



FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY SPRAY USING CALENDULA

Rushikesh Ramesh More¹, Vishal Ashok Bhise², Vidya R. Kale³, Gulab Munjaji Punjare⁴

Students of Yashodeep Institute of Pharmacy, Chhatrapati Sambhaji Nagar, Maharashtra, India,
Under the Dr Babasaheb Ambedkar Technology University, Lonere.

Email Id: Rushimore266@gmail.com

Mobile No: 8999424952

ABSTRACT:

Inflammation is a vital immune response that helps the body fight off harmful stimuli and initiate the healing process. When tissues are damaged by injury, infection, or irritants, the immune system releases signaling molecules, such as cytokines and chemokines, to recruit immune cells to the affected area. These immune cells, including white blood cells like neutrophils and macrophages, help to remove pathogens and damaged cells, as well as initiate tissue repair. There are two main types of inflammation: acute and chronic. Acute inflammation is a short-term response to injury or infection and typically resolves once the threat is eliminated. It is characterized by symptoms such as redness, swelling, heat, and pain at the site of injury. Chronic inflammation, on the other hand, persists over a longer period and can contribute to the development of various diseases, including rheumatoid arthritis, inflammatory bowel disease, and cardiovascular disease. While inflammation is a natural and necessary process for healing, chronic inflammation can be harmful to health. Lifestyle factors such as diet, exercise, stress management, and smoking can all influence the body's inflammatory response. Managing inflammation through healthy lifestyle choices and, when necessary, medical interventions can help prevent chronic inflammation and reduce the risk of associated diseases.

Objective: The objective of this study was to formulate and evaluate Spray utilizing Calendula flower as a key ingredient, aiming to harness its potential therapeutic benefits for inflammation conditions.

Purpose: The purpose of this research was to develop a spray that offers anti-inflammatory effect, utilizing Calendula flower as a natural and potentially effective active ingredient. This capsule could serve as a safer and more accessible alternative for managing inflammation condition.

Result: The formulated spray demonstrated promising activity against anti-inflammatory in both in vitro assays and in vivo experiments, exhibiting inhibition of inflammation markers and reducing injuries inflammation in animal models. Additionally, the spray showed favorable physical characteristics, stability, and safety profiles, making it suitable for spray application.

Conclusions: This study concludes that the developed anti-inflammatory spray holds potential as a novel therapeutic option for managing inflammation conditions. Further research and clinical trials are warranted to explore its efficacy, safety, and clinical applications in greater depth.

Keywords: Anti-inflammatory, spray Formulation, Active ingredients, Delivery system, Solubility, Stability, Bioavailability, safety profile.

INTRODUCTION: -

Inflammation:

Anti-inflammatory drugs are a class of medications designed to reduce inflammation in the body, alleviating pain and swelling associated with various conditions. Inflammation is a natural response of the immune system to injury, infection, or irritation, characterized by redness, heat, swelling, and pain. While acute inflammation is a necessary process for healing, chronic inflammation can contribute to various diseases, including arthritis, cardiovascular disease, and autoimmune disorders.

The discovery and development of anti-inflammatory drugs have revolutionized the treatment of inflammatory conditions, providing relief to millions of people worldwide. These drugs work by inhibiting specific enzymes or pathways involved in the inflammatory response, thereby suppressing the production of inflammatory mediators such as prostaglandins, cytokines, and leukotrienes.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used anti-inflammatory medications. They work by inhibiting the enzyme cyclooxygenase (COX), which is responsible for producing prostaglandins, lipid compounds that promote inflammation, pain, and fever. Common

NSAIDs include aspirin, ibuprofen, and naproxen. These drugs are effective for relieving pain and reducing inflammation associated with conditions such as arthritis, muscle strains, and menstrual cramps.

Another class of anti-inflammatory drugs is corticosteroids, which mimic the effects of cortisol, a hormone naturally produced by the adrenal glands. Corticosteroids exert their anti-inflammatory effects by suppressing the immune response and reducing the production of inflammatory substances. These drugs are commonly used to treat inflammatory conditions such as asthma, rheumatoid arthritis, and inflammatory bowel disease. They are available in various forms, including oral tablets, topical creams, and inhaled formulations.

Biologic therapies represent a newer class of anti-inflammatory drugs that target specific molecules or cells involved in the inflammatory process. These medications are typically used to treat autoimmune diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Biologics work by blocking the action of pro-inflammatory cytokines or by inhibiting the activity of immune cells responsible for causing tissue damage.

In recent years, research has focused on the development of novel anti-inflammatory agents with improved efficacy and safety profiles. One area of investigation involves the discovery of natural compounds with anti-inflammatory properties, such as curcumin from turmeric, resveratrol from grapes, and omega-3 fatty acids from fish oil. These compounds have shown promising results in preclinical studies and clinical trials for various inflammatory conditions.

Additionally, researchers are exploring the potential of targeting novel molecular pathways involved in inflammation, including the Janus kinase (JAK) pathway, the nuclear factor kappa B (NF- κ B) pathway, and the inflammasome complex. By developing drugs that specifically target these pathways, scientists aim to achieve more precise and potent anti-inflammatory effects with fewer side effects.

Despite their efficacy, anti-inflammatory drugs are not without risks. NSAIDs, for example, can cause gastrointestinal ulcers and bleeding, cardiovascular events, and kidney damage with long-term use. Corticosteroids may lead to adverse effects such as osteoporosis, weight gain, and increased susceptibility to infections. Biologic therapies carry risks of infections, infusion reactions, and autoimmune phenomena.

In conclusion, anti-inflammatory drugs play a crucial role in managing inflammatory conditions and improving the quality of life for millions of people worldwide. From traditional NSAIDs to biologic therapies and emerging treatments, these medications offer relief from pain and inflammation while driving advancements in medical research and drug development. However, their use must be carefully monitored to minimize risks and maximize benefits for patients.

Symptoms

1. Fever
2. Redness
3. Fatigue
4. Swelling
5. Loss of function
6. Heat

CAUSES :

- **Injury:** Physical trauma, such as cuts, burns, or fractures, can damage tissues and trigger an inflammatory response as the body works to repair the injured area.
- **Irritants:** Exposure to irritants like chemicals, pollutants, or allergens can cause inflammation in sensitive tissues, such as the skin, lungs, or digestive tract.
- **Infection:** Pathogens such as bacteria, viruses, and fungi can trigger an immune response, leading to inflammation. as the body tries to eliminate the invading microorganisms.

◆ GENERAL TREATMENTS :

Medications: Nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen or aspirin can help reduce pain and inflammation. Corticosteroids may be prescribed for more severe inflammation or autoimmune conditions. **Topical treatments:** Creams, ointments, or patches containing anti-inflammatory medications can be applied directly to the skin to relieve localized inflammation and pain.

◆ TYPES OF Inflammation

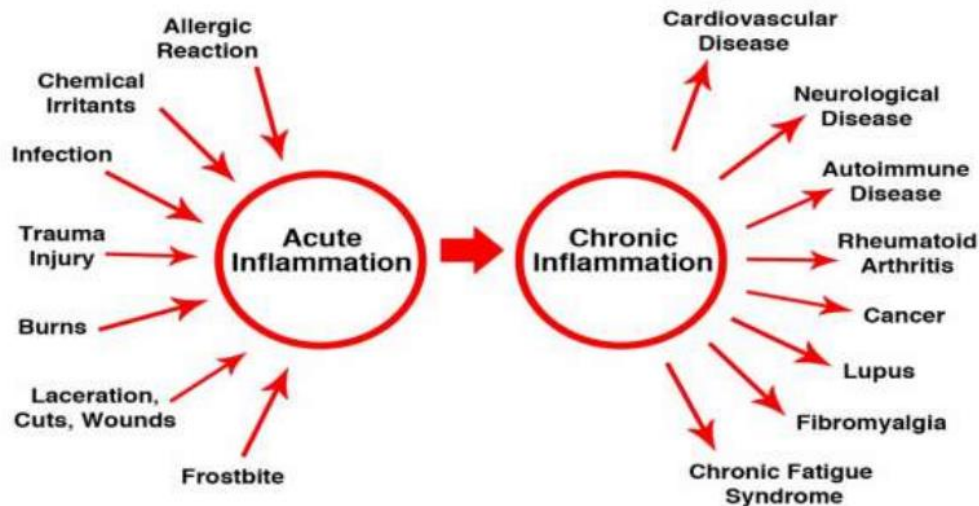
There are 2 main types of inflammation

- 1) Acute Inflammation.
- 2) chronic inflammation

1. Acute inflammation

Acute inflammation is a rapid and short-term response to injury, infection, or tissue damage. It is characterized by symptoms such as redness, swelling, heat, and pain at the site of injury. Ex. cuts, bruises, sprains, and infections like the common cold or a sore throat.

2) Chronic inflammation Chronic inflammation is a prolonged and persistent immune response that can last for weeks, months, or even years. It may occur low-grade and asymptomatic or be associated with symptoms such as fatigue, joint pain, and recurrent infections. Chronic inflammation can damage tissues and organs over time, leading to tissue destruction, scarring, and impaired function.



Mechanism of inflammation:

Inflammation is a complex process involving various cells and molecules in the body's immune response. One of the key mechanisms is the release of inflammatory mediators such as cytokines, chemokines, and prostaglandins in response to tissue injury or infection. These mediators attract immune cells to the site of injury or infection, leading to vasodilation, increased vascular permeability, and the recruitment of leukocytes. This immune cell infiltration helps to remove pathogens, clear damaged tissue, and initiate the healing process. Additionally, inflammation can be modulated by various signaling pathways, including the NF- κ B pathway, which regulates the expression of genes involved in inflammation.

Resolution of inflammation :

1. **Anti-inflammatory mediators:** Specialized pro-resolving mediators (SPMs), such as lipoxins, resolvins, protectins, and maresins, are produced during inflammation resolution. They actively dampen inflammation and promote tissue repair.
2. **Removal of inflammatory signals:** Inflammatory mediators, such as cytokines and chemokines, are degraded or neutralized to reduce their activity. This helps to halt the recruitment of immune cells to the inflamed site.
3. **Apoptosis of immune cells:** Immune cells recruited to the site of inflammation undergo programmed cell death (apoptosis) once their task is complete. This helps to resolve the inflammatory response and prevent excessive tissue damage.
4. **Phagocytosis of cellular debris:** Macrophages and other phagocytic cells engulf and digest cellular debris, pathogens, and apoptotic immune cells. This process clears the inflammatory site and promotes tissue repair.
5. **Tissue repair and regeneration:** Inflammation resolution is accompanied by the activation of pathways involved in tissue repair and regeneration. Fibroblasts lay down new extracellular matrix, and stem cells differentiate into specialized cell types to replace damaged tissue.

Mechanism of action:

1. **Reduction of inflammatory mediators:** Anti-inflammatory sprays often contain corticosteroids, which work by inhibiting the production of inflammatory mediators such as prostaglandins and leukotrienes. This helps to decrease inflammation and associated symptoms like pain and swelling.
2. **Inhibition of immune cell activation:** Some sprays contain non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen or diclofenac, which block the activity of enzymes called cyclooxygenases (COX). COX enzymes play a role in the production of prostaglandins, which are key mediators of inflammation. By inhibiting COX enzymes, NSAIDs reduce inflammation and pain.
3. **Local vasodilation:** Certain sprays contain ingredients that cause local vasodilation, leading to increased blood flow to the affected area. This can help to reduce inflammation by promoting the delivery of anti-inflammatory agents and oxygen to tissues, as well as enhancing the removal of inflammatory by-products.

4. **Direct inhibition of inflammatory pathways:** Some sprays may contain specific inhibitors targeting particular inflammatory pathways, such as inhibitors of the NF- κ B pathway or specific cytokines involved in inflammation. These agents work by directly blocking the activity of key molecules involved in the inflammatory process.

Components of Anti-inflammatory spray:

1. **Corticosteroids:** These are powerful anti-inflammatory agents that work by suppressing the immune response and reducing inflammation. Examples include hydrocortisone, triamcinolone, and fluticasone.
2. **Nonsteroidal anti-inflammatory drugs (NSAIDs) :** Some sprays may contain NSAIDs like ibuprofen or ketoprofen, which work by inhibiting the production of prostaglandins, chemicals that cause inflammation and pain.
3. **Local anesthetics:** These numb the affected area, providing immediate pain relief. Common examples include lidocaine and benzocaine.
4. **Counterirritants:** Ingredients like menthol, camphor, or capsaicin create a cooling or heating sensation that distracts from the pain and may help reduce inflammation.
5. **Topical analgesics:** These ingredients, like salicylates, provide pain relief by blocking the transmission of pain signals from the nerves.
6. **Emollients and carriers :** These are substances like alcohol, glycerin, or propylene glycol, which help deliver the active ingredients and may also have soothing properties.

Advantage of Anti-inflammatory :

1. **Localized Relief :** They provide targeted relief to the specific area of inflammation or pain, minimizing systemic effects.
2. **Quick Absorption :** Sprays are often quickly absorbed through the skin, providing rapid relief compared to oral medications.
3. **Convenience :** They are easy to apply and carry, making them convenient for use on the go.
4. **Reduced Side Effects :** Since the medication is applied locally, the risk of systemic side effects is lower compared to oral medications.
5. **Non-Invasive :** Unlike injections or surgeries, using a spray is non-invasive and generally painless.
6. **Suitable for Sensitive Stomachs :** For individuals who have sensitive stomachs or gastrointestinal issues, anti-inflammatory sprays offer an alternative to oral medications that may cause irritation.
7. **Reduced Risk of Drug Interactions :** Topical application reduces the likelihood of drug interactions compared to oral medications that are metabolized through the liver.
8. **Precise Dosage :** Sprays allow for precise dosage control, ensuring that the right amount of medication is applied to the affected area.
9. **Applicability to Various Conditions :** Anti-inflammatory sprays can be used to treat a wide range of conditions, including arthritis, tendonitis, bursitis, and muscle strains.
10. **Improved Compliance :** The ease of use and targeted relief provided by sprays can improve patient compliance with treatment regimens, leading to better outcomes.

Application of Anti-inflammatory :

1. **Arthritis :** Anti-inflammatory sprays can be applied directly to the affected joints to reduce pain and inflammation associated with arthritis, such as osteoarthritis or rheumatoid arthritis.
2. **Muscle Strains :** Sprays can be used to alleviate pain and inflammation caused by muscle strains or overuse injuries, providing localized relief to the affected muscles.
3. **Tendonitis :** When tendons become inflamed due to overuse or injury, anti-inflammatory sprays can help reduce swelling and pain, promoting healing.
4. **Bursitis :** Bursitis, inflammation of the fluid-filled sacs (bursae) that cushion joints, can be relieved by applying anti-inflammatory sprays directly to the affected area.
5. **Sports Injuries :** Athletes often use anti-inflammatory sprays to manage pain and inflammation resulting from sports-related injuries, such as sprains, strains, or contusions.
1. **Back Pain :** Anti-inflammatory sprays can provide relief for individuals suffering from acute or chronic back pain by targeting inflammation in the affected area of the spine or surrounding muscles.

Challenges and Consideration:

1. **Skin Sensitivity :** Some individuals may experience skin irritation or allergic reactions to the ingredients in anti-inflammatory sprays. It's essential to perform a patch test and discontinue use if any adverse reactions occur.
2. **Potential Systemic Absorption :** While anti-inflammatory sprays are intended for topical use, there is a risk of systemic absorption, especially if applied to large areas of the body or broken skin. This can increase the risk of systemic side effects, such as adrenal suppression with corticosteroids.
3. **Limited Depth of Penetration :** Sprays may not penetrate deeply enough to reach inflammation occurring deep within tissues or joints, limiting their effectiveness for certain conditions.
4. **Dosage Control :** Ensuring proper dosage control can be challenging with sprays, as it may be difficult to accurately measure the amount of medication applied with each spray.

5. **Avoiding Contact with Mucous Membranes** : Care must be taken to avoid spraying the medication near the eyes, mouth, or other mucous membranes to prevent irritation or accidental ingestion.
6. **Interaction with Other Medications** : Some anti-inflammatory sprays may interact with other medications or topical products. It's essential to consult with a healthcare professional before using them concurrently with other treatments.
7. **Overuse and Dependency** : Long-term or excessive use of anti-inflammatory sprays may lead to dependency or tolerance, where the body requires higher doses to achieve the same level of pain relief.
8. **Not Suitable for All Conditions** : While anti-inflammatory sprays can provide relief for many inflammatory conditions, they may not be suitable for all types of inflammation or injuries. Some conditions may require alternative treatments such as oral medications, physical therapy, or surgery.
9. **Cost** : Depending on the formulation and brand, anti-inflammatory sprays can be relatively expensive compared to other topical or oral medications, which may impact accessibility for some individuals.
10. **Regulatory Compliance** : Manufacturers must adhere to regulations regarding the formulation, labeling, and marketing of anti-inflammatory sprays to ensure they meet safety and efficacy standards. Consumers should purchase products from reputable sources to minimize the risk of counterfeit or substandard product

PLANT PROFILE (CALENDULA)

Botanical Classification:

- **Kingdom: Plantae**

- **Clade: Tracheophytes**

- **Clade: Angiosperms**

- **Clade: Eudicots**

- **Clade: Asterids**

- **Order: Asterales**

- **Family: Asteraceae**

- **Genus: Calendula**

- **Species: Calendula officinalis**



Botanical Description :

Calendula (Calendula officinalis):

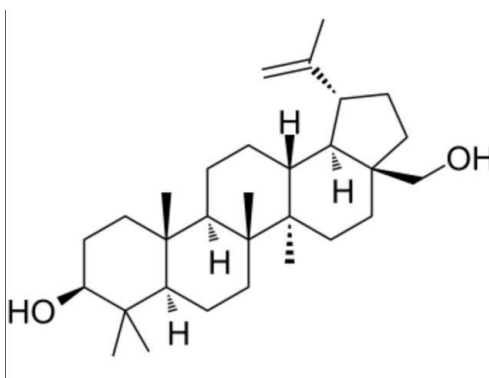
1. **Habit:** Calendula is an herbaceous annual or short-lived perennial plant, typically growing to a height of 12 to 24 inches (30 to 60 centimeters). It has a bushy, upright growth habit with multiple branching stems.
2. **Leaves:** The leaves of Calendula are lanceolate to oblong in shape, arranged alternately along the stems. They are typically 2 to 6 inches (5 to 15 centimeters) long, with a slightly hairy texture and serrated margins.
3. **Flowers:** Calendula produces large, vibrant, daisy-like flowers that are typically 1 to 3 inches (2.5 to 7.5 centimeters) in diameter. The flowers come in a range of colors, including bright yellow, orange, and apricot. Each flower head consists of multiple ray florets surrounding a central disc floret.
4. **Inflorescence:** The flowers of Calendula are solitary and terminal, meaning they are borne singly at the ends of the stems.
5. **Fruit:** After flowering, Calendula produces small, cylindrical fruit known as achenes. Each achene contains a single seed and is often surrounded by a ring of bristles.
6. **Roots:** Calendula has a fibrous root system, with shallow roots that spread laterally in the soil.
7. **Fragrance:** The flowers of Calendula have a mild, pleasant aroma that is sometimes described as herbal or citrus-like.
8. **Blooming Period:** Calendula typically blooms from late spring to early fall, with flowers appearing continuously throughout the growing season under favorable conditions.

9. **Cultivation:** Calendula is easy to grow and thrives in a variety of soil types, preferring well-draining soil with moderate fertility. It prefers full sun but can tolerate partial shade, especially in hot climates. Seeds can be sown directly into the garden after the danger of frost has passed, or started indoors several weeks before the last frost date.
10. **Uses:** Calendula is valued for its ornamental, culinary, and medicinal uses. The petals are edible and can be used fresh or dried in salads, teas, and other culinary creations. Medicinally, Calendula is used in herbal remedies, salves, and creams to soothe skin irritations, wounds, and inflammation. In the garden, Calendula is often grown for its cheerful blooms, which attract pollinators such as bees and butterflies.

Chemical constituents:

1. **Flavonoids:** Flavonoids such as quercetin, kaempferol, and isorhamnetin are typically present in Calendula at concentrations ranging from 0.1% to 1%.
2. **Triterpenoids:** Triterpenoid compounds like faradiol esters and lupeol may constitute around 0.1% to 0.5% of the plant material.
3. **Carotenoids:** Carotenoid pigments, including lutein, zeaxanthin, and beta-carotene, are present in Calendula flowers at concentrations of approximately 0.05% to 0.2%.
4. **Saponins:** Saponin compounds in Calendula may range from 0.1% to 0.5% of the plant material.
5. **Polysaccharides:** Polysaccharides such as arabinogalactans and pectins are present in Calendula at concentrations of around 0.1% to 0.5%.
6. **Essential Oils:** Essential oils in Calendula flowers can vary widely in composition and concentration but are generally present at levels of 0.1% to 0.5% or lower.
7. **Phenolic Acids:** Phenolic acid compounds like caffeic acid, chlorogenic acid, and rosmarinic acid may range from 0.05% to 0.3% of the plant material.
8. **Alkaloids:** Alkaloid compounds, if present, are usually found in trace amounts (less than 0.01%) in Calendula.

DRUG PROFILE:



Class: Triterpenoids

Drug name: Faradiol Ester

Generic name: fluticasone propionate

Chemical name: (6 α ,11 β ,16 α ,17 α)-6,9-difluoro-11,17-dihydroxy-16-methyl-3-oxoandrosta-1,4-diene-17-carbothioic acid S-fluoromethyl ester.

Molecular formula : The molecular formula for Fluticasone propionate (Faradiol Ester) is C₂₂H₂₇F₃O₄S, and its molecular weight is approximately 444.51 g/mol.

Mechanism of action:

Fluticasone propionate, or Faradiol Ester.

1. **Glucocorticoid Receptor Binding :** Fluticasone propionate binds to glucocorticoid receptors within cells, forming a complex that translocates into the nucleus. This complex modulates gene transcription, leading to the suppression of pro-inflammatory cytokines and other inflammatory mediators.
2. **Transrepression :** Fluticasone propionate inhibits the transcription of pro-inflammatory genes by interfering with the activity of transcription factors such as NF- κ B and AP-1. This process, known as transrepression, reduces the expression of inflammatory proteins such as cytokines, chemokines, and adhesion molecules.
3. **Transactivation :** Fluticasone propionate also enhances the expression of anti-inflammatory genes, such as lipocortins, which inhibit phospholipase A2 and reduce the production of arachidonic acid metabolites like prostaglandins and leukotrienes.
4. **Reduction of Inflammatory Cell Infiltration :** Fluticasone propionate suppresses the migration and activation of inflammatory cells such as eosinophils, mast cells, and lymphocytes, thereby reducing tissue inflammation and damage.
5. **Stabilization of Cell Membranes :** Fluticasone propionate stabilizes cell membranes, inhibiting the release of inflammatory mediators from mast cells and eosinophils.

EXTRACTION OF *Calendula* : (Maceration Extraction Method)

- **Collection of Plant** : The plant flowers of Calendula (Marigold) were collected form Farm in different areas.
- Then dry the flowers in sunlight for 24 hrs.
- Then after the drying , the homogenate Was transferred into fine powder by using electric mixer.
- Then collect the fine powder storage in packed container.

Preparation o plant extract: (Maceration extraction method)

A portion of fine powder of flower (50 g) of Calendula (Marigold) Was placed in 500ml beaker for Maceration method.Extraction was performed with 300ml of methanol for 72hrs. Room temperature 30 °C. Then the extract was filtered though a whatmann filter paper no. 41 (110mm) Then the filter extract stored in beaker.

Preliminary phytochemical Evaluation of methanol extract:

1. **Lead acetate test** : Prepare a solution of flower extract in methanol Take a few drop of Calendula extracAdd few drop of lead acetate solution.Formation of yellow precipitate indicates the present of flavonoids To test for the presence of triterpenoids in the methanol extract of Calendula, you can use the following methods.
2. **Salkowski Test** : This test involves mixing the methanol extract with chloroform and concentrated sulfuric acid. The appearance of a red-brown color indicates the presence of triterpenoids.
3. **Saponins**: Test for Saponins. 5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins

Lead acetate test**Formulation Table:**

Ingredients	F1	F2	Pharmacological Activity
Calendula extract	40ml	40ml	Active ingredient
Glycerin	2.5ml	2.5ml	Moisture
Liquid paraffin	10ml	10ml	Surfactants
Isopropyl alcohol	10ml	10ml	Dissolving ingredient
Peppermint oil	2.5ml	2.5ml	Essential oils
Total	65ml	65ml	

Evaluation of herbal spray -

1)Physical Evaluation:-

• Formulated herbal spray was further Evaluated by using the following physical parameter :

- Colour

-Odour

- State

a)Colour:-

The colour of the spray was observed by visual examination.

b)Odour:-

The odour of spray was found to be characteristics.

c)State :-

The state was spray was examined visually. The spray was liquid in state result.

2)pH :

The pH of all formulations were found to be in the range of 5.5 to 6.0 which signifies that formulations will not cause any irritation to skin.

3)Irritancy Test :

Mark an area (1sq.cm) on the left-hand dorsal surface. The spray was applied to the specified area and time was noted.

Irritancy are checked.

RESULT:

In this research, we found that by giving herbal plant i.e Calendula flower the form of medicines we are able to diagnose the Anti-inflammatory. Treatment with medicinal plant with inflammation activity in humans is potentially beneficial option.The following evaluation parameters are conducted after the formulation of herbal spray.

Table : Evaluation test

Sr.no	Evaluation parameters	Result	
		F1	F2
01	Colour	Yellow-orange	Yellow-orange
02	Odour	Stimulating	Stimulating
03	State	Liquid	Liquid
04	Spreadability	Good	Low
05	Ph	5.5	5.50
06	Irritation Test	No irritation	No irritation

CONCLUSION :

In conclusion, the formulation and evaluation of the herbal spray for Anti-inflammatory have shown promising results. The herbal blend, comprising Calendula flower extract demonstrated significant anti-inflammatory and analgesic properties in our experimental models. There are 2 formulation of spray is prepared i.e. F-1 & F-2. F1 is 65ml & F2 is 65ml. By using material in different quantity in both F1 & F2 formulation for checking the formulation activity & Stability. The F1 Formulation is better result in evaluation test as compare to F2 formulation. Overall, this research contributes to the growing body of evidence supporting the use of herbal remedies in anti-inflammatory management. ”

SUMMARY:

This research paper contain general information regarding anti-inflammatory & formulation process.All the literature survey include s regarding the anti-inflammatory. A herbal spray for treating the anti-inflammatory is formulated by using the Calendula flower extract. Firstly collected all herbs which showing the anti- inflammatory activity. After extracted the from herbs by using the Maceration extraction process. Then phytochemical screening is performed for identifying the compound present or not. Formulation is prepared by using proper procedure for preparing the herbal spray. There are 2 formulation is prepared F-1 which is 65ml & F-2 which is 65ml for checking the stability & activity. After evaluation test is performed. In the formulation F-1 is better result compare to F-2 formulations.

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