A Research on: Formulation and Evaluation of Anti-inflammatory Cream by using Moringa Oleifera Seed Oil

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Abstract:

This study evaluated the chemical composition and anti-inflammatory activity of moringa seed oil, formulated a cream, and assessed its efficacy in vivo. Moringa oleifera seed oil is receiving global attention due to several commercial interests, namely the nutritional, physical, chemical and pharmacological properties Moringa oleifera seeds. are a promising resource for food and non-food applications, due to their content of monounsaturated fatty acids with a high monounsaturated/saturated fatty acids (MUFA/SFA) ratio, sterols and tocopherols, as well as proteins rich in sulfated amino acids. Moringa oil, also known as Ben oil, is a topical treatment for skin and hair due to its high levels of Behenic acid, providing therapeutic benefits As well as large applies as food, lubricant, perfumes, drugs, skincare and biodiesel raw material. The oil detaches mainly by the great concentration of omega-9 and small quantities of polyunsaturated fatty acids that promote great oxidative resistance and lower susceptibility to ramification. Furthermore, it also synthetize tocopherol, bioactive compounds sterols, vitamin E and minerals. Several researches confirm antioxidant, anti-inflammatory and antimicrobial activities detected from the oil, representing quite important to health. However, it is still necessary more research to elucidate its compounds and action type in an organism.

Keyword: Moringa seed oil, Anti-inflammatory, Nutrition.

Introduction:

The plant Moringa oleifera is native to northern India, but it can also be found growing in subtropical and tropical regions of Asia and Africa. For generations, this plant's leaves, seeds, flowers, and roots have been utilized in traditional medicine. Because they are abundant in different phytochemical elements that treat a wide range of ailments, medicinal plants are thought to have therapeutic significance. Studies based on traditional folklore have demonstrated the possible advantages of plants. Redness, warmth, and swelling at the location are some of the main indicators of inflammation, which is a general mechanism by which the body responds to any injury, infection, or irritation. It is thought to have distinct mechanisms for every kind of infection. Currently, medications used to treat inflammation .Plant medicines for topical use in the form of creams or liniments are available. The tree Moringa oleifera, which is found all over India, is a member of the Moringaceae plant family. It is frequently referred to as the "drumstick tree" and is extensively utilized in herbal and culinary preparations. Leaves are said to have purgative qualities and can be used as a poultice on wounds or applied to the temples for migraines. Leaves, bark, and other aromatic materials are consumed to aid in digestion. It is widely utilized in Ayurveda medicine because of its many medical benefits. Because it has a high concentration of Behenic acid, moringa oil, which is prepared from M. oleifera seeds, is also known as Ben oil. It works wonders when applied topically to the skin and hair.

Figure No. 1 Moringa Oleifera Plant

Moringa oleifera seed oil:

The exceptional stability, favorable nutritional qualities, and therapeutic properties of moringa oleifera seed oil have attracted global attention, primarily due to its high content of natural monounsaturated fatty acids, particularly oleic acid. This oil, also known as "oil Ben" or "oil Behen," is rich in behanic acid, which contributes to its name. With over 70% of its composition being monounsaturated fatty acids, specifically omega-9 or oleic acid, it exhibits remarkable resistance to oxidative damage due to its low levels of polyunsaturated and saturated fatty acids. Additionally, the oil is abundant in tocopherols, sterols, vitamin E, minerals, and bioactive substances. Its versatile applications include use in food, treatment of rheumatism, foot drop, blood cleansing, and improvement of heart function. The literature highlights numerous nutritional, antibacterial, antioxidant, and anti-inflammatory qualities, making it suitable for both commercial and human use. Despite the importance of moringa oleifera seed oil, there is limited knowledge about its primary characteristics. Therefore, this study aims to comprehensively evaluate its nutritional, physicochemical, antibacterial, anti-inflammatory, and antioxidant properties, shedding light on its potential benefits.

Figure No.2 Moringa seed oil

Advantages :

1. Moringa oleifera seed oil is a good source of essential fatty acids, antioxidants, and vitamins such as A and E, which can help promote healthy skin and hair.
2. It has been shown to have anti-inflammatory properties, which can benefit conditions such as arthritis and skin inflammation.

3. The oil is lightweight and easily absorbed by the skin, making it a good moisturizer for dry or damaged skin.
4. Moringa oleifera seed oil has been found to have antimicrobial properties that can help fight off bacteria and fungi.

Disadvantages:

1. Some individuals may be allergic to moringa oleifera seed oil and may experience skin irritation or other allergic reactions when using products containing the oil.
2. While there are some studies on the benefits of moringa oleifera seed oil, more research is needed to fully understand its potential effects and safety.
3. Moringa oleifera seed oil may not be as readily available as other oils, and it can be more expensive compared to other common oils.
4. Moringa oleifera seed oil is sensitive to light and heat, which can affect its stability and shelf life if not stored properly.

Health Benefit:

Moringa oleifera is very nutritious:

Moringa oleifera is a fairly large tree native to North India.

- It goes by a variety of names, such as drumstick tree, horseradish tree or ben oil tree.
- Almost all parts of the tree are eaten or used as ingredients in traditional herbal medicines.
- This especially applies to the leaves and pods, which are commonly eaten in parts of India and Africa.

Literature review:

Author Name	Description
Vidyadhara Suryadevara (*et .Al)* (2018)	Title: "Formulation and Evaluation of Anti-inflammatory cream using Moringa Oleifera Seed Oil". The research focuses on developing an anti-inflammatory cream using

	Moringa seed oil, a plant with medicinal properties. The oil was extracted and tested for alkaloids, glycosides, tannins, and flavonoids. The cream was prepared through alkali saponification and underwent various tests, revealing its potency in anti-inflammatory properties.
Smith A, Johnson B,*(et.Al)*2019	Title: "Evaluation of Anti-Inflammatory Activity of Moringa oleifera Seed Oil in Topical Cream Formulation". The purpose of this study was to look at the anti-inflammatory activity of Moringa Oleifera oil in topical cream formulation. The researchers prepared a cream containing Moringa oil and tested its anti-inflammatory activity in a rat dermatitis model. The results showed that Moringa oil cream significantly decreased inflammation compared with the control group. This suggests that Moringa may be a natural treatment for inflammatory skin conditions.
Gomez C, Martinez D,*(et.Al)* 2022	Title: "Bioactive Compounds and Anti-Inflammatory Properties of Moringa oleifera Seed Oil-Based Creams". This research explored the bioactive compounds present in Moringa oleifera seed oil and their anti-inflammatory effects in topical cream formulations. Various creams containing different concentrations of Moringa oleifera seed oil were prepared and tested for their anti-inflammatory activity using in vitro and in vivo models. The study revealed that Moringa oleifera seed oil-based creams exhibited potent anti-inflammatory properties, suggesting their potential for the development of novel natural anti-inflammatory products.
Lee X, Wang S,*(et.Al)*2020	Title: "Comparative Analysis of Moringa oleifera Seed Oil Cream and Hydrocortisone Cream in Treating Inflammatory Skin Conditions". This comparative study investigated the effectiveness of Moringa oleifera seed oil cream versus hydrocortisone cream in managing

	inflammatory skin conditions. Patients with eczema and dermatitis were treated with either Moringa oleifera seed oil cream or hydrocortisone cream for a specified duration. Results demonstrated that both creams effectively reduced inflammation, but the Moringa oleifera seed oil cream showed comparable efficacy to hydrocortisone cream with fewer side effects, highlighting its potential as a safe and natural alternative for inflammatory skin conditions.

Plan of work:

- **Topic selection:** Provide an overview of Moringa oleifera, including its botanical characteristics, traditional uses, and current popularity in skincare products. Dive into the specific compounds present in Moringa oleifera seed oil that contribute to its anti-inflammatory properties. Explain how these compounds work to reduce inflammation in the skin.
- **Literature and Review**: Briefly introduce the topic of anti-inflammatory creams and the use of Moringa oleifera seed oil in skincare products. Provide an overview of the importance of addressing inflammation in skincare and the potential benefits of natural ingredients like Moringa oleifera seed oil.:
- **Material collection**:Gather all the necessary ingredients for the cream. In addition to Moringa oleifera seed oil, consider selecting other skin-friendly ingredients such as shea butter, coconut oil, beeswax, and essential oils like lavender or tea tree.
- **Formulation of Anti-Inflammatory cream:**Measure out the required amounts of each ingredient based on your chosen recipe. Ensure a clean and sanitized work area before proceeding. Combine the ingredients in a double boiler or a heat-safe container over low heat to melt and blend them together. Stir continuously to ensure even mixing.
- **Evaluation of cream**: If promising results are obtained from the in vivo studies, consider moving on to human clinical trials. Evaluate the cream's effectiveness and any potential side effects. Perform quality control tests to assess the consistency, texture, and overall quality of the cream. Proceed with in vivo studies on animal models to further evaluate the efficacy and safety of the anti-inflammatory cream.

- **Result and Conclusion**: Provide a concise summary of the main findings from the studies included in your review that investigated the use of Moringa oleifera seed oil in anti-inflammatory cream* Discuss the effectiveness of anti-inflammatory creams containing Moringa oleifera seed oil in reducing inflammation, based on the results of various in vitro and in vivo studies. Summarize the reported safety profile of Moringa oleifera seed oil-based skincare products, including any documented adverse reactions and overall tolerability.Compare the efficacy and safety of Moringa oleifera seed oil-based anti-inflammatory creams with conventional skincare products, highlighting the potential advantages of natural ingredients.

Discuss the implications of the reviewed studies for the development of anti-inflammatory creams utilizing Moringa oleifera seed oil, considering both efficacy and safety aspects.

Chlorogenic acid: Also found in high amounts in coffee, chlorogenic acid may help moderate blood sugar levels after meals .One study in women found that taking 1.5 teaspoons (7 grams) of moringa leaf powder every day for three months significantly increased blood antioxidant levels.

Moringa leaf extract may also be used as a food preservative. It increases the shelf life of meat by reducing oxidation.

Morphology and Taxonomy:

The tree exhibits rapid growth in loamy and well-drained sandy soils, showing a preference for elevations around 500 meters above sea level. Generally, it achieves a small to medium size, characterized by naturally trifoliate leaves and flowers that grow on an inflorescence measuring 10–25 cm in length. The fruits, commonly known as "pods," are typically trifoliate. Although the trunk usually grows straight, occasional instances of poor formation can be observed. The branches have a tendency to be disorganized, resulting in an umbrella-shaped canopy. The brown seeds have a semi-permeable hull, and each tree produces approximately 15,000–25,000 seeds per year.

Anti-inflammatory activity:

The anti-inflammatory effect of M. oleifera components, such as leaves, pods, flowers, and roots, was evident in the study. A compound isolated from Moringa, 4-[2-o-Acetyl-alpha-l-rahamnoslyloxy) benzyl] thiocynate, displayed inhibitory activity on nitric oxide and was effective in Raw264.7. The research delves into the anti-inflammatory properties of the Moringa oleifera ethyl acetate fraction in RAW264.7 cells. It was observed that the fraction decreased iNOS, COX-2, and NF-κB expression, while elevating IκBα levels. This indicates a promising therapeutic approach, as agents disrupting NF-κB activation have proven effective in treating inflammation-related conditions. Furthermore, the study revealed that the ethyl

acetate fraction's anti-inflammatory potential is associated with the suppression of NF-κB activation, prevention of IκBα degradation, and inhibition of NF-κB p65 protein translocation. Additionally, aurnatiamide acetate and 1, 3-dibenzylurea, derived from Moringa oleifera roots, were identified as supplementary compounds with inhibitory effects on tumor necrosis factor (TNF-α) production.

The active chemical constituents of Moringa oleifera, including tannins, phenols, alkaloids, flavonoids, carotenoids, β-sitosterol, vanillin, and moringin, have been identified for their anti-inflammatory properties. The extract from M. oleifera fruit has been observed to inhibit the translocation of nuclear factor kappa B (NF κB), while the chloroform extract has shown cytotoxicity at higher concentrations (500–1000 µg/mL). In an experimental mice model, the application of Moringa oleifera leaf extract has emerged as an effective treatment strategy for atopic dermatitis in human keratinocytes. This treatment strategy is accompanied by a significant decrease in the expression levels of retinoic acid-related orphan receptor γT, lymphopoietin thymic stromal, and mannose receptor mRNA in the ear tissues. Moringa oleifera bark extract has also exhibited notable efficacy in addressing urinary tract infections, achieving a complete cure rate of 66.67% within three weeks. When added to human monocyte-derived macrophages (MDM) along with lipopolysaccharide (LPS) and cigarette smoke extract (CSE), the extract shows modulatory effects by controlling inflammatory markers TNF-α, IL-6, and IL-8, as well as inhibiting the expression of RelA, a crucial gene involved in the NF-κB p65 signaling cascade. In rat models with acetic acid-induced acute colitis, oral administration of M. oleifera seeds leads to a reduction in distal colon weight, ulcer severity, and mucosal inflammation, highlighting the potential of M. oleifera in mitigating inflammation-related conditions through a multifaceted approach.

Cream:

Creams have been an integral part of cosmetic products for centuries due to their simple preparation methods and safety for public use. The skin is prone to injuries, but it possesses the ability to heal naturally. However, this healing process can be slow and there is a risk of infection, especially in the initial stages of an injury. Medicated creams can be applied to the affected area to accelerate healing and prevent infections. This review focuses on the use of pharmaceutical creams for wound healing, discussing the process, preparation methods, classification based on function, advantages, disadvantages, characteristics, types, ingredients, and evaluation parameters. Creams, with their high water content, are easily absorbed and can cover large skin areas. Their oil content helps them stay on the skin's surface, protecting against moisture loss.

Figure No.3 Cream

Inflammation:

Injury or infection elicits a typical bodily response known as inflammation. This process is initiated by the release of chemicals that stimulate the immune system to combat the infection or repair injured tissue. Once the injury or infection has been resolved, the inflammatory process ceases.

The term inflammation originates from the Latin word "inflammare," which means to burn. It is a natural response of living tissues to injury. Inflammation is not a disease itself, but rather a sign of an underlying disease. Diseases that involve an inflammatory reaction as a major component are categorized with a specific suffix. Typically, these diseases are named after the affected organ followed by the suffix '-itis'. For example, inflammation of the meninges is known as meningitis. However, there are exceptions to this rule, such as pneumonia and typhoid fever. Inflammation aims to remove the initial cause of cell injury and necrotic cells and tissues damaged by harmful agents. It serves a protective function by diluting, destroying, or neutralizing harmful agents like microbes or toxins. Ultimately, the process of inflammation leads to healing and the restoration of the injured sites. Inflammation is closely linked to repair processes, where damaged tissue is replaced by the regeneration of parenchymal cells or the formation of fibrous scar tissue to fill any residual defects. The cardinal signs of inflammation include swelling (tumor), redness (rubor), heat (calor), pain (dolor), and loss of function (functio laesa). Various types of cells expressing and reacting to different mediators are involved in the complex sequence of events during inflammation.

Inflammation is commonly categorized based on the lesion and histological appearances observed in acute and chronic inflammation. However, these fundamental forms of inflammation can overlap, and various factors can alter their progression and histological characteristics. The initial phase of acute inflammation involves a transient series of tissue reactions in response to injury, lasting for a short duration ranging from a few minutes to a few days. This phase is characterized by the exudation of fluid and plasma proteins, primarily the accumulation of neutrophil leukocytes. It is accompanied by increased vascular permeability, infiltration of capillaries, and the migration of leukocytes. The classical symptoms of acute inflammation include redness, heat, edema, and pain. On the other hand, chronic inflammation lasts for a longer duration, ranging from days to years, due to the persistence of the initial stimulus, interference with normal healing, repeated episodes of acute inflammation, or a low-grade smoldering caused by the continuous production of immune response mediators. Chronic inflammation is characterized by the influx of lymphocytes and macrophages, along with associated vascular proliferation and scarring.

Type of inflammation :

Acute:

Acute inflammation is a complex process triggered by various chemical mediators such as prostaglandins, leukotrienes, and platelet-activating factor. Anti-inflammatory agents work through a variety of mechanisms. The acute inflammatory response involves heightened vascular permeability and cellular infiltration, resulting in edema formation due to the leakage of fluid and proteins, along with the accumulation of leukocytes at the site of inflammation temporarily. Acute inflammation is a multifaceted process initiated by a variety of chemical mediators like prostaglandins, leukotrienes, and platelet-activating factor.

Anti-inflammatory agents operate through diverse modes of action. The acute inflammatory response is characterized by increased vascular permeability and cellular infiltration, leading to edema formation from the extravasation of fluid and proteins, as well as the accumulation of leukocytes at the inflammatory site for a brief period of time.

Chronic:

Chronic inflammation arises when the acute response is inadequate in removing pro-inflammatory agents. It involves the proliferation of fibroblasts and the infiltration of neutrophils, resulting in fluid exudation.

The persistence of infection or antigen, repetitive tissue damage, or the failure of endogenous anti-inflammatory mechanisms can lead to chronic inflammation. Macrophages are key players in mediating

chronic (or acute) inflammation by activating inflammatory or immune cells, regulating the production of pro-inflammatory cytokines and mediators.

Physiological Role of Inflammation:

Inflammation serves as a crucial physiological response triggered by various harmful agents like bacterial infections or physical injuries. Its primary goal is to minimize damage and facilitate tissue healing. This process offers advantages like eliminating invading microorganisms and isolating abscesses to prevent infection from spreading. The body's internal biochemical pathways, activated during defense mechanisms, can regulate inflammation and support recovery.

What does an anti-inflammatory cream do?

Topical anti-inflammatories, whether natural or over-the-counter medications, are known for their simplicity and are believed to have fewer side effects compared to oral anti-inflammatory drugs. Injuries or infections trigger an immune response that leads to inflammation, characterized by increased blood flow, redness, warmth, and swelling. While inflammation is a normal defense mechanism, chronic inflammation can lead to pain, prolonged swelling, and tissue damage in conditions like rheumatoid arthritis. Topical analgesics, such as anti-inflammatory lotions containing CBD or NSAIDs like ibuprofen, can be applied directly to affected areas to alleviate pain from sprains, strains, arthritis, and muscle aches. Unlike NSAIDs, CBD works by interacting with the Endocannabinoid System (ECS) to reduce pain and inflammation throughout the body. CBD binds to ECS receptors, inhibiting the release of inflammatory substances. Due to their effectiveness and natural ingredients, many individuals prefer CBD balms and creams over traditional NSAIDs.

The balm absorbs into the skin after application. After reaching the site of inflammation, it penetrates farther into the tissue. In the case of NSAIDs like ibuprofen, it acts by preventing the actions of enzymes that are involved in the production of prostaglandin. This substance exacerbates tissue pain and inflammation. Because heavy muscular tissue prevents anti-inflammatory creams from reaching the inflammatory site quickly, they work best when the irritated joint is relatively close to the skin.

Symptoms:

- Redness
- Joint pain
- Heat
- Swelling
- Pain

- Loss of function

Anti-inflammatory cream:

Anti-inflammatory or antichloristic is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs, also called anti-inflammatories, make up about half of analgesics. Anti-inflammatory creams can be used to treat several symptoms of pain and inflammation, including: short-term strain or sprains. Muscle aches or pain, Tendon pain.

Activities of seed oil:

Antioxidant activity –

Moringa oleifera seed oil offers a significant antioxidant activity that has a wide range of applications in the food, lubricant, and cosmetics industries, making it important for various sectors. The high presence of monounsaturated fatty acids in this oil reduces the risk of cholesterol and heart disease. Scientific evidence suggests that Moringa seed oil contains abundant natural antioxidants, which can provide health benefits by combating hypercholesterolemia effects. This is due to the presence of phytosterols and the elimination of free radicals from the body, as well as the presence of tocopherols, phenolic compounds, and carotenoids. Compared to other edible oils that contain oleic acid (such as sunflower, safflower, and almond oil), Moringa seed oil is less susceptible to oxidative damage thanks to its antioxidant potential. Additionally, it can be mixed with other oils to enhance the oxidative stability of commercial oils or margarines. Studies have shown that a proper mixture of Moringa oil with traditional edible oils like palm, soy, and sunflower oil improves the physical-chemical characteristics and oxidative stability of the oils. Furthermore, when mixed with 50% butter, the oil exhibits even greater stability, lower cholesterol content, and a melting temperature of 35.5º C. If cultivated on a large scale, Moringa seed oil could serve as a viable alternative to vegetable oil for various purposes and potentially replace olive oil. According to [1], Moringa oleifera seed oil shows potential as an effective therapy for xenobiotic-induced liver disorders. Its hepatoprotective action is attributed to its natural antioxidant compounds, including tocopherols, phenolic compounds, and carotenoids. In a study reported by, Moringa seed oil was found to attenuate gentamicin-induced nephrotoxicity in rats through its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. This protective action is attributed to the bioactive compounds present in the oil. Taken together, these findings suggest that Moringa seed oil can mitigate oxidative stress and neurotoxicity induced by MTX injection. Interestingly, supplementation with virgin coconut oil (VCO) and Moringa seed oil (MSO) has been shown to attenuate MTX-induced brain neurotoxicity in rats.

Material methodology:

Collection of Plant Material:

The collected fruits were sun-dried. After the moisture in it was removed, the seeds were separated and dried.The fruits of M. oleifera were collected from the farmers under ambient conditions. The external coat present on the seed was removed manually. The seeds were then crushed into powder or further use.

Extraction procedure:

By soxhlet Apparatus:

The Moringa oleifera seed powder was packed inside a muslin cloth placed in a thimble of the soxh let extractor. The extraction was carried out using thermostatic heating metal. The solvent in the extracted oil was evaporated and the resulting oil further dried to constant weight in the oven.

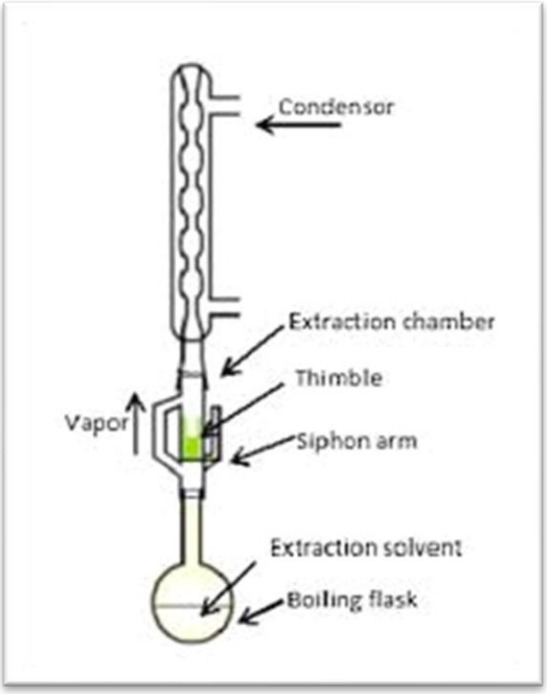

Figure No. 4 Soxhlet extraction

Formulation Table:

Ingredient	F1	F2	F3
Moringa seed oil	2gm	3gm	3.5gm
Lanolin	4gm	5gm	2.5gm
Stearic acid	1.5gm	1.5gm	1.5gm
Glycerine	3ml	3ml	3ml
Triethanolamine	2ml	2ml	2ml
Polyethylene glycol	0.8gm	0.8gm	0.8gm
Methyl paraben	0.5gm	0.5gm	0.5mg
Rose water	q.s	q.s	q.s
Purified water	q.s	q.s	q.s

Role of Ingredients:

Moringa seed oil: It is great for topical use on the skin and the hair. Moringa oil has many therapeutic benefits such as anti-Inflammatory action.

Stearic acid: Acts as thickner, help to soothe irritation.

Lanolin: Protectant and anti-inflammatory action.

Glycerine: Act as humectant.

Triethanolamine: Stabilizer and emulsifier

Polyethylene glycol: Cleansing agent

Methyl paraben: Preservative

Rose water: Perfume

Purified water: Volume makeup

Preparation of Moringa Oleifera Seed Oil Cream:

1. These w/o emulsion based preparations contain aqueous phase and an oil phase.
2. The Oil phase (A) containing suitable quantities of Moringa seed oil and Stearic acid, lanolin and gycerine were transferred in to a china dish and heated at 60°C–70°C on a water bath.

3. The aqueous phase (B) having suitable quantities of Triethanolamine, Methyl paraben, polyethylene gycol were dissolved in distilled water and heated at 60°C–70°C on a water bath .
4. When both aqueous and oily phases reach the same temperature, the oil phase was poured into a mortar and triturated continuously by adding alkali until the smooth cream is obtained.
5. The obtained cream was packed safely and stored.

Evaluation of cream:

The formulated creams were subjected to evaluation of various parameters as per the standard procedures.

Colour:

Colour is observered white or yellow and yellowish brown.

PH:

The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 ml of distilled water and its pH was measured.

Viscosity: The viscosity of a cream can be measured using various techniques such as a viscometer or rheometer. These instruments provide quantitative data on the cream's flow properties, including its consistency and thickness.

Dye test:

The scarlet red dye was mixed with the cream. A drop of cream was placed on a microscopic slide and covered with a coverslip. This was examined under microscope. The dispersed globules appear colorless in the red ground indicating the o/w type formulation.

Homogeneity:

The formulations were tested for homogeneity by visual appearance and by touch.

Appearance:

The appearance of the cream was judged by its color, pearlescence , roughness, and then graded.

Spread ability:

Adequate amount of sample is taken between two glass slide and weight of 100gm is applied on the slide for 5 min. Spreadability can be expressed as

S=m*l/t

Where, m=weight applied to upper slide

l= length moved on the glass slide

t= time taken

Irritancy test:

The cream was applied to the specified area and time was noted. Irritancy, erythema, and edema, were checked if any for regular intervals up to 24 h and reported.

In vitro Diffusion Studies:

Franz Cell apparatus was utilized to conduct in vitro diffusion studies. This apparatus is commonly employed in the development of formulations to assess skin permeation. The analysis of these studies involved the use of Fourier transform infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GCMS), and high performance thin layer chromatography (HPTLC).

Fourier transform-infrared spectroscopy analysis:

An extracted sample drop was deposited on the IR crystal port for analysis and compared with the standard sample analysis. Subsequently, the formulated cream and the in vitro diffused cream sample were analyzed using liquid FTIR. The spectra of all samples were gathered through a Bruker FTIR spectroscopy, with each spectrum acquired in the range of 4000-600 cm−1 by conducting 20 scans at a spectral resolution of 4 cm−1. The data collection was performed using OPUS 7.2.139.1294 software for peak height and area measurements.

Gas chromatography-mass spectrometry analysis:

The samples were analyzed on a JEOL GC MATE II gas chromatography with Quadra pole Double Focusing Mass Analyzer fitted with “Photomultiplier tube” detector and HP5MS column. Highly Pure Helium was used as a carrier gas with a flow rate of 1 mL/min and the oven temperature was maintained at 50°C to 250°C by increasing 100 C/min.

High-performance thin-layer chromatography analysis: The oily samples were dissolved in n-hexane quantitatively to strength of 0.1 µl/1 µl. 2 µl of the prepared solution was injected on a HPTLC Silica Gel GF254 (5 cm × 10 cm) plate. The plate was developed in Toluene: Ethyl acetate (9:1) mobile phase. Plate was derivatizied using 5% Methanol sulfuric acid and scanned at 620 nm under a UV Scanner.

Ex vivo anti-inflammatory activity:

Protein denaturation:

0.2 milliliters of egg albumin were introduced into vials containing 2.5 milliliters of 0.1M PBS at pH 6.4. Subsequently, 2 milliliters of sample and standard drug were added to the vials in triplicate to achieve

final concentrations of 50, 100, 150, and 20 micrograms per milliliter. Control vials received PBS (2.5 milliliters) and egg albumin. The mixture was then incubated at 37°C ± 2°C in a BOD incubator for 15 minutes, followed by heating at 70°C for 5 minutes. After cooling, the absorbance was measured at 660 nm using the vehicle as a blank. The percentage inhibition of protein denaturation of the test should be compared with the control.

% inhibition of denaturation =

$$\frac{\text{Absorbance in control group} - \text{Absorbance in test group} \times 100}{\text{Absorbance in control}}$$

In Vivo Anti-Inflammatory Activity:

Carrageenan-induced paw edema in rats:

The animals were divided into four groups, each consisting of five animals. Animals in Group 1 were administered normal saline, Group 2 received Ibuprofen cream, Group 3 was treated with Moringa seed oil, and Group 4 received Moringa seed oil cream. All groups were given 0.1 ml of 1% Carrageenan. Prior to Carrageenan injection on the right hind paw of the rats, the animals were pre-treated with vehicle/Ibuprofen/extracts 30 minutes earlier. Paw volumes were assessed by measuring the water column displacement in a plethysmometer at 1, 2, 3, and 4 hours post administration of the test substances. The reduction in paw volume compared to the control animals was considered as the anti-inflammatory response.

% Inhibition of edema =

$$\frac{\text{Absorbance in control group} - \text{Absorbance in test group} \times 100}{\text{Absorbance in control group}}$$

Result:

Evaluation of test	No. 1 cream	No.2 cream	No.3 cream
Colour	White	Yellowish brown	white
pH	6.4	6.3	6.5
Viscosity	9.1	7.8	8.6
Dye test	o/w	o/w	w/o
Appareance	Greasy	Smooth	Smooth
Homogeneity	Good	Good	Excellent
Spread ability	Complete	Complete, moderate	Complete
Irritancy test	Non irritant	Non irritant	Non irritant

Conclusion:

We successfully developed an M. oleifera seed oil cream. Moringa seed oil cream possessed anti-inflammatory activity, enhanced the skin hydration level, and reduced skin erythema, but did not affect the melanin content and skin visco-elasticity. There was no report of skin irritation from the application of the cream, suggesting that the moringa seed oil cream developed in this study is appropriate for pharmaceutical and cosmetic uses.

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