An In Vitro Comparative Assessment of The Antibacterial Potential of Darvyadi Churna Lepa and Khadiradi Vati Against Escherichia Coli and Staphylococcus Aureus

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Abstract
Mukhapaka is a common inflammatory condition of the oral cavity that causes pain, burning sensation and ulcers, making eating and speaking difficult. Bacterial infection can further delay the healing process and increase discomfort. In Ayurveda, Darvyadi Churna Lepa and Khadiradi Vati are traditionally used to treat Mukhapaka for their antimicrobial, anti-inflammatory and wound-healing properties. The present in vitro study was carried out to evaluate and compare the antibacterial activity of these two formulations against Escherichia coli and Staphylococcus aureus using the Agar Well Diffusion Method. Aqueous extracts of both formulations were prepared and tested at concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml. Mueller–Hinton agar was used for the study and distilled water was used as the negative control. After incubating the plates at 34°C for 24 hours, the antibacterial activity was assessed by measuring the zones of inhibition around the wells. Both formulations showed antibacterial activity against the tested bacteria and the size of the inhibition zone increased as the concentration increased, indicating a concentration-dependent effect. Darvyadi Churna Lepa showed better antibacterial activity than Khadiradi Vati at all tested concentrations. The highest zone of inhibition was observed with Darvyadi Churna Lepa against Escherichia coli (26 mm) at 100 mg/ml, while Khadiradi Vati showed a maximum inhibition zone of 23 mm against the same organism. No antibacterial activity was observed with negative control. Darvyadi Churna Lepa demonstrated comparatively better antibacterial activity and may be considered a promising herbal antimicrobial agent for oral infections.

Keywords: Darvyadi Churna Lepa, Khadiradi Vati, Antibacterial activity, Agar Well Diffusion Method.

Introduction

According to acharya Sushruta, Mukhapaka is a disorder chiefly caused by aggravated Pitta Dosha, along with the involvement of Vata, Kapha and Rakta. Sushruta systematises it into Vataja, Pittaja and Kaphaja types based on major Doshic influence[1]. Ayurveda describes many herbal formulations for the management of Mukhapaka. Darvyadi Churna Lepa[2] and Khadiradi Vati[3] are commonly used because of their antimicrobial, anti-inflammatory and wound-healing properties.
These formulations have been used in Ayurvedic practice for a long time; there is limited scientific evidence to support their antibacterial activity. Laboratory studies can help provide scientific validation for their traditional use. The present in-vitro study was carried out to evaluate and compare the antibacterial activity of Darvyadi Churna Lepa and Khadiradi Vati against Escherichia coli and Staphylococcus aureus using the Agar Well Diffusion Method. The findings of this study may help strengthen the scientific evidence for the use of these Ayurvedic formulations in the management of Mukhapaka.

AIM
To evaluate and compare the antibacterial activity of Darvyadi Churna Lepa and Khadiradi Vati against Escherichia coli and Staphylococcus aureus using the Agar Well Diffusion Method.

OBJECTIVES
- To assess the antibacterial activity of Darvyadi Churna Lepa against Escherichia coli and Staphylococcus aureus.
- To assess the antibacterial activity of Khadiradi Vati against Escherichia coli and Staphylococcus aureus.

Material and methods
The present in-vitro study was carried out under controlled laboratory conditions using the Agar Well Diffusion Method to evaluate the antibacterial activity of Darvyadi Churna Lepa and Khadiradi Vati. Aqueous extracts of both formulations were prepared and tested against Escherichia coli and Staphylococcus aureus. The materials used in

the study included Darvyadi Churna Lepa, Khadiradi Vati, distilled water, Mueller–Hinton agar, Whatman filter paper, sterile Petri plates, sterile cotton swabs, micropipettes, an 8 mm sterile cork borer, a measuring scale or Vernier caliper, an incubator and fresh bacterial cultures. All culture media, glassware and instruments were properly sterilized before use and the entire procedure was carried out under aseptic conditions to ensure accurate and reliable results.

EXPERIMENTAL PROCEDURE

1. Preparation of Mueller–Hinton Agar Plates[4]

Mueller–Hinton agar (MHA) was prepared according to the manufacturer's instructions. The required amount of MHA powder was dissolved in distilled water and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, the medium was allowed to cool to about 45–50°C. Approximately 20–25 ml of the molten agar was poured into sterile Petri plates under aseptic conditions. The plates were left undisturbed until the agar solidified and were then stored in a refrigerator until use.

2. Preparation of Bacterial Inoculum[5,6]

Fresh cultures of Escherichia coli and Staphylococcus aureus were used for the study. A few well-isolated bacterial colonies were transferred into sterile normal saline and mixed thoroughly to prepare a uniform bacterial suspension. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, which is approximately equivalent to 1.5×10^8 CFU/ml, to ensure that the same amount of bacteria was used in all tests.

3. Inoculation of Agar Plates

A sterile cotton swab was dipped into the prepared bacterial suspension and the excess liquid was removed by gently pressing it against the inner wall of the tube. The swab was then evenly spread over the entire surface of the Mueller–Hinton agar plate in three directions to obtain a uniform bacterial lawn. The inoculated plates were left undisturbed for 5–10 minutes to allow the bacterial suspension to absorb into the agar.

4. Preparation of Wells

Using a sterile cork borer, 8 mm diameter wells were made in the inoculated agar plates. The agar plugs were carefully removed with sterile forceps to create clean and uniform wells. Adequate space was maintained between the wells to prevent the zones of inhibition from overlapping.

5. Addition of Test Extracts

The extracts of Darvyadi Churna Lepa and Khadiradi Vati were tested at concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml. Using a sterile micropipette, 100 µl of each extract was carefully added into the respective wells. Care was taken to avoid spilling or overflowing the extracts. Distilled water was used as the negative control.

6. Incubation

After the extracts were added, the plates were kept at room temperature for about 15–20 minutes to allow the extracts to diffuse into the agar. The plates were then incubated in an inverted position at 34°C for 24 hours under aerobic conditions.

7. Observation and Measurement

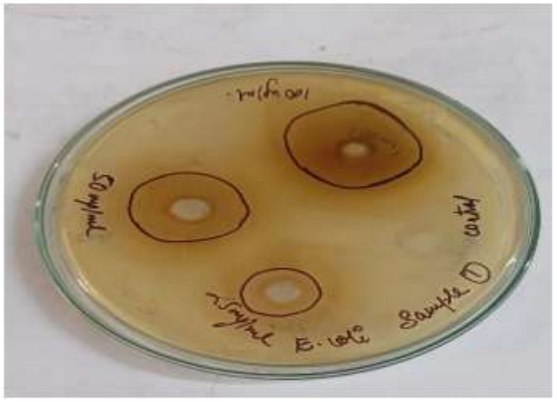
After incubation, the plates were examined for the presence of clear zones around the wells, indicating inhibition of bacterial growth. The diameter of each zone of inhibition was measured in millimeters (mm) using a ruler or Vernier caliper. The measurements were recorded carefully for concentration against both bacterial strains.

Sample – 1, 2

Extract Preparation

Culture Media

Wells for pour Sample



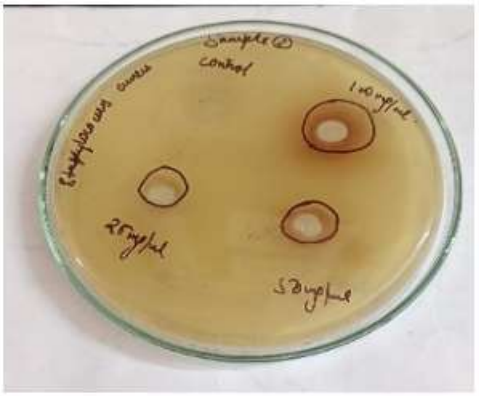


Image No. 01 – Methodology of Invitro Study

OBSERVATION AND RESULTS

Both Darvyadi Churna Lepa and Khadiradi Vati showed antibacterial activity against the tested microorganisms. No zone of inhibition was observed with the negative control (distilled water), confirming that the solvent itself had no antibacterial effect. In both formulations, the antibacterial activity increased with increasing concentration, indicating a clear concentration-dependent response. Among the two formulations, Darvyadi Churna Lepa demonstrated comparatively greater antibacterial activity than Khadiradi Vati against the tested bacteria.

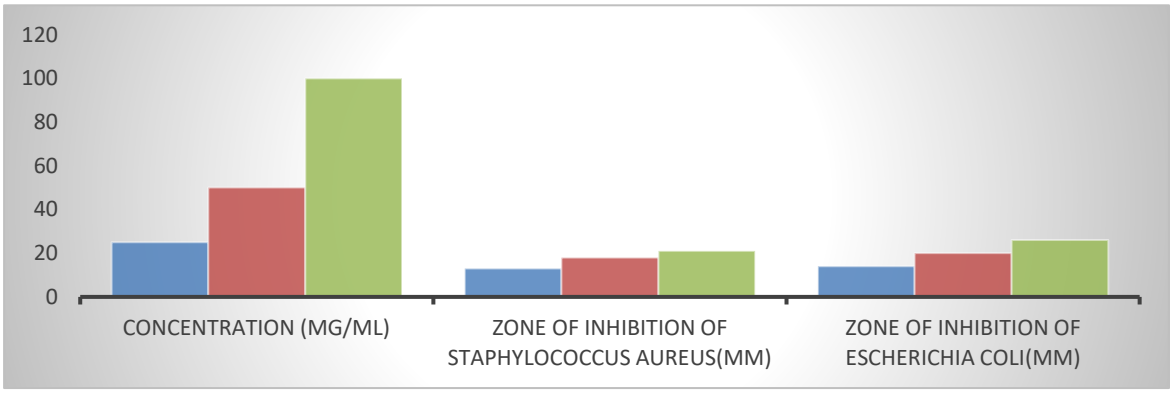


Figure No. 01- Antimicrobial Activity of Sample 1 (Darvyadi Churna Lepa)

Table No. 1 – Antimicrobial Activity of Sample 1 (Darvyadi Churna Lepa)

Concentration (mg/ml)	Zone of Inhibition of Staphylococcus aureus(mm)	Zone of Inhibition of Escherichia coli(mm)
25	13	14
50	18	20
100	21	26

Image No. 02- Antimicrobial Effect of Sample 1

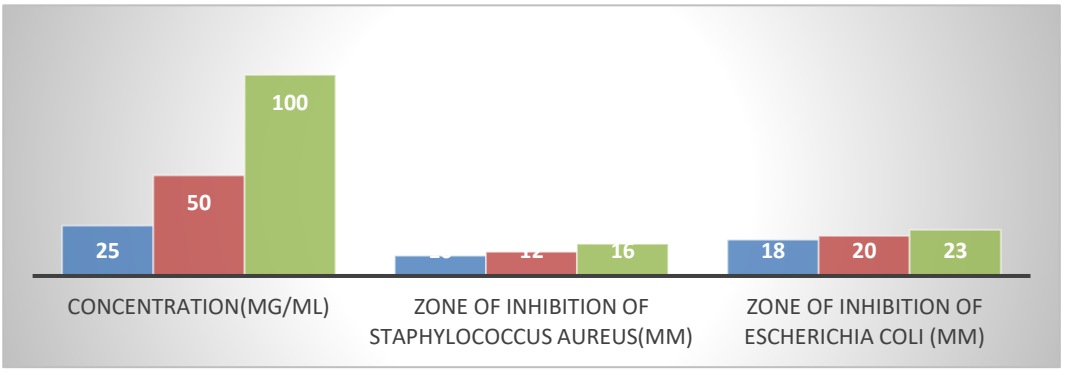


Figure No. 02– Anti-Microbial Activity of Sample 2 (Khadiradi Vati)

Table No. 2 – Anti-Microbial Activity of Sample 2 (Khadiradi Vati)

Concentration(mg/ml)	Zone of inhibition of Staphylococcus aureus(mm)	Zone of Inhibition of Escherichia coli (mm)
25	10	18
50	12	20
100	16	23

Image No. 03- Antimicrobial Effect of Sample 2

Discussion

The present in-vitro study demonstrated that both Darvyadi Churna Lepa and Khadiradi Vati possess significant antibacterial activity against Escherichia coli and Staphylococcus aureus. The zone of inhibition increased with increasing concentration (25, 50 and 100 mg/ml), indicating that both formulations showed a concentration-dependent antibacterial effect. No inhibition was observed in the negative control (distilled water), confirming that the antibacterial activity was due to the phytoconstituents present in the formulations rather than the solvent.

Darvyadi Churna Lepa produced larger zones of inhibition against both test organisms, suggesting comparatively stronger antibacterial activity. This may be attributed to the synergistic action of its ingredients—Daruharidra[7], Yashtimadhu[8], Haritaki[9] and Jati[10]. These drugs contain several bioactive compounds, including berberine, tannins, flavonoids, gallic acid, glycyrrhizin and rutin, which are known for their antimicrobial, anti-inflammatory, antioxidant and wound-healing properties.

The antibacterial effect of Darvyadi Churna Lepa mainly depends on the action of berberine, the major alkaloid

present in Daruharidra. Berberine first interacts with the bacterial cell wall and cell membrane, making them unstable and increasing their permeability. As a result, essential intracellular substances such as proteins, ions and nucleic acids leak out of the bacterial cell, leading to loss of cellular integrity. Berberine also enters the bacterial cell and interferes with DNA replication and protein synthesis, preventing bacterial multiplication. At the same time, tannins present in Haritaki and Jati bind with bacterial proteins and enzymes, causing protein precipitation and reducing the activity of enzymes required for bacterial growth. Flavonoids further enhance this effect by disturbing membrane function, inhibiting energy metabolism and reducing bacterial enzyme activity. The combined action of these phytochemicals results in effective inhibition of bacterial growth.

The antibacterial mechanism differs slightly between the two test organisms because of differences in their cell wall structure. Staphylococcus aureus is a Gram-positive bacterium with a thick peptidoglycan layer. The phytochemicals in Darvyadi Churna Lepa directly damage this layer, weaken the cell membrane and interfere with intracellular metabolic activities, ultimately causing bacterial death.

In contrast, Escherichia coli is a Gram-negative bacterium that possesses an additional outer lipopolysaccharide membrane. Berberine and flavonoids increase the permeability of this outer membrane, allowing the active compounds to enter the bacterial cell more easily. Once inside, they inhibit DNA replication, protein synthesis and essential metabolic pathways, resulting in inhibition of bacterial growth and eventual cell death. In the present study, E. coli showed larger zones of inhibition than Staphylococcus aureus, indicating greater sensitivity to the phytochemicals present in both formulations.

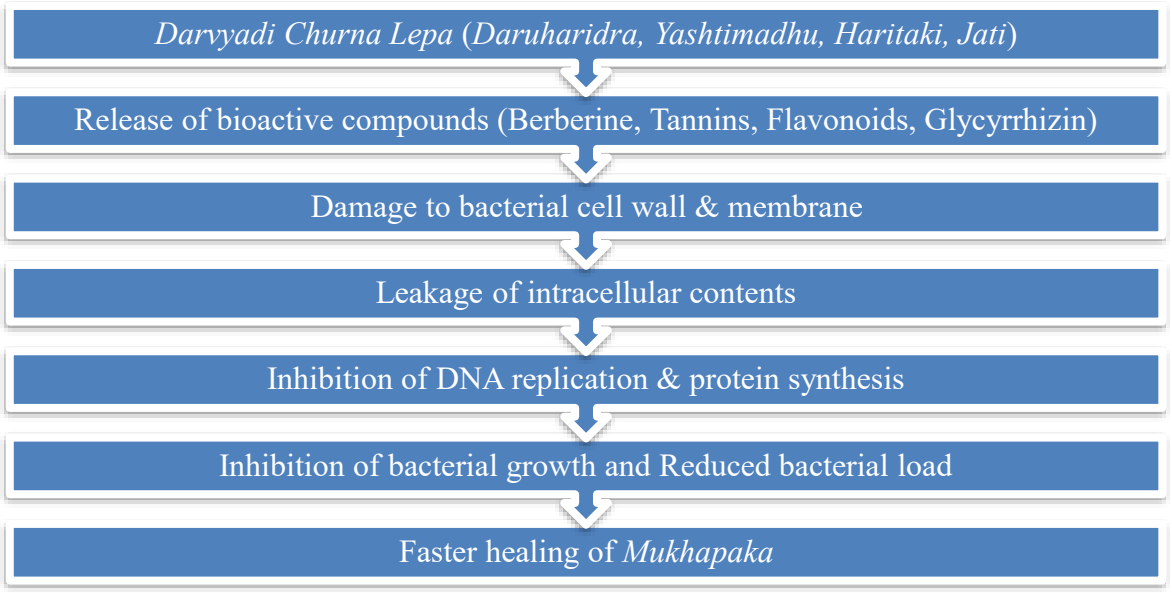


Figure No. 03- Mode Of Action of Darvyadi Churna Lepa

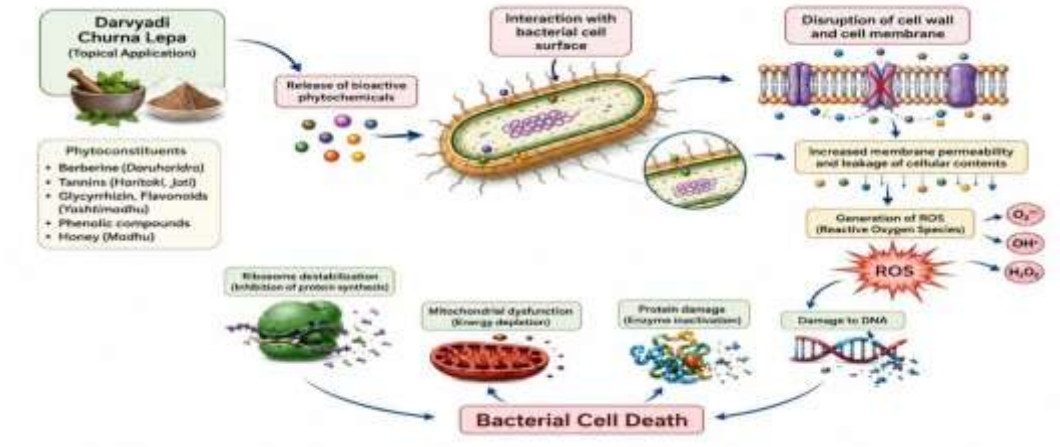


Figure No. 04- Mode of Action of Darvyadi Churna Lepa

Khadiradi Vati contains Khadira[11], Karpura[12], Jatiphala[13], Javitri[14], Puga[15] and Sitalchini[16]. Khadira contains catechin and tannins, which disrupt the bacterial cell wall, increase membrane permeability and inhibit enzymes involved in bacterial metabolism. The essential oils present in Karpura, Jatiphala and Javitri are lipophilic in nature and easily penetrate bacterial cell membranes. These volatile compounds disturb membrane integrity, alter cellular metabolism and interfere with enzyme activity, leading to inhibition of bacterial growth. The combined action of catechin, tannins, flavonoids and essential oils explain the antibacterial effect of Khadiradi Vati against both Gram-positive and Gram-negative bacteria.

Mukhapaka is characterized by painful oral ulcers that are often susceptible to secondary bacterial infection. Such infections may delay healing and increase local inflammation and discomfort. The antibacterial activity demonstrated by both formulations suggests that they can reduce bacterial colonization at the ulcer site, thereby minimizing the risk of secondary infection. Besides their antimicrobial action, the phytoconstituents also possess anti-inflammatory and antioxidant properties. Berberine helps suppress inflammatory mediators, flavonoids reduce oxidative stress, tannins produce an astringent effect that forms a protective layer over the ulcer surface and honey promotes tissue repair by maintaining a moist environment and supporting epithelial regeneration. These combined actions create favorable conditions for faster ulcer healing.

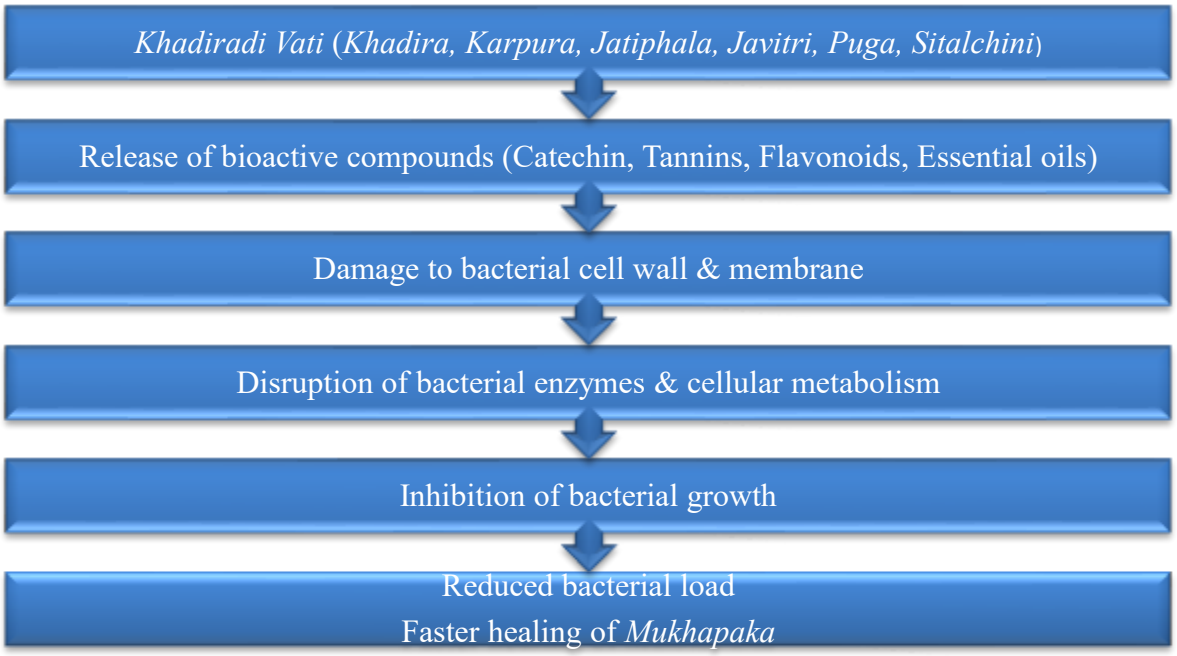


Figure No. 05- Mode Of Action of Khadiradi Vati

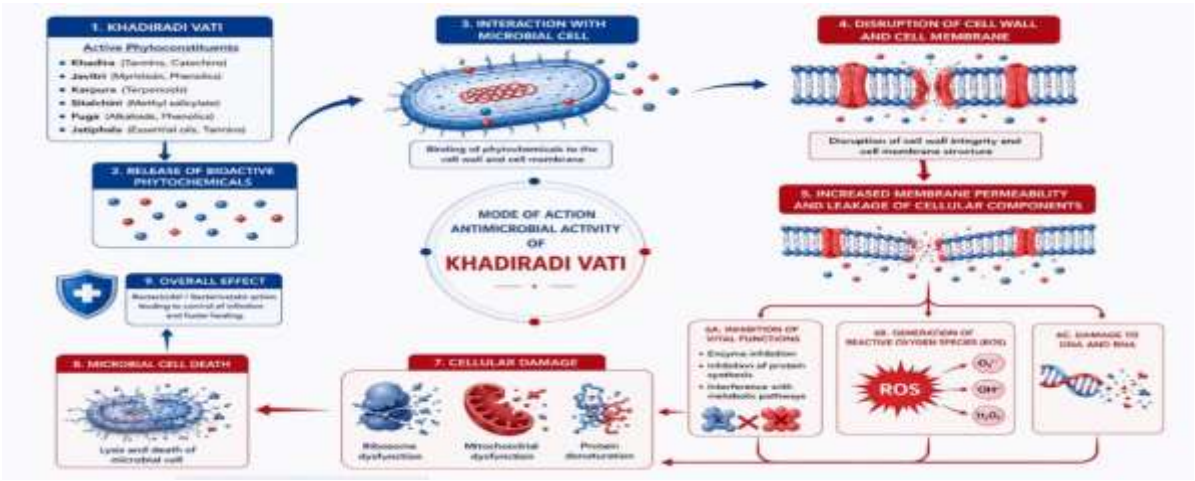


Figure No. 06- Mode of Action of Khadiradi Vati

The findings of the present study provide scientific evidence supporting the traditional use of Darvyadi Churna Lepa and Khadiradi Vati in the management of Mukhapaka. The comparatively superior antibacterial activity of Darvyadi Churna Lepa may be due to the synergistic action of berberine, tannins, flavonoids and honey, whereas the antibacterial effect of Khadiradi Vati is mainly mediated by catechin, tannins, flavonoids and essential oils. These results indicate that both formulations have promising antimicrobial potential and may help prevent secondary bacterial infection while promoting the healing of oral ulcers.

Conclusion

The present in-vitro study showed that both Darvyadi Churna Lepa and Khadiradi Vati possess significant antibacterial activity against Escherichia coli and Staphylococcus aureus. Darvyadi Churna Lepa demonstrated better antibacterial activity than Khadiradi Vati, with the highest zone of inhibition (26 mm) observed against Escherichia coli at a concentration of 100 mg/ml. No antibacterial activity was seen with the negative control, confirming the reliability of the experimental findings.

The study provides scientific support for the traditional use of Darvyadi Churna Lepa and Khadiradi Vati in the

management of Mukhapaka. The findings suggest that both formulations have good antibacterial potential, with Darvyadi Churna Lepa showing comparatively better efficacy. These formulations may be useful as natural herbal antimicrobial agents for the management of oral infections and may help promote faster healing of oral lesions.

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