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Pharmacological and phytochemical screening of *Murraya koenigii* oil base herbal cream

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Abstract: *This study explores the pharmacological and phytochemical properties of a topical herbal cream formulated with essential oil extracted from* Murraya koenigii *(curry leaves). Using hydrodistillation, essential oil was obtained from leaves collected in Dehradun, India, and subsequently incorporated into a cream base. Phytochemical screening revealed the presence of key bioactive compounds such as alkaloids, flavonoids, phenolics, and terpenoids. Gas Chromatography and Mass Spectrometry (GC-MS) analysis confirmed the presence of major components like sabinene, β-pinene, and limonene. The antifungal activity of the cream was evaluated against* Candida albicans *and* Aspergillus brasiliensis *using the agar well diffusion method, demonstrating notable zones of inhibition. These findings suggest that the formulated cream possesses significant antifungal properties and could serve as a promising natural alternative to conventional topical agents. Further clinical studies are recommended to confirm its therapeutic potential and safety.*

INTRODUCTION

Infectious diseases are a major global health concern and are among the leading causes of illness and death (World Health Organization, 1998). In recent years, fungal infections have become more common, especially among people with weakened immune systems—such as those who have had organ transplants, or are living with cancer or HIV/AIDS.This growing problem, along with increasing resistance to existing antifungal drugs and the harmful side effects of long-term treatment, has led researchersto search for new and safer medicines to treat fungal infections (Giordani et al., 2001).[1] Superficial fungal infections happen when certain types of fungi grow on the surface of the body, especially in areas like the skin, hair, and nails. These fungi usually affect the outermost layers, where there is dead skin or keratin. Some of the main fungi responsible include dermatophytes, Candida, Malassezia, Trichosporon, and Hortae species. Dermatophytes are the most common group and can live on the skin, nails, and hair. They don't go deep into the body—they stick to the outer, non-living parts. When they infect the skin, it's called epidermomycosis; when they affect the hair, it's called trichomycosis; and when they infect the nails, it's called onychomycosis. There are three main types of dermatophytes: Trichophyton, Microsporum, and Epidermophyton. Among them, Trichophyton rubrum is the one that most often causes skin and nail infections. Candida usually grows in warm, moist areas of the body, while Malassezia needs a moist and oily environment to grow. Each of these fungi prefers certain conditions and parts of the body, but they all stay on the surface without going deeper into tissues.[2,3,4]

Fungal infections, also known as mycoses, happen when yeast or mold grows on or inside the body. These infections are most often seen on the skin or nails, but fungi can also cause problems in areas like the mouth, throat, lungs, urinary tract, and other parts of the body.When a fungal infection appears on the skin, it might look red, swollen, or bumpy, and sometimes it looks like a rash or a lump under the surface. If the infection is in the nails, the nails can change color, become yellow, brown, or white, and might also get thick or break easily. Infections in the mouth or throat may show up as white patches or a coating on the inside. Anyone can get a fungal infection, especially in areas of the body that stay warm and damp, or where there's a lot of rubbing or friction. People with certain health conditions are more likely to get these infections. For example, those with diabetes, poor blood flow, or weakened immune systems—such as people living with HIV, cancer, or those taking medicines that lower the immune system—are more at risk.While skin and nail infections caused by fungi are usually not dangerous, they can be more serious in people whose immune systems are not strong. In those cases, the infection can spread or become harder to treat. [5].

Anti-fungal activity:-

Curry leaves (Murraya koenigii) have shown strong antifungal properties in many studies. Extracts made using alcohol, methanol, and ethanol were tested on harmful fungi that affect both humans and crops. These extracts were found to fight fungi like *Candida tropicalis* and *Candida albicans*, which can cause

infections in people. They also worked against fungi such as *Aspergillus fumigatus*, *Aspergillus niger*, and *Microsporum gypseum*, which are linked to breathing and skin problems. The alcoholic extract was effective in stopping crop-damaging fungi like *Rhizoctonia solani* and *Colletotrichum falcatum*. Methanolic and ethanolic extracts also reduced the growth of *Rhizoctonia solani* and *Fusarium oxysporum*, which harm plant roots. Though the strength varied, all extracts showed some antifungal action. This means curry leaves could be useful in making natural antifungal products for farming or herbal medicine. More research is needed to find out which parts of the leaf give these benefits.[6]

Curry leaves contain natural compounds like P-gurjunene, P-caryophyllene, and alkaloids such as furoquinoline and carbazole, which have antioxidant, antimicrobial, and cholesterol-lowering effects. Essential oils from plants are rich in compounds like terpenes, phenols, and alcohols. They offer health benefits due to their antimicrobial and antioxidant properties. Natural essential oils are now preferred over synthetic ones like BHA and BHT, which may harm the liver. These oils are also used in food to reduce spoilage and extend shelf life.[7]

Murraya koenigii (L.) Spreng, commonly known as the curry leaf plant, is a small tree or shrub that belongs to the Rutaceae family. It can grow up to 6 meters tall. This plant is native to India, Sri Lanka, Pakistan, Bangladesh, and the Andaman Islands. It is also grown in many parts of Southeast Asia, the United States, and Australia. In tropical regions of Africa, such as Nigeria, Kenya, and Tanzania, as well as in several Indian Ocean islands, the plant is commonly found, especially in areas where Indian communities have settled. Curry leaves have been used in Indian cooking for centuries, not only for their flavor but also for their importance in traditional medicine.[8,9] Out of the 14 species of the Murraya genus found around the world, only two — *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack — are present in India. Among these, *M. koenigii* is more commonly used due to its wide range of medicinal benefits and its long-standing use as a flavoring agent in Indian cooking.[10] In traditional medicine, *M. koenigii* is considered to have many healing properties. It has been used as a pain reliever, cooling agent, anti-nausea remedy, and for treating intestinal worms, diarrhea, fever, digestive issues, and inflammation. It is also used in managing conditions like skin problems, blood disorders, piles, kidney pain, vomiting, and snakebites. In Ayurveda, its leaves are known to help in managing diabetes.[11] Studies have identified many active compounds in every part of the plant. The leaves are rich in a variety of phytochemicals such as phenols, alkaloids, flavonoids, saponins, tannins, steroids, quinones, proteins, carbohydrates, and essential oils. Carbazole alkaloids found in the plant are especially known for their potential to fight cancer, bacteria, fungi, and even viruses like HIV.[11,12]. The bark contains carbazole alkaloids like mukoenine-A, B, and C, murrastifoline-F, mahanimbine, and many others. The leaves are known to include compounds such as koenimbine, mahanine, isomahanine, girinimbine, and many more, which contribute to the plant's strong aroma and healing properties. These leaves are also a source of essential nutrients like protein, fiber, minerals, carotene, vitamin C, and nicotinic acid.[13] Roots and fruits also contain powerful natural compounds, including murrayanol, koenimbine, girinimbine, and others. Seeds are found to have furocoumarins and various minor compounds that also show health benefits.The main aromatic compounds in the leaves, responsible for their strong scent, include p-gurjunene, p-caryophyllene, p-elemene, and o-phellandrene. These essential oils are widely used in personal care and wellness products like soaps, perfumes, lotions, hair treatments, and aromatherapy items.[14,15] The plant's chemical makeup has shown it to possess several therapeutic effects such as anticancer, liver-protective, fever-reducing, antioxidant, anti-obesity, antimicrobial, antifungal, and insect-repelling properties. Studies have shown that the high antioxidant activity in curry leaves is mainly due to compounds like mahanimbine, murrayanol, and mahanine, which also help fight bacteria and fungi. This particular study focuses on analyzing the chemical composition of essential oils from *Murraya koenigii* leaves collected in Dehradun, Uttarakhand, and on testing their antioxidant and antifungal properties.[16,17]

Macroscopic Description:

Murraya koenigii is a shrub or small tree that usually grows between 2.5 to 6 meters in height and has a width of about 15 to 40 cm. It has a short trunk with smooth bark that can be grey or brown in color. The leaves of the plant are highly aromatic and about 30 cm long. They are made up of 9 to 25 small leaflets, arranged alternately, with visible net-like veins. The plant produces small, white, funnel-shaped flowers that have a pleasant fragrance and five petals. Its fruits grow in tight clusters and are small, round or oval in shape. These fruits have a thin outer skin and contain one or two seeds that are green, similar in color to spinach. Due to its strong aroma and fruit features, this plant is commonly considered a spice or herb and belongs to the Rutaceae family.[18,19]

MATERIAL AND METHOD:

Collection of plant: -

The plant Murraya koenigii, commonly called the curry leaf plant, is an important medicinal and culinary herb native to India. For the purpose of research and study, fresh samples of Murraya koenigii were collected from the region of Suddhowala and Koti, located in Dehradun, Uttarakhand. These areas are known for their rich biodiversity and healthy natural environment, which makes them ideal for collecting medicinal plant specimens. The collection was carried out carefully, ensuring that healthy and mature leaves were picked during their active growing season. The collected samples were then kept in clean,

labeled containers to avoid contamination or misidentification.

Figure 1: Curry leaves (Murraya koeingii or Bergera koenigii)

Extraction of **Murraya koenigii:-**

Extraction of essential oil from Murraya koenigii (commonly known as curry leaves) can be done using a method called hydrodistillation with the help of a Clevenger apparatus. This is a common and effective method to separate the oil from the leaves in a natural way using water and heat.

Hydro-distillation Extraction:-

Shade-dried curry leaves are cleaned to remove dust, then lightly crushed to increase surface area. All parts of the Clevenger apparatus are sterilized in a hot air oven at 80°C for 20 minutes.Weigh 30 g of prepared leaves and place them in a round-bottom flask (RBF) with 300 ml of distilled water. Apply petroleum jelly to the mouth of the chamber for a tight seal, then connect the RBF to the Clevenger apparatus. Attach the condenser and connect the inlet and outlet pipes.Heat the setup. As water boils, steam passes through the leaves, carrying essential oils. The steam-oil mixture condenses back to liquid, and the essential oil separates from water. After extraction, turn off the heat and let the system cool. Collect the oil in Eppendorf tubes and discard excess water.

Figure no. 2,3: Hydro-distillation Extraction

Formulation of cream:

A **cream** is a type of semi-solid product that is applied to the skin. It usually contains both oil and water and is used to moisturize, protect, or deliver medicine to the skin.

Creams are made by mixing two main parts:

Oil phase (contains oils, waxes, and emulsifiers)

Water phase (contains water and water-soluble ingredients)

These two components are joined using heat and mixing to create a smooth, stable product.

Figure no. 4 Formulation of cream

Selection of fungi: **Candida albicans**

Candida albicans is a fungus that normally lives in the human body, especially in moist areas like the mouth, gut, and vagina, without causing harm. But if the immune system is weak, it can cause infections called **candidiasis**. These can be mild, like mouth or vaginal infections, or serious, affecting the blood or organs. Candida can grow in two ways: as round yeast cells or as long thread-like forms called **hyphae**, which help it spread and infect more easily.[20,21]

Aspergillus brasiliensis:

Aspergillus brasiliensis is a fungus with black spores commonly found in the environment. It belongs to a large group of molds called **Aspergillus**, which includes many species. Some are useful in industry for making enzymes and acids. However, certain types can cause infections, especially in people with weak immune systems. This infection is called **aspergillosis**. A related fungus, **Aspergillus flavus**, can grow on food like peanuts and make dangerous toxins that may cause liver cancer.[22,23]

Phytochemical analysis: -

Phytochemical analysis is the process of studying the natural chemical compounds found in plants. These compounds are not essential nutrients like vitamins or minerals, but they often have health benefits. When scientists analyze *Murraya koenigii*, or curry leaves, they are looking to identify these special plant chemicals.

Phenolic compounds: These are strong antioxidants that support overall health.

Flavonoids: These are antioxidants that protect the body from damage caused by harmful molecules called free radicals.

Protein : Curry leaves have a good amount of plant-based protein. This protein can help in body repair and growth. Although not very high, the protein present is beneficial in a vegetarian diet.

Carbohydrates: Curry leaves also have carbohydrates, which give us energy. The amount is moderate, and these carbs are often in the form of natural sugars and fibers.

Amino Acids: Amino acids are the building blocks of protein. Some important amino acids found in curry leaves include: Leucine, Isoleucine, Valine

Alkaloids: These are natural compounds that may have pain-relieving or other medicinal effects.

Quantitative analysis:

S NO	RT	COMONENTS	IN%
1	9.11	α-pinene	1.62
2	9.63	Camphene	0.21
3	10.53	Sabinene	26.63
4	10.66	β-Pinene	12.02
5	12.58	Limonene	4.08
6	29.18	Trans-Caryophyllene	13.81
7	35.52	Caryophyllene oxide	5.21

Table no. 1

Culture Preparation: Freshly prepared slant of C. albicans & A. brasiliensis was used and washed the slant by using 10 mL of sterile Normal saline solution.

Method: Cylinder Plate Method:

Media Preparation: Sabroud Dextrose Agar (SDA) was used for determining the activity of C. albicans. Potato Dextrose Agar (PDA) was used for determining the activity of A. brasiliensis. Media was prepared

as per Manufacturer's Instruction. The media was then autoclaved at 121˚C temp. & 15lbs pressure for 20 minutes.

Standard Preparation:

Take 1 gm of Miconazole cream into 100 ml volumetric flask. Add 10 ml DMSO & 10 ml MeOH & sonicate the sample for 10 mins. Makeup the final volume 100 ml with Methanol. Mix it well & filter the sample by Whatman filter paper. Pipette accurately 2.5 ml of sample from filtrate & add into 100 ml sterile volumetric flask. Make up the final volume 100 ml with Methanol. Mix it well. Pipette accurately 1 ml of sample into 10 ml volumetric flask. Make up the final volume 10 ml with Methanol. Mix it well and used the final dilution as a standard for activity.

Sample Preparation:

Weight approx. 2gm sample in to 150 ml flask. Add 3 ml Dimethyl sulfoxide, 5 ml of methanol & 7 ml of water in it & sonicate it for 10 mins. After sonication, sample was refluxed on water bath at 90˚C for 1 hr. Filter the sample by Whatman filter paper & evaporate the samples up to 5 ml of sample remaining and used as a test sample for activity.

Testing Procedure:

Invitro Anti-Fungal activity:

Cool down sterile media SDA & PDA up to 55°C and add 10µl of yeast culture in to SDA flask. 10µl of fungal culture in to PDA flask. Mixed it slowly. labelled the plates & then poured 25 ml of media by sterile measuring cylinder. The plate was solidified and made required wells at proper distance by sterile borer on plates. Add test samples, standard & blank in respected labeled well. When samples were diffused completely, incubate SDA & PDA plate into Biological Oxygen Demand incubator at 25°C for 48 hours observe the zone of inhibition. [24]

Gas Chromatography and Gas Chromatography–Mass Spectrometry analysis

Gas Chromatography (GC) was done using an Agilent 8890 system with a 7693A Auto Sampler. A split/splitless injector and FID detector were used, with nitrogen as the carrier gas. The column was an HP-5 capillary (30 m × 0.32 mm × 0.25 µm). The temperature started at 60°C (held for 2 min), increased by 3°C/min to 240°C, and held there for 5 min. The injector and detector were set at 210°C and 250°C. Sample volume was 0.5 µL. Gas Chromatography–Mass Spectrometry (GC-MS) was also carried out on the same Agilent 8890 system, using a PAL RSI 85 Auto Sampler and a split/splitless injector (1:50 split). An HP-5MS UI column (30 m × 0.25 mm × 0.25 µm) was used, with helium as the carrier gas at 0.80 mL/min. The GC was connected to an Agilent 7010B MS system, operating in EI+ mode with an ionization voltage of 70 eV. The injector, ion source, and transfer line were all kept at 280°C. Mass spectra were recorded over a range of m/z 40–450.

Compound Identification:

Compounds were identified by comparing the mass spectra with NIST 2.3 and Wiley libraries, and using reference data (Adams, 2017).

RESULT AND DISCUSSION

Sr no.	Phytochemical Test	Procedure	Result
1.	Test for Phenolic compounds	Iodine Test:1mL extract + some drop of dil. Iodine sol.	Light yellowish colour appears.
2.	Test for Flavonoids	Alkaline reagent test: 1mL extract + 2mL 2% NaOH solution (+ some drops dil. HCl)	A dark red colour appears.
3.	Test for Protein	Millon's test: 2mL filtrate + few drops of Millon's reagent	White precipitate found
4.	Test for Carbohydrates	Molish's test: 2mL filtrateb + 2 drops of alcoholic α–naphthol + 1mL conc.H2SO4 (along the sides of test tube)	Light blue colour appears.
5.	Test for Amino acids	Millon's test: 2mL filtrate + few drops of Millon's reagent	White precipitate found
6.	Test for Alkaloids	Dragendroff test: test Few mL filtratea +1-2mL Dragendorff's reagents	A dark red colour appaers

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Table no. 2

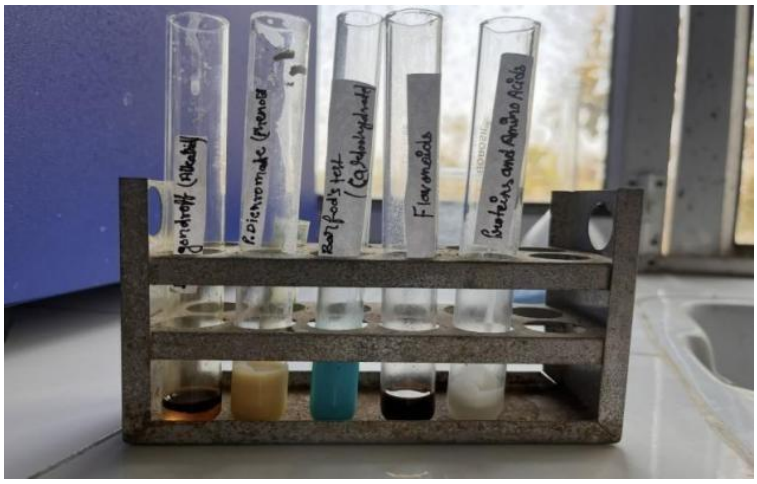

Figure no. 4 Phytochemical analysis

In Vitro Antifungal Activity

Sterile SDA and PDA media were cooled. To SDA, 10 µL of yeast culture was added and warmed to 55°C. Similarly, 10 µL of fungal culture was added to PDA. Both were gently stirred. After labeling, 25 mL of each medium was poured into sterile Petri dishes using a measuring cylinder. Wells were made using a sterile borer. Test samples, standard, and blank were added into the wells. Plates were incubated at 25°C for 48 hours in a BOD incubator. Zones of inhibition were then observed to assess antifungal activity.

Observations:

C. albicans *A. brasiliensis*

Figure no.5 C.albians and A. brasiliensis

GC and GC-MS Analysis

GC was performed using an Agilent 8890 with a 7693A Auto Sampler, FID detector, and HP-5 column. Nitrogen was the carrier gas. The oven was set from 60°C to 240°C at 3°C/min. Injector and detector were at 210°C and 250°C. Sample volume was 0.5µL.

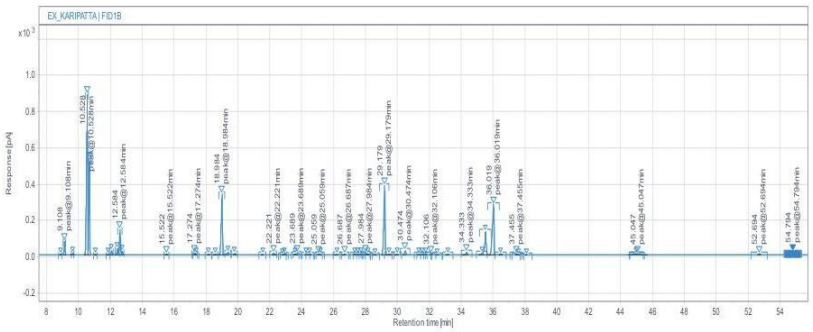

FIGURE 1. Typical chromatogram (GC-MS on HP-5MS, 30 m) of curry patta essential oil.

GC-MS used the same system with a PAL RSI 85 Auto Sampler and HP-5MS UI column. Helium was the carrier gas at 0.80 mL/min. The system ran in EI+ mode (70 eV), with all components at 280°C. Mass range was m/z 40–450.

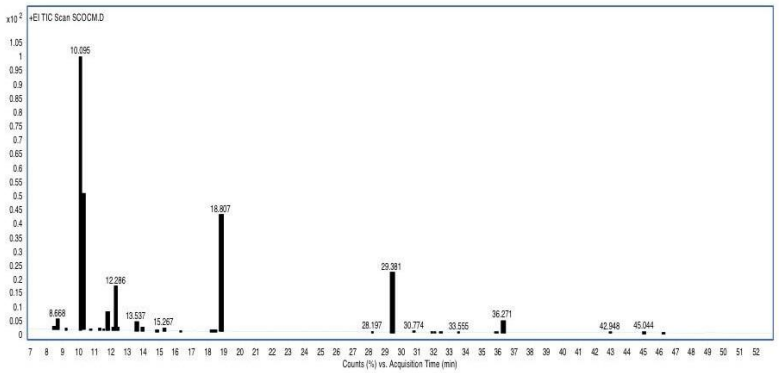

FIGURE 2. Typical chromatogram (GC-MS on HP-5MS, 30 m) of ointment based on curry patta essential oil.

S.N.	Retention Time Range (min)	Peak Intensity (Oil → Cream)	Probable Compound	% in Oil	% in Cream	Interpretation
1	9.108 – 8.668	Small → Minor	α-Pinene	1.62	0.13	Retained at lower %
2	10.528 – 10.095	Major → Major	Sabinene	26.63	34.85	Increased in cream
3	12.584 – 12.286	Small → Small	Camphene (or B-Pinene)	0.21 / 12.02	— / 17.47	Likely β-Pinene
4	15.522 – 13.537	Minor → Minor	Possibly Caryophyllene oxide	5.21	—	Present only in oil
5	17.274 – 15.267	Minor → Minor	trans- Caryophyllene	13.81	9.49	Retained, slightly reduced
6	18.984 – 18.807	Small → Major	Limonene	4.08	7.97	Increased in cream
7	22.221 – 28.197	Minor → Minor	Possibly p-Cymene or Thujene	—	1.29 / 2.87	New in cream
8	23.689 – 29.381	Minor → Small	Possibly 1,3,8-Menthatriene	—	1.39	New in cream
9	25.059 – 30.774	Minor → Minor	Possibly Butanoic acid	—	0.61	New in cream

10	26.687 – 33.555	Minor → Minor	Possibly Diethyl phthalate	—	2.20	Cream excipient
11	27.984 – 36.271	Minor → Minor	Unknown – likely oil residue	—	—	Trace volatile
12	29.179 – 42.948	Small → Minor	Unknown	—	—	Low concentration compound
13	30.474 – 45.044	Minor → Minor	Unknown	—	—	Consistent trace
14	32.106	Minor → —	Possibly oil-only compound	—	Absent	Not in cream
15	34.333	Minor → —	Possibly oil-only compound	—	Absent	Not in cream
16	36.019	Small → —	Possibly degradation product	—	Absent	Not in cream
17	37.455	Minor → —	Possibly Caryophyllene oxide	—	Absent	Missing in cream
18	45.047	Minor → —	Possibly Diethyl phthalate	—	Present	Cream excipient
19	52.694	Minor → —	Unknown high MW compound	—	Absent	Likely cream-unabsorbable
20	54.794	Minor → —	Unknown high MW compound	—	Absent	Present only in oil

Table no. 3

The present study focused on evaluating the pharmacological and phytochemical properties of a herbal cream formulated using *Murraya koenigii* (curry leaves) oil. Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids, which are known for their medicinal benefits. These constituents are likely responsible for the observed therapeutic effects.The herbal cream showed promising pharmacological activities, particularly antimicrobial and anti-inflammatory actions. The presence of natural phytoconstituents in the cream may enhance skin healing and protection against infections. The results support the traditional use of *Murraya koenigii* in skin-related treatments. Overall, the cream formulation was stable, easy to apply, and showed potential as a natural alternative to synthetic topical agents. Further research including clinical trials would help validate its safety and efficacy in broader applications.

CONCLUSION

The results of the GC-MS analysis clearly show that many important natural compounds from the curry leaf (*Murraya koenigii*) essential oil were successfully carried over into the prepared herbal cream. Notably, key compounds like **Sabinene**, **β-Pinene**, **Limonene**, **α-Pinene**, and **trans-Caryophyllene** were found in both the oil and the cream. Interestingly, the levels of **Sabinene** and **Limonene** increased in the cream, suggesting that the process of making the cream may have helped concentrate or stabilize these compounds.On the other hand, some components such as **Camphene** and **Caryophyllene oxide** were not detected in the cream. This might be because these compounds are more volatile (easily evaporate) or may have broken down during heating or processing. The GC-MS results also showed some new compounds in the cream that were not present in the original oil. These included **p-Cymene**, **Thujene**, **1,3,8-Menthatriene**, and **Diethyl phthalate**. These new peaks might be due to interactions between ingredients in the cream, natural changes during formulation, or added substances from the cream base (like fragrance or stabilizers).Overall, the study shows that the main active ingredients from curry leaf oil were successfully included in the cream, and many of them remained stable or even became stronger. This suggests that the herbal cream could be effective for treating infections or other skin issues. The presence of these stable and beneficial compounds supports the idea that the cream can deliver the medicinal benefits of the essential oil in a safe and convenient form for use on the skin

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