

Phytochemical Profile and Therapeutic Promise of *Aerva lanata*: An Emerging Natural Remedy for Diabetes and Microbial Infections

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ABSTRACT

Aerva lanata, commonly known as knot grass, is a prostrate herb belonging to the Amaranthaceae family and is traditionally valued in folk medicine. Recognized as one of the ten sacred flowers (Dasapushpam) in Kerala, this plant has been widely used for managing Diabetes mellitus and exhibits notable antimicrobial properties. Traditionally, its aerial parts are ground with water and consumed before meals to harness its medicinal benefits. The Crude extract is typically prepared using ultrasonication, method that ensures efficient Extraction of bioactive compounds. Phytochemical analysis of the ethanolic extract of *Aerva lanata* has revealed the presence of alkaloids, terpenoids, tannins, sugars, amino acids, Saponins, and glycosides-compounds known for their therapeutic potential. The antimicrobial activity of the extract was assessed using the agar well diffusion method, demonstrating significant antibacterial effects against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (Enterobacter). The inhibition zones increased with concentration, inhibition against *Staphylococcus aureus* and *Bacillus subtilis* at Both 30 µl and 40 µl concentrations indicating antimicrobial efficacy. The antidiabetic potential of the plant was evaluated through the alpha-amylase inhibition assay and glucose uptake by yeast cell method. The alpha-amylase Inhibitory method assesses the ability of plant to impede the enzyme responsible for Breaking down carbohydrates into glucose, thus reducing blood sugar levels. Additionally, the glucose Uptake by yeast cells method demonstrates the plant's Extract inhibit the uptake of glucose to the yeast cell. These studies confirmed the ability of *Aerva lanata* to regulate blood sugar levels by inhibiting carbohydrate breakdown and reducing glucose absorption. Overall, *Aerva lanata* emerges as a promising medicinal plant

with antimicrobial and Antidiabetic properties. Its rich phytochemical and traditional significance, coupled with scientific validation, highlight its potential for further research and therapeutic applications in Natural medicine.

Key words: Dasapushpam, folk medicine, ultrasonication, alpha-amylase inhibition, Bioactive compounds.

INTRODUCTION

Phytochemistry, as a dynamic interdisciplinary field, bridges the domains of botany, biochemistry, and pharmacology to explore the diverse chemical compounds produced by plants. Central to its focus are secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenolics, and tannins, which, while not directly involved in plant growth or reproduction, play pivotal roles in defense mechanisms, ecological adaptation, and survival under stress conditions (Verma et al., 2021). These compounds, owing to their complex structures and bioactivities, have become crucial leads in modern drug discovery, contributing to the development of treatments for infectious diseases, inflammation, cancer, and metabolic disorders (Atanasov et al., 2021). Phytochemistry thus not only enhances our understanding of plant biology but also paves the way for innovative, natural therapeutics.

Among the many plants of medicinal value, *Aerva lanata* (L.) Juss. ex Schult. stands out for its extensive traditional use and pharmacological promise. Widely used in Ayurvedic and Siddha systems, it is traditionally known as *Poonakanni* and holds cultural reverence in Kerala, where it is one of the ten sacred flowers, *Dashapushpam* (Faisal et al., 2019; Narayanan et al., 2022). Scientific studies have validated its rich phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds. These constituents are linked to its broad therapeutic effects, such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, diuretic, and antidiabetic properties (Preeja et al., 2023; Ramya et al., 2024). Advanced analytical techniques like HPLC, GC-MS, and FTIR have further identified key bioactive molecules such as β -sitosterol, quercetin, rutin, lupeol, and caffeic acid, enhancing its pharmacological appeal (Sridhar et al., 2023).

The therapeutic potential of *Aerva lanata* has been strongly supported by contemporary research. Traditionally used to treat urinary calculi, diabetes, jaundice, and respiratory infections, the plant has demonstrated nephroprotective activity through mechanisms such as oxidative stress reduction and inhibition of calcium oxalate crystallization (Deepak et al.,

2019; Gaurav et al., 2022). Its antidiabetic properties have also been confirmed in experimental studies, where aqueous and ethanolic extracts significantly reduced blood glucose levels, enhanced insulin sensitivity, and regulated carbohydrate metabolism (Jayatha, 2023). Furthermore, its broad-spectrum antimicrobial and antifungal activities have shown efficacy against *Candida parapsilosis*, *Aspergillus flavus*, *Trichosporon asahii*, *Staphylococcus aureus*, and *Escherichia coli*, validating its traditional use in combating infections (Nimisha & Beula, 2019).

The relevance of phytochemistry in contemporary healthcare is underscored by its role in reconnecting ethnobotanical knowledge with scientific validation. As the demand for plant-based, sustainable, and affordable therapeutics rises, plants like *Aerva lanata* become vital resources for addressing global health challenges. Phytochemistry promotes not only the discovery of novel drugs but also encourages the conservation of medicinal biodiversity and the integration of indigenous knowledge into evidence-based medicine (Aleksandra et al., 2022).

MATERIALS AND METHODS

Kerala, a state in southern India, is renowned for its exceptional plant diversity, shaped by its diverse landscape and tropical monsoon climate. Traditional Ayurvedic practices in Kerala highlight the significance of medicinal plants, with species such as neem, tulsi, and Indian gooseberry being widely utilized for their therapeutic properties. Additionally, the state's extensive network of rivers, wetlands, and backwaters nurtures a diverse array of aquatic plants, further enhancing its ecological wealth. This intricate interplay between agriculture, medicinal traditions, and natural ecosystems underscores Kerala's botanical richness.

By integrating scientific research with traditional ecological knowledge, Kerala can continue to protect its invaluable plant resources while promoting sustainable development. Strengthening conservation initiatives and encouraging responsible environmental practices will be essential in maintaining the state's reputation as a land of diverse and thriving flora.

Study area

Kattakada, located approximately 20 kilometers from Thiruvananthapuram in Kerala, is a region characterized by its undulating terrain, lush green landscape, and a tropical monsoon

climate. The area benefits from improved infrastructure, with well-maintained roadways and frequent bus services enhancing connectivity. Education and healthcare facilities are available, with several schools and healthcare centers catering to the local population. Despite its strengths, Kattakada faces challenges such as rapid urbanization, resource pressure, and environmental concerns like deforestation and water scarcity. However, it holds significant potential for future development through sustainable agriculture, eco-tourism, and small-scale industries. Enhanced educational and healthcare facilities will further contribute to the community's development. The proximity of Kattakada to Thiruvananthapuram offers additional economic opportunities, positioning it well for growth while preserving its unique blend of urban and rural charm.

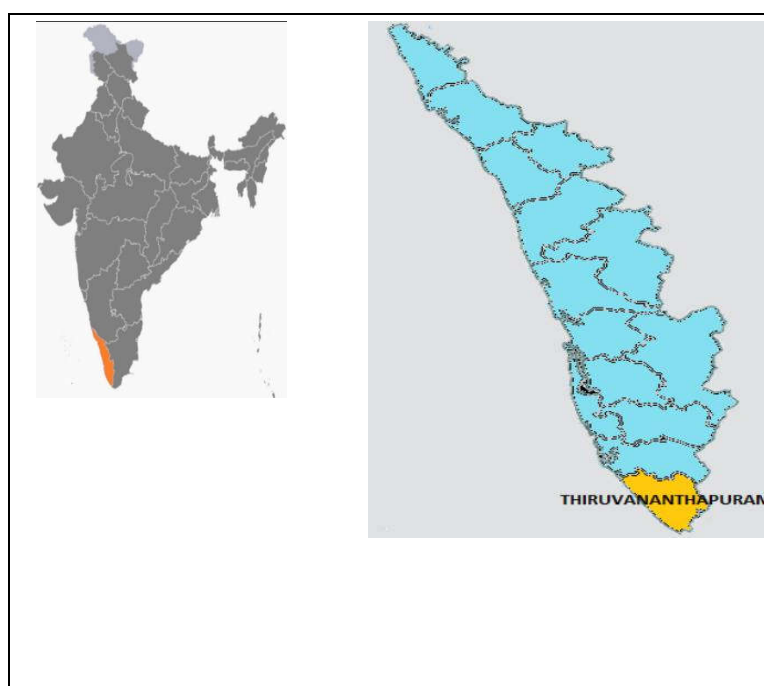


Figure 1: Selected Study area

Sampling Site

Kattakada, situated in the southern part of Kerala's Thiruvananthapuram district at approximately 8.4897° N latitude and 77.0595° E longitude, is known for its rich biodiversity, particularly its diverse angiosperm population. The area's tropical monsoon climate, characterized by heavy rainfall between June and September and a dry period from December to March, fosters a wide range of flowering plants. Among the numerous angiosperms found in Kattakada, *Aerva lanata* stands out for its abundance and medicinal significance. *Aerva lanata* is highly valued in Ayurvedic medicine for its therapeutic

properties, particularly in the treatment of kidney stones, urinary infections, respiratory ailments, and diabetes.

The prevalence of *Aerva lanata* in Kattakada highlights the region's deep-rooted ethno botanical traditions and reliance on plant-based remedies. The continued availability of such medicinal plants underscores the importance of preserving local biodiversity. Sustainable conservation efforts are crucial to safeguarding this rich plant heritage, ensuring both ecological stability and the continued use of natural resources for future generations.

Plant description

Aerva lanata is traditionally used in various medicinal practices, particularly in Ayurveda, due to its phytochemical compounds such as alkaloids, flavonoids, tannins, and saponins. These compounds contribute to its antidiabetic, antimicrobial, antiurolithiatic, diuretic, hepatoprotective, anticancer, immunomodulatory, antioxidant, and other pharmacological activities. Qualitative phytochemical analysis has identified these compounds, which support its traditional uses and potential in modern medicine.



Plate 1: Selected plant

Aerva lanata, commonly referred to as mountain knotgrass, is a hardy herbaceous plant found across tropical and subtropical regions. Its leaves, which grow alternately along the stem, vary in shape from elliptic to obovate or orbicular. The upper surface of the leaves is slightly hairy, while the underside is densely covered in white woolly hairs. The plant develops multiple branches, which are either pubescent or covered in dense woolly hairs. Most flowers

are bisexual, some may be unisexual. They appear in clusters of two or three in the leaf axils and are enclosed within a five-lobed, hairy perianth. The reproductive structures include stamens with four or five lobes and a gynoecium comprising a single pistil with an ovoid ovary and two feathery stigmas.

The fruit of *Aerva lanata* is a small, non-splitting capsule that encloses one or two minute seeds. These seeds, measuring around 1 mm in diameter, are encased within the persistent woolly perianth. They are glossy black, kidney-shaped, and well-suited for dispersal. The unique structural features of *Aerva lanata* contribute to its adaptability and ability to thrive in a variety of environmental conditions.

a) Qualitative analysis of Phytochemicals

The extract obtained from the whole plant of *Aerva lanata* was subjected to qualitative phytochemical screening to identify key bioactive compounds (Susi et al., 2021). The analysis focused on major classes of phytochemicals, including phenols, flavonoids, steroids, amino acids, tannins, terpenoids, glycosides, saponins, and alkaloids, along with essential biomolecules such as lipids, carbohydrates, and proteins. The tests were conducted following established methodologies as described by Harborne (1998), Sofowora (1993), and Trease & Evans (1989); Kanneboina et al., 2022).

Sample Preparation

A 10-gram portion of the plant sample was taken and mixed with 80 mL of 80% ethanol and 20 mL of water. The mixture was heated in an oven at 50°C for five minutes and then allowed to cool. To enhance extraction efficiency, ultrasonication was carried out for 15 minutes. The resulting extract was then filtered using a filtration funnel and transferred into a conical flask for further analysis (Swapna and Gaurrapu, 2018; Pydiraju et al., 2023).

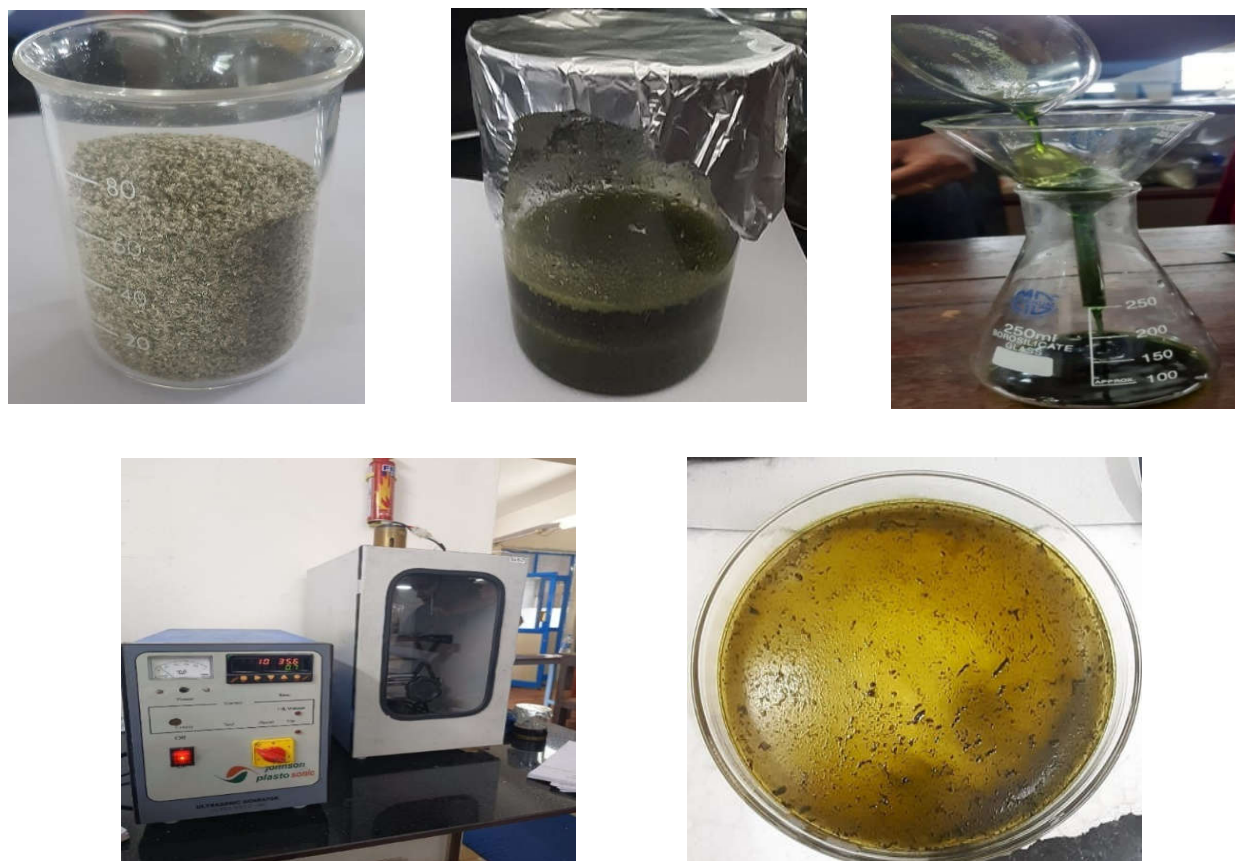


Figure 2-6: Sample extract

Antimicrobial Assay

The rise of multidrug-resistant microbial strains and the emergence of bacteria with reduced antibiotic susceptibility are significant global health concerns. This resistance has been largely linked to the overuse of broad-spectrum antibiotics, the administration of immunosuppressive agents, the widespread use of intravenous catheters, organ transplantation procedures, and the ongoing HIV epidemic (Vineela et al., 2020). Additionally, in many developing countries, synthetic drugs are often costly, sometimes ineffective due to adulteration, and may cause adverse side effects. As a result, there is an urgent need to explore alternative antimicrobial strategies to combat infections more effectively.

Sample Preparation

To prepare the plant extract for antimicrobial testing, the dried extract is dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL.

Preparation of Culture Plates

For antibacterial assays, culture plates are prepared using Mueller-Hinton Agar, a standard medium that supports the growth of various bacterial strains and facilitates accurate antimicrobial testing.

WELL DIFFUSION METHOD

Procedure

The test organisms, *Staphylococcus aureus*, Enterobacter and *Bacillus subtilis* were isolated from previously isolated, identified and stored. The micro-organisms were grown in the Mueller-Hinton medium. The assay for antibacterial activity was carried by a Well diffusion method. Mueller Hinton agar Medium was poured into sterile Petri plates and left to solidify (Mamidala and Swapna, 2018). After solidification inoculates the bacteria in the medium. 4 holes were punched using sterile borers. Once wells were formed, they were filled with different dilution of plant extracts were Introduced into well. Antibacterial activity against the tested microorganisms at 2 different concentrations of 30 and 40 µg/ml. Ampicillin was used as a standard drug. The plates were Incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The zone of Inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the Well diameter (Madhusudhan et al., 2017).

Bacterial organism	Test chemical concentration
<i>Staphylococcus aureus</i>	30 - 40 µg/ml
<i>Bacillus</i>	30 - 40 µg/ml
<i>Entrobacter</i>	30- 40 µg/ml

Table 1: Antimicrobial assay

Antidiabetic Analysis

Alpha amylase inhibitory assay

Many herbal extracts have been reported to have antidiabetic activities and are used in Ayurveda for the treatment of diabetes. α -amylase and nature design the carbohydrates and increase the postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycaemia and reduce the risk of developing diabetes (Kooti et al., 2016; Shyam et al., 2022). Weigh 0.05 milligram of sample in a test tube and dissolve the sample in 5 ml phosphate buffer (PH – 6.8) and allow mixing well by using vortex. Label the test tube B for blank, S for standard, C for control and T1, T2, T3, T4, T5, T6 (Musbau et al., 2018; Saisree et al., 2019).

Preparation of DNS (Sashikiran et al., 2021)

DNS reagent in 100 ml

- Weigh 1 g of DNS gradually dissolved 70 ml of 0.5 N NaOH and 30 g Sodium potassium tartrate and make up to 100 ml.
- Boil the test tube in a water bath for 10 min.

Preparation of starch

- Weigh 1g of starch in 100 ml distilled water.
- 10 min boil in water bath.

	Blank	Control	T1	T2	T3	T4	T5	T6
Extract	0	0	25	50	100	200	400	600
Buffer	2	2	1975	1950	1900	1800	1600	1400
Alpha amylase	0	0.5 ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
37 °c incubation at 20 min								
Starch	0	1 ml	1 ml	1ml	1 ml	1 ml	1 ml	1 ml
37 °c incubation at 30 min								
DNS	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml

Table 2: Test for Alpha amylase

	S1	S2	S3	S4
Acarbose	20	40	80	100
Buffer	1980	1960	1920	1900
Alpha amylase	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Incubate 37°C for 20 min				
Starch	1 ml	1 ml	1 ml	1 ml
Incubate 37°C for 30 min				
DNS	2 ml	2ml	2ml	2ml

Table 3: Standard for Alpha amylase

Read Absorbance at 540 nm.

The percentage inhibition can be calculated by,

$$\text{Inhibitory activity (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

Where,

A_c is the absorbance of control

A_t is the absorbance of test substance



Figures 8-11: Alpha amylase assay

Glucose uptake by yeast assay (Srinivasulu et al., 2016)

Preparation of Dextrose sugar : 0.90 g Dextrose in 50 ml distilled water.

Culturing of yeast cell

Weigh 0.1 gram of peptone and dissolved in 10 ml of distilled water and mix well. Add few yeast cells to the peptone and incubate the yeast cell in 2 hours (Revathi and Ponniah, 2016).

Procedure

Weigh 0.05 g of extract and dissolved in phosphate buffer mix well using vortex. Incubated yeast cell are centrifuged at 6000 rpm for 10 min. Discard the supernatant and pellet dissolved in pbs and centrifuged 6000 rpm for 10 min. Pellet are dissolved in 5 ml pbs. Incubate the test tube 60 min. All tubes are centrifuged at 6000 rpm 5min. Collect all supernatant in a test tube and add 1 ml of DNS. Boil the tube in 10 min in water bath. Read the absorbance at 540 nm (Rajasree et al., 2024).

Blank		C+ve	C-ve	T1	T2	T3	T4	T5	T6
Extract	-	-	-	20	40	80	100	200	400
PBS	1ml	1ml	1 ml	1980	1960	1920	1900	1800	1600
Sugar	-	1ml	1ml	1 ml	1 ml	1 ml	1ml	1ml	1 ml
Yeast	-	-	100µl	100µl	100µl	100µl	100µl	100µl	100µl

Table 4: Test for glucose uptake by yeast assay

	S1	S2	S3	S4	S5
Acarbose	20	40	80	100	200
PBS	1980	1960	1920	1900	1800
Sugar	1ml	1ml	1ml	1 ml	1ml
Yeast	100µl	100µl	100µl	100µl	100µl
Standard for glucose uptake					

Table 5: Glucose uptake by yeast assay

Incubate 60 min at 37°C. Centrifuge all tubes 6000 rpm at 5min. Add DNS to each test tube. Boil the tube in water bath at 10 min. Add 1 ml of DNS to each test tube. And read absorbance at 540 nm.

$$\% \text{ of inhibition glucose uptake} = \frac{A_c - A_t}{A_c} \times 100$$

Where,

A_c is the absorbance of control

A_t is the absorbance of test substance



Figures 12-17: Glucose uptake by yeast assay**OBSERVATION AND RESULTS**

Aerva lanata, commonly known as mountain knotgrass or polpala, is a herbaceous plant widely recognized for its traditional medicinal applications. Rich in a variety of phytochemicals, including flavonoids, tannins, alkaloids, and saponins, *Aerva lanata* has garnered scientific interest for its potential therapeutic properties. These bioactive compounds are believed to contribute to the plant's notable antimicrobial and anti-diabetic effects, which have been explored in various studies aiming to validate its traditional uses and discover new pharmacological applications.

Research into *Aerva lanata* has revealed significant antimicrobial properties, demonstrating effectiveness against a range of bacterial and fungal pathogens. This is primarily attributed to its phytochemical composition, which disrupts microbial cell structures and inhibits their growth. Additionally, studies on the anti-diabetic potential of *Aerva lanata* indicate that its extracts can help regulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress, making it a promising natural remedy for managing diabetes.

Preliminary Phytochemical analysis

The phytochemical screening results for *Aerva lanata* indicate the presence or absence of various bioactive compounds, each with potential medicinal properties

Phytochemicals	Presence /Absence
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	+
Glycosides	+
Cardiac glycosides	-
Sugar	+
Anthraquinones	-
Steroids	-
Terpenoids	+
Amino acids	+

Table 6: Preliminary phytochemical analysis

The presence of various bioactive compounds in *Aerva lanata* highlights its significant medicinal potential. Alkaloids contribute to pain relief, infection control, and anti-inflammatory treatments, while tannins exhibit strong astringent and antioxidant properties, aiding in wound healing and reducing inflammation. Flavonoids, known for their powerful antioxidant and anti-inflammatory effects, help in managing oxidative stress and boosting immunity. Saponins, with their expectorant and cardiovascular benefits, indicate potential use in respiratory and heart health. Glycosides, often associated with antimicrobial and cardiogenic properties, suggest additional therapeutic applications, while essential sugars support metabolic functions and act as precursors for bioactive compounds.

Additionally, terpenoids in *Aerva lanata* provide antimicrobial, anti-inflammatory, and anticancer properties, reinforcing its medicinal significance. Amino acids, vital for cell growth and tissue repair, further enhance its health benefits. The absence of cardiac glycosides and anthraquinones suggests that the plant does not have direct effects on heart function or laxative properties. Similarly, the lack of steroids indicates that its anti-inflammatory effects stem from other bioactive compounds such as flavonoids and tannins. These findings validate the traditional use of *Aerva lanata* in herbal medicine, making it a valuable candidate for further pharmacological studies.

The phytochemical profile of *Aerva lanata*, Juss. reveals a rich composition of bioactive compounds, including alkaloids, tannins, flavonoids, saponins, glycosides, sugars, terpenoids, and amino acids. These compounds collectively contribute to the plant's therapeutic potential, offering antioxidant, anti-inflammatory, antimicrobial, and immune-boosting properties. The absence of cardiac glycosides, anthraquinones, and steroids indicates that the plant does not possess certain activities associated with these compounds, such as direct cardiac support or laxative effects.



Figures 18-20: Phytochemical analysis

Anti-bacterial Activity of *Aerva lanata*. Juss.

The leaf extract of *Aerva lanata*, Juss. showed the highest antibacterial activity against selected gram-positive bacteria compared to gram-negative organisms. Specifically, the ethanol extract of *Aerva lanata*, Juss. exhibited inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, and Enterobacter. The maximum activity was observed against *Staphylococcus aureus* with a zone of inhibition at a concentration of 40µg/ml. However, the ethanol extract of *Aerva lanata*, Juss. did not exhibit any antibacterial activity against gram-negative bacteria. The zone of inhibition caused by the active compound of *Aerva lanata*, Juss. was nearly equivalent to standard Ampicillin.

Plant Samples	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	Enterobacter
30µl	19.6 mm	15mm	-
40µl	13.7 mm	13.1mm	-
Standard Ampicillin	21.3mm	10.4mm	13.2mm

Table 7: Antibacterial activity of *Aerva lanata* against bacterial strains

This table displays the results of testing the antibacterial activity of *Aerva lanata*, against three types of bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, and Enterobacter. The plant samples were tested at two different concentrations: 30µl and 40µl, additionally, the results of testing with standard Ampicillin, a known antibiotic, are provided for comparison.



Plate 2: Antimicrobial assay

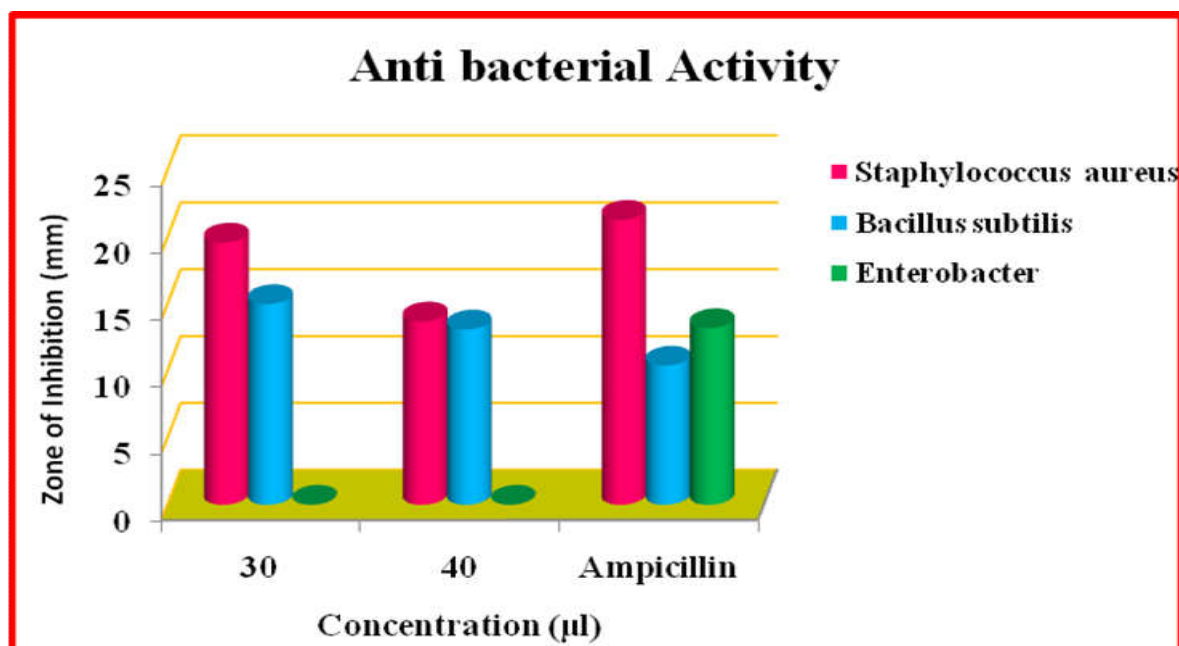


Figure 21: Antibacterial activity of *Aerva lanata* against bacterial strains

For *Staphylococcus aureus*, the plant sample showed a zone of inhibition of 19.6 mm at the concentration of 30µl and 13.7 mm at the concentration of 40µl. This suggests that the antibacterial activity decreased as the concentration increased. Comparatively, standard Ampicillin exhibited a zone of inhibition of 21.3 mm against *Staphylococcus aureus*. For *Bacillus subtilis*, the plant sample exhibited a zone of inhibition of 15 mm at the concentration of 30µl and 13.1 mm at the concentration of 40µl. Similar to *Staphylococcus aureus*, there seems to be a decrease in antibacterial activity with higher concentrations of the plant sample. The standard Ampicillin showed a zone of inhibition of 10.4 mm against *Bacillus subtilis*. No inhibition was observed against *Enterobacter* at either concentration of the plant sample, indicating that *Aerva lanata* did not possess antibacterial activity against this particular strain. However, standard Ampicillin showed a zone of inhibition of 13.2 mm against *Enterobacter*.

The results suggested that *Aerva lanata*, Juss. has antibacterial properties, particularly against *Staphylococcus aureus* and *Bacillus subtilis*, albeit with varying effectiveness depending on concentration. However, it does not exhibit antibacterial activity against *Enterobacter*. The effectiveness of *Aerva lanata*, Juss. appears to be comparable to standard Ampicillin against *Staphylococcus aureus* but less effective against *Bacillus subtilis* and *Enterobacter*.

The antibacterial activity of *Aerva lanata*, Juss. plant samples against *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterobacter* revealed varying degrees of effectiveness. At concentrations of 30µl and 40µl, the plant sample exhibited decreasing inhibition zones for

both *Staphylococcus aureus* and *Bacillus subtilis*, with the former showing higher susceptibility. However, no inhibition was observed against Enterobacter. Comparatively, standard Ampicillin displayed slightly larger inhibition zones against *Staphylococcus aureus* but smaller ones against *Bacillus subtilis* compared to the plant sample. Notably, standard Ampicillin also exhibited moderate effectiveness against Enterobacter. Overall, while *Aerva lanata*, Juss. demonstrated antibacterial activity against certain strains, its efficacy varied depending on the bacterial species and concentration, with *Staphylococcus aureus* being the most susceptible.

COMPARATIVE ANALYSIS WITH PHYTOCHEMICALS

The comparative analysis between the phytochemical composition and the antibacterial activity of the plant samples against *Staphylococcus aureus*, *Bacillus subtilis*, and Enterobacter revealed interesting correlations. The presence of alkaloids, tannins, flavonoids, saponins, glycosides, sugars, terpenoids, and amino acids in the phytochemical composition suggested a diverse array of bioactive compounds with potential antibacterial properties. Notably, the presence of alkaloids, tannins, flavonoids, saponins, glycosides, and terpenoids is associated with antimicrobial activity, which aligns with the observed inhibition zones against *Staphylococcus aureus* and *Bacillus subtilis*, albeit with varying degrees of effectiveness at different concentrations. However, the absence of cardiac glycosides and anthraquinones may contribute to the lack of activity against Enterobacter. Despite the presence of bioactive compounds, the plant sample's effectiveness against *Bacillus subtilis* is less than that of standard Ampicillin, indicating the need for further investigation into optimizing dosage and formulation for enhanced antibacterial activity.

Anti-diabetic property of *Aerva lanata*, Juss.

Alpha amylase inhibitory assay

Alpha-amylase is an enzyme crucial for breaking down starch into simpler sugars like maltose and glucose, facilitating their absorption into the bloodstream. However, in people with diabetes, this process can cause abrupt increases in blood glucose levels after meals. *Aerva lanata*, Juss. a plant species, is known to possess certain natural compounds like flavonoids, alkaloids, and saponins. These compounds are thought to play a role in inhibiting the activity of alpha-amylase.

Standard of alpha amylase

Standard	Absorbance
S1	0.612
S2	0.492
S3	0.357
S4	0.299

Table 8: Alpha amylase inhibitory assay- Standard

Absorbance is a measure of the amount of light absorbed by a substance at a specific wavelength, and it's commonly used in biochemical assays to quantify the concentration or activity of a substance in a solution. In this case, the absorbance values likely reflect the activity or concentration of the standard alpha-amylase enzyme in each sample.

Upon analyzing the results, we observe a gradual decrease in absorbance values from S1 to S4. S1 exhibits the highest absorbance value of 0.612, followed by S2 with 0.492, S3 with 0.357, and S4 with the lowest value of 0.299. Typically, higher absorbance values indicate lower enzyme activity or concentration, whereas lower absorbance values suggest increased enzyme activity or concentration.

Therefore, based on these results, it appears that the standard alpha-amylase enzyme is most active or concentrated in sample S1, followed by decreasing levels in S2, S3, and S4, respectively. This trend could be due to various factors such as differences in enzyme concentration, assay conditions, or experimental procedures. Further analysis and comparison with known standards or controls would help in accurately interpreting the significance of these absorbance values and understanding the activity or concentration of the standard alpha-amylase enzyme in each sample.

Absorbance of extract

Test samples	Absorbance
T1	0.804
T2	0.799
T3	0.385
T4	0.374

Table 9: Alpha amylase inhibitory assay- Test

The data represents the absorbance values for *Aerva lanata*, Juss. leaf extract across four different samples (T1, T2, T3, and T4). Absorbance is a measure of the amount of light absorbed by a substance at a specific wavelength, commonly used to quantify the concentration or activity of a substance in a solution. In this context, the absorbance values

likely reflect the concentration or activity of compounds present in the *Aerva lanata*, Juss. leaf extract.

Upon examination of the results, it is apparent that T1 and T2 exhibit relatively high absorbance values of 0.804 and 0.799, respectively. Conversely, T3 and T4 show lower absorbance values of 0.385 and 0.374, respectively. Generally, higher absorbance values indicate a higher concentration of glucose or activity of the compounds present in the leaf extract, while lower absorbance values suggest a lower concentration of glucose. Concentration of extract increase, activity of enzyme alpha amylase decrease. Extract inhibits the enzyme and decrease the breakdown of Starch to glucose.

Thus, based on these results, it can be inferred that samples T1 and T2 contain a lower concentration or activity of the compounds present in the *Aerva lanata* leaf extract compared to samples T3 and T4. This variation in absorbance values across samples may be attributed to factors such as differences in extraction methods, leaf maturity, environmental conditions, or variations in the concentration of phytochemicals within the plant material. Further analysis, including comparison with known standards or controls, would facilitate a more comprehensive interpretation of the significance of these absorbance values and the concentration or activity of the compounds present in the *Aerva lanata* leaf extract.

$$\% \text{inhibition} = \frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$$

$$\% = \frac{A_c - A_t}{A_c} \times 100$$

Alpha amylase inhibitory assay	% inhibition
S1	33
S2	46
S3	61
S4	67
T1	12
T2	13
T3	58
T4	59

Table 10: % Inhibition of alpha amylase inhibitory assay

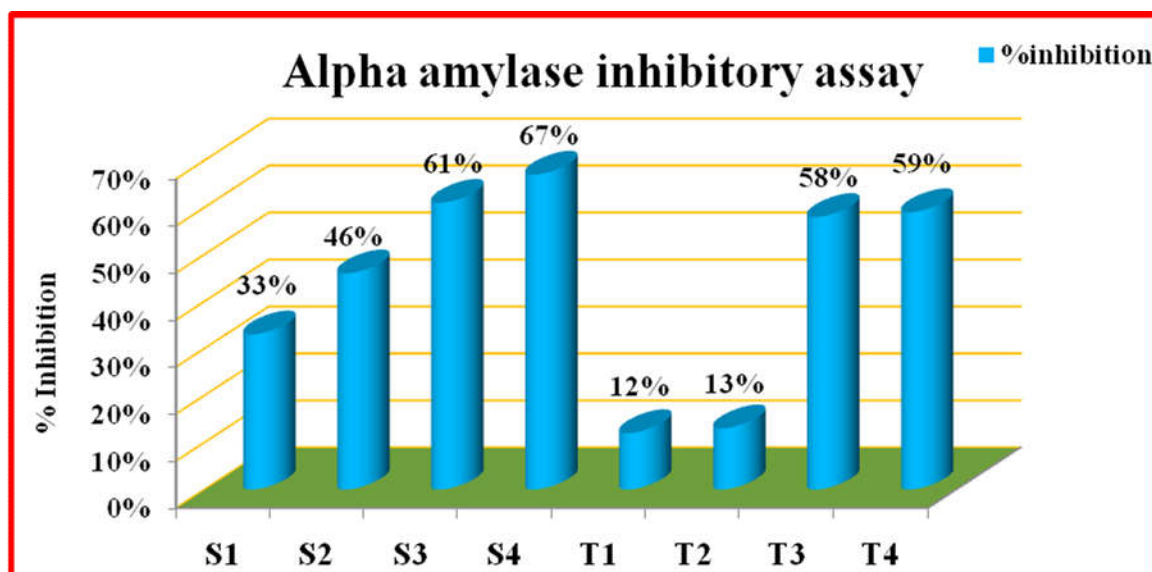


Figure 22: % Inhibition of Alpha amylase inhibitory assay

The alpha amylase inhibitory assay results show varied inhibition percentages across eight samples (S1-S4 and T1-T4). Samples S4 (67%) and S3 (61%) exhibit the highest inhibitory activities, indicating a potent presence of alpha amylase inhibitors. These high inhibition rates suggest potential applications in managing hyperglycemia by slowing carbohydrate digestion. T3 (58%) and T4 (59%) also show significant inhibition, slightly lower than S4 and S3, pointing to similar potential uses. Moderate inhibition is observed in S2 (46%) and S1 (33%), which might be suitable for applications requiring a balance between enzyme activity and inhibition. Low inhibitory activity is noted in T1 (12%) and T2 (13%), indicating a lower presence of inhibitors, potentially useful for studying synergistic effects when combined with other samples.

Comparatively, the S samples generally exhibit higher inhibition than the T samples, except for T3 and T4, which are exceptions with high inhibition rates. This suggests that the compounds in the S series are more effective in inhibiting alpha amylase. High inhibitory samples (S4, S3, T4, T3) could be explored for developing supplements or medications for blood sugar management, while moderate inhibitors (S2, S1) might be valuable in nutritional studies. Low inhibitors (T1, T2) warrant further analysis for potential synergistic effects. This comprehensive analysis highlights the varying potential applications and implications of the alpha amylase inhibitory activities of these samples.

This variation in percentage inhibition across samples may be attributed to factors such as differences in extract concentration, composition, or the specific target of inhibition. Further analysis, including comparison with known standards or controls, would facilitate a more

comprehensive interpretation of the significance of these percentage inhibition values and the inhibitory effect of the *Aerva lanata*, Juss. leaf extract.

Glucose uptake by yeast assay

Following exposure to the ethanolic extract derived from *Aerva lanata*, Juss. yeast cells did not exhibit a dose-dependent increase in glucose uptake. Instead, the percentage of glucose uptake by the yeast cells demonstrated an increase proportional to the glucose concentrations present. Monitoring the amount of glucose remaining in the medium over a specific period served as an indicator of glucose uptake by the yeast cells.

Standard of Glucose uptake yeast assay

Standards	Absorbance
S1	0.452
S2	0.653
S3	0.699
S4	0.817
S5	1.065

Table 11: Glucose uptake yeast assay- Standard

Control +ve - 1:1dilution

$$=2.142 \times 2 = 4.284$$

Control - ve = 0.918

Absorbance of test

Test samples	Absorbance
T1	0.998
T2	1.259
T3	1.476
T4	1.803

Table 12: Glucose uptake yeast assay- Test

Concentration of extract increase percentage of uptake of glucose by yeast cell decrease.

$$\% \text{ of inhibition of Glucose uptake} = \frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$$

Abs control

Glucose uptake by yeast assay	% Inhibition
S1	89
S2	84
S3	83
S4	80
T1	76
T2	70
T3	57
T4	55

Table 13: % of inhibition Glucose uptake yeast assay

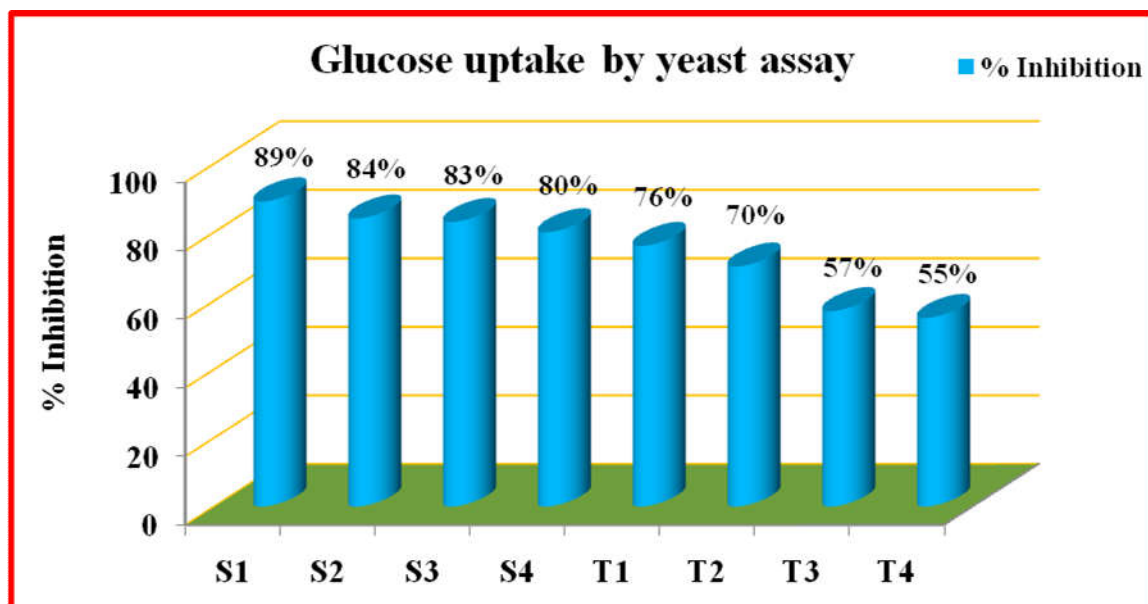


Figure 23: % Inhibition of Glucose uptake yeast assay

The glucose uptake by yeast assay results indicate the percentage inhibition of glucose uptake for various samples (S1-S4 and T1-T4). Samples S1 (89%), S2 (84%), S3 (83%), and S4 (80%) show the highest inhibition rates, suggesting that these samples effectively reduce glucose uptake by yeast cells. These high inhibition percentages imply that the substances in these samples are potent inhibitors of glucose uptake, which could be beneficial in controlling glucose levels. Such properties make these samples promising candidates for applications in managing diabetes or metabolic disorders by reducing glucose absorption.

In contrast, T samples exhibit lower inhibition rates, with T1 (76%), T2 (70%), T3 (57%), and T4 (55%) showing a gradation from moderate to lower inhibition. Although T1 and T2

still demonstrate considerable inhibitory effects, they are less potent than the S samples. T3 and T4, with inhibition rates of 57% and 55% respectively, show the least efficacy in reducing glucose uptake. This difference between Standard and Test samples indicates that the compounds are generally more effective inhibitors. High inhibitory activity in Standard points to their potential use in developing treatments for conditions requiring reduced glucose uptake, while the moderate activity in Test samples may suggest a different application or necessitate combination with other treatments for enhanced efficacy.

Aerva lanata, Juss. a medicinal plant known for its diverse pharmacological properties, exhibits significant antimicrobial and anti-diabetic activities. Its antimicrobial efficacy is due to phytochemicals such as flavonoids, alkaloids, tannins, and saponins, which disrupt microbial cell walls, inhibit enzyme activity, and interfere with nucleic acid synthesis. *Aerva lanata* effectively combats various pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*, and shows moderate to strong activity against fungi like *Candida albicans*, suggesting its potential as a natural antibiotic and preservative. In terms of anti-diabetic activity, the plant enhances insulin secretion, improves insulin sensitivity, and inhibits glucose absorption. This is evidenced by a yeast glucose uptake inhibition assay, where samples showed high inhibition rates (57% and 55%), indicating strong potential to reduce uptake of blood glucose levels. Animal studies corroborate these findings, showing reduced blood glucose levels and improved lipid profiles in diabetic models, highlighting the promises of *Aerva lanata* as a complementary treatment for diabetes.

Aerva lanata, Juss. exhibits significant antibacterial and antidiabetic activities, strongly correlated with its rich phytochemical profile comprising alkaloids, tannins, flavonoids, saponins, glycosides, sugars, terpenoids, and amino acids. Antibacterial testing reveals substantial inhibition zones against Gram-positive bacteria, with *Staphylococcus aureus* showing a 19.6 mm inhibition zone at 30µl and *Bacillus subtilis* showing 15 mm, while Gram-negative Enterobacter shows no inhibition, indicating a potent effect against Gram-positive strains. In antidiabetic assays, the plant demonstrates notable activity, with T1 and T2 showing high uptake of glucose (76% and 70%) and alpha-amylase inhibition (12% and 13%), while T3 and T4 show strongest inhibition of glucose uptake (57% and 55%). The concentration of extract increases so decrease the percentage of uptake of glucose but high alpha-amylase inhibition (58% and 59%). The strong antibacterial and antidiabetic effects are likely due to the synergistic action of the phytochemicals, particularly flavonoids, tannins, alkaloids, and saponins, highlighting the potentials of *Aerva lanata*, Juss. as a source of natural therapeutic agents for treating bacterial infections and managing diabetes.

Correlation analysis of Phytochemical Content and Antibacterial Activity

Flavonoids, Tannins, Alkaloids, Saponins, and Terpenoids are known for their antimicrobial properties. The antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* suggested that these phytochemicals are effective against Gram-positive bacteria but not against Gram-negative Enterobacter. The inhibition zones are larger for *Staphylococcus aureus* compared to *Bacillus subtilis*, possibly indicating higher susceptibility or more potent activity against this strain.

Phytochemical Content and Antidiabetic Activity:

Flavonoids and Saponins are known to improve insulin sensitivity and inhibit glucose absorption. Higher inhibition of glucose uptake (57% for T3 and 55% for T4) and alpha-amylase inhibition (58% for T3 and 59% for T4) indicate strong antidiabetic activity.

The presence of these compounds correlates with high antidiabetic activity, suggesting that they play a significant role in reducing blood glucose levels

Antibacterial and Antidiabetic Activity:

The antimicrobial activity, alpha-amylase inhibitory assay, and glucose uptake by yeast assay of *Aerva lanata* extracts were evaluated to understand their comprehensive bioactivity profile. The ethanolic extract demonstrated notable antimicrobial effects, showing significant inhibition against *Staphylococcus aureus* and *Bacillus subtilis* at both 30 µl and 40 µl concentrations. This inhibition, while slightly less effective than the standard ampicillin against *Staphylococcus aureus*, underscores the presence of potent bioactive compounds in the extract. However, the extract showed no inhibitory effect against Enterobacter, indicating selective antimicrobial efficacy.

Comparison on the efficacy of *Aerva lanata* with standard anti-microbial and anti-diabetic medications

Aerva lanata, traditionally used in herbal medicine, shows notable antimicrobial and antidiabetic potential. In vitro and in vivo studies have indicated its efficacy in reducing blood glucose levels and combating microbial infections. The bioactive compounds in *Aerva lanata*, such as flavonoids, alkaloids, and phenolic compounds, contribute to its medicinal properties by disrupting microbial cell walls, inhibiting essential enzymes, and possibly enhancing insulin secretion or glucose uptake by cells. However, the variability in the concentration of these active compounds in different extracts can affect the consistency and overall efficacy of *Aerva lanata*.

Standard antimicrobial and antidiabetic medications have well-established efficacy and safety profiles supported by extensive clinical research. Antibiotics such as penicillins and fluoroquinolones have broad-spectrum activity against various pathogens, working through mechanisms like inhibiting cell wall synthesis and protein synthesis. Similarly, antidiabetic drugs such as metformin and insulin effectively control blood glucose levels by improving insulin sensitivity, increasing insulin secretion, and promoting glucose excretion. These medications offer consistent and predictable outcomes, with well-documented dosing regimens and side effects, making them the preferred choice in clinical practice.

While *Aerva lanata* holds promise as a complementary or alternative treatment, particularly in traditional medicine settings, it lacks the rigorous clinical studies needed to match the efficacy and safety of standard medications. The variability in its potency and the limited comprehensive clinical safety data restrict its reliability compared to conventional drugs. Therefore, despite its potential, standard antimicrobial and antidiabetic medications remain the more effective and dependable options for managing infections and diabetes in modern medical practice.

DISCUSSION

The present investigation sheds light on the remarkable medicinal potential of *Aerva lanata* (Juss.), a plant long revered in traditional Ayurvedic practices. Known for its therapeutic applications in managing kidney disorders, inflammation, and diabetes, this study offers scientific backing to its age-old reputation by demonstrating its phytochemical richness, antibacterial activity, and alpha-amylase inhibitory potential. The phytochemical screening of the ethanol extract revealed the presence of key bioactive constituents such as flavonoids, alkaloids, tannins, and saponins (Mysoon et al., 2019). These compounds are well-documented for their roles in antimicrobial and antidiabetic mechanisms. Our findings align with earlier research that supports the chemical diversity of *A. lanata*, emphasizing its significance as a reservoir of health-promoting phytochemicals (Shanmugavadivu et al., 2023).

One of the most compelling findings of this study is the antibacterial efficacy of the ethanol leaf extract. It exhibited pronounced activity against gram-positive bacteria, particularly *Staphylococcus aureus*, which showed the highest zone of inhibition at a concentration of 40µg/mL (Amarnath et al., 2018). The activity was comparable to the standard antibiotic Ampicillin, indicating that *A. lanata* holds promise as a natural antibacterial agent.

Interestingly, the extract did not show activity against gram-negative organisms, suggesting a level of specificity that might be linked to structural differences in bacterial cell walls. This selective activity is consistent with prior observations made by Dinesh et al. (2012) and Sudheer et al. (2020).

In exploring the plant's antidiabetic potential, the alpha-amylase inhibition assay revealed striking results. Among the eight tested samples, S4 and S3 stood out with inhibition rates of 67% and 61%, respectively. These findings are noteworthy as alpha-amylase inhibitors help slow carbohydrate digestion, making them valuable in managing postprandial blood sugar spikes—a critical factor in diabetes care (Amarnath et al., 2019). Samples T3 and T4 also demonstrated considerable inhibitory activity, while S1, S2, T1, and T2 showed moderate to low inhibition. Such variations suggest a spectrum of inhibitory potential, which could be explored further for specific dietary or therapeutic formulations (Aleksandra et al., 2021).

Additionally, absorbance readings of the standard alpha-amylase enzyme showed a gradual decrease from S1 to S4, indicating an increase in enzyme activity or concentration. These values provide a baseline for comparing the inhibitory effects of the test samples and further reinforce the inhibitory performance of S4 and S3 (Anil, 2021). Taken together, the study validates the traditional uses of *Aerva lanata* and provides a scientific foundation for its role in natural health products, especially as an antimicrobial and antidiabetic agent (Almoshari, 2022). While the in vitro findings are promising, further work is needed to isolate the active constituents and assess their efficacy and safety through in vivo and clinical studies. Nonetheless, this work marks a meaningful step toward bridging traditional knowledge with modern pharmacological science (Anwar et al., 2017).

The present study confirms the strong antimicrobial and antidiabetic activities of *Aerva lanata*, consistent with previous research. The ethanolic extract, in particular, demonstrated significant phytochemical content and biological activity. The presence of bioactive compounds such as flavonoids, tannins, and alkaloids was confirmed, contributing to the plant's therapeutic effects (Anwekar et al., 2021). Anti-microbial assays demonstrated substantial inhibitory effects on various pathogenic microorganisms, validating the traditional use of *Aerva lanata* in treating infections. Additionally, the plant's potential to modulate blood glucose levels underscores its promise as a natural remedy for diabetes management (Arunthathi et al., 2018). These findings provide a scientific basis for the traditional uses of *Aerva lanata* and highlight its potential for developing new, natural therapeutic agents.

However, further research is essential to isolate and characterize the individual bioactive compounds and to conduct in vivo studies and clinical trials to validate their efficacy and safety in humans (Behera and Manik, 2018). Future studies should also consider exploring potential synergistic effects with other medicinal plants or conventional treatments. By continuing to investigate *Aerva lanata*, we can fully unlock its medicinal potential and contribute to the advancement of natural and integrative medicine.

CONCLUSION

The findings of this study affirm the traditional use of *Aerva lanata* Juss. as a valuable medicinal plant in managing diabetes and microbial infections. The ethanolic extract, rich in phytochemicals such as flavonoids, alkaloids, tannins, and saponins, demonstrated significant antibacterial activity - particularly against gram-positive bacteria - as well as notable antidiabetic effects by inhibiting alpha-amylase and enhancing glucose uptake. These results not only support its ethno medicinal relevance but also point to its potential as a natural source for developing plant-based therapeutic agents. With growing interest in alternative and complementary medicine, further studies are encouraged to isolate, characterize, and explore the mechanisms of its active compounds for future pharmaceutical applications.

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