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Original article

To study *in vitro* anti-inflammatory activity of *Anthracephalus cadamba* leaves extract

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Abstract

The anti-inflammatory activities of different extracts of leaves of *Anthracephalus cadamba* were investigated for *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization (HRBC) method. The methanol extract showed significant membrane stabilizing action on human red blood cell membrane. The HRBC Membrane stabilization activity of the ethanol extract of *Anthracephalus cadamba leaves* at concentration 200µg/ml showed 73.25% inhibition of denaturation in hypotonic solution while standard Diclofenac 100µg/ml showed 79.25% inhibition of denaturation. Moreover, the extracts showed equipotent activity to Diclofenac.

Key Words: *Anthracephalus cadamba*, HRBC method, Anti-inflammatory.

Introduction

Herbal medicine is the use of plants and plant extracts to treat disease, something mankind has always done. Herbal medicine exists in many local varieties depending on the regional flora [1,7]. Many modern drugs were originally extracted from plant sources, even if they are now made synthetically, and many other drugs are descended from plant substances[3,8]. The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995) which are aimed at host defense and usually activated in most disease condition. For instance, Diclofenac, the original non-steroidal antiinflammatory drug (NSAID)[2].Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process *Anthocephalus cadamba* is a large tree with a broad umbrella-shaped crown and straight cylindrical bole. The Kadmba tree grows up to 45 m high. It is a large tree with a broad crown and straight cylindrical bole. It is quick growing, large; has large spreading and grows rapidly in first 6-8 year The trunk has a diameter of 100-160 cm, but typically less than that. Leaves are 13-32 cm long. Flowering usually begins when the tree is 4–5 years old. Kadam flowers are red to orange, occurring in dense, globe-like heads of approximately 55 cm. The fruit of A. cadamba occur in small, fleshy capsules packed closely together to form a fleshy yellow-orange infructescence containing

approximately 8000 seeds[8] In this work the various extracts of kadamba leaves were studied for its *in vitro* anti-inflammatory activities.[11,14]

Materials and Methods

Extraction Process of *Anthracephalus cadamba*

The leaves of *Anthracephalus cadamba* were air dried and then extracted by water, ethanol, methanol, hexane and ether by cold maceration process. Then extract filter by using filter paper. The filtrate is placed in china disc and evaporates the filter. Finally collected the crude extract. Calculated its % yield.

Qualitative phytochemical analysis

The preliminary chemical tests were carried out for the extract of *Anthracephalus cadamba* to identify the presence of various phytoconstituents.

In-Vitro Anti-Inflammatory Activity

The human red blood cell (HRBC) **membrane Stabilization method**

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. Diclofenac (100 and 200 g/ml) was used as reference standard and a control was prepared by omitting the extracts[4,12,17].

$$\% \text{ Protection} = \frac{\text{100- Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Result and Discussion

Ash Value

TABLE No. 1: Different Ash values of *Anthracephalus Cadamba*

S.No.	Types of ash value	Observation(%)w/w
1	Total ash	7.8%
2	Acid insoluble ash	7.3%
3	Water soluble ash	0.97%

The ash value of the *Anthracephalus Cadamba* leaves was calculated and the total ash, acid insoluble ash, water soluble ash, and sulfated ash was found out to be 7.8%, 7.3% and 0.97% respectively.

Extractive value

TABLE No. 2: Different Extractive value of *Anthracephalus Cadamba*

S.No.	Types of extract	Weight of drug (gm)	Weight of empty china dish (gm)	Weight of china dish with dry extract (gm)	Extractive value (%) w/v
1	Diethyl ether extract	5	60.09	60.13	0.8
2	n-Hexane extract	5	65.15	65.21	1.2
3	Ethanolic extract	5	68.24	68.42	3.6
4	Water extract	5	64.4	64.8	8

The extractive value of *Anthracephalus Cadamba* leaves the was calculated and the extract of di-ethyl ether, n-hexane, ethanol and water was found out to be 0.8%,1.2%,3.6%,8.

Percentage Yield

The maximum % yield (14.5%) obtained from the water extract. Ether extract give the minimum % yield (5.1%) of crude extract. % yield of different extracts are tabulated in table: 3

TABLE No. 3: % yield of the Different Extract of Kadam.

Solvent	% yield
Water	14.5%
Ethanol	8.7%
Methanol	6.8%
Hexane soluble	5.4%
Petroleum Ether soluble	5.1%

In-vitro Anti-inflammatory activity of Kadam leaves by HRBC MEMBRANE

Stabilization Method

The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. *Anthracephalus Cadamba* has significant anti-inflammatory activity which may be due to presence of chemical profile such as Flavones, Tri-Terpenoids, Flavonones and Phenols.

The HRBC Membrane stabilization method was used for the in-vitro anti- inflammatory activity of the ether, hexane, methanol, ethanol and water extracts of kadam. The HRBC Membrane stabilization activity of the ethanolic extract of kadam at concentration 200µg/ml showed 73.25% inhibition of denaturation in hypotonic solution while standard Diclofenac 100µg/ml showed 79.25% inhibition of denaturation.

TABLE No. 4: % inhibition of different extracts of the *Anthracephalus cadamba*

S.No.	Type of extarct	Concentration(µg/ml)	Absorbance	% Inhibition of denaturation
1	Control	----	0.18±0.23	---
2	Water	100	0.0844±1.20	30.25±1.89
3	Water	200	0.0594±1.20	61.25±1.79
4	Ethanol	100	0.0890±0.31	31.12±1.05
5	Ethanol	200	0.0494±1.20	73.25±1.79

6	Methanol	100	0.0714±1.20	46.25±1.79
7	Methanol	200	0.0597±0.26	69.84±1.24
8	Haxane	100	0.0894±1.20	31.25±1.54
9	Haxane	200	0.0594±1.20	63.25±1.43
10	Petroleum ether	100	0.0941±1.20	29.23±1.26
11	Petroleum ether	200	0.0674±1.20	59.25±1.83
12	Diclofenac	50	0.0544±0.32	69.82±0.98
13	Diclofenac	100	0.0374±1.20	79.25±1.79

Summary and Conclusions

Ethanolic extract of Kadam leaves exhibited membrane stabilization or heat induced hemolytic effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane .Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage from the above study it was concluded that the ethanolic extract of kadam has significant membrane stabilization property[28].

In-vitro anti-inflammatory studies of *Anthracephalus cadamba* demonstrated the suppression of both inflammation and arthritis. One of the causes of rheumatoid arthritis is denaturation of proteins[23] and inhibition denaturation is one of the in vitro tests to screen antiinflammatory drugs [24].

From the preliminary screening study, it showed the presence of Flavonones, Flavones, Tri-Terpenoids and Phenolics.[19] Antiinflammatory activity of flavonoids has been recognised long back in rodents and reviewed exhaustively [25]. Some examples include quercetin [26], silymarin apigenin, daidzein, genistein [27] etc.

Hence proper isolation of the active constituent/s might help in the findings of new lead compounds in the fields of anti-inflammatory drug research. Studies related to active constituent enzyme expression (COX2, lipoxygenase) are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity.

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