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ELUCIDATION OF *IN VITRO* ANTI- INFLAMMATORY ACTIVITY OF CINNAMOMUM ZEYLANICUM BY HRBC MEMBRANE STABILIZATION AND PROTEIN DENATURATION

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Abstract

The bark extract of *Cinnamomum zelanicum* were assessed for In-vitro Anti-Inflammatory activity by HRBC membrane stabilization method and Inhibition of protein denaturation method. The presence of flavanoids has been reported earlier in *C.zeylanicum*. Since the flavanoids have remarkable anti-inflammatory activity, so the present work aims at evaluating the anti-inflammatory activity of *C.zeylanicum*. Different concentrations of extract were compared against standard Diclofenac sodium. Maximum stabilization in our study is 68.41 % at 1000 µg/ml and maximum inhibition is 78.30% at 1000 µg/ml. Therefore, our studies support the use of *C.zeylanicum* in treating inflammation.

KEY WORDS: *Cinnamomum zelanicum,* HRBC, Diclofenac sodium, Anti-Inflammatory.

1. INTRODUCTION

Cinnamon (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India. The flowers, which are arranged in panicles, have a greenish colour and have a distinct odour. The fruit is a purple one-centimetre berry containing a single seed. Its flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition [1].

In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. *Cinnamon* is high in antioxidant activity. The essential oil of *Cinnamon* also has antimicrobial properties, which aid in the preservation of certain foods. "*Cinnamon*" has been reported to have remarkable pharmacological effects in the treatment of type II diabetes. *Cinnamon* has traditionally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold and aid digestion. [2]

Inflammation is a physiologic series of responses generated by the host in response to infection or other insults. Inflammation can have rapid onset and last a short period of time (acute inflammation), or it can persist due to a continuous stimulus or injury (chronic inflammation). The initial events of inflammation are derived from vascular reactions at the site of injury. Vascular changes are important for the induction of the response and are characterized by redness, heat, and swelling, usually accompanied by pain and loss of function, and collectively represents the "cardinal signs" of inflammation. These signs of inflammation are the result of vasodilatation and increased vascular permeability, leading to exudation of fluid and plasma proteins and recruitment of leukocytes to the site of injury. [3]

2. MATERIALS AND METHODS

2.1 Extract Preparation

Samples were collected and shade dried for 4 weeks until they show consistent weight. The dried parts were later grinded to powder. The dried parts were used for ethanolic extract using Soxhelet Apparatus. The extracts were filtered using Whatmann's No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Powdered extract was stored in air tight container for further use. [4]

2.2 In Vitro Anti-Inflammatory Activity

2.2.1 HRBC Membrane Stabilizing Method

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was

incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (500 µg/ml) was used as reference standard and a control was prepared by omitting the extracts and using distillled water. Product control lacks the red blood cells. [1, 5, 6, 7, 8] The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$percentage\ stabilization = 100 - \frac{O.D.of\ test - O.D.of\ product\ control}{O.D.\ \ of\ control} * 100$$

2.2.2 Inhibition of Protein Denaturation method

The reaction mixtures (0.5ml) consisted of 0.45ml bovine serum albumin (BSA) (5% aqueous solution) and 0.05ml of test compound (100 and 250 mg/ml of final volume). The pH was adjusted at 6.3 using a small amount of IN HCl. The samples were incubated at 37°C for 3 min. After cooling the samples 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. For control tests 0.05ml distilled water was used instead of extracts while product control lacked bovine serum albumin [1, 4, 9, 10 & 11]. The percentage inhibition of protein denaturation was calculated as follows-

$$percentage\ inhibition = 100 - \frac{O.D.of\ test - O.D.of\ product\ control}{O.D.\ \ of\ control} * 100$$

2.2.3 Statistical Analysis

Statistical Analysis was done by one way ANOVA following TURKEY test.

3. Results

From the result of present study, the bark extract of *C. zeylanicum* was subjected to In vitro anti-inflammatory activity in various concentrations i.e. 100, 200, 400, 600, 800, 1000 µg/ml and the percentage stabilization of different extracts by HRBC membrane stabilization method is shown in Table 1. The extract demonstrated a significant ($P<0.001$) anti-inflammatory activity at all the doses tested compared to control. The percentage membrane stabilization shows increase with the increase in concentration of the extract.

Table-1: Effect of *C.zeylanicum* bark extract on membrane stabilization.

Ethanol Extract Concentration (µg/ml)	% stabilization by ethanol extract	% stabilization by Diclofenac sodium at 500 µg/ml
100	30.16	73.49
200	41.29	
400	43.77	
600	54.71	
800	60.43	
1000	68.41	

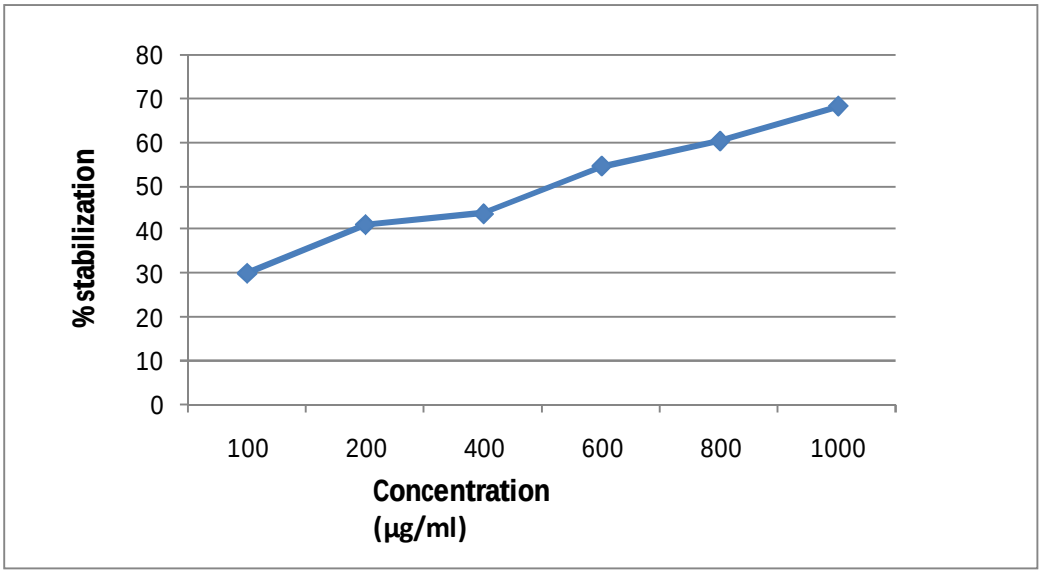

Figure-1: Effect of *C.zeylanicum* bark extract on membrane stabilization

The percentage inhibition of different extracts by Inhibition of Protein Denaturation method is shown in Table 2. The percentage inhibition is 32.81 at 100 µg/ml; 49.88 at 200 µg/ml; 63.13 at 400 µg/ml; 76.73 at 600 µg/ml and 85.71 at 800 µg/ml. The percentage inhibition shows increment with the increase in extract concentration. The extract concentration from 100 µg/ml to 600 µg/ml demonstrated extremely significant ($P<0.001$) anti-inflammatory activity at the doses tested compared to control. The concentration 800 µg/ml showed a significant ($P<0.01$) anti-inflammatory activity at the dose tested compared to control.

Table-2: Effect of *C.zeylanicum* bark extract on inhibition of protein denaturation

Extract Concentration (µg/ml)	% inhibition by ethanol extract	% inhibition by Diclofenac sodium at 500 µg/ml
100	32.81	87.46
200	49.88	
400	63.13	

600	76.73	
800	85.71	

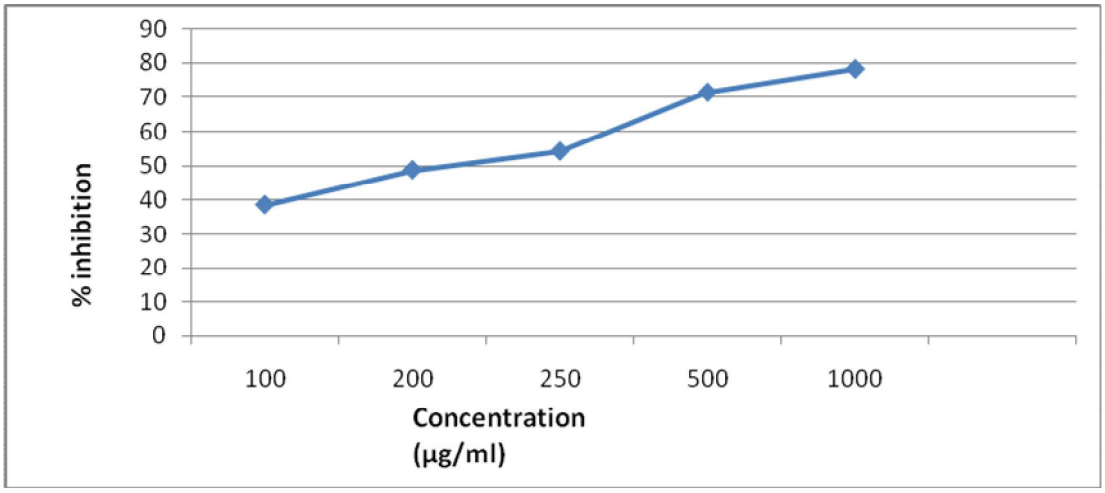

Figure 2: Effect of *C.zeylanicum* bark extract on inhibition of protein denaturation

4. DISCUSSION

Cinnamomum zeylanicum has been reported with the presence of flavanoids which has a remarkable anti-inflammatory activity. Therefore different concentrations of its extract have been taken for assessing the in vitro anti-inflammatory activity.

Bark of *Cinnamomum zeylanicum* exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. [12]

Denaturation of protein is one of the causes of rheumatoid arthritis that is documented. Production of auto antigen in certain arthritis disease may be due to denaturation of protein. [3] Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. From the present study, it can be stated that the extract of *C.zeylanicum* is capable of controlling the production of auto antigen and thereby it inhibit the denaturation of protein and its effect was compared with the standard drug.

5.CONCLUSION

The In vitro study on bark of C.zeylanicum showed the presence of significant anti inflammatory activity. Due to the presence of active principles such as flavonoids and tritrepenoids and related polyphenols may responsible for this activity. Further investigations are anticipated to identify the active components and lead to their further clinical use.

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