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Research Article

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Evaluation of Ethanolic *Momordica charantia L.* Leaf Extract Against Acne-Associated Bacteria

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ABSTRACT

Acne vulgaris is a multifactorial inflammatory skin disorder in which *Cutibacterium acnes* may contribute to disease in a strain- and context-dependent manner. This study screened ethanolic *Momordica charantia L.* leaf extract for in-vitro antibacterial activity against *C. acnes* and *Staphylococcus aureus* using disk diffusion. The extract was tested at reported concentrations of 5%, 7%, and 10%, with tetracycline and sterile distilled water as positive and negative controls. Qualitative phytochemical screening detected flavonoids. The largest reported mean net inhibition-zone measurement at 24 h was 6.67 mm for the 10% extract against each organism. Post-hoc groupings indicated a difference between the 10% extract and other treatments for *S. aureus*, whereas extract concentrations were not significantly different for *C. acnes*. These preliminary findings are limited by incomplete reporting of strain identity, replicate independence, variability, and assay standardization. The results warrant confirmation using a validated organism-appropriate method and additional endpoints before any therapeutic implication is considered.

1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit and a common skin disease worldwide. Its development reflects interactions among sebum production, follicular changes, host inflammation, and the skin microbiome (Vasam et al., 2023; Legiawati et al., 2023). *Cutibacterium acnes* and staphylococci are common members of facial skin microbial communities; their presence alone does not establish disease, and microbial effects may vary with strain and host context (Hamann et al., 2025).

Cutibacterium acnes (formerly Propionibacterium acnes) is a slow-growing, anaerobic, Gram-positive bacterium that commonly colonizes sebaceous skin. It is also an opportunistic pathogen in some settings, and strain-level variation in virulence and host interaction is relevant to its role in acne (Mayslich et al., 2021; Hamann et al., 2025). Antibiotic resistance in acne-

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associated bacteria has been reported and may complicate treatment (Legiawati et al., 2023). Accordingly, *C. acnes* is an appropriate organism for preliminary in-vitro screening, but should not be described as intrinsically pathogenic in all individuals.

Staphylococcus aureus is an important skin pathogen, and studies have reported its presence in some acne lesions and its possible association with acne vulgaris (Khorvash et al., 2012; Hegazy et al., 2023). This evidence supports examining S. aureus as an acne-associated organism, while recognizing that acne is multifactorial and that the present assay does not establish a causal role for the tested strain.

Antibiotics such as clindamycin, erythromycin, and tetracyclines are used in acne management, but antimicrobial resistance is a concern (Legiawati et al., 2023). Plant-derived extracts are therefore investigated as sources of compounds for preliminary antimicrobial screening. However, natural origin does not establish safety, clinical effectiveness, or suitability for topical use; these require separate chemical, toxicological, formulation, and clinical evaluation.

Momordica charantia L. (bitter melon) leaves have shown antibacterial activity in prior studies, including against *S. aureus* (Kamal et al., 2019; Meinasti, 2019). Evidence for activity against *C. acnes*, and the comparative response of both organisms across extract concentrations under a clearly characterized assay, remains limited in the cited literature.

This study conducted a preliminary in-vitro screen of ethanolic M. charantia L. leaf extract against *S. aureus* and *C. acnes* using disk diffusion. The primary outcome was the measured net inhibition-zone width at reported extract concentrations of 5%, 7%, and 10%; qualitative phytochemical screening was a secondary descriptive outcome. We expected larger extract concentrations to produce wider zones, while recognizing that disk diffusion alone cannot establish minimum inhibitory or bactericidal concentrations, a mechanism of action, or therapeutic efficacy.

2. MATERIALS AND METHODS

This preliminary in-vitro study evaluated ethanolic *Momordica charantia L.* leaf extract against the acne-associated bacteria *Staphylococcus aureus* and *Cutibacterium acnes*. Procedures comprised leaf extraction, qualitative phytochemical screening, bacterial culture preparation, disk-diffusion testing, and statistical analysis. The source manuscript reports three replicates per treatment in the results tables but does not specify whether these were independent cultures/plates or technical replicates; the experimental unit therefore requires confirmation.

a. Preparation of Momordica charantia L. Leaf Extract

Fresh M. charantia leaves were washed, cut into small pieces, oven-dried at 60°C, ground, and sieved. A 250-g portion of leaf powder was macerated in 96% ethanol at a 1:3 ratio for 24 h with occasional stirring, followed by repeated maceration over three 24-h cycles (Setyowati, 2016). The mixtures were filtered, and the filtrate was concentrated using a rotary evaporator at 40–50°C to obtain a crude extract. The crude extract was treated as the stock preparation and diluted with sterile distilled water to the

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tested concentrations of 5%, 7%, and 10%.

b. Phytochemical Screening

Qualitative phytochemical screening was conducted for alkaloids, flavonoids, and terpenoids. The reagents, observations, and positivity criteria for the reported tests are summarized in Table 1. The flavonoid test used magnesium powder and hydrochloric acid; a reddish-yellow color was recorded as positive (Septiningsih et al., 2017).

c. Preparation of Bacterial Cultures

Nutrient agar medium (Merck) was prepared in distilled water, sterilized at 121°C for 15 min, and poured aseptically into sterile Petri dishes. Cultures identified as *S. aureus* and *C. acnes* were streaked onto the medium. *S. aureus* was incubated aerobically at 37°C for 24 h, while *C. acnes* was incubated anaerobically in an anaerobic jar at 37°C for 24 h. Strain identifiers, culture sources, and inoculum standardization should be reported to support reproducibility. Because *C. acnes* is anaerobic and slow-growing, the medium, incubation period, and reading criteria should be justified against an organism-appropriate susceptibility-testing protocol.

d. Antibacterial Activity Assay

Antibacterial activity was screened by disk diffusion, following the cited procedure (Rizqiana and Pambudi, 2021). Bacterial suspensions were swabbed onto solidified agar. Sterile 6-mm paper disks were loaded with extract at the tested concentrations of 5%, 7%, and 10%; tetracycline and sterile distilled water served as positive and negative controls, respectively. Plates were incubated at 37°C, and measurements were reported at 12, 24, and 48 h. The inoculum density, volume loaded per disk, agar depth, disk spacing, tetracycline potency, and whether the same plates were read repeatedly should be reported for reproducibility. This assay is treated as a preliminary screen, not a standardized susceptibility test.

e. Measurement and Data Analysis

At each reading, the vertical and horizontal clear-zone measurements were averaged and the 6-mm disk diameter was subtracted, as described by Toy et al. (2015). The resulting values are net halo widths, not conventional total zone diameters. The categorical weak/moderate/strong thresholds are not applied because no validated interpretive framework for crude-extract measurements using this definition was provided. The tables list n = 3; the replicate unit and its independence should be clarified. The reported analysis used normality and homogeneity checks, one-way ANOVA at each incubation time, and Duncan's multiple range test in SPSS, with $p < 0.05$. Because the replicate structure and handling of repeated readings are unclear, inferential results are interpreted cautiously.

3. RESULTS AND DISCUSSION

a. Results

Phytochemical Screening

Qualitative screening recorded a positive color reaction for flavonoids and negative results for alkaloids and terpenoids (Table 1). These screening reactions indicate broad chemical classes only; they do not identify individual compounds, quantify their abundance, or

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establish which compounds account for any observed antibacterial activity.

Table 1. Qualitative phytochemical screening results of M. charantia L. leaf extract

Chemical compound	Reagent	Observation	Result
Alkaloid	H_2SO_4 + Mayer's reagent	No white precipitate was formed	Negative
Alkaloid	H_2SO_4 + Wagner's reagent	No brown precipitate was formed	Negative
Alkaloid	H_2SO_4 + Dragendorff's reagent	No red precipitate was formed	Negative
Flavonoid	Mg + 1 mL HCl	A reddish-yellow color change was observed	Positive
Terpenoid	HCl + H_2SO_4	No purple or green color change was observed	Negative

Antibacterial Activity Assay

The antibacterial activity of ethanolic M. charantia L. leaf extract was assessed by measuring net halo width at 12, 24, and 48 h of incubation.

The source tables report means and Duncan groupings for each treatment and time point. The reported one-way ANOVA indicated treatment differences for both organisms ($p < 0.05$), but exact F statistics, degrees of freedom, exact p-values, effect sizes, and dispersion measures were not included. The tables list n = 3; because the independence and type of these replicates are unspecified, the inferential results should be interpreted cautiously.

For *S. aureus*, the negative control showed no visible zone at any reading. Reported mean net halo widths for the 5% extract were 3.00, 1.00, and 0.83 mm at 12, 24, and 48 h; for 7%, they were 3.83, 3.00, and 2.50 mm; and for 10%, they were 7.00, 6.67, and 6.17 mm. The reported Duncan groupings place the 10% treatment separately from the other treatments at each time point, whereas 5%, 7%, and the positive control share a group. These are descriptive means without reported variability, and the unusually small positive-control values warrant verification of disk potency, strain identity, and quality-control conditions.

Table 2. Reported mean net halo widths against *S. aureus* at each incubation time

Treatment	n	12 h	24 h	48 h
Negative control (K−)	3	0.00^a	0.00^a	0.00^a
5%	3	3.00^b	1.00^b	0.83^b
7%	3	3.83^b	3.00^b	2.50^b
10%	3	7.00^c	6.67^c	6.17^c
Positive control (K+)	3	2.83^b	1.50^b	0.67^b

For *C. acnes*, the negative control showed no visible zone. Reported mean net halo widths for the 5% extract were 3.00, 2.50, and 1.67 mm at 12, 24, and 48 h; for 7%, they were 3.33, 2.83, and 2.00 mm; and for 10%, they were 7.50, 6.67, and 6.67 mm. The reported Duncan groupings do not distinguish the three extract concentrations, so the numerically larger values at 10% do not demonstrate a statistically significant concentration response. The tetracycline control had the largest reported zones; its disk potency and assay conditions should be verified.

Table 3. Reported mean net halo widths against *C. acnes* at each incubation time

Treatment	n	12 h	24 h	48 h
Negative control (K−)	3	0.00^a	0.00^a	0.00^a
5%	3	3.00^b	2.50^b	1.67^b
7%	3	3.33^b	2.83^b	2.00^b

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10%	3	7.50[b]	6.67[b]	6.67[b]
Positive control (K+)	3	16.17[c]	15.33[c]	9.67[c]

Across the reported readings, the measured net halo widths generally decreased between 12 and 48 h. This is a temporal pattern in the recorded measurements and cannot, by itself, establish loss of antibacterial activity, bacterial regrowth, or a bacteriostatic or bactericidal mechanism. The 10% extract had the largest reported mean among extract treatments for both organisms, but the post-hoc comparison distinguished it from lower concentrations for *S. aureus* only; the extract concentrations were not significantly different for *C. acnes*.

The 24-h values are summarized in Table 4 as reported net halo widths. They are not assigned weak, moderate, or strong categories because no validated interpretive framework for these crude-extract measurements was provided. The positive-control result for *S. aureus* was much smaller than that for *C. acnes* and should be reviewed as an assay quality-control issue before comparative conclusions are drawn.

Table 4. Reported net halo widths and observations at 24 h

Treatment	***S. aureus* net halo width (mm)**	**Observation**	***C. acnes* net halo width (mm)**	**Observation**
Negative control	0.00	No visible halo	0.00	No visible halo
5% extract	1.00	Not categorized	2.50	Not categorized
7% extract	3.00	Not categorized	2.83	Not categorized
10% extract	6.67	Not categorized	6.67	Not categorized
Positive control	1.50	Not categorized	15.33	Not categorized

b. Discussion

The disk-diffusion results provide preliminary evidence of visible growth inhibition under the reported assay conditions. The largest extract-associated mean was observed at 10%; however, the values are net halo widths after subtraction of the disk and are not directly comparable with standardized clinical zone diameters. No MIC, MBC, time-kill, or viability-recovery assay was performed, so the present data do not establish antibacterial potency, bactericidal action, or a mechanism.

The reported one-way ANOVA and Duncan groupings suggest treatment differences in the measured zones, but the absence of exact test statistics, dispersion estimates, and a definition of the experimental unit limits assessment. The tables indicate a difference between the 10% extract and lower treatments for *S. aureus*, while the 5%, 7%, and 10% extract groups were not significantly different for *C. acnes*. The latter pattern should be discussed as uncertainty in the concentration comparison, not as a confirmed dose-response relationship. Statistical significance also does not, by itself, establish biologically meaningful potency.

The 10% extract produced the largest reported mean net halo width among extract concentrations, but the strength of evidence differed by organism. For *S. aureus*, the reported post-hoc groupings separated 10% from the lower extract concentrations; for *C.*

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acnes, they did not. The data therefore support a concentration-related difference only for *S. aureus* under the reported analysis. Because replicate variability and independence are not specified, the size and reproducibility of these differences remain uncertain.

The reported net halo widths differed between *C. acnes* and *S. aureus*, but the comparison cannot be attributed to inherent species susceptibility from the information provided. Strain identity, inoculum density, culture medium, growth rate, aerobic versus anaerobic conditions, and compound diffusion may all affect disk-diffusion measurements. The manuscript provides no susceptibility profile or resistance phenotype for the tested *S. aureus* strain; resistance mechanisms should therefore not be inferred from these results.

Measured net halo widths generally declined across the reported reading times. Disk-diffusion measurements alone cannot distinguish bacteriostatic from bactericidal activity or demonstrate bacterial regrowth; these interpretations require viability-based assays such as time-kill or MBC testing. Differences in growth conditions and the timing of readings may also influence apparent zone size. The 12-, 24-, and 48-h observations should be interpreted as time-specific measurements under this protocol.

Tetracycline served as the reported positive control, but the disk potency and quality-control results were not provided. The relatively small *S. aureus* control measurement should be verified against the strain identity, inoculum, disk content, and a validated susceptibility-testing framework. It does not, on its own, demonstrate tetracycline resistance or an efflux mechanism. The substantially larger *C. acnes* control value also requires interpretation in light of the organism-specific test conditions.

4. CONCLUSION

Under the reported disk-diffusion conditions, ethanolic *Momordica charantia L.* leaf extract produced visible net halos against *Staphylococcus aureus* and *Cutibacterium acnes*. The 10% extract had the largest reported mean among extract concentrations; the post-hoc groupings distinguished it from lower concentrations for *S. aureus* but not for *C. acnes*. These observations are preliminary and do not establish a validated susceptibility category, MIC, MBC, mechanism, safety, or therapeutic efficacy. Interpretation is limited by incomplete reporting of strain identity, assay standardization, replicate independence, and variability. Further testing with confirmed strains, an organism-appropriate standardized method, and independent replicates is needed.

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