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Formulation Development & Characterization Of Anti-Inflammatory Ointment Of Brassica Oleracea.

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ABSTRACT

Brassica oleracea var. capitata L. (white cabbage) is a valuable vegetable with diverse nutraceutical benefit. Brassica phytochemicals exert a broad spectrum of health-promoting activities. The aim of this study was to investigate the possible beneficial effects of a cauliflower leaf powder (CLP)-enriched diet to prevent inflammation. This study highlights the potential utility of these compounds in the prevention or amelioration of chronic inflammatory diseases and encourage studies to identify selected member of Brassicaceae family able to provide the right quota of bioactive compounds to be included in dietary intervention. Brassica vegetables have also anti-inflamatory activity that has been known from a long time ago and have been used to different irritations of the human body. While the specific modes of action have not yet been defined, phytochemical research and pharmacological experiments performed over the past few decades on white cabbage juice or extract offer fair evidence for its numerous conventional uses. Study on the phytochemicals of white cabbage has been based on the well-known health promotion of components such as glucosinolates, polyphenols, and various vitamins correlated with anticarcinogenic, anti-inflammatory, and antioxidant activities.

KEYWORDS: anti-inflammatory; Brassica oleracea, cabbage, herbal ointment, inflammation, Isothiocyanate, Anthocyanin.

INTRODUCTION

Human skin

The skin is the body's largest and heaviest organ. It is made up of several different types of cells and its main purpose is to protect the inside of the body from the environment. Alongside its role as a protective barrier, the skin helps people maintain the right internal temperature and allows them to sense the world through nerve endings.Skin is a complex organ. An average square inch of skin contains 650 sweat glands, 20 blood vessels, and more than 1,000 nerve endings. Despite being just a few millimeters thick, the skin makes up around one-seventhTrusted Source of a person's body weight.

Skin layers

The skin has three basic layers — the epidermis, the dermis, and the hypodermis.

Epidermis

The epidermis is the outermost layer. It is a waterproof barrier that gives skin its tone. It's main roles are:

- to make new skin cells
- to give the skin its color
- to protect the body from the external environment

The five layers of the epidermis are:

- stratum corneum
- stratum lucidum
- stratum granulosum
- stratum spinosum
- stratum germinativum

Dermis

The dermis serves as connective tissue and protects the body from stress and strain. It also gives the skin strength and elasticity. In addition, its main roles are:

- to make sweat and oil
- to provide sensation and blood to the skin
- to grow hair

The reason the dermis can perform these functions is that it houses the hair follicles, blood vessels, and lymphatic vessels. It is home to a number of glands, including sweat glands and sebaceous glands, which produce sebum, an oil that lubricates and waterproofs hair.

Functions of the skin

- Provides a protective barrier against mechanical, thermal and physical injury and hazardous substances.
- Prevents loss of moisture.
- Reduces harmful effects of UV radiation.
- Acts as a sensory organ (touch, detects temperature).
- Helps regulate temperature.
- An immune organ to detect infections etc.
- Production of vitamin D.

Skin disease

1. Atopic dermatitis: Also known as eczema, this is an inflammatory skin disease characterized by dry, red, itchy patches of skin.
2. Acne: This is perhaps the most common skin disorder. It occurs when hair follicles become clogged with dead skin cells and oil.
3. Melanoma: A type of skin cancer caused by exposure to excess sunlight.
4. Rosacea: A common rash found in middle-aged people. They have a tendency to flush and have small red bumps on the center of the face.
5. Psoriasis: This is an auto-inflammatory skin disease. It causes red, flaky patches to appear on the skin.
6. Scabies: An itchy skin condition caused by the human scabies mite.
7. Shingles: Also called herpes zoster, it is a painful, blistering rash caused by a virus.

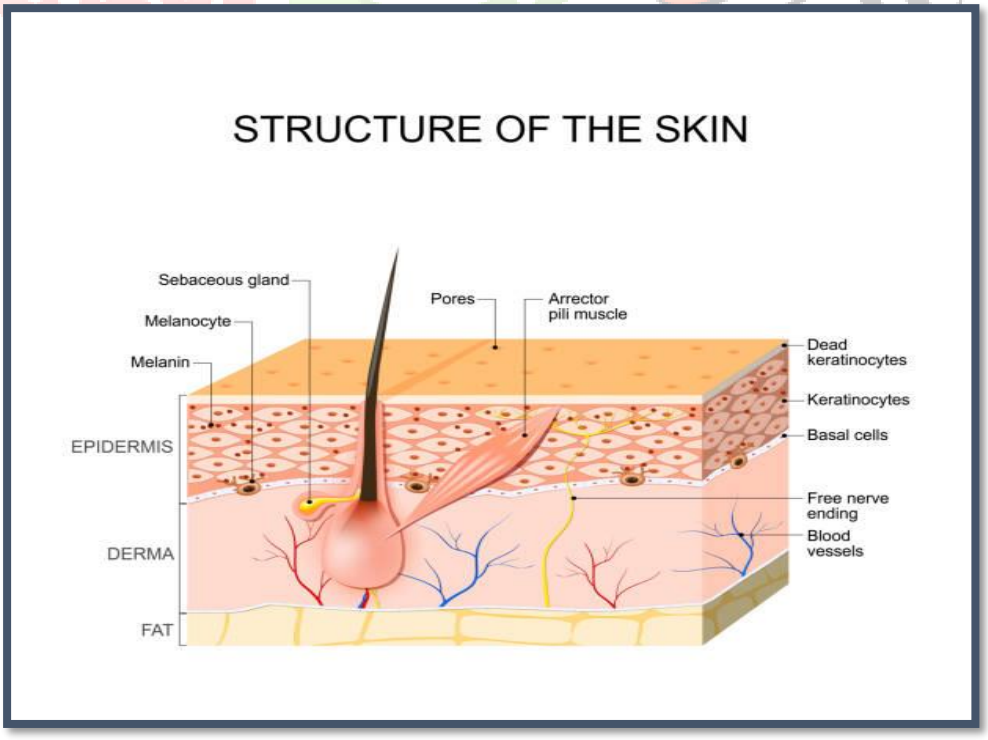

Fig : 1 Skin Anatomy[17]

Anti-inflammatory ointment

Topical antimicrobial agents are chemical substances that, directly applied to the skin, inhibit the growth or destroy any microorganism, fungi, viruses or bacteria. Within this term, we generally refer to those that are active against the latter. They are divided into antiseptic sand antibiotics. Antiseptics are directly applied to a living organism to eradicate the existing microorganism on the mucocutaneous surfaces, preventing their proliferation. Topical antibiotics are either produced by living organisms or manufactured through synthesis and are mainly used to fight infections.

Brassica oleracea

Cabbage (Brassica oleracea var. capitata L.), which belongs to the family Brassicaceae, is one of the most common vegetables grown worldwide. Cabbage is often referred to as green cabbage to distinguish it from red cabbage, which has the same scientific name. It is a biennial, herbaceous vegetable grown as an annual crop that produces a swollen bulb-like stem at the base of the plant. It was described by Percival as a form of cabbage with a turnip-like stem that stands quite above the ground that is generally utilized as a cooked vegetable, but the raw grated stem forms an acceptable component of winter salads. Due to its antioxidant and anti-inflammatory properties. cabbage has been widely used as an herbal medicine to treat gastrointestinal disorders such as gastritis, peptic and duodenal ulcers, and irritable bowel syndrome, as well as wounds and mastitis. Many people have believed cabbage can ameliorate various skin ailments such as xeroderma, skin troubles, and acne. In the theory of traditional medicine, malfunction of gastrointestinal tract is closely related in skin problems. In addition, cabbage is recognized as good supplement for both gastrointestinal disorders and skin problems in Korea.

Cabbage is one of the most popular Brassica vegetables in the human diet due to its affordability, availability, and range of health benefits and thus it forms a huge part of worldwide cuisines and diets. Many epidemiological studies and interventional trials have shown this cruciferous vegetable to be beneficial in reducing the risk of several types of cancer as well as other chronic diseases such as cardiovascular disease, cataracts, Alzheimer's disease, and diabetes, which is owed to its nutritious composition with its wide array of antioxidants and phytochemicals including carotenoids, glucosinolates, isothiocyanates, phenolic compounds, and vitamins E and C.

Phytochemical composition

Phytochemicals are non-nutritious chemicals that are derived from plants and provide defense against diseases in humans. They are oxidation preventive and sweep out free radicals, the byproducts of biochemical processes. Brassica plants are the rich source of phytochemical compounds of medicinal importance. The bioactive phytochemical compounds commonly found in most of the Brassica species include polyphenols, phenolic acids, flavonoids, carotenoids (zeaxanthin, lutein, β-carotene), alkaloids, tannins, saponins, anthocyanins, phytosterols chlorophyll, glucosinolates, phytosteroids, terpenoids, glycosides, vitamin C, Vitamin E and aliphatic and aromatic amines. Due to the presence of these compounds, Brassica plants show biological activities against various diseases and have been found to effective in treating various diseases in human. The edible parts of these plants show antimicrobial,

antibacterial, antidiabetic, antimalarial, antiaging, antiulcer, anti-hyperglycemic, anti-hyperlipidemic, anti-proliferative, neuroprotective, antidiabetic, anti-genotoxic and antioxidant activities.

Anti-inflammatory activity

Growing observations in various cellular models suggest that also single biomolecules isolated from in Brassicaceae can exert anti-inflammatory effects reducing, at different levels, the production of pro-inflammatory cytokines. The pure compounds of biomolecules present in Brassica that many of them (as glucosinolates, isothiocyanates, hydroxycinnamic acids, flavonols and anthocyanins) showed high anti-inflammatory activities reducing the production of the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β). A variety of Brassicaceae-derived phytochemicals seem to act as NF-κB inhibitors leading to down-regulation of inflammatory mediators. For instance, phytochemicals contained in watercress as SFN, phenethyl-isothiocyanate (PEITC), 8-methylsulphinyloctyl isothiocyanate (MSO) and indole-3-carbinol (I3C) can downregulate activation of NF-κB induced by LPS and suppress COX-2, iNOS, and prostaglandins expression in cultured mouse macrophages.

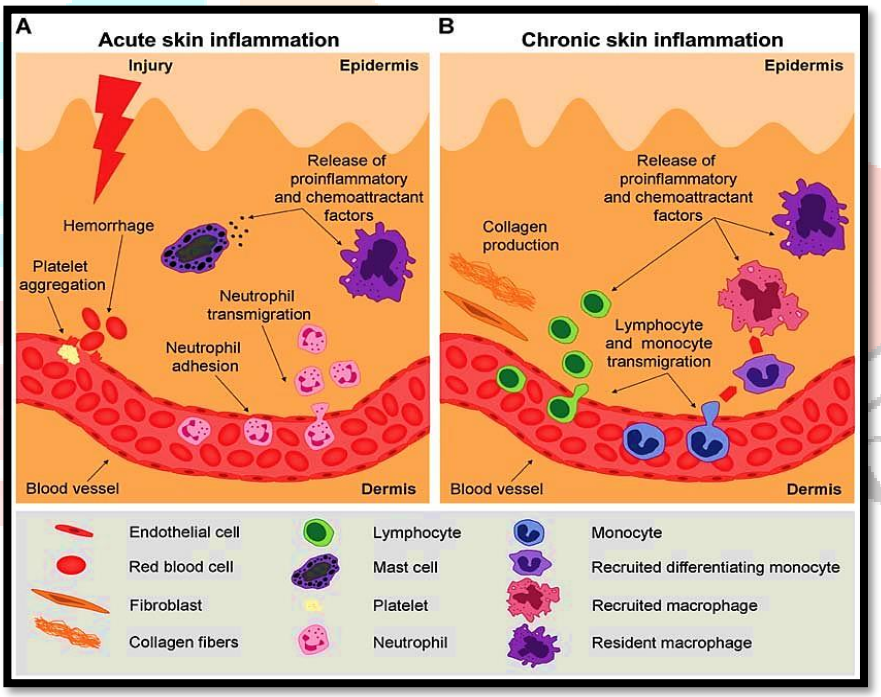

Fig: 2 Inflammatory view of skin[16]

Antibactorial activity

the antibacterial activity of different compounds from Brassicaceae [14] against bacteria, such as Helicobacter pylori, Escherichia coli, Bacillus cereus, B. subtilis, Listeria monocytogenes, S. aureus and yeasts such as C. albicans.

Anticancer activity

Epidemiological and multiple in vitro and in vivo studies indicate that the consumption of cruciferous vegetables can reduce the overall risk of cancer and provide protection at all stages of cancer progression Other essential and really well studied glucosinolate's hydrolysis products like sulforaphane and indol-3-carbinol, they are also present in white cabbage (Sivakumar et al., 2007; Park et al.,

2013). From the hydrolysis of glucoraphanin under the control of myrosinase enzymes is formed sulforaphane. This isothiocyanate, sulforaphane, is capable of targeting many cancer growth steps and is an extremely impressive nutritional chemoprevention and therapeutic tool.

Antioxidant activity

Consumption of food or nutritional supplements rich in antioxidants improves the pathways of defences against free radicals and reactive oxygen species (ROS) and thereby helps to protect against chronic diseases. The presence of biological activities with confirmed antioxidant capacity, like vitamin C, carotenoids, polyphenolics, flavonoids, glucosinolates, hydrolysis products, etc., has been predominantly associated with the health benefits of Brassica vegetable

Antiobesity, hypolipidemic and hypoglycemic effect

The effects of a canned blended green vegetable and fruit beverage containing broccoli and cabbage on serum lipid levels in hypercholesterolemic patients were tested in a clinical research by Suido et al. (2002). Thirty-one adults were offered two canned beverages (160 g/can) a day for 3 weeks, and their serum total cholesterol and low-density lipoprotein cholesterol levels decreased substantially. In several other animal studies, the anticholesterol, hypoglycemic and anticoagulant effects of white cabbage were examined.

APPLICATIONS

Brassica oleracea has been traditionally used in ayurvedic system for various pueposes.in the field of pharmacy, ointment made from Brassica oleracea may have potential application ,including:

1)Herbal medicine

2)Traditional medicine

3)Compounding pharmacy

4)Herbal skincare products

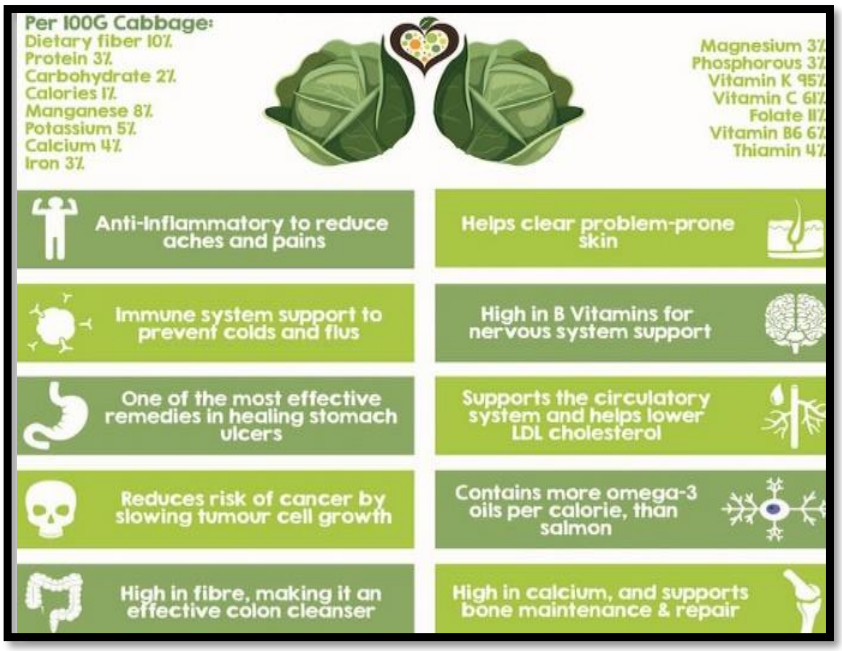

Fig : 3 Uses of brassica oleracea[18]

DRUG PROFILE

Brassica oleracea were purchased from local market and identified and contains following chemicals such as, glucosinolates, isothiocyanates, hydroxycinnamic acids, flavonols and anthocyanins, phenethyl-isothiocyanate (PEITC), 8-methylsulphinyloctyl isothiocyanate (MSO) and indole-3-carbinol.

Brassica oleracea

Cabbage (Brassica oleracea var. capitata) is a popular vegetable in many countries, which has been reported with high nutritional value and distinct health benefits [5] There have also been recent reports on cabbage's anti-inflammatory, anti-pyretic, analgesic, and antioxidant qualities. In traditional medicine, cabbage is frequently used to treat small wounds and wounds, mastitis, and symptoms related to gastrointestinal problems (e.g., gastritis, peptic and duodenal ulcers, irritable bowel .The center of origin of cabbage is most often considered to be the coastal areas of the Mediterranean, the British Isles, and western Europe. Cabbage is thought to have been first grown by the Greeks for medicinal purposes over 3,000 years ago. syndrome).

Botanical description

Brassica oleracea is a valuable garden and commercial crop as it is easy to grow, transport and store

Cultivation

- Elevation: grows best in highlands above 800 m (2624 ft)
- Rainfall: 380-500 mm (15-30 in); a shallow root system needs moisture throughout its growing season. Heavy mulch is recommended to retain soil moisture.
- Soil: Grows best in soil with high organic content, pH of 6.5-7.5. Acid soils need lime prior to planting.
- Temperature: Best head formation at average daily temperatures of 15-20° C (59-68° F) with night temperatures at least 5° C (41° F) or lower; light frost is tolerated. Heat may cause heads to split.

Harvesting and Seed Production

Mature cabbage plants can remain in the ground, covered by leaves or straw until after frost but before a hard freeze. Pull up the whole cabbage plant, stack heads close together, bottoms-up. Cover plants with a 7.5 cm (3 in) layer of fresh, banana leaves and 20 – 25 cm (8-10 in) of soil. Water the pit lightly twice a week. Cabbage can be kept for 6 months. Cabbage rarely sets seed in the tropics as low temperatures are required for many weeks. Seed stalks may be formed the second season when temperatures between the seasons are below 10° C (50° F) for 30 days or 15° C (60° F) for 60 days.Harvest the dry pods, hang in the shade, over a cloth for one week. After winnowing, seeds will store for 4-6 years in dry, cool conditions.

Taxonnomy

Kingdom	Plantae
Phylum	Angiosperms
Class	Magnoliopsida
Order	Brassicales
Family	Brassicaceae
Genus	Brassica
Species	B. oleracea

Table: 1-taxonomy of Brassica oleracea

Macroscopy :

Color : white /Greenish

Taste :bitter

Odour : characteristic

Chemical constituents

Brassica oleracea contains abundant polyphenols, flavonoids, anthocyanins, glucosinolates, fiber, and vitamins . Among these, phenolic acids and flavonoids possess important health benefits, including anticancer and antioxidant activities. In addition to phenolic acids and flavonoids, structurally and functionally diverse glucosinolates and isothiocyanates are abundant in cabbage. Brassica plants are the rich source of phytochemical compounds of medicinal importance. The bioactive phytochemical compounds commonly found in most of the Brassica species include polyphenols, phenolic acids, flavonoids, carotenoids (zeaxanthin, lutein, β-carotene), alkaloids, tannins, saponins, anthocyanins, phytosterols chlorophyll, glucosinolates, phytosteroids, terpenoids, glycosides, vitamin C, Vitamin E and aliphatic and aromatic amines. Due to the presence of these compounds, Brassica plants show biological activities against various diseases and have been found to effective in treating various diseases in human. The pure compounds of biomolecules present in Brassica that many of them (as glucosinolates, isothiocyanates, hydroxycinnamic acids, flavonols and anthocyanins) showed high anti-inflammatory activities reducing the production of the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β).

Therapeutic uses

Antidiabetic

Anticholesterolemic

Anticancer

Antihypertensive

Antioxidant

Antibacterial

Antiobesity

Anticoagulant

Hepatoprotective

MATERIAL AND METHOD

Methanol:

Methanol (also called methyl alcohol and wood spirit, amongst other names) is an organic chemical and the simplest aliphatic alcohol, with the formula CH3OH (a methyl group linked to a hydroxyl group. It is a light, volatile, colourless and flammable liquid with a distinctive alcoholic odour similar to that of ethanol. Completely mixes with water. Methanol is a colourless liquid that boils at 64.96 °C (148.93 °F) and solidifies at −93.9 °C (−137 °F)

Molecular formula: CH3 OH

Synonyms: Methanol, Methyl alcohol

Molecular weight: 32.042

Appearance: Colourless liquid

Melting point: -97.6 °C

Uses: It is used as a chemical building block for various everyday products such as plastics, beans, car parts and construction materials. Given its property as a clean energy resource it is used to fuel cars, trucks, ships, boilers and cook stoves etc.

Fig:4 Methanol

Cetostearyl Alcohol:

Stearyl alcohol, or 1-octadecanol, is an organic compound classified as a saturated fatty alcohol with the formula CH3 (CH2)16CH2OH. It takes the form of white granules or flakes, which are insoluble in water. It has a wide range of uses as an resins, perfumes, and cosmetics. ingredient in lubricants, Stearyl heptanoate, the ester of stearyl alcohol and heptanoic acid (enanthic acid), is found in most cosmetic eyeliners.19

Molecular formula: CH3 (CH2)17OH or $C_{18}H_{38}O$

Synonyms: octadecanol, stearyl alcohol

Molecular weight: 270.5

Appearance: White solid Odor: a faint, characteristic sweet odor

Melting point: 55 - 60 °C

Uses: uses as an ingredient in lubricants, resins, perfumes, and cosmetics. It is used as an emollient, emulsifier, and thickener in ointments, and is widely used as a hair coating in shampoos and hair conditioners.

Fig:5 Cetostearyl alcohol

Hard Paraffin :

White wax is like yellow wax that has been bleached by being rolled thinly and exposed to light and air, or bleached by chemical oxidants. Like yellow wax, white wax is also used in preparing ointments, cerates, plasters, and suppositories. Most waxes used in the pharmaceutical industry are also used for tablet coatings and binding. In cosmetics/personal care and pharmaceuticals, our products are used in skin creams, lotions, pastes, ointments and pharmaceutical base creams. Paraffin wax is mostly found as a white, odorless, tasteless, waxy solid.

Chemical formula: CnH2n+2

Synonyms: white beeswax

Appearance:: White solid

Odor: odorless

Melting point: 48° to 66° C

Uses: used as thickeners, emulsifiers, and as stiffening agents in cosmetics. Beeswax is a skincare ingredient with natural moisturizing properties and waxy texture.

Fig:6 Hard paraffin wax

Rose Water:

Rose water also has anti-inflammatory properties, which means it can be put on the skin to soothe the irritation caused by conditions. The antioxidants in rose water protect the cells in the skin against damage. The antibacterial properties may help reduce acne. Because of this, rose water is often included in a variety of natural and medicinal treatments. Rosewater contains about 10%-50% of rose oil, one of the major by-products obtained by distilling rose flowers. It has anti-inflammatory, antimicrobial, anti-anxiety, and antioxidant properties.

Biological name: Rosa sinuses

Kingdom: Rosaceous

Order: Rosales

Appearance: clear liquid

Uses: Soothes skin irritation, Soothes sore throats, Reduces skin redness, Helps prevent and treats infections.

Fig:7 Rose water

Wool fat

Lanolin , purified form of wool grease or wool wax used either alone or with soft paraffin or other fat as a base for ointments, emollients, skin foods, super fatted soap , and for dressing . it is used by humans comes from domestics sheep breeds that are raised specifically for their wool . lanolin waterproofing property aids sheep in shedding water from their coats .

Synonyms: Anhydrous lanolin , hydrous wool fat , lanolin

Chemical formula: C34H68O2

Melting point: 34° to 44° C

Odor: faint and characteristic

Colour : whitish yellow

Uses: as water absorbable ointment base .

As common ingredient and base for water soluble cream and cosmetics . can be allergic also .

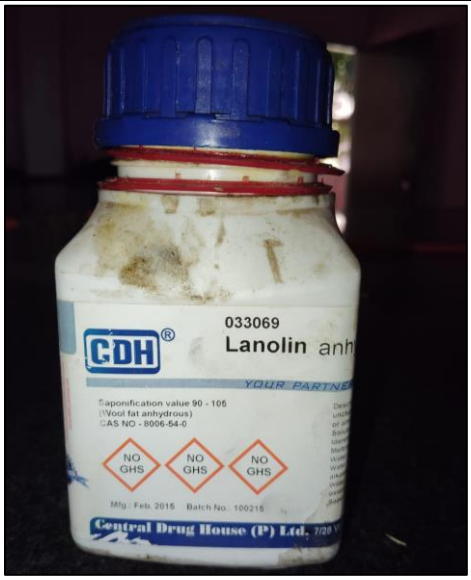

fig : 8 Wool fat (Lanoline)

White soft paraffine

It a homogenous mixtures of oily and waxy long chain , non polar hydrocarbon . odourless and tasteless, colour in white . This is a purified mixture of semisolid hydrocarbons that are derived from petroleum. White soft paraffin consist of microcrystals embedded in a gel composed of liquid and amorphous hydrocarbons that are themselves dispersed in a gel phase containing liquid and amorphous hydrocarbon.

Synonyms : White petroleum jelly, Vaseline.

Melting point: 38° to 56° C

Chemical formula: CnH2n+2

Appearance: White solid

Odour: Odourless

Uses: Acting to instantly sooth and soften dry, peeling and chapped skin.

Used as a barrier cream by providing a layer of oil on the surface of the skin to prevent water evaporating from the skin surface.

It is an emollient, some time known as skin lubricant. It is used to soothe, smooth and hydrate the skin.

Fig :9 White soft paraffin

EXPERIMENTAL WORK:

1) Preparation of anti-inflamatory ointment by using herbal extract of Brassica oleracea.

Table: 2 Ingredient

Sr.no	Ingredient	Qty. taken in (gm)	Role of ingredient
1)	Herbal extract	0.5	Active pharmaceutical ingredient
2)	Wool fat	1.17	emollient
3)	Hard paraffin	1.17	emollient
4)	Cetostearyl alcohol	1.17	Emulsifying agent
5)	Rose water	Qty sufficient	Perfume/ fragrance
6)	White soft paraffin	20	Ointment base

2)Preparation of herbal extract

a) Soxhlet extraction

soxhlet extraction is a continuous process of extraction with a hot organic solvent. it allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.it was invented in 1879 by a scientist named FRANZ VON SOXHLET. it was first invented for the Quantification of fat in milk.

A Soxhlet extractor has three main sections:

1)percolator (boiler and reflux) which circulates the solvent.

2)Thimble (made of thick filter paper) which retains the solid to be extracted,

3)Siphon mechanism which periodically empties the thimble.

Fig :10 Soxhlet apparatus.

Procedure

Solid material placed in a thimble.

Soxhlet extractor is placed onto a flask containing the extraction solvent.

Soxhlet equipped with a condenser.

The solvent is heated to reflux.

The solvent vapours travels up a distillation arm and floods into the chamber housing the thimble of solid.

Solid material in chamber slowly fills warm solvent.

When a soxhlet chamber is almost full, the chamber is emptied by the siphon. the solvent running back down to distillation flask.

This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the compound is concentrated in distillation flask.

After many cycle (72 hours) the desired compound is concentrated in distillation flask.

After extraction the solvent is removed yielding the extracted compound. the non-soluble portion of the extracted solid remain in the thimble, and is usually discarded.

Fig :11 Extract of Brassica Oleracea

Fig: 12 Filtrate of Brassica Oleracea

Procedure of ointment:

Collect the all ingredient and calibrate analytical balance.

Then weigh all bases as mention in the formula.

After that mixed all bases with gentle heat and prepare uniform mixture.

The extract was mixed with above base mixture by the process called levigation.

Prepare uniform mixture of extract and base.

Fill the above preparation in container.

Formulated Ointment

EVALUATION TEST

Organoleptic evaluation

Appearance, colour, odour texture and nature of the herbal ointment was evaluated by application of the ointment on the healthy skin.

Parameter	Observation
Appearance	Semisolid and smooth
Colour	Yellowish to brownish
Odour	Aromatic
Texture	Sticky
Nature	Soft fresh, clean from dirt

Table:3 Organoleptic evaluation

Physicochemical evaluation

Smoothness

Smoothness test was carried out by applying ointment formulation by topically on skin surface it gives soothing effect.

pH

For determine pH of antimicrobial ointment formulation a pH meter is used. Put 50.0 ml water in a beaker and dissolved the weighed amount of ointment (5 g) in it and its pH was measured.

Water solubility

If the contents should be soluble in 9 parts of water and in 1.7 parts of hot water then it's soluble in water and consistency was maintained.

It contains parameter such as Smoothness, pH, Water solubility, Alcohol solubility, etc.

Parameter	Obeservation
Smoothness	Smoother
pH	5.7
Water solubility	Soluble
Alcohol solubility	Soluble

Table:4 Physicochemical evaluation

Skin Irritancy Test:

There was no irritation was observed when the formulation was applied on skin. This formulation is very safe to use with no irritation as well as no side effect to skin is produced and is suitable to all type of skin.

Parameter	Obeservation
Irritation	NIL
Redness	NIL
Swelling	NIL
Photo Irritation	NO Irritation

Table:5 Irritancy test

OBSERVATION & RESULT

Result

Sr.No	Evaluation Parameter	Observations
1.	Organoleptic Properties	
	Appearance	Semisolid & Smooth
	Colour	Brownish
	Odour	Aromatic
	Texture	Sticky
	Nature	Soft fresh, Clean from dirt
2.	pH	5.7
3.	Smoothness	Smoother
4.	Skin irritancy Test	No irritation

Discussion

The main goal of this study was to formulate a simple, effective, compatible, and inexpensive Anti-inflammatory ointment from brassica oleracea. The pure compounds of biomolecules present in Brassica that many of them (as glucosinolates, isothiocyanates, hydroxycinnamic acids, flavonols and anthocyanins) showed high anti-inflammatory activities reducing the production of the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β).

CONCLUSION

The study proposed that anti-inflammatory effect of anthocyanine is present in *Brassica* vegetables at lower concentrations.

Natural preparation are prepared and more acceptable in recent forms which are mostly recommended as having less side effect and safe dosage form. The idea to be submerging this formulation was its antimicrobial property. Herbal ointment is used for the stimulation of healing inflammation , for giving natural heal and provide antiinflammatory effect with topical use.

In this context, Brassicaceae, already demonstrated to reduce cancer risk, cardiovascular disorders and diabetes , could represent a good support in IBD treatment, being an excellent sources of bioactive metabolites with high health-promoting benefits. Indeed, based on the various in vivo and in vitro studies, Brassica vegetables and other cruciferous foods have relevance in planning dietary recommendations for the prevention or relief of the pathological conditions due to chronic inflammatory diseases. Further studies should be addressed to investigate the differences among individuals, in terms as intestinal absorption, metabolism, age, gender and lifestyles, which can affect the beneficial proprieties. Moreover, although some patients suffering from Crohn's disease can tolerate different Brassicaceae vegetables, they are in generally avoided as they are believed to worsen their symptoms.

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