

Antimicrobial Effect of *Euphorbia hirta* on Common Oral Pathogens: *In Vitro* Study

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ABSTRACT

Background: *Euphorbia hirta* is a tropical plant common throughout India. It is used to treat cough, fever, asthma, etc. It has anti-inflammatory and antimicrobial properties. This study was conducted to investigate the antimicrobial activity of *E. hirta* on common oral pathogens.

Materials and methods: An *in vitro* study was conducted to determine the antimicrobial effect of the ethanol extract of *E. hirta* on the bacteria (*Streptococcus mutans*, *Lactobacillus acidophilus*, and *Escherichia Coli*) and fungi (*Candida albicans*). The agar well-diffusion method was used to determine the mean zone of inhibition (ZOI).

Results: *Euphorbia hirta* has antibacterial and antifungal activity against *S. mutans* (ZOI = 11 ± 0.54 mm), *L. acidophilus* (ZOI = 10 ± 0.04 mm), *E. coli* (ZOI = 13 ± 0.05 mm), and *C. albicans* (ZOI = 14 ± 0.59 mm) at concentration of 5 mg/mL.

Conclusion: *Euphorbia hirta* has antibacterial and antifungal properties against common oral pathogens.

Keywords: *Candida albicans*, *Escherichia coli*, *Euphorbia hirta*, *Lactobacillus acidophilus*, *Streptococcus mutans*.

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INTRODUCTION

Euphorbia hirta L. (Euphorbiaceae), a wild herb, is widely distributed in all tropical countries, including India. The stems are slender and often reddish in color and covered with yellowish bristly hairs, especially in the younger parts. The leaves are arranged opposite, lanceolate, and usually greenish or reddish, about 5 cm long on the underside. A white or milky sap is obtained from the stem and leaves when cut.¹ The plant is widely recognized in traditional medicine for the treatment of cough, hay fever, bronchial infections, intestinal disorders, worm infestation, and kidney stones.² *E. hirta* has antibacterial, anti-inflammatory, anthelmintic, antiasthmatic, sedative, antispasmodic, antifungal, and antimalarial properties.³

The aim of the study was to investigate the antimicrobial activity of extracts from *E. hirta* with the aim of finding the possibilities of new antimicrobial substances against some human pathogens. In this study, the antibacterial and antifungal activity of *E. hirta* is evaluated.

MATERIALS AND METHODS

An *in vitro* study was conducted to determine the ZOI of the ethanol extract of *E. hirta* on common oral pathogens. The approval was obtained from the Institutional Scientific Review Committee (ISRC) of Asan Memorial Dental College and Hospital.

Plant Identification

The plant *E. hirta* (Tamil name: Amman Pacharisi) was obtained from the herbal farm of an NGO named Irula Tribal Women's Welfare Society (ITWWS), Thandarai, Chengalpattu District, Tamil Nadu, India. The plant was authenticated by the Department of Botany, Madras Christian College, East Tambaram, Chennai, India.

Preparation of Plant Extract

The collected plant was washed under running water to remove mud and impurities. The aerial part of the plant was

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dried in the shade for 7–10 days. The dried plant was ground to a powder in an electric mill to a mesh size of 60. The ethanol extract was obtained by the Soxhlet method described by Jensen.⁴ About 20 gm of the ground sample was placed in a thimble and kept in a Soxhlet extractor with ethanol as a solvent. The solvent is heated in the jacket and the vapors enter the condenser. Condensed vapors drip into a reservoir containing a thimble. Once the solvent reaches the siphon, it flows back into the flask and the cycle begins again. The cycle was repeated for 48 hours and the temperature was maintained at 75°C. The extract was concentrated in an oven at 37°C to obtain a dry extract.

Table 1: ZOI of extract on oral bacteria

Bacteria	ZOI (in mm)				Amoxicillin (10 mg)
	Concentrations (μ l)				
	25	50	75	100	
<i>Streptococcus mutans</i>	–	–	10 \pm 0.48	11 \pm 0.54	21 \pm 1.06
<i>Lactobacillus acidophilus</i>	–	–	8 \pm 0.67	10 \pm 0.04	14 \pm 0.71
<i>Escherichia coli</i>	–	–	9 \pm 0.16	13 \pm 0.05	18 \pm 0.92

Table 2: The ZOI of *C. albicans*

Microorganism	ZOI (in mm)				Ketoconazole (20 μ l)
	Concentration (μ l)				
	25	50	75	100	
<i>Candida albicans</i>	–	–	9 \pm 0.42	14 \pm 0.59	20 \pm 0.21

ANTIBACTERIAL ACTIVITY

Agar Diffusion Method

Preparation of Inoculation

Stock cultures were maintained at 4°C on a nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of cells from stock cultures to tubes containing bacterial culture medium that were incubated at 37°C for 24 hours.

The antibacterial activity of the sample was determined by the well-diffusion method on Mueller–Hinton agar (MHA) medium. MHA medium was weighed to 3.8 gm and dissolved in 100 mL of distilled water, and then 1 gm of agar was added. The medium was then kept for sterilization. After sterilization, the medium was poured into sterile Petri dishes and allowed to solidify for 1 hour. After solidification of the medium, the inoculum was spread on to solid plates with a sterile swab moistened with the bacterial suspension. The boreholes were made with a cork borer. The sample (plant ethanol extract) was added to the respective wells at four different concentrations (25, 50, 75, and 100), and Amoxicillin (10 mg, Disk) was used as a control. These plates were incubated at 37°C for 24 hours, after which microbial growth was determined.

RESULTS

Antimicrobial effect is observed with the ethanolic extract of *E. hirta* on oral microorganisms. Table 1 depicts the ZOI of plant extract at different concentrations on the following bacteria: *S. mutans*, *L. acidophilus*, and *E. coli*. The ZOI was highest at the concentration of 100 μ L for all the test bacteria. Table 2 depicts the ZOI of plant extract on *C. albicans*. The highest inhibition was observed at 100 μ L of the extract against *C. albicans*.

DISCUSSION

The phytochemistry of *E. hirta* has shown the presence of the active substances such as heneicosane⁵ and tetracosane,⁶ which are responsible for the antimicrobial activity of the plant. A study conducted by Rao et al.² and Saravanan et al.⁷ demonstrated that the ethanol extract of *E. hirta* was effective against the following microorganisms: *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and the

fungal species: *Aspergillus niger*, *Aspergillus fumigatus* or *Aspergillus flavus*, *Rhizopus oryzae*.

This study revealed that *E. hirta* has antibacterial activity against oral pathogens such as *S. mutans* and *C. albicans*, similar to neem aqueous extract in a study by Bansal et al.,⁸ where the mean ZOI for *S. mutans* was 11.4 mm and for *C. albicans* it was 5.8 mm, indicating that *E. hirta* has better antifungal activity than neem.

Lactobacillus acidophilus is responsible for the progression of tooth decay. *Euphorbia hirta* extract had an average ZOI of 10 mm in the current study, which is less than aqueous neem extract of 25 mm in a study conducted by Sharma et al.⁹

Periodontitis, a multipathogen disease, has the latest addition to the family, namely *E. coli*, as a potential and emerging periodontal pathogen that is more potent than *Porphyromonas gingivalis* due to its lipopolysaccharide, making it a new threat in periodontal disease.¹⁰ In a study by Mauti et al., *E. hirta* extract was found to be effective against *E. coli* with an average ZOI of 13 mm, similar to *Tulsi* extract with 15 mm and less than garlic extract with 20 mm.¹¹

Euphorbia hirta has antimicrobial activity against common oral pathogens. Therefore, further studies may be conducted to determine its effect on oral biofilm, and the herb may eventually be included in a toothpaste or mouthwash.

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