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Phytochemical analysis and antimicrobial potential of Himalayas *Zanthoxylum armatum essential oil infused herbal cream.*

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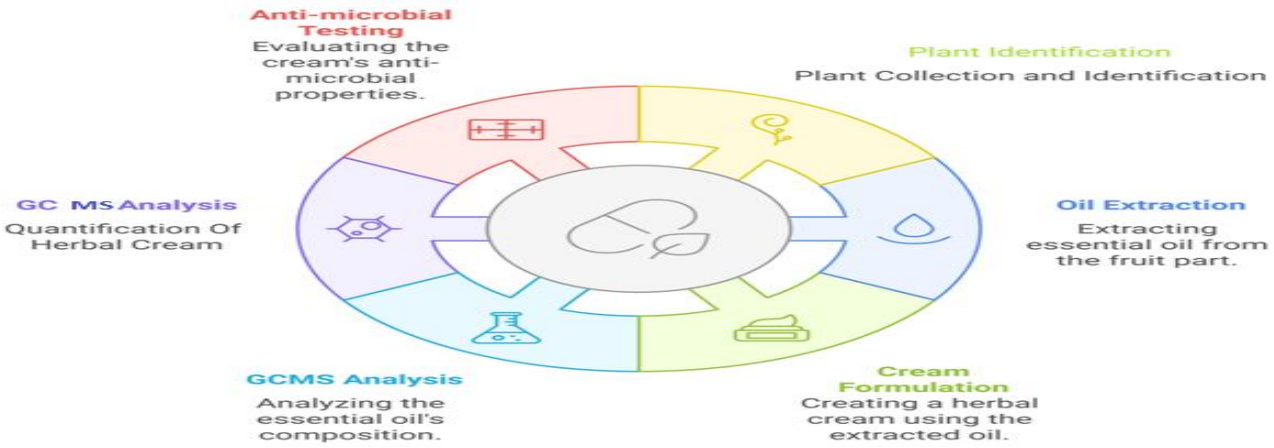
Abstract:*Background: Zanthoxylum armatum, a medicinal plant native to the Himalayas, is traditionally used for its antimicrobial properties. This study investigates the antimicrobial potential of an infused herbal cream formulated with the essential oil extracted from the fruit of Z. armatum.*
Methods: The essential oil was obtained through hydrodistillation using a Clevenger apparatus and subsequently characterized by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. To determine its phytochemical profile. The formulated herbal cream was analyzed using GC-MS to confirm the presence and stability of bioactive constituents. The antimicrobial efficacy of the herbal cream was evaluated against Candida albicans (for antifungal activity) and Staphylococcus aureus (for antibacterial activity) using Cylinder Plate Method microbiological assays.
Results: GC-MS analysis revealed the presence of major biologically active constituents, including Linalool (60.22%) and Limonene (30.52%). GC-MS confirmed the retention of essential oil constituents in the herbal cream. Most phytochemicals were retained in the cream formulation with slight reduction, except α-Thujene and α-Pinene, which were absent post-formulation. Antimicrobial testing demonstrated that the herbal cream exhibited notable antibacterial activity against Staphylococcus aureus (13 mm) comparable to Mupirocin. It also showed antifungal activity against Candida albicans (10 mm) relative to Miconazole cream. indicating potent antifungal and antibacterial activity.
Conclusion: The study highlights the potential of Z. armatum essential oil as an effective antimicrobial agent in topical formulations. The infused herbal cream exhibited promising antifungal and antibacterial activity, suggesting its potential application in managing microbial infections. Further studies on formulation optimization and clinical efficacy are recommended.

Keywords*: Zanthoxylum armatum, essential oil, infused herbal cream, antimicrobial activity, GC-MS, Candida albicans, Staphylococcus aureus.*

Graphical Abstract



INTRODUCTION:

Antimicrobials are among the most impactful medical advancements, helping to manage infectious diseases that pose a threat to human health. The word "antimicrobial" originates from Greek, combining "anti" (against), "mikros" (small), and "bios" (life). These substances work by either eliminating microorganisms or preventing their growth. Antibiotics, a specific type of antimicrobial, are naturally produced by certain microorganisms to inhibit or kill other microbes [1]. Since their introduction, antimicrobials have played a vital role in modern medicine. Sulfonamides were first used in 1937, followed by penicillin in 1942 for treating streptococcal infections and streptomycin in 1944 for tuberculosis. These discoveries have been among the most important medical advancements, greatly lowering the rates of infection-related illness and death. However, despite their effectiveness, the use of antimicrobial drugs can contribute to the emergence of resistant microorganisms over time [2]. Experts predict that by 2050, antimicrobial resistance (AMR) and infectious diseases could lead to 10 million deaths worldwide. Antimicrobial-resistant organisms (AMROs) are spreading and evolving more rapidly than new treatments are being developed. This rising challenge emphasizes the urgent need to combat AMR as a critical global health concern [3]. A frequently encountered and clinically important bacterial pathogen is *Staphylococcus aureus*. It is present in both hospital and community environments, leading to various health issues. However, treatment has become more Particularly challenging due to the rise of multidrug-resistant strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), which reduce the effectiveness of traditional antibiotics [4]. *Staphylococcus aureus* is a Gram-positive bacterium that takes on a purple color when subjected to a Gram stain test. It has a spherical shape (cocci) and usually forms clusters that look like bunches of grapes. It is a common bacterial infection that spreads easily and often causes minor skin conditions. However, it can also lead to severe and invasive infections, affecting hundreds of thousands to millions of people globally each year [5]. *Staphylococcus aureus* can cause skin infections such as boils, abscesses, and wound infections, which are usually not life-threatening but may lead to pain and discomfort. These infections are widespread, affecting millions of individuals annually in the U.S., and are considered a significant public health issue due to their frequent occurrence [6]. It is also associated with development of atopic dermatitis [7].

On the other hand, *Candida albicans* is an opportunistic fungal pathogen that resides as a commensal in the human mucosa but it can lead to infections ranging from mild cases of oral and vaginal candidiasis to severe, life-threatening systemic candidiasis. in immunocompromised individuals [8]. The increasing incidence of antifungal resistance in *C. albicans* infections, particularly against azole-based drugs, poses a serious clinical challenge [9]. Azole antifungal resistance has been most thoroughly investigated in *Candida albicans*. A key resistance mechanism in this species involves point mutations in the ERG11 gene. This gene encodes an enzyme that azole drugs typically target to disrupt the fungal cell's growth. However, when mutations develop in ERG11, they can alter the shape of the enzyme, reducing the drug's ability to bind to it properly. Consequently, the effectiveness of azole antifungals decreases, allowing the fungus to continue growing despite treatment [10]. A recent study investigated 63 clinical isolates of *Candida albicans* that showed resistance to the antifungal drug fluconazole. The researchers analyzed mutations in the ERG11 gene, which is important for the fungus's response to this medication. They found that 55 of these isolates had at least one mutation in ERG11, leading to changes in the amino acid sequence of the enzyme it encodes. These alterations can interfere with the drug's ability to bind and work effectively. Notably, the study also identified nine previously unreported amino acid changes, indicating that *C. albicans* can develop drug resistance through several different and newly emerging mechanisms [11]. An uncommon mechanism by which *Candida albicans* resists azole antifungal drugs involves the loss or deactivation of the ERG3 gene. This gene encodes the enzyme Erg3p, which is involved in the final steps of producing ergosterol, a vital component of the fungal cell membrane. When azoles are used, non-toxic sterol intermediates tend to accumulate. Under normal conditions, Erg3p converts these intermediates into toxic sterols that damage the fungus. However, if the ERG3 gene is inactive, these harmful sterols are not produced, allowing the fungus to survive. This type of

azole resistance is rare and has been observed in only a limited number of clinical isolates [12], [13]. Resistance is more common in patients with long-term or repeated azole exposure.

Pathogenicity

Staphylococcus aureus.

It is a facultative anaerobic, Gram-positive coccus that exhibits significant pathogenic potential in humans. Its ability to cause disease stems from a sophisticated arrangement array of pathogenic traits that promote host colonization, evasion of immune response along with tissue injury [4]. One of the key immune evasion mechanisms is mediated through the action of protein A, which attaches to the Fc region of IgG antibodies, the bacterium inhibits opsonization and subsequent phagocytosis. [14]. The bacterium also expresses adhesion to host surfaces is mediated by surface-associated proteins such as clumping factors (ClfA, ClfB), fibronectin-binding proteins (FnBPs), and proteins that bind to collagen, which mediate attachment toward the host system tissues and indwelling medical devices [15]. Exotoxins, which are harmful proteins released by *Staphylococcus aureus*, play a big role in how dangerous it is. One of them, alpha-toxin, damages human cells by creating holes in their membranes.

Another toxin known as Panton-Valentine leukocidin (PVL) targets and kills white blood cells. that normally fight infections. The bacteria also produce enterotoxins, which act in a strong way on the immune system and can cause food poisoning. In severe cases like toxic shock syndrome, the TSST-1 toxin triggers an extreme immune reaction by overstimulating immune cells, which can lead to serious illness [16]. *Staphylococcus aureus* also produces tissue-degrading enzymes such as coagulase, which induces clot formation around the bacterial colony, and hyaluronidase, which breaks down connective tissue, facilitating deeper invasion [17]. Additionally, Its tendency to establish biofilm communities on biotic and abiotic surfaces enhances reduced susceptibility to antimicrobial agents and host defenses, particularly in chronic and device-related infections [18]. The rise of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has further elevated its clinical importance, as these strains often exhibit resistance to multiple classes of antibiotics while maintaining high levels of virulence [19].

Candida albicains

Candida albicans is a common fungal species that normally survives harmlessly within the human body, especially in places like the mouth, gut, and skin. However, it can cause infections when a person's immune system is weak or when the natural balance of microbes is disturbed. Its ability to cause disease is mainly due to its flexibility in changing shape, its production of tissue-damaging enzymes, its ability to stick to body surfaces, and its skill in avoiding the immune system [20]. A central feature of *C. albicans* virulence is its ability to switch between yeast, pseudohyphal, and hyphal forms. This morphogenetic transition is critical for tissue invasion, biofilm formation, and evasion of host immune responses [21]. The hyphal form, in particular, facilitates deeper penetration into epithelial and endothelial barriers.

The pathogen also expresses a variety of adhesins such as agglutinin-like sequence (Als) proteins, which allow adherence to host cells and abiotic surfaces. This adhesion is a prerequisite for colonization and subsequent invasion [22]. *Candida albicans* secretes a range of hydrolytic enzymes, including aspartyl proteinases (Sap family), phospholipases, and lipases, which degrade host tissues and promote dissemination. These enzymes contribute to damage of mucosal surfaces and systemic spread in invasive candidiasis [23]. Immune evasion is also a crucial aspect of its pathogenicity. *C. albicans* can avoid recognition by masking pathogen-associated molecular patterns (PAMPs), and it can survive inside phagocytic cells by resisting oxidative stress and modulating host signaling pathways [24].

Epidemiology

Staphylococcus *aureus*

Staphylococcus aureus is a widely distributed bacterial species present all over the world and is known to cause many types of infections in both hospitals and communities. It often lives harmlessly on the skin, inside the nose, and on other body surfaces in healthy people. About 20% to 30% of people carry this bacterium in their noses without showing any signs of illness. However, having *S. aureus* on the body can increase the risk

of developing infections, especially in people who are undergoing surgery, have weakened immune systems, or use medical devices like catheters or implants [25].

This bacterium (*Staphylococcus aureus*) is a common cause of many serious infections, including skin infections and Severe conditions like bloodstream infections, pulmonary infections, and bone involvement (osteomyelitis), and heart infections (endocarditis) [26] . In hospitals, it is one of the main germs responsible for infections that patients get during their stay, especially after surgeries or when using medical devices like catheters. A major concern is the rise of MRSA, a resistant form of *S. aureus* that shows limited susceptibility to commonly prescribed antibiotics. Both types—those picked up in hospitals (HA-MRSA) and those acquired in the community (CA-MRSA)—are causing serious illness and even death around the world [27].

Epidemiological surveillance has shown geographical variability in resistance patterns, with higher MRSA prevalence in certain regions, particularly in Asia and parts of the Americas. Effective infection control practices and antibiotic stewardship remain essential in limiting transmission and managing outbreaks in healthcare and community settings [28].

Candida albicans

Candida albicans is a widespread fungus that can cause infections, especially when the body's defenses are weak. It is normally present in the human body, living harmlessly in places like the mouth, digestive system, vagina, and on the skin. In healthy people, it usually doesn't cause any problems. However, if a person's immune system becomes weak or if the natural balance of microbes in the body is disrupted (such as by antibiotics), *Candida albicans* can overgrow and lead to infections. These infections can affect the surface of the body or become more serious and spread inside the body [29].

C. albicans is still the most common fungus that causes candidiasis, a type of yeast infection seen around the world. It is responsible for most cases of infections in the mouth and throat (oropharyngeal), food pipe (esophageal), vagina, and serious internal infections. People in hospitals are at higher risk, especially those in intensive care units (ICUs), those receiving chemotherapy, or those being treated with strong antibiotics. In these patients, the fungus can enter the bloodstream, leading to a condition called candidemia, which is very dangerous. This type of infection can be life-threatening, with death rates ranging from 30% to 60%, depending on how sick the patient is and how quickly treatment is started [30].

Although other types of *Candida*, like *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*, are being found more often in recent years, *Candida albicans* is still the most common type seen in many parts of the world. Studies show that the types of *Candida* and their resistance to antifungal medicines can vary depending on the location and healthcare facility. This highlights the need for regular monitoring and careful use of antifungal drugs to prevent the spread of resistant strains [31].

Role of Plant in disease:

The northern Indian Himalayan region, though characterized by a harsh climate and a short growing season, is rich in medicinal flora. The Indian Trans-Himalayan mountain range, in particular, sustains limited vegetation. This region is home to more than 8,000 species of flowering plants (angiosperms), 44 species of cone-bearing plants (gymnosperms), and around 600 species of ferns and their allies (pteridophytes). Significantly, 1,748 of these plant species are recognized for their medicinal value [32]. Among them *Zanthoxylum armatum*, Belonging to the Rutaceae family, is regarded as a highly significant medicinal plant owing to its notable therapeutic benefits. Also known as Indian Prickly Ash, Nepal Pepper, or the Toothache Tree, it is very widespread in India, covering almost every part from Kashmir to Bhutan, up to elevations of 2,500 meters and more. This species is also found in Himalayan ranges of northeastern India and extends its reach to many more countries like China, Taiwan, Nepal, Philippines, Malaysia, Pakistan, and Japan, often thriving at elevations between 1,300 and 1,500 meters. [33]. Its essential oils and extracts are known to exhibit significant antimicrobial properties, due to the existence of bioactive chemical entities like alkaloids, flavonoids, and terpenoids. Several studies have demonstrated its efficacy effective toward various pathogenic bacteria and fungi. Phytochemical studies have shown that *Zanthoxylum armatum* contains a wide range of secondary metabolites, including alkaloids (such as berberine), flavonoids, lignans, terpenoids, and essential

oils. These bioactive constituents are largely responsible for the plant's strong antimicrobial properties. The essential oils extracted from its fruits and leaves have exhibited Broad-spectrum antimicrobial potential, capable of inhibiting both types of bacteria and a diverse group of fungal species. Several investigations have highlighted its significant inhibitory effects against clinically important pathogens [34]. In vitro studies indicate that the antimicrobial action of *Zanthoxylum armatum* may work through multiple mechanisms, including damaging microbial cell walls, blocking nucleic acid synthesis, and altering membrane permeability. These effects are largely attributed to the combined activity of terpenoids and phenolic compounds. Due to the growing threat of antibiotic resistance, An increasing focus has been placed on discovering plant-derived antimicrobial agents, and *Z. armatum* Appears to be a strong contender for the formulation of new therapeutic approaches [35]. In addition, essential oils derived from *Zanthoxylum armatum* have demonstrated significant synergistic antimicrobial effects when used alongside standard antibiotics. This interaction may help reduce the necessary dosage of conventional drugs, thereby minimizing associated side effects [36].

MATERIALS AND METHODS

Collection and identification of Material:

The fruits of *Zanthoxylum armatum* DC. were collected from the Chakrata region of Uttarakhand, India. A plant taxonomist confirmed the identity of the specimen, and the corresponding voucher specimen was submitted for future documentation.

Essential Oil Extraction:

The collected fruits were shade-dried and coarsely powdered. Essential oil was extracted using hydrodistillation with a Clevenger-type apparatus for 3–4 hours. The oil obtained was separated and stored in an amber-colored glass vial at 4 °C until further analysis.

Cream Formulation:

Table 1: Composition of cream.

Additives	F1 (g)	F2 (g)	F3 (g)
Glycerol monostearate	1.5	1.2	1.0
Stearic acid	2.0	2.5	1.5
Tween 80	1.0	0.8	1.5
Light liquid paraffin	3.0	2.5	2.5
Carbopol 940	0.5	0.6	0.6
EDTA	0.05	0.05	1.0
Sodium benzoate	0.1	0.1	1.0
Glycerin	3.0	4.0	2.5
Zanthoxylum armatum oil	1.0	1.0	5.0
Distilled water (q.s.)	up to 50 g	up to 50 g	Up to 50g

A water-based herbal cream was formulated using essential oil extracted from *Zanthoxylum armatum* fruits via hydrodistillation. The oil phase consisted of stearic acid, light liquid paraffin, glycerol monostearate, and *Zanthoxylum armatum* essential oil. Tween 80 was used as a non-ionic emulsifying agent to stabilize the emulsion. The aqueous phase included distilled water, glycerin as a humectant, Carbopol 940 as a gelling and thickening agent, and disodium EDTA as a chelating agent. Sodium benzoate was incorporated as a

preservative. Both phases were heated separately to around 70–75 °C and then mixed with continuous stirring to form a uniform cream. The final formulation was allowed to cool at room temperature and stored in airtight containers for further evaluation. The formula for the cream is given in table 1.

GC-MS Analysis of isolated Essential Oil and formulated Antimicrobial cream

The essential oil was found to be predominantly composed of Linalool (60.22%) and Limonene (30.52%), which are known for their strong antimicrobial and aromatic properties. Other notable constituents included Myrcene (2.18%), Methyl-E-Cinnamate (4.82%), and Trans-Caryophyllene (0.65%). Minor constituents such as α-Thujene (0.12%), α-Pinene (0.16%), β-Pinene (0.16%), Sabinene (1.20%), γ-Terpinene (0.21%) .

The GC-MS analysis of the cream formulated using the essential oil of *Zanthoxylum armatum* showed the retention of most key phytochemicals, albeit at altered concentrations. Linalool (60.08%) remained the dominant component, followed by Limonene (22.70%), indicating good stability of these two major constituents during the formulation process. Myrcene (1.25%), Methyl-E-Cinnamate (1.97%), Sabinene (0.50%), Trans-Caryophyllene (0.33%), γ-Terpinene (0.34%), and β-Pinene (0.11%) were also present in measurable amounts.

Notably, Benzoic acid (1.06%) was detected in the cream, though absent in the original essential oil. This was attributed to chemical interactions between the oil and excipients during formulation or possible degradation by-products. Compounds such as α-Thujene and α-Pinene were not detected in the final cream, possibly due to volatilization or transformation during the emulsification and heating processes.

Evaluation Of Cream.

pH Determination of the Cream:

The pH of the cream was checked using a digital pH meter, which was first calibrated with standard buffer solutions to ensure accuracy. About 0.5 g of the cream was gently weighed and blended into 50 ml of distilled water. The pH of the resulting mixture was then measured and noted.

Viscosity Measurement:

To check the thickness and flow of the cream, its viscosity was measured using a Brookfield viscometer, operated at 100 rpm with spindle number 7.

Dye Test:

To identify the type of emulsion, a small amount of scarlet red dye was mixed with the cream. A drop of this mixture was then placed on a microscope slide, covered with a cover slip, and observed under the microscope. If the cream is an oil-in-water (O/W) type, red globules will be visible in a clear background. On the other hand, if it is a water-in-oil (W/O) emulsion, the globules will appear clear while the background takes on a red color.

Homogeneity:

The formulations were assessed for homogeneity through both visual inspection and tactile evaluation.

Appearance:

The cream's color, pearlescence, and texture were observed and graded based on their uniformity and smoothness.

After-Feel:

The emollient properties, slipperiness, and the amount of residue remaining on the skin after applying a fixed quantity of cream were evaluated.

Type of Smear:

Following application, the nature of the film or smear left on the skin was examined.

Removal:

The ease with which the cream could be removed was tested by washing the applied area with tap water.

Irritancy Test:

A small area of about 1 cm^2 was marked on the back of the left hand. The cream was gently applied to this spot, and the time of application was noted. The area was then checked regularly over the next 24 hours for any signs of irritation, redness, or swelling, and observations were recorded.

Antimicriobial potential of Formulated herbal cream:

Selection of Microbes : For the evaluation of antimicrobial potential, two clinically relevant microorganisms were selected: *Staphylococcus aureus* (Gram-positive bacteria) and *Candida albicans* (fungus). *Staphylococcus aureus* is a common pathogen responsible for a wide range of skin and soft tissue infections and is known for its resistance to multiple antibiotics, making it a suitable model for assessing antibacterial efficacy. *Candida albicans* was selected for antifungal evaluation due to its role as an opportunistic fungal pathogen frequently associated with cutaneous, mucosal, and systemic candidiasis. The selection of these organisms provides a relevant biological model to assess the broad-spectrum antimicrobial potential of *Zanthoxylum armatum* essential oil, particularly in the context of herbal cream formulations intended for topical application.

Sample Used:

Name of the Sample

Reference Standard – Miconazole Cream (*Staphylococcus aureus*)

Reference Standard – Mupirocin Cream (*Candida albicans*)

Test Herbal Cream HF- Essential oil of *Zanthoxylum armatum* based herbal cream (*Staphylococcus aureus, Candida albicans*)

Culture used:

Name of the Culture	ATCC Number
Staphylococcus aureus (*S. aureus*)	ATCC 6538
Candida albicans (*C. albicans*)	ATCC 10231

Table2: Name of Culture used.

Media Used:

Media	Lot No.	Make
Muller Hinton Agar (MHA)	0000509408	Hi-media
Sabroud Dextrose Agar (SDA)	0000508784	Hi-media

Table 3: Name of media used

Muller Hinton Agar (MHA) for *S. aureus*

Sabroud Dextrose Agar (SDA) for *C. albicans*

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

Method: Cylinder Plate Method

Method for:

1) Media Preparation: Muller Hinton Agar (MHA) was used for determining the activity of S. aureus. Sabroud Dextrose Agar (SDA) was used for determining the activity of C. albicans. Media was prepared as per Manufacturer's Instruction. The media was then autoclaved at 121˚C temp. & 15lbs pressure for 20 minutes.

2) Standard Preparation: Miconazole Cream Take 1 gm of cream sample 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.

3) Standard Preparation: Mupirocin Cream Take 1 gm of cream sample 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.

4) 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:

For Anti-microbial activity: Cool down sterile media MHA, SDA up to 55°C and add 10µl of bacterial cultures in to MHA flasks, 10µl of yeast culture in to SDA 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 Standard & test samples in respected labeled well.When samples were diffused completely, incubate MHA plate into Bacteriological incubator at 35°C for 24 hours. SDA plate into Biological Oxygen Demand incubator at 25°C for 48 hours observe the zone of inhibition.

Observations:

C. albicans

Figure 1: Zone of inhibition of formulated cream and Standard cream (Antibacterial)

S. aureus

Figure 2: Zone of inhibition of formulated cream and Standard cream (Antifungal)

Results:

Quantification of phytochemicals present in Essential oil VS Formulated Cream by GCMS.

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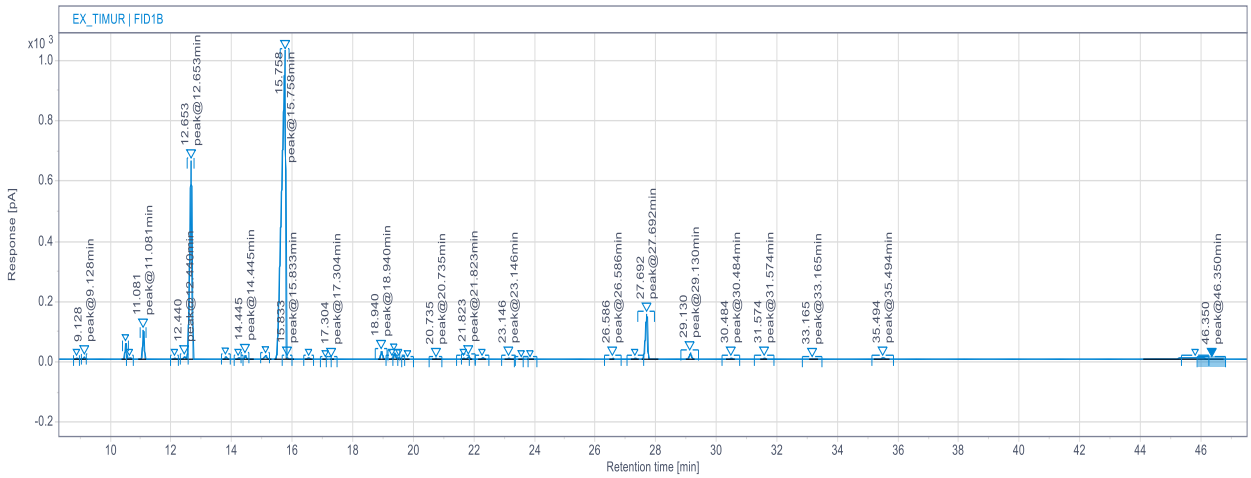

Figure 3: Typical chromatogram (GC-MS on HP-5MS, 30 m) of isolated essential oil.

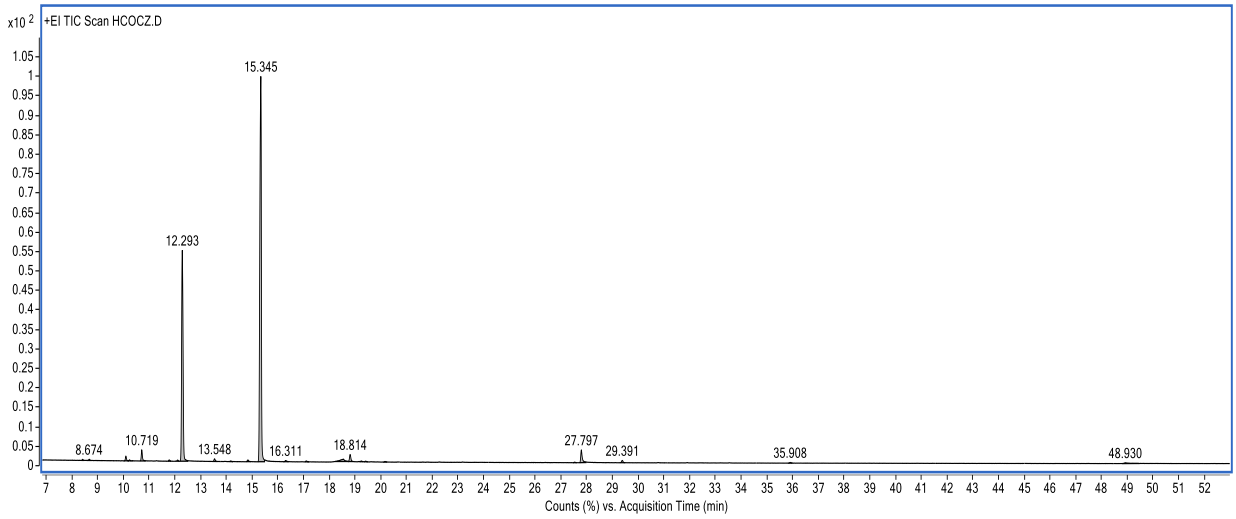

Figure 4. Typical chromatogram (GC-MS on HP-5MS, 30 m) of Herbal cream based on *Zanthoxylum armatum* essential oil

Sr. No.	Phytochemical	Retention Time (Oil – Cream)	Presence Isolated essential oil	Presence in Herbal Cream	Interpretation
1	α-Thujene	9.128 min (Oil only)	0.12%	Absent	Volatile monoterpene lost in cream due to instability
2	α-Pinene	11.081 min (Oil only)	0.16%	Absent	Not retained; lost during cream preparation
3	β-Pinene	10.719 min (Oil) – Cream RT included in same range	0.16%	0.11%	Retained in smaller quantity; moderately stable

4	Limonene	12.653 – 12.293 min	22.52%	30.70%	Major active; well retained and slightly concentrated
5	Myrcene	14.445 – 13.548 min	2.18%	1.25%	Retained partially; some loss due to formulation conditions
6	Linalool	15.758 – 15.345 min	60.22%	60.84%	Main bioactive; excellent retention
7	γ-Terpinene	17.304 – 16.311 min	0.21%	0.34%	Retained and slightly increased; thermally stable
8	Sabinene	18.940 – 18.814 min	1.20%	0.50%	Significant loss, but still detectable
9	trans-Caryophyllene	20.735 – 27.797 min	0.65%	Present	Long-chain sesquiterpene preserved well
10	Methyl cinnamate (E)	21.823 – 29.319 min	4.82%	Present	Delayed elution; retained in cream matrix
11	Benzoic acid	23.146 – 35.980 min	Not listed	1.06%	Possibly added or formed; not present in oil
12	Unknown – high boiling component	26.586 – 48.930 min	Trace	Present	Likely benzoates or thermal derivatives
13	Unknown (lost volatile)	27.586 min (Oil only)	Small	Absent	Not stable in formulation
14	Unknown (volatile)	29.130 min (Oil only)	Minor	Absent	Evaporated or degraded
15	Unknown	31.574 min (Oil only)	Minor	Absent	Heat-sensitive compound lost
16	Unknown	33.165 min (Oil only)	Minor	Absent	Not compatible with cream base
17	Unknown high molecular compound	35.494 min (Oil only)	Minor	Absent	Likely too large or non-polar for cream matrix

Table 4: Retention time of essential oil and formulated cream

pH Determination of the Cream:

The cream had a pH between 6.5 and 6.8, which is ideal for the skin's natural balance. All the formulations showed pH levels that were close to what the skin normally requires, making them gentle and safe for regular use. (Table 4)

Formulation	pH
F1	6.8
F2	6.5
F3	6.7

Table 5: pH of cream

Viscosity:

The cream's viscosity was found to be between 27,019 and 27,023 cps, suggesting it spreads easily without much effort. Among all the samples, formulations F1 and F3 were noticeably smoother and easier to apply than the others.

Formulation	Viscosity (in cps)
F1	27019
F2	27023
F3	27021

Table 6: Viscosity of Cream

Dye Test: The dye test indicated that all the cream formulations were oil-in-water (O/W) type emulsions.

Irritancy test: During the irritancy test, formulations F1, F2, and F3 did not cause any redness, swelling, inflammation, or irritation, suggesting that they are safe and gentle for use on the skin.

Formulation	Irritant	Erythema	Edema
F1	NIL	NIL	NIL
F2	NIL	NIL	NIL
F3	NIL	NIL	NIL

Table 7: Type of adverse effect of formulations

Homogeneity:

All the formulations showed a uniform distribution of extracts throughout the cream, as confirmed by their consistent appearance and feel upon touch (Table 8).

Appearance:

The creams maintained their original color even after prolonged storage, indicating good stability (Table 8).

After-feel:

The creams demonstrated desirable qualities such as smoothness, softness, and minimal residue left on the skin after applying a fixed amount (Table 8).

Type of Smear:

Upon application, the creams formed a non-greasy layer on the skin, enhancing user comfort (Table 8).

Removal:

The formulations were easily washable with plain tap water, leaving no sticky residue behind (Table 8).

Days	Temperature	Formulation	pH	Homogeneity	Appearance	Spreadability	After feel	Type of smear	Removal
0	RT	F1	6.6	**	NCC	**	E	NG	ES
0	RT	F3	6.5	**	NCC	**	E	NG	ES
0	40 °C	F1	6.7	*	NCC	**	E	NG	ES
0	40 °C	F3	6.6	**	NCC	**	E	NG	ES
5	RT	F1	6.8	**	NCC	**	E	NG	ES
5	RT	F3	6.5	**	NCC	**	E	NG	ES
5	40 °C	F1	6.7	*	NCC	**	E	NG	ES
5	40 °C	F3	6.5	**	NCC	**	E	NG	ES
10	RT	F1	6.6	**	NCC	**	E	NG	ES
10	RT	F3	6.6	**	NCC	**	E	NG	ES
10	40 °C	F1	6.8	*	NCC	**	E	NG	ES
10	40 °C	F3	6.7	**	NCC	**	E	NG	ES

Table 8: Physical parameter of F1 and F3 cream on room and accelerated temperature

Abbreviation: RT: Room Temprature, **: Good, *: Satisfactory, P: Pearlescent, E: Emollient, NG: Non greasy, ES: Easy, NCC: Not change in colour

Zone of Inhibition of Herbal Cream Versus Marketed Standard:

Sr. No.	Name of sample	Zone of Inhibition	
		S. aureus	*C. albicans*
1	Blank (MeOH: DMSO)	NZI	NZI
2	Ref. Std. – Mupirocin Cream	27 mm	NA
3	Ref. Std. – Miconazole Cream	NA	20 mm
4	Herbal Cream - HF	13mm	10mm

Table 9: Zone of Inhibition

Abbreviation: NZI: No Zone of Inhibition, NA: Not applicable

Compared to the reference standard Mupirocin cream, the in-vitro study of the herbal cream HF demonstrated good antibacterial activity against Staphylococcus aureus, with a zone of inhibition of 13 mm. Compared to the reference standard antifungal Miconazole cream, the herbal cream HF demonstrated antifungal activity against Candida albicans, with a zone of inhibition of 10 mm.

DISCUSSION

The herbal face cream developed in this study was an oil-in-water (O/W) emulsion, which means it could be easily washed off with plain water—making it more convenient and comfortable for everyday use. Using the phase inversion technique during preparation helped create a finer internal phase, which contributed to better physical stability over time. The creams maintained a steady pH and had a smooth, uniform texture. They felt soft and non-greasy on the skin and could be removed easily after use. Stability testing showed that the formulations were gentle and safe, with no signs of irritation or allergic response. Observations related to viscosity and shear behavior gave useful information about how easily the cream could be processed and applied. Although a slight initial breakdown in structure was noted, the viscosity remained stable, confirming that the cream held up well during use and handling.

Microorganisms such as bacteria and fungi are major contributors to a wide range of infectious diseases in humans. Pathogenic microbes can invade the body, disrupt normal cellular functions, and produce toxins that lead to illness. Common bacterial infections include skin infections, respiratory diseases, and food poisoning, while fungi are responsible for conditions like candidiasis and ringworm. The rise of antibiotic-resistant strains has further intensified the challenge of managing microbial infections, highlighting the urgent need for safe and effective antimicrobial agents.

In this context, *Zanthoxylum armatum* essential oil presents a promising natural alternative due to its potent antimicrobial properties. The oil exhibits significant activity against a variety of microbial strains, which can be attributed to its rich phytochemical profile. Major constituents such as linalool, limonene, trans caryophyllene, and are known for their ability to disrupt microbial cell membranes, interfere with protein synthesis, and inhibit enzyme activity. These bioactive compounds collectively contribute to the oil's ability to inhibit the growth of both gram-positive bacteria like *Staphylococcus aureus* and fungal pathogens such as *Candida albicans*. Thus, the essential oil of *Z. armatum* holds great potential as a natural antimicrobial agent for use in herbal formulations aimed at treating skin infections and other microbial conditions.

Therefore, the essential oil of *Zanthoxylum armatum* appears to be a promising natural ingredient for developing antimicrobial creams. In our study, formulations F1 and F3 demonstrated greater physical stability over time, while the other formulations showed signs of emulsion breakdown during extended storage. Among the stable formulations, F1 and F3 maintained a consistent pH, exhibited uniform texture, felt smooth and non-greasy on the skin, and were easy to wash off after application. Additionally, these formulations were well-tolerated, showing no signs of skin irritation or allergic responses, indicating their suitability for topical use.

CONCLUSION

The present study highlights the promising potential of *Zanthoxylum armatum* essential oil in the development of an effective herbal antimicrobial cream. The formulated creams, particularly F1 and F3, demonstrated desirable physical attributes, pH stability, and safety for topical application. In vitro antimicrobial evaluation showed that the herbal formulation HF exhibited noteworthy antibacterial and antifungal activity, with a zone of inhibition of 13 mm against *Staphylococcus aureus* and 10 mm against *Candida albicans*, comparable to standard Mupirocin and Miconazole creams, respectively.

GC-MS analysis revealed the presence and stability of key bioactive constituents during formulation. Major compounds such as linalool (60.22% in oil; 60.84% in cream) and limonene (22.52% in oil; 30.70% in cream) demonstrated excellent retention and are likely responsible for the observed antimicrobial effects. Several minor monoterpenes like α-thujene and α-pinene were lost during cream preparation, indicating their instability. However, thermally stable components such as γ-terpinene, trans-caryophyllene, and methyl cinnamate were retained or even slightly increased, contributing to the cream's overall efficacy. Interestingly, benzoic acid and certain high-boiling or unknown components were detected only in the cream, possibly formed during formulation or due to interactions with the base.

Overall, the study confirms that *Zanthoxylum armatum* essential oil, when appropriately formulated, retains its antimicrobial activity and stability, making it a viable natural alternative to conventional synthetic topical agents for the treatment of skin-related microbial infections.

It can be concluded that the essential oil of *Zanthoxylum armatum*, when combined in varying ratios with suitable additives, offers multiple beneficial effects such as antibacterial, and antifungal activity. Considering the growing concern of microbial resistance, the development of this herbal antimicrobial cream represents a promising alternative to conventional topical agents. Among the tested formulations, F1 and F3 demonstrated greater physical stability, consistent pH, and overall safety for topical application. These results suggest that the selected composition of active extracts and cream base in F1 and F3 may serve as an effective and stable formulation for combating skin-related microbial infections.

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