

Evaluation of *in-vitro* anti-inflammatory activity of *Citrus macroptera* Montr.

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Abstract

Objective: The use of naturally occurring medicines dependent on essential oils (EO) is now a days of great interest. In addition, within the human body, EO shows high efficacy as antioxidants and anti-inflammatory drugs. The present experiment was conducted to access the anti-inflammatory activity of essential oil obtained from the fruit peels of *Citrus macroptera* Montr. (Rutaceae) against the denaturation of protein *in-vitro* model.

Materials and Methods: The test sample (essential oil) was incubated under controlled laboratory conditions at varying concentrations with egg albumin and was subjected to absorbance determination for the anti-inflammatory property analysis. Diclofenac sodium was used as the standard reference drug for the experiment.

Results: The results show a concentration dependent inhibition of protein (albumin) denaturation by the test oil. This was concluded by comparing their IC₅₀ average values. *Citrus macroptera* Montr. essential oil possessed IC₅₀ average value 54.6±0.07 µg/mL whereas that of diclofenac sodium was found to be 52.89±0.06 µg/ml. The result shows that the test oil is more effective than the standard drug.

Conclusion: From the above experimental finding it can be concluded that the *Citrus macroptera* Montr. essential oil has significance anti-inflammatory effect against the denaturation of the protein *in-vitro* model. The activity may be due to the presence of terpene polyphenolic component or some other active compound present in the oil. The provided information was first of its kind of knowledge to keep the scientific data for future reference.

Keywords: Anti-inflammatory, essential oil, *Citrus macroptera*, protein denaturation, polyphenolic compounds.

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1. Introduction

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defence, the response of a tissue to an

accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [1]. The inflammatory responses are elicited as a defense mechanism by an organism or tissues; however, sustained inflammation can lead to undesired health effect as a consequence of interplay of various bio-molecules that are secreted during the process of inflammation. Inflammation has been indicated in several diseases including cancer [2]. Chronic pain induced by inflammatory processes is a major clinical problem worldwide, Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used treatments in these chronic pain states. NSAIDs, such as

Diclofenac, aspirin and indometacin, block the biosynthesis pathway of prostaglandins by inhibiting the cyclooxygenase (COX) enzymes, producing anti-inflammatory, analgesic and antipyretic effects [3]. Drugs currently used for management of pain and inflammatory conditions present toxic side effects on chronic administration. Therefore, attempts are being taken to study promising plants which may lead to develop newer or safer drugs [4].

2. Materials and methods:

2.1 Collection, Identification and Authentication of Plant

The fruits of the plant *Citrus macroptera* were collected locally from East Khasi Hills, Meghalaya. The plant was identified, confirmed and authenticated by Botanical Survey of India, Shillong, Meghalaya.

2.2 Extraction of Essential Oil

The fruit peels of *Citrus macroptera* were thoroughly washed with distilled water, cut into small pieces and about 200 gms in four fractions were subjected to hydro-distillation using a clevenger apparatus for about 4 hours. The steamed and vaporized oil were condensed into liquid by a vertical condenser and collected in a measuring cylinder. Being immiscible and lighter than water, the volatile oil is separated out as an upper layer. The oil was collected. Finally it was dried over anhydrous sodium sulphate and kept in an airtight container at 4-8°C until further analysis. **Standard Drugs:** Diclofenac sodium was purchased from SIGMA-ALDRICH Co., and purity was labelled to be 98% respectively.

2.3 Preparation of Crystallized Egg Albumin:

The crystallized albumin obtained from twenty-four eggs was dissolved in about 500ml of distilled water and poured into 5 liters of boiling distilled water with rapid stirring so that the coagulated albumin was finely divided. It was then poured on several large filters and washed until the washings were entirely free of sulphates. The substance was then allowed to drain thoroughly, transferred to a flask containing about 10 volumes of 95 per cent boiling alcohol for an hour, and filtered. This was repeated once with 5 volumes of 95 per cent alcohol and once with 5 volumes of absolute alcohol. The material was filtered and then stirred

with 3 volumes of a good grade of ether and again filtered. This process was repeated twice and the protein was finally dried in a vacuum desiccator over sulphuric acid. This gave a dry white product which could easily be reduced to a powder. The yield was about 1 gm. per egg [5].

3. Anti-inflammatory bioassay in vitro:

3.1 Inhibition of albumin denaturation:

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of the essential oil of *Citrus macroptera* Montr. so that final concentrations become (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500µg/ml)[6-8]. Similar volume of distilled water served as control. Then the mixtures were incubated at 37±2°C in an incubator for 15minutes and then heated at 70°C for 5 minutes. After cooling down, their absorbance was measured at 660 nm by using vehicle as blank.

Diclofenac sodium at the final concentration of (100-500µg/ml) was used as reference drug and treated similarly for determination of absorbance [9-13]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times [\text{abst} / \text{absc} - 1]$$

Where, abst = absorbance of test sample, absc = absorbance of control.

The oil /drug concentration for 50% inhibition (IC₅₀) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

3.2 Statistical analysis:

Statistical analysis was done using one way analysis using ANOVA where P<0.05 were considered as significant. Values are expressed as Mean±SD (n=3).

4. Results:

The present investigation reports the *in vitro* bioassay of anti-inflammatory effect of essential oil obtained from the fruit peels of *Citrus macroptera* against denaturation of egg albumin. The results were given in the table 01 and IC₅₀ values summarized in Table 02.

Table 01: In vitro anti-inflammatory activity of *Citrus macroptera* Montr. essential oil (Egg albumin)

SL.NO	Concentration (µg/ml)	<i>Citrus macroptera</i> essential oil	Standard(Diclofenac sodium)
1.	100	19.5±0.9	14.77±0.6
2.	200	26.3±1.2	32.78±2.5
3.	300	44.7±1.9	41.88±3.8
4.	400	87.6±2.1	75.43±4.3
5.	500	95.1±3.4	99.60±5.30
IC50 Average	300	54.6±0.07	52.89±0.06

IC₅₀: inhibitory concentration, µg/ml=microgram/ml.

Table 02: Average IC₅₀ of *Citrus macroptera* Montr. essential oil and Diclofenac sodium against protein denaturation.

S.No	Treatment	IC ₅₀ values
1.	<i>Citrus macroptera</i> essential oil	240
2.	Standard (Diclofenac sodium)	580

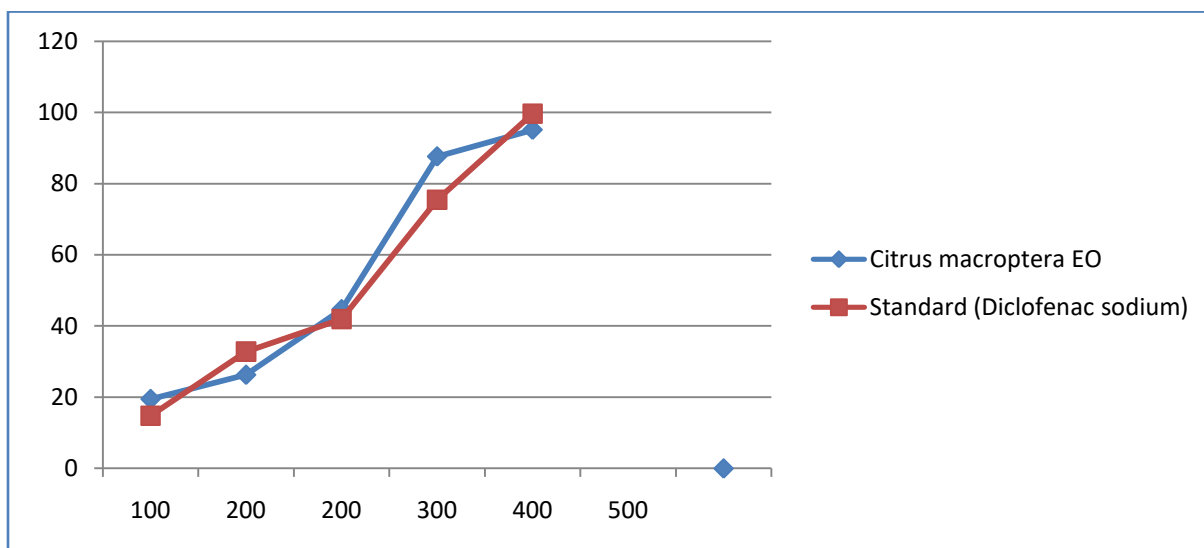


Figure 1: In-vitro anti-inflammatory activity of *Citrus macroptera* Montr. (Egg albumin)

5. Results

The present investigation reports the *in vitro* bioassay of anti-inflammatory effect of essential oil obtained from the fruit peels of *Citrus macroptera* against denaturation of egg albumin. The results were given in the table 01 and IC₅₀ values summarized in Table 02. As per the results, the test sample (essential oil) was found to be effective and comparable to the standard drug i.e, Diclofenac sodium. This was concluded by comparing their IC₅₀ average values. *Citrus macroptera* Montr. essential oil possessed IC₅₀ average value 54.6±0.07 µg/mL whereas that of diclofenac sodium was found to be 52.89±0.06 µg/ml.

6. Conclusion

Eventually, the findings of this investigation concluded that extracts of EO derived from *Citrus macroptera* Montr were determined and have anti-inflammatory function and show prominent results as opposed to normal diclofenac sodium. As we all know the protein denaturation is a well-documented cause of inflammation. As a part of the investigation into the anti-inflammation activity mechanism EO has been successful in inhibiting heat-induced denaturation of albumin. The EO tested in this study could practically be used as a natural medication or as a candidate for pharmaceutical and medical use.

Reference

- [1]. Leelaprakash G, Dass SM, Road B. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *International Journal of Drug Development and Research* 2011; 3(3): 189-196.
- [2]. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Investigation of the anti-inflammatory and analgesic activities of ethanol extract of stem bark of Sonapatha *Oroxylum indicum in vivo*. *International journal of inflammation*. 2016 Jan 26; 2016.
- [3]. De Almeida Barros TA, De Freitas LA, Filho JM, Nunes XP, Giulietti AM, De Souza GE, Dos Santos RR, Soares MB, Villarreal CF. Antinociceptive and anti-inflammatory properties of 7-hydroxycoumarin in experimental animal models: potential therapeutic for the control of inflammatory chronic pain. *Journal of Pharmacy and Pharmacology*. 2010; 62(2):205-13.
- [4]. Divya TS, Latha PG, Usha K, Anuja GI, Suja SR, Shyamal S, et al. Properties of *Wattakaka volubilis* (Linn. F.) Stapf. 2009; 8(2): 137-141.
- [5]. Physiological H, Arbor. Crystallized Egg Albumin. 1931; (3).
- [6]. Djihane B, Mihoub ZM. Evaluation of *in-vitro* anti-inflammatory activity of *Helichrysum italicum* (Roth) G. Don essential oil. 2016; 8(4): 41-44.

- [7]. Williams LAD, Connar AO, L Latore, Dennis O, Ringer S, Whittaker JA, et al. *West Indian Med J*, 2008; 57: 327-331.
- [8]. Perrone MR, Artesani MC, Viola M, Gaeta F, Caringi M, Quaratino D, Romano A. Tolerability of Rofecoxib in patients with adverse reactions to nonsteroidal anti-inflammatory drugs: a study of 216 patients and literature review. *Int. Arch Allergy Immunol.* 2003; 132:82–86.
- [9]. Lourens AC, Reddy D, Başer KH, Viljoen AM, Van Vuuren SF. In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species. *Journal of ethnopharmacology*. 2004; 95(2-3): 253-8.
- [10]. Chou ST, Lai CP, Lin CC, Shih Y. Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. *Food chemistry*. 2012; 134(1):262-8.
- [11]. Siani AC, Ramos MD, Menezes-de-Lima Jr O, Ribeiro-dos-Santos R, Fernandez-Ferreira E, Soares RO, Rosas EC, Susunaga GS, Guimarães AC, Zoghbi MD, Henriques MD. Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of *Protium*. *Journal of Ethnopharmacology*. 1999; 66(1):57-69.
- [12]. Al-Reza SM, Yoon JI, Kim HJ, Kim JS, Kang SC. Anti-inflammatory activity of seed essential oil from *Zizyphus jujuba*. *Food and chemical toxicology*. 2010 Feb 1; 48(2): 639-43.
- [13]. Baricevic D, Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, Zupancic A. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. *Journal of ethnopharmacology*. 2001; 75(2-3):125-32.