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Research Article

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In Vitro Anti-inflammatory Properties of Leaf Extract of *Polyalthia longifolia* Extract

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Abstract This study shows the *in vitro* anti-inflammatory effect of leaf extracts of *Polyalthia longifolia*. These were analyzed in different assays to determine their anti-inflammatory effects. Protein denaturation and RBC membrane stabilization were assayed. The results were reported as % inhibition in triplicate determination and were subjected to statistical also diclofenac sodium and Aspirin were used as standards. The result of the % inhibition of protein denaturation showed that at 1mg/ml diclofenac, ethanolic extract, fresh leaf aqueous extract and dry leaf aqueous extract of *Polyalthia longifolia* inhibited 15.03 ± 1.09, 20.47 ± 2.75, 17.01 ± 0.49, 12.56 ± 2.90 respectively and at 9 mg/ml diclofenac, ethanolic extract, fresh leaf aqueous extract and dry leaf aqueous extract of *Polyalthia longifolia* inhibited 69.49±3.19, 56.74±0.99, 55.57±0.22, 35.59±3.39 respectively. The result of % inhibition heat induced hemolysis showed that at 1mg/ml aspirin, ethanolic extract, fresh leaf aqueous extract and dry leaf aqueous extract of *Polyalthia longifolia* inhibited 18.78±3.10, 7.84±3.10, 25.39±3.22, 14.44±4.16 respectively. From the above results it is obvious that the extracts of *Polyalthia longifolia* showed greater anti-inflammatory effects.

Keywords *Polyalthia longifolia*, anti-inflammatory

Introduction

Inflammation is generally referred to as a complex biological response of vascular tissues to harmful stimuli. Inflammation is associated with pain, and it involves in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, among others [1]. Inflammation is also described as the body response to inactivate or eliminate the invading stimuli or organisms, to remove the irritants and set the stage for tissue repair, and the process is accelerated by the release of chemical mediators from injured cells or tissues and migrating cells [2].

The *in-vitro* erythrocyte haemolysis assay is generally used for screening anti-inflammatory activity of drugs. Majority of the anti-inflammatory drugs stabilize the plasma membrane of mammalian erythrocytes and thereby inhibit the heat-induced and the hypo tonicity-induced haemolysis [3]. The plasma membrane of mammalian red blood cells (erythrocytes) has been particularly useful as a model for studies of membrane structure. Mammalian red blood cells do not contain nuclei or internal membranes, so they are used as a source from which pure plasma membranes can be easily isolated for biochemical analysis. The erythrocyte plasma membrane resemblances to the lysosomal membrane and hence the stabilizing effect of drugs on erythrocyte membrane may correlate with its lysosomal membrane stabilizing effect [4]. The lysosomal membrane stabilization leads to the inhibition of release of the inflammatory mediators and consequent inhibition of the process of inflammation [5]. In the membrane stabilization assay, the erythrocytes are challenged with different haemolytic stimuli like heat, osmotic shock and free radicals [6]. The heat-induced and hypotonicity induced haemolysis of erythrocytes is extensively used as a

rapid, simple, economic and sensitive tool in determining the anti-inflammatory property of drugs. Medicinal plants used in traditional medicine in developing countries contain a wide range of phythochemicals that can be used to treat chronic as well as infectious diseases in the treatment of present or future diseases [7]. One such plant belonging to the genus Polyalthia and known to have curative value is Polyalthialongifolia (Sonn.) Thwaites (PL) from Annonaceae family (Figure 1). Polyalthiais the Greek word for poly, meaning much or many and althia from àltheo, meaning to cure, which showed its multiple health benefit. The genus Polyalthia includes about 120 species occurring mainly in Africa, South and South-Eastern Asia, Australia, and New Zealand [8]. *P. longifolia* is one of the most important indigenous medicinal plants in Indian medicinal Literature is found throughout Malaysia and widely used in traditional medicine as febrifuge and tonic. Almost all parts of this plant are used in Indian traditional system for the treatment of various ailments and the significant medicinal properties was further reported through scientific investigation.

Materials and Methods

Preparation of Plant Extract

After the collection of leaves, the leaves were shade dried for nineteen (19) days (10th Nov, 2018 to 29th Nov, 2018) and then grounded. A total weight of 1388.55g was obtained.

Preparation of Ethanolic Extract

692.85g of grounded leaves were transferred into a glass container and 2.5 L of 100% ethanol was added. The mixture was stirred with a stirring rod and allowed to soak for 48 hours (2 days). After 48 hours, the mixture was separated using a funnel and a cheese cloth. The filtrate was then evaporated using a water bath at a temperature of 65 °C for three days. The weight obtained after evaporation of the ethanolic extract was 49.84g.

1g of ethanolic extract was then dissolved in 10mls of distilled water (0.1g/ml). This stock solution was stored were dilutions of 1 mg/ml to 9 mg/ml were made.

Percentage Yield was 9.17 %

Preparation of Dry Leaves Aqueous Extract

10g of dry grounded leaves were dissolved and the volume made up to 100ml with distilled water. The mixture was allowed to soak for 2 hours. After 2 hours, it was filtered using a cheese cloth. The filtrate was centrifuged at 3000rpm for 10minutes. The supernatant was stored in the refrigerator for further analysis.

Preparation of Fresh Leaves Aqueous Extract

Fresh leaves were collected from the plant (*Polyalthia longifolia*). 10g of fresh leaves were grounded in a mortar and pestle. The grounded fresh leaves were soaked in 100ml of distilled water. The extract was filtered using a cheese cloth and transferred into tubes for centrifugation. The extract was centrifuged at 3000rpm for 10 minutes. The supernatant was stored in the refrigerator for further analysis.

Results

The result obtained from the analysis carried out on the various assays in the determination of the *in vitro* anti-inflammatory effect of leaf extract of *Polyalthia longifolia* are showed in tables below. Protein denaturation assay and membrane stabilization assay were all carried out and results obtained are tabulated after determining the %inhibition to its concentration. Also the plant extracts were Ethanolic extract, Fresh aqueous leaf extract and dry aqueous leaf extract.

Table 1: % inhibition of protein denaturation assay of the extracts of *Polyalthia longifolia* and standard drug

Conc. (mg/ml)	Diclofenac	Ethanolic Extract	Fresh leaf Aqueous Extract	Dry leaf Aqueous Extract
1	15.03 ± 1.09	20.47 ± 2.75	17.01 ± 0.49	12.56 ± 2.90
3	27.86 ± 0.23	39.78 ± 0.06	22.77 ± 3.19	15.66 ± 2.20
5	33.64 ± 2.09	45.11 ± 0.54	37.82 ± 3.09	20.69 ± 1.10
7	41.88 ± 1.59	48.597 ± 0.11	46.32 ± 1.45	28.85 ± 2.49
9	69.49 ± 3.19	56.74 ± 0.99	55.57 ± 0.22	35.59 ± 3.39

Data are represented as mean ± standard deviation of triplicate determination

Table 2: RBC membrane stabilization assay of extracts of *Polyalthia longifolia* and standard drug

Conc. (mg/ml)	Aspirin	Ethanolic Extract	Fresh Plant Aqueous Extract	Dry Plant Aqueous Extract
1	18.78 ± 3.10	7.84 ± 3.10	25.39 ± 3.22	14.44 ± 4.16
3	23.87 ± 1.23	17.64 ± 2.18	33.40 ± 0.19	17.79 ± 5.17
5	32.35 ± 1.12	21.27 ± 3.18	44.64 ± 0.10	20.38 ± 2.17
7	40.25 ± 3.37	30.99 ± 0.80	52.66 ± 2.10	31.43 ± 3.14
9	45.12 ± 3.18	41.35 ± 1.15	60.09 ± 3.10	39.43 ± 0.12

Data are represented in mean ± standard deviation of triplicate determination

Discussion

Protein Denaturation

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, the ability of extracts to prevent protein denaturation was studied; it was effective from the leaf extracts of *Polyalthia longifolia* at different aqueous extracts and ethanol extract. Using diclofenac sodium the standard drug, comparing the in vitro anti-inflammatory effect of the extracts, diclofenac at 9 mg/ml exhibited the highest effect with 69.49±3.19 %, followed by ethanolic extract at 56.74±0.99 %, fresh leaf aqueous extract had 55.57±0.22 %, finally dry leaf aqueous extract 35.59±3.39 %

The reason for the increase in inhibition with ethanolic could be that ethanol is a strong hydrophilic solvent that extracts better than water. These results are in line with the works of Anosike *et al* [9] who reported the effect of garden egg extract on Membrane stabilization as a mechanism of the anti-inflammatory activity.

RBC Membrane Stabilization Test

Stabilization of RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of extracts of *Polyalthia longifolia.* The extracts effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. (Table 2), the Aspirin as standard drug showed 18.78±3.10 % , ethanolic extract shows 7.84±3.10 %, fresh leaf aqueous extract shows 25.39±3.22 % and dry leaf aqueous extract had 14.44±4.16 % all at 1 mg/ml concentration respectively. The effect increases as the concentration increased down the column for the standard drug, and all the extracts. The highest inhibition was showed by the fresh aqueous extract at 9 mg/ml 60.09±3.10 %. These results are in line with the recent works of Amujoyegbe *et al* [4].

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