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Anti-inflammatory activity and phytochemical analysis of *Moringa oleifera* ethanol and acetone leaves extract

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ABSTRACT

This present investigation stated that acetone and ethanol extract of *M,oleifera* leaves was estimated that presence of phytochemical constituents by biochemical test and evaluated for anti-inflammatory activity. The anti-inflammation activity was assessed by calculating inhibition of protein denaturation, proteinase activity and membrane stabilization activity at different concentration of extract. The plant extract highly protective activity against heat induced protein denaturation and the IC50 results values 271.25±2.74 and 304.25±2.33µg/ml, for acetone and ethanol extract respectively. Heat induced haemolysis was 50% inhibited for acetone and ethanol extract at the concentration of 271.43±0.73 and 322.10±1.34 µg/ml, respectively. The membrane stabilization activity (IC_{50}) was assessed by hypotonicity induced haemolysis at a concentration of 216.98±1.84 and 259.65±1.83µg/ml for acetone and ethanol extract, respectively. The results obtained in the present study indicate that ethanol extracts of *M.oleifera* leaves can be a potential source of anti-inflammatory agents compared than acetone extract and standard drug.

Keywords: Antinflammatory, plant extract, phytochemicals

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INTRODUCTION

Inflammation is associated with pain and includes increased vascular permeability, protein denaturation and membrane alteration. Inflammation is occurs when tissue damage, heat or burn, and microbial invasion [1]. Inflammation caused stress characterized by pain, redness, swelling, heat and loss of function in the damaged or injured area [2,3].

Nowadays, most of the drugs are discovered and developed from naturally available medicinal plants. Medicinal plants are having variety of secondary metabolites and phytochemical constituents are responsible for treatment of newly emerging diseases [4]. Synthetic drugs for treatment occasionally cause side effects as well low effectiveness. Plant derived drugs are fulfil the need of discovery of new drugs for newly emerging diseases [5] Hence, plant derived drugs may offer alternative medicine fir inflammation. Screening of phytochemicals available in the plants has efficient defence and treatment for various diseases.

Moringa oleifera is a tree grow up to 5 -12 m belongs to the family Moringaceae. Native of this plant is India, Pakistan and Afghanistan where it is mostly used in folk medicine [6]. All the part of this plant is very culturally important for its nutritional and pharmacological values [7,8]. The leaves contain trace elements, proteins, vitamins and β-carotene [9]. Leaves are contain rich bioactive compounds such as carotenoids, tocopherols, ascorbic acid, highest proportion of essential aminoacids with a high content of proteins and trace minerals [10]. Several studies indicated that *M.oleifera* leaves exhibit anticancer, anti-oxidant, antimicrobial, anti-nutritional, anti-diabetic and anticonvulsant activity [11].

MATERIALS AND METHODS

Preparation of acetone and ethanol extract of *M.oleifera*

Fresh leaves of *M.oleifera* was collected and washed with tap water and double distilled water. Leaves were shade dried at room temperature for 3 days and grind into fine powder. 25 g was weighed and extracted with 80% ethanol and acetone in separate soxhlet's apparatus for 24 h at 60°C. The extracted samples were evaporated to obtain concentrated sample and used for further analysis.

Phytochemical screening of leaves extract

Acetone and ethanol extracted leaves of *M.oleifera* was examined to preliminary phytochemical analysis. The presence of various Phytoconstituents was analyzed by carried out using the standard methods described by Harborne [12], Trease and Evans [13] and Sazada et al [14].

Assessment of in-vitro anti-inflammatory activity

Inhibition of protein denaturation

The anti-inflammatory activity of acetone and ethanol extract of *M.leifera* was studied by determined based on the inhibition of albumin denaturation technique [15]. In this assay, 2.0 ml of different concentrations (100 – 500µg/ml) of acetone and ethanol leaf extract was mixed with 0.2 ml of 1% egg albumin and 2.8 ml phosphate buffer. The reaction mixture was incubated at 37 ºC for 20 min and heated 70 ºC for 5 min. Then the samples were subsequently cooled down and measure the turbidity at 660 nm using UV- Visible Spectrophotometer. Experiment was conducted in triplicate. The Percentage of inhibition of protein denaturation was calculated as follows:

% inhibition = (Abs Control –Abs Sample) X 100/ Abs control

The extracts concentration for 50% inhibition (IC50) was determined using linear graph by plotting percentage inhibition with respect to control against treatment concentration.

Proteinase inhibition activity

The test of proteinase inhibition activity of acetone and ethanol extract was assayed according to the method of Oyedepo and Femurewa [16] with slight modifications. The reaction mixture containing 0.06 mg of trypsin, 1 ml of 20mM Tris HCl buffer and 1 ml of acetone and ethanol extract of *M.oleifera* prepared at different concentrations (100 – 500µg/ml). Then the reaction mixture was incubated at 37ºC for 5 min. After incubation 1 ml of 0.8% casein was added and incubated for 20 min. To stop the reaction 2 ml of 70% perchloric acid was added and the mixture was centrifuged and collects the supernatant. The supernatant was observed absorbance at 210 nm against buffer as blank. Acetyl salicylic acid was used as positive control. The experiment was carried out in triplicates. Percentage inhibition of proteinase activity was calculated as follows

% inhibition = (Abs control –Abs sample) X100/ Abs control

Analysis of membrane stability

Heat induced haemolysis

Fresh human blood was collected and centrifuged at 3000 rpm for 10 min and the cells washed with saline water in three times. Then RBC layer was dilute to 10% v/v suspension with saline solution [17, 18]. A 100 µL of 10% RBC suspension was mixed with 100 µL of acetone and ethanol extract prepared at different concentrations (100 - 500 µg/ml). Blank was maintained with saline solution and acetyl salicylic acid was used as positive control. All the solutions were heated at 56°C for 30 min followed by centrifugation at 2500 rpm for 5 min. collect the supernatant and read the absorbance at 560 nm and calculate percentage of inhibition of haemolysis [19].

% inhibition = (Abs control –Abs sample) X 100/ Abs control

Hypotonicity-induced haemolysis

In this assay, the reaction mixture consists of 0.5 ml of different concentrations of acetone and ethanol extract (100-500µg/ml), 1ml of phosphate buffer (pH 7.4), and 2ml of hyposaline and 0.5ml of human RBC suspension. The mixture was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. Collect the supernatant and measure the absorbance at 560 nm and calculate the haemoglobin content in hypotonic treated blood suspension [20]. Experiment was made triplicate and percentage of membrane stabilization was calculated as follows:

% protection = 100- (OD sample/OD control) x 100

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical constituents of acetone and ethanol extract of *M. oleifera* were listed in the Table 1. The results showed the presence or absence of certain phytochemicals in acetone and ethanol extract of *M.oleifera* leaves. From Table 1, the results reveal that the phyto-constituents present in the acetone extract contains anthroquinone, flavonoids, glycosides, protein, tannins, and terpenoids. Although, the ethanol extract contains alkaloids, flavonoids, saponin, steroid, tannin, and terpenoids. Alkaloids, reducing sugars, saponin, and steroid were absent in acetone extract which are confirmed by the chemical tests.

Table 1:. Phytochemical analysis of *M. oleifera* leaves solvent extracts

Phyto-constituents	Test	Presence/Absence	
		Acetone	Ethanol
Alkaloids	Wagner's test	_	+
Anthraquinone	Borntranger test	+	_
Flavonoids	Lead acetate test	+	+
Glycosides	Keller Killani test	+	_
Protein	Biuret test	+	_
Reducing sugar	Fehling's test	_	_
Saponin	Frothing Test	_	+
Steroid	Salkowski Test	_	+
Tannin	Ferric chloride test	+	+
Terpenoids	Salkowski test	+	+

+ = presence, - = absent

Anti-inflammatory activity

Inhibition of albumin denaturation

The inhibition of protein denaturation was assessed using acetone and ethanol extract of *M.oleifera* leaves. The inhibition was studied in heat induced albumin denaturation in concentration dependent manner and compared with standard acetyl salicylic acid (Figure 1). Both the extract has the ability to control protein denaturation during the inflammation. The 50% of inhibition (IC_{50}) was observed at 271.25±2.74 and 304.25±2.33 µg/ml for both acetone and ethanol extract, respectively.

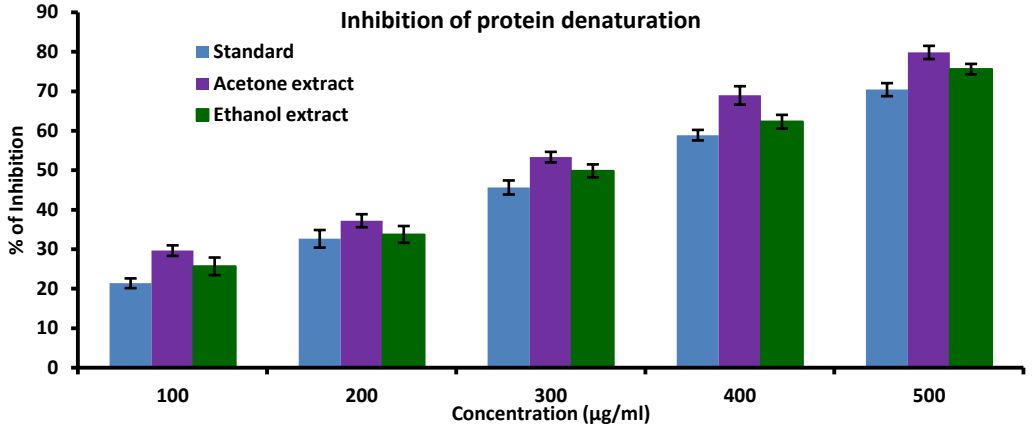

Figure 1: Effect of different concentration of acetone and ethanol extract on inhibition of protein denaturation

Proteinase Inhibitory Action

The proteinase enzyme inhibition of acetone and ethanol extract of M.oleifera was shown in Figure 2. The activity was exhibited at different concentrations show significant antiproteinase property. This property was increase with increasing the dose concentration and showed 50% inhibition in Table 3. IC50 was observed at 274.43±0.73 and 322.10±1.34 µg/ml, respectively. From the results, acetone extract exhibit more activity than ethanol extract of leaves.

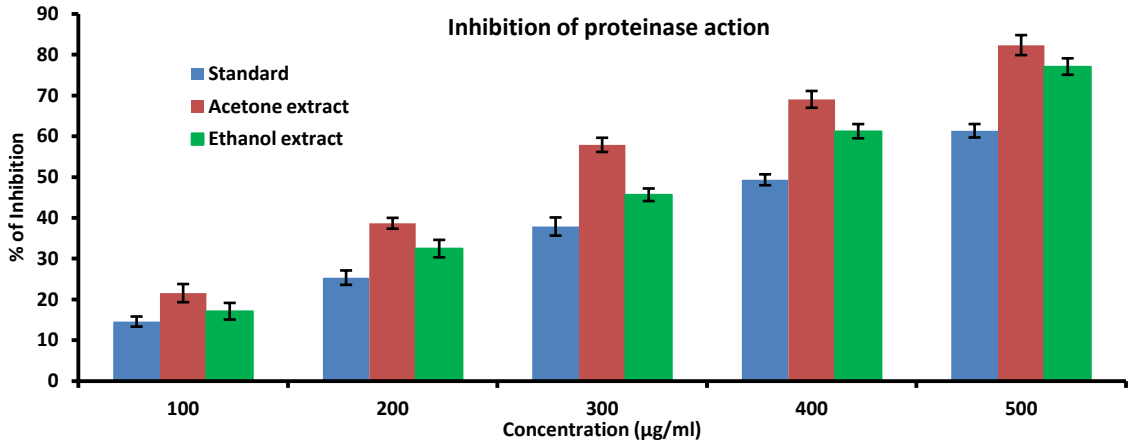

Figure 2: Effect of different concentration of acetone and ethanol extract on inhibition of proteinase

Membrane stabilization

The study of membrane stabilization of RBC was the best method to assess the in-vitro anti- inflammatory activity of acetone and ethanol extract of *M.oleifera* leaves. The extract significantly exhibit stabilization of heat induced lysosomal membrane at different concentrations. The results showed that IC_{50} value of acetone and ethanol extract at concentration 224.96±2.34 and 249.70±1.82 µg/ml, respectively protect significantly the erythrocyte membrane against heat induced lysis (Figure 3).

Another method of analysis of membrane stabilization activity in erythrocyte of ethanol and acetone extract assessed against lysis induced by hypotonic solution. The heamolysis inhibition was increase as increasing the concentration of acetone and ethanol extract, hence the activity was observed in dose dependant manner. Moreover, IC_{50} value of anti-inflammatory activity of both the extracts was observed at 216.98±1.84 and 259.65±1.83µg/ml (Figure 4). Acetone extract shows a significant protection activity against damaging effect from heat solution. Among these extracts acetone shows higher anti-inflammatory activity due to the presence of alkaloids, flavonoids and tannins. These phytochemicals are strongly responsible for antioxidants, antimicrobial and anti-inflammatory properties [21-24].

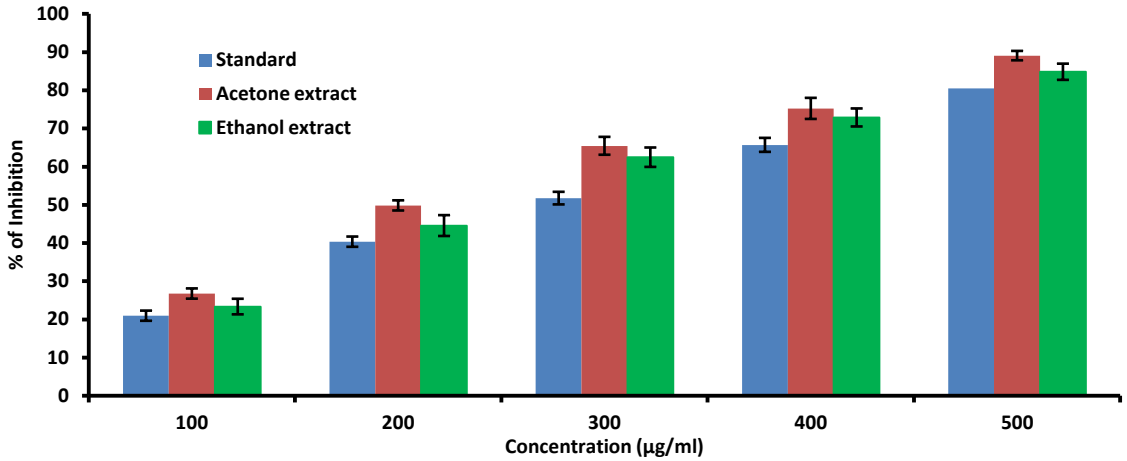

Figure 3: Effect of different concentration of acetone and ethanol extract on inhibition of heat induced haemolysis

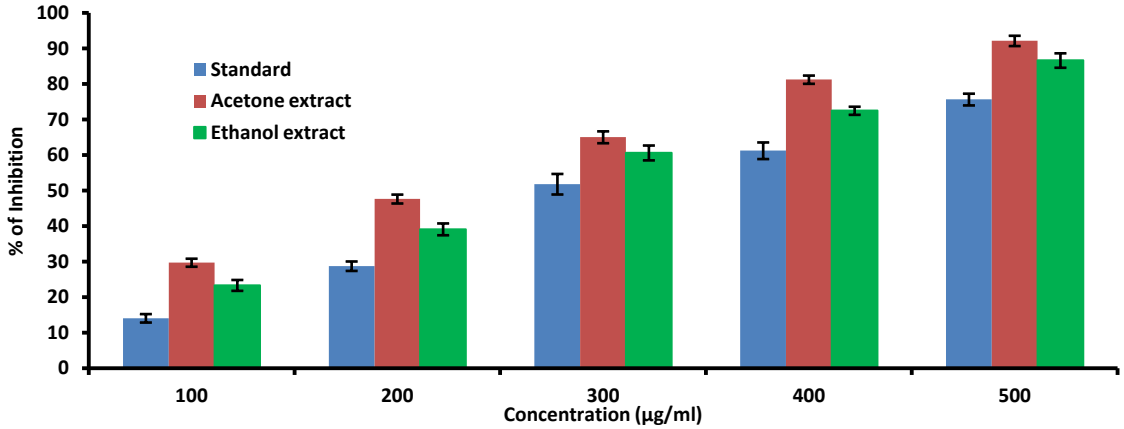

Figure 4: Effect of different concentration of acetone and ethanol extract on inhibition of Hypotonicity Induced Haemolysis

CONCLUSION

This present investigation indicates that the acetone and ethanol solvent extracts of *M.oleifera* leaves exhibits anti-inflammatory property. Phyto-chemical constituent analysis revealed the presence of secondary metabolites in both the extract. Acetone extract exhibit more significant anti-inflammatory activity than the ethanol extract. As a result, this medicinal plant by *in-vitro* results proved that *M.oleifera* leaves extract effective source to formulation of anti-inflammatory drugs.

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