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In-Vitro Anti-Inflammatory Activity of Chloroform Extract of *Malaxis rheedii Sw* by Using Albumin Denaturation Assay and HRBC Membrane Stabilization Method

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ABSTRACT

This research work explores the Anti-inflammatory effect of phytocompounds identified from chloroform extract of *Malaxis rheedii Sw* using the *in-vitro* pharmacological screening methods for anti-inflammatory agents. The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation technique which was studied according to the modifications. HRBC membrane stabilization method using the lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. This method is the stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. Since HRBC (human red blood cell) membrane is similar to the lysosomal membrane; the study was undertaken to check the stability of the HRBC membrane by the extracts to predict the *in vitro* anti-inflammatory activity. The denaturation of protein is one of the causes of Rheumatoid arthritis. Alternation in electrostatic, hydrogen, hydrophobic, and disulfide bonding causes denaturation of proteins. The inhibition of albumin denaturation from the three different sources that are mainly used 5% Bovine serum albumin. The standard Aspirin concentration range of inhibition was shown in the percentage at the different concentrations provide a significant protection agent of denaturation protein.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a diverse group of compounds with similar biological capabilities all NSAIDs reduce or eliminate the erythema, swelling, elevated temperature, and pain caused by a variety of inflammatory stimuli. The mechanisms of action of NSAIDs have not yet been fully elucidated, but evidence suggests that their anti-inflammatory effects are primarily achieved through inhibiting Prostaglandin production. This mode of action is common to all NSAIDs. The cyclo oxygenize enzyme was first identified as the therapeutic target of NSAIDs by Vane in 1971, showing that these anti-inflammatory substances block the biosynthesis of prostaglandins (PGs) that contribute to a variety of physiological and path physiological functions. The most prominent NSAIDs are aspirin, and aproxen, all available over the countries in most countries. Paracetamol (acetaminophen) is generally not considered an NSAID because it has only little anti-inflammatory activity. It treats pain mainly by blocking COX-2 mostly in the central nervous system, but not much in the rest of the body. Cyclooxygenase (COX) inhibitors, commonly called nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, diclofenac, and naproxen, have anti-inflammatory and analgesic/antipyretic properties across a wide range of dosing regimens[1-4].

All NSAIDs inhibit COX, an enzyme that converts arachidonic acid to prostaglandins, thereby mediating pain, inflammation, and fever. In the process, prostaglandin H2 is converted to five primary prostaglandins, including thromboxane A2 (which stimulates platelet aggregation and blood clot formation) in platelets and Prostacyclin (a vasodilator that inhibits platelet aggregation) in the endothelium. Two COXES is enzymes (COX-1 and COX-2) are commonly recognized[5]. In general, COX-1 is constitutively expressed and is involved in gastroprotection from stomach acid and in thromboxane formation by platelets. COX-2 is inducible by inflammatory mediators in a wide range of issues and has been associated with inflammation; however, it may also be constitutively expressed, where it contributes to renal physiology, reproductive function, bone resorption, and neurotransmission[6].

MATERIALS AND METHODS

Selection of the plant:

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethno pharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compounds have not been isolated or may involve taxa collected randomly for a large screening program. Based on an intensive literature survey; *Malaxis rheedii sw, the* whole plant was selected for the present study.

Collection and Authentication of Plant Material:

The whole plant of *Malaxis rheedii sw* was collected from the surrounding areas of hills of yercaud salem district, Tamilnadu, India. The plant has been taxonomically identified and authenticated by the botanist Dr. S. Radha Msc. Ph D.Central Siddha Medicinal Plant Garden Mettur Dam Tamilnadu.

Extraction of Plant Material:

The coarsely powdered whole plant of *Malaxis rheedii sw.* were extracted with water for 48 hours at room temperature. After extraction, the extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for further analysis.

Method of extraction: Continuous hot percolation process

Solvents used: Petroleum ether, Chloroform. (60-80C), Alcohol90%v/v.(75-78C).

Extraction procedure:

The dried powder of leaves of *Malaxis rheedii sw.* was defatted with petroleum ether in Soxhlet apparatus by hot percolation. The defatted powder material (marc) thus obtained was further extracted with Chloroform and Alcohol90%v/v. The solvent was removed by distillation under reduced pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator[7].

PHARMACOLOGICAL SCREENING METHODS

In Vitro Anti-Inflammatory Activity

Inhibition of Albumin Denaturation

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin de-naturation technique which was studied according to the modifications[8].

Procedure:

The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. Different concentrations of plant extract was added to the reaction mixture and were incubated at 37ºC for 20 min and then heated at 57º C for 5 min after cooling the samples, 2.5 mL of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 600 nm. The percentage inhibition of protein denaturation was calculated[9].

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

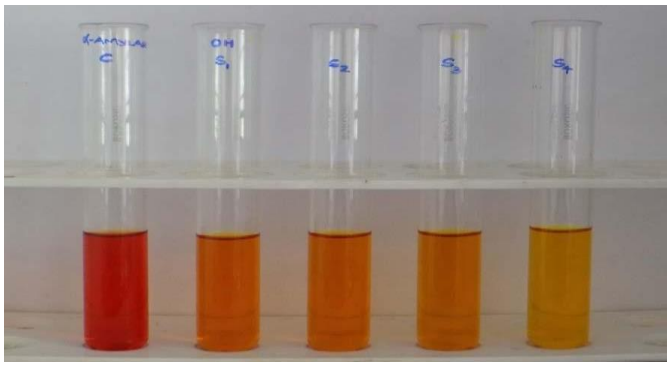

Figure No: 1 Inhibition of Albumin Denaturation activity of Chloroform extract of ***Malaxis rheedii Sw***

HRBC Membrane Stabilization Method

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation.

The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane[10].

Procedure:

Blood was collected (2 mL) from healthy volunteers and was mixed with an equal volume of sterilized Alsevers solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid, and 0.42 % NaCl in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10 % v/v suspension was prepared with normal saline and kept at 4ºC undisturbed before use[11]. Different concentrations of plant extract (50, 100, 200, 400 and 800 µg /0.5 ml) in normal saline, Aspirin as standard (50, 100, 200, 400 and 800 µg / 0.5 ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of 10% HRBC suspension was added to prepared. All the assay mixtures were incubated at 37º C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula[12].

$$\text{Percentage stabilization} = \frac{(Abs_{Control} - Abs_{sample})}{Abs_{Control}} \times 100$$

Figure No: 2 Hrbc Membrane Stabilization activity of Chloroform extract of ***Malaxis rheedii Sw***

RESULTS

Inhibition of Albumin Denaturation

The denaturation of protein is one of the causes of Rheumatoid arthritis. Alternation in electrostatic, hydrogen, hydrophobic, and disulfide bonding causes denaturation of proteins. The percentage of inhibition at different concentrations provides significant protection against in denaturation of proteins was showed in **Table No.1**. The standard Aspirin concentration range of inhibition was shown in the percentage at the different concentrations provide significant protection agent of de-naturation protein[13-15].

The maximum inhibition was observed at 500µg/ml of chloroform extract of *Malaxis rheedii sw* is 60.50± 0.60 and the Standard Aspirin 67.20±0.45. IC_{50} values for standard Aspirin were found to be 370.81 and Chloroform extract of *Malaxis rheedii sw* was found to be 432.99.

Table No: 1 ALBUMIN DENATURATION ASSAY OF CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*

Concentration (µg/ml)	CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*	Aspirin (Standard)
100	11.10 ± 0.50	13.30 ± 0.50
200	24.20 ± 0.70	27.20 ± 0.10
300	34.50 ± 0.65	42.50 ± 0.30
400	45.40 ± 0.50	53.10 ± 0.20
500	60.50 ± 0.60	67.20 ± 0.45
IC_{50}	432.99	370.81

Values are expressed as mean ± SD (n=3).

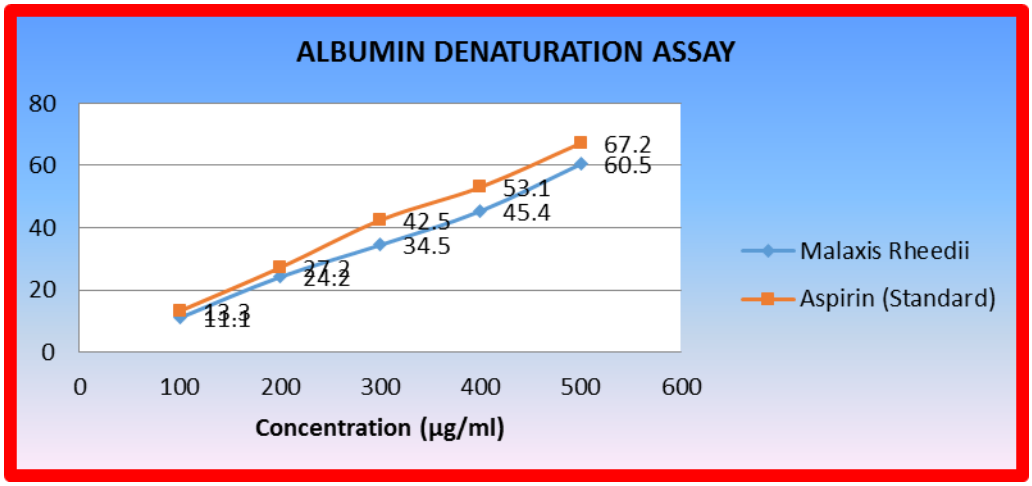

Figure No: 3 ALBUMIN DE-NATURATION ASSAY OF CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*

HRBC Membrane Stabilization Method

Inhibiting these lysosomal enzymes by stabilizing the lysosomal membrane. Concerned in this method is the stabilization of human red blood cell membrane by hypotonicity-induced membrane lyses. Since HRBC (human red blood cell) membrane is similar to the lysosomal membrane the study was undertaken to check the stability of HRBC membrane by the extracts to predict the *in vitro* anti-inflammatory activity of *Malaxis rheedii sw* extracts at the different concentrations. The results showed chloroform extract in **Table No.2** a dose-dependent anti-inflammatory activity in HRBC membrane assay.

The maximum inhibition was observed at 500µg/ml of chloroform extract of *Malaxis rheedii sw* is 66.20± 6.30 and the Standard Aspirin 71.60±0.30. IC_{50} values for standard Aspirin was found to be 323.13 and Chloroform extract of *Malaxis rheedii sw* was found to be 354.99.

Table No: 2 HRBC MEMBRANE STABILIZATION ASSAY OF CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*

Concentration (µg/ml)	CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*	Aspirin (Standard)
100	14.89 ± 0.25	16.10 ± 0.20
200	29.55 ± 0.45	36.25 ± 0.10
300	43.35 ± 0.40	47.20 ± 0.30
400	55.10 ± 0.10	60.45 ± 0.30
500	66.20 ± 0.30	71.60 ± 0.30
IC_{50}	354.99	323.13

Values are expressed as mean ± SD (n=3).

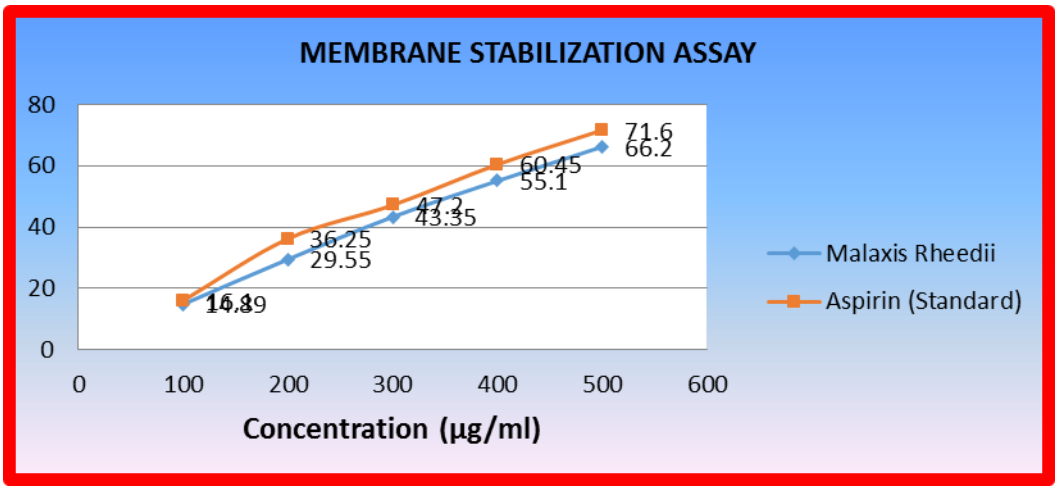

Figure No: 4 HRBC MEMBRANE STABILIZATION ASSAY OF CHLOROFORM EXTRACT OF *MALAXIS RHEEDII SW*

CONCLUSION

From the results, we concluded the chloroform extract of *Malaxis rheedii sw* exhibited good anti-inflammatory activity by the ability to inhibition of albumin de- maturation and HRBC stabilization membrane method. This study gives the idea that chloroform extract of M*alaxis rheedii sw* can be used as a lead compound for designing a potent anti-inflammatory drug that can be used for the treatment of various diseases such as cancer, neurological disorder, aging, and inflammation.

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