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THE COMPARISON OF IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF ABELMOSCHUS ESCULENTUS & CAMELLIA SINENSIS

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ABSTRACT

Ethyl acetate extract of Abelmoschus esculentus & Camellia sinensis was assessed for its anti-inflammatory activity by invitro methods. In-vitro anti-inflammatory activity was evaluated using membrane stabilization, and Protein denaturation activity at different concentrations. Diclofenac sodium (100 µg/ml) were used as standard drugs. The results showed that both the plants have significant anti-inflammatory activity, but EAECS have more activity than EAEAE. In the heat induced protein denaturation EAECS showed % inhibition 68.42% at concentration 500 µg/ml. Which is close to % inhibition of standard i.e. 65.78% at concentration of 100 µg/ml. and EAEAE showed % inhibition 55.26 % at concentration 500 µg/ml which is near about % inhibition by EAECS at concentration of 400 µg/ml. In the heat induced hemolysis of erythrocyte**,** EAECS showed % inhibition 69.52% at concentration 500 µg/ml. Which is near about % inhibition of standard i.e. 70.47% at concentration of 100 µg/ml and EAEAE showed % inhibition 58.09% at concentration 500 µg/ml which is near about % inhibition by EAECS at concentration of 400 µg/ml. In the hypotonicity induced haemolysis of erythrocyte, EAECS showed % inhibition 67.56% at concentration 500 µg/ml which is more than % inhibition of standard i.e. 54.05% at concentration of 100 µg/ml. hence present study conclude that EAECS have more potent constituents responsible for anti-inflammatory activity than that of EAEAE.

INTRODUCTION

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells.[1] The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.[2] Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived effect and low cost. Polyphenols rich plant have anti-inflammatory activity but present study aim to compare anti-inflammatory activity of polyphenol rich plant so one could able to select best anti-inflammatory activity rich plant here in-vitro anti-inflammatory activity evaluated by Inhibition of albumin denaturation, Membrane stabilization Method.

MATERIALS AND METHODS

Plant material

*Camellia sinensis.*was ordered from Agro Industries Limited, Deckiajuli tea Estate, Sonitpur, Assam-784110.and *Abelmoschus esculentuswas* collected from local market of Akluj, Taluka-Malshiras, Dist- Solapurand authenticated from Department of Botany, D.B.F.Dayanand College of Arts and Science, Solapur.

Preparation of extracts

Initially, 3 g of ground leaves *Camellia sinensis*, & powder of *Abelmoschus esculentuswas* seed.was extracted with 60 ml of pure water at temperatures of 80^0C, 40 min. under continuous stirring at 300 rpm. Each extraction was filtered by Muslin cloth. The filtered samples were initially partitioned with water/chloroform (1:1 vol.%). Then the water phase was collected and the impurities associated with the chloroform phase were discarded. As a second partition, water/ethyl acetate (1:1 vol.%) was used. Polyphenols moved into the ethyl acetate layer. Collected product is evaporated and recrystallize with ethanol.[3]

Assessment of in-vitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of *Camellia sinensis* and *Abelmoschus esculentuswas* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al[4] and Sakat et al[5] followed with minor modifications. The reaction mixture was consists of test extracts of concentraction 100, 200, 300, 400, 500 µg/ml for both the plants. Diclofenac sodium (100µg/ml) was used as a standard drug, and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm by using Photoelectric colorimeter. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension[6],[7]

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. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis[6], [8]

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows: Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control.

Hypotonicity-induced haemolysis[9]

Different concentration of extract (100-500µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 370c for 30minutes and centrifuged at 3000rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%. Percentage protection = 100- (OD sample/OD control) x 100

Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by OneWay Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test) using the soft ware Graph Pad Instat.

RESULTS

Inhibition of albumin denaturation

In the process of Protein Denaturation, protein breaks tertiary and secondary structure is by application of external stress or by compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most proteins biologically nonfunctional when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 68.42% was observed at 500 µg/ml dose of EAECS & 55.26% inhibition was observed in EAEAE.at 500 µg/ml dose. Diclofenac sodium, a standard anti-inflammation drug showed the maximum inhibition 65.78 % at the concentration of 100 µg/ml compared with control (Table 1).

Table 1: Effect of EAECS & EAEAE on heat induced protein denaturation.

Sr no	Treatment (s)	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of protein denaturation
1	Control		0.38 ±0.006***	
2	standard	100	0.13 ±0.006***	65.78
3	EAECS	100	0.29 ±0.006***	23.68
4	EAECS	200	0.23 ±0.006***	39.47
5	EAECS	300	0.2 ±0.006***	47.36
6	EAECS	400	0.16 ±0.006***	57.89

7	EAECS	500	0.12 ±0.006***	68.42
8	EAEAE	100	0.34 ±0.006**	10.52
9	EAEAE	200	0.3 ±0.006***	21.05
10	EAEAE	300	0.26 ±0.006***	31.57
11	EAEAE	400	0.21 ±0.006***	44.73
12	EAEAE	500	0.17 ±0.006***	55.26

Each value represents the mean ± SD. N=3, Experimental group were compared with control ***p< 0.001 considered extremely significant **p< 0.01 considered more significant

EAECS- Ethyl Acetate Extract of *Camellia sinensis*

EAEAE- Ethyl Acetate Extract of *Abelmoschus esculentus*

Membrane stabilization

The HRBC membrane stabilization has been used as a method to study the invitro anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane[10], [11] and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane.[12]

Heat Induced Haemolysis

The extract was effective in inhibiting the heat induced haemolysis at different concentrations. The results showed all the concentrations showed significant anti-inflammatory activity as compare to control but maximum inhibition i.e. 69.52% was showed by EAECS at 500µg/ml dose and which is nearly equals to standard (Diclofenac sodium) inhibition which is 70.47% at concentrations of 100 µg/ml. EAEAE also showed significant anti-inflammatory activity as compare to control but not as effective EAECS. EAEAE showed maximum inhibition of 58.09% at concentration of 500 µg/ml.

Table 2: Effect of EAECS & EAEEA on heat induced hemolysis of erythrocyte.

Sr no	Treatment (s)	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of protein denaturation
1	Control	-	1.05 ± 0.0033***	-
2	standard	100	0.31± 0.005***	70.47
3	EAECS	100	0.75±0.005***	28.57
4	EAECS	200	0.62±0.005***	40.95
5	EAECS	300	0.60±0.005***	42.85
6	EAECS	400	0.42±0.005***	60
7	EAECS	500	0.32±0.005***	69.52
8	EAEAE	100	0.71±0.005***	32.38
9	EAEAE	200	0.61±0.005***	41.9
10	EAEAE	300	0.55±0.005***	47.61
11	EAEAE	400	0.50±0.005***	52.38
12	EAEAE	500	0.44±0.005***	58.09

Each value represents the mean ± SD. N=3, Experimental group were compared with control ***p< 0.001 considered extremely significant **p< 0.01 considered more significant

EAECS- Ethyl Acetate Extract of *Camellia sinensis*

EAEAE- Ethyl Acetate Extract of *Abelmoschus esculentus*

Hypotonicity Induced Hemolysis

The results showed both the plant have significant anti-inflammatory action but maximum inhibition showed by EAECS i.e. 67.56% at concentration of 500 µg/ml. and which is more than standard. EAEAE showed minimum inhibition as compare to standard and EAECS. hence EAECS have more potent than EAEAE.

Table 3: Effect of EAECS & EAEAE on hypotonicity induced hemolysis of erythrocyte.

Sr no	Treatment (s)	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of haemolysis
1	Control		0.36±0.008***	
2	standard	100	0.16±0.005***	54.05
3	EAECS	100	0.22±0.005***	40.54
4	EAECS	200	0.21±0.005***	43.24
5	EAECS	300	0.17±0.003***	51.35
6	EAECS	400	0.15±0.005***	56.75
7	EAECS	500	0.11±0.005***	67.56
8	EAEAE	100	0.25±0.003***	32.43
9	EAEAE	200	0.22±0.005***	29.72
10	EAEAE	300	0.20±0.003***	35.13
11	EAEAE	400	0.18±0.003***	29.72
12	EAEAE	500	0.17±0.003***	45.94

Each value represents the mean ± SD. N=3, Experimental group were compared with control ***p< 0.001 considered extremely significant **p< 0.01 considered more significant

EAECS- Ethyl Acetate Extract of *Camellia sinensis*

EAEAE- Ethyl Acetate Extract of *Abelmoschus esculentus*

DISCUSSION

In the present study, results indicate that both the plants (*Camellia sinensis, Abelmoschus esculentus*) have significant anti-inflammatory properties. These activities in both plants may be due to the strong occurrence of polyphenol compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. In the heat induced protein denaturation EAECS showed % inhibition 68.42% at concentration 500 µg/ml. which is close to % inhibition of standard i.e. 65.78 % at concentration of 100 µg/ml. and EAEAE showed % inhibition 55.26 % at concentration 500 µg/ml which is near about % inhibition by EAECS at concentration of 400 µg/ml. In the heat induced hemolysis of erythrocyte**,** EAECS showed % inhibition 69.52% at concentration 500 µg/ml. which is near about % inhibition of standard i.e. 70.47% at concentration of 100 µg/ml. and EAEAE showed % inhibition 58.09 % at concentration 500 µg/ml which is near about % inhibition by EAECS at concentration of 400 µg/ml. In the hypotonicity induced hemolysis of erythrocyte, EAECS showed % inhibition 67.56% at concentration 500 µg/ml which is more than % inhibition of standard i.e. 54.05% at concentration of 100 µg/ml. But the Purification of each bioactive compound is necessary and this purified form of the compound can be used which may show increased activity. This study gives on idea that *Camellia sinensis* have more potent active ingredients responsible for anti-inflammatory activity than *Abelmoschus esculentus*.

CONCLUSION

From above study it was concluded that *Camellia sinensis & Abelmoschus esculentus* have significant anti-inflammatory activity. But EAECS have more potent activity as compare to standard drug (Diclofenac Sodium). 500 µg/ml dose of EAECS showed equal effect as that of standard drug (Diclofenac Sodium) at conc. 100 µg/ml.and *Camellia sinensis* better anti-inflammatory activity than that of *Abelmoschus esculentus.* Hence it was concluded that

Camellia sinensis have more potent ingredients responsible for Anti-inflammatory action. This study gives on idea that the compound of the plant *Camellia sinensis* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of many diseases.

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