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Antibacterial Activity of Ethanol Extract of Srikaya Leaves (*Annona squamosa* Linn) Against the Growth of Acne Vulgaris-Causing Bacteria *Staphylococcus epidermidis*

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A B S T R A C T

Introduction: Acne vulgaris, a prevalent skin condition, is primarily triggered by the proliferation of *Cutibacterium acnes* within pilosebaceous units. However, *Staphylococcus epidermidis*, a commensal skin bacterium, can exacerbate acne inflammation upon follicular invasion. This study investigates the antibacterial potential of ethanol extract derived from srikaya leaves (*Annona squamosa* Linn) against *S. epidermidis*. **Methods:** Srikaya leaves underwent ethanol extraction via maceration. The extract's antibacterial efficacy was assessed using the agar well diffusion method against *S. epidermidis* at varying concentrations (25%, 50%, 75%). Zones of inhibition were measured, and minimum inhibitory concentrations (MICs) were determined. Phytochemical screening of the extract was conducted to identify potential bioactive compounds. **Results:** The ethanol extract of srikaya leaves exhibited significant antibacterial activity against S. epidermidis. Increasing extract concentrations led to larger zones of inhibition, indicating a dose-dependent effect. The MIC of the extract against *S. epidermidis* was determined to be 50%. Phytochemical analysis revealed the presence of flavonoids, phenols, and alkaloids, which are known for their antimicrobial properties. **Conclusion:** Ethanol extract of srikaya leaves demonstrates promising antibacterial activity against *S. epidermidis*, suggesting its potential as a natural therapeutic agent for managing acne vulgaris. Further research is warranted to elucidate the mechanisms of action and evaluate the extract's efficacy in clinical settings.

1. Introduction

Acne vulgaris, a prevalent inflammatory skin disorder affecting a vast majority of adolescents and a significant proportion of adults, continues to pose a considerable challenge in dermatology. This chronic condition is primarily characterized by the formation of comedones, papules, pustules, and nodules, resulting from the complex interplay of various factors, including sebum production, follicular hyperkeratinization, inflammation, and bacterial colonization. While the exact etiology of acne remains multifaceted, the proliferation of *Cutibacterium acnes* within pilosebaceous units is widely recognized as a key initiating factor. This Gram-positive anaerobic bacterium thrives in the lipid-rich environment of the pilosebaceous unit, where it metabolizes sebum triglycerides into free fatty acids. These free fatty acids, along with bacterial cell components, trigger an inflammatory cascade, leading to the characteristic lesions of acne. Although *C. acnes* is the primary causative agent, other microorganisms, including *Staphylococcus epidermidis*, can exacerbate acne inflammation upon follicular invasion. *S. epidermidis*, a commensal bacterium residing on the skin's surface, typically coexists harmoniously with the host. However, under certain conditions, such as follicular occlusion or compromised skin barrier integrity, *S. epidermidis* can penetrate the follicles and trigger an inflammatory response, contributing to acne pathogenesis. This bacterium produces various

virulence factors, including biofilm formation, adhesion molecules, and toxins, which can amplify the inflammatory process and worsen acne lesions. Biofilm formation, in particular, is a significant concern as it enhances bacterial survival and resistance to antimicrobial agents, making the eradication of *S. epidermidis* from acne lesions more challenging.[1-3]

The management of acne vulgaris often involves the use of topical or systemic antibiotics, which aim to reduce the bacterial load and mitigate inflammation. However, the indiscriminate use of antibiotics has led to the emergence of antibiotic-resistant strains of *S. epidermidis*, posing a significant challenge in acne treatment. Antibiotic resistance not only compromises the efficacy of conventional acne therapies but also raises concerns about the potential spread of resistance to other pathogenic bacteria. Therefore, there is a growing interest in exploring alternative therapeutic approaches, particularly those derived from natural sources, to combat *S. epidermidis* and manage acne vulgaris effectively. Natural products, derived from plants, have been used for centuries in traditional medicine for their diverse therapeutic properties. These products offer a rich source of bioactive compounds with potential antimicrobial, anti-inflammatory, and antioxidant activities. In the context of acne vulgaris, natural products present an attractive alternative to conventional antibiotics due to their lower risk of inducing antibiotic resistance and their potential to address multiple aspects of acne pathogenesis. Srikaya (*Annona squamosa* Linn), a tropical fruit tree native to the Americas, has been traditionally used for various medicinal purposes. The leaves of srikaya are known for their rich phytochemical content, including flavonoids, phenols, alkaloids, and terpenoids. These compounds have been reported to possess diverse biological activities, including antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties.[4,5]

Flavonoids, a class of polyphenolic compounds, are known for their ability to disrupt microbial metabolism by damaging cell walls and inhibiting nucleic acid synthesis. They can also interfere with bacterial adhesion and biofilm formation, which are crucial virulence factors of *S. epidermidis*. The ability of flavonoids to modulate bacterial virulence factors makes them promising candidates for developing novel anti-acne therapies. Phenols, another group of phytochemicals found in srikaya leaves, exhibit antimicrobial activity by denaturing bacterial proteins and disrupting cell membrane integrity. The disruption of cell membrane integrity leads to leakage of cellular contents and ultimately bacterial death. Phenols also possess antioxidant properties, which can help to mitigate the oxidative stress associated with acne inflammation. Alkaloids, nitrogen-containing compounds, have been shown to possess antibacterial properties by interfering with bacterial DNA replication and protein synthesis. The ability of alkaloids to target essential bacterial processes makes them potent antimicrobial agents. Additionally, alkaloids have been reported to possess anti-inflammatory properties, which can further contribute to their therapeutic potential in acne management. Terpenoids, a diverse class of organic compounds, are known for their antimicrobial, anti-inflammatory, and antioxidant activities. While the presence of terpenoids in srikaya leaves is not well-documented, their potential contribution to the overall therapeutic effect of srikaya leaf extract cannot be overlooked.[6-8] Given the rich phytochemical profile of srikaya leaves and the reported antimicrobial activities of its constituents, this study aims to investigate the antibacterial potential of ethanol extract derived from srikaya leaves against *S. epidermidis*.

2. Methods

Fresh, mature srikaya leaves (*Annona squamosa* Linn) were harvested from Sukoharjo, Indonesia. The selection criteria for the leaves included a vibrant green color, absence of visible damage or disease, and a mature stage of development. The leaves were carefully handpicked to ensure the quality and consistency of the plant material. Upon collection, the leaves were subjected to a thorough washing process using deionized water to remove any dust, debris, or potential contaminants. This step was crucial to maintain the purity of the extract and prevent the

introduction of extraneous substances that could interfere with the subsequent analysis and testing.

Following the washing step, the leaves were thinly spread on clean drying racks and allowed to air dry under shade. The drying process was conducted in a well-ventilated area with low humidity to facilitate the removal of moisture from the leaves. The leaves were turned regularly to ensure uniform drying and prevent the growth of mold or other microorganisms. The drying process continued until the leaves reached a brittle texture, indicating a significant reduction in moisture content. Once dried, the leaves were carefully separated from the stems and other extraneous plant parts. The dried leaves were then pulverized using a mechanical grinder to obtain a fine powder. The grinding process was performed in multiple batches to ensure uniform particle size and maximize the surface area available for extraction. The powdered leaves were stored in airtight containers in a cool, dark place until further use. The extraction of bioactive compounds from the powdered srikaya leaves was achieved using ethanol as the solvent. Ethanol was chosen due to its ability to effectively dissolve a wide range of phytochemicals, including flavonoids, phenols, alkaloids, and terpenoids, which are known to be present in srikaya leaves. The extraction process employed the maceration technique, a simple and widely used method for extracting plant materials. In the maceration process, a pre-determined quantity of the powdered srikaya leaves was placed in a clean glass container. A specific volume of ethanol, calculated based on the desired solvent-to-solid ratio, was then added to the container. The mixture was thoroughly agitated to ensure proper wetting of the plant material and to facilitate the dissolution of the bioactive compounds into the solvent. The container was then sealed tightly and stored in a cool, dark place for a specific duration, during which the solvent gradually penetrated the plant material and extracted the desired compounds. After the maceration period, the mixture was filtered through a Whatman filter paper to separate the liquid extract from the solid plant residue. The filtration process was repeated multiple times to ensure complete removal of the solid particles and obtain a clear extract. The filtered extract was then concentrated using a rotary evaporator. This instrument operates under reduced pressure and controlled temperature, allowing for the efficient removal of the solvent while preserving the integrity of the extracted compounds. The concentrated extract was further dried under vacuum to remove any residual solvent, resulting in a semi-solid or viscous mass. The obtained ethanol extract of srikaya leaves was then subjected to various analyses and tests to determine its phytochemical composition and antibacterial activity. The extract was stored in airtight containers in a cool, dark place until further use.

A clinical isolate of *Staphylococcus epidermidis* was obtained from a reputable microbiological laboratory. The isolate was selected based on its relevance to acne vulgaris and its potential to exacerbate inflammation in acne lesions. The bacterium was carefully subcultured on nutrient agar (NA) plates to ensure its purity and viability. The NA plates were prepared by dissolving a pre-determined quantity of nutrient agar powder in distilled water. The mixture was then heated and sterilized by autoclaving to eliminate any potential contaminants. The sterilized agar was poured into sterile Petri dishes and allowed to solidify. The *S. epidermidis* isolate was then streaked onto the surface of the NA plates using a sterile inoculation loop. The plates were incubated at 37°C for 24 hours to allow for bacterial growth. The incubation temperature of 37°C was chosen as it mimics the physiological temperature of human skin, providing optimal conditions for the growth of *S. epidermidis*. The incubation period of 24 hours was sufficient to allow for the formation of visible colonies on the NA plates. The bacterial colonies were then examined for their morphological characteristics, such as size, shape, color, and texture, to confirm the identity of *S. epidermidis*.

The antibacterial activity of the ethanol extract of srikaya leaves against *S. epidermidis* was evaluated using the agar well diffusion method. This method is a widely used and reliable technique for assessing the antimicrobial activity of plant extracts and other substances. The principle of the agar well diffusion method is based on the diffusion of the test substance through the agar medium and its subsequent interaction with the bacteria. In this study, NA plates

were prepared as described previously. The *S. epidermidis* isolate was then swabbed uniformly onto the surface of the NA plates using a sterile cotton swab. This ensured an even distribution of the bacteria on the agar surface, allowing for consistent interaction with the test substance. Wells were then punched into the agar using a sterile cork borer. The wells were carefully spaced to prevent overlapping of the zones of inhibition, which could lead to inaccurate measurements. Different concentrations of the ethanol extract of srikaya leaves (25%, 50%, 75%) were prepared by dissolving the extract in sterile distilled water. A fixed volume of each concentration was then added to the respective wells using a micropipette. Chloramphenicol, a broad-spectrum antibiotic commonly used in the treatment of bacterial infections, was used as a positive control. A fixed volume of chloramphenicol solution was added to a separate well to serve as a reference for comparing the antibacterial activity of the srikaya leaf extract. Sterile distilled water was used as a negative control to ensure that any observed inhibition was due to the extract and not the solvent.

The plates were then incubated at 37°C for 24 hours. During this period, the extract diffused through the agar medium and interacted with the *S. epidermidis* bacteria. If the extract possessed antibacterial activity, it would inhibit the growth of the bacteria, resulting in a clear zone of inhibition around the well. The size of the zone of inhibition was directly proportional to the antibacterial potency of the extract. After the incubation period, the plates were examined for the presence of zones of inhibition. The diameter of each zone of inhibition was measured using a digital caliper and recorded. The measurements were taken in triplicate for each concentration of the extract to ensure accuracy and reproducibility. The mean zone of inhibition for each concentration was then calculated and compared to that of the positive and negative controls.

The minimum inhibitory concentration (MIC) of the ethanol extract of srikaya leaves against *S. epidermidis* was determined using the broth microdilution method. This method is a quantitative technique that allows for the determination of the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism in a liquid medium. In this study, a series of two-fold dilutions of the ethanol extract of srikaya leaves were prepared in Mueller-Hinton broth (MHB). MHB is a standardized medium commonly used for antimicrobial susceptibility testing. The dilutions were prepared in sterile 96-well microtiter plates. A standardized suspension of *S. epidermidis* was then added to each well containing the diluted extract. The final concentration of bacteria in each well was adjusted to approximately 5 x 10^5 colony-forming units (CFU)/mL. The plates were then incubated at 37°C for 24 hours. After the incubation period, the plates were examined for visible bacterial growth. The MIC was defined as the lowest concentration of the extract that prevented visible turbidity in the well, indicating the inhibition of bacterial growth. The MIC value was recorded and used as a measure of the extract's antibacterial potency.

Phytochemical screening of the ethanol extract of srikaya leaves was conducted to identify the presence of various classes of bioactive compounds, including flavonoids, phenols, alkaloids, and terpenoids. Standard qualitative tests were employed for each class of compounds. The presence of flavonoids was determined using the Shinoda test. In this test, a small amount of the extract was dissolved in ethanol and treated with magnesium ribbon and concentrated hydrochloric acid. The development of a pink or red color indicated the presence of flavonoids. The presence of phenols was determined using the ferric chloride test. In this test, a small amount of the extract was dissolved in water and treated with a few drops of ferric chloride solution. The formation of a bluish-black color indicated the presence of phenols. The presence of alkaloids was determined using the Dragendorff's test and Mayer's test. In the Dragendorff's test, a small amount of the extract was dissolved in dilute hydrochloric acid and treated with Dragendorff's reagent. The formation of an orange or reddish-brown precipitate indicated the presence of alkaloids. In the Mayer's test, a small amount of the extract was dissolved in water and treated with Mayer's reagent. The formation of a creamy-white

precipitate indicated the presence of alkaloids. The presence of terpenoids was determined using the Salkowski test. In this test, a small amount of the extract was dissolved in chloroform and treated with concentrated sulfuric acid. The development of a reddish-brown color at the interface of the two layers indicated the presence of terpenoids.

3. Results and Discussion

Table 1 presents the antibacterial activity of srikaya leaf ethanol extract against *Staphylococcus epidermidis*, a bacterium implicated in acne vulgaris. The extract's efficacy is evident in the formation of zones of inhibition, and clear areas around the application sites where bacterial growth is hampered. A clear dose-dependent relationship is observed, with larger zones of inhibition corresponding to higher extract concentrations. At 25%, the extract demonstrates a modest inhibitory effect, classified as "resistant" based on Clinical and Laboratory Standards Institute (CLSI) guidelines. However, at 50% and 75%, the zones of inhibition expand, transitioning into the "intermediate" category. This suggests that while these concentrations may not completely eradicate the bacteria, they significantly impede its growth. Chloramphenicol, a standard antibiotic, serves as a positive control, exhibiting the largest zone of inhibition and achieving "susceptible" status. This highlights the extract's potential as a natural alternative, although not as potent as the pharmaceutical option. The negative control, lacking any antibacterial agent, expectedly shows no inhibitory effect. The variations in zone sizes, represented by standard deviations (SD), underscore the inherent variability in biological systems. These fluctuations could be attributed to factors like bacterial density, agar consistency, or minor experimental variations. Despite this variability, the overall trend of increasing inhibition with higher concentrations remains robust.

Table 1. Antibacterial activity of ethanol extract of srikaya leaves against *Staphylococcus epidermidis*.

Extract concentration (%)	**Zone of inhibition (mm)±SD**	**Interpretation**
25	10.82±2.73	Resistant
50	14.46±2.43	Intermediate
75	16.80±2.21	Intermediate
Chloramphenicol (30mcg)	21.13±3.21	Susceptible
Negative control	0	No effect

Table 2 unveils the intricate chemical composition of the ethanol extract derived from srikaya leaves, providing a glimpse into its potential therapeutic properties. The presence of flavonoids, revealed by the vibrant pink hue in the Shinoda test, hints at the extract's antioxidant and anti-inflammatory potential. These versatile compounds are renowned for their ability to scavenge harmful free radicals and modulate inflammatory pathways, suggesting a possible role in mitigating the oxidative stress and inflammation associated with acne vulgaris. The bluish-black coloration observed in the ferric chloride test confirms the presence of phenols, a class of compounds celebrated for their antimicrobial prowess. These natural defenders act as molecular saboteurs, disrupting bacterial cell walls and proteins, potentially hindering the growth and survival of *Staphylococcus epidermidis* and other acne-inducing bacteria. The positive results in both Dragendorff's and Mayer's tests, indicated by the formation of characteristic precipitates, unequivocally establish the presence of alkaloids. These nitrogen-containing compounds are renowned for their diverse biological activities, including antibacterial, antifungal, and anti-inflammatory effects. The presence of alkaloids in the srikaya leaf extract further strengthens its potential as a multifaceted therapeutic agent against acne, potentially targeting multiple aspects of its pathogenesis. Interestingly, the Salkowski test yielded a negative result, indicating the absence of terpenoids in the extract. While terpenoids are known for their antimicrobial and anti-inflammatory properties, their

absence does not diminish the overall therapeutic potential of the extract, as the presence of flavonoids, phenols, and alkaloids provides a robust foundation for its antibacterial and anti-inflammatory effects.

Table 2. Phytochemical screening of ethanol extract of srikaya leaves.

Phytochemical class	Test	Result
Flavonoids	Shinoda test	+
Phenols	Ferric chloride test	+
Alkaloids	Dragendorff's test	+
Alkaloids	Mayer's test	+
Terpenoids	Salkowski test	-

Table 3 provides a comprehensive overview of the characteristics of both the srikaya leaf simplicia (dried, powdered leaves) and the ethanol extract derived from it. The simplicia exhibits a brownish-green hue, indicative of the chlorophyll and other pigments naturally present in the leaves. Its characteristic srikaya leaf odor suggests the retention of volatile aromatic compounds during the drying process. The loss on drying (LOD) value of 71.43% signifies a substantial reduction in moisture content during the drying process. This is crucial for preserving the simplicia and preventing microbial growth. However, the LOD falls short of the ideal range of 85-90%, suggesting potential for further drying to optimize storage stability. The moisture content of the simplicia, at 7.99%, aligns with the standard requirement of less than 10%. This low moisture level further supports the simplicia's stability and resistance to spoilage. In contrast, the ethanol extract boasts a higher moisture content of 11.72%. This is expected, as extracts often retain some solvent and may absorb moisture during the concentration process. The yield of 10.13 grams signifies the efficiency of the ethanol extraction process in drawing out the bioactive compounds from the simplicia. This yield can be influenced by various factors, including the solvent used, extraction time, and the simplicia's inherent phytochemical content. The absence of ethanol contamination in the extract, confirmed by the negative test result, is a critical quality control measure. Ethanol, while an effective solvent, can interfere with subsequent analyses and biological assays. Its removal ensures the purity of the extract and the accuracy of further investigations into its properties.

Table 3. Characteristics of srikaya leaf simplicia and extract.

Characteristic	Simplicia	Extract
Organoleptic (Color)	Brownish green	Green
Organoleptic (Odor)	Characteristic srikaya leaf odor	Characteristic srikaya leaf odor
Loss on drying (LOD)	71.43%	Not applicable
Moisture content	7.99%	11.72%
Yield	-	10.13 g
Ethanol contamination	-	Negative

The present study delves into the antibacterial potential of ethanol extract derived from srikaya leaves (*Annona squamosa* Linn) against *Staphylococcus epidermidis*, a bacterium implicated in the exacerbation of acne vulgaris. The findings illuminate a compelling narrative of the extract's dose-dependent inhibitory effect on *S. epidermidis* growth, underpinned by its rich phytochemical composition. The agar well diffusion assay, a cornerstone of antimicrobial susceptibility testing, revealed a clear correlation between the extract's concentration and its inhibitory prowess. At 25%, the extract displayed a

modest zone of inhibition, classifying it as "resistant" according to CLSI guidelines. This suggests that while the extract may possess inherent antibacterial properties, its efficacy at this concentration is limited. However, a significant shift was observed at 50% and 75% concentrations, where the zones of inhibition expanded, transitioning into the "intermediate" category. This escalation in antibacterial activity underscores the extract's dose-dependent nature, implying that higher concentrations can more effectively impede *S. epidermidis* growth. The observed dose-dependent response aligns with the fundamental principles of pharmacology, where the biological effect of a substance often correlates with its concentration. In the context of antibacterial agents, higher concentrations typically lead to increased interactions with bacterial targets, resulting in enhanced growth inhibition or outright bactericidal effects. This phenomenon is particularly relevant in the case of plant extracts, which often contain a complex mixture of bioactive compounds with varying potencies and mechanisms of action.[9-11]

The phytochemical screening of the srikaya leaf extract unveiled a treasure trove of bioactive compounds, including flavonoids, phenols, and alkaloids. These compounds, renowned for their antimicrobial properties, likely contribute synergistically to the extract's inhibitory effect on *S. epidermidis*. Flavonoids, a diverse class of polyphenolic compounds, are known to disrupt bacterial cell walls, inhibit nucleic acid synthesis, and interfere with essential enzymatic processes. Their ability to target multiple bacterial pathways makes them potent antimicrobial agents. Phenols, another group of phytochemicals abundant in srikaya leaves, exert their antibacterial effects through various mechanisms, including disruption of cell membrane integrity, inhibition of protein synthesis, and interference with energy metabolism. The presence of phenols in the extract may explain its ability to create a hostile environment for *S. epidermidis*, leading to growth inhibition. Alkaloids, nitrogen-containing compounds with diverse biological activities, have been shown to possess antibacterial properties by targeting bacterial DNA replication, protein synthesis, and cell wall formation. The presence of alkaloids in the srikaya leaf extract further strengthens its antimicrobial arsenal, potentially contributing to its observed inhibitory effect on *S. epidermidis*.

The absence of terpenoids in the extract, as indicated by the negative Salkowski test, is noteworthy. Terpenoids are a class of organic compounds known for their antimicrobial and anti-inflammatory properties. While their absence may seem counterintuitive, it is important to consider that the antibacterial activity of plant extracts is often a result of the synergistic interaction of multiple compounds rather than the isolated effect of a single constituent. Therefore, the absence of terpenoids does not necessarily diminish the overall therapeutic potential of the srikaya leaf extract.[12-14]

The observed antibacterial activity of the srikaya leaf extract against *S. epidermidis* aligns with previous research findings. Several studies have reported the antimicrobial efficacy of srikaya leaf extracts against various bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Propionibacterium acnes*. These studies have attributed the antibacterial activity of srikaya leaf extracts to their rich phytochemical content, particularly flavonoids, phenols, and alkaloids.

The current study builds upon this existing body of knowledge by specifically focusing on the inhibitory effect of srikaya leaf extract against *S. epidermidis*, a bacterium known to play a role in acne pathogenesis. The findings of this study provide further evidence of the broad-spectrum antimicrobial potential of srikaya leaf extract and its potential as a natural therapeutic agent for acne management. The clinical implications of these findings are significant. Acne vulgaris, a prevalent skin condition affecting millions worldwide, is often associated with a dysbiosis of the skin microbiome, characterized by an overgrowth of *C. acnes* and other opportunistic pathogens like *S. epidermidis*. The ability of srikaya leaf extract to inhibit the growth of *S. epidermidis* suggests its potential to restore microbial balance on the skin and mitigate the inflammatory response associated with acne.[15-17]

Furthermore, the dose-dependent nature of the extract's antibacterial activity suggests that it could be formulated into topical preparations with varying concentrations to cater to different severities of acne. Lower concentrations may be suitable for mild cases, while higher concentrations could be employed for more severe or recalcitrant acne. However, it is important to acknowledge the limitations of this study. The agar well diffusion method, while a valuable tool for screening antimicrobial activity, does not provide a comprehensive understanding of the extract's mechanism of action. Further research is needed to elucidate the precise molecular targets and pathways involved in the extract's inhibitory effect on *S. epidermidis*. Additionally, in vivo studies are warranted to evaluate the extract's efficacy and safety in clinical settings.[18-20]

4. Conclusion

The present study provides compelling evidence of the antibacterial activity of ethanol extract of srikaya leaves against *Staphylococcus epidermidis*. The extract's dose-dependent inhibitory effect, coupled with its rich phytochemical profile, suggests its potential as a natural therapeutic agent for managing acne vulgaris.

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