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Phytochemical screening and evaluation of anti-inflammatory activity of methanolic extract of *Abroma augusta* Linn

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ABSTRACT

Objective: To evaluate the Phytochemical and anti-inflammatory property of the different parts of methanolic extracts of *Abroma augusta* Linn. **Materials and methods:** *Abroma augusta* Linn (Family-Malvaceae) commonly known as Ulatkambal in Hindi and Devil's cotton in English, found in tropical Asia, South and eastern Africa, and Australia. It is mainly used for dysmenorrhoea, ammenorrhoea, sterlilty and other menstrual disorder. The present study aimed at evaluation of Phytochemical and anti-inflammatory study of different parts of *Abroma augusta* Linn methanolic extract by the carrageenan induced rat paw oedema method. **Results:** The result showed significant anti-inflammatory property of different parts of *Abroma augusta* Linn methanolic extract. **Conclusions:** The methanolic extract of different parts of *Abroma augusta* Linn showed potent activity comparing with the standard drug diclofenac sodium perhaps due to the alkaloids and flavonoids present in the plant.

1. Introduction

Inflammation plays an important role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally. During an inflammatory response, mediators, such as pro-inflammatory cytokines, including interleukin IL-1, tumour necrosis factor (TNF), interferon (INF)-c, IL-6, IL-12, IL-18 and the granulocyte-macrophage colony-stimulating factor, are released; this response is antagonised by anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, IFN-a and the transforming growth factor. The nuclear factor-jB (NF-jB), transcription factor, also plays an important role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX-2) [1, 2] inducible nitric oxide synthase (iNOS) and COX-2 both stimulate the production of large amounts of pro-inflammatory mediators. In chronic inflammation, the negative regulatory mechanism appears to be dysfunctional. Although inflammation is primarily a protective response (against micro-organisms, toxins or allergens, for example), inflammation that is chronic and uncontrolled becomes detrimental to tissues [3].

Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant-derived formulations [4, 5]. The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated.

Abroma augusta Linn (Family-Malvaceae) commonly known as Ulatkambal in Hindi and Devil's cotton in English. A genus of evergreen plant large, spreading, quick-growing hairy shrub or a small tree with velvety branches, found in tropical Asia, South and eastern Africa, and Australia. It is mainly used for dysmenorrhoea, ammenorrhoea, wound healing, sterlilty and other menstrual disorder. Powdered root act as anabortifacient and anti-fertility agent. Leaves are useful in treating uterine disorders, diabetes, rheumatic pain of joints, and headache with sinusitis. Leaves and stem are demulcent and an infusion of fresh leaves and stem in cold water is very efficacious in gonorrhea. The root-bark is used as an emmenagogue and uterine tonic. [6, 7, 8]

However this plant has not been studied for anti-inflammatory activity. This study was aimed at providing pharmacologic basis for its folkloric use in inflammation. Based on this an attempt has been made to evaluate the inflammatory potency of *Abroma augusta* Linn with their phytoconstituents.

2. Materials and methods

2.1. Collection of plant materials

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The leaves, bark, root of *Abroma augusta* was collected from Siliguri, Raigang, West Bengal, India. A herbarium sheet was prepared & it was sent to A.J.C.B INDIAN BOTANIC GARDEN, Shibpur, Howrah and West Bengal, India for authentication. The authentication no. of the study plant is" CNH/111/2011/Tech.II/627". The leaves, bark, root of *Abroma augusta* was collected and dried under shade. These dried materials were mechanically powdered, sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further Phytochemical and anti-inflammatory study (Figure 1 and 2).

Figure 1: Picture showing the *Abroma augusta* plant.

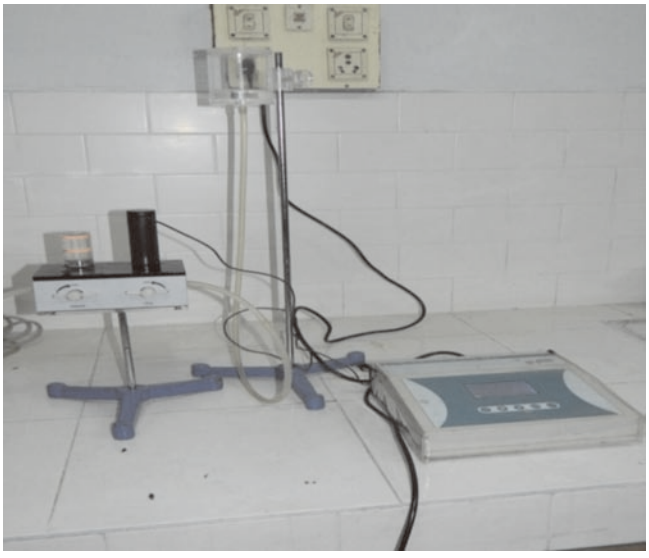

Figure 2: Picture of digital Plethysmometer(ORCHID SCIENTIFICS, PFM-01)

2.2. Preparation of extract

The air dried crushed leaves, bark and roots (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w/) of the residue, which was stored at 20 ℃ until use. The concentrate was suspended in 5% w/v Tween 80 and given at dose 1ml/100gm body weight.

2.3. Treatment of animals

Healthy male and female rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioural veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within±20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA).

Sixty young adult male Wistar rats, weighting 120-150 g were obtained from the Institutional Animal House of Gupta College of Technological Sciences. The rats were housed in polyethylene cages in the Animal House. The rats were housed in polyethylene cages, allowed one week of acclimatization, and maintained on standard rat chow and standard laboratory conditions throughout the experiment.

2.4. Phytochemical Screening

The concentrated extracts were used for preliminary screening of various phytoconstituents viz. carbohydrate, amino acid, alkaloids, tannins and flavonoids were detected by usual methods prescribed in standard tests. [9]

2.5. Acute toxicity test

Acute toxicity study was performed as per OECD guidelines 423. [10] (Acute toxicity class method).

2.6. In-vivo anti-inflammatory activity

Male or female Sprague-Dawley rats with a body weight between 120 -180 g were used. The animals were starved overnight. The animals were fasted for 18 hours prior to the experiment. Animals were divided into five groups of six animals each and marked. Group I received 1% Tween-80 (1%, i.p.) and served as control. Group II received Diclofenac sodium 5mg/kg b.w. i.p. and served as standard. Group III, group IV,group V received the leaf, bark and root extract respectively at dose of 250mg/kg b.w. i.p each. One hour after the administration (as per the experimental protocol), 0.1ml of 1% carrageenan solution was injected beneath the sub-plantar surface of the right hind paw of all animals. For the assessment of the anti-inflammatory activity, the volume of the paw was measured with the help of digital plethysmometer at 0h and at 1h interval for a period of three hours after the carrageenan treatment. The results are tabulated by % of inhibition. [11]

2.7. Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison test. Comparison between control and drug treated groups were considered to be significant $P<0.01$, $P<0.001$. All values are expressed as mean±SEM.

3. Results

3.1. Acute toxicity studies

The extracts of *Abroma augusta* did not show any sign

of toxicity up to 2000 mg/kg body weight and hence it was considered to be safe.

3.2. Phytochemical studies

From the Phytochemical study, it has evaluated the presence of alkaloid, carbohydrate, flavonoid and tannin in leaves and bark. But roots contain lower alkaloids, carbohydrate and tannins. (Table 1)

3.3. In-vivo anti-inflammatory study

Methanolic Extracts were evaluated for Anti-inflammatory activity. In Carrageenan induced paw oedema the intraperitoneally administration of leaves, bark, root produced a significant anti-inflammatory activity in a dose-dependent manner respectively in the rats. Bark extract has shown significant anti-inflammatory effect than root and leaves. (Table 2)(Figure 3 and 4)

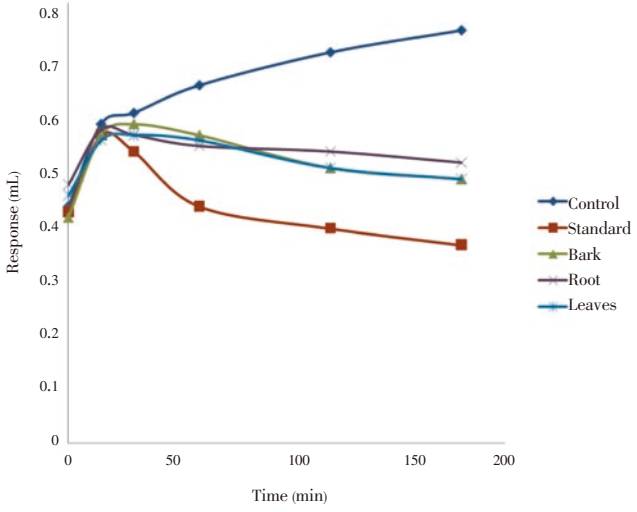

Figure 3: This graphical representation shows the various response on rat in carrageenan induced paw oedema in rat in 15min, 30 min, 60min, 120min, 180min after drug administration. Graph is plotted between. Time (min) in X axis and Response (ml) in Y axis. Data are shows onset of response time significantly increase by extract of Root than Bark and Leaves from Control. $P<0.01$,$P<0.001$.

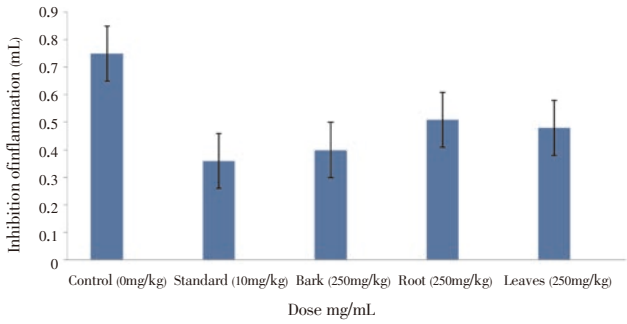

Figure 4: This histrogram showing the inhibition of carrageennan indused paw oedema in rats. Histrogram is poltted between control, doses of standard drug Diclofenac sodoum (100mg/kg), extracts of bark, root, leaves (250mg/kg) in X axis and inhibition of paw oedema in Y axis. Data are shows inhibition of paw oedema significantly decrease by extract of Bark than Root and Leaves from Control. Data are Mean S.E.M. indicates significant decrease in inflammation form control and indicates highly significant decrease, $P<0.01$, $P<0.001$.

4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. *Abroma augusta* of the family Malvaceae is a common plant of Asia, South and eastern Africa, and Australia. Phytochemical evaluation of the various extracts of *Abroma augusta* reveals the presence of flavonoids, carbohydrate, tannins and alkaloids. Here anti-inflammatory activity was performed based on the folk lore information using rat paw oedema method.

Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the like [12-13]. There is good evidence that the early or first phase of transient permeability is due to the release of histamine and can thus be suppressed by antihistamines. The mediation of the delayed or second phase of exudation is more controversial and complex,

Table 1

Preliminary Phytochemical screening of the various extracts of the leaves of *Abroma augusta*

Chemical Constituent	Solvent			
	Test	Methanolic root extract	Methanolic bark extract	Methanolic leaves extract
Alkaloid	1.Dragendroff's Reagent	–	–	–
	2.Mayer's Reagent	+	+	+
	3.Wagner's Reagent	+	+	+
	4.Hager Reagent	–	–	–
Amino acid	Millon's Test	–	–	–
Carbohydrate	1.Molish Test	+	+	+
	2.Barfoed's Test	+	+	+
Flavonoid	Sample + Lead acetate	+	+	+
Tanin	Ferric Chloride Test	+	+	+

Table 2

Effect of Methanolic extract of *Abroma augusta* on carrageenan induced inflammation

GROUP	DOSE mg/kg	Paw volume after Carrageenan injection									
		15min		30min		60min		120min		180min	
		EV(ml)	EI%	EV(ml)	EI%	EV(ml)	EI%	EV(ml)	EI%	EV(ml)	EI%
Control	–	0.58± 0.0011		0.60± 0.0008		0.65± 0.0006		0.71± 0.0001		0.75± 0.0002	
Standard	100	0.56± 0.0006	3.97	0.53± 0.0095	11.11	0.43± 0.0009**	34.5	0.039± 0.0010**	45.11	0.36± 0.0015**	51.33
Bark	250	0.56±0.0005	3.97	0.58± 0.0007	4.58	0.54± 0.0010*	14.20	0.50± 0.0005*	29.30	0.40± 0.0008**	46.90
Root	250	0.57± 0.0007	1.7	0.54±0.0010	7.14	0.55± 0.0002*	16.9	0.53± 0.0009*	26.03	0.51± 0.0009*	31.42
Leaves	250	0.58± 0.0003	5.67	0.55± 0.0002	6.66	0.56± 0.0007*	15.73	0.50± 0.0001*	30.22	0.48± 0.0001*	35.40

The data are expressed as mean±S.E.M. Significant differences in each group versus the control were as follows: * $P < 0.05$. ** $P < 0.01$.

and has been attributed in part to kinins, prostaglandins, neutrophils, and lipoxygenase products of arachidonic acid metabolism. The probable mechanism of anti-inflammatory action of Extract may be due to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than the lipoxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan[14, 15].

Different parts of methanolic extracts of *Abroma augusta* showed significant anti-inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the alkaloids and Flavonoids [16] present in the extract. The present result indicates the efficacy of *Abroma augusta* as an effective therapeutic agent in the treatment of acute inflammations [17]. The result of present study authentifies the folk lore information on the anti-inflammatory property of the leaf extract of *Abroma augusta*. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to idendification of the possible mechanism of its anti inflammatory property.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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