Short Research Article

Phytochemical Analysis and Antibacterial Assessment of Pineapple (*Ananas comosus*) Peel Extract

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ABSTRACT

Aims: This study assessed the phytochemical composition and antibacterial properties of pineapple peel *(Ananas comosus),* a significant by-product of pineapple production, which is often discarded despite its potential health benefits. Pineapple peels constitute 30-42% of the fruit's weight, leading to substantial waste and environmental challenges when improperly disposed of.

Study design: The researchers utilized a qualitative research approach to achieve their desired results. The research was experimental as it involved different trials in terms of Alkaloids, Flavonoids, Saponins, Tannins, and Terpenoids and three trials against *P. aeruginosa, S. aureus, and E. coli.*

Place and Duration of Study: Botany Department, Central Mindanao University (Sayre Highway, Maramag, Bukidnon), Wet Laboratory of San Isidro College (Impalambong, Malaybalay City, Bukidnon), and the Microbiology Section of Bukidnon Medical Provincial Center (Sayre Highway, Casisang, Malaybalay City, Bukidnon), between the timeframe of April 2024 to May 2024

Methodology: Pineapple peel samples were collected from local fruit stands, processed into ethanolic extracts, and subjected to phytochemical analysis using colorimetric tests for alkaloids, flavonoids, saponins, tannins, and terpenoids. The antibacterial activity was assessed through the Kirby-Bauer disk diffusion method against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

Results: Phytochemical analysis confirmed the presence of alkaloids and terpenoids while revealing the absence of flavonoids, saponins, and tannins. The antibacterial testing showed a zone of inhibition of 0 mm against all tested pathogens, indicating limited antibacterial efficacy.

Conclusion: Although the pineapple peel extract demonstrated low antibacterial activity, its rich phytochemical content suggests potential antioxidant and anti-inflammatory properties that warrant further investigation. This study emphasizes the need for sustainable utilization of agricultural waste by exploring the health benefits of pineapple peels, thereby contributing to environmental conservation and resource optimization in the food industry. Future research should focus on varying extraction methods and concentrations to enhance the antibacterial properties and explore additional applications in pharmaceuticals and nutraceuticals.

Keywords: Ethanolic extract, Kirby-Bauer's test, pineapple peel, phytochemical analysis, zone of inhibition

1. INTRODUCTION

Renowned for its exquisite flavor and juicy texture, the pineapple (Ananas comosus) holds the title of "Queen of Fruits" and is a widely cultivated fruit worldwide. Its popularity is further boosted by its health-promoting properties, such as anti-inflammatory and antioxidant effects, benefits to the nervous system, and support for healthy digestion (Mohd Ali et al., 2020). Owing to the aforementioned qualities, pineapples are currently the most widely produced crop of the Bromeliaceae family, which boasts around 2500 species (Wali, 2019). "Smooth Cayenne" is the most widely available commercial variety of pineapple, which can be consumed either directly or by processing. Nowadays, pineapples have been incorporated into a variety of products, including canned fruit, jams, crystallized fruit, juice concentrate, and dehydrated snacks (Campos et al., 2020). Typically, only its pulp is consumed. Its byproducts, including the peel, crown, core and the pomace are usually left discarded as waste products.

The Philippines, a major producer of pineapples, generates substantial volumes of pineapple peel waste during processing and harvesting. Approximately 60 grams of peel waste is produced from 400-gram pineapple fruit, amounting to about 15% of the total fruit weight (Saraswaty et al., 2017). With the Philippines processing large quantities of pineapples, the total pineapple peel waste generated is significant. However, the disposal of this pineapple peel waste poses environmental challenges.

Currently, much of the disposed peel waste is burned, contributing to air pollution and haze (Bansod et al., 2023). This practice not only harms the environment but also represents a wasteful utilization of resources. The lack of effective management and valorization of pineapple peel waste in the Philippines results in environmental and economic losses (Bansod et al., 2023). Pineapple peel waste contains valuable bioactive compounds and antioxidants that could be extracted for various applications (Saraswaty et al., 2017). Pineapple peels represent a rich source of bioactive compounds and the valuable bromelain protein (Bansod et al., 2023). Recognizing this potential, researchers are exploring their utilization, which could contribute to a circular economy and the development of sustainable products (Sarangi et al., 2023).

Pineapple peel is a potent source of bioactive compounds, such as phenolic compounds, ferulic acid, and vitamins A and C, all of which contribute to its notable antioxidant properties (Fitriyanti et al., 2019). This study can help in utilizing agricultural waste sustainably, which could benefit the economy and the environment. Secondly, by identifying the specific phytochemicals present, this study provides valuable insights for future applications in pharmaceuticals, nutraceuticals, and cosmetics. Furthermore, the result of the antibacterial testing is important as it demonstrates that not all pineapple extracts exhibit antibacterial properties, which expands our knowledge of natural remedies. Moreover, the identified phytochemicals may offer other health benefits, such as antioxidants or anti-inflammatory properties, which deserve further investigation. Pineapple peel waste has been shown to contain a variety of bioactive compounds with potential therapeutic applications (Owoeye et al., 2022). Overall, this study highlights the importance of thorough scientific inquiry and the potential of agricultural by-products across various industries.

This study aimed to investigate the phytochemical properties and antibacterial components of pineapple (*A. comosus)* peel, specifically on the effectiveness of pineapple (*A.comosus)* peel extract against *P. aeruginosa, S.aureus,* and *E. coli*. The researchers examined the phytochemical compounds present in pineapple peel. Subsequently, the researchers conducted efficacy tests on the extract against two strains of gram-negative bacteria and one strain of gram-positive bacteria. Furthermore, by exploring the phytochemical compounds in pineapple (*A. comosus)* peels and the efficacy of the pineapple peel against bacteria, the study can encourage more researchers and professionals to initiate actions for the usage of pineapple (*A. comosus)* peels. This can lead to reduced food waste since the frequently discarded peel will be turned into useful value-added products.

2. MATERIALS AND METHODS

2.1 Research Design

The study sought to investigate the phytochemical properties of the Pineapple (*A. comosus*) peel extract and its antibacterial activity in regards with *P. aeruginosa, S. aureus,* and *E. coli*. Accordingly, the researchers utilized a qualitative research approach to achieve their desired results. The research was experimental as it involved different trials in terms of Alkaloids, Flavonoids, Saponins, Tannins and Terpenoids and three trials against *P. aeruginosa, S. aureus,* and *E. coli*.

2.2 Locale of the Study

The pineapples were obtained from local fruit stands located along the Sayre Highway, Mailag, Valencia City, Bukidnon, 8709. The researchers then forwarded select pineapples to the Botany Department of the Central Mindanao University located at Sayre Hwy, Maramag 8714, Bukidnon, for further identification. Moreover, powdered samples were prepared along with the maceration and extraction of the extract in the Wet Laboratory of San Isidro College located at Impalambong, Malaybalay City, Bukidnon, 8700. Subsequent phytochemical analysis testing was also conducted in the Wet Laboratory of San Isidro College. Finally, the researchers delivered the extract to the Microbiology Section of the Bukidnon Medical Provincial Center located at Sayre Highway, Impalampong, Bukidnon, 8700 for the antibacterial assessment.

2.3 Collection and Preparation of Specimen

The researchers purchased the pineapple from a local fruit stand located along the Sayre Highway, Mailag, Valencia City, Bukidnon, 8709. After consuming the pulp, the peel was cleaned with neutralized water and carefully brought to the wet laboratory located in San Isidro College, Impalambong, Malaybalay City 8700, Bukidnon for extraction.

2.4 Identification of the Pineapple Specimen

The specimen was taken to Central Mindanao State University located at Maramag, Bukidnon, for identification. Botany experts were consulted to ensure the accurate classification and verification of plant species.

2.5 Maceration and Extraction Process

The sample was air-dried by placing it outside the house for two weeks. After drying, the sample was pulverized and crushed into a fine powder using an electric blender. This fine powder was then stored in a clean, pristine container to maintain its sterility. To extract the desired compounds, the pineapple peel powder was mixed with a 98% ethanol solvent in a

1:10 ratio. The mixture was soaked for three days to allow thorough extraction. After the soaking period, the solution was filtered using filter paper and a funnel to separate the liquid extract from the solid residue.

2.6 Phytochemical Analysis

The Phytochemical Analysis was carried out at the Science Laboratory of San Isidro College, Impalambong, Malaybalay city after the specimen was prepared and collected.

2.6.1 Test for Alkaloids

Two milliliters of dilute hydrochloric acid (HCl) were mixed with an equal volume of the sample's ethanolic extract. After thoroughly mixing the solutions, a drop of Wagner's reagent was carefully added. The appearance of a reddish-brown color in the solution served as a positive indicator, confirming the presence of alkaloids (Sicalan et al., 2023).

2.6.2 Test for Flavonoids

To identify flavonoids, the researchers performed an alkaline reagent test. This involved adding a few drops of sodium hydroxide to the test solution. The presence of flavonoids was indicated by the development of an intense yellow color, which subsequently disappeared upon the addition of a few drops of dilute acid (Pant et al., 2017).

2.6.3 Test for Saponins

A 20 mL solution is prepared by diluting 50 mg of extract with distilled water. The mixture was then shaken in a graduated cylinder for 15 minutes. The formation of a 2 cm foam layer after this procedure suggests the presence of saponins (Banu et al., 2015; Murray et al, 2023).

2.6.4 Test for Tannins

The presence of tannins was detected using the ferric chloride test. Specifically, they added 1g of the extract to 2ml of a 1% HCl solution. The subsequent formation of a red precipitate indicated the presence of tannins (Sheel et al., 2014).

2.6.5 Test for Terpenoids

The Salkowski test was used to detect the presence of terpenoids. In this test, five milliliters of the extract were combined with two milliliters of chloroform, followed by the addition of three milliliters of concentrated sulfuric acid, forming a distinct layer. The appearance of a reddish-brown coloration at the interface was observed, indicating the presence of terpenoids (Das et al., 2014).

2.7 Preparation and Implementation of Antibacterial Analysis

Bacteria *E. coli* and *S. aureus* are well-suited for educational settings due to their predictable and well-documented zones of inhibition (Bio, 2023). The antibacterial analysis was performed using a method used by Hudzicki et al. (2016) with minor modifications. To begin the antibacterial analysis, filter paper discs are saturated with the test extract and left for 24 hours. Simultaneously, a bacterial suspension is prepared. Subsequently, Mueller-Hinton agar plates are inoculated with the bacterial suspension. Antibiotic discs, serving as positive controls, are carefully placed on the agar to ensure that the resulting zones of inhibition do not overlap. After a 24-hour incubation at 37 °C, the diameters of the zones of inhibition around each disc are measured and recorded. This method allows for effective control and clear observation of bacterial inhibition, making it suitable for educational purposes (Abdon et al., 2024a).

2.8 Data Gathering Procedure

To ensure the success of the study, the researchers distributed several letters requesting permission for the testing to be conducted in three specific locations. After which, the researchers prepared the necessary materials needed for the following tests. The phytochemical analysis was carried out at San Isidro College's Wet Laboratory. Analysis of phytochemicals, in particular alkaloids, flavonoids, saponins, tannins, and terpenoids, was covered. For alkaloids, Wagner's test was utilized, and for flavonoids, the Alkaline Reagent Test, Frothing test was utilized for saponins. For tannins, use the ferric chloride test; for terpenoids, use the Salkowski test. The Bukidnon Provincial Medical Center's Microbiology Section carried out the antibacterial assessment in the interim. As part of the data gathering process, the antibacterial assessment comprised disk diffusion utilizing the Kirby-Bauer method in an antibacterial test.

3. RESULTS AND DISCUSSION

3. 1 Phytochemical Analysis

Secondary metabolites are essential for plant growth and development, as they store essential phytochemicals and protect plants from environmental stress (Nikolić, 2012). These compounds help plants defend against herbivores and other species. Humans also use secondary metabolites in medicine, food flavoring, pigments, and even recreational drugs (Abdon et al., 2024b). They have been utilized in the development of safe and effective medicines, either as individual compounds or in combination (Seca & Pinto, 2019). These substances allow plants to detect herbivore attacks and respond quickly to environmental changes. Additionally, plant metabolites produce useful natural compounds that offer various benefits (Yang et al., 2018).

Table 1. Phytochemical Analysis using Colometric Tests

Phytochemicals and Tests Used	Trial	Sample	Remarks
Alkaloids using Wagner Test	1	Reddish-brown	Positive
	2	Reddish-brown	Positive
	3	Reddish-brown	Positive
	4	Reddish-brown	Positive
	5	Reddish-brown	Positive
Flavonoids using Alkaline Reagent Test	1	Light yellow	Negative
	2	Light yellow	Negative
	3	Light yellow	Negative
	4	Light yellow	Negative
	5	Light yellow	Negative
Saponins using Frothing Test	1	No foam for at least 1 cm	Negative
	2	No foam for at least	Negative

		1 cm	
	3	No foam for at least 1 cm	Negative
	4	No foam for at least 1 cm	Negative
	5	No foam for at least 1 cm	Negative
Tannins using Ferric Chloride Test	1	Yellow coloration	Negative
	2	Yellow coloration	Negative
	3	Yellow coloration	Negative
	4	Yellow coloration	Negative
	5	Yellow coloration	Negative
Terpenoids using Salkowski Test	1	Reddish-brown	Positive
	2	Reddish-brown	Positive
	3	Reddish-brown	Positive
	4	Reddish-brown	Positive
	5	Reddish-brown	Positive

Table 1 shows the results gathered from the phytochemical analysis of pineapple peel extract. This study's phytochemical analysis of pineapple peel extract offered important findings on its possible medicinal benefits. To begin with, the consistent presence of alkaloids across all trials, as indicated by the reddish-brown coloration in Wagner's test, underscores the significance of these compounds in the extract. Wagner's test is a well-known method for identifying alkaloids. It involves adding Wagner's reagent to the sample, which produces a reddish-brown precipitate if alkaloids are present (Gutiérrez-Grijalva et al., 2020). The observed coloration in this study suggests the extract contains alkaloids, which have historically been integral to traditional medicine and possess diverse pharmacological activities, including antibacterial effects. Additionally, the absence of flavonoids in the extract, as indicated by the negative results of the Alkaline reagent test, is noteworthy. The Alkaline reagent test involves the addition of sodium hydroxide solution followed by dilute acid to the sample, with a positive result indicated by a transition from yellow to colorless (Biodiversitas, 2017). The absence of this color change in all trials suggests the extract lacks detectable levels of flavonoids, which are renowned for their antimicrobial properties. In addition, the absence of saponins in the extract, as demonstrated by the negative results of the Frothing test, highlights another aspect of its phytochemical composition. The Frothing test involves vigorously shaking the sample with water to produce foam, with the presence of saponins indicated by the formation of a stable foam layer (Kumari & Jain, 2012). The absence of foam formation in all trials suggests the extract does not contain measurable quantities of saponins, which also possess antimicrobial properties. Moreover, The Ferric chloride test for tannins revealed consistent negative results across all trials, indicating the absence of detectable traces of tannins in the extract. This test is performed by adding ferric chloride solution to the sample, where the formation of a red precipitate indicates a positive result (Ogwu et al., 2019). The consistent yellow coloration observed suggests the absence of tannins, which are known for their antioxidant properties and various health benefits. The consistent presence of terpenoids in the extract, shown by the reddish-brown interface layer in the Salkowski test, suggests its potential medicinal use. This test detects terpenoids by mixing the sample with chloroform and concentrated sulfuric acid, where a positive result is indicated by the formation of a reddish-brown interface layer (Das et al., 2014). The uniformity of this coloration in all trials suggests the extract is a reliable source of terpenoids, which encompass a diverse group of compounds with various biological activities, including antiviral and anticancer properties.

3.2 Antibacterial Analysis

Table 1 below summarizes, records, and measures the zone of inhibition of *A. comosus* extract on *E. coli, S. aureus, and P. aeruginosa*.

Table 2. Zone of Inhibition Using Ethanolic Pineapple (*A. comosus*) peel extract

Bacteria	Zone of Inhibition	Interpretation
E. coli	0 mm	Resistant
P. aeruginosa	0 mm	Resistant
S. aureus	0 mm	Resistant

Table 2 presents the results of an antibacterial assessment of pineapple peel extract against *E. coli, S. aureus*, and *P. aeruginosa*, showing a mean ZOI of 0 mm for all three bacterial strains. This indicates no antibacterial activity under the experimental conditions, suggesting that the extract either lacks sufficient antibacterial compounds or the concentrations used were ineffective (Fitriyanti et al., 2019; Ogwu et al., 2019). The resistance observed could be due to the absence of key compounds like flavonoids, tannins, and saponins, which are known for their antibacterial properties (Ogwu et al., 2019; Okoh et al., 2019). The presence of terpenoids and alkaloids, while bioactive, may not have been potent enough to inhibit these bacterial strains effectively. The resistance of *E. coli*, *S. aureus*, and *P. aeruginosa* to the pineapple peel extract emphasizes the role of specific bioactive compounds and their concentrations in determining antibacterial effectiveness. In addition, factors such as the extraction method and the solvent used can significantly impact the presence of these compounds (Lubaina et al., 2019). Further research is needed to optimize the concentration and composition of bioactive compounds, explore synergistic effects, and elucidate specific mechanisms of action against different bacterial strains (Punbusayakul, 2018). By enhancing the extract's antibacterial efficacy through targeted modifications, future studies may overcome bacterial resistance and improve its potential as a natural antibacterial agent (Gunwantrao et al., 2016).

4. CONCLUSION

Through various means of phytochemical analysis testing, the pineapple *(A. comosus)* extract was demonstrated to be positive in the presence of Alkaloids and Terpenoids. However, tests indicated the absence of flavonoids, saponins, and tannins. Data variations may occur due to factors such as the type of solvent used, extraction method, and climatic conditions of the area where the plant samples were collected. Accordingly, the results obtained by the researchers are contrary to results obtained from similar studies (Gunwantrao et al., 2016). In this study, their testing methods revealed a positive indication for the presence of Saponins and Tannins; while also indicating a negative presence for Terpenoids; which is contrary to the results obtained in the study. However, despite these discrepancies, both studies indicate a positive presence of Alkaloids and a negative presence of Flavonoids. As for the antibacterial activity, the zone of inhibition was recorded at 0mm. Thus, the extract did not display any inhibitory effects on *E. coli, S. aureus,* and *P. aeruginosa.* Therefore, the following results would suggest that the pineapple *(A. comosus)* peels possess low antibacterial activity.

5. RECOMMENDATION

Based on the study's findings, several recommendations were proposed to further explore the phytochemical composition and antibacterial properties of pineapple (*A. comosus*) peel and its extract.A more thorough investigation into its antibacterial properties is needed, particularly using varying concentrations during assessments. Researchers suggest using different extracts, such as methanol, to modify concentrations and achieve different results. Additionally, including other noteworthy pathogens could broaden the scope of the study. Exploring different testing methods for both the plant's phytochemical content and antibacterial capabilities is also advised. Future research should examine the phytochemical content and antibacterial properties of unripe pineapple peels to ensure comprehensive coverage and pave the way for more detailed studies.

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