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DETERMINATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF COMBINATION OF HERBAL PLANTS

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ABSTRACT

Inflammation is a healthy process resulting from some disturbance or disease. The signs of inflammation are redness, elevated heat, swelling, pain, loss of function. Inflammation process plays a protective role in our body and in some conditions produces some negative effects such conditions include the inflammatory disorders rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis. For overcoming this problem, search of newer drugs is very requisite and necessary and there are many of phytoconstituents present in plants which are playing a very important role in the treatment of inflammation. The present study shows some plant phytochemicals which having anti-inflammatory activity that have been tested *in vitro* inflammatory methods using modern scientific technique.

KEYWORDS

Diclofenac, HRBC suspension, Alseiver solution and Prostaglandins.

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INTRODUCTON

Plants are the main stay of medicine credited with mystical and almost supernatural powers of healing. Herbs play a significant role especially in modern medicines. The growing interest in herb is based on the belief that the plants have a vast potential for use as a curative medicine and the basis that "natural" equal 'harmless'. Medicinal plants are very promising for the development of new drugs traditionally different plants are grown to have different efficacy for testing various types of diseases.

Herbal drug development is an aspect of pharmacology to know how plants are used for their medicinal potential. From the point of view of drug

development, the important aspects of which are to be justified include the correct part of the plant which have the active chemical constituent essential for the pharmacological activity, preliminary phytochemical screening, the selection of solvents, extraction process, and the other methodology are the important aspect to evaluate the pharmacological activity of the plants. In this point of view, we have selected the plants of genus *Zingiber* and *Curcuma* for the development of successful herbal drug formulations. The genus *Curcuma and Zingiber* belongs to family *Zingiberaceae* is a family of flowering plants made up of about 50 genera with a total of about 1600 known species of aromatic perennial herbs with creeping horizontal or tuberous rhizomes distributed throughout tropical Africa, Asia and the Americas

Inflammation

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair.

MATERIAL AND METHODS

Reagents and Chemicals

Dextrose, Sodium Chloride, Sodium Citrate, Citric Acid, Methanol

Apparatus

Funnel, China dish, Test Tubes

Instruments

Centrifuge, U.V- VISIBLE Spectrophotometer

Methods for Anti-Inflammatory Studies

***In-Vivo* Methods**

Carrageen an induced paw oedema in rats

Oxazolone induced ear oedema in mice

***In-Vitro* Methods**

Human red blood cell (HRBC) membrane stabilization method

Protein denaturation method.

***In-vitro* Anti-inflammatory activity: The human red blood cell (HRBC) membrane stabilization method**

The blood is collected from healthy human volunteer who have not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alseiver solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl in water) and centrifuged at 3,000 rpm for 10mins. The packed cells are washed with isosaline and a 10% suspension is made.

Various concentrations of extracts are prepared (200 and 400µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension is added. It is incubated at 37C for 30 min and centrifuged at 3,000rpm for 20 min and the haemoglobin content of the supernatant solution is estimated on UV spectrophotometer at 560nm. Diclofenac (200 and 400µg/ml) is used as reference standard and a control is prepared by omitting the extracts.

%Inhibition of haemolysis = {(OD control-OD test samples)/OD control} x 100

RESULTS AND DISCUSSION

From the results, it is concluded that the combination of Methanolic extracts of turmeric (*C.longa*) and ginger (*Z. officinale*) possess greater anti-inflammatory activity than individual plant extract. Here, the anti-inflammatory activity was assessed by in vitro screening method such as HRBC method. During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with variety of disorders. 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 extracellular release. It is reported that the ethanolic extract of *C.longa* showed 25.17%, 38.92% and

Z.officinale showed 9.52%, 18.24% and in combination, it showed 24.84%, 58.45% inhibition of haemolysis at a concentration of 200µg/ml, 400µg/ml respectively.

Phytochemical Screening

Table No.1: Data showing preliminary phyto-chemical screening of the various extracts of *Curcuma longa and Zingiber officinale*

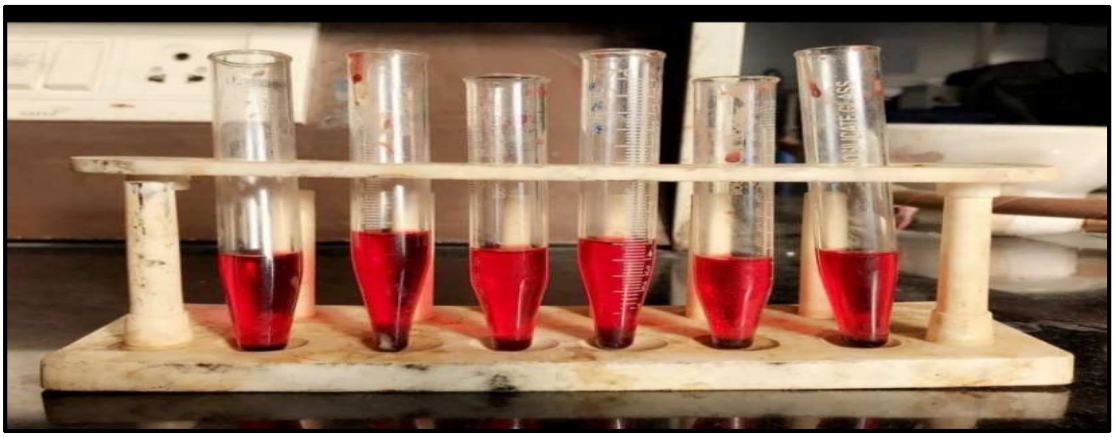
S.No	Contents	*Turmeric* Extract	*Ginger* Extract
1	Alkaloids	+	+
2	Saponins	-	+
3	Glycosides	-	+
4	Carbohydrates	-	+
5	Flavonoids	-	+
6	Steroids	+	+
7	Terpinoids	+	+

(+) = Present, **(-)** = Absent

Table No.2: Effect of Methanolic extract of various plants on heat induced haemolysis of erythrocytes

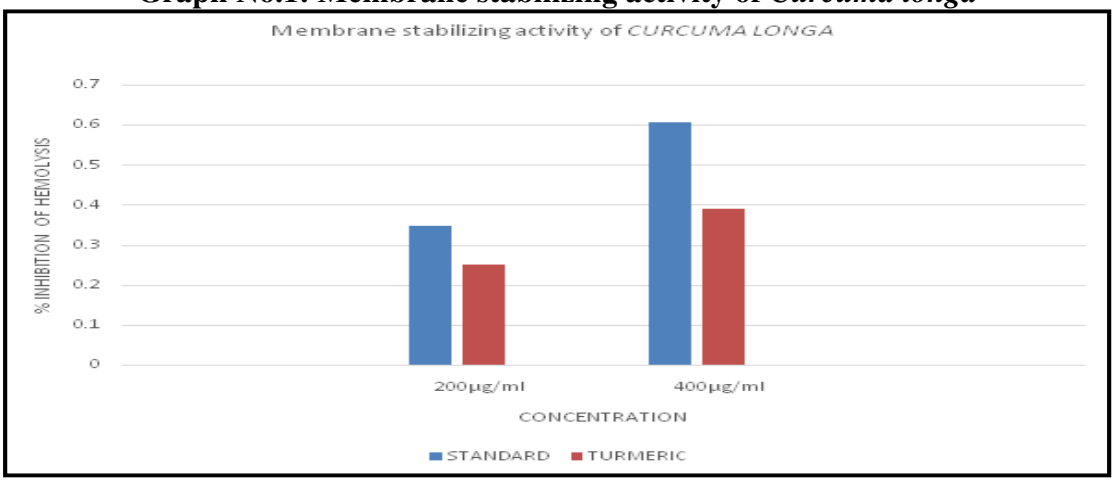
S.No	Treatment	Concentration (µg/Ml)	Absorbance at 560nm	% Inhibition of Haemolysis
1	Control	-	0.147±0.035	_
2	Diclofenac (standard)	200	0.096±0.003	34.69%
		400	0.058±0.002	60.54%
3	*Curcuma*	200	0.11±0.004	25.17%
		400	0.089±0.015	38.92%
4	Zingiber	200	0.133±0.010	9.52%
		400	0.12±0.003	18.24%
5	Combination	200	0.11±0.001	24.84%
		400	0.061±0.004	58.45%

Each value represents the mean ± SD.



Different concentrations of turmeric, ginger and combination of HRBC suspension

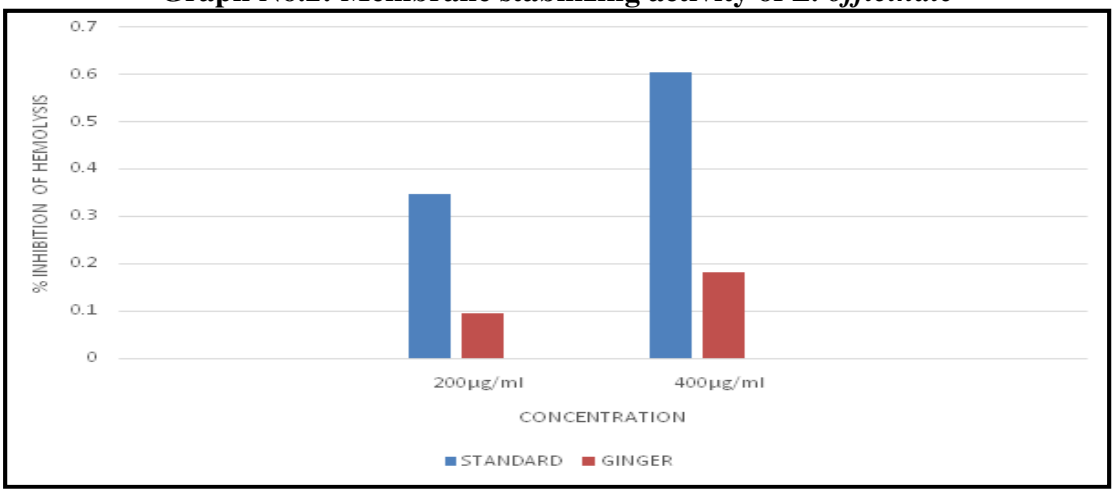
Graph No.1: Membrane stabilizing activity of *Curcuma longa*



X-axis:- Concentration

Y-axis:- % inhibition of haemolysis

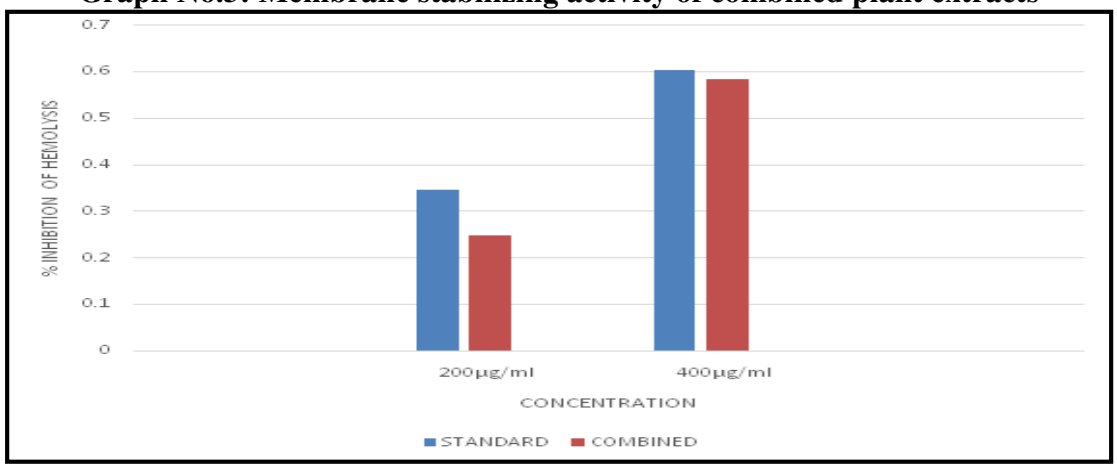
Graph No.2: Membrane stabilizing activity of *Z. officinale*



X-axis:- Concentration

Y-axis:- % inhibition of haemolysis

Graph No.3: Membrane stabilizing activity of combined plant extracts



X-axis:- Concentration
Y-axis:- % inhibition of haemolysis

CONCLUSION

In the present investigation, the results indicate that the ethanolic leaf extracts of *C.longa* and *Z.officinale* possess anti-inflammatory activity properties. The protective effect against membrane stabilization is known to be a good index of anti-inflammatory activity. From the present study, it is concluded that combination of *C.longa* and *Z.officinale* (400µg/ml) possesses the highest anti-inflammatory activity when compared with individual extracts of 200µg/ml 400µg/ml.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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