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IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF *CARALLUMA UMBELLATA* HAW (ASCLEPIADACEAE)

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ABSTRACT: Ethanol extract of *Caralluma umbellata* Haw whole plant was assessed for its anti-inflammatory activity by in-vitro methods. Qualitative analysis of whole plant extract revealed the presence of alkaloid, flavonoid, saponins, steroid, phenol, tannin, glycoside and terpenoid. In-vitro anti-inflammatory activity was estimated using proteinase inhibitory activity, albumin denaturation assay, membrane stabilization and antilipoxygenase activity at different concentrations. Aspirin was employed as standard drug. The results showed that *Caralluma umbellata* ethanol extract at a concentration of 500 µg/ml significantly ($p<0.01$) protects the heat induced protein denaturation. At the concentration of 500 mg/ml, *Caralluma umbellata* whole plant extract showed significant ($p<0.01$) inhibition of 73% of protease inhibitor action. Heat induced haemolysis of erythrocyte was significantly ($p<0.01$) inhibited at the concentration of 500 µg/ml. Hypotonicity induced haemolysis and lipo-oxygenase activity were significantly ($p<0.01$) inhibited at the concentration of 500 µg/ml. From the results it is concluded that flavonoid, phenols and terpenoids present in the *Caralluma umbellata* extract may be responsible for the anti-inflammatory activity.

KEYWORDS: *Caralluma umbellata*, Albumin, Hypotonicity, Lipoxygenase.

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1.INTRODUCTION

Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. It is triggered by the release of chemical mediator from injured tissue and migrating cells. Inflammation is a complex process, which is frequently associated with pain and involves occurrence such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compounds such as strong acid or base a concentrated inorganic salt an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well documented cause of inflammation [1, 2]. Inflammation can be classified as either acute or chronic. Acute inflammation is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes. Chronic inflammation is associated with infiltration of mononuclear immune cells, macrophages, monocytes neutrophils, fibroblast activation, proliferation (angiogenesis) and fibroblasts. Inflammation may be potentially harmful, causing life threatening, hypersensitivity reactions and progressive organ damage [3]. Non-steroidal anti-inflammatory drugs (NSAIDs) are reported to possess prevention of the denaturation of proteins, which act as antigens and leads to acute immune disease [4]. Medicinal plants are the main sources of chemical substances with potential therapeutic effects. Now-a-days herbal drugs are routinely used for curing diseases rather than chemically derived drugs having side effects. As a result a reach for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drug [5]. According to World Health Organization (WHO), about three-quarters of the world population depends on traditional medicines (mainly herbs) for their healthcare. Ayurveda and Chinese medicinal systems are the most acceptable traditional system which has a considerable amount of research on pharmacognocy, chemistry, pharmacology and clinical therapeutics [6, 7]. The renewed interest in medicinal plant research has focused on herbal cures among indigenous populations around the world. *C. umbellata* belongs to the Asclepiadaceae family. It is a thick, erect, leafless, branching, succulent thorny perennial herb. Preganes, Pregane, glycosides, saponins, flavonoids, triterpenoids, flavonoes, and steroidal glycosides are the phytochemical constituents' which are present in *C. umbellata*. The stem juice, mixed with turmeric is used to cure stomach disorder and abdominal pains. It is also used for treatment of obesity and diabetes. The stem of the plant burn in direct fire, eaten for five days regularly in empty stomach will cure ulcer problems [8]. Hence, the present investigation was carried out to evaluate the in-viro anti-inflammatory potential of *C. umbellata* using different models.

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2. MATERIALS AND METHODS

Caralluma umbellata Haw whole plant was collected in fresh from Parvathipuram, Kanyakumari District, Tamil Nadu, India. The plant sample was found out with the help of local flora and confirmed by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of composed plants was deposited in the Ethnopharmacology Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu, India.

Solvent extraction

Ethanol was used as solvent to prepare the plant extracts. The whole plant was directly soaked for 12 hrs in 500 ml ethanol and then subjected to extraction by reflexing for 6 to 8 hrs below the boiling point of the solvent. The ethanol extracts were concentrated by evaporating at a reduced pressure using rotary evaporator. The concentrated extracts were further dried at 37^0C for 3 to 4 days in order to facilitate complete evaporation of the solvents. The concentrated extracts were used in qualitative analysis of phytochemicals with standard protocols [9, 10, 11, 12].

Assessment of in-vitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of *C.umbellata* was premeditated by using inhibition of albumin denaturation technique. This was calculated according to Mizushima *et al.* [4] and Sakat *et al*. [13] followed with minor modifications. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was altered using small amount of 1N HCl. The sample extracts were protected at 37 ºC for 20 min. Then it is heated to 51 º C for 20 min. After cooling the samples the turbidity was calculated at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was computed as follows:

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

Antiproteinase action

The test was performed according to the modified method of Oyedepo and Femurewa, [14] and Sakat *et al*. [13]. The reaction mixture (2 ml) contained 1 ml 20 mM Tris HCl buffer (pH 7.4), 0.06 mg trypsin, and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was kept warm at 37ºC for 5 min. To this 1 ml of 0.8% (w/v) casein was added. The mixture was kept warm for an extra 20 min. 2 ml of 70% perchloric acid was added to it in order to arrest the reaction. Followed by this the cloudy suspension was centrifuged. Then the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was repeated thrice. The percentage inhibition of proteinase inhibitory activity was computed.

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

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Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension [13, 15]

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. At 3000 rpm for 10min the tubes were centrifuged and were washed three times with equal volume of normal saline. The volume of blood was determined and re constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis [13, 16]

The reaction mixture (2ml) consisted of 1 ml test sample of dissimilar concentrations (100 - 500 µg/ml) and 1 ml of 10% RBCs suspension, in its place of test sample, only saline was adjoined to the control test tube. As a standard drug Aspirin was used. All the centrifuge tubes enclosing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled using running tap water. At 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm the reaction mixture was centrifuged. The experiment was completed in triplicates for all the test samples.

The Percentage inhibition of Haemolysis was computed as follows:

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

Hypotonicity-induced haemolysis [17]

Different reference sample, different concentration of extract (100-500µg/ml), and control were individually mixed with 2ml of hyposaline, 1ml of phosphate buffer, and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were kept warm at 370 ºC for 30 minutes. Then centrifuged at 3000rpm. The supernatant liquid was poured and the haemoglobin content was approximated by a spectrophotometer at 560nm. The percentage haemolysis was calculated approximately by assuming the haemolysis produced in the control as 100%.

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

Anti-lipoxygenase activity [16]

Anti-Lipoxygenase activity was considered using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and additional 0.25ml of lipoxidase enzyme solution (20,000U/ml) is added and kept warm for 5 min at 250C. After which, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard.

The percent inhibition was computed from the following equation,

% inhibition= [{Abs control- Abs sample}/Abs control] x 100

A dose response curve was plotted to establish the IC_{50} values. IC_{50} is defined as the concentration sufficient to get 50% of a maximum scavenging capacity. All tests and analyses were repeated

thrice and averaged.

Statistical analysis

Results are articulated as Mean ± SD. The difference between experimental groups be compared by One Way Analysis Of Variance (ANOVA). This is followed by Dunnet Multiple comparison test (control Vs test) making use of the soft ware Graph Pad Instat.

3. RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of ethanol extract of *C. umbellata* whole plant revealed the presence of alkaloid, flavonoid, saponin, steroid, phenol, tannin, glycoside and terpenoid.

Inhibition of albumin denaturation

Denaturation of proteins is a well certified cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, capability of extract to inhibit protein denaturation was studied. It was helpful in inhibiting heat induced albumin denaturation (Table 1). Maximum inhibition was observed at the concentration of 500 µg/ml and showed the inhibition 72%. Aspirin, a standard drug showed the maximum inhibition 65% at the concentration of 100 µg/ml compared to control.

Table 1: Effect of CU Extract on heat induced protein denaturation

Treatment	Concentration Ug/ml	Absorbance at 660 nm	% of inhibition of heat induced protein denaturation
Control	-	0.36±0.03	-
CU Extract	100	0.22±0.04	39
CU Extract	200	0.19±0.03**	47
CU Extract	300	0.16±0.02**	56
CU Extract	400	0.12±0.05**	67
CU Extract	500	0.10±0.02**	72
Aspirin	100	0.12±0.05**	65

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant.

Proteinase inhibitory action

Neutrophils is a rich resource of serine proteinase and is localized at lysosomes. It was previously reported that leukocytes proteinease play an important role in the development of tissue damage during inflammatory reactions and significant level of proteinase was provided by proteinase inhibitors [18]. Ethanol extract of *C. umbellata* whole plant exhibited significant antiproteinase

activity at different concentration as shown in table 2. It showed maximum inhibition of 73% at 500 µg/ml. Aspirin showed the maximum inhibition 62% at 100 µg/ml.

Table 2: Effect of CU Extract on proteinase inhibitory action

Treatment	Concentration Ug/ml	Absorbance at 210 nm	% of inhibition of proteinase action
Control	-	0.37±0.06	-
CU Extract	100	0.30±0.04	19
CU Extract	200	0.25±0.02	32
CU Extract	300	0.21±0.04**	43
CU Extract	400	0.14±0.04**	62
CU Extract	500	0.10±0.03**	73
Aspirin	100	0.14±0.01**	62

Each value represents the mean ± SD. N=3,Experimental group were compared with control **p<0.01, considered extremely significant.

Heat induced haemolysis

The lysosomal enzyme released during inflammation creates a variety of disorder. The extracellular activity of these enzymes is said to be released to acute or chronic inflammation. The non-steroidal drugs act either by inhibitory or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane the study was undertaken to check the stability of HRBC membrane by ethanol extract of *C. umbellata* whole plant. The extract was effective in inhibitory the heat induced haemolysis at different concentrations. The results showed that ethanol extract of *C. umbellata* whole plant at concentration 500 µg/ml protect significantly ($p < 0.01$) the erythrocyte membrane against lysis induced by heat (Table 3). Aspirin 100 mg/ml showed significant ($p < 0.01$) protection against damaging effect of heat solution.

Hypotonicity induced haemolysis

Ethanol extract *C. umbellata* whole plant at concentration of 100 – 500 mg/ml protected the human erythrocyte membrane against lysis induced by hypotonic solution (Table 4). During inflammation there are lyses of lysosomes which release their component enzymes that moderate a variety of disorders. Exposive of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat, methyl salicylate or phenylhydrazine results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin [19]. Since human red blood cells (HRBC) membranes are similar to lysosomal membrane competent [20], the inhibition of hypotonicity red blood cells membrane lysis was taken as a measure of the mechanism of anti-

inflammatory activity of *C. umbellata* extract. The haemolytic effect of hypotonic solution related to excessive accumulation of fluid within the cell results in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to remedy damage through free radical induced lipid peroxidation [21]. Membrane stabilization leads to the prevention of release of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory medication [22]. Ethanol extract of *C. umbellata* perhaps stabilized the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation.

Table 3: Effect of CU Extract on heat induced haemolysis of erythrocyte

Treatment	Concentration Ug/ml	Absorbance at 560 nm	% of inhibition of heat induced haemolysis of erythrocyte
Control	-	0.33±0.02	
CU Extract	100	0.23±0.01	30
CU Extract	200	0.20±0.07	39
CU Extract	300	0.16±0.03**	51
CU Extract	400	0.10±0.07**	70
CU Extract	500	0.08±0.004**	76
Aspirin	100	0.07±0.01**	79

Each value represents the mean ± SD. N=3,Experimental group were compared with control **p<0.01, considered extremely significant.

Antilipoxygenase activity

Lipoxygenase (LOXs) are the family of the key enzyme in the biosynthesis of leukotrienes which plays an important role in the pathogenecity of reveal inflammatory diseases. Lipoxygenase are receptive to antioxidants and the most of their action may include inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipidperoxy-radicals formed increase of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX [23]. Ethanol extract of *C. umbellata* whole plant has been checked at 100, 200, 300, 400 and 500 mg/ml. From these result the strongest inhibition was obtained at concentration 500 mg/ml. The standard aspirin showed an 82% inhibition at a concentration of 100 mg/ml (Table 5).

Table 4: Effect of CU Extract on hypotonicity induced haemolysis of erythrocyte

Treatment	Concentration Ug/ml	Absorbance at 560 nm	% of inhibition of hypotonicity induced haemolysis of erythrocyte
Control	-	0.32±0.04	-
CU Extract	100	0.30±0.02	6
CU Extract	200	0.26±0.07	19
CU Extract	300	0.21±0.01	34
CU Extract	400	0.16±0.03**	50
CU Extract	500	0.14±0.07**	56
Aspirin	100	0.10±0.03**	69

Each value represents the mean ± SD. N=3,Experimental group were compared with control **p<0.01, considered extremely significant.

Table 5: Effect of CU Extract on lipoxygenase inhibitory action

Treatment	Concentration Ug/ml	Absorbance at 234 nm	% of inhibition of lipoxygenase action
Control	-	0.40±0.06	-
CU Extract	100	0.29±0.04	27
CU Extract	200	0.26±0.05	35
CU Extract	300	0.21±0.04**	47
CU Extract	400	0.16±0.03**	60
CU Extract	500	0.11±0.06**	72
Aspirin	100	0.07±0.01**	82

Each value represents the mean ± SD. N=3,Experimental group were compared with control **p<0.01, considered extremely significant.

4. CONCLUSION

The results obtained from the present study on ethanol extract of *C. umbellata* whole plant have shown a potential anti-inflammatory activity. These activities may be due to the strong occurrence of phenolic compounds alkaloids, flavonoids, tannins and steroids. This study gives an idea the compound of the plant *C. umbellata* can be used as lead compound for designing a potent anti-inflammatory drug.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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