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PHYTOCHEMICAL AND ANTI INFLAMMATORY EVALUATION OF *ALANGIUM SALVIIFOLIUM* ROOT EXTRACT

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ABSTRACT

The main purpose of present study was to perform phytochemical screening and explore anti-inflammatory properties of *Alangium salviifolium* root extract. The *Alangium salvifolium* root was studied for phytochemical characteristics and thin layer chromatography (TLC) was studied. The percentage inhibition of carrageenan induced paw oedema was studied in rats. *Alangium salvifolium* root gave maximum extractive values of 6.4 % w/w with Ethanol and the Loss on Drying value, total Ash value, acid-insoluble ash, water soluble Ash values were within limits. The ethanol extract gave total solid content of 91.96 % w/w. The extract gave positive tests for Phytosterols, Triterpenes, Flavonoids, Carbohydrates and Alkaloids. The extract was free from Glycosides, Saponins, Tannins, Proteins and Amino Acids. The TLC of *Alangium salviifolium* ethanolic extract was clearly developed with 5% H_2SO_4 in methanol as spraying agent and Chloroform: Methanol (8:2) solvent system. The *Alangium salvifolium* root gave significant per cent inhibition of the maximal paw Oedema in 6h and very highly significant per cent inhibition of total paw Oedema during 6h. This study revealed that *Alangium salvifolium* root has good anti-inflammatory actions when compared with Diclofenac sodium.

KEYWORDS: *Alangium salviifolium,* ethanolic extract, paw Oedema, anti-Inflammation

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INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants[1]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. Anti-inflammatory agents are used in the treatment of inflammation of various types. Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat different arthritis, other inflammatory diseases and in management of different postoperative conditions. The anti-inflammatory effect of NSAIDs is due to their ability to inhibit activities of cycylooxygenases (COX), enzymes which mediate the production of prostaglandins. There are two isoforms of COX viz; COX-1 and COX-2. It is thought that anti-inflammatory actions of NSAIDs are caused by the inhibition of COX-2, whereas the unwanted side effects, such as gastrointestinal and renal toxicity, are caused by the inhibition of COX-1[2].

The family *Alangiaceae* consists of twenty-two species out of which *Alangium salviifolium* (*Linn.f*) Wang is mainly used as medicine in India, China and Phillipines[3]. Different parts of this plant are reported to possess acrid, astringent, emollient, anthelmintic, diuretic and purgative properties. It is also used externally in acute case of rheumatism and leprosy. The leaf juice can be applied externally and taken internally in case of rabid dog bite. Root bark is an antidote for several poisons. Fruits are sweet, cooling and purgative and used as a poultice for treating burning sensation and haemorrhage[4]. The leaves

are used as a poultice in rheumatism. However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. The taxonomical classification of *Alangium salviifolium Linn* was shown in Table 1. *Alangium salviifolium Linn* plant and root were shown in Figures 1 and 2.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The entire plants and roots of *Alangium salviifolium Linn* were collected from Visakhapatnam, Andhra Pradesh, South India. Prof. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam, identified the plants.

Materials

Carrageenan, Sodium CMC and Diclofenac sodium were gift samples from Waksman and Selman Pharmaceuticals, Anantapur, India. Methanol, 95% Ethanol were procured from SD fine chemicals Mumbai, India. All the chemicals used were of AR grade and deionized water was used throughout the experiment.

Preparation and Extraction of Plant Sample

The fresh root of *Alangium salviifolium Linn* was chopped into pieces and sun dried for a period of two weeks, reduced to fine powder with the aid of a mechanical grinder. The milled powder sample was collected and stored in glass jars, tightly covered and kept for further studies in refrigerator (-4^{0}C). Extraction of the plant material (800g) with ethanol (3 l) by maceration for 48 h and filtration of the extract was carried out at room temperature 25^{0}C. The reddish brown extract was concentrated to dryness using a rotary evaporator at 30^{0}C at reduced pressure. The dried extract was stored in a refrigerator at -4^{0}C until use[5].

Processing and Storage

Fresh plant materials were used for the pharmacognostic evaluation. The collected plant materials were dried in shade for about 15 days and powdered coarsely in the mill. The powder obtained was passed through # mesh 40 and then used for physicochemical evaluation. The powders were extracted with Ethanol (95%) and the ethanolic extracts were used for phytochemical evaluation (**Table 2**).

Pharmacognostic Evaluation

A systematic pharmacognostic study was carried out on the herbal drugs selected, to describe them more scientifically and to identify specific characteristics, if any, which will be helpful in the quality assurance and standardization of these plant drugs[6-11].

Phytochemical Screening

Phytochemical tests were carried out on the powdered sample using standard experimental procedures.

Selection and Maintenance of Animals

Wistar albino rats of either sex weighing 200-250 g were employed for the study. There were procured from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The rats were maintained under standard laboratory conditions at 25 ± 2^{0}C, relative humidity 50 ± 15% and normal photo period (12 h dark / 12 h light) were used for the experiment. Commercial pellet diet (Ratan Brothers, India) supplied by and water were provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no.516/01/A/CPCSEA).

Preparation of Sodium CMC Suspension

Stock suspension of Sodium CMC was prepared by triturating 1g sodium CMC in 100 ml of distilled water and used for suspending the test and standard drugs.

Preparation of Carrageenan Suspension

Suspension of carrageenan sodium salt, 1% was prepared by sprinkling 100 mg of carrageenan powder on 10 ml of saline (0.9% NaCl) solution and set aside to soak for 1 h. A homogenous suspension was then obtained by thorough mixing with a magnetic stirrer.

Preparation of Drug Suspension

Drug concentrations were prepared to maintain uniform dose volume, which was always given in a total equivalent to 0.1 ml per 100gm rat. For instance, for a dose of 5mg/kg a 5 mg/ml suspension was prepared. Accordingly, a rat weighing 250gm would be given 0.25ml.

Preparation of Ethanolic Extracts

Shade dried powdered material were extracted in soxhlet extraction apparatus. The extracts were concentrated in a rotary flash evaporator (Roteva Equitron, Medica Instrument manufacturing company, Mumbai) under vacuum at a temperature not more than 50^{0}C and dried in a desiccator over anhydrous calcium chloride.

Preparation of Plates

100 g of Silica gel-G was weighed and made into a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10 x 2, 10 x 5, 20 x 5, 20 x 10 cm etc.). The coated plates were allowed to dry in air, followed by heating at 100 – 105^o C for 1 h, cooled and stored in a dry atmosphere to protect from moisture. Before using, the plates were activated by heating at 100^o C for 10 minutes.

Acute Toxicity Studies

The acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD), draft guidelines 423 received

from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Acute toxicity studies on ethanolic extract of the root under the study are carried out at dose range of 50mg to 3000mg/kg body weight orally and the number of animals that died in a 7 day period after a single dose was recorded. The animals were also closely examined for signs of intoxication, lethargy, behavioural modification and morbidity.

Carrageenan Induced Oedema Model

Acute hind paw oedema was induced either in mice or in rats by injecting 0.05 ml to 0.1 of 1 % w/v carrageenan which reaches a peak level at 3-5 h of carrageenan injection. Although oedema can be induced by many other phlogestic agents like dextrin, formaldehyde, 5-hydroxytryptamine, histamine bradykinin and prostaglandin E1 etc., for routine screening, acute carrageenan induced oedema test was employed.

Anti-inflammatory activities of the formulations were tested in either albino rats (weighing 160-180 g). Animals in group (A) received 1% Sodium CMC. Other groups of animals received the test formulations, one each as shown above. The rats in this groups received single daily dose corresponding formulation (0.1ml/kg). (This dose corresponds to an adult human dose).

Statistical Analysis

The observations are represented as Mean ± S.E.M. Statistical analysis was carried out using student's "t" - test. The results were judged Significant if $P< 0.05$.

Induction of Paw Oedema

The rats were pre dosed orally with extracts at different dose levels 18 h and 2 h (unless otherwise mentioned) prior to the induction of carrageenan subcutaneously (SC) into the sub plantar tissue of the hind paw of each rat, 0.1 ml of 1% carrageenan suspension[12].

The drug effects were estimated by comparing the maximal oedema response during 6 h in the drug as extract treated group with that of vehicle treated group as control. Group I normal rats treated with vehicle (1% Sodium CMC) and served as normal control and Group III-V rats were treated with methanolic extract of *Alangium salviifolium* at a doses of 100, 250 and 500mg/kg body weight respectively, Group II rats were treated with Diclofenac sodium 5 mg/kg body weight. All the doses were administered orally according to their body weight.

RESULTS AND DISCUSSION

The coarse powder of *Alangium salvifolium* (root) powder was brown in colour, which has characteristic odour and has bitter in taste. The extractive values of *Alangium salvifolium* root was 4.5% w/w with Petroleum-ether, 3.2 % w/w with Chloroform, 6.4 % w/w with Ethanol and 4.6 % w/w with aqueous solvents. The Loss On Drying value was 12.8% w/w, the total Ash value was 2.84% w/w, the acid-insoluble ash and water soluble Ash were 1.24 and 0.8 % w/w respectively. The extracts did not show any Fluorescence. The ethanol extract gave Total Solid Content of 91.96 % w/w. The pre cent yield with ethanolic extract gave 4.72 % w/w, which was semi solid, light green in colour with a bitter taste. It gave positive tests for Phytosterols, Triterpenes, Flavonoids, Carbohydrates and Alkaloids. The extract was free from Glycosides, Saponins, Tannins, Proteins and Amino Acids. The phytochemical characteristics of *Alangium salvifolium* root were shown in **Table 2**.

Diclofenac Sodium (Standard) showed the percentage inhibition of the maximal paw oedema during 6h was 52.88 ± 4.55%, whereas the percentage inhibition of total (AUC) paw oedema during 6h was 51.83 ± 0.53. The percentage inhibition of the maximal paw oedema during 6h with *Alangium Salviifolium*-100, 250 and 500 mg was 27.01 ± 6.65, 36.24 ± 7.06 and 66.79 ± 3.93 respectively. On the other hand the percentage inhibition of total (AUC) paw oedema during 6h was 25.18 ± 3.46, 37.46 ± 0.56 and 60.76 ± 0.39 respectively. These values were highly significant ($P*** < 0.001$) and shown in **Table 3**. .

The TLC of *Alangium salviifolium* ethanolic extract with 5% H_2SO_4 in methanol as spraying agent and Petroleum ether: Chloroform (1:1) as solvent system and 5% H_2SO_4 in methanol as spraying agent and Chloroform: Methanol (8:2) as solvent system was shown in **Figure 3 A and 3B**.

Carrageenan has produced significant oedema in the left hind paw of the vehicle treated group and the paw oedema was significantly reduced ($P<0.001$) in the standard drug, Diclofenac sodium (5mg/kg) treated group.

Ethanolic extract of *Alangium salviifolium* at the dose of 500mg/kg exhibited significant reduction ($P<0.05$) in paw thickness when compared to control group treated with standard drug Diclofenac sodium (5mg/kg) at third and fourth hour. The other two doses of *Alangium salviifolium* at 100, 250mg/kg also produced significant reduction which is comparable with that of standard drug Diclofenac sodium (5mg/kg). The effect produced by the extracts is dose dependent. The results were given in **Table 3** and **Figures 4 and 5**.

The Percentage inhibition of the maximal paw oedema volume with all the three different doses of *Alangium salviifolium* were 27.01 ± 6.65 with 100 mg/kg, 36.24 ± 7.06 with 250 mg/kg and 66.79 ± 3.93 (significant value) with 500 mg/kg against standard drug Diclofenac sodium

5mg/kg which produced 52.88 ± 4.55 after 6 h. All these values were represented in Table 3 and shown in Figure 4.

The Percentage inhibition of the total paw oedema with all the three different doses of *Alangium salviifolium* were 25.18 ± 3.46 with 100 mg/kg, 37.46 ± 0.56 with 250 mg/kg and 60.76 ± 0.39 with 500 mg/kg against standard drug Diclofenac sodium 5mg/kg which produced 51.83 ± 0.53 after 6 h. All these values were very highly significant and tabulated in Table 3 and shown in Figure 5.

CONCLUSION

This study revealed that *Alangium salviifolium* roots gave maximum extractive values and good per cent yield values with Ethanol. The *Alangium salvifolium* root gave significant per cent inhibition of the maximal paw Oedema in 6h and very highly significant per cent inhibition of total paw Oedema during 6h. This study concluded that *Alangium salvifolium* root has good anti-inflammatory actions when compared with Diclofenac sodium.

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Table 1: Taxonomical Classification

Botanical Name	*Alangium salviifolium*
Synonyms	*Alangium lamarckii*
Class	Dicotyledons
Sub Class	Polypetalae
Series	Calyciflorae
Order	Cornales
Family	Alangiaceae

Table 2: Phytochemical characteristics of *Alangium salvifolium* (Root)

Physical Tests of Crude Drugs	***Alangium salvifolium* (Root)**
Nature	Coarse Powder
Colour	Brown
Odour	Characteristic
Taste	Bitter
Extractive values	4.5(Petroleum-ether)
(% w/w)	3.2 (Chloroform)
	6.4 (Ethanol)
	4.6 (Aqueous)
Loss On Drying(% w/w)	12.8
Total Ash(% w/w)	2.84
Acid-insoluble ash (% w/w)	1.24
Water-soluble Ash (% w/w)	0.8
Fluorescence Analysis	No Fluorescence
Total Solid Content (% w/w) *	91.96 (Ethanolic Extracts)
Yield % w/w	4.72
Nature	Semi Solid
Color	Light Green
Odour	Characteristic
Taste	Bitter
Ethanolic extracts	
Phytosterols	+
Triterpenes	+
Glycosides	-
Saponins	-
Flavonoids	+
Tannins	-
Proteins & Amino Acids	-
Carbohydrates	+
Alkaloids	+
+ = Positive; - = Negative	

Table 3: Percentage inhibition of carrageenan induced paw oedema in rats

Treatment	**% inhibition of the maximal paw Oedema during 6h.**	**% inhibition of total (AUC) paw Oedema during 6h.**
Drug Vehicle	0.0 ± 3.046	0.0 ± 2.21
Diclofenac Sodium (Standard)	52.88 ± 4.55	51.83 ± 0.53***
Alangium Salviifolium-100	27.01 ± 6.65	25.18 ± 3.46***
Alangium Salviifolium-250	36.24 ± 7.06	37.46 ± 0.56***
Alangium Salviifolium-500	66.79 ± 3.93*	60.76 ± 0.39***
Values are expressed as Mean ± SEM; P* < 0.05; P* < 0.001)**		

Fig 1: *Alangium salviifolium* Linn plant

Fig 2: *Alangium Salviifolium Linn* root

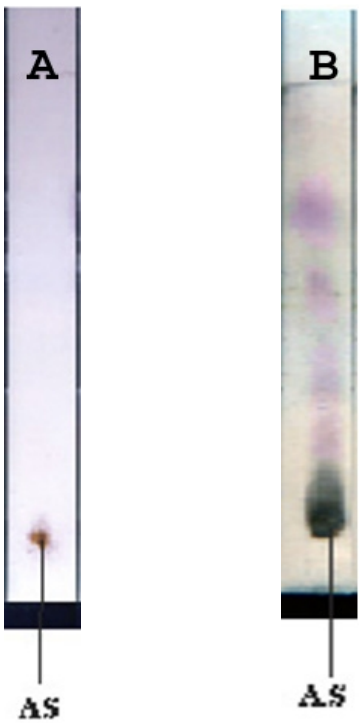

Fig 3: TLC of *Alangium salviifolium* ethanolic extract A. With 5% H_2SO_4 in methanol as spraying agent and Petroleum ether: Chloroform (1:1) as solvent system; B. With 5% H_2SO_4 in methanol as spraying agent and Chloroform: Methanol (8:2) solvent system; AS = *Alangium salviifolium*

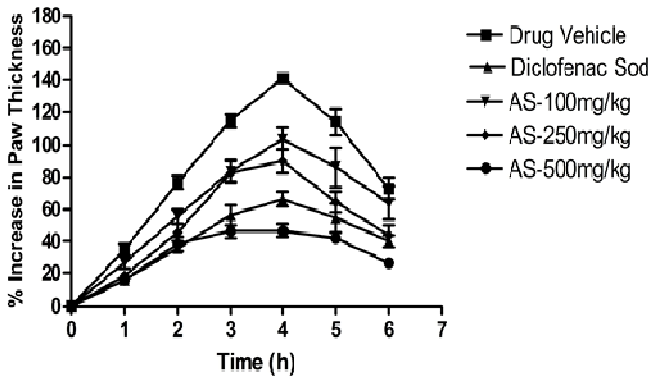

Fig 4: Effects of the ethanolic extract of *Alangium salviifolium* 100, 250 and 500 mg/kg respectively along with standard- maximal paw oedema in Carrageenan induced rats

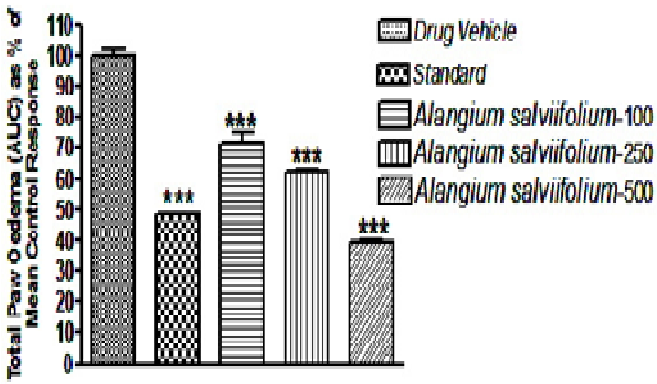

Fig 5: Effects of the ethanolic extract of *Alangium salviifolium* 100, 250 and 500 mg/kg respectively along with standard- total paw oedema in Carrageenan induced rats (P* < 0.001)**