

**EVALUATION OF *IN VITRO* ANTIACNE ACTIVITY OF *PUPALIA LAPPAGEA*
(L.) JUSS.**

ABSTRACT

Acne vulgaris is caused by *Propionibacterium acnes*, subsequently aggravated by *Staphylococcus epidermidis*, and *Staphylococcus aureus*. *Pupalia lappacea* (L.) Juss. It is a common weed found in Asia and tropical Africa. The current investigation is aimed to explore its antiacne property targeting the aforementioned gram-positive strains. Disc diffusion method was adopted to screen the antibacterial property of the ethanol extract at various concentrations (12.5, 25, 50 & 100mg/ml) compared with Ciprofloxacin using Mueller Hinton Agar medium. The diameter of the clear zone of inhibition and minimum inhibitory concentration (MIC) values were calculated. From the results, it is apparent that the zone of inhibition was high for *P. acnes* (19mm), followed by *Staphylococcus epidermidis* (17mm) and *Staphylococcus aureus* (12mm), and was more sensitive to *Pupalia lappacea* (L.) Juss. ethanol extract at 100mg/ml concentration. Similarly, from the MIC values, it is found that the plant extract completely halted the growth of *Propionibacterium acnes* more efficiently at 0.32mg/ml, *Staphylococcus aureus* at 0.25mg/ml, and 0.28mg/ml for *Staphylococcus*

epidermidis. Either alone or in combination, the phytochemicals may be responsible for the observed scavenging property.

KEYWORDS: *Pupalia lappacea* (L.) Juss., Amaranthaceae, disc diffusion method, minimum inhibitory concentration, antiacne.

INTRODUCTION

Pupalia lappacea (L.) Juss. of the Amaranthaceae family, commonly called Tella Uttareni, is a plant found in India as a weed that can spread from 2 to 3 feet as a creeper [1]. It has elliptic to ovate type leaves with acute apex and a hairy base. Flowers are bracteate with terminal spikes that bloom from August and can bear fruits by December [2]. In Indian folklore medicine, the leaves are applied to set the bone fracture, paralysis, jaundice, fevers, gastrointestinal disorders, and other inflammatory diseases. The fruits have wound healing activity and are helpful in treating skin infections [3]. The phytochemical review reveals that *Pupalia lappacea* (L.) Juss. is rich in glycosides, alkaloids, saponins, steroids, and steroid glycosides [4]. *Pupalia lappacea* (L.) Juss. was also reported for antioxidant, cytotoxic, antidiabetic, hypolipidemic, and antidiarrheal activities on various *In vitro* and *In vivo* models [5-6].

Acne vulgaris is a common complaint throughout the globe; a most vexatious chronic inflammatory skin condition affecting adults, especially adolescents [7]. Symptomatically it can be illustrated by pimples, oily skin, and skin abrasions and associated with pathogenic bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. *Propionibacterium acnes* is an opportunistic facultative anaerobic bacterium that causes acne vulgaris and other skin infections. Accumulation of inflammatory mediators such as neutrophils due to the formation of decomposed sebum products (fatty acids) causes swelling, and topically, *Propionibacterium* damages the skin, and *Staphylococcus* will aggravate further. All these organisms collaborate to form acne lesions on the skin [8-9].

Various herbal remedies were practiced worldwide to get rid of acne. Herbs that are potent antibacterial in nature will target these microbial environments to prevent acne.

The present investigation is aimed to screen the antibacterial activity of *Pupalia lappacea* (L.) Juss. against acne-causing bacteria.

MATERIALS AND METHODS:

Plants Material

Aerial parts of *Pupalia lappacea* (L.) Juss. were collected from Osmania University campus, Hyderabad, and authenticated by Dr. L. Rasingam, Scientist-E, Botanical survey of India, Deccan section, Hyderabad, India. A voucher specimen (BSI/DRC/2021-22/Tech/369) was deposited in the Herbarium for future reference.

Reagents and chemicals

All the chemicals, reagents were analytical grade, procured from Sigma Aldrich (laboratory grade), and the media were purchased from Hi-media.

Preparation of extracts

The shade-dried plant was powdered and subjected to Soxhlet extraction with ethanol. The solvent was evaporated to dryness to get the solid extract, and percentage yield was calculated.

Phytochemical analysis

The preliminary phytochemical investigation of ethanolic extract of *Pupalia lappacea* (L.) Juss. was carried out by employing standard protocols.

Microorganisms & Media

Three gram-positive bacterial strains were selected for the screening; namely, *Propionibacterium acnes* (MTCC 1951), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermidis* (MTCC 931), Bacterial strains were procured from the IMTECH (MTCC), India. The microbes were sub-cultured using Mueller Hinton Broth (MHB). After 24 hours of incubation, the microbial cultures were adjusted to a concentration of 0.5 McFarland values and resuspended in the broth medium to get 1.5×10^6 CFU/ml [10].

Antibacterial screening by disc diffusion method

For *Propionibacterium*, Mueller Hinton Agar medium was prepared according to the procedures and autoclaved at temperature 121°C for 15 minutes. The bacterial suspension with adjusted Mc Farland values was inoculated on the agar plates by the spread plate technique. Sterile filter paper discs absorbed with the plant extracts and antibiotic (Ciprofloxacin) of known concentrations were aseptically transferred on the media. The Petri plates were allowed to stand for 30 minutes for diffusion at room

temperature and incubated for 72 hours. The incubation was conducted using anaerobic bags incorporated with the gas pack and indicator tablet at around 37°C. Similarly, *Staphylococcus aureus* & *Staphylococcus epidermidis*, inoculated with 24 hours old bacterial culture, and incubated in the medium under aerobic conditions for 24 hours at 37°C. The experiment was done in triplicates, and the antibacterial activity was determined by measuring the diameter of the clear zone of inhibition around the discs impregnated with the samples [11-12].

Determination of MIC values

MIC values of the extract were determined by agar dilution method using various concentrations of the extract (10, 5, 2, 1, 0.5, 0.25, 0.05 mg/ml). The prepared extracts were added to the agar medium, mixed gently for a few seconds, and transferred to sterile Petri plates. After 30 minutes of standing, microbes were inoculated by spread plate method and incubated at 37°C according to the procedure followed for the antibacterial experiment. The presence or absence of the microbial growth is observed, and the MIC was calculated in mg/ml as the lowest concentration of the sample that stopped the microbial growth in the given medium and conditions [13].

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

The preliminary phytochemical study of ethanolic extracts of *Pupalia lappacea* (L.) Juss. exposed that the extracts are instituted with various secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, saponins (Table 1).

Table 1. Phytochemical screening of *Pupalia lappacea* (L.) Juss.

Phytochemicals	Ethanol extract of <i>P. lappacea</i>
Alkaloids	+
Glycosides	-
Flavonoids	+
Terpenoids	+
Steroids	+
Tannins	+
Proteins	-
Carbohydrates	-

Amino acids	-
Saponins	+

+ indicates the presence and - indicates the absence of phytochemicals

Antibacterial activity

From table 2. It is clear that the ethanol extract of *Pupalia lappacea* (L.) Juss. is inhibiting the selected gram-positive bacterial strains in a dose-dependent manner. The zone of inhibition is clear evidence for the antibacterial activity and recorded against *P. acnes* (11mm) followed by *Staphylococcus epidermidis* (9mm) and *Staphylococcus aureus* (7mm) for *Pupalia lappacea* (L.) Juss. at 50mg/ml concentration (Figure1&2). Whereas, Ciprofloxacin developed the highest clear zone of inhibition among all (Table 2). The results also suggest that the *P. acnes* (19mm) followed by *Staphylococcus epidermidis* (17mm) and *Staphylococcus aureus* (12mm) were more sensitive to *Pupalia lappacea* (L.) Juss. ethanol extract at 100mg/ml concentration.



Figure 1. Antibacterial activity of *Pupalia lappacea* (L.) Juss.

Table 2. Antibacterial activity of *Pupalia lappacea* (L.) Juss.

Treatment	Zone of inhibition (mm)		
	<i>Propionibacterium acnes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
12.5mg/ml	4±0.12	2±0.03	2±0.12
25mg/ml	7±0.11	4±0.07	5±0.09
50mg/ml	11±0.06	7±0.1	9±0.11
100mg/ml	19±0.04	12±0.06	17±0.12
Ciprofloxacin	33±0.04	28±0.07	31±0.21

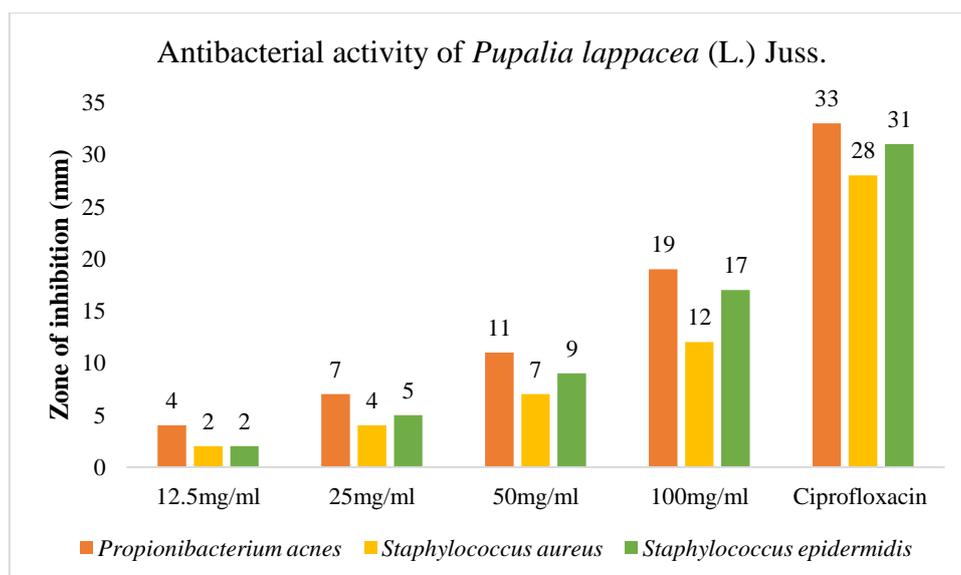


Figure 2. Antibacterial activity of *Pupalia lappacea* (L.) Juss.

Diversified secondary metabolites present in the aerial parts of the ethanol extract of *Pupalia lappacea* (L.) Juss. such as polyphenolic compounds may directly contribute to the antibacterial activity. The results are in accordance with the results of Elin Julienti et al., 2017. The zone of inhibition is comparatively high for the *P. acnes* due its sensitivity towards the plant extracts followed by the two species of *Staphylococcus* strains. This indicates the specificity of the plant extracts towards this acne causing bacteria and can be developed as a herbal formulation or can be further subjected to chromatographic isolation to identify the specific compound/s responsible for the activity [14].

Determination of MIC values

The MIC values were taken as the minimum concentration of the extract of *Pupalia lappacea* (L.) Juss. that inhibit the growth of the selected strains of the bacteria. Results suggest that the extract does not allow microbial growth at a concentration of 0.32mg/ml for *Propionibacterium acnes*, at 0.25mg/ml *Staphylococcus aureus*, and 0.28mg/ml for *Staphylococcus epidermidis*.

Table 3. MIC of *Pupalia lappacea* (L.) Juss. on the selected bacterial strains

Treatment	Minimum inhibitory concentration (mg/ml)		
	<i>Propionibacterium acnes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
<i>P. lappacea</i> extract	0.32	0.25	0.28

Ciprofloxacin	0.002	0.005	0.004
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Plant secondary metabolites such as polyphenolic compounds and other phytochemicals are proven effective *In vitro* antibacterial compounds that can target various pathways. Secondary metabolites such as alkaloids, saponins, flavonoids, steroids, and terpenoids present in *Pupalia lappacea* (L.) Juss. ethanol extract may be responsible for the reported antibacterial activity. Few Amaranthaceous plants such as *Aerva lanata*, *Acalypha indica*, *Amaranthus hypochondriacus*, *Amaranthus cruentus* etc. are already proved to have antiacne properties [15-16].

CONCLUSION

The ethanol extract of *Pupalia lappacea* (L.) Juss. was found to be effective in hindering the growth of acne-causing organisms viz., *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* in disc diffusion method assay. The MIC values suggest that the plant extracts are antibacterial and can clutch acne vulgaris effectively. Further research is in progress in designing the plant extract as a topical formulation to treat acne.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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