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Anti-Inflammatory activity of *Anaphyllum wightii* Schott by membrane stabilization method

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Abstract

Anaphyllum wightii Schott (Family-Araceae) commonly known as Keerikizhagu in Malayalam, found in Southern Western Ghats. *Anaphyllum wightii* Schott is listed as an endemic and threatened species of South India. Tribal communities (Kani Tribes, Malasars, Kadars, Pulaiyars, Madhuvars) use mainly the rhizome of this plant as food and as an antidote against snake bite. The present study aimed at preliminary evaluation of phytochemical and anti-inflammatory study of various extracts of tuber of *Anaphyllum wightii* Schott by in vitro HRBC membrane stabilizing activity. Chloroform and aqueous extracts of *Anaphyllum wightii* were found to have significant anti-inflammatory activity. The chloroform and aqueous extract of *Anaphyllum wightii* showed potent anti-inflammatory activity when comparing with the standard drug Indomethacin, perhaps due to the presence of secondary metabolites like alkaloids, steroids, flavonoids, phenols and saponins.

Key-Words: *Anaphyllum wightii*, Araceae, Anti-inflammatory, HRBC membrane stabilization

Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is a defensive mechanism of the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. In absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism1. Many components are involved in the inflammation process to name few are edema formation; leukocyte infiltration and granuloma formation are widely noticeable[2]. 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. The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated. Genus –*Anaphyllum beddomei* Engl and *Anaphyllum wightii* Schott were reported from the high ranges of Western Ghats. The two species in this genus are similar in appearance to those in the genus *Anaphyllopsis*.

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Genus *Anaphyllum wightii* is listed as an endemic and threatened genus of South India (Nayar, 1987)[4]. The species of the genus *Anaphyllum* are found in marshes. They are characterized by pinnate leaves and twisted spathe for the spadix. *Anaphyllum* is a genus of flowering plants in the Araceae family. Leaves of *Anaphyllum beddomei* form a part of tribal diet. Arun et al. (2007) reported the use of the corms of *Anaphyllum wightii* (Keerikizhangu), as an antidote to snake bite along with some medicinal plants[5].

Material and Methods

Plant material

Plant tubers were collected from Wynad hills, Wynad district, Kerala, India. The taxonomical identification of the plant was done by Dr. N. Sasidharan, Scientist-F, Programme co-ordinator, FE& BC division, Kerala Forest Research Institute, Peechi, Trissur. The voucher specimen (NOPOCL/02/2014/APSC) was preserved in Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

Preparation of plant extract

The collected plant tubers were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 200g of powdered materials were extracted with chloroform and water by successive solvent extraction method. The extracts

were then concentrated using vacuum evaporator and dried under reduced pressure.

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[6,7,8].

In vitro anti-inflammatory activity by Human red blood cell (HRBC) membrane stabilizing activity[9,10]

This method evaluates the membrane stabilizing activity of various agents on red blood cells against the osmotic pressure exerted by Alsever solution. Alsever solution is prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05%citric acid and 0.42% of sodium chloride in distilled water followed by sterilization. Blood was collected from Pariyaram Medical College Blood Bank. The collected blood was mixed with equal volumes of Alsevers solution. The blood was centrifuged at 3000 rpm and the packed cells were washed with isosaline and 10%(v/v) suspension was made. The drug samples ranging from a concentration of 50µg/ml-250µg/ml were prepared by suspending the residue in hot water. The assay mixture contained the drug, 1ml phosphate buffer, 2ml hyposaline (0.25%w/v), 0.5ml HRBC suspension. Indomethacin was used as the reference drug and 2ml of distilled water as control. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. Percentage of protection was calculated using the following equation.

Percentage Protection = 1 - (OD Sample / OD Control) x100

Results and Discussion

Phytochemical studies

Preliminary phytochemical analysis of chloroform and aqueous extracts showed the presence of secondary metabolites such as alkaloids, phenols, saponins, steroids, flavonoids. (Table 1)

In vitro anti-inflammatory activity (HRBC membrane stabilization)

The membrane stabilizing activity of chloroform and aqueous extract of *Anaphyllum wightii* possess significant membrane stabilizing activity when compared with the control group. Inhibitory concentration (IC 50%) values ranged from 277.83 to 214.93 with values for Indomethacin (121.68 µg/ml), chloroform extract (214.93 µg/ml) and aqueous extract (277.83µg/ml). (Table 2).

Preliminary phytochemical analysis of *Anaphyllum wightii* indicated the presence of alkaloids, steroids, flavonoids, phenols, saponins[11]. Reported that all secondary metabolites could impart medicinal properties to the plant and has specific healing properties, healthy action and non-toxic effects. It is well reported that phytochemicals are found to have a broad range of activities which may help in protection against chronic diseases[12,13]. The present study on preliminary phytochemical analysis provides a better understanding of this less explored medicinal plant. This analysis gave important information in the identification and authentication of the plant material.

Lysosomes are single membrane structures that contain digestive enzymes. When certain white blood cells engulf bacteria, the bacteria are digested and destroyed by these lysosomal enzymes. Worn out cell parts and dead cells are also digested by these enzymes. This is a beneficial process and is necessary before tissue repair can begin. But it does not have a disadvantage in that lysosomal digestion contributes to inflammation in damaged tissues. An excessive inflammation can start a vicious cycle, actually a positive feedback mechanism that results in extensive tissue damage[14].

Various methods are employed to screen and study drugs, chemicals, herbal preparations that inhibit the inflammation. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation[15]. Human Red Blood Cell stabilization against hypotonicity induced lysis was selected for the assessment of anti-inflammatory activity of *Anaphyllum wightii* due to its simplicity and reproducibility. HRBC membrane is similar to the lysosomal membrane. During inflammation, histamine from damaged tissues makes capillaries more permeable and the lysosomes of damaged cells release their enzymes which help breakdown damaged tissue but may also cause destruction of nearby healthy tissue. Some of the NSAIDS and glucocorticoids stabilize lysosomes in tissue cells and there by prevent release of lysosomal enzymes into the cytoplasm of the cells, thus preventing deterioration from this source[16]. Stabilization of lysosomal membrane therefore can control inflammatory response and therefore stabilization of human red blood cell (HRBC) from hypotonicity induced lysis can be correlated with the anti-inflammatory potential of a drug.

Some of the Indian medicinal plants like *Cassia grandis* Linn[17], *Ficus carica*[18], *Abroma augusta* Linn[19] have been screened for their anti-inflammatory activity by various researchers so far. The assay method involves incubation of RBCs into a hypotonic solution (less than 282 mOsm/L), so that water will diffuse into the cell, causing it to swell; water will continue to diffuse into the cell, resulting in lysis of the cell. In the present study various extracts of *Anaphyllum wightii* possess significant stabilizing of HRBC, the probable mechanism of protection of hypotonicity induced lysis is shrinking of the cell membrane and involves processes that prevent the migration of these intracellular components outside the cell. It has been shown that cell deformability and cell volumes of erythrocytes are closely related to their intracellular content of calcium[20]. Thus the membrane stabilization effect by these agents may be due to alteration of the influx of calcium into the erythrocytes. The precise mechanism for these effects remains to be elucidated.

Table 1: Phytochemical screening of tubers of *Anaphyllum wightii*

Phytochemical constituents	Chloroform extract	Aqueous extract
Carbohydrates	–	–
Amino acids	–	–
Proteins	–	–
Fats	–	–
Steroids	+	+
Alkaloids	+	+
Saponins	–	+
Cardiac glycosides	–	+
Flavonoids	+	–
Tannins	+	+
Phenols	+	+
Starch	–	–
Quinone	–	–
Sugar	–	–

(+): Present, (-): Absent

Table 2: In vitro anti-inflammatory activity of ethyl alcohol and aqueous extract of *Anaphyllum wightii*

Sample	Concentration (µg)	Absorbance at 560nm	% Inhibition	EC_{50} (µg/ml)
Control	-	0.912±0.012	-	
Indomethacin	50	0.593±0.006	34.98	121.68
	100	0.496±0.007	45.62	
	150	0.406±0.008	55.49	
	200	0.305±0.005	66.56	
	250	0.199±0.008	78.18	
Chloroform extract	50	0.686±0.007	24.79	214.93
	100	0.619±0.004	32.13	
	150	0.536±0.003	40.68	
	200	0.469±0.001	48.58	
	250	0..414±0.004	54.61	
Aqueous extract	50	0.773±0005	13.35	277.83
	100	0.733±0.005	20.54	
	150	0.665±0.007	24.99	
	200	0.620±0.004	31.20	

Values are expressed as mean ± SEM.,n=6 in each groups.

Conclusion

Present study reveals that *Anaphyllum wightii* tuber could be useful resource as bio therapeutic agents. In vitro results indicates that it posses anti-inflammatory activity. So these efforts could open up the possibility of finding new clinically useful bio therapeutic agents.

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