

RESEARCH ARTICLE

***In vitro* anti-inflammatory activity of different extracts of  
Citrus lemon peel**

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**ABSTRACT**

The in-vitro anti-inflammatory activities of different extracts of *Citrus lemon* peel (Rutaceae) were investigated by human red blood cell membrane stabilization (HRBC) method. The alcoholic extract showed significant membrane stabilizing action on human red blood cell membrane. The HRBC Membrane stabilization activity of the alcoholic extract of *Citrus lemon* at concentration 400µg/ml showed 63% inhibition of denaturation in hypotonic solution while standard Aspirin 100µg/ml showed 75.34% inhibition of denaturation. Moreover, the extracts showed equipotent activity to Aspirin.

**Keywords:** Anti-inflammatory activity, *Citrus lemon*, HRBC method, leaves.

**INTRODUCTION**

Herbal medicine is the use of plants and plant extracts to treat disease, something mankind has always done. Herbal medicine exists in many local varieties depending on the regional flora. Many modern drugs were originally extracted from plant sources, even if they are now made synthetically, and many other drugs are descended from plant substances.<sup>[1,2]</sup> The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition. For instance, Aspirin, the original non-steroidal anti-inflammatory drug (NSAID).<sup>[3,4]</sup> Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process.<sup>[5,6]</sup> *Citrus lemon* (Rutaceae) or its constituents exhibited various biological activities such as antibacterial, antifungal, antiviral, antitumor and antidiabetic effects.<sup>[5,6]</sup> The greatest interest, however, has been focused on their anti-cholesterolemic and antithrombotic activities. In this work the various extracts of *Citrus lemon* peel were studied for its *in vitro* anti-inflammatory activities.<sup>[7, 8, 18]</sup>

**MATERIALS AND METHODS**

**Preparation of extracts**

*Citrus lemon* peel was collected from Bahuwala, Dehradun and authenticated by Mr. S. K. Srivastava, Department of Botanical Survey of India, and Dehradun, U.K. The peel of lemon dried, crushed in motor pestle and passed through sieve no. 44 and then extracted by water, ethanol and hydroalcohol by cold maceration process. Then extract filter by using filter paper. The filtrate is placed in china disc and evaporates the filtrate. Finally collect the crude extract. Calculate its % yield.

**Qualitative phytochemical analysis**

The preliminary chemical tests were carried out for the extract of lemon to identify the presence of various phytoconstituents.<sup>[7]</sup>

***In-Vitro* Anti-Inflammatory Activity**

**The Human Red Blood Cell (HRBC) Membrane Stabilization 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 (200 and 400 µg/ml) 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 on UV spectrophotometer at 560 nm. Aspirin was used as reference standard and a control was prepared by omitting the extracts. [8, 9]

$$\% \text{ Protection} = \frac{100 - \text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

## RESULT AND DISCUSSION

Inflammation is the integral part of the body's defense mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation. Tissue infection is a prototype of inflammatory response. Anti-inflammatory drugs inhibit different stages of inflammation. Lemon is one of the most frequently used drug in the traditional and folklore systems of medicine. The maximum % yield (11.5%) obtained from the water extract. The HRBC Membrane stabilization method was used for in-vitro anti-inflammatory activity of the water, ethanol and hydroalcohol extracts of Lemon. The HRBC Membrane stabilization activity of the ethanolic extract of lemon at concentration 400µg/ml showed 81.81% inhibition of denaturation in hypotonic solution while standard Aspirin 100µg/ml showed 89.61% inhibition of denaturation. The above result revealed that ethanolic extract of lemon peel has some active principles which shows promising anti inflammatory activity.

TABLE No. 1: % yield of the different extract of lemon peel.

Solvent	% yield
Water	11.5%
Ethanol	5.7%
Hydroalcohol	8.5%

TABLE No. 2: % inhibition of different extracts of the lemon peel.

S. No.	Type of extract	Concentration(µg/ml)	Absorbance	% Inhibition
1	Control	----	0.154	
2	Water	10	0.093	39.61038961
3	Water	25	0.082	46.75324675
4	Water	50	0.072	53.24675325
5	Water	100	0.064	58.44155844
6	Water	200	0.041	73.37662338
7	Water	400	0.038	75.32467532
8	Alcohol	10	0.086	44.15584416

9	Alcohol	25	0.066	57.14285714
10	Alcohol	50	0.045	70.77922078
11	Alcohol	100	0.039	74.67532468
12	Alcohol	200	0.032	79.22077922
13	Alcohol	400	0.028	81.81818182
14	Hydroalcohol	10	0.087	43.50649351
15	Hydroalcohol	25	0.076	50.64935065
16	Hydroalcohol	50	0.064	58.44155844
17	Hydroalcohol	100	0.058	62.33766234
18	Hydroalcohol	200	0.047	69.48051948
19	Hydroalcohol	400	0.031	79.87012987
20	Aspirin	50	0.025	83.76623377
21	Aspirin	100	0.016	89.61038961

## SUMMARY AND CONCLUSION

Different extracts of lemon peel, dose dependant, exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extracts may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage. From the above study aqueous extract exhibited more potent membrane stabilization property than other extracts and the above results established that lemon peel has potential anti-inflammatory activity. [10]

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## REFERENCES

1. Arya V and Arya ML, In vitro anti-inflammatory studies of Sphaeranthus indicus, International Journal of Pharm Tech Research, 2010; 3(2): 899-908.
2. Chatterjee A, The Treatise of Indian medicinal plants, National Institute of Science and Communication CSIR, New Delhi; 1997: 4, 212-22
3. Das S, Mukherjee H, Ahmed SM, Haldar PK, Mandal AB, Mahapatra A, Mukherjee PK, Chakraborti S and Chattopadhyay D, Evaluation of an ethnomedicinal combination containing Semecarpus kurzii and Hernandia peltata used for the management of inflammation,

Pharmaceutical Biology, 2013; 51(6): 677-685.

4. Jarald EE and Jarald SE, Textbook of Pharmacognosy and Phytochemistry. 1<sup>st</sup> ed. CBS Publishers & Distributors; 2007: 5, 12, 125.
5. Faizul H, Habib A and Mukhtar A, Tradional uses of medicinal plants of Nandiar khuwarr catchment (district Battagram), Pakistan. Journal of Medicinal Plants Research 2011; 5(1): 39-44
6. Masuma S, Kaiser H, Mohammad HAR, Sakina A and Choudhuri MSK, Changes in lipid profile of rat plasma after chronic administration of Laghobanondo rosh (LNR) – an Ayurvedic formulation, Biology and Medicine, 2010; 2(3): 58-63.
7. Nakayoma J and Yamada M, Isolate three chemical constituents from Anthracephalus cadamba, Biochemical Pharmacology, 1995; 45: 265-267.
8. Polterait O, Characterization of different chemical constituents from Anthracephalus cadamba. Organic Chemistry, 1997; 1: 415-440.
9. Rahmat A, Edrini S, Ismail P, Yap T and Fadzelly M, Chemical constituents, antioxidant activity and cytotoxic effects of essential oil from Strobilanthes crispus and Lawsonia inermis, Journal of Biological Sciences, 2006; 6(6): 1005-1010.
10. Reviews of Indian Medicinal Plants, Indian Council of Medicinal Research, New Delhi, 2011, 10, 2011 ,729.