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## ANTI-INFLAMMATORY POTENTIAL OF $\alpha$ -FENCHOL AND $\alpha$ -GURJUNENE: AN *IN VITRO* STUDY

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**Abstract:**

**Objective:** Chronic inflammatory diseases are still one of the main health complications acknowledged globally. At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail, cannot be ruled out; hence the search for newer therapeutic strategies with lesser side effects has always been a matter of research. The present study aims to evaluate the *in vitro* anti-inflammatory effects of two essential oils  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene.

**Methodology:** *In vitro* anti-inflammatory activity of both the essential oils was evaluated using albumin denaturation assay.

**Results:** At various concentrations *viz.* 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00  $\mu$ g/ml,  $\alpha$ -Fenchol exhibited inhibition of protein denaturation in the range of 180-1400% and  $\alpha$ -Gurjunene in the range of 290-360%. The present findings corroborated the anti-inflammatory property of essential oils,  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene *in vitro*.

**Conclusion:** Both the essential oils possess *in vitro* anti-inflammatory effects, further definitive studies are necessary to ascertain the mechanisms behind their anti-inflammatory actions *in vivo* using suitable experimental models.

**Keywords:**  $\alpha$ -Fenchol,  $\alpha$ -Gurjunene, Anti-inflammatory, Protein denaturation

## **Introduction**

Inflammation is regarded as multifaceted process linked with pain, redness, heat and swelling at the affected area. When microbes, any other physical or chemical agent damage body cells, affected tissue began to experiences increase of vascular permeability, increase of protein denaturation and membrane alteration depending upon the extent and site of injury [1]. The inflammatory response involves a complex array of enzyme activation, mediator (kinins, prostroglandins and histamine) release, vasodilation and increased permeability of the capillaries, fluid extravasations, cell migration, tissue breakdown and repair, which are aimed at host, defense and usually activated in most disease condition [2]. Chronic inflammatory diseases are still one of the main health complications acknowledged globally. At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration [3]. Currently much interest have been paid in the search 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 amplifies the disease process.

An essential oil is an aromatic oily volatile liquid characterized by a strong odor, obtained as the product by hydro-, steam- or dry-distillation or by a suitable mechanical process without heating of a plant or some parts of it (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root). Essential oils are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes in various parts of plants [4]. Essential oils have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils includes terpenes (oxygenated or not), with monoterpenes and sesquiterpenes prevailing. Nevertheless, allyl- and propenylphenols are also important components of some essential oils [5]. In Nature, essential oils play an important role in the attraction of insects to promote the dispersion of pollens and seeds or to repel other ones, also act as anti-microbial, insecticides, herbicides or have feeding deterrent effects against herbivores by reducing their appetite for such plants [6]. The detection of some of these biological properties needed for the survival of plants has also been the base for

searching similar properties for the combat of several similar or other diseases in humans and animals. Essential oils only represent a small fraction of plant's composition; nevertheless, they confer the characteristics by which aromatic plants are used in the food, cosmetic and pharmaceutical industries [7]. During past years, the search for anti-inflammatory potential of various essential oils has been increased and well documented from various plants such as *Tanacetum vulgare*, *Cymbopogon validus*, *Cinnamomum osmophloeum* and *Trachydium roylei* etc [8]. The search for newer therapeutic strategies with lesser side effects has always been a matter of research, the present study aims to evaluate the *in vitro* anti-inflammatory effects of two essential oils  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene, as their this property has not been evaluated so far.

## **Methodology**

### ***Chemicals and solutions***

A descriptive cross-sectional study design was used to assess prevalence of depression  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene (Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India) and diclofenac sodium (Zenith Pharma Pvt. Ltd., Indore, India) were used in the present study. All other chemicals and reagents used were of analytical grade.

### ***Assessment of in vitro anti-inflammatory activity***

*In vitro* anti-inflammatory activity of  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene was evaluated using albumin denaturation assay. The reaction mixture (5 ml) consisted of 0.2 ml of fresh egg albumin from hen's egg, 2.8 ml phosphate buffered saline (pH 6.4) and 2.0 ml of varying concentrations of essential oils ( $\alpha$ -Fenchol and  $\alpha$ -Gurjunene separately in different reaction mixtures) so that final concentration becomes 31.25, 62.50, 125.00, 250.00, 500.00, 1000.00  $\mu$ g/ml. A similar volume of double-distilled water served as control. The mixtures were incubated at  $37 \pm 2$  °C for 15 min and kept for denaturation at 70 °C for 5 min in an incubator. After cooling, absorbance was measured at 660 nm (Shimadzu UV-1700, Kyoto, Japan) against blank. Diclofenac sodium in concentrations of 78.125, 156.25, 312.50, 625.00, 1250.00, 2500.00  $\mu$ g/ml was used as standard [9]. Percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percent inhibition (\%)} = 100 \times [V_t / V_c - 1]$$

Where,  $V_t$  = absorbance of the test sample,  $V_c$  = absorbance of control

The extract concentration for 50% inhibition (IC<sub>50</sub>) was determined by the dose-response curve.

## Results

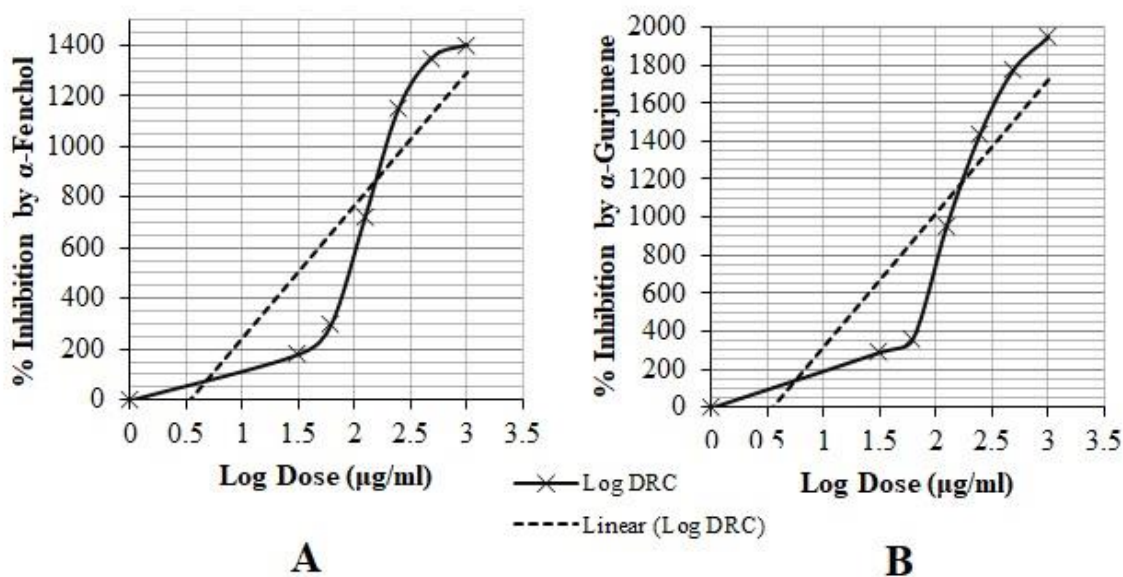
The present findings exhibited a concentration dependent inhibition of albumin protein denaturation by both the essential oils  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene. Throughout the concentration range of 31.25 to 1000.00  $\mu$ g/ml,  $\alpha$ -Fenchol exhibited inhibition of protein denaturation in the range of 180-1400% and  $\alpha$ -Gurjunene in the range of 290-360% (Table 1). Standard drug diclofenac sodium at the concentrations of 78.125, 156.25, 312.50, 625.00, 1250.00 and 2500.00  $\mu$ g/ml exhibited concentration dependent inhibition of protein denaturation as 12.50, 12.50, 25.00, 50.00, 212.50 and 812.50 respectively. Figure 1 represents the sigmoid log-dose response curve of percent inhibition by both the essential oils. The inhibition by both the essential oils was further confirmed by comparing their IC<sub>50</sub> values (Table 2).

**Table 1. Effect of  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene against protein denaturation**

Concentration ( $\mu$ g/ml)	Percent inhibition (%)	
	$\alpha$ -Fenchol	$\alpha$ -Gurjunene
31.25	180	290
62.50	300	360
125.00	720	950
250.00	1150	1440
500.00	1350	1780
1000.00	1400	1950

**Table 2. IC<sub>50</sub> values of  $\alpha$ -Fenchol,  $\alpha$ -Gurjunene and diclofenac sodium against protein denaturation**

Treatments	IC <sub>50</sub> ( $\mu$ g/ml)
$\alpha$ -Fenchol	3.98
$\alpha$ -Gurjunene	5.65
Diclofenac sodium	625.00



**Figure 1. Log dose-response curve indicating percentage inhibition of protein denaturation by (A)  $\alpha$ -Fenchol and (B)  $\alpha$ -Gurjunene**

## Discussion

Inflammation is a bodily response to harmful stimuli such as injury and infection. Various inflammatory models allow evaluation of test compounds and provide further understanding about the inflammatory process [10]. There are certain problems associated with animal use in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of both the essential oils  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene.

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat [11]. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* [12] Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

As natural volatile substances from plants, essential oils may represent an alternative source of anti-inflammatory agents because they are not usually extracted as chemically pure substances, but these oils consist of mixtures containing many bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programs [12]. In the present study, the anti-inflammatory effects of two essential oils were investigated.

The increments in the absorbance of the test samples, with respect to the control, indicated stabilization of protein, that is, inhibition of protein (albumin) denaturation or an anti-denaturation effect by essential oils and the reference drug, diclofenac sodium [9]. It has been reported that one of the features of several non-steroidal, anti-inflammatory drugs, is their ability to stabilize (prevent denaturation) heat-treated albumin at the physiological pH (pH: 6.2 – 6.5) [13]. Therefore, from the results of the present preliminary study, it can be concluded that both the essential oils  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene possess a marked anti-inflammatory effect against the denaturation of protein *in vitro*. Previous researchers have reported the anti-inflammatory activity of plants *Alpinia galangal* and *Cyperus rotundus* that are the rich source of  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene respectively [14,15], our findings are supported by these studies.

### **Conclusion**

The present findings corroborated the anti-inflammatory property of essential oils,  $\alpha$ -Fenchol and  $\alpha$ -Gurjunene *in vitro*. Further definitive studies are necessary to ascertain the mechanisms behind their anti-inflammatory action both *in vivo* and *in vitro* using other experimental models.

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