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## ***In vitro* studies on methanol extract of whole fruit of *Cucumis melo*. Linn. (F1 hybrid) with a focus on antiinflammatory activity**

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### **Abstract**

*Cucumis melo*; Linn, family: Cucurbitaceae, (Commonly known as Musk melon or sweet melon) is an annual creeper or climber, cultivated in various parts of the world including India for its edible fruits. The fruits are reported to have several benefits. They act as tonic, laxative, galactagogue, diuretic, strengthens the heart, the brain. In the current study, we evaluated the antiinflammatory potential of the methanol extract of whole fruit of *Cucumis melo*. The anti-inflammatory effects of various extracts of the fruits of this plant are already evaluated. The present study was aimed to explore some of the mechanism contributing to the anti-inflammatory activity of *Cucumis melo*, (F1 hybrid). We observed that the extract can inhibit trypsin, as well as the proinflammatory enzymes like cyclooxygenase and lipoxygenase *in vitro* (P<0.001). The extract also possess stabilizing potential on erythrocyte membrane *in vitro*. We also evaluated the potential to inhibit thermal degradation of proteins *in vitro*, but it was found to be insignificant.

**Keywords:** *Cucumis melo*, proteinase inhibition, denaturation of protein, Cyclo oxygenase inhibition, 5-Lipoxygenase

### **Introduction**

Plant based medicines are having an important role in treatment of many ailments. Most of the people rely on plant based drugs due to its easy availability decreased side effects and ease of administration compared to its synthetic counter parts. *Cucumis melo* Linn (Musk melon) is an annual climber or creeper plant belonging to cucurbitaceae family [1, 2]. It is cultivated in various parts of the world for its fruits. In india it is cultivated in many states, especially in hot and dry norther parts. Main parts used are pulp, root, seeds and seed oil [3, 4]. The fruits of the plant is reported to contain Carbohydrates, Fatty acids and phospholipids amino acids, Vitamin C, glycolipids, chromones, volatile compounds like terpenoids, apocaretenoids, several minerals, and reported to have many medicinal properties like asthma, auto immune diseases, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel disease, pelvic inflammatory disease, rheumatoid arthritis etc [4, 5] Some of these conditions are chronic in nature and requires long term pharmacotherapy. Synthetic drugs targets symptoms caused by specific diseases by assessing its pathophysiology scientifically, at the same time a herbal medicine boost up the body's own healing process. Herbal medicines act gently, by supporting the natural systems and mechanisms and make them most powerful. Symptom relief forms only a part of therapeutic strategies of medicinal plants [6].

Pharmacological study conducted on *Cucumis melo* indicated its effectiveness in the treatment of ailments like cardiovascular disorders, liver diseases, pain and inflammation, malignancy, and dysuria. Potent analgesic activity was reported for the methanolic extract of *Cucumis melo* seeds [7, 8]. It is reported that *Cucumis melo* can inhibit the influx and reduced LTB4 levels [9]. In the present study, we studied the effects of methanol extract of whole fruits of *Cucumis melo* (MECM) in carragenen induced rat hind paw oedema model and in various *in vitro* models like inhibition of total COX in LPS activated macrophages, *in vitro* inhibition of proteinases and inhibition of protein denaturation *in vitro*.

### **Materials and methods**

*Cucumis melo* fruits were procured from Vadanerkulam part of Tindivanam area in Villuparam district of Tamil Nadu state India. The entire fruits were washed, cut in to slices with stainless steel knife and the entire material including seeds were dried, communitated and.

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extracted with absolute methanol by Soxhlet extraction. The dried soft extract (MECM) was used for the study.

#### Inhibition of haemolysis<sup>[10, 11]</sup>

The test measures the ability of the extract to stabilize erythrocytes membrane and protect from haemolysis induced by hypotonic saline. We used the method explained by Shinde *et al* (1989), with slight modifications. 10 ml of bovine blood was collected in anticoagulant solution (ACD), from which erythrocytes were separated by centrifuging at 300 rpm for 10 minutes. The pellet were re suspended in 10 ml isosaline. 0.5 ml aliquotes of this solution were pipetted in to tubes (in triplicates) containing 12 ml phosphate buffer (2 g of NaH<sub>2</sub>PO<sub>4</sub>, 1.15 g of Na<sub>2</sub>HPO<sub>4</sub> and 9 g of NaCl in 1 litre of distilled water, final pH 7.2) and then added various concentrations of the extract in 1 ml isosaline. Diclofenac sodium was used as standard. Hyposaline was used as positive control and isosaline saline was used as the normal control. After incubation for 30 minutes at 37.5 °C, haemolysis was induced by addition of 2.5ml hyposaline and the contents of the tubes were centrifuged. The optical density of the supernatant was measured at 540 nm.

#### Inhibition of trypsin *in vitro*<sup>[12, 13]</sup>

To tubes containing 1 ml of MECM in concentrations of 100, 250 and 500 µg/ml (each in triplicate), added 1.0 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 0.06 mg trypsin. The final pH was adjusted to 6.3 using 1N HCl. The reaction mixtures were then incubated at 37°C for 5 min followed by addition of 1 ml of 0.8% (w/v) casein and further incubated for 20 minutes. After the incubation period, the reaction was stopped by the addition of 2 ml of 70% perchloric acid. The cloudy suspension was then centrifuged at 1000 rpm for 3 minutes and the absorbance of the supernatant was read at 280 nm against the buffer as blank. Protease inhibition cocktail was used as standard, tested at the same concentrations in a similar manner. The control consisted of all the reagents except the test or standard.

The percentage inhibition of proteinase inhibitory activity was calculated using the formula:

$$\text{Protein inhibitory activity (\%)} = [1 - (C-T)/C] \times 100$$

Where, C and T are the optical densities of Control and Test respectively.

#### Inhibition of denaturation of proteins *in vitro*<sup>[14]</sup>

The ability of MECM to inhibit the thermal denaturation of albumin was evaluated *in vitro* at varying concentrations. The reaction mixture consisted of 1 ml 1% w/v solution of bovine serum albumin (BSA) and the test solutions in concentrations of 100, 250 and 500 µg/ml. The standard used was acetyl salicylic acid in the same concentrations. For control, 1 ml of distilled water was used. All the concentrations were tested in triplicates.

The mixture of (BSA) and test compounds was incubated initially for 37°C for 20 minutes. Then the temperature was increased to 51°C and maintained at that temperature for another 30 minutes. After cooling to room temperature the optical density of the solutions were measured at 660 nm.

The inhibition of protein denaturation (%) was calculated by the following formula:

$$\% \text{ inhibition of albumin denaturation} = [1-T/C] \times 100$$

Where, C and T are the absorbance of control and test respectively.

#### *In vitro* assay of total COX and 5- LOX in LPS activated macrophages<sup>[16-18]</sup>

RAW 264.7 cell lines (NCCS) were cultured in DMEM containing 10% heat inactivated foetal bovine serum (FBS), L-glutamine, antibiotics and 1.5% Na<sub>2</sub>CO<sub>3</sub>, and the culture was activated by adding 1µl LPS by further Incubation for further 24 hours. The cells were then exposed to 100 µl of solution of MECM in DMSO, from solutions of 25,50 and 100 µg/ml solutions and incubated for 48 hours. The standard used was Diclofenac sodium at similar concentration. The cells the isolated and lysed by cell lysis buffer, centrifuged and the pellet suspended in a small amount of supernatant.

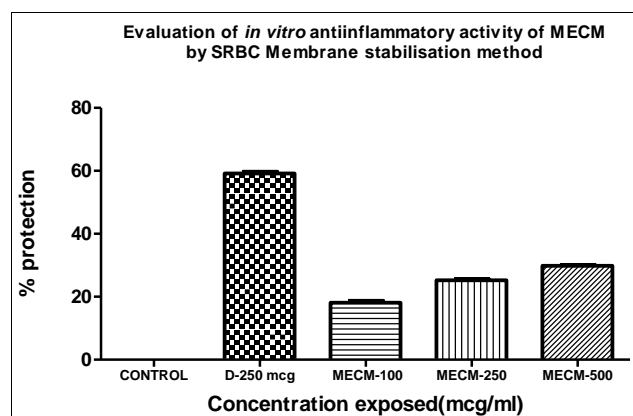
The pellet was then incubated with Tris-HCl buffer (pH 8), 5 mM glutathione and 5 m M haemoglobin for 1 min at 25°C. The reaction was initiated by the addition of 200 µM arachidonic acid and terminated after 20 min incubation at 37°C by addition of 10% TCA in 1N HCl, following centrifugal separation and addition of 1% thiobarbituric acid (TBA). COX activity was determined by reading absorbance at 632 nm.

For estimating total LOX, the cell lysate was suspended in Tris-HCl buffer (pH 8.1), and added solution of sodium linoleate (10 mM). The LOX activity was monitored as an increase of the absorbance at 234 nm which reflects the formation of hydroperoxy linoleic acid.

## Results

### Evaluation of erythrocyte membrane stabilization activity of MECM *in vitro*

It was observed that methanol extract of *Cucumis melo* (MECM) have a potential to stabilize erythrocyte membrane *in vitro* and prevent hyposaline induced haemolysis. It was found that the percentage inhibition produced by Diclofenac sodium in concentration of 250 µg of 59.2 ± 0.514 (Mean ± SEM). The percentage inhibition of MECM at concentrations of 100,250 and 500 µg were 18.1 ± 0.614 %, 25.3 ± 0.292 % and 29.8 ± 0.293 respectively. (Significant, p<0.01, against control).



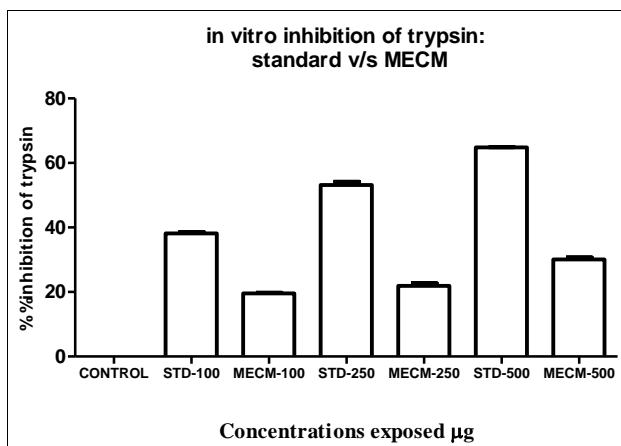
**Fig 1:** Comparison of % inhibition of hyposaline induced haemolysis of bovine erythrocytes *in vitro*. X- axis shows concentration, Y- axis shows % inhibition of haemolysis. MECM versus standard (Diclofenac sodium).

### Inhibition of trypsin *in vitro*

It was observed that both MEM and the standard protease inhibition cocktail inhibited trypsin activity *in vitro* at significant levels. (p<0.01). The results are plotted in fig.2.

The percentage inhibition of trypsin activity exhibited by MECM at concentrations of 100,250 and 500 µg were 38.15 ± 0.395 %, 53.15 ± 1.033% and 64.812 ± 0.109% respectively.

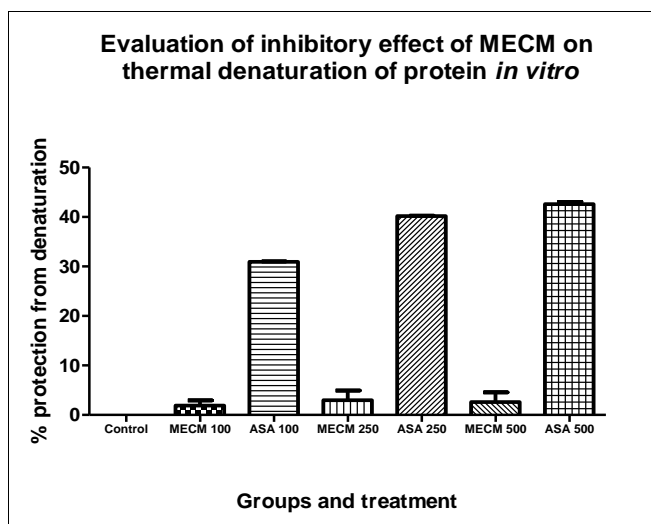
The inhibition shown by MECM for similar concentrations were 19.59± 0.151%, 21.92 ±0.783% and 30.13 ± 0.592% respectively.



**Fig 2:** Comparison of inhibitory activity on trypsin *in vitro*: X- axis depicts concentrations of both standard protease inhibitor and MECM, Y- axis shows % inhibition of trypsin activity calculated from change in optical density.

**Inhibition of denaturation of proteins *in vitro***

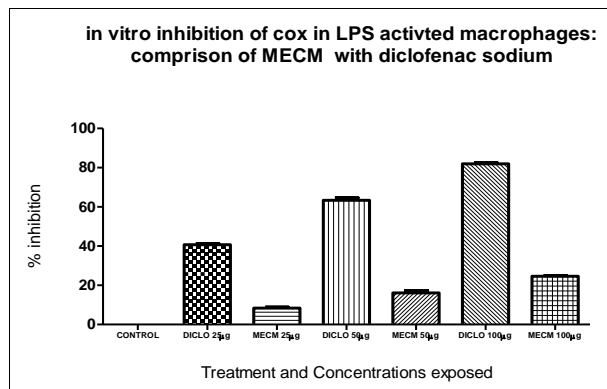
The test for evaluation of inhibition of protein denaturation by heat *in vitro* showed that MECM has an insignificant (p<0.05) effect. The results of the study is plotted in fig.3



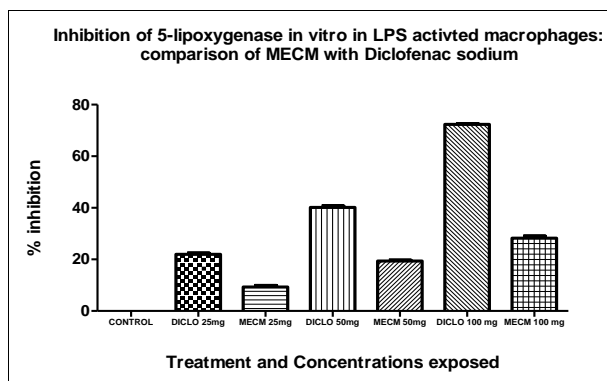
**Fig 3:** Inhibition of heat induced denaturation of proteins *in vitro*: omparison of % inhibition exhibited by MECM versus acetyl salicylic acid>X- xis shows concentrations exposed, Y- axis shows % inhibition of denaturation, calculated from change of Optical density.

**Inhibition of COX and 5-LOX in LPS activated macrophages *in vitro*.**

The levels pro-inflammatory enzymes OX and LOX in macrophages activated by bacterial lipopolyscharides were found to be significantly reduced after exposure to MECM. The results of the study is shown in table 1 and fig 4(a) and 4(b).



**Fig 4(a):** *In vitro* inhibition of COX in LPS activated cells: a comparative depiction. X- axis shows concentrations of MECM and diclofenac sodium exposed. Y- axis shows percentage inhibition of enzyme activity.



**Fig 4(b):** *In vitro* inhibition of 5-LOX in LPS activated cells: a comparative depiction. X- axis shows concentrations of MECM and diclofenac sodium exposed. Y- axis shows percentage inhibition of enzyme activity.

**Table 1:** Comparison of percentage inhibition of COX and 5-Lipoxygenase *in vitro* in lipo polysaccharide activated macrophage cells by *Cucumis melo* extract (methanol) and Diclofenac sodium at different concentrations.

Concentration (µg/ml)	% inhibition of Total COX (Mean ± SEM)		% inhibition of Total LOX (Mean ± SEM)	
	MECM	DICLOFENAC	MECM	DICLOFENAC
25	8.395 ± 0.451	40.71 ± 0.531*	9.286 ± 0.619*	21.97 ± 0.470
50	16.13 ± 1.276	63.39 ± 1.2*	19.40 ± 0.323*	40.15 ± 0.619
100	24.61 ± 0.390	81.97 ± 0.599*	28.25 ± 0.078*	72.36 ± 0.268

Significant, p<0.001, N=3. ANOVA and post hoc test by Dunnett's multiple comparison with control group.

## Discussion

In the present study we have tried to evaluate the anti-inflammatory potential of methanol extract of musk melon in various *in vitro* models. Inflammation is one of the body's response to various noxious stimuli having immunological basis. It involves multiple and complex pathways of cascades and activation, producing various acute and long lasting effects in the body. These immunological reactions are triggered and progressed by several mediators like histamine, kinins, Leukotrienes, prostaglandins etc. The first steps of inflammatory process involves activation of immunological cells leading to release of these mediators, which cause vasodilation and increased vascular permeability. These changes result in exudation of fluids, proteins and leukocytes in to the site of inflammation<sup>[19, 20]</sup>.

In the present study we observed that methanol extract of *Cucumis melo* significantly protect erythrocytes from haemolysis ( $P < 0.001$ ). One of the important mediators of inflammation is histamine, which plays a key role in the vascular events of inflammation. Mast cell is the store house of histamine, and is released through a G-protein mediated transduction mechanism<sup>[21]</sup>. Agents which stabilize mast cell membrane will reduce mast cell degranulation and release of histamine. NSAIDs and steroids can stabilize the lysosomal membrane and prevent release of pro-inflammatory cytokines. Human erythrocyte membrane behaves in a similar manner to that of lysosomal membrane, and agents that can stabilize human erythrocyte membrane are thought to stabilize lysosomal membrane and hence possess anti-inflammatory activity<sup>[22]</sup>.

Flavonoids can interact with cell membranes in multiple ways can affect cell signalling mechanisms<sup>[23]</sup>, many plants containing flavonoids exhibit membrane stabilization potential and is found to possess anti-inflammatory activity<sup>[24]</sup>. So the flavonoids present in the extract must be contributing significantly to the membrane stabilizing potency of MECM and hence to its anti-inflammatory activity.

*In vitro* inhibition of proteinases like trypsin is considered to be a simple technique to evaluate anti-inflammatory as well as anti-arthritis activity. The role of proteinases in the pathogenesis and progression of inflammatory process is well established. Neutrophils carry many serine proteinases in their granules. The role of proteinase in inflammation is well established. Proteinases can produce damage to tissues in inflammation. It has been reported that protease inhibitors can interfere with liposomal functions and activation of inflammatory cells by microbial lipopolysaccharides<sup>[25, 26]</sup>.

We found out that MECM did not afford any protection against thermal denaturation of albumin *in vitro*. But it significantly inhibited the action of trypsin *in vitro* ( $p < 0.001$ ) in a concentration dependent manner. It is well established that *Cucumis melo* contain an anti protease principles and such a compound named Cucumisin is already isolated from the melon fruits<sup>[27]</sup>.

Phospholipases have a cardinal role in generation of two important groups of inflammatory mediators: Prostaglandins and leukotrienes. Leukotrienes are potent proinflammatory mediators that contribute to pathophysiology of inflammatory conditions. They mediate eosinophile migration, neutrophile function and micro vascular permeability<sup>[28]</sup>. Leukotrienes are synthesised from arachidonic acid by the enzyme Lipoxygenase. Our study showed that methanol extract of *Cucumis melo* can inhibit both cyclo oxygenase and lipoxygenase *in vitro*, in concentration dependent

manner. (Significant,  $p < 0.001$ ). Many studies suggest that polyphenols can inhibit cyclooxygenase and lipoxygenase<sup>[29, 30]</sup>. Many workers suggest that terpenes and terpenoids can inhibit expression of pro-inflammatory cytokines and mediators through NF-kappa B<sup>[31, 32]</sup>.

Our present study suggests that multiple mechanisms are operational in anti-inflammatory potential of *Cucumis melo*. This must be indeed, attributed to the presence of multiple pharmacologically active phytochemicals, especially terpenoids, polyphenols and flavonoids.

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