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ANTI-INFLAMMATORY ACTIVITY OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO

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Keywords:

Anti-inflammatory activity, Bovine albumin, *Cucumis melo* L. subsp. agrestis (Naudin) Pangalo, Cucurbitaceae

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ABSTRACT: The present study was aimed to evaluate the *in-vitro* anti-inflammatory activity of fruits and leaves extract of *Cucumis melo* L. subsp. agrestis (Naudin) Pangalo (Family Cucurbitaceae). The ethanol and aqueous extracts of fruits and leaves of the plant were subjected to preliminary phytochemical screening and *in-vitro* anti-inflammatory activity. Extracts were incubated with bovine albumin under controlled conditions for denaturation, and protein denaturation was calculated by determination of their absorbance. Since, the erythrocyte membrane is analogous to the lysosomal membranes and its stabilization implies that the extract may well stabilize lysosomal membranes thus human red blood cell membrane stabilization was used as a method to study the *in-vitro* anti-inflammatory activity. The results showed that the plant extracts showed anti-inflammatory activity in a concentration-dependent manner, and the activity was increased on increasing the concentration of extracts. The ethanol extract was found to be more effective than other extracts of fruit and leaf. The present study reveals that the fruit of *Cucumis melo* L. subsp. agrestis (Naudin) Pangalo possesses a higher anti-inflammatory than the leaf. The activity may be due to the presence of phenols, tannins, and flavonoids present in the plant.

INTRODUCTION: The genus Cucumis is one of the economically most important genera of flowering plants and includes many commonly grown vegetables as well as ornamentals [1, 2]. Species of Cucumis are characterized by a trailing, climbing, or bushy growth habit. It is native to dry areas of India being common throughout South America and other parts of tropical Asia. *Cucumis callosus* is a variety of the melon that is *Cucumis melo* var agrestis (family Cucurbitaceae) [3, 4].

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Inflammation is a normal protective response to tissue injury and characterized by redness, swelling, and pain, stiffness of joints, and loss of joint function [5-8]. Membrane alterations, increase in vascular permeability, and protein denaturation is associated with Inflammation [6, 7].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. In *the in-vitro* anti-inflammatory human red blood cell (HRBC) membrane stabilization method, the erythrocyte membrane is analogous to the lysosomal membranes; its stabilization implies that the extract may well stabilize lysosomal membranes [9].

The present study has been performed as an attempt to prove the anti-inflammatory potential of *Cucumis melo* L subsp. Agrestis (Naudin) Pangalo fruit and leaf parts.

MATERIALS AND METHODS:

Plant Material: *Cucumis melo* L. subsp. *agrestis* (Naudin) Pangalo was collected from the widely grown region of Southern Haryana in the month of June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium, and Museum Division, NISCAIR, New Delhi, vide reference number NHCP/NBPGR/2016-15.

A voucher specimen of the same has been retained in the Department for the future reference. The leaves and fruits of the plant were air-dried at room temperature and ground into a coarse powder for further use for the study.

Drugs and Chemicals: All organic solvents and other reagents were procured from SD Fine chemicals Ltd. Mumbai and were of analytical grade. Diclofenac sodium was obtained as a gift sample from Horizon bioceuticals Pvt. Ltd. Kala-amb (Himachal Pradesh).

Preparation of Extract: The powdered plant material (500 g) was extracted with ethanol using Soxhlet apparatus [10]. The extract obtained was concentrated by distilling off the solvent and recovering the same. The total aqueous extract was prepared by using the cold maceration method. The drug was macerated with distilled water for 24 h and then filtered.

The filtrates were evaporated to dryness. The dried extracts were kept in a desiccator. The percentage yield of *Cucumis melo* fruit ethanol and aqueous extracts were 8.41% w/w and 9.94% w/w respectively, and leaf ethanol and aqueous extracts were 12.72% w/w and 11.35% w/w respectively these extracts were further used for evaluation of the *in-vitro* anti-inflammatory activity.

Preliminary Phytochemical Screening: Various chemical tests were performed [10, 11] using dried ethanol and aqueous extracts to detect the presence of phytoconstituents like carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids, and saponins.

***In-vitro* Anti-inflammatory Activity:**

1. **Inhibition of Albumin Denaturation:** The different concentrations of plant extracts ranging from 100-500 µg/ml were prepared. The reaction mixture was consisting 1ml of test extracts and 1% aqueous solution of bovine albumin solution. These prepared solutions were incubated at 27 ± 1 °C for 15 min. Then, the reaction mixtures were kept at 70 °C in a water bath for 10 min to induce denaturation. The solutions were cooled, and turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as a standard drug in the concentration of 100-500 µg/ml and treated similarly as test extracts. Percentage inhibition of denaturation was calculated using the control in which no drug was added. Each experiment was done in triplicate, and the average was taken. The percentage inhibition of protein denaturation was calculated by following equation [12, 13].

$$\% \text{ Inhibition of protein denaturation} = 100 \times [A_1 - A_2 / A_1]$$

Where: A_1 = Absorbance of control; A_2 = Absorbance of test/standard sample with albumin solution.

2. **Membrane Stabilization Test:**

Preparation of Human Red Blood Cells (HRBCs) Suspension: Fresh whole human blood (5 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with an equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline [12, 14].

Hypotonicity Induced Haemolysis: The reaction mixture (4.5 ml) was containing of 1 ml of phosphate buffer, 2 ml of hypo saline, and 0.5 ml of HRBC suspension and 1 ml test extracts of different concentrations (100-500 µg/ml). Diclofenac sodium 100 µg/ml was used as a standard drug. In control instead of test extracts, the only saline was added. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm [5-17]. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectro-photometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in control as 100%.

Percentage protection = 100 - (Absorbance sample / Absorbance control) × 100

Statistical Analysis: Data was analyzed by ANOVA, followed by Dunnett's t-test. The values were represented as mean ± S. E. M (standard error of mean).

RESULTS:

Preliminary Phytochemical Screening: Preliminary phytochemical screening of the extracts revealed the presence of carbohydrates, phenols, tannins, saponins, and flavonoids.

***In-vitro* Anti-inflammatory Activity:** The extracts were found to be effective as an anti-inflammatory agent and showed significant activity as compared to the standard drug. The anti-inflammatory activity was also shown in a concentration-dependent manner, and the activity was increased on increasing the concentration of extracts. Hence, maximum activity was reported at the highest concentration taken for evaluation. The ethanol extract was found to be more effective than aqueous extract and showed 78.23 ± 0.53 (leaf), 115.26 ± 0.34 (fruit) % inhibition of protein denaturation while 68.03 ± 0.02 (leaf), 93.34 ± 0.26 (fruit) % inhibition was shown by aqueous extract respectively at the concentration of 500 µg/ml which was the highest concentration evaluated.

The percentage inhibition by the extracts (fruit and leaf) at different concentrations and their comparison with the standard drug are shown in **Table 1** and **Table 2**. IC_{50} values for ethanol (fruit and leaf) extracts were 155 and 180 µg/ml. IC_{50} values for aqueous extract (fruit and leaf) were 300 and 360 µg/ml, respectively, which further, confirmed that ethanol extract was most effective.

TABLE 1: PERCENTAGE INHIBITION OF PROTEIN DENATURATION BY STANDARD DRUG AND LEAF EXTRACTS OF *CUCUMIS MELO L.* SUBSP. *AGRESTIS* (NAUDIN) PANGALO

Conc. (µg/ml)	Percentage inhibition of protein denaturation		
	Diclofenac sodium	Ethanol extract	Aqueous extract
50	62.51 ± 0.25**	20.63 ± 0.14*	13.41± 0.08*
100	76.31 ± 0.33**	34.7± 0.32*	23.41 ± 0.12*
200	92.83 ± 0.22***	52.04 ± 0.25**	34.13 ± 0.29**
300	110.05 ± 0.07***	69.26 ± 0.05**	43.05 ± 0.18**
400	130.31 ± 0.14***	83.21 ± 0.04***	54.11± 0.01**
500	150.26 ± 0.17***	91.34 ± 0.03***	68.03 ± 0.02**
IC50	35.20 ± 0.24***	180 ± 0.02*	360 ± 0.05*

All data were expressed as mean ± standard error mean (SEM) data was analyzed by ANOVA followed by Dunnett's t-test at *** P<0.001, ** P<0.01, * P<0.05

TABLE 2: PERCENTAGE INHIBITION OF PROTEIN DENATURATION BY STANDARD DRUG AND FRUIT EXTRACTS OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO

Conc. (µg/ml)	Percentage inhibition of protein denaturation		
	Diclofenac sodium	Ethanol extract	Aqueous extract
50	62.51 ± 0.25**	32.31 ± 0.12*	18.11 ± 0.15*
100	76.31 ± 0.33**	44.13 ± 0.41*	27.01 ± 0.32*
200	92.83 ± 0.22***	58.24 ± 0.05**	39.51 ± 0.21**
300	110.05 ± 0.07***	72.42 ± 0.21**	50.15 ± 0.10**
400	130.31 ± 0.14***	96.51 ± 0.10***	71.26 ± 0.52**
500	150.26 ± 0.17***	115.26 ± 0.34***	93.34 ± 0.26***
IC50	35.20 ± 0.24***	155 ± 0.02**	300 ± 0.05*

All data were expressed as mean ± standard error mean (SEM) data was analyzed by ANOVA followed by Dunnett's t-test at *** P<0.001, ** P<0.01, * P<0.05.

TABLE 3: PERCENTAGE INHIBITION OF HYPOTONICITY INDUCED HAEMOLYSIS BY STANDARD DRUG AND LEAF EXTRACTS OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO

Conc. (µg/ml)	Percentage inhibition of hypotonicity induced hemolysis		
	Diclofenac sodium	Ethanol extract	Aqueous extract
50	52.12 ± 0.04**	20.31 ± 0.12*	13.32 ± 0.05*
100	60.32 ± 0.02**	31.11 ± 0.01*	20.30 ± 0.02*
200	76.27 ± 0.01***	48.36 ± 0.05*	36.71 ± 0.17*
300	92.26 ± 0.07***	65.72 ± 0.01**	53.03 ± 0.05**
400	108.35 ± 0.04***	83.01 ± 0.16**	70.20 ± 0.02**
500	125.62 ± 0.03***	97.63 ± 0.04***	88.04 ± 0.06***
IC_{50}	48.27 ± 0.04***	230 ± 0.14*	290 ± 0.05*

All data were expressed as mean ± standard error mean (SEM) data were analyzed by ANOVA followed by Dunnett's t-test at *** P<0.001, ** P<0.01, * P<0.05.

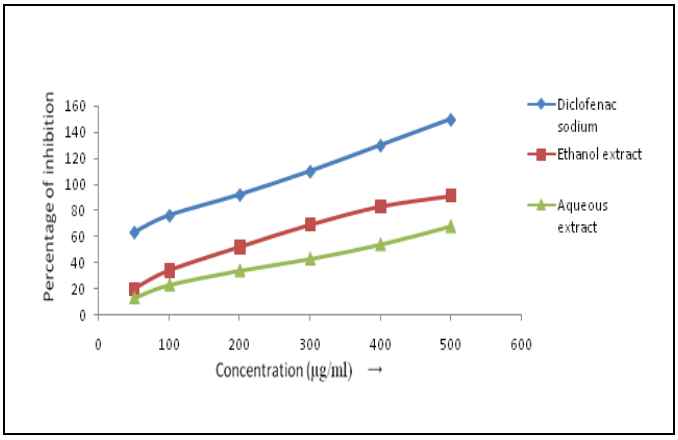

FIG. 1: EFFECT OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO (LEAF) ON PROTEIN DE-NATURATIO

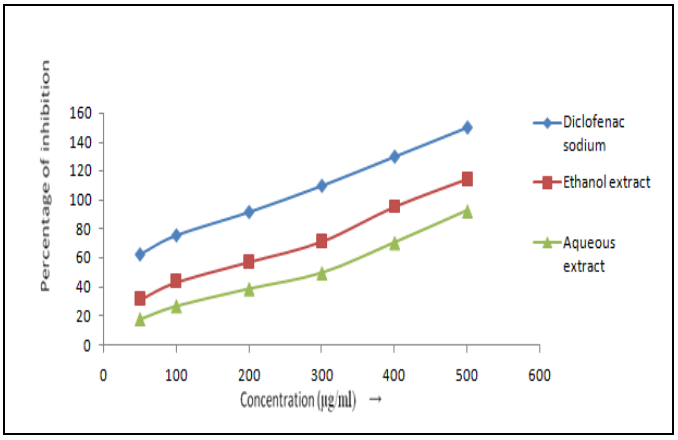

FIG. 2: EFFECT OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO (FRUIT) ON PROTEIN DENATURATION

TABLE 4: PERCENTAGE INHIBITION OF HYPOTONICITY INDUCED HAEMOLYSIS BY STANDARD DRUG AND FRUIT EXTRACTS OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO

Conc. (µg/ml)	Percentage inhibition of hypotonicity induced haemolysis		
	Diclofenac sodium	Ethanol extract	Aqueous extract
50	52.12 ± 0.04**	32.06 ± 0.01*	19.24 ± 0.02*
100	60.32 ± 0.02**	41.73 ± 0.05*	28.51 ± 0.21*
200	76.27 ± 0.01**	56.08 ± 0.12*	42.13 ± 0.03*
300	92.26 ± 0.07***	72.43 ± 0.04**	55.47 ± 0.02**
400	108.35 ± 0.04***	92.54 ± 0.05***	69.15± 0.31**
500	125.62 ± 0.03***	109.33 ± 0.04***	90.05 ± 0.11***
IC_{50}	48.27 ± 0.04***	175 ± 0.02**	260 ± 0.03*

All data were expressed as mean ± standard error mean (SEM) data were analyzed by ANOVA followed by Dunnett's t-test at *** P<0.001, ** P<0.01, * P<0.05.

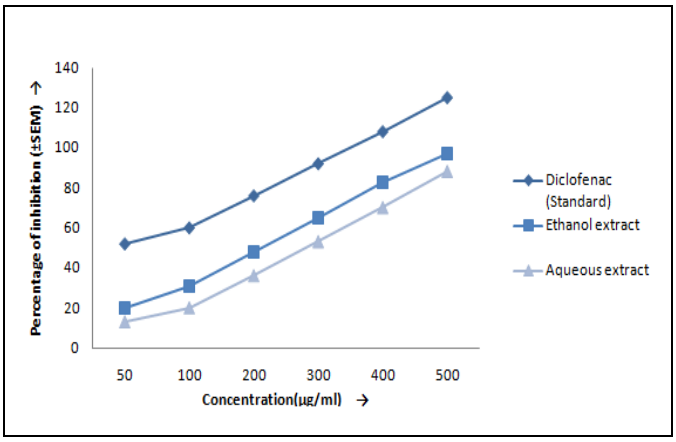

FIG. 3: EFFECT OF *CUCUMIS MELO* L. SUBSP. *AGRESTIS* (NAUDIN) PANGALO (LEAF) ON HYPOTONICITY INDUCED HAEMOLYTIC METHOD

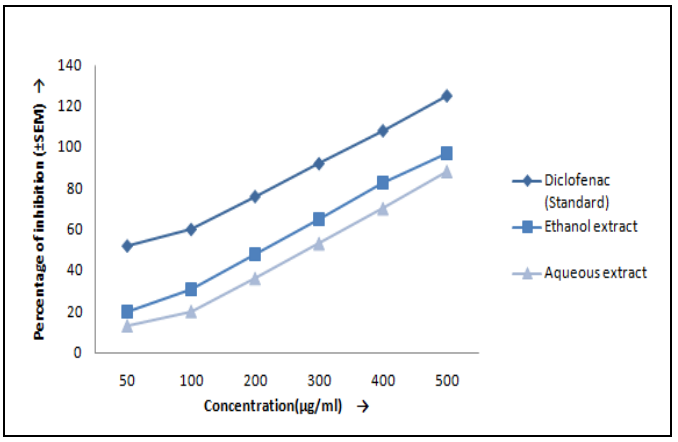

FIG. 4: EFFECT OF *CUCUMIS MELO* L. SUBSP. AGRESTIS (NAUDIN) PANGALO (FRUIT) ON HYPOTONICITY INDUCED HAEMOLYTIC METHOD

DISCUSSION: Most biological proteins lose their biological function when denatured. Denaturation of proteins is one of the well-defined causes of inflammation [18, 19]. In the present study, ethanol extract (fruit and leaf) have shown inhibition of thermally induced protein (albumin) denaturation in a dose-dependent manner. The IC_{50} values of ethanol extract of the fruit and leaf show the highest inhibition in albumin denaturation, and hypotonicity induced hemolysis method than the aqueous extract of fruit and leaf but their activity was less than standard diclofenac sodium 35.20 µg **Table 1, 2** and 48.27 µg **Table 3, 4** respectively. The lysosomal enzymes released during inflammation produce various disorders. The extracellular activity of lysosomal enzymes is 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.

CONCLUSION: The study revealed that *Cucumis melo* L. subsp. *agrestis* (Naudin) Pangalo leaves and fruits exhibit anti-inflammatory activity. The ethanol extract of fruit *Cucumis melo* L. subsp. *agrestis* (Naudin) Pangalo showed good anti-inflammatory activity than aqueous extracts. The future scope of study involves the extraction of the hydroalcoholic extract by novel extraction technique and isolation of phytoconstituents. Find out the mechanism responsible for the anti-inflammatory activity.

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CONFLICTS OF INTEREST: Nil

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