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ANTIBACTERIAL EFFECT OF *EUPHORBIA HIRTA* ON *PROPIONIBACTERIUM ACNES* ISOLATED FROM ACNE PUS

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Abstract

Acne vulgaris is a chronic inflammatory condition of the pilosebaceous unit, frequently associated with *Propionibacterium acnes* (currently *Cutibacterium acnes*). Due to increasing antimicrobial resistance, medicinal plants such as *Euphorbia hirta* are gaining attention for their therapeutic potential. This study aimed to isolate *P. acnes* from acne pus samples and evaluate the antibacterial effect of *E. hirta* leaf extracts prepared using ethanol, methanol, and chloroform. Samples were cultured on Brain Heart Infusion Agar, followed by Gram staining and biochemical characterization. Phytochemical screening of *E. hirta* leaf extracts showed the presence of saponins, flavonoids, tannins, alkaloids, terpenoids, and carbohydrates. Antibacterial activity determined by the well diffusion method revealed that methanol extract exhibited the highest inhibition zone (37 mm), followed by ethanol extract (30 mm), whereas chloroform extract showed no activity. The findings suggest that *E. hirta* possesses significant antibacterial properties and may serve as a potential natural therapeutic agent for acne management.

Keywords: Acne vulgaris, *Propionibacterium acnes*, *Euphorbia hirta*, antibacterial activity, phytochemicals.

I. INTRODUCTION

Acne vulgaris is one of the most common dermatological disorders worldwide and is characterized by inflammation of the pilosebaceous unit (Adityan *et al.*, 2009). The primary clinical features include seborrhoea, comedones, papules, pustules, nodules, cysts, and scarring (Degitz *et al.*, 2007). Although acne does not pose a threat to general health, it greatly impacts psychological well-being due to its visible nature (Aydemir, 2002; James *et al.*, 2008). During adolescence, increased androgen production stimulates sebaceous glands to produce excess sebum, leading to follicular blockage and creating an anaerobic environment suitable for *P. acnes* growth (Yosipovitch *et al.*, 2007; Meixner *et al.*, 2008). Acne pustules contain a variety of microorganisms including *P. acnes*, *Staphylococcus epidermidis*, *Kocuria varians*, *Acinetobacter lowffii*, and others (Niyan Innam *et al.*, 2015). These organisms contribute significantly to acne pathogenesis.

P. acnes is a gram-positive, anaerobic skin commensal that thrives on fatty acids in sebum, playing a major role in inflammatory acne. Its scientific classification places it under the genus *Cutibacterium*. Plants have been used in medicine since ancient times, and many modern drugs are derived from plant secondary metabolites (Maciel *et al.*, 2002). One such plant is *Euphorbia hirta*, traditionally used for treating respiratory, gastrointestinal, dermatological, and infectious diseases (Lind *et al.*, 1971; Akomas *et al.*, 2015). It possesses antibacterial, antifungal, antiasthmatic, antimalarial, and anti-inflammatory properties (Ogbulie *et al.*, 2007). Given its medicinal significance, this study aimed to examine the antibacterial effect of *E. hirta* leaf extract against *P. acnes* isolated from acne pus.

II. RESEARCH METHODOLOGY

Sample Collection

This study was conducted from August 2023 to February 2024 at Malankara Catholic College. Acne pus samples were obtained from individuals aged 18–23 years. Samples were collected using sterile swabs and streaked directly onto Brain Heart Infusion (BHI) Agar plates.

Gram Staining

Gram staining was performed following standard protocol. Smears were stained sequentially with crystal violet, iodine, alcohol, and safranin before microscopic observation.

Isolation and Preservation of *P. acnes*

BHI plates were incubated at 37°C for 48–72 hours. Small, circular, creamy, raised colonies indicative of *P. acnes* were sub-cultured and preserved in nutrient broth.

Biochemical Characterization

Biochemical tests such as Catalase test, Indole test, Nitrate reduction test, Sugar fermentation test, Methyl red test were carried out for the characterization of the bacterial isolate.

Collection of *Euphorbia hirta*

Fresh leaves were collected, washed thoroughly, and shade-dried for one week.

Preparation of Plant Extracts

Five grams of dried leaf powder were extracted using 50 ml each of methanol, ethanol, and chloroform via cold extraction. Extracts were filtered after one week and used for phytochemical and antibacterial assays.

Phytochemical Screening

Tests for the detection of saponins, flavonoids, steroids, tannins, alkaloids and carbohydrates were performed.

Antibacterial Activity

The antibacterial activity of *E. hirta* extracts against *P. acnes* was tested using the well as well as disc diffusion method.

III RESULTS AND DISCUSSION

Isolation of *P. acnes*

Colonies were small, circular, raised, and creamy off-white, consistent with *P. acnes* morphology.

Biochemical Results

All biochemical tests (catalase, indole, nitrate reduction, sugar fermentation, methyl red) were positive, confirming the organism as *P. acnes*.

Phytochemical Findings

Phytochemical screening indicated the presence of saponins in ethanol and methanol extracts, terpenoids in ethanol and chloroform extracts, tannins in methanol and chloroform extracts, alkaloids, flavonoids and carbohydrates all extracts were detected.

Antibacterial Activity

Methanol extract demonstrated the highest antibacterial activity (30 mm), followed by the Ethanol extract (30 mm) and No inhibition was produced by the Chloroform extract.

The bacterial profile of acne pus in this study aligns with earlier findings showing *P. acnes*, *Staphylococcus epidermidis*, and *Micrococcus* species as predominant pathogens (Hassanzadeh *et al.*, 2008; Biatecka *et al.*, 2005). The strong antibacterial activity exhibited by ethanol and methanol extracts of *E. hirta* corresponds with previous studies demonstrating the plant's antimicrobial potential (Rautela *et al.*, 2020; Ogbulie *et al.*, 2007). Methanol extract performed best, likely due to better extraction of phytochemicals such as flavonoids, tannins, and alkaloids. The lack of inhibition in the chloroform extract may be attributed to the absence of saponins and lower solubility of active compounds.

IV TABLES

Table 1. Biochemical Analysis of *P. acnes*

Test	Response
Catalase	Positive
Indole	Positive
Nitrate	Positive
Sugar Fermentation	Positive
Methyl Red	Positive

Table 3. Antibacterial Activity Against *P. acnes*

Extract Volume	Solvents		
	Ethanol	Methanol	Chloroform
50 µg	5 mm	25 mm	-
100 µg	23 mm	30 mm	-
150 µg	30 mm	37 mm	-

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