

FORMULATION OF BIOACTIVE OINTMENT FOR PSORIASIS MANAGEMENT

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ABSTRACT

Background: Herbal medicine is the oldest kind of health care known to humanity. The skin is an important organ of communication with the external world and has a close interaction with the mind. Psoriasis is a persistent skin disease and one of the most common dermatological disorders. Plant-based alternatives are highlighting the need for safety. *Cleome viscosa* originates from the Capparadiceae family. It is commonly called as wild or Dog mustard. The plants parts are used to treat various illnesses in traditional system.

Objective: To formulate and develop bioactive ointment using *C. viscosa* seed oil for its potential psoriasis management.

Methodology: The plant seed was collected, extracted using n-hexane and the phytoconstituents were identified and analyzed using Gas Chromatography-Mass Spectrometry (GCMS) and its safety efficacy was tested by MTT cytotoxicity assay using Human melanoma A375 cells. Then the extracted oil was incorporated into an ointment base and then evaluated.

Results: Extracted oil using n-hexane shows a high yield. GC-MS analysis showed the presence of 13 bioactive compounds with its anti-inflammatory properties. Cytotoxicity testing showed that the oil was safe. The formulated ointment exhibited with a texture of smooth spreadability, homogeneity and a favorable pH value of 5.4 which falls within the range for topical application.

Conclusion: The study successfully formulated a bioactive ointment incorporating the

C. viscosa seed oil. This study paves the way for future investigation, including in vivo, in vitro assays, animal models, and clinical trials to establish the therapeutic effects and safety.

Keywords: Psoriasis, bioactive ointment, seed oil, GC-MS, cytotoxicity.

I. INTRODUCTION

Numerous natural medicinal herbs can be utilized to treat a wide range of skin disorders (Rout et al., 2017). Psoriasis, a common skin illness, is a chronic inflammatory skin disease that is mediated by the immune system. It forms thick, silvery-scaled skin patches. Millions of individuals worldwide suffer from the common ailment known as psoriasis (Gazi et al., 2012). Psoriasis is a skin condition that affects up to 2.5% of the global population, making it one of the most common dermatologic diseases (Singh et al., 2015). Skin inflammation caused by psoriasis is a chronic illness that has detrimental effects on a person's physical, mental, and social well-being (Mermin et al., 2016). Psoriasis is a prevalent, long-lasting skin condition characterized by the development of clearly defined, scaly, red plaques (Kimmel and Lebwohl., 2018). Typically, patients with psoriasis have distinct, long-lasting erythematous plaques on their knees, elbows, scalp, umbilicus, and lower back that are covered in silvery white scales (Owen et al., 1996).

In psoriasis, the skin's life cycle is shortened to 1.5 - 3 days, whereas normal skin takes 30 days to mature (Menter et al., 2018). Although psoriasis is thought to be a non-life-threatening condition, it still costs the healthcare system a fortune and causes social hardship for individuals. An important factor in the pathophysiology of psoriasis is inflammation (Krueger., 2002). Red, flaky patches of skin covered in silvery scales and discomfort are the hallmarks of psoriasis. Genital sores, cracked skin, small scaling patches, itching, soreness, pitted or swollen nails, stiff, inflammatory joints, and an abundance of dandruff on the scalp are further indications of psoriasis (Rapalli et al., 2020). Psoriasis is caused by many factors such as stress, skin injuries, adverse weather, strep throat, excessive alcohol use, irregular hormone levels, and smoking. a wound, scrape,

sunburn, or insect bite that affects the skin. prescribed drugs, such as antibiotics, lithium, NSAIDS, an antimalarial drug, and other tranquilizers (Salunke et al., 2023).

C. viscosa, referred to as "wild or dog mustard," locally known as "Jakhiya," is an annual, sticky plant that is a prevalent weed throughout the tropical regions of the world and the plains of India. It is a member of the Capparaceae family (Thatte and Dahanukar., 1986) The plant is more widely distributed and is frequently found growing as a weed in Northern, Southern, and Central India (Chatterjee., 1991). All plant parts, including leaves, roots, and seeds, are frequently utilized for their traditional and folk medicinal uses (Mali.,2010). Fever and diarrhea can be treated using *C. viscosa* seeds (Chopra and Nayar., 1956). For mental sickness and infantile seizures, the rural people consume the fresh juice of the crushed seeds of this plant (Nadkarni and Nadkarni., 1976). The *C. viscosa* seed's protein, amino acid, vitamin, mineral, fatty acid, and taxicol content were all investigated. Seeds have thioglycosylation in them (Olatunji et al., 2005) (Wake et al.,2011). It has been found that providing *C. viscosa* seeds orally and intraperitoneally to rats, mice, and Guinea pigs does not pose any health risks. The main 12 ingredients in the aerial parts of the seeds were monoterpene hydrocarbons and some oxygenated derivatives (Singh and West., 1991). Moreover, the seeds contain viscosain and viscousaic acid. *C. viscosa* seed oil is high in linoleic acid. Studies have shown that the fatty components of *C. viscosa* edible seed oil are stearic acid, palmitic acid, oleic linoleic acid, and palmitic acid (Rukmini.,1978). Salicyclic acid and luperol are also present in the seed (Ahmad and Malik., 2022)

Research on pharmacology has revealed that *C. viscosa* has biological activities that are noteworthy. These includes activities such as antibacterial, analgesic, immunomodulatory, antipyretic, and hepatoprotective (Mali.,2010) (Zimmerman.,1982) hepatobiliary (Higa et al.,2000), Antioxidant (Pillai and Nair., 2013)(Prakash et al.,2018) antidiarrheal (Devi et al., 2003) antiemetic (Ahmed et al.,2011), anticancer (Govindan et al.,2018) (Yerragunta et al., 2012), antifibrotic (Kumar et al.,2009) antiulcer (Lalita et al., 2013), antimicrobial (Kavitha et al., 2010), anti-inflammatory (Senthamilselvi et al.,2012) , Central Nervous System Depressant (Takagi et al., 1971), antidiabetic (Suresh et al., 2020), wound healing (Hsu., 200).

The primary objective of this study was to formulate a bioactive ointment of *C.viscosa* Linn seeds extract for advancing natural-based therapies for psoriasis management.

II. MATERIALS AND METHODS

Collection of the plant

The entire plant sample were collected in and around Mettuplayam, Tamilnadu, India. The taxonomical identification of the plant was done by Dr B.D Sheeja., Taxonomist, Department of Botany, Government Arts College, Ooty. The herbarium voucher was submitted and deposited in School of Life Sciences (Ooty Campus), JSSAHER Library. The whole Plant was dried and the pods were isolated, dried under sunlight, then dehusked and dehulled. The seeds were separated according to color and appearance and then the seeds were powdered in the grinder to a coarse powder. Finally, the powder was preserved at room temperature.

Preparation Of the Extract

The powdered plant material was extracted using n-hexane solvent (40-60) in a Soxhlet extractor, 200g of powdered *C. viscosa* seeds were extracted for 15 hours. Solvent was extracted at lower pressure. 4 milliliters of unrefined n-hexane were separated via TC. An oily, yellowish compound known as SR was obtained from the n-hexane extraction process and constituted 0.12% w/w of the fresh plant material. TLC analysis was used to look at various bioactive ingredients in the n- hexane extract of *C. viscosa*. The crude oil was extracted and stored for further analysis.

Phytochemical Screening

GC-MS Analysis (Susikumar et al., 2021)

The equipment expresses its GC/MS Clarus 500 Perkin Elmer by mass spectrometer interfacing and a GC. This is also part of the specifications. The device used 70 eV electron impact mode, the carrier gas was helium with purity of 99.999% at a flow rate of 1 ml/min, the split ratio for this work stands at 10:1. When using the injector device, we employed an injected volume of 10 µl once upon a time during this work. The temperature of ionization source was set at two hundred degrees centigrade but the injector's temperature increased to two hundred and ninety degrees Celsius. Electronic thermostat initiated heating process at fifty degrees celsius rising eighty-seven degrees celsius in one minute until it reached two hundred and twenty degrees Celsius at which point it stayed five minutes and finally rose to two hundred and eighty degrees Celsius in eight minutes.

In the present, GCMS analysis was performed on n-hexane fraction extracted from seeds of *C. viscosa*. In GC analysis of the *C. viscosa* volatiles packed column with 15% SE-32 on Chromosorb HP 80/100 with temperature programmer, the following thirteen components were found: The major component which contributed up to 55% of the extract was identified eluting at 46 mins in RT at high temperature condition minute retention duration.

Mass spectra of fragment ions range from 40 to 600 Da were obtained at 70 eV, with a 0.2 second scan interval. The major component, 56.4% of the sample, had a retention time (RT) of 54.73 minutes and eluted at a high temperature. In the investigation of the minor constituents, linalool, was discovered to make up about 3% of the extract and had a 13.5- minute retention duration. Four other compounds were present in modest amounts, with the remaining components being negligible. The absence of trustworthy reference samples prevented the positive identification of the other components.

Cytotoxicity Analysis (MTT Assay)

TRIzol reagent was purchased from Sigma Aldrich Chemicals, United States of America. DMEM (Dulbecco's Modified Eagle Medium), FBS (Fetal Bovine Serum), MTT, and primer were obtained from GIBCO-BRL, USA, Himedia, India, Biogene, India, respectively. The experiments involved the use of analytical grade chemicals and reagents, as stated in

other sections. Test drug QKP 23, formulated as an aqueous gel developed according to ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines, was dissolved in 0.1% Dimethyl sulfoxide (DMSO) for the study. This result was in a good agreement with the dissolution of other compounds in DMSO with medium solubility, as well as with slight differences that may be caused by the fact that every compound was unique, and its solubility may be influenced by certain factors.

Maintenance of cell lines

Human melanoma A375 Primary mouse embryonic fibroblast (MEF) cells were provided by National Centre for Cell Science (NCCS) Pune, India. The cells were cultured, passage in high glucose DMEM containing 10% heat inactivated FBS, 100IU/ml of penicillin, 100µg/ml of streptomycin. These cells were then moved to 75 cm³ culture flask and cultured with some medium for several days at 37°C in CO incubator with CO₂ at a concentration of 5%.

Procedure

Primary MEFs were obtained from NCCS, Pune, India. The cells were cultured and passage in high glucose DMEM containing 10% heat inactivated FBS, 100IU/ml of penicillin and 100µg of streptomycin. Such cells were then transferred to 75 cm³ culture flask and maintained in a number of the medium for several days in CO incubator at 37°C with 5% CO₂.

Preparation of Ointment Formulation

Oil in water (o/w) emulsion consisted of stearic acid, Cetyl Alcohol, Isopropyl Myristate, Liquid paraffin, Quercetin, Seed oil Extract as shown in the figure 3A was dissolved that is heated to 75°C Oil phase. Borax, Methyl paraben, Quercetin and beetroot powder were dissolved in the aqueous phase (B) shown in the table 3 and figure 3B as shown in the table 3 and heated about 75°C.

Phase B was then added with stirring into phase A at intervals. It was observed that at relatively high concentrations of both the aqueous phase and the oil phase a very stable emulsion was obtained whereas when both the water and the oil phase being in relatively lower concentration an unstable emulsion was formed. In case of color purpose, the time required is 90 minutes and the value of 0.3 g of beetroot powder was added while stirring the mixture using a magnetic stirrer to attain color till emulsion become cool.

Evaluation Of Ointment

All the prepared ointments were characterized for the parameters such as appearance, odor, color, homogeneity, pH, Spreadability, washability.

Organoleptic characteristics

The formulated ointment was tested for physical appearance, color, texture, smell, state and homogeneity. These characters were evaluated by visual observation. Homogeneity and texture were tested by pressing a small quantity of the formulated ointment between the thumb and the index finger. The consistency of the formulations and the presence of coarse particles were used to evaluate the texture and homogeneity of the formulations.

pH

About 0.5 g of the formulatated ointment were taken in dry beaker and 45 ml of water was added. Beaker containing ointments was heated on water bath at 60–70°C. The pH of ointments determined using a pH meter.

Washability

Small amount of ointment was applied onto a hand and rubbed until it spreads and run a cold tap water over it again.

Spreadability

Two glass slides were used to measure the spreadability of the ointment. 1g of ointment awas sandwiched between the glass slides. A 1kg weight was placed at the top of the glass

slides for 5mins to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The spreadability was observed and the time was noted. Spreadability was calculated using the following formula.

$$S = M \times L / T$$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide and

T = Time (in seconds) taken to separate the slide completely each other

III. RESULTS AND DISCUSSION

GC-MS Analysis

Mass spectra of fragment ions range from 40 to 600 Da were obtained at 70 eV, with a 0.2 second scan interval. The major component, 56.4% of the sample, had a retention time (RT) of 54.73 minutes and eluted at a high temperature. In the investigation of the minor constituents, linalool, was discovered to make up about 3% of the extract and had a 13.5- minute retention duration. Four other compounds were present in modest amounts, with the remaining components being negligible. The absence of trustworthy reference samples prevented the positive identification of the other components.

These compounds were confirmed quantitatively and qualitatively using the GCMS by determining their peak area and the time they eluted. Altogether, a total of 13 compounds were found to be present of which flavone (8.3%), E,E,6,8 Tridecadien-2 ol-acetate (11.2%), 31 Methyl ester of 3,7,11,15-tetramethyl hexadecanoic acid, methyl ester (28.5%), Pentadecanoic acid, 14 methyl- methyl ester (40%), phytol (52%), Phenol,2,4,6-

trimethyl, (1.57%), Bicyclo (3,1,1), heptane,2,6,6-trimethyl (0.21%), Humulane-1,6-diene-3-ol, (0.58%), Octadecanal (0.62%), 1-(1 Butynyl) cyclopentanol (13.47%), Urs-12-en-28-al (17.58%), Linalool (11.21%) .The retention time, molecular weight and composition percentage of the sample materials of *C.viscosa* were recorded in Table 1.

Among these 13 compounds the major peak was obtained for the compound is Linalool ($C_{10}H_{18}O$) in the retention time of 28.5 as shown in the figure 1. The linalool through in vitro and in vivo investigations in health science in the last five years has proved that the compound is not toxic in its pure form. Linalool is another monoterpene compound found in the essential oil of several aromatic species under investigation (Pereira et al., 2018). Also, it contains attributes that are analgesic, anti-inflammatory, and sunscreen in nature, particularly against Ultraviolet (UV) lesions on the skin (Tsai et al., 2023). Aside from it being an antioxidant compound and being effective against cancer cells, linalool also showed antifungal and antibacterial activity (Herman et al., 2016).

Cytotoxicity Testing (MTT Assay)

To evaluate the cytotoxic effects of the sample on normal cell lines, various concentrations were tested against human melanoma cell lines. The results showed in the figure 2, that at all these concentrations they observed lack of cytotoxicity effect using the MTT assay. It also does not trigger any cell mortality to a high level. when the cells were exposed to the sample throughout the experimental duration. This suggests that the sample does not exhibit harmful effects on the viability of normal human melanoma cells, thereby confirming its non-toxic nature under the conditions tested and IC_{50} value were represented in Table 2. These findings are crucial for further development and potential therapeutic applications of the sample.

Preparation of ointment formulation

The final product ointment was formulated and exhibited a pinkish color, smooth consistency, and homogeneous appearance without phase separation as shown in the Figure 4. The soft texture facilitates easy application over psoriatic plaques without causing any irritation. The pinkish hue may enhance patient acceptability by masking lesion discoloration.

Evaluation of ointment

The formulated ointment was physically evaluated for color, odor, smell, and State in the Table 4.

pH

The formulated ointment pH was found to be 5.4. This falls in the normal pH range of the skin.

Washability

The formulated ointment was smeared onto a hand and then washed with normal water to conduct the washability test. It rinsed off easily.

Spreadability

In this study, the ointment found to have good spread ability in the range of 6.5 – 7.5. The values of spread ability indicate that the ointment was easily spreadable by small amount of shear.

IV. CONCLUSION

The current study effectively created a pink topical ointment with *Cleome viscosa* seed oil for possible psoriatic symptoms. The GC-MS analysis, of the oil included bioactive components such [Linalool, Humulane-1,6-diene-3-ol, octadecanal etc], many of which are said to have antioxidant, anti-inflammatory, and skin barrier-protective qualities. High cell viability across tested concentrations was confirmed by the MTT assay results on the [A375 Cell line], indicating a good safety profile for topical application. A physiologically

suitable pH of 5.4 was demonstrated by the prepared ointment, suggesting a low risk of skin irritation and appropriateness for long-term topical use. Together, these results imply that the ointment has prospective qualities for treating psoriatic symptoms by combining low cytotoxicity, therapeutic phytochemicals, and suitable physicochemical properties. However future research including, stability studies, testing for in vitro anti-psoriatic efficacy, and clinical trials to validate its efficacy.

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Tables

Table 1: *Phytochemical constituents identified in Cleome viscosa seed oil using GC-MS. These compounds are primarily associated with anti-inflammatory and antioxidant properties, which may contribute to the oil's potential anti-psoriatic effect.*

Peak	Retention Time	Area (%)	Name of the compound	Molecular Formula	Molecular weight (g/mol)
1	13.5	8.3	Flavone	$C_{15}H_{10}O_2$	215.62
2	15.4	11.2	E, E,6,8 Tridecadien-2-ol-acetate	$C_{16}H_{28}O_2$	252.39
3	16.8	28.5	11 Hexadecanoic, methyl ester	$C_{17}H_{32}O_2$	274.32
4	17.9	40	Pentadecanoic acid, 14 methyl methyl esters	$C_{17}H_{34}O_2$	278.54
5	19.25	52	Phytol	$C_{20}H_{40}O$	296.78
6	21.2	7.2	Methyl ester 3,7,11,15-Tetramethylhexadecanoic Acid	$C_{21}H_{42}O_2$	326.24
7	15.75	1.57	Phenol,2,4,6-trimethyl	$C_9H_{12}O$	136.19
8	16.67	0.21	Bicyclo(3,1,1)heptane,2,6,6-trimethyl	$C_{10}H_{18}$	138.24
9	33.09	0.58	Humulane-1,6-diene-3-ol	$C_{15}H_{26}O$	222.36
10	22.13	0.62	Octadecanal	$C_{18}H_{36}O$	268.48
11	32.28	13.47	1-(1, Butynyl) Cyclopentanol	$C_9H_{14}O$	138.21

12	32.39	17.58	Urs-12-en-28-al	$C_{35}H_{50}O_3$	482.73
13	28.57	11.21	Linalool	$C_{10}H_{18}O$	154.25

Table 2: IC_{50} value of *Cleome viscosa* seed oil on A375 human melanoma cells as determined by MTT assay

Sample	Cell line	Assay used	IC_{50} ($\mu\text{g/mL}$)
<i>C.viscosa</i> seed oil	A375 (Melanoma)	MTT Assay	58.52 ± 4.25

Table 3: The formulated bioactive ointment, organized into oil and aqueous phases.

Phase	Component	Quantity
Oil Phase	Stearic Acid	10 g
	Cetyl Alcohol	2 g
	Isopropyl Myristate	2 ml
	Liquid Paraffin	5 ml
	Quercetin	50 μg
	<i>C.viscosa</i> Seed Oil	3 ml
Aqueous Phase	Borax	2 g
	Methyl Paraben	0.10 g
	Beetroot Powder	0.3 g

Table 4: The formulated ointment demonstrates consistent physical properties of the final product.

Parameters	Formulated Ointment
Color	Pink

Odor	Pleasant
Texture	Smooth
State	Semi- solid
Homogeneity	Homogenous

FIGURES

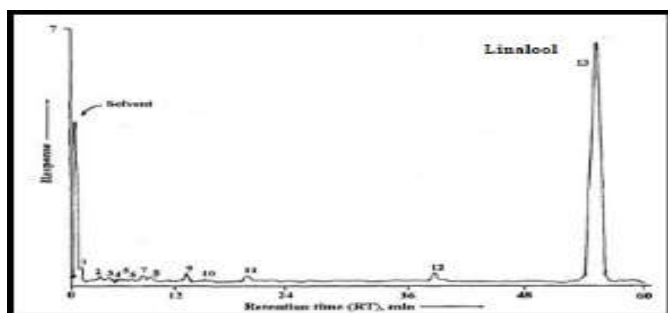


Figure 1: GCMS of the n-hexane extract seeds of the plant *Cleome viscosa* Linn



Figure 2: Cytotoxic effect of seed oil extract of *C. viscosa* at different concentration in A375 cells in terms of percentage viability after exposure as determined through MTT assay.



Figure 3A: Oil Phase



Figure 3B : Aqueous Phase

Visual representation of the oil (Fig 3A) and aqueous (Fig 3B) phases before emulsification. The separation of phases highlights the immiscibility prior to homogenization.



Figure 4: Formulated ointment containing C.viscosa seed oil showing a smooth, homogeneous texture with a characteristic pink color. The uniform appearance indicates successful incorporation of the oil into the ointment base.