

In-vitro Antibacterial Screening of an Ayurvedic Polyherbal Topical Formulation: Epi-Cure Gel

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Abstract

Background: Bacterial skin and wound infections caused by harmful microorganisms like *Staphylococcus aureus* and *Escherichia coli* are a significant health issue worldwide. Rising antimicrobial resistance due to prolonged and improper antibiotic use has led to a greater need for safer and effective alternative antimicrobial agents. Herbal and Ayurvedic topical formulations that include medicinal plants with antimicrobial and wound-healing properties may offer promising treatment options. **Aim:** To assess the antibacterial activity of Epi-Cure Gel against *Staphylococcus aureus* and *Escherichia coli* using the cylinder plate method. **Materials and Methods:** This in-vitro study took place at an AYUSH-approved Ayurvedic testing lab. Epi-Cure Gel is a polyherbal Ayurvedic formulation that includes *Rubia cordifolia*, *Curcuma longa*, *Terminalia chebula*, *Tinospora cordifolia*, *Pongamia pinnata*, and other traditional ingredients. We evaluated its antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) using Mueller Hinton Agar medium. We prepared the test sample with dimethyl sulfoxide, methanol, and distilled water, followed by sonication and reflux extraction. We measured antibacterial activity using the cylinder plate method according to Indian Pharmacopoeia 2014 guidelines. The zone of inhibition was measured after incubating at 35°C for 24 hours. **Results:** Epi-Cure Gel showed antibacterial activity against both tested microorganisms. The formulation created a zone of inhibition of 14 mm against *Staphylococcus aureus* and 12 mm against *Escherichia coli*. The blank control, made of methanol and dimethyl sulfoxide, showed no inhibitory activity. We observed stronger antibacterial activity against *Staphylococcus aureus*. **Conclusion:** This study showed that Epi-Cure Gel has considerable antibacterial activity against both gram-positive and gram-negative bacteria. The antibacterial action is likely due to the combined effects of the herbal and mineral ingredients that have antimicrobial and wound-healing properties. The results provide initial scientific evidence supporting the potential use of Epi-Cure Gel as a topical antimicrobial Ayurvedic formulation. Further studies on physicochemical standardization, additional microbiological testing, and clinical evaluation are needed.

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Introduction

Bacterial skin and wound infections are a significant global healthcare issue. They are often linked to harmful microbes like *Staphylococcus aureus* and *Escherichia coli*. These bacteria are commonly found in postoperative wound infections, cellulitis, abscesses, infected ulcers, and other skin conditions. *Staphylococcus aureus* is one of the most common gram-positive bacteria that cause skin and soft tissue infections. In contrast, *Escherichia coli* is an important gram-negative opportunistic pathogen that leads to wound contamination and delayed healing.¹ The rise of antibiotic resistance, caused by the careless and prolonged use of standard antibiotics, has greatly diminished the effectiveness of current treatments. Antibiotic-resistant strains lead to longer hospital stays, slower wound healing, frequent infections, higher healthcare costs, and increased illness. Thus, there is a pressing need to look for alternative antimicrobial agents that are effective, affordable, safer, and can overcome microbial resistance.²

Herbal and Ayurvedic remedies have been used for ages to manage wounds, skin diseases, inflammation, and infections.³ Ayurveda identifies many medicinal plants known for their wound healing, cleansing, antimicrobial, anti-inflammatory, and pain-relieving properties. Herbal topical treatments are becoming more recognized in both research and commerce because they come from natural sources, are better accepted by patients, have fewer side effects, and offer a wide range of pharmacological benefits.⁴ Medicinal plants contain various compounds such as flavonoids, alkaloids, tannins, terpenoids, glycosides, phenolic compounds, and essential oils that show antibacterial, antioxidant, anti-inflammatory, and wound healing effects. Numerous studies have shown that herbal preparations can effectively aid tissue regeneration, collagen production, new blood vessel formation, skin layer growth, and microbial control during the healing process.⁵

Although Ayurvedic topical treatments have a long history of use, there is still limited scientific validation and microbiological testing for many of

these formulations. It is crucial to standardize and assess herbal remedies based on evidence to ensure their quality, safety, effectiveness, and acceptance worldwide.⁶ In-vitro antibacterial tests are vital for the initial evaluation of herbal formulations against harmful microbes and provide scientific backing for their traditional uses. Modern microbiological methods, such as agar diffusion tests and cylinder plate techniques, are commonly used to assess the antimicrobial properties of herbal products. Experimentally validating Ayurvedic formulations can bridge the gap between traditional knowledge and modern scientific research, supporting their practical application in medicine.

Epi-Cure Gel is a polyherbal Ayurvedic topical formulation made from traditional ingredients like *Manjistha* (*Rubia cordifolia*), *Haridra* (*Curcuma longa*), *Padma* (*Prunus cerasoides*), *Haritaki* (*Terminalia chebula*), *Vibhitaki* (*Terminalia bellirica*), *Amalaki* (*Emblia officinalis*), *Vidanga* (*Embelia ribes*), *Kutki* (*Picrorhiza kurroa*), *Guduchi* (*Tinospora cordifolia*), *Karanja* (*Pongamia pinnata*), and *Tuttha* (Copper sulphate).⁷ These ingredients are recognized for their antimicrobial, anti-inflammatory, antioxidant, and wound healing properties. *Rubia cordifolia* has shown significant anti-inflammatory and wound healing effects,⁸ while *Curcuma longa* is known for its antibacterial and tissue regeneration properties.⁹ Therefore, the current study was conducted to assess the in-vitro antibacterial activity of Epi-Cure Gel against *Staphylococcus aureus* and *Escherichia coli*.

Material & Methods:

Study Design and Site of Study

The current in-vitro experimental study took place at Vasu Research Centre in Vadodara, Gujarat. This is an AYUSH-approved Ayurvedic testing lab. The study aimed to assess the antibacterial activity of Epi-Cure Gel against specific harmful microorganisms.

Composition and Preparation of Epi-Cure Gel

Table no. 1 Content of Epi-Cure Gel

Sr. No.	Ingredient	Botanical Name	Part Used
1	<i>Manjistha</i>	<i>Rubia cordifolia</i>	Rhizome
2	<i>Haridra</i>	<i>Curcuma longa</i>	Rhizome
3	<i>Haritaki</i>	<i>Terminalia chebula</i>	Fruit
4	<i>Vibhitaki</i>	<i>Terminalia bellirica</i>	Fruit
5	<i>Amalaki</i>	<i>Emblia officinalis</i>	Fruit

6	<i>Vidanga</i>	<i>Embelia ribes</i>	Leaf
7	<i>Guduchi</i>	<i>Tinospora cordifolia</i>	Stem
8	<i>Karanja</i>	<i>Pongamia pinnata</i>	Seeds
9	<i>Kutki</i>	<i>Picrorhiza kurroa</i>	Root
10	<i>Padma</i>	<i>Prunus cerasoides</i>	Bark
11	<i>Tuttha</i>	Copper sulphate	<i>Tuttha drava</i>

Epi-Cure Gel is a herbal Ayurvedic topical formula made from traditional ingredients found in Sushruta Samhita. It includes medicinal plants like *Manjistha* (*Rubia cordifolia*), *Haridra* (*Curcuma longa*), *Padma* (*Prunus cerasoides*), *Haritaki* (*Terminalia chebula*), *Vibhitaki* (*Terminalia bellirica*), *Amalaki* (*Emblia officinalis*), *Vidanga* (*Embelia ribes*), *Kutki* (*Picrorhiza kurroa*), *Guduchi* (*Tinospora cordifolia*), *Karanja* (*Pongamia pinnata*), and *Tuttha* (Copper sulphate), as listed in table no. 1. These ingredients are known for their wound healing, cleansing, anti-parasitic, anti-inflammatory, and pain-relieving properties.

All raw ingredients for Epi-Cure Gel were sourced from trusted suppliers and verified before use. Equal amounts of all herbal components, except for *Tuttha*, were ground into a coarse powder. Six parts of water were added and boiled, then filtered. The resulting liquid was mixed with the coarse powder and *Tuttha drava* and stirred well. The mixture was left overnight to allow for proper extraction and blending of active ingredients.

After 24 hours, filtered polyethylene glycol (PEG) was collected. Carbapol (1.8%) was added and let sit for another 24 hours to form the gel. Then, 3 mL of the PEG filtrate and 1% sodium benzoate were incorporated and mixed thoroughly to achieve the final gel with the right consistency and stability.

Microorganisms and Sample Preparation

Table no. 2 Test Microorganisms

Sr. No.	Microorganism	ATCC Number
1	<i>Staphylococcus aureus</i>	ATCC 6538
2	<i>Escherichia coli</i>	ATCC 8739

The antibacterial activity of Epi-Cure Gel was tested against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739), as shown in table no. 2. These bacterial strains were chosen because they are often linked to skin and wound infections. The

study used Mueller Hinton Agar (MHA) medium from Hi-media for the antibacterial assay. Fresh bacterial slants were rinsed with 10 mL of sterile normal saline solution to create a microbial suspension.

To prepare the test sample, about 2 g of Epi-Cure Gel was measured into a 150 mL flask. Then, 3 mL of dimethyl sulfoxide (DMSO), 7 mL of methanol, and 3 mL of distilled water were added to the flask. The mixture was sonicated for 10 minutes. After sonication, the sample was heated on a water bath at 90°C for 1 hour to ensure proper extraction of phytoconstituents. The extract was filtered using Whatman filter paper and concentrated to 5 mL to create the test sample for the antibacterial activity.

Antibacterial Activity Procedure

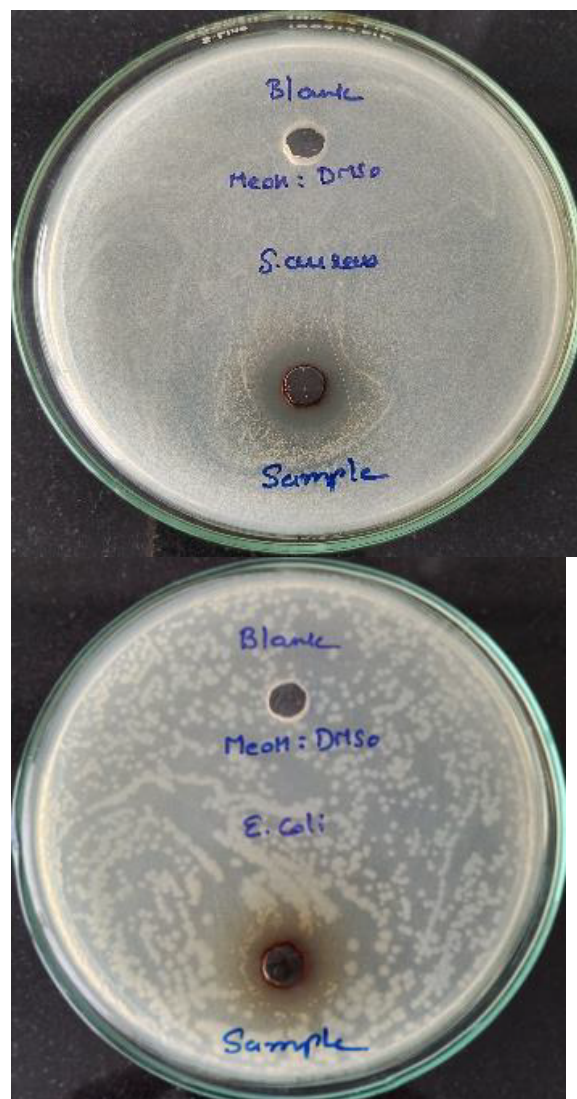
The antibacterial activity was evaluated using the cylinder plate method based on Indian Pharmacopoeia 2014 guidelines.¹⁰ Mueller Hinton Agar medium was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C under 15 lbs of pressure for 20 minutes to keep it sterile. The sterile medium was allowed to cool to around 55°C, and 10 µL of bacterial culture suspension was added to separate flasks. The inoculated media were mixed gently and poured into sterile petri plates using a sterile measuring cylinder. After the medium solidified, wells were created using a sterile borer at suitable distances.

The prepared test sample, blank solution, and standard were placed into their respective labelled wells. The plates were incubated in a bacteriological incubator at 35°C for 24 hours. After incubation, antibacterial activity was measured by the zone of inhibition around the wells in millimetres. If there was no inhibition, it was recorded as NZI (No Zone of Inhibition). Photographs of the inhibition zones for both microorganisms were taken during the study for observation and record-keeping.

Results

The antibacterial activity of Epi-Cure Gel was tested against *Staphylococcus aureus* and *Escherichia coli* using the cylinder plate method. The antibacterial effectiveness was measured by the diameter of the zone of inhibition around the wells containing the test sample after incubation at 35°C for 24 hours. The blank solution, made of methanol and dimethyl sulfoxide (DMSO), showed no inhibitory activity against either of the tested microorganisms.

Figure no. 1 *S. aureus*
Figure no. 2 *E. coli*



Clear zones of inhibition were seen around the wells with Epi-Cure Gel, showing the antibacterial potential of the formulation against both gram-positive and gram-negative bacteria. The formulation had an inhibitory effect on *Staphylococcus aureus*, with a zone of inhibition of 14 mm. Against *Escherichia coli*, the zone of inhibition was 12 mm as we seen in figure no. 1 and 2.

Table 3: Antibacterial Activity of Epi-Cure Gel

Sr. No.	Sample	Zone of Inhibition against <i>S. aureus</i>	Zone of Inhibition against <i>E. coli</i>
1	Blank (MeOH: DMSO)	NZI	NZI
2	Epi-Cure Gel	14 mm	12 mm

NZI: No Zone of Inhibition

The results showed that Epi-Cure Gel had more antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*. The larger zone of inhibition against *Staphylococcus aureus* indicates that gram-positive bacteria are more vulnerable to the plant compounds in the formulation. While there was lower activity against *Escherichia coli*, the formulation still effectively inhibited these gram-negative bacteria.

No zone of inhibition appeared in the blank control, confirming that the antibacterial effect came from the active ingredients in Epi-Cure Gel and not from the solvents used during preparation. The study suggests that Epi-Cure Gel has broad-spectrum antibacterial potential against common bacteria found in wounds.

Photographic evidence from the culture plates further supported the antibacterial effectiveness of the formulation, showing clear inhibition zones around the wells containing the test sample.

Discussion

This in-vitro study aimed to assess the antibacterial activity of Epi-Cure Gel against *Staphylococcus aureus* and *Escherichia coli* using the cylinder plate method. The study found that Epi-Cure Gel showed significant antibacterial activity against both bacteria, as seen by the distinct zones of inhibition around the wells with the test sample. The formulation displayed a 14 mm zone of inhibition against *Staphylococcus aureus* and a 12 mm zone against *Escherichia coli*, indicating greater activity against the gram-positive organism.

The observed antibacterial effects may arise from the combined action of several herbal and mineral ingredients in Epi-Cure Gel. The formulation includes various medicinal plants traditionally used in Ayurveda for wound cleansing, healing, infection prevention, pain relief, and skin conditions. The combination of different herbs may provide a wide range of effects due to various phytochemical constituents that work in multiple ways.

Manjistha (*Rubia cordifolia*), a key ingredient in Epi-Cure Gel, is recognized in Ayurvedic texts for its healing properties. Modern studies have highlighted its anti-inflammatory, antioxidant, antimicrobial, and wound-healing abilities.¹¹ The wound-healing effects of *Manjistha* may be linked to its role in collagen production, reducing oxidative stress, and promoting tissue repair. Similarly, *Haridra* (*Curcuma longa*) has strong antibacterial, anti-inflammatory, antioxidant, and tissue-repairing properties, mainly due to curcumin. Curcumin can inhibit bacterial growth, lower inflammation, and speed up wound healing.¹²

Other ingredients, like *Haritaki* (*Terminalia chebula*), *Vibhitaki* (*Terminalia bellirica*), and *Amalaki* (*Embellica officinalis*), are known for their antimicrobial, antioxidant, and wound-cleansing properties. These plants contain tannins and phenolic compounds, which may help inhibit

microbial growth and prevent wound contamination. *Guduchi* (*Tinospora cordifolia*) has immune-boosting, antimicrobial, and anti-inflammatory benefits, while *Vidanga* (*Embelia ribes*) is traditionally used for its effects against parasites. *Karanja* (*Pongamia pinnata*) and *Kutki* (*Picrorhiza kurroa*) also show antimicrobial and anti-inflammatory properties, potentially enhancing the formulation's effectiveness.

The formulation also includes *Tuttha* (Copper sulphate), known in Ayurvedic texts for its antibacterial properties. Copper compounds can kill a wide range of bacteria and may significantly contribute to the antibacterial effects seen in this study. The combination of herbal and mineral ingredients in Epi-Cure Gel likely creates a stronger antimicrobial effect.

The higher antibacterial activity against *Staphylococcus aureus* than *Escherichia coli* can be explained by differences in their cell walls. *Staphylococcus aureus*, as a gram-positive bacterium, has a simpler peptidoglycan cell wall, making it easier for the plant compounds to penetrate. In contrast, *Escherichia coli* has a gram-negative structure with an outer lipopolysaccharide membrane that acts as a barrier, limiting the entry of antimicrobial agents. This structural difference often results in lower susceptibility of gram-negative bacteria to herbal extracts and antimicrobial substances. The study findings align with typical patterns observed in many herbal antimicrobial studies.

No inhibition zone was found in the blank control with methanol and dimethyl sulfoxide, confirming that the antibacterial activity was due to the active ingredients in Epi-Cure Gel, not the solvents. The clear inhibition zones around the test wells showed that active compounds diffused into the agar medium, suppressing bacterial growth.

The cylinder plate method used in this study is a recognized microbiological test outlined in the Indian Pharmacopoeia for measuring antimicrobial activity. It is commonly accepted for screening antibacterial agents because it's simple, reproducible, and reliable for assessing bacterial inhibition. Using standard ATCC bacterial strains adds to the credibility and consistency of the study results.

The antibacterial activity shown by Epi-Cure Gel suggests its potential use in treating wound infections, skin disorders, and postoperative care. Since bacterial contamination and infection are key factors in delayed wound healing, antimicrobial formulations capable of lowering microbial load can significantly improve healing rates. Additionally, the anti-inflammatory and wound-healing properties of the ingredients may further aid in tissue recovery, reduce swelling, relieve pain, and prevent secondary infections.

The findings from this study support earlier research on the antibacterial and healing properties of medicinal plants in Ayurvedic formulations. Several components in Epi-Cure Gel have individually shown inhibitory effects against common pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*. Formulating these ingredients into a gel may also improve local retention, ease of application, acceptance by patients, and the sustained release of active ingredients at the application site.

However, this study has its limitations. It focused only on preliminary in-vitro antibacterial tests against two bacterial strains and did not explore minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill assays, or antibiotic sensitivity testing. Statistical analysis and repeated trials were also not conducted. Moreover, the study only assessed antibacterial activity and did not examine antifungal actions, cytotoxicity, physical and chemical properties, stability, or phytochemical standardization. Further research is needed to include detailed microbiological evaluations, physical and chemical analyses, HPTLC fingerprinting, phytochemical profiles, toxicity tests, and controlled clinical trials to fully validate Epi-Cure Gel. Such studies may help confirm its effectiveness, safety, quality, and potential use as an Ayurvedic topical antimicrobial treatment.

Conclusion

This in-vitro study demonstrated that Epi-Cure Gel has antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli* using the cylinder plate method. The formulation showed more inhibition against *Staphylococcus aureus*. The lack of inhibition in the blank control confirmed that the antibacterial activity was due to the active constituents in the formulation.

The antibacterial activity likely stems from the combined effects of the herbal and mineral ingredients with healing and infection-fighting properties. The results provide initial scientific evidence supporting the potential use of Epi-Cure Gel as a topical antimicrobial Ayurvedic formulation. Additional studies on physical and chemical evaluations, standardization, and clinical testing are recommended.

Abbreviations

ATCC – American Type Culture Collection, DMSO – Dimethyl Sulfoxide, MHA – Mueller Hinton Agar, PEG – Polyethylene Glycol, NZI – No Zone of Inhibition, MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, IP – Indian Pharmacopoeia, mm – Millimeter, °C – Degree Celsius.

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