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THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF PANDANUS AMARYLLIFOLIUS LEAF EXTRACTS: A PROMISING SOURCE OF BIOACTIVE COMPOUNDS

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ABSTRACT

Pandanus amaryllifolius, also known as pandan, is a tropical plant with distinctively fragrant leaves that is utilized extensively in Southeast Asian cuisine and traditional medicine. The antibacterial and antioxidant properties of leaf extracts made with different solvents (methanol, acetone, chloroform, and distilled water) were examined in this work. Using the disc diffusion method, the antibacterial activity was evaluated against five harmful bacteria: *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus sp.*, and *Klebsiella pneumoniae*. The findings showed that the methanol extract had the greatest overall antibacterial activity, with the widest zone of inhibition against *B. subtilis* (1.8 cm) and *E. coli* (1.9 cm). *S. typhi* (1.2 cm) and *K. pneumoniae* (1.0 cm) were significantly inhibited by the distilled water extract. The reducing power method was used to assess the antioxidant capability; higher absorbance was indicative of higher reducing power. The distilled water extract had the lowest antioxidant activity, whereas the acetone extract had the greatest, indicating a significant presence of reducing chemicals. These results support *P. amaryllifolius*'s traditional usage and demonstrate its potential as a natural source of antioxidants and antibacterial agents for use in food and medicine.

KEYWORDS: *Pandanus amaryllifolius*, Pandan, Antibacterial activity, Disc diffusion, Antioxidant activity, Reducing power, 2-acetyl-1-pyrroline.

INTRODUCTION

Pandanus amaryllifolius, also referred to as aromatic screw pine or pandan, is a perennial plant in the Pandanaceae family that is important both economically and culturally. Its long, thin, blade-like leaves, which are native to tropical Southeast Asia, are prized for their distinctively sweet, flowery scent. The volatile substance 2-acetyl-1-pyrroline is mainly responsible for this distinct scent.[1,2] P. amaryllifolius is a common culinary ingredient in Indonesia, Malaysia, Thailand, and the Philippines. It is used to give rice, desserts, and savory meals flavor, scent, and a natural bright green color.[3,4] For generations, pandan has been an essential component of traditional medicine in addition to its culinary use. Conventional uses include as a general tonic, to cure skin conditions, and to lower fever.[5]

Research on the bioactive chemicals present in plants has increased due to the growing interest in natural products around the world as substitutes for medications and synthetic additives. Plant-based phytochemicals frequently have potent antioxidant and antibacterial properties.[6,7] Alkaloids, flavonoids, glycosides, and terpenoids are among the chemicals found in *P. amaryllifolius* leaves that are frequently linked to their biological activity.[8,9] Finding novel and potent natural antimicrobial agents is crucial because antimicrobial resistance (AMR) poses a serious threat to global health.[10] Similarly, the pathophysiology of many chronic diseases, including as cancer, cardiovascular problems, and neurodegenerative ailments, is linked to oxidative stress, which arises from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses.[11] Antioxidants generated from plants can lessen this stress.[12] This study sought to determine the antioxidant activity using the reducing power method and to scientifically assess the antibacterial activity of *P. amaryllifolius* leaf extracts prepared with solvents of varying polarity (methanol, acetone, chloroform, and distilled water) against a panel of Gram-positive and Gram-negative pathogenic bacteria, given its widespread traditional use. The goal of this study is to determine the best solvent for extracting pandan's powerful bioactive chemicals and to offer scientific support for the plant's therapeutic uses.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh *Pandanus amaryllifolius* leaves were gathered, cleaned, allowed to air dry, and then ground into a fine powder. Each of the four solvents- methanol, acetone, chloroform, and distilled water, was used to cold macerate a mass of 20 g of the powdered leaves. By allowing the mixtures to soak, different bioactive chemicals based on their polarity might be extracted. The corresponding leaf extracts were then obtained by filtering each suspension through a funnel with filter paper. [13] To obtain the crude extracts for analysis, the filtrates were concentrated using a rotary evaporator (for organic solvents) or air-drying (for water extract).

Antibacterial Activity Assay

Bacterial Strains and Culture

The Gram-positive bacteria *Streptococcus sp*. and *Bacillus subtilis*, as well as the Gram-negative species *Salmonella typhi*, *Klebsiella pneumoniae*, and *Escherichia coli*, were acquired from the MCC BT lab.[14] For later investigation, the cultures were kept in nutrient broth as turbid growth and kept at 4°C.[15]

Disc Diffusion Method (Kirby-Bauer)

The disc diffusion method was used to evaluate the antibacterial activity.[16, 17] Each test organism's grass was evenly distributed throughout the surface of nutrient agar plates.[18] To reach the final test concentration, the concentrated plant extracts were dissolved in dimethyl sulfoxide (DMSO). The obtained extract solutions were impregnated into sterile

filter paper discs with a diameter of 5 mm, allowing the solvent to fully evaporate after each addition.[19] After that, the discs were carefully positioned on the agar surface that had been air-dried. For a whole day, the plates were incubated at 37°C.[20] The diameter of the zone of inhibition (ZOI) of bacterial growth surrounding each disc was used to measure the antibacterial activity; the size of the zone correlated with the organism's sensitivity to the extract.[21]

Evaluation of Antioxidant Activity

Reducing Power Method

The reducing power method was used to assess the extracts' antioxidant capability.[22] One milliliter of distilled water was used to generate distinct solutions of each leaf extract at a concentration of 100 g/mL. 2.5 mL of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$) and 2.5 mL of 0.2 M phosphate buffer (pH 6.6) were added to this.[23] For 20 minutes, the mixture was incubated at 50° C.[24] After the reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid (TCA), the liquid was centrifuged for 10 minutes at 1500 × g. 0.5 mL of 0.1% ferric chloride ($FeCl_3$) and 2.5 mL of distilled water were combined with the upper layer solution (2.5 mL).[25,26] Spectrophotometric measurements of the resultant solution's absorbance at 700 nm were made. [27] A higher reducing power, which is indicative of increased antioxidant activity, was evidenced by an increase in absorbance.[28] To evaluate the dose-dependent effect, the experiment was conducted at five distinct concentration levels (T1-T5).[29]

RESULTS AND DISCUSSION

Antibacterial Activity

The findings of the experiment showed that the antibacterial properties of *Pandanus amaryllifolius* leaf extracts varied significantly across the four solvents utilized. Table 1 and Figure 1 provide a summary of the findings. The presence of potent polar antimicrobial compounds was confirmed by the methanol extract's greatest antibacterial activity, especially against the Gram-positive bacterium *Bacillus subtilis* (1.8 cm) and the Gram-negative bacterium *Escherichia coli* (1.9 cm zone of inhibition).[1, 19] Methanol's enhanced action is in line with studies that highly polar solvents are effective at extracting secondary metabolites, such as flavonoids and phenolics, which are well-known antibacterial agents.[30,31] Interestingly, the distilled water extract showed significant, selective activity against *Salmonella typhi* (1.2 cm) and *Klebsiella pneumoniae* (1.0 cm), suggesting that certain water-soluble compounds, possibly tannins or specific glycosides, have a selective action against these Gram-negative pathogens, an observation supported by studies showing plant extracts' varying efficacy based on microbial membrane characteristics.[32, 33] The overall greater sensitivity of Gram-positive bacteria (*B. subtilis*) compared to Gram-negative bacteria (*E. coli*) to most organic extracts aligns with the generally higher resistance attributed to the complex outer membrane of Gram-negative strains.[33]

Table 1: Antibacterial activity of methanol, acetone, chloroform, and distilled water extracts of Pandanus amaryllifolius against selected microbes (Zone of Inhibition in cm)

Sl. N	Test organism	Methanol	Acetone	Chloroform	Distilled water
1	*Escherichia coli*	1.9	0.6	0.9	-
2	*Bacillus*	1.8	1.6	0.7	0.5
3	*Streptococcus*	0.9	0.7	-	-
4	*Salmonella typhi*	1.3	-	0.8	1.2
5	*Klebsiella*	1.1	-	0.6	1

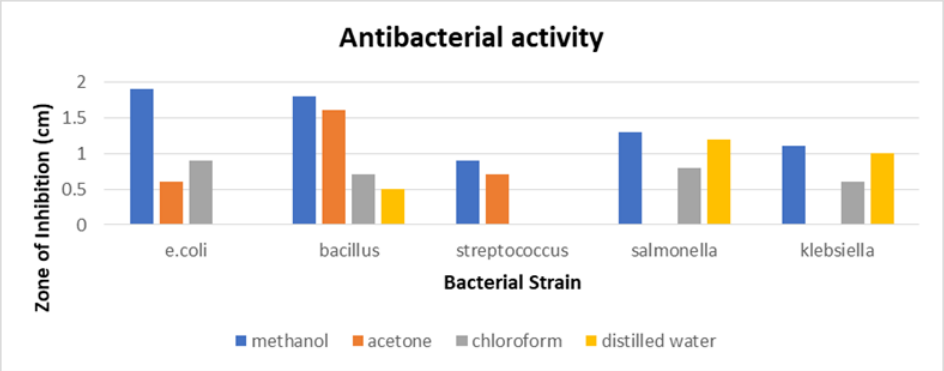

Figure 1: Graphical representation of the zone of inhibition (cm) for methanol, acetone, chloroform, and distilled water extracts of Pandanus amaryllifolius against five pathogenic bacterial strains.

Antioxidant Activity

The acetone extract showed the highest absorbance values across all doses (e.g., 2.371 at T5), far surpassing the commercial standard, in the assessment of antioxidant capability using the reducing power method. This was closely followed by the methanol extract (2.12 at T5). Table 2 and Figure 2 provide a summary of the findings. This significant reducing power validates *P. amaryllifolius's* function as a natural antioxidant source by demonstrating a robust ability to donate electrons and stabilize free radicals.[34, 39] In line with other plant studies where solvent polarity significantly affects the yield of particular bioactive compounds, the discovery that the moderately polar solvent acetone produced the highest antioxidant activity implies that non-polar or moderately polar compounds, such as carotenoids or lipophilic flavonoids, are the primary contributors to the leaf's reducing capacity.[35- 37, 40] On the other hand, the distilled water extract's low activity confirms that this plant's strongest reducing agents are not very soluble in water.[38] The plant's dual therapeutic potential as an antibacterial and an antioxidant agent is thus confirmed by the diverse results across the solvents, which emphasize the crucial need for optimizing extraction techniques to maximize the output of specific bioactive chemicals.[2]

Table 2: Antioxidant activity of Pandanus amaryllifolius extracts by reducing power method (Absorbance at 700 nm)

Sample extract	T1	T2	T3	T4	T5
Standard	0.313	0.627	0.847	1.15	1.47
Methanol	0.938	1.365	1.639	2.057	2.122
Acetone	1.292	1.858	2.094	2.235	2.371
Chloroform	0.332	0.515	0.692	0.755	0.849
Distilled water	0.258	0.311	0.368	0.481	0.553

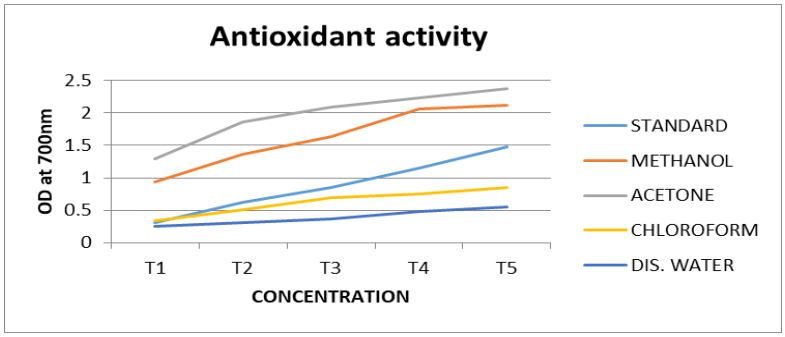

Figure 2: Dose-dependent antioxidant activity of Pandanus amaryllifolius extracts and standard measured by the reducing power method (Absorbance at 700 nm).

CONCLUSION

In summary, the study provides scientific support for the extensive usage of *Pandanus amaryllifolius* leaf extracts in traditional medicine by confirming their strong antibacterial and antioxidant properties. With the largest zone of inhibition against *E. coli* (1.9 cm), the methanol extract proved to be the most effective antibacterial agent against a wide range of tested pathogens. According to the reducing power experiment, the acetone extract simultaneously demonstrated greater antioxidant activity, indicating that it is the most effective solvent for separating the substances that scavenge free radicals. The noteworthy biological activities noted highlight the importance of *P.amaryllifolius* as an easily accessible and sustainable source for the creation of food-grade antioxidants and natural antimicrobial medications, providing promising substitutes for synthetic compounds in the battle against diseases linked to oxidative stress and antibiotic resistance.

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