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ANTI-INFLAMMATORY ACTIVITY OF SECONDARY METABOLITES IN THYME OIL (THYMUS VULGARIS) AND SESAME OIL (SESAMUM INDICUM) INCORPORATED TOOTHPASTE

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ABSTRACT
The essences of plants are secondary metabolites with a variety of pharmacological characteristics. Since ancient times, researchers have been studying natural bioactive compounds derived from various aromatic plants because of their special qualities, which are crucial to the pharmaceutical, cosmetic, and food sectors. For example, particular chemicals present in thymus have been linked to a number of advantageous effects, including anti-inflammatory, antioxidant, antibacterial, and antiseptic qualities. Furthermore, these substances can be added to a variety of food products because they are categorized as Generally Recognized as Safe (GRAS). These substances and their derivative forms can be extracted from thyme leaves using well-established conventional methods. Thymus vulgaris is the term given to thyme in botany. Thyme belongs to the family Lamiaceae, which includes mint plants. Originating in southern Europe, it is grown all over the world. Commonly referred to as garden thyme or common thyme, Thymus vulgaris thrives on dry, gravelly, or rocky soils that have adequate drainage and a pH range of neutral to alkaline. The primary component of thyme, thymol, is an essential oil that is widely recognized for its antibacterial, anti-inflammatory, and antioxidant properties. Hydrocarbon and phenolic components such as borneol, carvacrol, cymol, linalool, thymol, tannin, apigenin, luteolin, saponins, and triterpenic acid are the main substances that are active in thyme oil.

Keywords: *Anti-inflammatory, pharmaceutical, secondary metabolites, aromatic plants.*

1. INTRODUCTION

1.1 Anti-inflammatory Activity

An essential physiological reaction, inflammation serves to shield the body against damage, infection, and other damaging stimuli. Acute inflammation is necessary for immune response and tissue repair, but chronic inflammation can also play a role in the etiology of a number of illnesses, such as inflammatory bowel diseases, arthritis, and cardiovascular disorders.

The investigation of natural substances with the ability to alter inflammatory processes has resulted from the search for potent anti-inflammatory drugs. Of these, numerous plant-derived chemicals and botanical extracts have attracted a lot of attention due to their alleged anti-inflammatory qualities [1].

The context for exploring the wider field of anti-inflammatory agents—with an emphasis on natural sources like plants and their derivatives—is established by this introduction. The goal of this study is to present a thorough overview of the various treatment approaches that could be used to treat inflammation by clarifying the mechanisms that underlie inflammation and the reasons for investigating natural cures [2]. In addition, issues pertaining to the effectiveness, safety, and future paths of this field of study will be examined, providing valuable perspectives on the function of natural anti-inflammatory medicines in contemporary medical procedures.

1.1.1 Types of Anti-inflammatory assays

Cell Based Assays

Thrombokine Assays Determine the concentrations of pro- or anti-inflammatory substances (e.g., IL-10, TNF-α, IL-1β, and IL-6) released by immune cells in reaction to an inflammatorystimuli. Anti-inflammatory compounds are those that either increase the secretion of anti-inflammatory cytokines or decrease the generation or release of cytokines that are pro-inflammatory in nature. NF-κB Reporter Evaluations: One important transcription factor that controls the expression of genes related to inflammation is NF-κB. Reporter assays quantify NF-κB activity in response to stimuli that are inflammatory and evaluate a compound's capacity to prevent NF-κB activation, therefore mitigating inflammation. Assays for Cell Viability: Determine if immune cells, such as macrophages and lymphocytes, are still viable after being exposed to inflammatory factors and

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possible anti-inflammatory drugs. Anti-inflammatory compounds are those that shield cells from cytotoxicity or apoptosis brought on by inflammation [3].

Enzyme Inhibition Assays

Cyclooxygenase (COX) Inhibition Assay: Prostaglandins are synthesised by COX enzymes and are a major factor in inflammation. Compounds that inhibit COX activity can lower inflammation and prostaglandin synthesis. Compounds' capacity to suppress COX enzyme activity is measured by COX inhibition tests.

Lipoxygenase (LOX) Inhibition Assay: Leukotrienes, which contribute in inflammatory processes, are formed by LOX enzymes. Inhibitors of LOX activity can reduce inflammation and the synthesis of leukotrienes. Compounds' capacity to suppress LOX enzyme activity is measured using LOX inhibition tests.

1.2 Thyme (*thymus vulgaris*)

Thyme (Thymus vulgaris) is a widely used herb with a unique perfume that has been utilized for culinary and medicinal purposes in many cultures. Although it is native to the waters of the Mediterranean, it is grown all over the world and is a member of the Lamiaceae family. Thyme's antibacterial, antioxidant, and anti-inflammatory qualities led to its widespread usage

In traditional medicine in ancient Egypt and Greece [4] .The immune system's natural reaction to damaging stimuli like infections, wounds, or irritants is inflammation. Acute inflammation is necessary for human immune processes, but persistent inflammation can cause a number of illnesses, including cancer, arthritis, and cardiovascular problems.

The anti-inflammatory properties of thyme isolates and its bioactive components, such as thymol and carvacrol, have been the subject of numerous studies. Both in vitro and in vivo studies have shown that these substances significantly suppress pro-inflammatory mediators and pathways. Furthermore, in animal models, thyme extracts have demonstrated encouraging outcomes in reducing inflammatory diseases such dermatitis, colitis, and rheumatoid arthritis[5].

The ability of thyme to modulate different inflammatory pathways, such as inhibiting pro-inflammatory enzymes like cyclooxygenase, also known as COX, and lipoxygenase (LOX) and suppressing pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), is thought to be the mechanism behind its anti-inflammatory activity[5].

Researchers are becoming more interested in investigating alternative medicines derived from natural sources, such as thyme, due to the rising incidence of chronic inflammatory disorders and the shortcomings of traditional anti-inflammatory medications. Comprehending the mechanisms underlying thyme's anti-inflammatory properties and its possible therapeutic uses can yield important insights for the creation of innovative preventive and therapeutic approaches against inflammatory diseases. As a result, the goal of this review is to thoroughly assess the body of research on thyme's ability to reduce inflammation while emphasizing both its mode of action and possible therapeutic uses.

1.3 Sesame Oil (*sesamum Indicum)*

From the seeds of the Sesamum indicum plant, sesame oil has been used for millennia in traditional medical and cooking methods throughout the world. Sesame oil, well-known for its rich flavor and wide range of uses, also contains a multitude of bioactive components that offer various health advantages, such as possible anti-inflammatory effects. A complex biological reaction to damaging stimuli, inflammation is a key player in the pathophysiology of many chronic diseases, including arthritis, neurological diseases, and cardiovascular ailments. As a result, there is increasing interest in finding natural substances that help control inflammatory processes; sesame oil stands out as a possible contender[6]. The context for investigating sesame oil's anti-inflammatory properties is established in this introduction, which also highlights the oil's historical applications and the scientific basis for their prospective health benefits. This study attempts to provide an in-depth knowledge of sesame oil's involvement in inflammation modulation by clarifying the mechanisms by which it exerts its anti-inflammatory benefits and analyzing the increasing amount of proof from both preclinical as well as clinical studies. The viability of incorporating sesame oil into upcoming therapeutic approaches for inflammatory illnesses will also be clarified by understanding the safety profile, the bioavailability and possible synergy relationships with other anti-inflammatory drugs. Numerous bioactive substances, such as fatty acids (like oleic and linoleic acid), lignans (like sesaminol and sesamolin), and antioxidants (like sesamol and sesamin) are abundant in sesame oil. These ingredients may have an impact on how components of sesame oil are absorbed, distributed, metabolized, and eliminated from the body [7]. The bioavailability of sesame oil might be impacted by the extraction procedure used. In contrast to refined sesame oil, which goes through

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procedures such refining, bleaching, and and deodorizing that may remove some beneficial components, cold-pressed sesame oil, which is made by crushing dry sesame seeds without heat or solvents of any kind, may retain more of its biologically active substances[8].

2. MATERIALS AND METHODOLOGY

Anti-inflammatory activity

Anti-haemolysis assay:

1. Red blood cells (RBCs) - typically sourced from fresh whole blood of appropriate species
2. Phosphate-buffered saline (PBS), pH 7.4
3. Test substance (the compound or extract being evaluated for anti-haemolytic activity)
4. Positive control (a known anti-haemolytic agent)
5. Negative control (e.g., PBS or buffer)
6. Haemolysis-inducing agent (e.g., hydrogen peroxide, hypotonic solution)
7. Microcentrifuge tubes
8. Spectrophotometer
9. Pipettes and tips
10. Vortex mixer
11. Water bath or incubator
12. Disposable cuvettes or microplate

Preparation of Red Blood cells (RBCs) suspension**:**

Gather new whole blood and place it in a tube with an anticoagulant (EDTA, for example). To separate the plasma and the shiny coating from the red blood cells, centrifuge the blood for five minutes at low speeds (e.g., 1000 rpm). Take care not to disrupt the RBC pellet when removing the supernatant, which includes the buffy coat and plasma. Resuming the pellet and concentrating it once more allows you to wash the red blood cell pellet three times with PBS. Re-suspend the RBC pellet in PBS following the last wash to reach the target concentration (typically between 2 and 4% v/v) [10].

Preparation of test samples

Prepare test and positive control solutions in PBS or an acceptable solvent, with the substances at the proper concentrations. Provide a negative control that is only composed of buffer or PBS.

Treatment with Red blood cells

Transfer the RBC suspension in aliquots to microcentrifuge tubes. Fill each tubes with the test material, haemolysis-inducing drug, positive control, and negative control. Use a vortex to ensure even mixing.

Treatment of RBC

Transfer the RBC suspension in aliquots to microcentrifuge tubes. Fill each tubes with the test material, haemolysis-inducing drug, positive control, and negative control. Use a vortex to ensure even mixing.

Incubation

It is necessary to incubate the tubes at a certain temperature (e.g., 37°C) for a predetermined amount of time (typically 1-2 hours) in order to allow the test chemicals to protect the tubes and induce haemolysis[9].

Centrifugation

Centrifuge the tubes slowly to remove debris and unbroken RBCs after the incubation time. The samples are centrifuged at 3000 RPM for 5 mins and the supernatant is isolated.

Measurement of Haemolysis

Place the supernatants in the cuvettes or microplate wells of each tube. Using a spectrophotometer, determine each sample's absorbance at an appropriate wavelength (540 nm, for example).

Data Analysis

To assess the test substance's anti-hemolytic activity, compare the proportion of hemolysis in the sample being tested to that found in the positive control.

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Reporting

Record all experimental parameters, outcomes, and statistical evaluations. Analyze the data and make deductions about the test substance's anti-hemolytic efficacy.

Formula

% of inhibition = (Abs. control –Abs. sample) X 100 / Abs. control

3. RESULTS AND INTERPRETATION

Table1: Anti-inflammatory activity

S.No.	Concentration(µg/ml)	Absorbance @560nm
1.	CONTROL	0.244
2.	20	0.216
3.	40	0.209
4.	60	0.185
5.	80	0.164
6.	100	0.148
7.	120	0.112

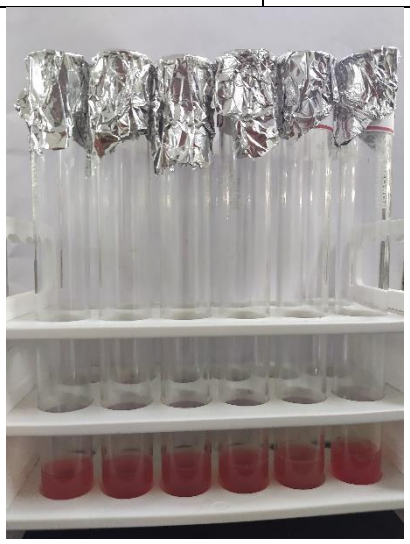

Figure 1: Drug infused blood samples

CONCLUSION

The Anti-inflammatory activity of thyme and sesame oil that is infused in toothpaste has been taken in different concentrations (20 µg/ml,40 µg/ml,60 µg/ml,80 µg/ml,100 µg/ml,120 µg/ml) and the highest inhibition effect of absorbance 0.112 is recorded in the 120 µg/ml concentration.

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