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### Formulation and *Invitro* Anti-Inflammatory Activity of Polyherbal Oral Gummies

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#### Abstract:

**Background:** Herbal gummies represent an emerging chewable oral dosage form that combines the therapeutic value of medicinal plants with improved palatability and patient acceptance. Clove (*Syzygium aromaticum*) and cardamom (*Elettaria cardamomum*) are traditionally used spices reported to possess anti-inflammatory activity, but their combined use in a single polyherbal gummy formulation has been little explored. Objective: This study aimed to formulate polyherbal oral gummies containing clove and cardamom extracts and to evaluate their physicochemical properties and in vitro anti-inflammatory activity. Methods: Authenticated clove and cardamom were extracted by cold maceration in ethanol and screened for phytoconstituents. Three gummy formulations (F1–F3) were prepared by the moulding method using gelatin as the base and evaluated for weight variation, pH, moisture content, thickness, physical appearance, stability and disintegration time. Anti-inflammatory activity of the aqueous extracts was assessed by the albumin denaturation inhibition assay over a concentration range of 100–500 µg/mL, using diclofenac sodium as the standard. Results: Phytochemical screening revealed alkaloids, flavonoids, tannins, saponins and terpenoids in clove extract, and alkaloids, terpenoids, tannins, glycosides and carbohydrates in cardamom extract. The optimized formulation (F2) showed an average weight of 4.696 g (deviation within  $\pm 7.5\%$ ), pH 4.05, moisture content 16.17% w/w, average thickness 4.89 mm, disintegration time 9.83 min, and physical stability over four weeks. Both extracts produced concentration-dependent inhibition of protein denaturation, reaching  $82.16 \pm 1.62\%$  (clove) and  $79.24 \pm 1.30\%$  (cardamom) at 500 µg/mL, compared with  $89.24 \pm 2.80\%$  for diclofenac sodium. The estimated IC<sub>50</sub> values were 151.9 µg/mL for clove and 168.3 µg/mL for cardamom. Conclusion: *Syzygium aromaticum* and *Elettaria cardamomum* can be successfully formulated into a stable, palatable polyherbal gummy with demonstrable in vitro anti-inflammatory activity, supporting their potential as a natural adjunct for

managing mild inflammatory conditions, particularly of the oral cavity, pending further in vivo validation.

**Keywords:** Polyherbal gummies; Syzygium aromaticum; Elettaria cardamomum; Anti-inflammatory activity; Albumin denaturation assay; Novel oral drug delivery; Phytochemical screening.

## 1.0 INTRODUCTION

### 1.1 Traditional medicinal system:

It is widely recognized that traditional systems of medicine have always been important in addressing global healthcare needs. They are still doing this now and will have an important role in the future as well. The system of medicines that are from India or those that came to India from other countries and were incorporated into Indian culture are called Indian Systems of Medicine (Prasad, 2002). India is the only country that has six officially recognized systems of medicine in this group. They are Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy. Even though Homoeopathy arrived in India during the 18th century, it fully merged with the Indian culture and grew stronger, just like other traditional systems. So, it is now seen as one of the Indian Systems of Medicine (Prasad, 2002). Besides these systems, there are many healers in the folklore tradition who haven't been grouped into any specific category.<sup>[1]</sup>



**Fig 1:** Traditional Medicinal System

#### 1.1.1 AYURVEDA:

Ayurveda is a well-known traditional medicine system that has lasted and grown over a long time. Because of the deep understanding of natural medicine, how the human body and its functions connect with nature and the elements in the universe that work together and influence living things, this system will keep growing and thriving for a long time in the future. There are still lots of areas that need to be studied by researchers, professionals, and experts in the field. These people have the duty of making sure traditional medicine systems stay alive and help them grow in the future.<sup>[2]</sup>



**Fig 2: Ayurveda**

### 1.1.2 SIDDHA:

According to the Siddha system, matter and energy are the two main forces that greatly affect the nature of the universe. In the siddha system, they are known as Siva and Sakthi. Matter cannot exist without energy and vice-versa. Thus, both are inseparable (Narayanaswamy, 1975).



**Fig 3: Siddha**

According to SSM, a human is seen as both a small version of the universe and the universe itself. A living person is made up of three humors, called vaatham, pitham, and kabham, seven basic tissues known as saptha dathukkal, and five main elements, which are earth, water, fire, air, and space. A proper mix of these five elements, working together, helps create the different parts and ways the body works.<sup>[3]</sup>

### 1.1.3 UNANI:

According to Unani medicine, problems in an organ are mainly caused by an imbalance in the body's temperament and the number of humours, which leads to the buildup of harmful substances known as mawad-e-fasida. Therapeutic methods work to bring balance back to the body by using medicines and food to counteract the unhealthy tendencies that are present during illness. These treatments are also supported by a special way of living called Ilaj bit-tadbeer, and they help remove harmful substances, known as raddi akhlat, from the body through a process called istafraag. This helps in bringing back the normal balance of the body's humours. body.<sup>[4]</sup>



**Fig 4: Unani**

### 1.1.4 HOMEOPATHY:

Homeopathy is a unique type of medicine that is practiced all around the world. The system has become a natural part of many countries' traditions and roots, and it helps take care of health for a big number of

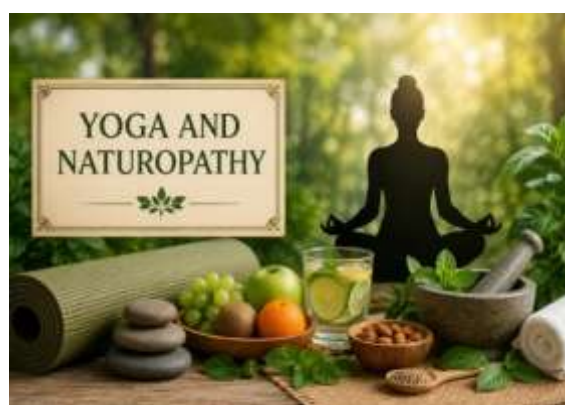
people. It is commonly used as the first choice for treating many not very serious illnesses, often by people taking medicine on their own. It is also a main choice for treating several serious illnesses with expert medical advice. The power of homeopathy comes from how well it works for specific health issues where there are few other treatment choices available.<sup>[5]</sup>



**Fig 5:** Homoeopathy

### 1.1.5 YOGA AND NATUROPATHY:

Yoga and naturopathy are holistic system of health that emphasize the promotion of well-being through natural and lifestyle-based approaches. Naturopathy is founded on the principle that the body possesses an inherent ability to heal itself when supported by healthy living practices. Yoga, an ancient Indian discipline, aims to achieve harmony between the body, mind, and spirit. Classical yoga is based on the eight limbs (ashtanga yoga): ethical restraints (Yama) personal observation (niyama), physical posture (asana), breathing techniques.



**Fig 6:** Yoga and Naturopathy

In recent years, there has been growing interest in reviving and promoting traditional system of medicine. To support their development and regulation, the department of Indian systems of medicine and homeopathy (ISM&H) was established by the ministry of health and family welfare, government of India, in 1995 as a separate department one of its primary objectives was to formulate standards and promote the quality, safety and efficacy of medicines used in ayurveda, yoga, naturopathy, Unani, siddha, and homeopathy, systems.<sup>[6]</sup>

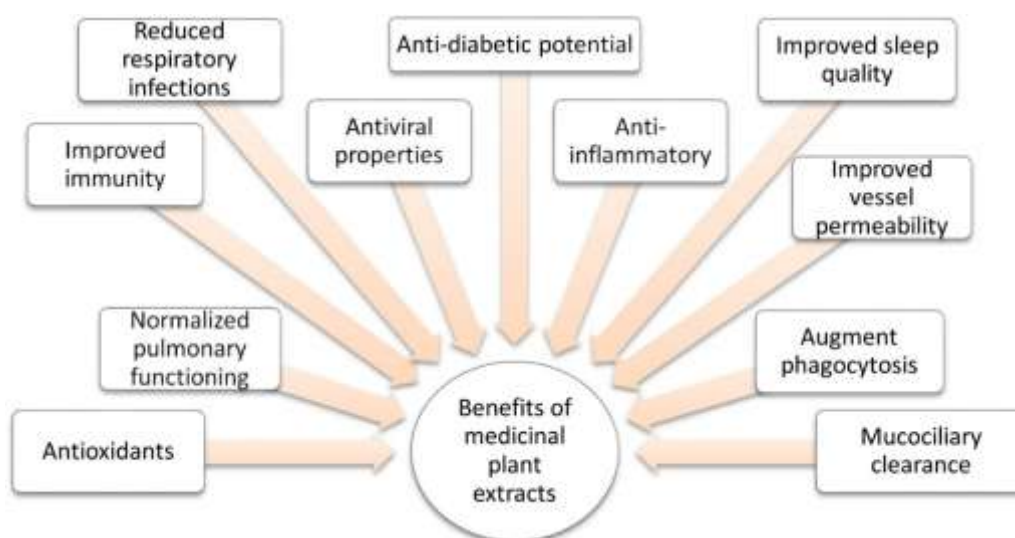
### 1.2 HERBAL MEDICINAL PLANTS:

Medicinal plants have been used in traditional medicine since ancient times. These plants contain bioactive compounds that possess therapeutic properties and are used to prevent, manage, or treat various diseases. In addition to their medicinal value, some medicinal plants can inhibit the growth of bacteria, fungi, and other harmful microorganisms. They are widely used in traditional healthcare systems, particularly in developing countries, due to their affordability, accessibility, and cultural acceptance. Medicinal plants are defined as plants or plant parts that contain substances with potential therapeutic, nutritional, or health promoting benefits.<sup>[7]</sup>

Throughout history, humans have depended on nature to meet their basic requirements, including food, shelter, clothing, transportation, fertilizer, Flavors, fragrance, and medicines. Among these natural resources, plants have been an essential source of therapeutic agents for thousands of years. These traditional remedies laid the foundation for the development of modern remedies and continue to contribute significantly to healthcare systems and world wide.<sup>[8]</sup>

Both wild and cultivated plant species are recognized for their medicinal value. Among medicinal plants, herbs constitute the largest group, while shrubs, trees, and climbers also contribute significantly to traditional and modern healthcare systems. Various plant parts, including leaves, roots, flowers, bark, fruits, seeds, and rhizomes, are utilized for their therapeutic properties and are incorporated into numerous medicinal preparations. Plant extracts and their active constituents serve a valuable resource for the development of novel drug formulation that can be used in the prevention and treatment of a wide range of diseases. Medicinal plants play a crucial role in promoting both individual and community health by providing accessible and cost-effective healthcare solutions.

Different types of herbs are used to prevent and treat diseases various organs in both humans and animals. Herbs contain a wide range of chemical constituents, some of which provide therapeutic benefits, while others may be harmful if used improperly. Indigenous medicinal plants, which occur naturally in their native environments, are valuable natural resources. As these species have evolved and adapted to specific climatic and ecological conditions, their conservation is essential. Many native plants possess unique medicinal properties that have contributed to the discovery and development of life-saving medicines.<sup>[9]</sup>



**Fig 7: Benefits of Medicinal Plant Extract**

### 1.2.1 CURRENT STATUS OF HERBAL MEDICINAL PLANT

Herbal medicinal plants have been used for centuries in traditional systems of medicine such as Ayurveda, Siddha, Unani, Traditional Chinese Medicine, and many indigenous healthcare practices. According to the World Health Organization (WHO), a large proportion of the global population relies on traditional herbal medicines for their healthcare needs.<sup>[10]</sup>

In recent years, the demand for herbal medicinal plants has increased significantly due to growing awareness of the side effects associated with some synthetic drugs and the preference for natural products. The pharmaceutical, nutraceutical, cosmetic, and food industries are increasingly using plant-based ingredients, leading to rapid growth in the global herbal medicine market. Many countries, including India, are promoting the cultivation and commercialization of medicinal plants through research institutions and government initiatives.<sup>[11]</sup>

To address these challenges, governments, research organizations, and conservation agencies are promoting sustainable cultivation, biodiversity conservation, good agricultural and collection practices (GACP), and scientific research. These efforts aim to ensure the long-term availability, quality, and safe use of medicinal plants.<sup>[12]</sup>

### 1.2.2 FUTURE IMPORTANCE OF HERBAL MEDICINAL PLANTS

The future of herbal medicinal plants is highly promising due to the increasing global demand for natural healthcare products. As people become more health-conscious, there is growing interest in herbal medicines that are considered safer, environmentally friendly, and suitable for long-term wellness. This trend is expected to continue in both developing and developed countries.

Advances in biotechnology, phytochemistry, molecular biology, and pharmaceutical research are creating new opportunities for the discovery of plant-derived medicines. Scientists are identifying bioactive compounds from medicinal plants that may help in the prevention and treatment of chronic diseases such as diabetes, cancer, cardiovascular diseases, and infectious diseases. Modern research is also improving the quality, safety, and standardization of herbal medicines.<sup>[13]</sup>

In the coming years, conservation of medicinal plant diversity, sustainable harvesting, cultivation technologies, and scientific validation will become increasingly important. By integrating traditional knowledge with modern science, herbal medicinal plants can contribute to global healthcare, biodiversity conservation, and sustainable economic growth.<sup>[12]</sup>

### 1.3 HERBS AND SPICES:

Herbs and spices have been an essential part in human life for thousands of years that is used at a domestic and industrial level as flavouring, preservation, and colouring agent in nutraceutical, pharmaceutical, and cosmetics products. Spice crop is being affected by insect pests, fungal, and non-pathogenic diseases causing production constraints. Therefore, the quality of spices is very low.<sup>[14]</sup>

Most of the known herbs and spices originate from Europe, Africa, and Asian countries. Spices and herbs are obtained from non-woody and flowering plants. Spices are obtained from the dry part of the plant, such as creeping rootstalk, twigs and leaves, fruits, vegetables, nuts, flower buds, whole and ground seeds, and outermost layers of stems and roots, while herbs are always derived from the leaves. It provides a delectable, distinctive aroma, and taste to food products.<sup>[15]</sup>

Most commonly spices and herbs are used in the preparation of different processing products like pickles, flavour sauces, salad dressings, bakery products, vinegar, beverages, meat products, and sausages. Furthermore, spices and herbs are excellent sources of phenolic and polyphenolic compounds with bioactive functions and phytochemicals. Over the past few decades, there has been a significant increase in research into their health benefits, protect the spice crop, and ways to use them in the diet, as many herbs and spices have possessed properties associated with reducing the risk of chronic diseases.<sup>[16]</sup>



**Fig 8:** Herbs and Spices

### 1.4 GUMMIES:

In recent years, oral drug delivery systems have gained significant importance due to their ease of administration and improved patient acceptability. Among various novel oral dosage forms, gummies have emerged as a promising dosage form that combines therapeutic benefits with pleasant taste and better patient compliance. Oral gummies are chewable formulations designed to deliver active pharmaceutical ingredients, herbal extracts, vitamins, and minerals in an attractive and convenient form. They provide an alternative approach to conventional tablets and capsules, especially for paediatric and geriatric patients who have trouble in swallowing. The gummy matrix provides an effective platform for incorporating multiple herbal extracts into a single formulation, thereby improving therapeutic convenience.<sup>[17]</sup>

The transformation of traditional herbal medicines into gummy dosage forms represents an innovative approach in modern pharmaceuticals. This dosage form enhances the acceptability of herbal therapy without altering the therapeutic value of medicinal plants.

The growing demand for convenient and attractive dosage forms has increased research interest in herbal gummies, making them a promising platform for the future development of patient-centric herbal formulations.<sup>[18]</sup>



**Fig 9: Gummies**

Gummies are generally prepared using suitable gelling agents such as gelatin, pectin, agar, and carrageenan, along with sweeteners, flavours, and colouring agents to achieve desirable texture, stability, and palatability. Due to their chewable nature and acceptable taste, gummies can improve patient adherence and provide a flexible platform for incorporating various active ingredients. Proper formulation and evaluation are necessary to ensure quality, stability, and effectiveness of the final product.<sup>[17]</sup>

Compared with conventional liquid preparations, gummy formulations simplify drug administration by eliminating the need for dose measurement and reducing the possibility of accidental spillage. Their chewable nature allows gradual release of herbal ingredients during mastication, which may prolong the contact time of active compounds within the oral cavity.<sup>[19]</sup>

The increasing interest in herbal-based formulations has encouraged the development of novel dosage forms containing medicinal plant extracts. Medicinal plants contain various bioactive phytoconstituents responsible for their therapeutic activities. Herbal gummies provide an effective and acceptable delivery system for these natural compounds and may improve patient compliance compared with conventional formulations.<sup>[20]</sup>

Medicated gummies have gained attention as a patient-friendly dosage form for delivering therapeutic agents, particularly herbal extracts. Incorporation of plant-derived active ingredients into gummy formulations provides a convenient method of administration while maintaining the benefits of natural compounds. Herbal gummies are considered an emerging approach that combines traditional herbal medicine with modern pharmaceutical dosage forms.<sup>[21]</sup>

Polyherbal gummy formulations have gained importance because they can deliver different phytoconstituents with complementary pharmacological activities in a single dosage form. In addition, gummies offer a stable base that helps maintain the quality and integrity of herbal constituents during formulation and storage, making them suitable for long-term administration.<sup>[22]</sup>

#### **1.4.1 TYPES OF HERBAL GUMMIES**

##### **1. For Stress and Sleep:**

Gummies with ashwagandha (adaptogen, lowers stress) Gummies with valerian root (promotes sleep)  
Gummies made with chamomile (calming, helpful with anxiety and sleep) Gummies with lavender (relaxation)

##### **2. For Immunity:**

Elderberry candies (boosting the immune system) Echinacea gummies (to prevent colds and the flu)  
Gummies with turmeric and ginger (anti-inflammatory, immunological support).

### 3. For Digestion:

Ginger gummies (help with digestion, alleviate nausea) Gummies with peppermint (calms the stomach)  
Gummies made from licorice root (gut health).

### 4. For Energy and Focus:

Ginseng gummies (improves mental clarity and vitality).

### 5. For Diabetes:

Paneer Doda Gummies (manage Diabetes, Improves Digestive Health), Star  
Fruit Gummies (Helps Prevents Blood sugar spikes), Noni Fruit Gummies (Reduce Insulin Resistance).

### 6. For Anti-inflammatory:

Gummies with clove and cardamom (used for reduction of inflammation).<sup>[18]</sup>

## 1.4.2 COMPOSITION OF GUMMIES

### 1. Gelling Agent (Base Polymer)

- Ex. Gelatine, Pectin, Agar, Carrageenan, Gum Arabic
- Function: Provides the Structural Matrix; Gives Chewiness and Texture

### 2. Sweeteners

- Ex. Sucrose, Glucose Syrup, Fructose, Xylitol, Sorbitol, Stevia
- Function: Provides Sweetness, Mouth feel, And Helps with Texture

### 3. Plasticizers / Humectant

- Ex. Glycerol, Sorbitol
- Function: Retain Moisture, Improve Flexibility, And Prevent Brittleness

### 4. Active Pharmaceutical Ingredient (API)

- Ex. Vitamins, Herbal Extracts, Metformin, Minoxidil, Ascorbic etc.
- Function: Provides Therapeutic or Nutritional Benefits

### 5. Acidulants

- Ex. Citric Acid, Malic Acid, Lactic Acid
- Function: Adjust pH, Enhance Flavour, And Prevent Microbial Growth.

### 6. Flavours

- Ex. Fruit Essences (Orange, Lemon, Strawberry, Etc.)
- Function: Enhance Palatability

### 7. Colorants Natural Colours

- Ex. Beetroot Extract, Curcumin, Chlorophyll
- Function: Provide Attractive Appearance

### 8. Preservatives

- Ex. Sodium Benzoate, Potassium Sorbate
- Function: Extend Shelf-Life and Prevent Microbial Contamination

### 9. Coating Agents

- Ex. Carnauba Wax, Beeswax
- Function: Prevent Sticking a water Purified Water Solvent for All Ingredients; Affects Texture and Firmness.<sup>[23]</sup>

### 1.4.3 TYPES OF GELLING AGENTS

#### 1. Gelatine (Animal-based)

- Source: Collagen from animal skin, bone.
- Gelling Temperature: 20–25°
- Characteristics:
  - a) Forms Elastic
  - b) Chewy

**Limitations:** Not suitable for vegans/vegetarians; sensitive to heat.

#### 2. Pectin (Plant-based)

- Source: Citrus fruits, apple pomace.
- Types: High Methoxyl (HM), Low Methoxyl (LM)
- Characteristic:
  - a) Forms soft to firm gels
  - b) LM pectin gels in presence of calcium ions

#### 3. Agar-Agar

- source: Red seaweed
- Characteristics:
  - a) Strong, brittle gels
  - b) High melting point (~85°C)

#### 4. Carrageenan

- Types:  $\kappa$ -carrageenan,  $\iota$ -carrageenan,  $\lambda$ -carrageenan
- Source: Sourced from red algae
- Characteristic:
  - a)  $\kappa$ -carrageenan forms firm and brittle gels in the presence of potassium ions.
  - b)  $\iota$ -carrageenan yields soft, elastic gels.
  - c) Commonly used in dairy gels and gummies.<sup>[24]</sup>

### 1.4.4 BENEFITS OF EATING GUMMIES:

#### 1. Easy to Consume

Gummies are soft, chewable, and do not require water, making them a convenient option for children, elderly individuals, and those with difficulty swallowing pills.

#### 2. Better Taste & Palatability

Unlike traditional tablets or capsules, gummies come in a variety of flavors, making them more enjoyable to consume.

#### 3. Enhanced Nutrient Absorption

Some gummies contain pre-dissolved nutrients, which may be easier for the body to absorb compared to solid pills.

#### 4. Customizable Formulations

Gummies can be fortified with vitamins, minerals, probiotics, collagen, CBD, melatonin, and other functional ingredients to support various health needs.

#### 5. Improved Compliance & Consistency

Their enjoyable taste and texture encourage regular consumption, reducing the likelihood of skipping doses in supplementation.

## **6. Gentle on the Stomach**

Unlike some pills, gummies are less likely to cause stomach irritation or discomfort.

## **7. Kid & Elderly-Friendly**

Gummies provide a fun and appealing alternative to traditional medications, making it easier for children and older adults to take their daily supplements.

## **8. Portable & convenient**

They are easy to carry and consume anywhere without the need for water, making them travel-friendly.<sup>[22]</sup>

### **1.4.5 ADVANTAGES:**

1. Gummies resemble traditional candies and can be consumed discreetly, which is particularly beneficial for individuals who may feel stigmatized or uncomfortable taking medication in public.
2. Gummies are a convenient dosage form that can be easily consumed and making them an attractive option for individuals who have difficulty swallowing pills or capsules.
3. Medicine-infused gummies can be formulated to contain a wide range of active ingredients, including vitamins, minerals, and herbal extracts, allowing for customization to meet individual health needs.
4. Gummies are often flavoured, making them more palatable and enjoyable to consume compared to traditional medication forms.
5. When consumed orally, the active ingredients in gummies are slowly released into the bloodstream, leading to longer-lasting effects compared to other forms of medication.

### **1.4.6 DISADVANTAGES**

1. Gummies can be difficult to cut or break into smaller doses, leading to potential inaccuracies in dosing.
2. The potency of gummies can vary depending on the manufacturing process, leading to inconsistencies in the amount of active ingredients present in each gummy.
3. The candy-like appearance and taste of gummies can increase the risk of overconsumption, particularly in children or individuals with impulse control issues.
4. The gelatine or pectin base used in gummies may not be compatible with certain drugs, leading to decreased efficacy or stability.
5. Gummies often contain high amounts of sugar, which can be problematic for individuals with diabetes or other health conditions.<sup>[21]</sup>

### **1.4.7 APPLICATIONS**

1. Herbal remedies and plant-based supplements are commonly incorporated into gummy form to facilitate consumption.
2. Gummies have become a favoured option in the confectionery sector because of their delicious taste and enjoyable texture. They are available in a wide range of Flavors, shapes, and sizes.
3. Gummies serve as a convenient way to consume vitamins and dietary supplements, offering crucial nutrients such as vitamin C, D, B12, omega-3 fatty acids, and probiotics in a pleasant and easy-to-take form.
4. Some medications are manufactured in gummy form to enhance their appeal, particularly for children and individuals who struggle with swallowing tablets.
5. Gummy candies have the potential to be enriched with extra nutrients or beneficial substances such as fibre or antioxidants, to amplify their health advantages beyond simply being a sweet indulgence.<sup>[25]</sup>

### **1.4.8 LIMITATIONS**

#### **1. High Sugar Levels:**

Gummies frequently contain sucrose or glucose, making them inappropriate for individuals with diabetes.

#### **2. Temperature Sensitivity:**

Gummies are prone to softening or melting in high temperatures, which can compromise their stability and shelf life.

### **3. Low Drug Loading Capacity:**

Only small doses of active pharmaceutical ingredients (Apis) can be included, as higher doses are not feasible.

### **4. Potential for Microbial Contamination:**

The moisture present in gummies can facilitate microbial growth if preservatives are insufficient.

### **5. Delayed Drug Release:**

The gelatin or pectin matrix may hinder the quick release of drugs, which is unsuitable for medications requiring rapid action.<sup>[26]</sup>

## **1.5 INFLAMMATION**

The US National Library of Medicine defines inflammation as “the body’s immune system’s response to stimulus,” and it derives from the Latin word “inflammo”, which means “blaze, burn.” The pathogen (bacteria, viruses, or fungi) infection, tissue injuries and remodelling, or non-physiological cell death frequently causes inflammation, which is generally regarded as a self-protective reaction and is crucial for wound healing.<sup>[27]</sup>

Inflammation is defined as a component of the intricate biological response of vascular tissues to unpleasant stimuli such as irritants, pathogens, or damaged cells. Loss of joint function, joint discomfort, stiffness, and red, swollen joints are among its characteristics. Inflammation is a typical protective reaction to tissue damage brought on by physical trauma, hazardous chemicals, or microbiological substances.<sup>[28]</sup>

### **1.5.1 ORAL INFLAMMATION**

Oral inflammation is a protective biological response of the oral tissues to microbial infection, mechanical injury, or chemical irritation. It is initiated by the activation of the host immune system, leading to the recruitment of inflammatory cells and the release of various inflammatory mediators such as cytokines, prostaglandins, and chemokines. The primary purpose of inflammation is to eliminate harmful stimuli and promote tissue repair. However, when the inflammatory response becomes excessive or persists for a prolonged period, it may result in tissue destruction and contribute to the development of oral diseases such as gingivitis, periodontitis, and dental pulp inflammation.<sup>[29]</sup>

Oral inflammation may be classified into

- Acute oral inflammation
- Chronic oral inflammation
- Acute Oral Inflammation

Acute oral inflammation is the initial and rapid immune response of the oral tissues to harmful stimuli such as bacterial infection, dental caries, trauma, chemical irritation, or dental procedures. It develops within a short period and functions as a protective mechanism to eliminate microorganisms, remove damaged tissue, and initiate tissue repair. During acute inflammation, vasodilation and increased vascular permeability allow neutrophils and other inflammatory cells to migrate to the affected site. These cells release inflammatory mediators that help control infection and promote healing.

Clinically, acute oral inflammation presents with redness, swelling, pain, tenderness, increased local temperature, and temporary impairment of oral function. Conditions such as pulpitis, dental abscess, traumatic ulcers, and inflammation following tooth extraction are common examples. When the causative factor is eliminated, acute oral inflammation usually resolves completely without causing permanent tissue damage.<sup>[30]</sup>

- Chronic Oral Inflammation

Chronic oral inflammation occurs when the inflammatory response persists because the underlying cause, such as persistent bacterial plaque, poor oral hygiene, or continuous irritation, is not removed. It is characterized by prolonged infiltration of macrophages, lymphocytes, and plasma cells, along with continuous production of inflammatory mediators that gradually damage the surrounding tissues. Chronic

inflammation affects the gingiva, periodontal ligament, alveolar bone, and other supporting structures of the teeth, leading to progressive tissue destruction.

Clinically, it is associated with persistent gingival swelling, bleeding on brushing, periodontal pocket formation, alveolar bone loss, tooth mobility, and, in advanced stages, tooth loss. Gingivitis and periodontitis are the most common chronic inflammatory diseases of the oral cavity. Therefore, early diagnosis and effective anti-inflammatory treatment are essential to prevent disease progression and preserve oral health.<sup>[30]</sup>

### 1.5.2 MEDIATORS OF INFLAMMATION:

Inflammatory mediators are diverse signalling molecules that play crucial roles in initiating and regulating the inflammatory response. Key mediators include histamine, released from mast cells, which increases vascular permeability and causes vasodilation; bradykinin, which contributes to pain, vasodilation, and edema; prostaglandins, synthesized by various cells, which promote pain, fever, and inflammation; leukotrienes, produced by leukocytes, which contribute to bronchoconstriction, vascular permeability, and chemotaxis; cytokines (such as tumour necrosis factor- $\alpha$ , interleukins), which modulate immune cell activities and inflammation; nitric oxide (NO), involved in vasodilation and pathogen killing; and platelet-activating factor (PAF), which promotes leukocyte aggregation and vascular permeability.

These mediators work together to orchestrate the complex biological processes of inflammation, but their excessive or uncontrolled production can lead to chronic inflammation and tissue damage.<sup>[31]</sup>

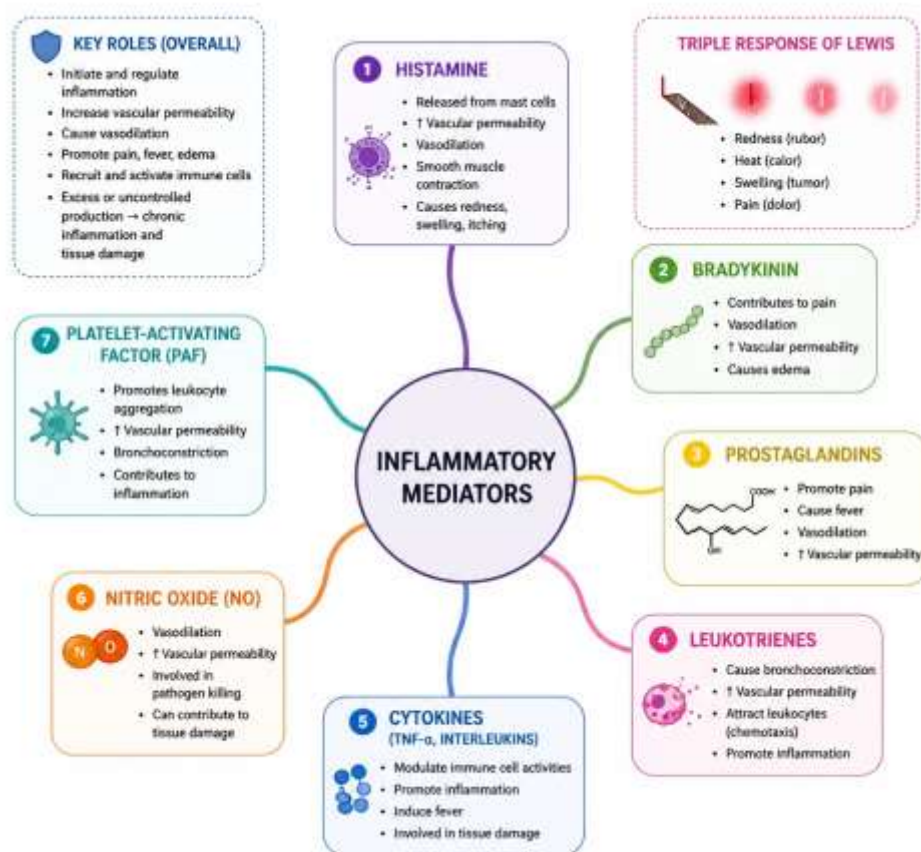


Fig 10: Inflammatory Mediators

### 1.6 ANTI-INFLAMMATORY ACTIVITY:

Anti-inflammatory action encompasses any substances, mechanisms, or methods that inhibit, control, or lessen an inflammatory response in the human body. Regulating chronic or excessive inflammatory processes without permanently impairing the immune system's protective functions is the main goal of anti-inflammatory methods.<sup>[32]</sup>

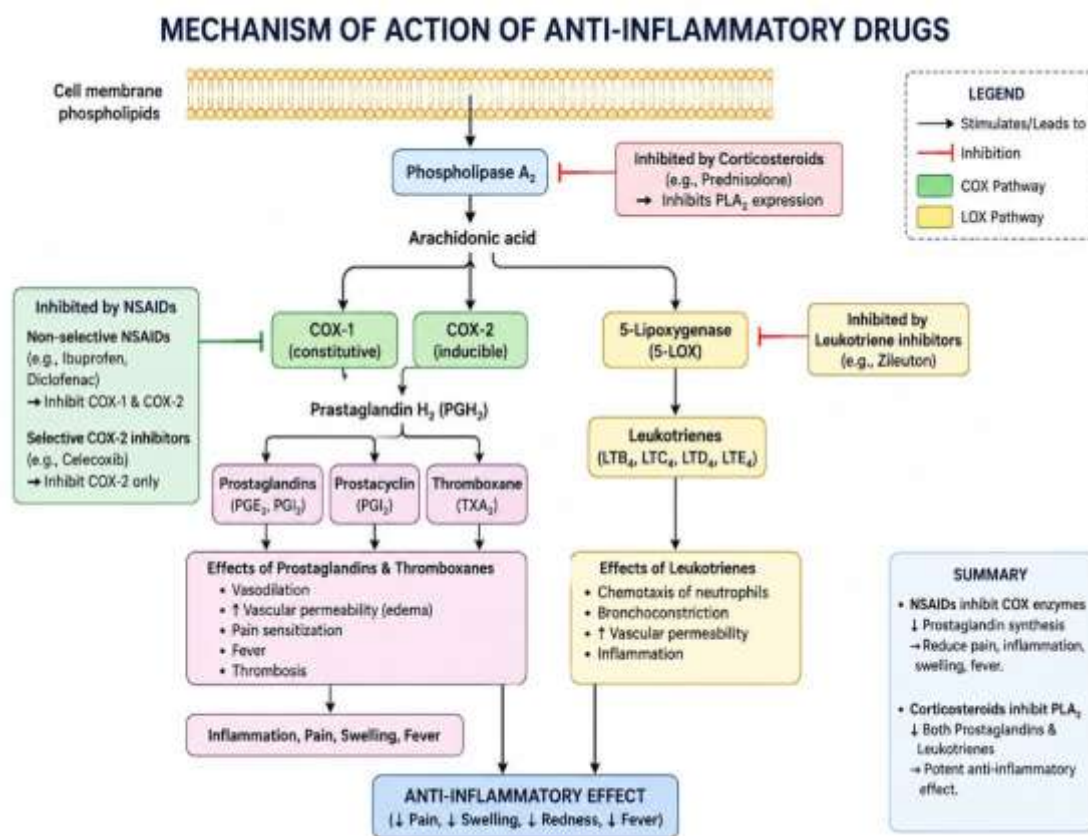
Anti-inflammatory activities are crucial for the healing process. In this process, keratinocytes, B lymphocytes, T lymphocytes, and macrophages produce immune-responsive chemicals (cytokinins and

interleukins) (Jacob et al., 2012). Inflammatory responses are caused by antibodies and enzymes released by the endocrine system. The mediators for inducing the healing processes (Satyavani et al., 2011) are cytokines, IL-1, and IL-2, which are the anti-inflammatory organs from primary immune organs. These mediators also regulate the biochemical process and prevent the illness from spreading.<sup>[33]</sup>

### 1.6.1 ANTI-INFLAMMATORY MECHANISMS

Anti-inflammatory treatments target several stages of the inflammatory process:

- Substances like tumour necrosis factor (TNF-alpha), interleukins, leukotrienes, and prostaglandins are inhibited or have their synthesis decreased.
- Inhibition of enzymes: Lipoxygenases and cyclooxygenases (COX-1, COX-2) are two examples of enzymes that are essential for the production of inflammatory mediators. Their inhibition reduces the inflammatory response.
- Modulation of the immune system: Some medications decrease the activity of immune cells like T-lymphocytes, neutrophils, or macrophages.
- Antioxidant properties: Inflammatory processes are facilitated by oxidative stress. Polyphenols, vitamin E, or vitamin C are a few examples of antioxidants that might indirectly block inflammation.<sup>[32]</sup>



**Fig 11:** Mechanism of action of Anti-inflammatory agents

### 1.6.2 ANTI-INFLAMMATORY AGENTS:

Anti-inflammatory agents or chemicals can interfere with the pathophysiology of inflammation to lessen tissue damage and make the patient more comfortable. Anti-inflammatory agents aim to effectively treat various diseases by targeting specific pathways involved in inflammation while minimizing side effects. Current drugs include nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and COX-2 inhibitors, which block the synthesis of inflammatory prostaglandins; glucocorticoids, which suppress multiple cytokine genes and reduce inflammation but have significant side effects; and biologics such as

monoclonal antibodies that neutralize specific cytokines like TNF- $\alpha$ , IL-6, IL-1, IL-12, and IL-17, used to treat autoimmune diseases like rheumatoid arthritis, Crohn's disease, and psoriasis.<sup>[24]</sup>

#### 1.6.2.1 CLASSIFICATION OF ANTI-INFLAMMATORY AGENTS:

Steroidal and Non-Steroidal Anti-Inflammatory Medications are classified as anti-inflammatory agents that are utilized to boost the innate immune response against inflammatory illnesses, and many of these agents have negative side effects when used over an extended period of time. The long-term usage of non-steroidal anti-inflammatory medications (NSAIDs), such as aspirin and coxibs, raises the likelihood of cardiovascular and gastrointestinal problems (Sostres et al., 2010). It has been suggested that preparations based on natural products may offer a possible answer to some of these issues.<sup>[26]</sup>

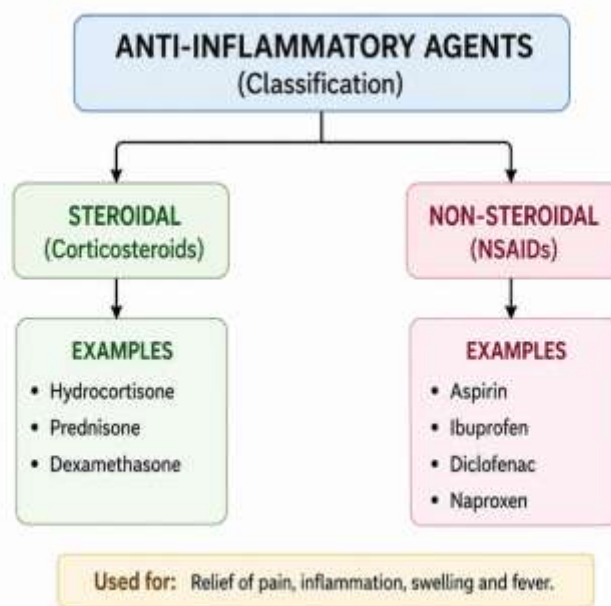


Fig 12: Anti-inflammatory agent

## 2.0 LITERATURE REVIEW

### 2.1 LITERATURE SURVEY – SYZYGIIUM AROMATICUM AND ELETTARIA CARDAMOMUM

**1. Mahasin Ahmed Wadi, et al., (2025):** The study evaluated the antibacterial activity of aqueous extracts of *Syzygium aromaticum* (clove) against ten Gram-positive and Gram-negative bacterial strains. The antibacterial activity was assessed using the well diffusion method, while the chemical composition of the extract was characterized by GC–MS analysis. The clove extract exhibited broad-spectrum antibacterial activity against all tested microorganisms, with *Escherichia coli* and *Klebsiella pneumoniae* showing the highest sensitivity. GC–MS analysis identified 58 bioactive compounds, among which eugenol was the major constituent. Overall, the findings demonstrated that clove extract possessed significant antibacterial potential and could be utilized as a natural antimicrobial agent.<sup>[34]</sup>

**2. Vinay Kumar Pandey, et al., (2026):** The review highlighted the medicinal and food processing applications of *Syzygium aromaticum* (clove) due to its rich composition of bioactive compounds, including eugenol, eugenol acetate, flavonoids, and gallic acid. It summarized the antibacterial, antifungal, anticancer, analgesic, and anti-inflammatory properties of clove essential oil. The review reported that the anti-inflammatory activity of clove was mainly attributed to eugenol and flavonoids, which helped reduce inflammation and pain. It also discussed the potential use of clove essential oil as a natural bioactive ingredient in food preservation and emphasized its importance in food processing and medicinal applications.<sup>[35]</sup>

**3. Avisek Majumder, et al., (2024):** The review summarized the medicinal and pharmacological properties of cardamom (*Elettaria cardamomum*), highlighting its antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities. It reported that various phytochemicals isolated from cardamom exhibited potential anticancer effects by reducing drug resistance, protecting against

chemotherapy-induced toxicity, and promoting programmed cell death in cancer cells. The review also discussed the therapeutic potential of major bioactive compounds, including limonene, cymene, pinene, linalool, borneol, and cardamomin. Overall, the findings suggested that cardamom possessed promising medicinal properties and could serve as a potential natural agent for cancer prevention and treatment, although further clinical studies were required.<sup>[36]</sup>

**4. Hossein Hosseinzadeh, et al., (2021):** The review evaluated the therapeutic potential of *Elettaria cardamomum* (cardamom) in the management of metabolic syndrome (MetS) and its associated complications. It summarized evidence from in vitro, in vivo, and clinical studies, highlighting the rich phytochemical composition of cardamom, including phenolic compounds and volatile oils. The review reported that cardamom exhibited antihypertensive, antioxidant, anti-inflammatory, antidiabetic, lipid-lowering, anti-obesity, and cardioprotective activities. Overall, the findings suggested that cardamom could be beneficial in the prevention and management of metabolic syndrome and its related disorders through multiple therapeutic mechanisms.<sup>[37]</sup>

**5. Giselle Teresa Martinez, et al., (2025):** The study investigated the effect of Instant Controlled Pressure Drop (DIC) technology on the fatty acid profile of *Elettaria cardamomum* (cardamom). The fatty acid composition was analyzed using GC–MS, and eight major fatty acids were identified. The results showed that DIC processing significantly influenced the fatty acid composition, with temperature and treatment time playing important roles in determining the levels of most fatty acids. The optimized DIC conditions improved the yield and quality of fatty acids, thereby enhancing the nutritional and sensory properties of cardamom. Overall, the findings demonstrated that DIC technology could be applied as an effective processing method to improve the quality of cardamom.<sup>[38]</sup>

## 2.2 LITERATURE SURVEY – ANTI INFLAMMATORY

**1. Abdullatif Azab, et al., (2016):** This article presents highlights of the published literature regarding the anti-inflammatory activities of natural products. Many review articles were published in this regard, however, most of them have presented this important issue from a regional, limited perspective. This paper summarizes the vast range of review and research articles that have reported on the anti-inflammatory effects of extracts and/or pure compounds derived from natural products. Moreover, this review pinpoints some interesting traditionally used medicinal plants that were not investigated yet.<sup>[39]</sup>

**2. Yilma Hunde Gonfa, et al., (2022):** The review evaluated the anti-inflammatory potential of phytochemicals derived from medicinal plants and their metal/metal oxide nanoparticles (M/MONPs). Various phytochemicals, including flavonoids, terpenoids, polyphenols, saponins, tannins, alkaloids, and anthraquinones, were reported to exhibit significant anti-inflammatory activities against a wide range of inflammatory disorders. The review summarized the therapeutic effects and mechanisms of action of these phytochemicals and their nanoparticles in treating skin, liver, cardiovascular, gastrointestinal, neurological, lung, and joint inflammatory diseases. Overall, the findings highlighted the potential of medicinal plant-derived phytochemicals and their nanoparticles as safe and effective anti-inflammatory agents with fewer side effects.<sup>[40]</sup>

**3. Chayanika Padumadasa, et al., (2026):** The review examined inflammation as a biological response and highlighted the limitations associated with the long-term use of conventional anti-inflammatory drugs, including NSAIDs, glucocorticoids, and immunosuppressants. The study emphasized the need for safer plant-based anti-inflammatory agents and summarized various in vitro assay methods used to evaluate their anti-inflammatory potential. It also discussed the principles, advantages, and limitations of these methods to facilitate the identification of plant-derived bioactive compounds with promising anti-inflammatory properties.<sup>[41]</sup>

**4. Xiuqing Huang, et al., (2024):** The review examined the role of chronic inflammation in the development of various chronic diseases and highlighted the limitations of currently available anti-inflammatory drugs for long-term treatment. It emphasized the potential of natural foods and plant extracts as safer alternatives for the prevention and management of inflammation-related disorders. The review summarized various in vitro assays used to evaluate anti-inflammatory activity, including the assessment of pro-inflammatory enzymes and cytokines. It also discussed the principles, advantages, and limitations of these methods for screening natural products with anti-inflammatory potential.<sup>[42]</sup>

**5. Bhanita Das, et al., (2024):** The study evaluated the anti-inflammatory potential of *Anemone rivularis* leaf extracts through phytochemical screening and protein denaturation inhibition assays. Methanolic leaf

extracts were prepared using maceration and hot percolation techniques and were analyzed for the presence of bioactive compounds. Phytochemical analysis revealed the presence of flavonoids, saponins, steroids, triterpenoids, tannins, and phenolic compounds. The extracts showed significant inhibition of protein denaturation, indicating strong anti-inflammatory activity compared to the standard. Overall, the findings suggested that *Anemone rivularis* possessed promising anti-inflammatory properties and could serve as a potential natural therapeutic agent for managing inflammation-related disorders.<sup>[43]</sup>

## 2.3 LITERATURE SURVEY – CHEWABLE GUMMIES:

**1. Mohammad Tarahi, et al., (2023):** Consumer awareness of healthy diets increased the demand for natural and nutritious food products, particularly for children. Consequently, the food industry focused on developing products with reduced sucrose and artificial additives while enhancing antioxidant, protein, and fiber contents. In addition, the demand for vegan, halal, and kosher-certified products increased. Conventional gummy candies gradually lost their popularity, leading researchers and manufacturers to develop plant-based and functional gummy candies using alternative gelling agents, sweeteners, natural flavors, and colorants. They also incorporated medicines, fiber, protein, and antioxidants to improve the techno-functional, sensory, and nutritional properties of gummy candies.<sup>[44]</sup>

**2. Edite Teixeira-Lemos, et al., (2021):** The study developed gummy jellies using natural ingredients without added sugars or artificial additives in two formulations: orange juice with honey (ORH) and mixed berry puree (BEM). The developed gummies were evaluated through physicochemical, microbiological, and sensory analyses. The results showed that both formulations were microbiologically safe and contained significantly lower caloric values than similar commercial products. In addition, the gummies exhibited higher antioxidant capacity and provided carbohydrates and proteins as major macronutrients. Sensory evaluation revealed that although the developed gummies were slightly less preferred than commercial products, they were well accepted by the panel, particularly the ORH formulation. Overall, the developed gummy jellies offered a healthier alternative to conventional gummy candies due to their lower calorie content and higher antioxidant properties.<sup>[45]</sup>

**3. Mozghan Roudbari, et al., (2024):** The study aimed to enhance the nutritional value of gummy candies by incorporating pistachio green hull extract (PGHE), stevia, and starch into the formulation. The gummy candy formulations were optimized using response surface methodology (RSM) with different concentrations of PGHE, stevia, and gelatin-to-starch ratios. The physicochemical and textural properties of the developed gummies were evaluated, and three optimal formulations were identified based on sensory acceptance. One selected formulation showed high total phenolic content, strong antioxidant activity, and good storage stability. Overall, the developed gummy candies provided a reduced-sugar product with high antioxidant activity while eliminating the need for artificial flavors and synthetic colorants.<sup>[46]</sup>

**4. Augustine Amalraj, et al., (2026):** The study aimed to develop a heat-stable liposomal vitamin C gummy using In Situ Soft Sphere Integrated (ISSI) technology with a xyloglucan/trehalose/citric acid polymeric matrix. The developed gummies were evaluated for their physicochemical properties, stability, and in vitro release behavior. The ISSI system improved the thermal, structural, and chemical stability of vitamin C during processing and storage. The developed gummies showed desirable physicochemical characteristics, enhanced vitamin C retention, and sustained intestinal release compared to conventional gummies. Overall, the liposomal vitamin C gummies provided a stable and consumer-acceptable delivery system for vitamin C in functional foods and nutraceutical applications.<sup>[47]</sup>

**5. Francesca Aiella, et al., (2024):** The study utilized hydroalcoholic extracts obtained from the peels of red and blonde oranges and lemons as sources of bioactive compounds for the development of functional gummies. The extracts were characterized for their antioxidant activity and bioactive composition, with lemon peel extract showing the highest antioxidant capacity. The extracted polyphenols were grafted onto gelatin chains to develop functional gummy formulations with improved antioxidant properties. The developed gummies exhibited good antioxidant activity and desirable rheological properties throughout the storage period. Overall, the findings demonstrated that citrus peel waste could be utilized as a sustainable source of bioactive compounds to produce functional gummies.<sup>[48]</sup>

## 3.0 AIM AND OBJECTIVES:

### 3.1 AIM

The aim of the present study was to formulate polyherbal oral gummies containing extracts of *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (cardamom) and to evaluate their in vitro anti-inflammatory activity using the albumin denaturation inhibition assay.

### 3.2 OBJECTIVES

1. To collect and authenticate the selected herbal materials, *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (cardamom).
2. To prepare extracts of clove and cardamom for further phytochemical and pharmacological investigation.
3. To perform preliminary phytochemical screening of the selected herbal extracts for identification of major phytoconstituents.
4. To formulate polyherbal oral gummies containing clove and cardamom extracts using suitable pharmaceutical excipients.
5. To evaluate the prepared gummies for physicochemical parameters such as weight variation, pH, moisture content, thickness, physical appearance, stability and disintegration time.
6. To assess the in vitro anti-inflammatory activity of clove and cardamom extracts by the albumin denaturation inhibition assay.
7. To compare the anti-inflammatory activity of the herbal extracts with diclofenac sodium as a standard drug.
8. To determine the IC<sub>50</sub> values of the herbal extracts and assess their potential anti-inflammatory activity.

### 4.0 PLAN OF WORK

- Literature survey
- Selection of herbs
- Authentication and collection of *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (cardamom)
- Extraction of the selected herbal materials
- Preliminary phytochemical screening
- Formulation of polyherbal oral gummies using clove and cardamom extracts
- Evaluation of the prepared gummies for physicochemical parameters
- In vitro anti-inflammatory activity by albumin denaturation inhibition assay
- Result and Discussion
- Conclusion

### 5.0 PLANT PROFILE

#### 5.1 CLOVE:

Clove (*Syzygium aromaticum* L.) is an aromatic spice belonging to the family Myrtaceae. It consists of the dried, unopened flower buds of the clove tree and has been widely used as a culinary spice and as an important medicinal plant in traditional systems of medicine. Owing to its rich phytochemical composition and broad spectrum of biological activities, clove has gained considerable attention in pharmaceutical and nutraceutical research.<sup>[49]</sup>

The major phytochemical constituents of clove include eugenol, eugenyl acetate, and  $\beta$ -caryophyllene, with eugenol being the principal component of clove essential oil. In addition, clove contains flavonoids, tannins, triterpenoids, and other phenolic compounds that contribute to its therapeutic potential. These bioactive constituents are responsible for the characteristic aroma, flavor, and pharmacological properties of clove.<sup>[50]</sup>

Clove possesses a wide range of pharmacological activities, including antioxidant, antimicrobial, antifungal, analgesic, antiseptic, and anti-inflammatory effects. Traditionally, it has been used for the treatment of toothache, oral infections, digestive disorders, and various inflammatory conditions. The presence of eugenol plays a significant role in providing these medicinal benefits.<sup>[51]</sup>



**Fig 13:** Clove

Among the various pharmacological properties of clove, its anti-inflammatory activity has attracted significant research interest. The major bioactive constituent, eugenol, has been shown to suppress the production of pro-inflammatory cytokines and inflammatory mediators by modulating signaling pathways such as NF- $\kappa$ B and MAPK. In addition, eugenol exhibits potent antioxidant activity by reducing oxidative stress and protecting cells from free radical-induced damage. These combined anti-inflammatory and antioxidant properties make clove a promising natural ingredient for the development of oral herbal formulations, including polyherbal gummies, intended for the management of oral inflammatory conditions.<sup>[52]</sup>

#### **SYNONYM**

- *Eugenia caryophyllus*
- *Caryophyllus aromaticus*
- Lavang
- Kirambu/Krambu

#### **BOTANICAL NAME**

- *Syzygium aromaticum* (L.) Merr. & L.M. Perry

#### **BIOLOGICAL SOURCE**

- It consists of the dried, unopened flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry, belonging to the family Myrtaceae.<sup>[49]</sup>

#### **TAXONOMIC CLASSIFICATION**

- Kingdom – Plantae
- Sub kingdom – Tracheobionta
- Super division – Spermatophyta
- Division – Magnoliophyta
- Class – Magnoliopsida
- Subclass – Rosidae
- Order – Myrtales
- Family – Myrtaceae
- Genus – *Syzygium*
- Species – *Syzygium aromaticum* (L.) Merr. & L.M. Perry.<sup>[53]</sup>

#### **HISTORY & ORIGIN**

Clove is one of the most ancient and valuable spices of the orient, with its origin as old as the first century, before Christ. The ancient Chinese Handy nasty lasting from 207 B.C. to 220 A.D. gives us our first clue to the use of fragrant clove. The use clove as a spice reached Europe around the 4<sup>th</sup> century A.D. when commercial trading really started with the Arabs, who in turn acquired these dried and fragrant buds from the cultures to the East in Asia. For over 2,000 years, both Indian and Chinese traditional medicine made extensive use of clove flowers and clove oil. Clove introduced in India around 1800AD by the East India company in its 'spice garden' in Court Allam, Tamil Nadu. Cloves are believed to be native to the Molucca Island of Indonesia. Although Indonesia is the largest producer of Clove, Zanzibar and Madagascar are the major exporters, where Clove trees cover thousands of acres of the island. Historically, Clove originating from Madagascar have been considered superior.<sup>[54]</sup>

## DESCRIPTION

Cloves are the aromatic dried buds of a tree (*Eugenia caryophyllata* also sometimes *Syzygium aromaticum*) used as a spice in virtually all the world's cuisine. The term 'Clove' is derived from the French word 'Clou' and the English word 'Clout', both meaning 'nail' from the likeliness of the flower bud of the clove tree to a broad-headed nail. The clove tree is an evergreen tree, which grows to a height ranging from 8-12m, having large square leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after which they are ready for collecting. Cloves are harvested when 1.5-2cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals, which form a small ball in the centre.<sup>[50]</sup>

## MORPHOLOGY OF CLOVE

### 1. TREE

- Height: 10-20 meters when fully matured.
- Type: Evergreen, medium-sized tree with a conical to cylindrical crown.
- Bark: Smooth and Gray with a light brown inner bark.
- Wood: Hard and durable with a light aromatic scent.

### 2. LEAF

- Arrangement: Opposite
- Shape: Elliptic to ovate
- Size: 7-12cm long, 3-6cm wide
- Color: Dark green, glossy on the upper surface; lighter underneath
- Texture: Leathery with a prominent midrib and aromatic oil glands
- Smell: Strongly aromatic when crushed

### 3. FLOWER

- Cloves are harvested as unopened flower buds
- Peduncle (flower stalk): Slender and short, about 1-2cm long
- Bud shape: Cylindrical or Slightly conical with a swollen base and a four-toothed calyx
- Size: 1.5-2cm in length
- Color: Green when immature, turning bright red as they mature, and dark brown when dried
- Calyx: Tubular with four prominent sepals forming a crown-like tip

- Petals: 4 small, pale petals covering the developing stamens, which drop off when the bud blooms
- Stamens: Numerous, enclosed within the petals in unopened buds
- Pistil: Single, centrally placed

#### 4. FRUIT

- Type: Oblong berry (rarely seen in commerce as buds are harvested early)
- Color: Dark purple to black when ripe
- Seeds: one or two

#### 5. ROOT

- Type: Tap root system with strong lateral branches
- Function: Anchors the tall tree and facilitates nutrient uptake in tropical, well-drained soils.<sup>[55]</sup>

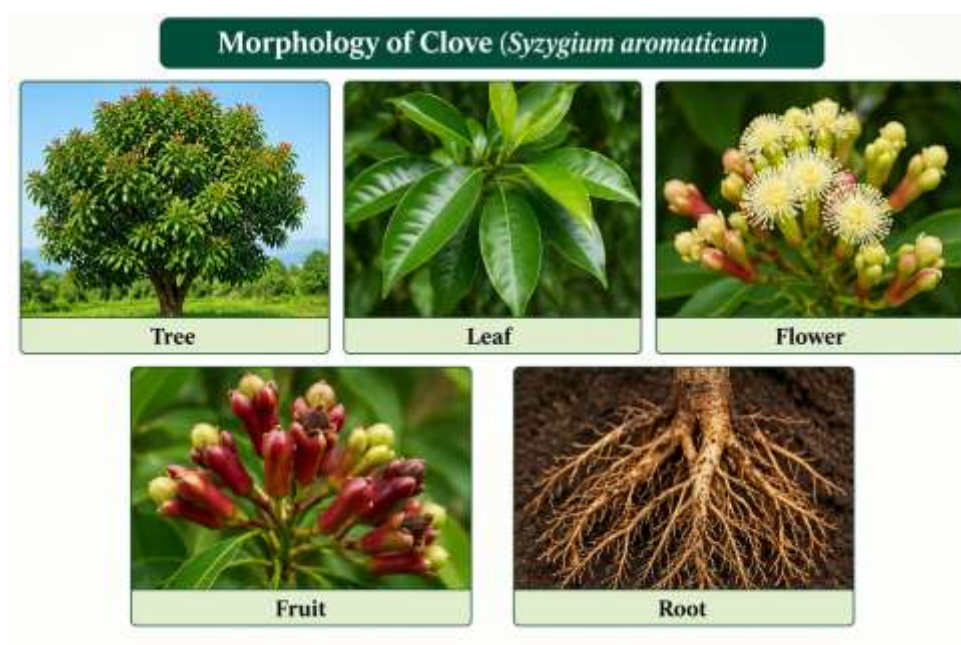


Fig 14: Morphology of clove

#### CULTIVATION AND COLLECTION

Clove is an evergreen tree and generally grows to a height of about 10-20m. It requires a moist, warm and equable climate with well-distributed rain fall, and an annual rainfall of about 150-250cm is suitable for its cultivation. Deep, rich organic soil with high humus content and good drainage is preferred. Clove is propagated mainly by seeds, which are generally sown from August to October in a nursery bed at a spacing of about 10cm, while well-drained suitable soil and a spacing of about 25cm. may also be used for sowing. The seeds germinate within four to five weeks, and the seedlings are transplanted into pots after about six months. Initially, the young plants require protection from direct sunlight and are therefore maintained under a greenhouse or under frames of about 1m height covered with banana leaves. As the banana leaves gradually decay, increasing amount of sunlight reach the seedling, allowing them to tolerate full sunlight at about nine months of age. When the seedlings attain a height of approximately 1m, they are transplanted to open fields at a spacing of about 6m, preferably just before the rainy season.

Young clove trees are protected from excessive sunlight for a longer period by planting banana trees between them. The plants should also be protected against pests and plant diseases. Suitable fertilizers such as ammonium sulphate, superphosphate and potash are applied in two doses, with the first dose given during May-June and the second dose in October.

The clove buds are collected when they change in colour from green to slightly pink. The buds are harvested either by handpicking or by beating the branches with bamboo sticks. After harvesting, the

collected cloves undergo suitable pre-treatment followed by drying, winnowing, packaging and storage. The drug may be collected annually from approximately six years of age and can continue to be harvested up to about 70 years.<sup>[56]</sup>

## DISTRIBUTION

Cloves are the second most valuable and significant spice traded worldwide. Clove requires damp tropical and sub-tropical environments for growth. The North Maluku Islands in Indonesia are home to the widely grown clove plant. Clove farming is only permitted in Kerala, Tamil Nadu, and Karnataka in India. Since different countries have distinct harvest seasons, cloves are accessible all year long.<sup>[55]</sup>

## CHEMICAL CONSTITUENTS

Numerous research has shown that *S. aromaticum* is one of the major vegetal sources of phenolic compounds, as well as terpenoids. Clove contains 14–21% of volatile oil. The other constituents present are the eugenol, acetyl eugenol, Gallo tannic acid, and two crystalline principles;  $\alpha$ - and  $\beta$ - caryophyllenes, methyl furfural, gum, resin, and fibre. Caryophyllene is odourless component and appears to be a phytosterol, whereas eugenol is a colourless liquid. Clove oil has 60–90% eugenol, which is the cause of its anaesthetic and antiseptic properties.<sup>[58]</sup>

## MEDICINAL USES

1. Since clove has antibacterial characteristics, it is used to kill bacteria in a variety of mouthwashes, throat sprays, dental creams, and toothpastes.
2. Additionally, it helps to enhance general oral health and relieves the discomfort of sore gums. Cloves are aphrodisiacs, which are substances that stimulate or heighten sexual arousal or potency.
3. Due to its high flavonoid concentration, clove is utilized as an anti-inflammatory.
4. Pure clove oil is used by aroma therapists to treat the signs and symptoms of arthritis and rheumatism. To promote peristalsis, raise stomach hydrochloric acid, and act as a carminative, clove is employed.
5. To treat acne, apply a paste made of clove powder and honey. Clove powder mixed with water to make a paste encourages quicker healing of bites and injuries. Many digestive issues can be easily treated with cloves. It has therapeutic properties that can treat nausea, indigestion, loose stools and flatulence.
6. Cloves can be used for easing the symptoms of diarrhoea, irritation in the stomach and vomiting. By cleaning the blood and assisting in the battle against numerous diseases, cloves and clove oil strengthen the immune system.
7. The treatment of athlete's foot and nail fungus with clove oil is successful.
8. Good expectorants like cloves encourage the release of mucus and secretions from the respiratory tract.
9. When inhaled, the fragrant clove oil can relieve several respiratory ailments like sinusitis, bronchitis, asthma, colds and coughs.
10. Because clove oil promotes blood flow and circulation, it is beneficial for persons with chilly extremities.<sup>[59]</sup>

## 5.2 CARDAMOM

Cardamom (*Elettaria cardamomum* (L.) Maton) is an aromatic spice belonging to the family Zingiberaceae and is commonly known as the "Queen of Spices." It is widely used as a flavouring agent in foods and beverages and has been traditionally used for various medicinal purposes. Cardamom has been used in traditional medicine for digestive disorders, respiratory problems and oral health conditions. Due to its pleasant aroma, flavour and medicinal properties, cardamom has gained considerable importance as both a culinary spice and a medicinal plant.<sup>[60]</sup>

Cardamom contains a wide range of biologically active phytoconstituents, including essential oils, terpenoids, flavonoids and phenolic compounds. The essential oil is mainly responsible for the characteristic aroma and flavour of cardamom. Important volatile constituents include 1,8-cineole,  $\alpha$ -pinene,  $\alpha$ -terpineol, linalool, linalyl acetate and  $\alpha$ -terpinyl acetate. These constituents contribute significantly to the biological and therapeutic properties of cardamom.<sup>[61]</sup>

Cardamom and its extracts have been reported to possess several pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, gastroprotective, antidiabetic and anticancer

activities. The presence of various bioactive compounds contributes to its ability to exhibit these diverse biological effects. Therefore, cardamom has attracted considerable scientific interest for its potential applications in pharmaceutical, nutraceutical and functional food formulations.<sup>[62]</sup>



**Fig 15: Cardamom**

Among its various pharmacological activities, the anti-inflammatory activity of cardamom is of particular interest. Studies have suggested that cardamom may help modulate inflammatory pathways and reduce the production of inflammatory mediators such as TNF- $\alpha$ , IL-6, NF- $\kappa$ B and COX-2. Clinical studies have also reported potential reductions in certain inflammatory markers following cardamom consumption. Hence, cardamom can be considered a promising natural source of bioactive compounds with potential applications in formulations intended for inflammation-related conditions.<sup>[63]</sup>

#### **Synonyms of Cardamom:**

- Elettaria cardamomum
- Elettaria repens
- Cardamomum
- Elakkai
- Elaichi

#### **BOTANICAL NAME:**

- Elettaria cardamomum (L.) Maton

#### **BIOLOGICAL SOURCE**

- It consists of the dried ripe seeds of Elettaria cardamomum Maton.<sup>[64]</sup>

#### **TAXONOMIC CLASSIFICATION**

- Kingdom – Plantae
- Subkingdom – Tracheobionta
- Super division – Spermatophyta
- Division – Magnoliophyta
- Class – Liliopsida
- Subclass – Zingiberidae
- Order – Zingiberales
- Family – Zingiberaceae
- Genus – Elettaria
- Species – Elettaria cardamomum (L.) Maton.<sup>[60]</sup>

#### **HISTORY & ORIGIN**

Cardamom is one of the oldest and most highly valued spices in the world. It is believed to have originated in the evergreen forests of the Western Ghats of South India, particularly in Kerala, Karnataka and Tamil Nadu. Historical evidence indicates that cardamom has been cultivated and traded in India for over 2,000 years. Ancient Indian texts such as the Charaka Samhita, Taittiriya Samhita, and Kautilya's Arthashastra mention cardamom as a medicinal plant, flavouring agent and ceremonial offering. The Greeks and Romans imported cardamom from India and used it as a spice and perfume. During the medieval period, Arab traders introduced cardamom to the Middle East and Europe, making it an important commodity in the international spice trade. Today, India is regarded as the native home of true cardamom, while Guatemala is the largest producer, and cardamom is also cultivated in Sri Lanka, Tanzania, Costa Rica and other tropical countries.<sup>[65]</sup>

## DESCRIPTION

Cardamom otherwise known as Malabar cardamom, true cardamom or small cardamom second only to pepper in its importance during the Renaissance period, is often qualified as the Queen of Spices because of its very pleasant aroma and taste. The Western Ghat forest of the Malabar coast, India, is the centre of origin and diversity for cardamom and it might have been nature's design that the King and Queen of spices (black pepper and cardamom) originated in the same forest.

This volume contains sixteen chapters, fourteen on cardamom and one each on large cardamom and false cardamoms. All aspects of the cardamom crop have been covered in this volume, and each chapter is written by experts in their respective fields. This volume is visualized as both a textbook and reference work for scientists and students of horticulture, plantation crops, botany and related fields, and will go on to serve as the main reference volume on cardamom for many years to come.<sup>[66]</sup>

## MORPHOLOGY OF CARDAMOM

### 1. PLANT

- Height: 2–4 meters.
- Type: Perennial, evergreen herbaceous plant.
- Stem: Short underground rhizome with erect leafy shoots.
- Habitat: Grows well in tropical, humid climates under partial shade.

### 2. LEAF

- Arrangement: Alternate, distichous (two rows).
- Shape: Lanceolate to linear-lanceolate.
- Size: 30–60 cm long, 5–9 cm wide.
- Color: Dark green above, lighter green below.
- Texture: Smooth, glabrous, aromatic when crushed.

### 3. FLOWER

- Type: Small, bisexual flowers in long panicles arising from the rhizome.
- Color: White to pale green with violet or purple streaks on the lip.
- Flowering: Mainly during the rainy season.

### 4. FRUIT

- Type: Trilocular capsule.
- Shape: Ovoid to oblong.
- Size: 1–2 cm long.

- Color: Green when fresh; pale green to yellowish after drying.

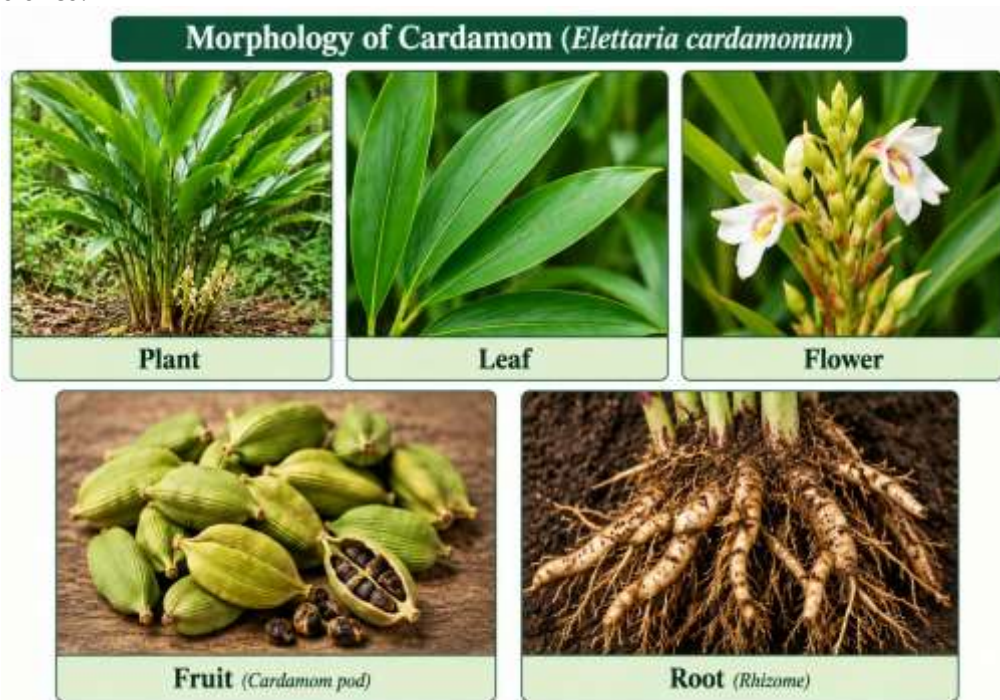
## 5. SEED

- Shape: Angular and irregular.
- Color: Dark brown to black.
- Surface: Rough and wrinkled.
- Odour: Strongly aromatic.
- Taste: Warm, sweet, slightly pungent.<sup>[67]</sup>

## CULTIVATION AND COLLECTION

It is a large perennial herb, largely cultivated in forests 2,500 to 5,000 feet above sea-level in North Canara, Coorgi, and Wynaad. It grows to a height of 6 to 10 feet from a thumb-thick, creeping rootstock. The seeds are first sown in nurseries and then transplanted into rich moist soil, when the seedlings are a year old or about 30 cm. small crops are obtained after the third year till six to seven years.

It flowers in April and May and the fruit gathering lasts in dry weather for three months, starting in October when the colour turns from green to yellow. (The methods of cultivating and preparing vary in different districts) The collected fruits are washed to remove the impurities like sand, and the fruits are dried quickly by putting them on trays in thin layers, exposed to sunlight, with occasional sprinkling of water and dried.<sup>[68]</sup>



**Fig 16:** Morphology of cardamom

## DISTRIBUTION

Cardamom production is concentrated mainly in India and Guatemala. India was traditionally a major producer, consumer and exporter of cardamom, while Guatemala later became the leading producer and exporter. Cardamom is also produced in smaller quantities in Sri Lanka, Nepal, Thailand, Indonesia, Vietnam and Tanzania. Guatemala produces approximately 20,000–30,000 tons annually, with most of its production being exported due to low domestic consumption. India produces about 11,000–12,000 tons annually, but because of high domestic consumption, only a small quantity is exported.<sup>[69]</sup>

## CHEMICAL CONSTITUENTS

The seeds contain 3 to 6% of volatile oil along with fixed oil, salts of potassium, a colouring principle, nitrogenous mucilage, an acrid resin, starch, ligneous fibre, and ash. The active constituent of the volatile oil is cineole. Other aromatic compounds present are terpinyl acetate, terpineol, borneol, terpinene, etc. The oil is colourless when fresh, but becomes thicker, more yellow and less aromatic on storage. It is soluble in alcohol and readily in four volumes of 70% alcohol, producing a clear solution. Its specific gravity at 25°C is 0.924 to 0.927. <sup>[70]</sup>

### MEDICINAL PROPERTIES

1. The major medicinal properties of cardamom essential oil are its antiseptic, carminative, digestive, diuretic, stimulant, stomachic, tonic and antispasmodic, antimicrobial and anti-inflammatory activities.
2. It has been used to treat bronchitis, haemorrhoids, stangury, renal and vesical calculi, anorexia, dyspepsia and gastropathy.
3. According to Ayurveda cardamom is used against heart disease, kidney disease, urinary disease, bacterial infection, teeth infection, pulmonary tuberculosis, asthma, food poisoning, eyelid inflammation, digestive disorders, sore throats, colds, bladder disease, snake bite, scorpion bite, and constipation.
4. Cardamom seeds are used as aromatic, acrid, sweet, abortifacient, cooling, alexiteric, carminative cardiac tonic, digestive, diuretic, expectorant, stimulant, and tonic.
5. Cardamom seeds are also giving beneficial effect in anorexia, dyspepsia, asthma, bronchitis, strangury, haemorrhoids, renal and vesical calculi, halitosis, gastropathy and
6. Burning sensation. <sup>[67]</sup>

## 6.0 METHOD OF PREPARATION

### 6.1 COLLECTION OF SYZGIUM AROMATICUM

The clove buds were collected for the present study. The plant material was botanically identified and authenticated by Dr. S. P. Anand, Associate Professor, Department of Botany, National College (Autonomous), Tiruchirappalli, Tamil Nadu. The collected plant material was identified as *Eugenia caryophyllus* Thunb. (Syn. *Syzygium aromaticum*) belonging to the family Myrtaceae. The collected material consisted of dried, unopened flower buds of clove. The buds were properly cleaned and shade dried at room temperature and used for further extraction studies.



**Fig 17: COLLECTION OF SYZGIUM AROMATICUM**

### 6.2 COLLECTION OF Elettaria CARDAMOMUM

The cardamom pods were collected for the present study. The plant material was botanically identified and authenticated by Dr. S. P. Anand, Associate Professor, Department of Botany, National College (Autonomous), Tiruchirappalli, Tamil Nadu. The collected plant material was identified as *Elettaria cardamomum* (L.) Maton belonging to the family Zingiberaceae. The collected material consisted of dried green seed pods containing seeds. The pods were properly cleaned and shade dried at room temperature and used for further extraction studies.



**Fig 18: COLLECTION OF ELETTARIA CARDAMOMUM**

### **7.0 EXTRACTION OF ACTIVE INGREDIENTS FROM CLOVE AND CARDAMOM (COLD MACERATION METHOD)**

The dried clove buds and cardamom seeds were cleaned and shade-dried separately. The dried sample were powdered individually using an electric grinder to obtain a coarse powder. Each powdered sample was transferred separately into a clean iodine conical flask containing 99.9% ethanol. The flasks were tightly closed and kept at room temperature for 3 days for cold maceration with occasional shaking. After the extraction period, the extracts were filtered through Whatman filter paper to obtain clear filtrates. The filtrates were concentrated using a rotary evaporator at 50° C. Finally, the concentrated extracts were transferred into separate, amber-coloured airtight containers and stored until further analysed.<sup>[71]</sup>



**Fig 19: Extraction of active ingredients**

### **8.0 PHYTOCHEMICAL SCREENING**

Phytochemical screening is a preliminary qualitative method used to identify the major bioactive chemical constituents present in medicinal plants and their extracts. Plants produce a wide variety of phytochemicals, particularly secondary metabolites, which may contribute to their biological and pharmacological properties. Commonly investigated phytochemical groups include alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids and glycosides.

Phytochemical screening is useful as an initial step in the investigation of medicinal plants because it is relatively simple, economical and accessible compared with advanced analytical techniques. The results obtained from preliminary screening can provide baseline information for further studies such as quantitative estimation, isolation and characterization of individual bioactive compounds.

In the present study, clove and cardamom extracts can be subjected to preliminary phytochemical screening to identify the major classes of phytoconstituents present in the extracts before their incorporation into the herbal gummy formulation. The information obtained from the screening can

support the selection and characterization of the herbal extracts used in the formulation. Thus, phytochemical screening serves as an important preliminary investigation for establishing the phytochemical profile of the selected herbal ingredients.<sup>[72]</sup>

## 9.0 CHEMICAL TEST



**Fig 20:** Chemical test for clove



**Fig 21:** Chemical Test for Cardamom

**Table 1: Chemical test for Clove**

| S.NO | EXPIREMENT                                                                                              | OBSERVATION                                     | REFERENCE          |
|------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------|--------------------|
| 1.   | Dragendorff's Test: 2 ml of the extract was taken, and a few drops of Dragendorff's reagent were added. | Orange or reddish-brown precipitate was formed. | Alkaloids present. |

|    |                                                                                                                                             |                                                   |                     |
|----|---------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|---------------------|
| 2. | Shinoda Test: 2 ml of the extract was taken, magnesium turnings and a few drops of concentrated HCl were added.                             | Pink, red or reddish colour was developed.        | Flavonoids present. |
| 3. | Ferric Chloride Test: 2ml of the extract was taken and a few drops of 5% ferric chloride solution were added.                               | Blue-black or greenish-black colour was formed.   | Tannins present.    |
| 4. | Foam Test: 2ml of the extract was mixed with 5ml of distilled water and shaken vigorously.                                                  | Persistent foam or froth was formed.              | Saponins present.   |
| 5. | Salkowski Test: 2ml of the extract was mixed with 2ml of chloroform. Then, 2ml of conc. H <sub>2</sub> SO <sub>4</sub> was added carefully. | Reddish-brown colour was formed at the interface. | Terpenoids present. |

**Table 2: Chemical test for Cardamomum**

| S.NO | EXPIREMENT                                                                                                                                                                                                                   | OBSERVATION                                                                                 | REFERENCE              |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------|
| 1.   | Dragendorff's Test: 2 ml of the extract was taken, and a few drops of Dragendorff's reagent were added.                                                                                                                      | Orange or reddish-brown precipitate was formed.                                             | Alkaloids present.     |
| 2.   | Salkowski Test: 2ml of the extract was mixed with 2ml of chloroform. Then, 2ml of conc. H <sub>2</sub> SO <sub>4</sub> was added carefully.                                                                                  | Reddish-brown colour was formed at the interface.                                           | Terpenoids present.    |
| 3.   | Lead Acetate Test: 2 ml of the extract was taken, and a few drops of lead acetate solution were added.                                                                                                                       | White precipitate was formed.                                                               | Tannins present.       |
| 4.   | Keller–Killiani Test 2 ml of the extract was taken and glacial acetic acid containing a trace of ferric chloride was added. Concentrated H <sub>2</sub> SO <sub>4</sub> was carefully added along the side of the test tube. | Brown ring was formed at the interface and bluish-green colour appeared in the upper layer. | Glycosides present.    |
| 5.   | Molisch's Test: 2 ml of the extract was taken and a few drops of Molisch's reagent were added. Concentrated H <sub>2</sub> SO <sub>4</sub> was carefully added along the side of the test tube.                              | Violet or purple ring was formed at the interface.                                          | Carbohydrates present. |

**10.0 PREPARATION OF GUMMIES:**

Gelatin was hydrated in purified water.

↓

The gelatin solution was heated with continuous stirring until completely dissolved.



Sucrose and sorbitol were added and mixed thoroughly to obtain a homogeneous gummy base.



Sodium benzoate, citric acid and calcium gluconate were incorporated with continuous stirring.



Clove extract (4 mL) and cardamom extract (4 mL) were added and mixed uniformly.



The prepared gummy mass was poured into pre-lubricated moulds (liquid paraffin).



The moulds were allowed to stand until the gummies were completely set.



The gummies were removed from the moulds and dried to obtain the desired consistency.



The prepared herbal gummies were packed in moisture-resistant containers and stored under appropriate conditions.<sup>[73]</sup>



**Fig 22:** Preparation of Gummies

**Table 3: Preparation of Gummies**

| S.NO | COMPOSITION          | F1    | F2    | F3    |
|------|----------------------|-------|-------|-------|
| 1.   | Syzygium aromaticum  | 3ml   | 4ml   | 5ml   |
| 2.   | Elettaria Cardamomum | 3ml   | 4ml   | 5ml   |
| 3.   | Gelatin              | 10g   | 10g   | 10g   |
| 4.   | Sucrose              | 30g   | 30g   | 30g   |
| 5.   | Sorbitol             | 15ml  | 15ml  | 15ml  |
| 6.   | Sodium Benzoate      | 0.25g | 0.25g | 0.25g |
| 7.   | Citric Acid          | 0.50g | 0.50g | 0.50g |
| 8.   | Calcium Gluconate    | 2.5g  | 2.5g  | 2.5g  |

|     |                 |              |              |              |
|-----|-----------------|--------------|--------------|--------------|
| 9.  | Liquid Paraffin | q.s          | q.s          | q.s          |
| 10. | Distilled Water | q.s to 100ml | q.s to 100ml | q.s to 100ml |

## 11.0 EVALUATION TEST:

### 11.1 Weight variation test

#### Procedure

- Select 10 gummies randomly.
- Weigh each individually on a digital balance.



**Fig 23:** Weight variation test

10 gummies were randomly selected and weighed individually using an analytical balance. Individual Deviation = Individual Weight – Average Weight Percentage Deviation = (Individual Deviation / Average Weight) × 100

**Table 4: Weight variation test**

| S.NO | Individual Weight(g) | Average Weight(g) | Individual Deviation(g) | Percentage Deviation |
|------|----------------------|-------------------|-------------------------|----------------------|
| 1    | 4.52                 | 4.696             | -0.176                  | -3.75%               |
| 2    | 4.61                 | 4.696             | -0.086                  | -1.83%               |
| 3    | 4.68                 | 4.696             | -0.016                  | -0.34%               |
| 4    | 4.73                 | 4.696             | +0.034                  | +0.72%               |
| 5    | 4.79                 | 4.696             | +0.094                  | +2.00%               |
| 6    | 4.84                 | 4.696             | +0.144                  | +3.07%               |
| 7    | 4.56                 | 4.696             | -0.136                  | -2.90%               |
| 8    | 4.70                 | 4.696             | +0.004                  | +0.09%               |
| 9    | 4.88                 | 4.696             | +0.184                  | +3.92%               |
| 10   | 4.65                 | 4.696             | -0.046                  | -0.98%               |

Result: All the gummies passed the weight variation test, as the percentage deviation of individual gummies was within the predetermined permissible limit of  $\pm 7.5\%$ .

### 11.2 PH Measurement

#### Procedure:

- One gummy was dissolved in 10 mL of distilled water.
- The pH meter was calibrated using appropriate standard buffer solutions. The electrode was rinsed thoroughly with distilled water and gently blotted dry.
- The pH of the prepared gummy solution was measured using the calibrated pH meter at 25°C.



**Fig 24:** pH Measurement

Result: The pH of the prepared gummies was determined using a calibrated pH meter. The observed pH value was approximately 4.05. The formulated gummies exhibited a slightly acidic pH.

### 11.3 Moisture Content / Loss on Drying

#### Procedure:

- A known quantity of the gummy sample was accurately weighed.
- The weighed sample was placed in a hot air oven maintained at 105°C.
- The sample was dried for 3–4 hours or until a constant weight was obtained.
- The dried sample was allowed to cool and weighed again.
- The percentage of moisture content was calculated from the loss in weight.

#### Moisture content formula

$$\text{Moisture Content (\%)} = [(W_1 - W_2) / W_1] \times 100$$

#### Calculation:

$W_1$  = Initial weight of the sample = 3.34 g

$W_2$  = Final weight of the sample after drying = 2.80 g

$$= [(3.34 - 2.80) / 3.34] \times 100$$

$$= (0.54 / 3.34) \times 100$$

$$= 16.17\% \text{ w/w}$$

Result: The moisture content of the formulated gummies was found to be 16.17% w/w.

### 11.4 Thickness test

#### Procedure:

The thickness of the prepared gummies was measured using a Vernier caliper. The thickness of each formulation was measured and recorded in millimetres (mm).

#### CALCULATION:

**Table 5: Thickness**

| FORMULATION | THICKNESS (mm) |
|-------------|----------------|
| F1          | 4.85           |
| F2          | 4.95           |
| F3          | 4.87           |

$$\begin{aligned} \text{Average Thickness} &= (4.85 + 4.95 + 4.87) / 3 \\ &= 14.67 / 3 \end{aligned}$$

$$= 4.89 \text{ mm}$$

$$\text{Average Thickness} = 4.89 \text{ mm}$$

Result: The thickness values of F1, F2 and F3 were found to be relatively close to each other, indicating good uniformity in the thickness of the prepared gummies.

### 11.5 Physical Appearance

The prepared polyherbal oral gummies were visually examined for their colour, shape, odour, texture and taste. The physical characteristics of F1, F2 and F3 formulations were observed and recorded.

**Table 6: Physical Appearance**

| PARAMETER | F1                                      | F2                                      | F3                                      |
|-----------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| Colour    | Light brown to brown                    | Light brown to brown                    | Light brown to brown                    |
| Odour     | Characteristic clove and cardamom odour | Characteristic clove and cardamom odour | Characteristic clove and cardamom odour |
| Shape     | Triangle                                | Triangle                                | Triangle                                |
| Texture   | Soft and chewy                          | Soft and chewy                          | Soft and chewy                          |
| Taste     | Sweet with slightly spicy               | Sweet with slightly spicy               | Sweet with slightly spicy               |

### 11.6 Stability Study

#### Procedure:

The formulated gummies were stored at room temperature ( $25 \pm 2^\circ\text{C}$ ) for a period of four weeks. The samples were observed periodically for any changes in appearance, colour and texture during the storage period.

**Result:** The formulated gummies were found to be physically stable during the four-week observation period. No significant changes were observed in the appearance, colour and texture of the gummies.

### 11.7 Disintegration time

The disintegration time of the prepared gummies was determined using a USP disintegration apparatus with purified water as the medium maintained at  $37 \pm 0.5^\circ\text{C}$ . The time required for each gummy to disintegrate was recorded.

**Table 7: Disintegration Time**

| S.NO | DISINTEGRATION   | TIME (MIN) |
|------|------------------|------------|
| 1    | Gummy (Basket 1) | 9.4        |
| 2    | Basket (2)       | 8.7        |
| 3    | Basket (3)       | 10.1       |
| 4    | Basket (4)       | 8.4        |
| 5    | Basket (5)       | 9.8        |
| 6    | Basket (6)       | 12.6       |
|      | AVERAGE TIME     | 9.83 (min) |

#### Calculation:

$$\begin{aligned} \text{Average Disintegration Time} &= (9.4 + 8.7 + 10.1 + 8.4 + 9.8 + 12.6) / 6 \\ &= 59.0 / 6 \\ &= 9.83 \text{ min} \end{aligned}$$

Average Disintegration Time = 9.83 min

### 12.0 In Vitro Anti-Inflammatory Activity of Clove (*Syzygium aromaticum*) and Cardamom (*Elettaria cardamomum*) Aqueous Extracts

The anti-inflammatory potential of the aqueous extracts of clove (*Syzygium aromaticum*) and cardamom (*Elettaria cardamomum*) was assessed in vitro by the albumin denaturation inhibition assay, a well validated model in which the ability of a test substance to prevent heat-induced denaturation of bovine serum albumin correlates with anti-inflammatory potential. Both extracts exhibited a clear, concentration dependent inhibition of protein denaturation over the tested range of 100–500 µg/mL.

At the lowest concentration tested (100 µg/mL), the clove aqueous extract inhibited protein denaturation by  $42.35 \pm 1.82\%$ , which progressively increased to  $55.64 \pm 2.14\%$  at 200 µg/mL,  $67.92 \pm 2.80\%$  at 300 µg/mL,  $75.84 \pm 1.75\%$  at 400 µg/mL, and reached a maximum of  $82.16 \pm 1.62\%$  at 500 µg/mL. The cardamom aqueous extract showed a comparable dose-dependent profile, producing  $40.28 \pm 1.65\%$  inhibition at 100 µg/mL,  $53.76 \pm 1.94\%$  at 200 µg/mL,  $65.83 \pm 1.94\%$  at 300 µg/mL,  $78.52 \pm 2.10\%$  at 400 µg/mL, and  $79.24 \pm 1.30\%$  at 500 µg/mL. This steady, near-linear rise in percentage inhibition with increasing concentration confirms a dose-dependent anti-inflammatory response for both extracts. The standard drug, diclofenac sodium, was included for comparison and recorded  $89.24 \pm 2.80\%$  inhibition of protein denaturation at 500 µg/mL, the highest concentration tested, indicating greater maximal protein-stabilizing activity than either plant extract at the same concentration.

The concentration required to produce 50% inhibition ( $IC_{50}$ ) was estimated from the regression line of each extract's dataset. The clove aqueous extract showed an  $IC_{50}$  value of approximately 151.9 µg/mL, while the cardamom aqueous extract recorded a slightly higher  $IC_{50}$  of approximately 168.3 µg/mL. Since a lower  $IC_{50}$  denotes greater potency, this comparison indicates that the clove extract was modestly more potent than the cardamom extract under the tested conditions. An  $IC_{50}$  value for diclofenac sodium could not be calculated, as only a single concentration (500 µg/mL) was recorded for the standard in this dataset. Nevertheless, the appreciable and consistent inhibitory activity shown by both extracts across all tested concentrations confirms that clove and cardamom aqueous extracts possess genuine protein-stabilizing, anti-inflammatory activity, albeit at a lower potency than the standard NSAID at the concentration compared.

**Table 8: Anti-Inflammatory activity of Clove and Cardamomum**

| S.No | SAMPLE                                   | EXTRACT         | CONCENTRATION (µg/ml) | % INHIBITION OF PROTEIN DENATURATION (Mean ± SD) |
|------|------------------------------------------|-----------------|-----------------------|--------------------------------------------------|
| 1.   | Clove ( <i>Syzygium Aromaticum</i> )     | Aqueous extract | 100                   | $42.35 \pm 1.82$                                 |
|      |                                          |                 | 200                   | $55.64 \pm 2.14$                                 |
|      |                                          |                 | 300                   | $67.92 \pm 2.80$                                 |
|      |                                          |                 | 400                   | $75.84 \pm 1.75$                                 |
|      |                                          |                 | 500                   | $82.16 \pm 1.62$                                 |
| 2.   | Cardamom ( <i>Elettaria cardamomum</i> ) | Aqueous extract | 100                   | $40.28 \pm 1.65$                                 |
|      |                                          |                 | 200                   | $53.76 \pm 1.94$                                 |
|      |                                          |                 | 300                   | $65.83 \pm 1.94$                                 |
|      |                                          |                 | 400                   | $78.52 \pm 2.10$                                 |
|      |                                          |                 | 500                   | $79.24 \pm 1.30$                                 |
| 3.   | Diclofenac sodium                        | Standard        | 500                   | $89.24 \pm 2.80$                                 |

$IC_{50}$  value: Clove aqueous extract  $\approx 151.9$  µg/mL; Cardamom aqueous extract  $\approx 168.3$  µg/mL (estimated by linear regression).  $IC_{50}$  for diclofenac sodium standard not determined from available single-point data.

#### RESULT:

Preliminary phytochemical screening of the ethanolic extracts confirmed the presence of alkaloids, flavonoids, tannins, saponins and terpenoids in the clove (*Syzygium aromaticum*) extract, and alkaloids, terpenoids, tannins, glycosides and carbohydrates in the cardamom (*Elettaria cardamomum*) extract. These phytoconstituents supported the selection of both extracts for incorporation into the polyherbal gummy formulation.

Three formulations, F1, F2 and F3, containing increasing concentrations of clove and cardamom extract, were successfully prepared by the moulding method and evaluated for their physicochemical parameters. The results obtained are summarised below.

| Parameter                           | Observation      | Remark                 |
|-------------------------------------|------------------|------------------------|
| Average weight                      | 4.696 g          | Uniform                |
| Weight variation                    | −3.75% to +3.92% | Within ±7.5% limit     |
| pH                                  | 4.05             | Mildly acidic          |
| Moisture content                    | 16.17% w/w       | Acceptable             |
| Average thickness                   | 4.89 mm          | Uniform (F1–F3)        |
| Disintegration time                 | 9.83 min         | Satisfactory           |
| Stability (4 weeks)                 | No change        | Physically stable      |
| IC <sub>50</sub> – Clove extract    | 151.9 µg/mL      | More potent of the two |
| IC <sub>50</sub> – Cardamom extract | 168.3 µg/mL      | Moderately potent      |

The physical examination of the formulations revealed an acceptable light brown to brown colour, a characteristic clove–cardamom odour, a soft and chewy texture, and a sweet, mildly spicy taste across F1, F2 and F3, with no visible change in appearance during the stability period. Among the three, formulation F2 (containing 4 mL each of clove and cardamom extract) was identified as the best formulation, showing the most balanced thickness and physical characteristics of the batches and corresponding to the extract concentration carried forward for the remaining evaluation studies (weight variation, pH, moisture content, disintegration time and stability).

The *in vitro* anti-inflammatory activity, assessed by the albumin denaturation inhibition assay, showed a clear concentration-dependent increase in percentage inhibition for both extracts across the range of 100–500 µg/mL. At 500 µg/mL, the clove extract produced  $82.16 \pm 1.62\%$  inhibition and the cardamom extract produced  $79.24 \pm 1.30\%$  inhibition, compared with  $89.24 \pm 2.80\%$  inhibition shown by the standard drug, diclofenac sodium, confirming the concentration-dependent anti-inflammatory potential of both herbal extracts.

## DISCUSSION:

The present study was undertaken with the aim of formulating polyherbal oral gummies containing extracts of *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (cardamom), and evaluating their physicochemical characteristics and *in vitro* anti-inflammatory activity. The results obtained indicate that the objectives framed for this project were successfully achieved.

The preliminary phytochemical screening revealed the presence of flavonoids, tannins, terpenoids and related phenolic constituents in both extracts. These classes of phytoconstituents have been widely reported to possess membrane-stabilising and free-radical scavenging properties, which are considered important mechanisms underlying anti-inflammatory action. Their presence in the selected extracts therefore provided a rational basis for evaluating them for anti-inflammatory potential and is consistent with earlier reviews on clove and cardamom.

The physicochemical evaluation indicated a formulation of acceptable pharmaceutical quality. The percentage weight deviation remained well within the pharmacopoeial limit of ±7.5%, and the mildly acidic pH of 4.05 favoured both palatability and resistance to microbial growth. The moisture content of 16.17% w/w fell within the range typical of chewable gummies and accounted for their soft, chewy consistency, while the uniform thickness across F1, F2 and F3 reflected the reproducibility of the mould-based method. The disintegration time of 9.83 minutes, though longer than that of a conventional tablet,

suits a gummy meant to be chewed rather than swallowed intact, and the absence of visible change over the four-week study suggests short-term compatibility between the extracts and excipients used.

The in vitro anti-inflammatory activity of both extracts increased in a concentration-dependent manner, characteristic of a genuine pharmacological effect. The lower IC<sub>50</sub> of the clove extract (151.9 µg/mL) compared with cardamom (168.3 µg/mL) indicates slightly greater potency, likely due to its higher eugenol content, a phenolic compound well documented for anti-inflammatory activity. Although both extracts showed lower maximal inhibition than diclofenac sodium, the substantial inhibition achieved supports their potential as safer, plant-based alternatives for managing mild oral and dental inflammation. As this activity was assessed using only the albumin denaturation assay, further in vitro and in vivo studies would be needed to substantiate any therapeutic claim.

## CONCLUSION:

The present project work was successfully carried out with the objective of formulating polyherbal oral gummies containing extracts of *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (cardamom), and evaluating their physicochemical properties and in vitro anti-inflammatory activity.

The selected herbal materials were properly collected, authenticated and extracted, and preliminary phytochemical screening confirmed the presence of pharmacologically relevant phytoconstituents, including flavonoids, tannins and terpenoids, in both extracts. These extracts were successfully incorporated into a gummy dosage form using standard pharmaceutical excipients such as gelatin, sucrose, sorbitol, sodium benzoate, citric acid and calcium gluconate.

Evaluation of the prepared formulations (F1, F2 and F3) showed acceptable weight uniformity (percentage deviation within  $\pm 7.5\%$ ), a mildly acidic pH of 4.05, a moisture content of 16.17% w/w, an average thickness of 4.89 mm, and satisfactory physical characteristics in terms of colour, odour, texture and taste. The formulated gummies also exhibited good physical stability over the four-week observation period and an acceptable average disintegration time of 9.83 minutes.

The in vitro anti-inflammatory activity, evaluated using the albumin denaturation inhibition assay, revealed that both clove and cardamom extracts produced concentration-dependent inhibition of protein denaturation across the tested range of 100–500 µg/mL. At 500 µg/mL, clove extract exhibited  $82.16 \pm 1.62\%$  inhibition and cardamom extract exhibited  $79.24 \pm 1.30\%$  inhibition, compared with  $89.24 \pm 2.80\%$  inhibition shown by the standard drug, diclofenac sodium. The estimated IC<sub>50</sub> values of 151.9 µg/mL for clove and 168.3 µg/mL for cardamom further confirmed the comparatively higher anti-inflammatory potency of the clove extract under the tested experimental conditions.

Based on these findings, it can be concluded that *Syzygium aromaticum* and *Elettaria cardamomum* possess promising in vitro anti-inflammatory activity and can be formulated into a stable, palatable polyherbal oral gummy. The formulation may serve as a convenient natural alternative for managing mild inflammatory conditions, particularly in the oral cavity, though further in vitro, in vivo and stability studies are recommended before clinical or commercial application.

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