

# Phytochemical and Invitro Anti-Inflammatory Activity Studies of Naregamia Alata Aerial Parts

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**Abstract:-**The article focused on the phytochemical and invitro anti-inflammatory activity studies of the methanolic extract of Naregamia alata. The plant aerial parts were extracted successively with Chloroform, methanol, ethanol and water in a Soxhlet extractor and the phytochemical screening of the extracts were carried out using this fraction. The ethanolic extract showed maximum active constituents like alkaloids, phenolics and glycosides. Invitro anti-inflammatory activity studies of the methanolic extract were carried out using Albumin Denaturation method, Heat Induced Hemolysis, HRBC membrane stabilization method and Ant proteinase assay. The standards used were Aspirin, Indomethacin and Diclofenac sodium. The results of various invitro anti-inflammatory studies showed that the methanolic extract of the plant aerial parts were effective at the concentration range of 100-500 µg/ml and was significant.

**Keywords:-**Anti Inflammatory, Methanolic, HRBC Membrane Stabilization, Albumin Denaturation.

## I. INTRODUCTION

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the most of the world's population [1]. WHO has emphasized the need to ensure the quality of medicinal plant products using modern techniques and suitable standards [2]. Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. [3] There is a major research emphasis on discovering plants with antioxidant and anti-inflammatory potential that may treat various kinds of injuries or protect against diseases. Cellular and tissue damage caused by oxidative stress is defined by the elevated levels of free radicals or other reactive oxygen species (ROS) that can elicit

direct or indirect damage to the body. It appears that the various roles of enzymatic antioxidants help to protect organisms from excessive generation of oxidative stress during inflammation process, which leads to studies focusing on the role for natural products in suppressing the production of oxidation by increasing enzymatic antioxidants in tissues [1, 2].

Inflammation is a protective response that is initiated either after injury, physical and chemical damage, or infection by microorganism, but persistent inflammation may cause chronic diseases. Inflammation is a complex process associated with pain and involves an increase of vascular permeability, protein denaturation and membrane alteration. Inflammation is a complex biological response of vascular tissues to harmful stimuli. It can be classified as either acute or chronic. Inflammation is caused by the body release of hormone-like substances called prostaglandins and leukotrienes. The mechanism of inflammation involves a series of reactions which involves metabolism of arachidonic acid or cyclooxygenase pathway or by 5-lipoxygenase. Protein denaturation is the process in which there will be loss of secondary and tertiary structure of protein by any external stress or by inorganic and organic compounds or heat. Inflammatory condition can be easily identified using denaturation studies. [4,5]

Indian medicinal plant Naregamia alata Wight belonging to family Meliaceae is a shrub which is mainly found in the rocky and grass slopes. In traditional medicine they can be used for the treatment of Chronic fever, Rheumatism, itch, wounds, emesis, halitosis, anaemia, ulcers, vitiated conditions of varta and pitta, pruritis, dysentery etc [6]. The root is a cholagogue, emetic and expectorant and contain an alkaloid called naregamin [7] The plant has antirheumatic activity and the leaf and stem are antibilious. The plant is used in prescriptions for malarial and chronic fevers, anaemia and enlarged spleen. The root and stem gave heneicosane, beta-sitosterol, stearic and palmitic acids. [8]

The macroscopical studies revealed that the leaves were alternate, three foliate with unrigged petioles, The leaflets were small, cuneate, obovate. The flowers were either solitary and axillary or two together in the axil. The flowers were pentamerous with 5 lobed sepals and 5 free petals which

are white in colour. Sameus were 10, fused in a column with free authens which are appendaged. Ovant was superior, three celled with 2 ovules in each cell. Fruits was a loculicidal capsule. Seeds were curved and truncate at both ends. <sup>[9]</sup>

## II. PLANT PROFILE

Scientific Name: *Naregamia alata*

Family: Meliaceae

Common Name:

Eng: *Goanese ipecac*

Mal: Nilanaragam

San: Amlavally

Propagation: Seeds, Stem Cutting

Odour: aromatic

Taste: acrid, pungent



Fig 1: *Naregamia Alata*

## III. MATERIALS AND METHODS

### A. Collection of Plant Material

The whole plant of *Naregamia alata* were collected from the village areas of Kooroppada, Kottayam and was authenticated by the botany department of NSS college. The plant was shade dried and ground into uniform powder and stored.

### B. Extraction of Plant Material

The powdered plant material of *Naregamia alata* (20g) were successively extracted with 250 ml of pet ether, Chloroform, methanol, ethanol and water in the increasing order of polarity using Soxhlet apparatus. The extracts were concentrated and the percentage yield of each extracts were noted and found to be 1.53g, 5.13 g, 3.15 g and 2.59 g respectively for the chloroform, methanol, ethanol and water extracts.

### C. Phytochemical Screening

The extracts were screened for the determination of active constituents present

#### a). Alkaloids

The extracts were treated with few drops of dil HCl and filtered. The filtrate was tested with the following reagents:

#### b). Mayer's Test

A few drops of the solution was added to Mayer's reagent. Formation of gelatinous white precipitate at the junction of two liquids shows the presence of alkaloids.

#### c). Dragendorff's Reagent

The extract was treated with few drops of Dragendorff's. Orange red precipitate shows the presence of alkaloids.

#### d). Hager's Test

To the extract add few drops of Hager's reagent (saturated solution of picric acid) gives a yellow precipitate.

#### e). Wagner's Test

To the extract add few drops of Wagner's reagent (iodine in potassium iodide) gives a reddish brown precipitate.

#### f). Glycosides

The extracts were hydrolysed with HCl for few hrs in a water bath and the hydrolysate was subjected to various tests.

#### g). Legal's Test

To the hydrolysate, 1 ml of pyridine and few ml of sodium nitroprusside solution were added and then it was made alkaline with NaOH solution. Appearance of pink/red colour.

#### h). Borntrager's Test

The hydrolysate was treated with chloroform and the layer was separated. To this equal quantity of dil. ammonia solution was added. Ammoniacal layer shows rose-pink colour.

#### i). Flavanoids

Extract was treated with few drops of aq. NaOH solution. A blue to violet colour was observed, yellow colour (flavones), yellow to orange (flavanones)

Extract was treated with conc. Sulphuric acid, yellowish to orange

#### j). Shinoda Test

The extract was dissolved in alcohol, to that pieces of Mg were added followed by Conc: HCl drop wise and heated. Presence of magenta colour shows the presence of flavanoids.

#### k). Tannins

Small quantities of various extracts were taken separately in water and tested for the presence of tannins.

- Extract was treated with dilute FeCl<sub>3</sub> solution. A blue-dark green or violet colour was obtained.
- Extract was treated with 10 % lead acetate solution. A white precipitate was obtained.

*l). Terpenoids*

The extract was treated with 1 ml of dihydrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange colour indicates the presence of terpenoids.

*m). Saponins*

The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 min. The formation of 1 cm foam shows the presence of saponins [10, 11].

**IV. PREPARATION OF EXTRACT**

The powdered plant material was packed in a soxhlet extractor and extracted successively with Chloroform, methanol, ethanol and water based on the increasing order of polarity. The extracts were then concentrated and refrigerated for the further studies.

**V. INVITRO ANTI-INFLAMMATORY ACTIVITY**

*A. Inhibition of Albumin Denaturation*

The reaction mixture for albumin denaturation study consist of 0.05ml of test extracts of *Naregamia alata* of concentrations ranging from 100-500 µg/ml and 1% aqueous solution of bovine albumin fraction (0.5 ml). The pH of the reaction mixture was adjusted with small amount of 1 N HCl. The sample extracts were incubated at 37<sup>0</sup> C for 20 minutes and at 57<sup>0</sup> C for another 5 minutes and then cooled . To the cooled mixture add 2.5 ml of phosphate buffer and measured the absorbance at 660 nm. The blank was prepared by replacing distilled water in place of test extracts without any bovine serum albumin .Diclofenac sodium at the concentration of 100 µg/ml served as the control. The experiment were performed in triplicate [12, 13 ]

Percentage inhibition of albumin denaturation = (Abs Control –Abs Sample) X 100/ Abs control

*B. Antiproteinase Assay*

The reaction mixture consist of 2 ml of 0.06 mg trypsin, 1 ml of 20 Mm Tris Hcl Buffer of P<sup>H</sup> 7. 4 and 1 ml of test solution of concentration ranging from 100- 500 µg/ml. The mixture was incubated at 37<sup>0</sup>C for 5-10 minutes. Then add 1 ml of 0.8% w/v casein . The mixture was incubated at 37<sup>0</sup> C for again 20 minutes. Add 2 ml of 70% perchloric acid and the obtained suspension is centrifuged and the absorbance was noted at 210 nm against buffer as the blank solution. [14, 15]

*C. HRBC Membrane Stabilization Method*

Blood was collected from the healthy volunteers who had not taken ant NSAID for 2 weeks prior to the experiment and mixed with equal volume of sterilized alsever solution. The blood was centrifuged at 3000 rpm and the separated packed cells were washed with isosaline and a 10 % v/v suspension was made with isosaline. Various concentrations of the extracts ranging from 100-500 µg/ml were prepared using distilled water and to each concentration add 1ml of phosphate buffer,

2 ml of hyposaline and 0.5 ml HRBC suspension. The assay mixtures were incubated at 37<sup>0</sup> C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The supernatant solution was decanted and the haemoglobin content was estimated spectrophotometrically at 560 nm. Indomethacin at a concentration of 100 µg/ml was used as the reference standard and the control was prepared by omitting the samples.

% HRBC Membrane stabilization= 100-[Absorbance of sample-Absorbance of control] × 100.

*D. Heat Induced Haemolysis*

The reaction mixture consist of 1ml test extract of concentrations ranging from 100-500µg/ml and 1 ml of 10 % RBC suspension. The control was prepared in the same concentration of test extract replacing the extract with saline. Aspirin was used as the standard drug. The reaction mixture was incubated at 56<sup>0</sup> C for 30 minutes. The mixture was then cooled and centrifuged at 2500 rpm for 5 minutes and the absorbance was noted at 560 nm. The experiment was performed in triplicate for all the test samples.

% Inhibition of Haemolysis = [Absorbance of Control- Absorbance of Sample] ×100/ Absorbance of control

**VI. RESULT AND DISCUSSION**

The plant extracts obtained after successive soxhletation were done the phytochemical screening for the active constituents present.

Phyto constituents	Chloroform	Methanol	Ethanol	Water
Alkaloids	-	++	+	+
Flavanoids	+	-	-	-
Glycosides	-	++	+	+
Phenolics	+	++	+	-
Terpenoids	-	+	-	-
Saponins	-	-	-	--

++ ....High + .... Moderate - ....Least

Table 1: Preliminary Photochemical Screening

The phytochemical analysis were carried out in chloroform, methanol, ethanol and aqueous extracts. The methanolic extract showed maximum active constituents and was selected for carrying out the invitro anti inflammatory activity studies.

### A. *In vitro* Anti inflammatory Activity

The anti inflammatory activity were carried out by four invitro methods (Inhibition of albumin denaturation, Antiproteinase assay, HRBC Membrane stabilization and Heat Induced Haemolysi) which are assessable. The methanolic extract of the plant showed good inhibition for various concentrations of the extracts.

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	% Inhibition
Control	-	0.36±0.03	-
Naregamia ethanolic extract	100	0.24±0.01	33
Naregamia ethanolic extract	200	0.18±0.02	50
Naregamia ethanolic extract	300	0.14±0.04	61
Naregamia ethanolic extract	400	0.11±0.01	69
Naregamia ethanolic extract	500	0.08±0.02	78
Diclofenac Sodium	100	0.16± 0.01	56

Table 2: Inhibition of Albumin Denaturation

Each value represent mean± SD. Experimental groups were compared with control with p value  $p \leq 0.01$  were considered extremely significant.

The albumin denaturation study was carried out using concentrations ranging from 100-500 µg/ml . The control was taken without the extracts and Diclofenac Sodium was taken as the standard. The maximum inhibition was found to be 78 % at 500 µg/ml.

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	% Inhibition
Control	-	0.30±0.03	-
Naregamia ethanolic extract	100	0.24±0.08	21
Naregamia ethanolic extract	200	0.21±0.07	30
Naregamia ethanolic extract	300	0.19±0.05	36
Naregamia ethanolic extract	400	0.17±0.01	43
Naregamia ethanolic extract	500	0.15±0.03	51
Aspirin	100	0.08± 0.05	71

Table 3: Antiproteinase Assay

Each value represent mean± SD. Experimental groups were compared with control with p value  $p \leq 0.05$  were considered extremely significant.

The antiproteinase assay was carried out by taking the methanolic extracts of the plant at concentration ranging from 100-500 µg/ml. The control was taken without the extract . Aspirin was used as the standard at the concentration of 100 µg/ml . In this method the maximum inhibition was found to be 71 % .

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	% Inhibition
Control	-	0.38±0.08	-
Naregamia ethanolic extract	100	0.30±0.05	21
Naregamia ethanolic extract	200	0.28±0.02	27
Naregamia ethanolic extract	300	0.24±0.07	36
Naregamia ethanolic extract	400	0.21±0.01	42
Naregamia ethanolic extract	500	0.18±0.03	53
Indomethacin	100	0.15± 0.03	55

Table 4: HRBC Membrane Stabilization Method

Each value represent mean± SD. Experimental groups were compared with control with p value  $p \leq 0.01$  were considered extremely significant.

The inhibition of HRBC Membrane Stabilization was taken as a measure of anti-inflammatory activity. In this method the standard used was Indomethacin at a concentration of 100 µg/ml. The different concentrations of the extract ranging from 100- 500 µg/ml were used as the test. The maximum % inhibition was found to be 55 %

Treatments	Concentration (µg/ml)	Absorbance (660 nm)	% Inhibition
Control	-	0.31±0.02	-
Naregamia ethanolic extract	100	0.22±0.01	30
Naregamia ethanolic extract	200	0.16±0.09	48
Naregamia ethanolic extract	300	0.14±0.03	54
Naregamia ethanolic extract	400	0.12±0.07	61
Naregamia ethanolic extract	500	0.07±0.02	75
Aspirin	100	0.15± 0.01	51

Table 5: Heat Induced Haemolysis

Each value represent mean± SD. Experimental groups were compared with control with p value  $p \leq 0.01$  were considered extremely significant.

The heat induced haemolysis method utilized aspirin as the standard. The % inhibition was found to be 51%.

### VII. CONCLUSION

The anti-inflammatory activity studies of the methanolic extract of *Naregamia alata* were carried out using four different methods .It was found that the plant extract were effective against inflammation due to the % inhibition properties which were compared using different methods. The highest % inhibition was shown at the concentration range of 500 µg/ml. The highest inhibition was shown by thye extracts in the albumin denaturation and antiproteinase assay methods and found to be significant.

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