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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NEPALESE ORIGIN *CINNAMOMUM ZEYLANICUM* BLUME LEAVES EXTRACT OINTMENT

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ABSTRACT

Even in areas where modern medicine is available, the interest in herbal drugs and their utilization has been rapidly increasing in recent years. Using plants for healing predates recorded history and forms the origin of modern medicine. This research explores the antibacterial and antifungal potential of an ointment formulated with Nepalese-origin *Cinnamomum zeylanicum* Blume leaves extract. The extraction was conducted using the Soxhlet method, and the phytochemical screening was carried out using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR). Two typesof formulation were prepared (naming F1 to F10) with two different bases (hydrocarbon base and emulsion base). The efficacy of the formulations, comprising 10 variations with different concentrations of extract, was evaluated through the disc diffusion method. Interestingly, the emulsion-based formulation demonstrated superior activity compared to the hydrocarbon- based counterpart. Specifically, the F10 emulsion exhibited a notable zone of inhibition (ZOI) of 1923±0.24 mm and 22±0.17 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively. Additionally, F10 displayed considerable antifungal activity with ZOIs of 19±0.72 mm and 23±0.92 mm against *Candida albicans* and *Aspergillus flavus*, respectively. Similarly,the minimum inhibitory concentration (MIC) of F5 and F10 formulation ranges from 150 to 200 µg/mL. These findings underscore the efficacy of the formulated ointment, especially the emulsion base, in inhibiting the growth of bacterial and fungal strains. The study provides valuable insights into the potential application of *Cinnamomum zeylanicum* Blume leaves extract for developing effective antimicrobial ointments, emphasizing its relevance in the context of pharmaceutical formulations.

KEYWORDS: *Cinnamomum zeylanicum*, herbal extract, antimicrobial activity, formulation.

1. INTRODUCTION

The use of herbal remedies has been an integral part of traditional medicine systems for centuries. *Cinnamomum zeylanicum* Blume, commonly known as Ceylon cinnamon, has been recognized for its medicinal properties and is traditionally used to treat various ailments. The leaves of *Cinnamomum zeylanicum* Blume possess bioactive compounds that exhibit anti- inflammatory, antimicrobial, and antioxidant properties.[1] Harnessing the therapeutic potential of these leaves in the formulation of herbal ointments can provide an effective and natural alternative for topical applications. Skin disorders, ranging from minor irritations to chronic conditions, are prevalent and can significantly impact an individual's quality of life.[2] The search for safe and effective topical treatments remains a continuous challenge. Existing pharmaceutical ointments carry side effects or contribute to the growing concern of antibiotic resistance. Hence, there is a pressing need to explore natural alternatives that can address skin ailments without compromising safety. The formulation of herbal ointments presents an opportunity to tap into the therapeutic potential of botanical extracts.[3],[4]

Modern pharmaceuticals often come with synthetic ingredients that may have adverse effects or cause skin irritations. Herbal formulations, on the other hand, have gained popularity due to their perceived safety and minimal side effects.[5] Developing an herbal ointment containing *Cinnamomum zeylanicum* Blume leaves extract could offer a promising solution for various skin conditions while embracing the principles of traditional medicine. However, there is a lack of comprehensive studies on the formulation and evaluation of herbal ointments containing *Cinnamomum zeylanicum* Blume leaves extract. Sim et al., explore the antimicrobial effect of cinnamon essential oil and Cinnamaldehyde against canine otitis externa pathogens. They found the significant antibacterial activity of Cinnamaldehyde (22 µg/mL) than cinnamon bark oil (41 µg/mL).[6] Tran at el., conducted an investigation of *Cinnamomum zeylanicum* bark and leaf essential oils against *Candida albicans* and *Candida auris* and reported the significant minimum inhibitory concentration (MIC) below 0.03% (v/v).[7] Shahina Z. at al., reported the presence of eugenol by Gas chromatography–mass spectrometry (GC-MS) analysis. They also revealed the significant antifungal activity of plant extract.[8] Julyana at al., conducted a clinical trial on the safety and tolerability of essential oil from *Cinnamomum zeylanicum* Blume leaves with action on oral Candidiasis, and suggested the anti-candida activity, with MIC of 625.0 μg/mL.[9] The imitated research on the formulation of ointment from *Cinnamomum zeylanicum* (especially from Nepalese origin), makes us curious to explore the antimicrobial effect by formulating different base ointments. Therefore, understanding the optimum formulation parameters, assessing the stability, and determining the efficacy of such herbal ointments are critical aspects that need thorough investigation.[10],[11]

This research aims to contribute to the scientific knowledge on herbal formulations for topical use, providing insights into the potential of *Cinnamomum zeylanicum* Blume leaves extract as a key ingredient in the development of safe and effective herbal ointments for various antibacterial and antifungal activity.

2. MATERIALS AND METHODS

2.1. Reagents and equipment

All the equipment and reagents used were lab-grade. The equipment includes; Weighing balance, Hot air oven, pH meter, Water bath, Glassware (petri plates, test tubes, beakers, volumetric flasks, pipette, glass rod), measuring cylinder, Soxhlet apparatus, Autoclave, Incubator and other: forceps, inoculating loop, and spatula. Reagents include; Mueller Hintonagar, Potato dextrose agar, Nutrient Broth, DPPH, Sulphuric acid, Ethanol, Ferric chloride, HCL, Acetic

acid, Chloroform, Lead acetate, NaOH, Wagner's reagent, Molisch reagent, Dragendorff's reagent, Hager's reagent, Mayers reagent, Methanol, SLS, White soft paraffin, Liquid paraffin, Propylene glycol, Glycerine, and Polyethylene glycol.

2.2. Ethical approval, plant collection and authentication

The ethical approval for the research was taken from the Internal Review Committee (IRC) at Sunsari Technical College (IRC No. ST43RE189). The leaves of plant *Cinnamomum zeylanicum* were locally collected from Jhapa district, Nepal in the months of March and April during the daytime and authenticated by the Department of Botany, Post Graduate Campus, Biratnagar, Nepal. The leaves were stored in the pharmacognosy lab of Sunsari Technical College at room temperature.

2.3. Macroscopical and microscopic study

Macroscopical study was carried out with the naked eye which gave details concerning the plant aspect like general appearance, colour and odor. For the microscopic study, fresh leaves were collected and preserved in glycerin and alcohol mixture (1:1) for softening. Sections of fresh leaves were cut in transverse plains, stained with crystal violet, and mounted on a microscope for microscopical characters.

2.4. Cinnamon extraction procedure

To create a homogenous powder with a particle size of 0.18 mm, the plant's leaves were dried at room temperature for around 20 days, then triturated in a grinder and filtered through a mesh 80 sieve. The powder was kept at room temperature (25°C) in an airtight plastic container. A thimble containing over 30 g of the powder was weighed and stored in the Soxhlet equipment. A sufficient volume (450 mL) of methanol and chloroform, used as solvents, were stored in a round-bottom flask to conduct the extraction operation for a full day each. A heating mantle with a temperature regulation held the entire assembly. After being heated, the solvent in the flask evaporated, rising to the distillation arm and then condensing to fall into the extraction unit that held the thimble. The plant material that had been pulverized was extracted using both methanol and chloroform. The extracts were then dried in an evaporator at a temperature that was chosen, and the extract yield % was computed.[12]

$$Extractive\ value = \frac{Weight\ of\ dried\ extract}{Weight\ of\ plant\ material\ taken}\ x\ 100\% \ldots\ldots\ldots\ldots (1)$$

2.5. Phytochemical screening

The presence of different primary and secondary metabolite in the extract was screened by different screening test. The screen test of alkaloid, glycoside, tannin, flavonoid, carbohydrate, protein, phytosterol, saponin, terpenoid, phlobatannins, anthocyanin and phenolic compounds were carried out by using published methodology.[13] Detailed explanation of test is given insupplementary file.

2.6. Fourier transform infrared spectroscopy (FT-IR)

FT-IR provides the insight of functional group present in sample. The department of central instrumental analysis, Nargund College of Pharmacy, Bengaluru, India provided the space and facility for spectral analysis. The IR spectra of methanolic extract was recorded on a FT-IR (Model Shimadzu 8700) in the range of 400-4000 cm-1 by KBr pellet method.[14]

2.7. Gas chromatography-Mass spectroscopy (GC-MS)

It was conducted in Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal for the analysis of compound present in the extract. The methanolic extracts from the leaves of *Cinnamomum zeylanicum* was subjected to GC-MS analysis. The Agilent 7890A GC system and Agilent 5975 C mass selective detector were used for the analysis. The HP-5MS GC column (5% phenyl methyl siloxane, Agilent 19091S-433, 30 m × 250µm internal diameter, 0.25µm film thickness) was utilized for the analysis. A 1.21 mL/min flow rate of helium was employed as the carrier gas. The device was run in the 50–500 m/z scan range in the electron impact (EI) mode at 70 eV and 230 °C for the ion source temperature. After being held at 40°C for two minutes, the temperature was ramped up to 270 °C at a rate of 4 °C per minute andmaintained for five minutes (a total of 65 minutes of run time). A split injection approach wasused to inject a volume of 2µL of a diluted sample solution of the extracts, which was producedin methanol. By comparing the mass spectra contained in an MS database (NIST 08), the ingredients were found.[15]

2.8. General manufacturing procedure of herbal ointment

2.8.1. Hydrocarbon base

First, the high-melting-point petroleum jelly was melted. Next, low melting materials suchas liquid paraffin, PEG 6000, and Ceto stearyl alcohol were melted. Following that, small amount of purified water was dissolved in different concentrations (0.25 to 5%) ofCinnamon extract, and the mixture was introduced to a large volume non-aqueous phase. After the liquid had cooled completely while being stirred, a soft mass of ointment was produced. The formulations were given name as F1, F2, F3, F4 and F5 having an extract concentration of 0.25%, 0.5%, 1%, 2.5%, and 5% respectively (supplementary file Table 1S and 2S).

2.8.2. Emulsion base

The mineral oil, stearic acid, and cetyl alcohol were mixed and melted over a water bath. The mixture was heated until it reached a temperature of roughly 70ºC. The water was boiled to 70ºC after the addition of the other ingredients, glycerine and triethanolamine. Stirring constantly, the wax mixture was added to the aqueous phase. After that, cinnamonextract was added in different concentrations (0.25 to 5%). The entire mixture was continually mixed until it solidified. The formulations were given name as F6, F7, F8, F9, and F10 having an extract concentration of 0.25%, 0.5%, 1%, 2.5%, and 5% respectively (supplementary file Table 3S and 4S).[16]

2.9. Evaluation of herbal ointment

1. Physical appearance: Physical parameters like appearance and colour were checked byvisual inspection on light.
2. Measurement of pH: The pH of formulation was measured by using digital pH meter. 1gm of ointment was taken which was dissolved on 10 mL water then left for two hoursthen, the measurement of pH was carried out for three times and the average values were calculated.
3. Homogeneity: Developed ointment formulations were tested for homogeneity by visual inspection after the ointments was kept into the container. Test done by their presenceand appearance of any aggregates.
4. Spreadability: Spreadability was guaranteed based on the ointment's slide and drag properties. The device, which is made up of a wooden block with a pulley at one end, was used to measure spredability. Using this technique, a 2gm ointment was applied onthe ground slide. The fed was fastened with a hooked structure and positioned betweenthis glass slide and another one that was the same length or size as a fixed ground slide.For five minutes, a one-kilogram weight was placed on top of the slides to release air and create a consistent layer of gel between the slides. The

excess gel was removed byscraping off the edges. Next, a 35.8 g weigh was placed on the top plate, and the slides were pulled with the aid of a striking device that was fastened to the hook. The amountof time (in seconds) needed by the top slide to travel 7.5 cm was recorded. A smaller interval exhibits superior dispersion. The formula used to calculate spreadability was as follows[17]:

$$Spreadability = \frac{M\ x\ L}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, S = spreadability

M= weight in pan (tied to upper slide). L= length moved by the glass slide.

T= time (in second and 0 taken to separate the slide completely each other).

5. Solubility test: Solubility test was performed by dissolving the ointments in varioussolvents (chloroform, ethanol, and water).
6. Consistency: For the consistency test of the ointment formulation, the formulations (F5 and F10) were placed at room temperature. A defined amount of the ointment (10 mg) was spread uniformly on a glass slide, and the spread was observed and measured for uniformity and smoothness. The ease of spreading was noted to assess the consistency of the ointment, and the sample was compared with a standard or control ointment for reference. Smooth and no greediness is observed.[18]
7. Stability study: For the stability test, the ointment formulations (F5 and F10) were stored under various environmental conditions, including room temperature, refrigerated, and elevated temperature (40°C) environments. Formulations were placed in suitable containers and sealed to prevent contamination. Observations were made at regular intervals (e.g., 1 week, 1 month, and 3 months), examining the formulations for changes in color, odor, consistency, and phase separation. The pH of the formulation was measured to detect any significant changes, and microbial testing was conducted periodically to check for contamination. The physical and chemical properties of the ointment were compared against initial values to determine stability over time.[19]

2.10. Assessment of Antimicrobial activity

The agar well diffusion method was used to assess the antibacterial activity of each batch of the herbal ointment as well as different extract concentrations. Using a sterile swab, approximately 100 µL of bacterial organisms (*Staphylococcus aureus* and *Escherichia coli*) were placed on each Muller Hinton Agar plate. The bacteria were allowed to air dry to eliminate any surface moisture. Using a sterile cork, nearly 6 mm diameter wells were punched into the agar plate. In separate petri dishes, an adequate 0.5 g of ointment with concentrations of 0.25% w/w, 0.5% w/w, 1% w/w, 2.5% w/w, and 5% w/w were poured to the pores and allowed to diffuse out into the agar medium. Finally, a scale was used to measure the diameter of the zone of inhibition (ZOI) in mm following a 24 hours incubation period at 37°C. The reference antibacterial agent ciprofloxacin (same concentration as sample formulations) was employed.[20]

2.11. Assessment of antifungal activity

Using the agar-well diffusion method, the antifungal activity of each batch of ointment and different quantities of extract were assessed. Through the use of a sterile swab, approximately 100 µL of fungal organism (*Candida albicans* and *Aspergillus flavus*) was dispersed on a potato dextrose agar plate, and the surface moisture was removed by air drying. A sterile cork borer with a 6 mm diameter was used to drill wells into the agar. Following a pre-incubation diffusionperiod of approximately one hour at room temperature, an adequate 0.5 g of ointment from each batch with

concentrations of 0.25% w/w, 0.5% w/w, 1% w/w, 2.5% w/w, and 5% w/w wasinjected into the holes. The plates were then incubated at 28-30°C for 48 hours. After 48 hours,the plates were checked for the zone of inhibition. Lastly, a scale was used to determine the zone of inhibition's (ZOI) diameter in mm. The common antifungal used was clotrimazole cream (same concentration as sample formulations).[21]

2.12. Minimum inhibitory concentration (MIC)

Through the use of the agar well diffusion method, the MIC for the crude extract was ascertained. The sodium propionate and cinnamon extract ointment (only F5 and F10 formulation) were first reconstituted in DMSO in order to create a two-fold serial dilution. Then, to get a decreasing concentration range of 400 to 12.5 µg/mL, it was diluted in sterile DMSO. Each dilution was aseptically injected in 50 µL volumes to the wells of Mueller Hintonagar plates that had been inoculated with 10^6 CFU/mL standardized inoculums of the test bacteria. For a whole day, the agar plates were incubated at 37°C. The sole control used was sodium propionate. Every experiment was run three times. For fungus, the same protocol was followed, except Sabouraud Dextrose Agar (SDA) plates were utilized and the plates were incubated at 28°C. The MIC was defined as the lowest concentration of oil and cinnamon extract that clearly displayed a zone of inhibition.[22]

3. RESULTS AND DISCUSSION

The purpose of this study was to create a topical herbal ointment using a leaf extract from *Cinnamomum zeylanicum*. The leaves were ground, sieved, and extracted with a solvent (methanol and chloroform). Following that, the extract from cinnamon leaves was tested for phytochemicals, antibacterial and antifungal activity. Additionally, an herbal ointment was formulated utilizing two distinct ointment bases and various components. Evaluation experiments were conducted on ten distinct formulations, five of which had an emulsion base and five of which had a hydrocarbon base.

3.1. Macroscopic identification

On observation, leaves are simple on short petiole small to medium in size with acute, ovate toelliptic leaf shape. The sizes of the leaves are 8-12 cm in length and 3-6 cm in width. The macroscopic identification is done by organoleptic test of fresh leaves of *Cinnamomumzeylanicum*. In our investigation of the macroscopic characteristics of plant leaves, particularlyfocusing on a specific plant species, we have observed distinct features contributing to its identification. The leaves exhibit a vibrant and characteristic deep green color, signifying the presence of chlorophyll and potential photosynthetic activity. Notably, the leaves emit a pleasant and aromatic odor, hinting at the presence of volatile compounds that may have aromatic or medicinal properties. Additionally, upon tasting, the leaves manifest a characteristic sweet flavor, which could be indicative of certain phytochemicals or sugars present in the plant. These macroscopic observations are crucial for the identification and classification of the plant, offering valuable insights into its potential uses in various applications, including herbal medicine or culinary purposes. Further microscopic and chemical analyses will complement these macroscopic findings, providing a comprehensive understanding of the plant's botanical profile.

3.2. Microscopic identification

The microscopic analysis involved the examination of transverse sections (T.S. sections) of fresh Cinnamon leaves, particularly focusing on the midrib, utilizing a microscope (supplementary file, Figure 1S). The observations revealed distinct characteristics. The lamina of both species exhibited a thick cuticle covering the upper epidermis, while the lower cells were characterized by a thin cuticle. In the case of *Cinnamomum zeylanicum*, the cuticle was nonlignified

and nonpapillose. Palisade cells were observed as single-layered, and the spongymesophyll displayed a variation in the number of layers, ranging from 4 to 5 in *C. zeylanicum*. Cavities beneath the epidermis contained volatile oils. Anomocytic stomata cells were identified, and isolated sclerenchyma cells were evident on both sides of the vascular bundles,providing a detailed microscopic insight into the anatomical features of the examined leaves.

3.3. Extractive value

About 13.13% and 24.8% yield was obtained from the extraction of plant by using chloroformand methanol as solvent respectively. The yield percentage from two solvents was calculated and yield was found to be the highest in case of methanol extract. The methanolic extract wasused for formulation and activity evaluation owing its higher yield.

3.4. Phytochemical screening

The extract demonstrates the presence of phytochemicals including alkaloids, glycosides, flavonoids, phenolic compounds and tannins, terpenoids, cellulose, and reducing sugars.

Table 1: Phytochemical screening of chloroform and methanolic extract.

Test	Methanol extract	Chloroform extract
Alkaloid	+	+
Glycoside	+	+
Carbohydrate	+	-
Proteins	-	-
Steroids	+	+
Terpenoids	+	+
Saponins	+	+
Phenolic compounds	+	+
Reducing sugar	+	-
Anthocyanin	-	-
Flavonoid	+	+
Cellulose	+	+
Tannins	+	+
Phlobatanins	-	-

Note: '+' represents presence and '-' represents absence.

3.5. GC-MS spectral analysis

The spectra suggest the presence of fatty constituents and alkaloid components. Some of the important constituents include Eugenol, Cyclohexane, Propyl glycol, Phthalic anhydride, and 2-Furancarboxaldehyde (Table 2 and Figure 1). The GC-MS analysis of the Cinnamon plant extract revealed a diverse chemical composition with 2-Isopropoxyethyl propionate (32.155%) as the predominant compound, indicating its significant presence. Other notable compounds include dihydroxyacetone dimer (10.336%) and 9,17-Octadecadienal (5.3262%), which together contribute substantially to the extract's profile. Moderately abundant compounds such as 3-Methoxy-2,2-dimethyloxirane, Trifluoroacetate, and n-Hexadecanoic acid further diversify the chemical makeup. The presence of aromatic and potentially therapeutic compounds like Eugenol and Eucalyptol suggests applications in flavoring, fragrance, and health-related industries. Lesser quantities of compounds like Cyclohexane and Nonane also influence the extract's properties. This varied composition underscores the extract's potential for use in industrial processes as chemical intermediates and highlights its multifaceted applications in different sectors.[23],[24] However, Aswathi K. et al., also reported the phytoconstituents like, linalool, cinnamaldehyde, caryophyllene, benzyl benzoate, eugenol, and humulene in their GC-MS analysis.[25] Hasitha W. et al., distinguished the wild type of Cinnamon from commercial

types by DNA bar coding and revealed the active phytoconstituents such as; Trans-cinnamaldehyde, β-Linalool, β-Phellandrene, Palmitic acid, and Camphor.[26]

Table 2: GC-MS spectra of methanolic extract of *C. zeylanicum*.

S. N.	R.T.	Reported Compounds	Peak Area %
1.	2.432	Cyclohexane	1.833
2.	4.5898	Propylene Glycol	3.135
3.	7.6969	dl-Glyceraldehyde	1.851
4.	10.189	Dihydroxyacetone dimer	10.336
5.	16.888	1,3,5-Triazine-2,4,6-triamine	2.847
6.	18.539	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	3.451
7.	18.971	3-Methoxy-2,2-dimethyloxirane	5.173
8.	21.225	2-Furancarboxaldehyde	3.653
9.	21.571	Butanoic acid, 3-oxo-, hexyl ester	3.0864
10.	22.865	Nonane	0.846
11.	23.372	Eugenol	1.4059
12.	25.595	Oleic acid	1.8371
13.	27.127	2-Isopropoxyethyl propionate	32.155
14.	30.773	3-Deoxy-d-mannoic lactone	4.124
15.	30.924	Eucalyptol	1.7831
16.	31.529	Benzyl benzoate	2.0087
17.	36.9125	Stearic acid hydrazide	1.8788
18.	38.0669	n-Hexadecanoic acid	4.0671
19.	41.2388	9,12-Octadecadienoic acid	3.0398
20.	41.3359	9,17-Octadecadienal	5.3262
21.	45.0148	Trifluoroacetate	4.2748

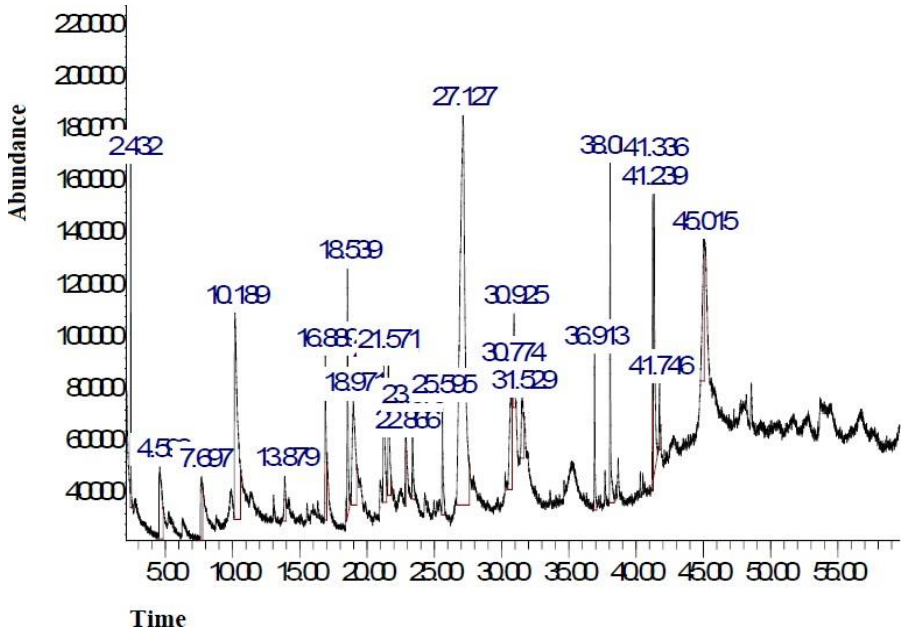

Figure 1: GC-MS spectra of ethanolic extract of *C. zeylanicum* extract.

3.6. FT-IR spectral analysis

FT-IR spectroscopy was used to identify the functional groups in the essential oil of *C. zeylanicum* (Figure 2). The peaks located at around 1640 cm^{-1} and 1604 cm^{-1} are attributed tothe vibration stretching of aldehyde carbonyl (C=O) groups, representing a high concentrationof aldehydes in *C. zeylanicum* leaves extract. A small sharp peak at 2383 cm^{-1} confirms the presence of nitrile group in the extract. Other significant peaks were observed at 621 cm^{-1} (vibration

absorption of alkanes), 764 cm^{-1} (benzene rings =CH), 941 cm^{-1} (C-H bond), 1120 cm^{-1} (C-O and C-OH bonds), 1221 cm^{-1} (C-O-C bond of aromatic acid ester and C-OH groups of phenolic compounds), 1276-1367 cm^{-1} (alkanes CH_2), 1431 cm^{-1} (alcohol C-OH bond), 1564 cm^{-1} (aromatic C=C bond), 2914 cm^{-1} (=C-H bond), and 3026 cm^{-1} (aromatic C-H bond).Similarly, a sharp peak is observed at 3284 cm−1 followed by broad peak at 3415 cm^{-1} represents the presence of amine and hydrogen bonded alcohol group respectively. All these characteristic peaks confirm that the methanolic extract is rich in phenolic, amine, carbonyl, and aromatic compounds.

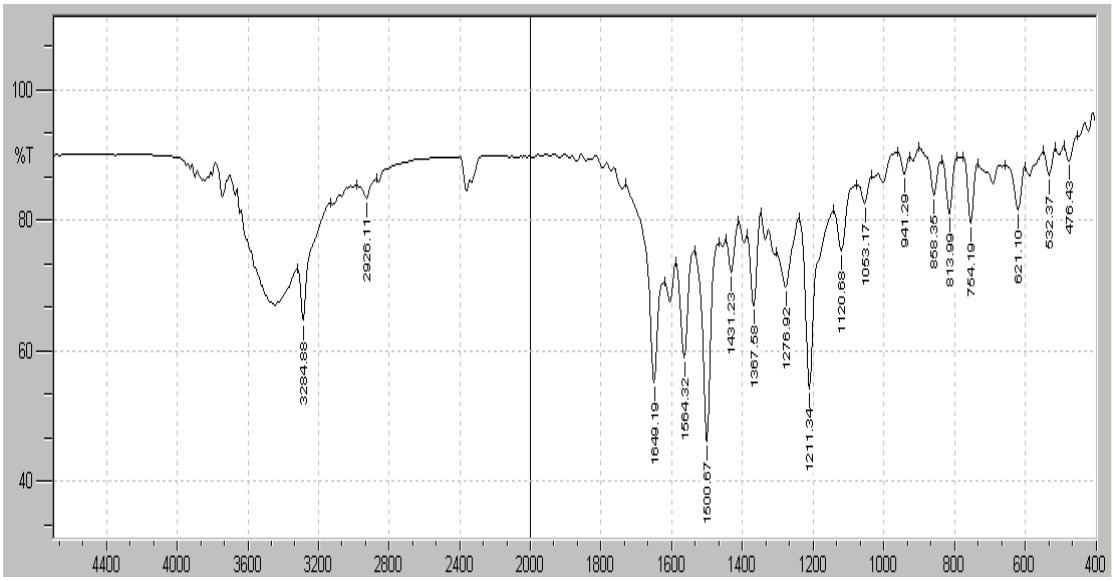

Figure 2: FTIR spectrum of *Cinnamomum zeylanicum* leaf extract.

3.7. Comparative study of different formulations

The parameters in several formulations are the same. After comparing the physical characteristics of each formulation, it is discovered that all the formulation are stable at room temperature with a pH range of 6.6–7.3 and good uniformity (Table 3 and 4). In contrast to the formulations made from emulsion bases, which were insoluble in chloroform but soluble in ethanol and warm water, the hydrocarbon base formulations were soluble in chloroform.

Washability refers to the ease with which an ointment can be removed from the skin. It is an important characteristic, especially for patient compliance and user comfort. An ointment withgood washability can be easily cleansed from the skin surface, preventing residue buildup. Thisis essential for dermatological applications, as excessive residue may lead to skin irritation or affect the patient's adherence to the prescribed treatment regimen. Formulating ointments with appropriate washability ensures a positive patient experience and facilitates the practicality of repeated applications. Hydrocarbon based formulation is not easily washable whereas emulsion-based formulation is easily washable. Phase separation in ointments refers to the physical separation of components, typically the oil and water phases in emulsion-based formulations. Achieving and maintaining stability against phase separation is crucial for the uniform distribution of active ingredients. Unwanted phase separation can lead to inconsistent dosing and compromise the efficacy of the formulation. Proper emulsification and the use of stabilizing agents help prevent phase separation, ensuring that the ointment maintains its homogeneity over time, enhancing its shelf life and therapeutic reliability.[27] Interestingly, inboth the formulation there is no phase separation.

Table 3: Physical parameters of hydrocarbon base formulation.

S. N.	Parameters	F1	F2	F3	F4	F5
1.	Colour	Lightgreen	Lightgreen	Greenish brown	Brown	Dark brown
2.	pH	7.2	6.9	7.1	7.0	7.3
3.	Homogeneity	Good	Good	Good	Good	Good
4.	Spreadability	5sec 53.7	5.43sec 49.44	4.98sec 53.9	5.28sec 50.83	5.3sec 50.66
5.	Phase separation	No phase separation				
6.	Washability	Not easily washable				
7.	Solubility	Soluble in chloroform, insoluble in ethanol and warm water				

Table 4: Physical parameters of emulsion base formulation.

S. N.	Parameters	F6	F7	F8	F9	F10
1.	Colour	Light pink	Light pink	Lightbrown	Brown	Dark brown
2.	pH	7.1	6.9	6.7	6.6	6.8
3.	Homogeneity	Good	Good	Good	Good	Good
4.	Spreadability	2.48sec 108.26	2.45sec 109.59	3.2sec 83.90	3.1sec 86.61	2.92sec 91.95
5.	Phase separation	No phase separation				
6.	Washability	Easily washable				
7.	Solubility	Soluble in ethanol and warm water and insoluble in chloroform				

Solubility is a critical factor influencing the dissolution and availability of active pharmaceutical ingredients in ointments. The solubility of the active components in the chosenbase determines the uniform distribution of therapeutic agents. Poor solubility can result in uneven dispersion, affecting the consistency and efficacy of the ointment. The choice of a suitable base, incorporating appropriate solvents or co-solvents, is essential to enhance the solubility of active ingredients. This ensures that the ointment delivers consistent and effective concentrations of therapeutic agents during application. Emulsion-based formulation is soluble in ethanol and warm water and insoluble in chloroform. Whereas hydrocarbon-base formulation is soluble in chloroform but insoluble in ethanol and warm water.[28]

3.8. Consistency and stability testing

Formulation F5 from hydrocarbon base and F10 from emulsion base were selected randomly for consistency and stability testing. Formulation F5 and F10 showed ease of spreading and uniformity similar to the control (supplementary file, Table 5S). The refrigerated formulation (F5 and F10) showed no significant changes over three months, indicating good stability. Similarly, at elevated temperature, both the formulation showed no significant changes in color, odor, consistency, and phase separation after three months, along with microbial growth, indicating good stability under those conditions (supplementary file, Table 6S).

3.9. Antibacterial activity of herbal ointment

The formulated herbal cream F10 (5% extract) exhibits antibacterial activity with zone of inhibition (ZOI) values of 19±0.24 mm and 22±0.17 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively (Table 5). In comparison, the reference drug ciprofloxacin demonstrates larger ZOI values of 33±1.20 mm and 3533±0.67 mm for *S. aureus* and *E. coli*, indicating a potentially higher antibacterial efficacy. Although F10 displays activity against both bacterial strains, its ZOI values are comparatively smaller than ciprofloxacin, suggesting a lesser potency (supplementary file Figure 2S and 4S). Further investigations into the specific antibacterial mechanisms and optimization of the herbal cream's formulation may enhance its efficacy against these pathogens. Kaur M. et al., formulated a nano sponge carrier of cinnamonoil and significance antibacterial activity with 50±1.2 mm ZOI against *S.*

aureus.[29] In comparison to a prior study reporting a ZOI of 55±1.2 mm against *S. aureus*, our research observed a reduced ZOI of 19±0.24 mm. This disparity could potentially be attributed to the utilization of a lower concentration of the extract, specifically at 5%. The variation in extract concentration may account for the differences in antibacterial efficacy observed between the two studies. In a study by Behbahani B. et al., they reported a ZOI of 26±0.4 mm against *S. aureus*, slightly surpassing our research findings where the ZOI was 19±0.24 mm.[30] This discrepancy in antibacterial efficacy might be attributed to the difference in the employed plant parts; they utilized bark essential oil, whereas our study utilized a leaves extract of Cinnamon. The choice of plant parts for extraction could potentially account for the observed variations in antimicrobial activity against *S. aureus* between the two studies. In a study conducted by Nita P. et al., the reported ZOI against *S. aureus* and *E. coli* was 15±0.8 mm and 9±0.05 mm, respectively, indicating lower activity compared to our research.[31] One potential explanation for this difference could be our utilization of an ointment formulation, potentially enhancing the penetration and release profile of the cinnamon extract within bacterial cells. Additionally, the origin of the cinnamon source might contribute to the variation; our research employed Nepalese-origin cinnamon, while Nita Parisa used cinnamon from a tropical region. These factors collectively underscore the multifaceted influences on antimicrobial outcomes in studies involving cinnamon extract. Various studies have been observed that cinnamon extract and their constituents inhibit bacteria by lipid profile modification, cell membrane damage, inhibition of ATPases, cell division, membrane porins, motility, and biofilm formation, as wellas through anti-quorum sensing actions.[32]

Since only F5 and F10 formulation showed some reasonable ZOI, we have determined MIC for them only. In our research, F5 exhibited a MIC of 200 µg/mL against both *S. aureus* and *E. coli*, while F10 demonstrated a more potent antimicrobial effect with an MIC of 150 µg/mL against the same bacterial strains. This suggests F10's superior efficacy in inhibiting bacterial growth compared to F5.

Table 5: Antibacterial property of herbal ointment.

Parameters	Zone of inhibition (mm)			MIC (µg/mL)	
	Bacteria / Formulations	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Hydrocarbon Base	F1	-	-		
	F2	-	-		
	F3	-	-		
	F4	-	-		
	F5	-	-	200	200
Emulsion Base	F6	-	-		
	F7	-	4±0.76		
	F8	11±0.32	13±1.02		
	F9	14±0.44	15±0.98		
	F10	19±0.24	22±0.17	150	150
Standard	Ciprofloxacin	33±1.20	35±0.67	12.5	12.5

3.10. Antifungal activity of herbal ointment

The antifungal activity of the formulated emulsion base herbal cream F10 is assessed through the measurement of ZOI against *Candida albicans* and *Aspergillus flavus*. The MIC of both F5 and F10 formulation for all the fungal strain reported same which is 200 µg/mL (Table 6). In case of fungal activity tested against *C. albicans* and *A. flavus*, the hydrocarbon base formulation F5 (concentration of extract 5%) shown weak activity which is reported by ZOI (12±0.43 mm and 11±0.81 mm against *C. albicans* and *A. flavus* respectively). The poor antibacterial activity observed in hydrocarbon base formulations may be attributed to several factors related to their inherent properties. Hydrocarbon

base formulations may create a physical barrier that hinders the diffusion of active antimicrobial agents. The occlusive nature of hydrocarbon could impede the penetration of extract into the bacterial cell membrane. Furthermore, the absence of aqueous components in hydrocarbon bases may limit the solubilityand dispersion of certain extract. However, emulsion base formulation F10 (5% extract concentration) exhibits ZOI values of 19±0.75 mm and 23±0.92 mm for *Candida albicans* and*Aspergillus flavus*, respectively (Table 6). In comparison, the reference drug clotrimazole displays larger ZOI values of 27±0.39 mm and 30±0.50 mm for the respective fungi, indicating potentially higher antifungal efficacy. While F10 demonstrates antifungal activity against both strains, its ZOI values are smaller than those of clotrimazole, suggesting a comparatively lesserpotency (supplementary file, Figure 3S and 5S).

In contrast to Hoang N. H. Tran et al., study reporting a ZOI of 53.00 mm against *Candida albicans*, our research observed a ZOI of 19±0.75 mm for F10 and 12±0.43 mm for F5 formulation.[7] These disparities indicate variations in antifungal activity, suggesting potentialdifferences in formulations or concentrations between the studies. In concordance with Sukatta U. et al., findings, our research reports a MIC of 200 µg/mL against Aspergillus species.[33] This consistency in MIC values underscores the reliability and reproducibility of the antimicrobial efficacy against Aspergillus observed in both studies.

Table 6: Antifungal activity of herbal ointment.

Parameters	Zone of inhibition (mm)			MIC (µg/mL)	
	Fungus / Formulation	*Candida albicans*	*Aspergillusflavus*	*Candida albicans*	*Aspergillus flavus*
Hydrocarbon Base	F1	-	-		
	F2	-	-		
	F3	-	-		
	F4	-	-		
	F5	12±0.43	11±0.81	200	200
Emulsionbase	F6	-	-		
	F7	-	12±0.78		
	F8	12±0.27	15±0.11		
	F9	14±1.77	18±0.56		
	F10	19±0.75	23±0.92	200	200
Standard	clotrimazole	27±0.39	30±0.50	50	50

The enhanced antibacterial and antifungal activity observed in an emulsion base ointment compared to hydrocarbon base formulations may be attributed to the unique properties of emulsions. Emulsions provide a stable platform for incorporating active ingredients, ensuring uniform distribution and sustained release. The oil-in-water or water-in-oil emulsion structures may facilitate better penetration of ointment into the microbial cell membrane, disrupting its integrity. Additionally, emulsions can create a protective film over the application site, prolonging the contact time between the active components and microorganisms. The presenceof emulsifying agents in the formulation may enhance solubility and bioavailability of antimicrobial compounds. Furthermore, the physical structure of emulsions may enhance adhesion to the skin, promoting prolonged therapeutic effects. These combined factors contribute to the superior antibacterial and antifungal efficacy observed in emulsion-based ointments, making them promising vehicles for delivering antimicrobial agents.[34]

The spreadability of an ointment, plays a crucial role in optimizing the application and contact dynamics of the formulation on the skin. In the context of emulsion-based ointments, their enhanced spreadability ensures a uniform and even distribution over the skin surface. This, inturn, contributes to better adhesion to the skin, facilitating prolonged

contact between the plant extact and the microbial population. The improved spreadability may also enhance the penetration of active ingredients into the skin, allowing for more effective interaction with microorganisms residing in deeper layers. Furthermore, the creation of a thin and continuous barrier on the skin surface by the emulsion base provides a protective shield against microbial invasion, potentially promoting sustained release and prolonged efficacy of the antimicrobial compounds. While spreadability itself does not dictate antimicrobial activity, its influence on application dynamics can optimize the overall effectiveness of emulsion-based ointments against bacterial or fungal growth.[35],[36]

4. CONCLUSION

From the present investigation, it was concluded that the formulated ointment from *Cinnamomum zeylanicum* leaves extract with emulsion base has better option to people with minimum skin irritation than that of hydrocarbon base which has shown very poor antimicrobial activity. In our investigation, F5 showed a 200 µg/mL MIC against E. coli and S.aureus, however F10 showed a more powerful antibacterial activity with a 150 µg/mL MIC against the same bacterial strains. The MIC of the F5 and F10 formulations for all fungal strains reported is 200 µg/mL. It can be formulated and marketed as an herbal formulation with furtherstudies and tests and can be used for wound infection since it has shown a good antibacterial, antifungal.

5. Data Availability

The data used to support the findings of this study are available from the corresponding authorupon request.

6. Conflict of interest

The authors declared no conflict of interest.

7. Funding

No funding was used in this study.

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9. Authors' contribution

Conceptualizing, method development, docking analysis, original draft writing, and article searching were done by Prakash Babu Dahal and Bipin Rajbanshi. Second draft preparation, data analysis was done by Sudishna Khanal, Nivya N, Amit Kumar Gupta. Editing, review, supervision, and finalizing by Shankar Thapa.

10. Supplementary Materials

Table 1S: Ingredients used to prepare hydrocarbon base, Table 2S: Formulations from hydrocarbon base, Table 3S: Ingredients used to prepare emulsion base, Table 4S: Formulations from emulsion base, Table 5S: Observation table for consistency test, Table 6S: Observation table for stability test , Figure 1S: T.S. of Cinnamon leaves, Figure 2S: Antibacterial activity of herbal ointment, Figure 3S: Antifungal activity of herbal ointment, Figure 4S: Well plate for antibacterial activity of herbal ointment, Figure 5S: Well plate for antifungal activity of herbalointment.

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