percentage inhibition of edema at different time intervals was calculated. The study has been approved by the institutional Animal Ethical Committee.

### Animal Used

Acute oral toxicity study was carried out suing Albino Wister rat of either sex weighing between 25 and 30 g while male Albino Wister rat weighing between 150 and 250 g were employed for assessment of anti-inflammatory activity.

### Materials Used

Extracts {Petroleum ether (40-60°C), Chloroform, Ethanol and Distilled Water extract} 300 mg/kg, Ibuprofen was used as standard (100 mg/kg), Carrageenan as phlogistic agent (irritants) and 1 % tween 80 as suspending agent.

### Acute Toxicity Studies<sup>11</sup>

Acute toxicity study was carried out according to OECD (Organization of Economic Cooperation and Development) guideline in albino rats. The acute toxicity study of various extracts of *Moringa oleifera* Lam. seeds was showed signs of toxicity like tremor, convulsion and deep breathing at 3000

mg/kg b.w. 1/10<sup>th</sup> of the same dose for all these exacts were taken as therapeutic dose i.e. 300 mg/kg.b.w<sup>9</sup>.

### Carrageenan Induced Paw edema Model in Rat<sup>12</sup>

Rat were randomly divided in to six groups of six animals in each and maintained on normal diet and water ad libitum.

Group I : Served as control (Inflammation induced)

Group II : Standard group Ibuprofen 100 mg/kg

Group III : Petroleum ether extract 300 mg/kg

Group IV : Chloroform extract 300 mg/kg

Group V : Ethanol extract 300 mg/kg

Group VI : Distilled Water extract 300 mg/kg

Thirty minutes after drug or test compound administration, 0.1 mL. of 1% carrageenan in distilled water was injected into the subplantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of plethysmographic by mercury displacement method, at zero hours. (Immediately after injecting carrageenan). The same procedure was repeated at 30 mins. 1, 2, 3 hours. The difference between 1 hours and subsequent hours reading was taken as actual edema volume. The percentage inhibition of paw edema in the various treated groups was then calculated by using the formula;

$$\text{Percentage inhibition} = (1 - V_t/V_c) \times 100$$

Where  $V_t$  = is the edema volume in the drug treated group.

$V$  = is the edema volume in the control group.

### Statistical Analysis

The mean values  $\pm$  SEM of changes in paw volume on 1, 2, 3 hr. after induction were evaluated for statistical significance by one-way ANOVA test followed by student-Newman Keul's post test. Differences of  $P < 0.05$  were taken as statistically significant.

## RESULTS AND DISCUSSION

The Table No. 1. Shows the successive solvent extraction like different extracts petroleum ether (40-60°C), chloroform, ethanol and distilled water of 35g, 15g, 66g and 10g respectively. Table No. 2 on qualitative chemical investigation of *Moringa oleifera* lam. has indicated the presence of Sterols, Carbohydrates, Alkaloids, Glycoside, Amino acid, flavonoids and Proteins, fats, oil. Tannins and phenolic compounds.

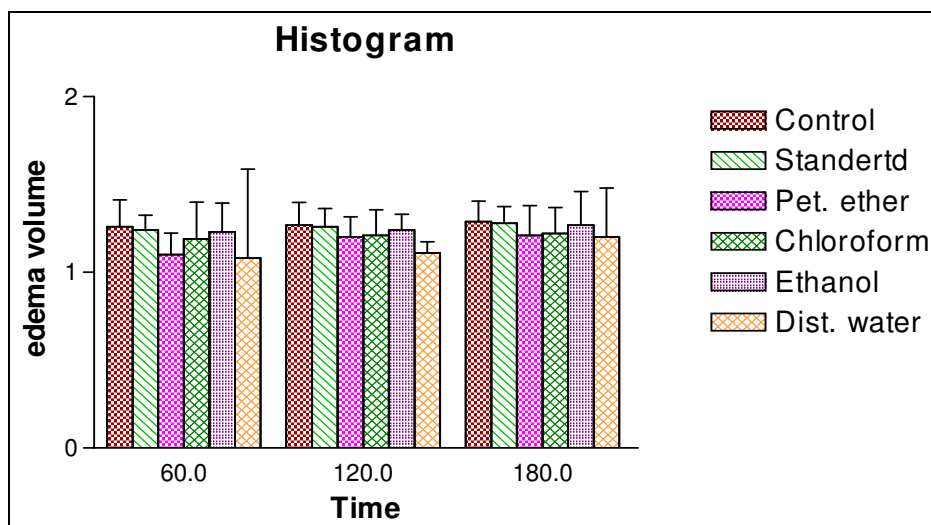
Presence of amino acid and sterols in Ethanolic extracts was identified by TLC. The extract shows two spot on TLC with  $R_f$  value 0.86 (pink) and 0.96 (violet) in solvent system water: ethanol: acetic

acid (1:5:0.5) solvent system for amino acid and  $R_f$  value 0.96 (pink) and 0.54 (Blue) in solvent system Chloroform: methanol (85:15) solvent system for sterol.

The anti-inflammatory activity of various extracts of *Moringa oleifera* lam. Seeds was assessed by carrageenan induced rat paw edema. Table No. 3 on the carrageenan- induced rat paw edema indicated that, ethanol extract shown good significant ( $p < 0.001$ ) reduction in paw edema to the extent of 52.7% at 300 mg/kg concentration, respectively from 3<sup>rd</sup> hour when compared to control group However, petroleum ether (60-80°C) extract, chloroform extract and distilled Water extract has reduced the paw edema to the extent of 23%, 24% and 22% respectively at 300 mg/kg concentration when compared with control group. Where as Ibuprofen also significantly ( $P < 0.001$ ) reduced paw edema form 3<sup>rd</sup> hours when compared to control group.

**Table No. 3:** Showing the effect of *Moringa oleifera* lam. seeds extracts on carrageenan induced rat paw edema method

Group	Test Material (dose)	Mean increase in paw volume and % inhibition		
		1 hr.	2 hr.	3 hr.
1.	Control	4.23 $\pm$ 0.06	4.44 $\pm$ 0.15	4.50 $\pm$ 0.09
2.	Standard (Ibuprofen I) (100 mg/kg)	3.89 $\pm$ 0.05 (9%)	3.05 $\pm$ 0.09 (32%)	1.94 $\pm$ 0.18 (56%)
3.	Petroleum ether extract (300 mg/kg)	4.16 $\pm$ 0.02 (2%)	3.90 $\pm$ 0.08 (13%)	3.50 $\pm$ 0.08 (23%)
4.	Chloroform extract (300 mg/kg)	4.13 $\pm$ 0.02 (3%)	3.88 $\pm$ 0.09 (13%)	3.45 $\pm$ 0.16 (24%)
5.	Ethanol extract (300 mg/kg)	4.07 $\pm$ 0.08 (4%)	3.33 $\pm$ 0.12 (25%)	2.13 $\pm$ 0.12 (52.7%)
6.	Distilled Water extract (300 mg/kg)	4.17 $\pm$ 0.01 (2%)	3.95 $\pm$ 0.07 (12%)	3.55 $\pm$ 0.07 (22%)



**Figure No. 1:** Anti-inflammatory activity of *Moringa oleifera* lam. Seeds extracts by using carrageenan induced rat paw edema method.

It appears from the study that ethanol extract of treated group showed good significant anti-inflammatory activity as compared to standard group, whereas petroleum ether extract, chloroform extract and distilled water extract group showed anti-inflammatory activity.

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