



Anti-inflammatory activity of the rhizomes of *Anaphyllum wightii* Schott.

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ABSTRACT

Plants have been used as an alternative remedy for the treatment of various ailments since ancient times. The *in vitro* anti-inflammatory activity of the alcoholic and aqueous extracts of the *Anaphyllum wightii* were studied. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The anti inflammatory activity of the ethanolic extract was comparable to that of the standard drug Diclofenac sodium. The ethanolic extract of the rhizomes of *Anaphyllum wightii* has significant anti inflammatory activity in comparison with the aqueous extract of the same plant.

Keywords: Anti-inflammatory, *invitro*, *Anaphyllum wightii* , Rhizomes, HRBC Membrane stabilisation.

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INTRODUCTION

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The major symptom of acute inflammation includes pain, heat, redness and swelling. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. In general we can say that inflammation is one of the responses of the organism to the pathogen⁸.

MATERIALS AND METHODS

Plant material

The genus *Anaphyllum* from Araceae family comprises of with two species like; *Anaphyllum beddomei* Engl. and *Anaphyllum wightii* Schott. These unexplored plant species from South Western Ghats are striving for their existence with treasures of genes with medicinal importance. *Anaphyllum wightii* are seen in the Western Ghats region throughout Kerala at an altitude of 650 – 1000 meters. *Anaphyllum beddomei* is observed only at an altitude of 1200 – 1500 meters from the Agusthya peaks of the South Western Ghats. Tribal communities and villagers of various parts of Kerala use these plants as an antidote to snake bite⁷.

The Fresh rhizomes of *Anaphyllum wightii* Schott were collected from the Ghatt section of Sakleshpur, Karnataka. The plant material was identified and a voucher specimen was deposited in the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram, Kerala.

Preparation of extracts

The rhizomes were sliced, dried under shade and finally powdered. The powder was transferred to soxhlet extractor and subjected to extraction with ethanol and Double distilled water . After extraction, the solvents were distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

ANTI-INFLAMMATORY ACTIVITY

The HRBC membrane stabilization has been used as a method to study the anti inflammatory activity¹. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution The blood was centrifuged at 4000 rpm and packed cells were washed with iso-saline and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration ,1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacine was used as the reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixtures were incubated

at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula

$$\text{Percentage Protection} = 100 - (\text{OD sample} / \text{OD Control}) \times 100$$

*OD=Optical Density

RESULTS AND DISCUSSIONS

Table 1: Anti-inflammatory activity (%Protection)

Sr No	Concentration mg/ml	Anti-inflammatory activity (%Protection)		
		Ethanollic Extract of <i>Anaphyllum wightii</i>	Aqueous extract of <i>Anaphyllum wightii</i>	Standard Diclofenac Sodium
1	Control	-----	-----	-----
2	100	77.2± 0.02	58.6 ± 0.07	84.13± 0.07
3	200	79.2 ± 0.04	69.2 ± 0.03	91.56 ± 0.08
4	300	83.3 ± 0.09	70.3 ± 0.01	96.11 ± 0.02
5	400	98.1 ± 0.06	73.1 ± 0.07	101.33 ± 0.01
6	500	101.2± 0.04	79.2 ± 0.07	122.10± 0.03

(Values are expressed as SEM of 3 readings)

HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release⁴. The alcoholic as well as aqueous extracts of *Anaphyllum wightii* were subjected to erythrocyte (RBC) membrane stabilization induced haemolysis by hypotonic solution. The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane².

During inflammation process lysosomal enzymes will get released which can even produce a series of diseases. The extra cellular activity of the lysosomal enzyme is because of acute or chronic inflammation. The major mechanism of action of non steroidal drugs are by inhibiting these lysosomal enzymes or by means of stabilizing the lysosomal membrane³. The components of HRBC membrane and lysosomal membrane are similar. The HRBC membrane lysis is induced by the the prevention of hypotonicity and which is taken as a measure of anti-inflammatory activity of the drug

It was observed from the table that the ethanolic extract shows significant anti inflammatory activity at the concentration of 500 micro gram/ml which is comparable to the standard drug , diclofenac sodium. The anti inflammatory activity of the alcoholic and aqueous extracts were concentration dependent, with the increasing concentration the activity was also increased. The alcoholic extract of the rhizomes of *Anaphyllum wightii* has significant anti inflammatory activity in comparison to the aqueous extract of the rhizome of the same plant.

CONCLUSION

Inflammation is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which can cause a decrease in peripheral blood lymphocyte counts. The ethanolic extract of the rhizome exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is an analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. From the above study it was concluded that the ethanolic extract of *Anaphyllum wightii* has significant membrane stabilization property and it was comparable to the standard drug Diclofenac sodium. Further the compound isolation, purification and characterization which is responsible for this particular activity, has to be evaluated for the usage of an anti-inflammatory agent.

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