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Evaluation Of *Musa Sapientum* (Banana) Leaf Extracts (BLE) As Natural Preservatives Against Three Common Spoilage Fungi

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Abstract
Fungal spoilage continues to poses monumental drawback to the sustainability and safety of fruits and vegetables particularly in developing countries. This study aimed to investigate the potential of banana leaf extracts (BLE) (Musa sapientum) as natural preservatives against common spoilage fungi, including Aspergillus niger, Fusarium oxysporum, and Rhizopus stolonifer, recovered from spoilt bakery products. The aqueous and ethanol BLEs were analyzed for their phytochemical compositions and antifungal activities against the three fungal isolates using standard protocols. Additionally, the minimum inhibitory concentrations (MIC) of the extracts were determined to ascertain the bio-effective concentrations. The ethanol BLE showed intense levels of saponins, steroids, terpenoids, and glycosides as against alkaloids, tannins, steroids and flavonoids in the aqueous BLE. The antifungal activity of the extracts was concentration-dependent, with poor susceptibility observed at lower concentrations (200mg/mL and 400mg/mL). However, the highest concentration (800mg/mL) exhibited significant antifungal activity against all tested spoilage fungi. Significantly better antifungal activity ($p<0.05$) was observed with the ethanol extract fraction particularly at lower concentrations. The MIC of the ethanol extract was 200mg/mL for A. niger, F. oxysporum, and R. stolonifer, 800mg/mL the aqueous extract on the three fungi. The findings of this study suggest that ethanol BLE hold promise as natural preservatives against spoilage fungi. Further research is necessary to refine the formulation and assess the practical application of BLF as natural food preservatives.

Keywords: *Banana leaf extracts, natural preservatives, phytochemical, Rhizopus, Fusarium, Apergillus*

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I. Introduction

The banana plant was reported to have originated from Southeast Asia and is now dominant in other parts of the world including Africa (Heuze and Tran, 2016). Its leaves are large, flexible, and have a unique shape, which makes them ideal for various applications. These versatile and widely available leaves have been used for various purposes in many cultures around the world for centuries. In many countries, such as India, Thailand, Malaysia, and the Philippines, banana leaves have been traditionally used for cooking, serving food, and for wrapping various items. Banana leaf extracts are derived from the leaves of the herbaceous banana plant, classified as *Musa sapientum*, found in the Musaceae family. They specifically fall under the genera *Musa*, *Musella*, and *Ensete* (Probojati *et al.*, 2021).The popular species consumed by humans are *Musa acuminata* and *M. balbisiana*, which produce a wide variety of bananas differing in color, taste, and nutritional contents (Venkataramana *et al*., 2015). Banana contains a rich composition of bioactive compounds, including polyphenols, flavonoids, tannins, and other phytochemicals. These compounds are known for their potential health benefits and have been studied for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Afzal *et al*., 2022). It also rich in potassium, magnesium, vitamins A, B, and C (Oyeyinka and Afolayan, 2019). One of the remarkable applications of banana leaf extracts is in traditional medicine. In Ayurveda, the Indian traditional medicine practice, banana leaves are believed to possess biological activities against diabetic, hypertensive, wound infections, skin disorders, digestive disorders, and respiratory ailments (Kumar *et al.*, 2012; Jyothirmayi and Rao, 2015). Different plant parts of the *Musa* spp., such as the stem juice, flowers, and fruits, have been utilized in traditional medicine across various cultures for the treatment of ailments like diarrhea, ulcers, and snakebites (Rao et al., 2014; Kamira *et al.*, 2015; Panda *et al*., 2020). The leaf, stem and flowers extracts of *Musa* spp. did not exhibit significant cytotoxicity towards healthy cells, indicating

their potential safety (Panda *et al.*, 2020). In most African countries, including Nigeria, the leaves of banana applied in treatment of diverse human diseases and packaging traditional delicacies (Kamira *et al.*, 2015; Ezeudu *et al.*, 2021). In addition to their medicinal uses, banana leaf extracts are also utilized in the cosmetic and skincare industry and holds great potential application in formulating natural skincare products to nourish and revitalize the skin (Yoo *et al.*, 2016).

Fungi include broad group microorganisms that are mostly involves in biodegradation and biomineralization of organic materials in the environment as source of energy and carbon (Yu *et al.*, 2023). They reproduce by producing spores, which are tiny reproductive structures that can be easily dispersed through the air, water, or other means. These spores can survive in unfavorable conditions and germinate when they encounter suitable environments, such as nutrient-rich substrates, causing spoilage and contamination (Lorenzo *et al.*, 2018). They possess broad range of enzymatic machinery to degrade different fruits, vegetables, cereals and other stored products (Garnier *et al.*, 2017; Snyder and Worobo, 2018; Bernardi *et al*., 2019; Karanth *et al.*, 2023) . Some common food spoilage fungi, including species of *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* often manifests as visible signs, such as mold growth, discoloration, off-odors, and texture changes (Pitt and Hocking, 2022; Sohlberg *et al.*, 2022). Fungal spoilage not only affects the appearance, taste, and texture of food but can also produce toxic metabolites called mycotoxins. Mycotoxins are bioactive metabolites produced by certain fungi and can pose serious health risks if consumed in significant amounts (Ntsama *et al.*, 2023). Some well-known mycotoxins include aflatoxins, produced by *Aspergillus* species, which have been linked to liver cancer, and *ochratoxin-A*, produced by *Aspergillus* and *Penicillium* species, which can cause kidney damage (Pandey *et al.*, 2023).

The direct or indirect existence of fungi in raw of processed food products is a major concern due to their detrimental effects on the quality and safety the foody (Sohlberg *et al.*, 2022; Pandey *et al.*, 2023). The use of synthetic fungicides raises concerns about potential health and environmental risks (Wu *et al*., 2023). Therefore, there is a need to explore natural alternatives for controlling fungal growth in food. The investigation of natural products as potential antifungal agents is of great significance in promoting food safety and reducing the reliance on synthetic fungicides (Teshome *et al.*, 2022). Banana leaves are a promising candidate due to their abundance, easy accessibility, and traditional usage for medicinal purposes. Investigating the antifungal properties of banana leaf extracts will provide valuable insights into their efficacy and potential application in the food industry. Therefore, this study was aimed at investigating the antifungal activities of *Musa sapientum* (banana) leaf extracts on selected fungi isolates obtained from bread samples. By exploring the potential antifungal properties of bananas, this research contributes to the broader objective of developing inexpensive and safe solutions aimed at food preservation and safety.

II. Materials And Methods

Study Area

The research was conducted at the Federal University Lokoja, located in Kogi state, Nigeria. Kogi state is situated between latitude 6.33 ^{o}N to 8.44 ^{o}N and longitude 5.40 ^{o}E to 7.49 ^{o}E (Figure 1). The region is characterized by Guinea Savannah as its natural vegetation (Odekunle, 2004). The average maximum temperature in the state is around 33°C, with an average minimum temperature of 23°C, resulting in hot conditions throughout the year (Sowomi and Akinola, 2010). The annual rainfall in Kogi state ranges from 1016-1524 mm (Olatunde and Adejoh, 2008).

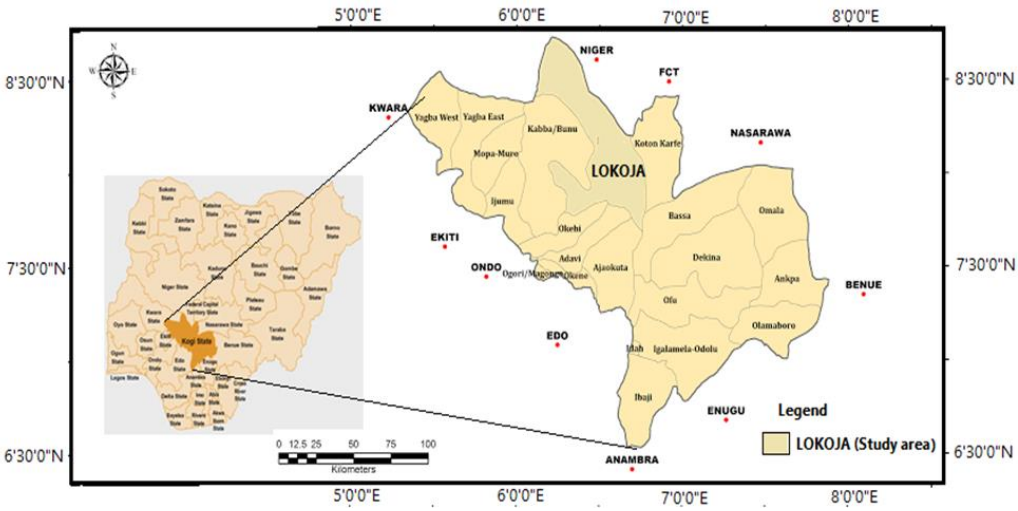

Figure 1: Location map of Lokoja, Kogi State, Nigeria

Collection of materials

Musa sapientum leaves were collected in clean bags from Mont Patti, Lokoja, Kogi State. Bread samples showing signs of spoilage were purchased from shops within the vicinity of Federal University Lokoja, Kogi State. The samples were properly package in clean containers, labeled and transported to laboratory for further processing and analyses.

Isolation of Test fungal from bread samples

The fungi isolation process from bread samples followed the methodology described by Samson et al. (2010). Initially, small portions of the bread samples were directly placed onto Potato Dextrose Agar (PDA) media supplemented with chloramphenicol, with each plate containing 5 bits in triplicate. Subsequently, the plates were incubated at a temperature of 25 °C for 5 to 7 days. After the incubation period, the fungal colonies obtained were sub-cultured on PDA and further incubated at the same temperature for an additional 5 to 7 days. The three predominant fungal isolates were subsequently characterized and identified. A loopful of lactophenol stain was placed on a clean grease-free slide before adding a small portion of the mycelium from the fungal cultures was carefully extracted using a mounted needle and placed into the stain. The mycelium was then evenly spread across the slide using two mounted needles, and a cover slip was gently placed on top. Finally, the slide was examined under a microscope, specifically using the high power objective (×40), to observe the morphological characteristics of the fungi, including the type of hyphae and asexual reproductive structures (Ogu *et al.*, 2017). The three major fungal isolates, namely *Aspergillus niger*, *Fusarium oxysporum*, and *Rhizopus stolonifer*, were recovered and used in this study. The isolates were purified and stored for characterization.

Processing of Plant Materials

The fresh banana leaves were freed of all extraneous materials by rinsing repeatedly in distilled water. Subsequently, the fresh leaves were preserved by cutting them in the morning when they were fully hydrated. This method maintained the flexibility, texture, and form of the leaves. The leaf samples were allowed to dry on the lab table at normal room temperature for duration of 14 days. Afterward, they were pulverized into a powdered form using a sterile mortar and pestle. The powdered plant materials were properly labeled and stored in separate sterile, airtight containers for further extraction processes.

Preparation of Aqueous and Ethanol Extract of the *M. sapientum* leaf

To obtain the aqueous extract, 50g of powdered *M. sapientum* leaves were weighed and placed in a 2000mL beaker. The beaker was labeled according to the sample contained within it. Thereafter, 200 mL of sterile distilled water was measured and used to soak the powdered leaf samples for 24 h duration. While standing, the content was manually shaken periodically to ensure thorough extraction process. The resulting mixture was filtered using a sterile 3-layer cheese cloth, and the filtrate extract was collected in a 250mL beaker. Two concentrations, 80 % and 60 %, were prepared by subjecting the extract to a water bath, allowing the aqueous solvent to evaporate. The same procedures as those used for the aqueous extract were followed to prepare the ethanol extract. The filtered ethanol extract was subjected to a water bath to evaporate the ethanol solvent, resulting in standardized extracts for the antifungal activity test.

Determination of Phytochemical Components of the Leaf Extract

Phytochemical screening was carried to qualitatively detect various active ingredients in the extract, including alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, phenols, and glycosides. The protocol adopted for the analysis of each phyto-compound was previously report (Hoang *et al.*, 2023).

Antifungal susceptibility testing

The method described by Ogu *et al.* (2013) was adopted for the susceptibility utilizing potato dextrose agar (PDA) media. The PDA was prepared following the manufacturer's instructions, autoclaved, and dispensed aseptically sterile petri dishes and left to gel. Next, a standardized suspension of fungi cells was streaked evenly on each agar plate. Using a sterile cork borer with a diameter of 5mm, four wells were bored on the solidified agar medium of each plate. The fourth well served as a positive control and was loaded with ketoconazole. The extract was double diluted to obtain different concentrations (800mg/mL, 400mg/mL, and 200mg/mL) for the antifungal assay. To prepare the extract concentrations, four test tubes were assigned for each extract. The first test tube received 2mL of sterile peptone water, while the other three test tubes received 1mL each. To convert 1 gram of each extract into 100 milligrams, it was multiplied by 1000. The resulting 1000mg was transferred and dissolved in the first test tube containing 2mL of peptone water. From the first test tube, 1mL was transferred to the second test tube containing 1mL of peptone water, resulting in a concentration of 800mg/mL. The same process was repeated to obtain a concentration of 400mg/mL in the third test tube and 200mg/mL in

the fourth test tube. The extracts were then added to the appropriately labeled wells and holes on the plates using a sterile syringe (2mL). The positive control well contained ketoconazole, while the negative wells contained the extracting solvents. After 30 min of pre-diffusion, the inculcated plates were incubated at 37°C for 24 h. The experimental set was done in triplicates and the diameter zones of inhibition were read with a clean, millimeters graded transparent ruler.

Determination of the minimum inhibitory concentration (MIC)

The broth dilution method described by Duke-Ndudim *et al*. (2016) was followed to carry out the minimum inhibitory concentration (MIC) of the plant extract. The extract was double diluted to obtain three different concentrations (800mg/mL, 400mg/mL, and 200mg/mL). A total of 24 sterile test tubes were arranged, with four test tubes allocated for each test organism. Each test tube was labeled according to the concentration of the extract and the specific fungi pathogen to be placed in them. Sterile peptone water (2mL) was added to each test tube labeled according to the extract concentration, while the control tubes received 2mL of the control solution. Then, 1mL of each extract was added to the corresponding test tubes based on their labeled concentrations. Additionally, 1mL of standardized suspensions of each test organism was inoculated into the test tubes containing dilutions (800mg/mL, 400mg/mL, and 200mg/mL) of each extract. The test tubes were properly stopped with cotton wools and incubated at 37°C for 24 h. The least test tube where absence of turbidity was found indicated with MIC for the extract concentration.

Data analysis

The data obtained from the antifungal susceptibility testing and determination of MIC was analyze descriptively and were expressed as mean ± standard deviation. The mean of the different extract concentrations were compared using one way ANOVA (p=0.05) and where there was significance, Tukey's HSD test was employed for further comparism. SPSS version 20 test package was used for the analyses.

III. Results

In this study, Ethanol and aqueous banana leaf extracts (EBLE and ABLE) were evaluated to determine the bioactive ingredients and antifungal efficacy against selected common spoilage fungal isolates. Table 1 shows the findings from the phytochemical analysis. The ethanol extract exhibited high quantities of saponins, steroids, terpenoids, and glycosides, while alkaloids, tannins, and flavonoids were present in lower quantities, with phenols being absent. On the other hand, the aqueous extract showed high quantities of alkaloids, tannins, and flavonoids, and lower quantities of saponins, steroids, and terpenoids, with phenols being absent. The antifungal activities of the ethanol banana leaves extract (EBLE) are presented in Table 2. It was observed that EBLE exhibited inhibition against all test microorganisms only at the highest concentration (800mg/mL). The positive control, ketoconazole, also showed inhibitory effects against the test microorganisms, with varying zone of inhibition diameters for *Rhizopus stolonifer*, *Aspergillus niger*, and *Fusarium oxysporum*. However, the aqueous banana leaves extract (ABLE) did not demonstrate any inhibitory activity against the test microorganisms, as indicated in Table 3. The positive control, ketoconazole, showed inhibition against the microorganisms, albeit with different zone of inhibition diameters for each. The minimum inhibitory concentration (MIC) of the ethanol banana leaves extract (EBLE) was determined and recorded in Table 4. For *Aspergillus niger*, *Fusarium oxysporum*, and *Rhizopus stolonifer*, the MIC of EBLE was found to be 200mg/mL. In contrast, the MIC of the aqueous banana leaves extract (ABLE) for all the test microorganisms was determined to be 800mg/mL, as shown in Table 5.

Table 1: Phytochemical screening of ethanol and aqueous extracts of banana leaves

Pyhtochemicals	Method Used	Ethanol leaf extract	Aqueous leaf extract
Alkaloids	Wagner's Reagents Test	(+)	(+ +)
Tannins	Ferric Chloride Test	(+)	(+ +)
Saponins	Frothing Test	(+ +)	(+)
Flavonoids	Ammonium Test	(+ +)	(+ +)
Steriods	Sarkowski's Test	(+ +)	(+ +)
Terpenoids	Sarkowski's Test	(+ +)	(+)
Phenols	Ferric Chloride Test	(-)	(-)
Glycosides	Fehling's Solution Test	(+ +)	(+)

Key: (++) = Highly present, (+) = moderately present, (-) = absent

Table 2: Mean diameter (mm) of zone of inhibition of EBLE and control on test microorganisms

Extracts	**Test Micro-organisms**		
Concentration	***A. niger***	***F. oxysporum***	***R. stolonifer***
800mg/mL	13.00 ± 1.00^{c}	11.00 ± 2.00^{b}	9.10 ± 1.00^{c}
400mg/mL	0.00 ± 0.00^{c}	0.04 ± 0.00^{c}	0.02 ± 0.00^{c}
200mg/mL	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.01^{c}
Ketoconazole	17.00 ± 2.00^{a}	14.00 ± 1.00^{a}	12.04 ± 1.00^{a}

*EBLE: Ethanol banana leaf extract, Mean in the same columns having the same alphabet are not significantly different at $p< 0.05$

Table 3: Mean diameter (mm) of zone of inhibition of ABLE and control on test microorganisms

Extracts	**Test Micro-organisms**		
Concentration	***A. niger***	***F. oxysporum***	***R. stolonifer***
800mg/mL	0.02 ± 0.00^{c}	0.00 ± 0.00^{c}	0.01 ± 0.00^{c}
400mg/mL	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
200mg/mL	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Ketoconazole	15.00 ± 1.05^{a}	9.00 ± 1.00^{a}	13.00 ± 2.00^{a}

*ABLE: Aqueous banana leaf extract, Mean in the same columns having the same alphabet are not significantly different at $p< 0.05$

Table 4: Minimum inhibiting concentration (MIC) of EBLE on test microorganisms

Test fungi	Concentration (mg/mL)				
	1600	800	400	200	100
Aspergillus niger	(-)	(-)	(-)	(+)	(+)
Fusarium oxysporum	(-)	(-)	(-)	(+)	(+)
Rhizopus stolonifer	(-)	(-)	(-)	(+)	(+)

Key: (+): Growth, (-): No growth, EBLE: Ethanol banana leaf extract

Table 5: Minimum inhibiting concentration (MIC) of ABLE on test microorganisms

Test fungi	Concentration (mg/mL)				
	1600	800	400	200	100
Aspergillus niger	(-)	(+)	(+)	(+)	(+)
Fusarium oxysporum	(-)	(+)	(+)	(+)	(+)
Rhizopus stolonifer	(-)	(+)	(+)	(+)	(+)

Key: (+): Growth (-): No growth, ABLE: Aqueous banana leaf extract

IV. Discussion

Over the years scientists have found natural resources, such as plants to possess potential sustainable photochemical compounds needed for management of diverse pathogens and spoilage causing microbes (Siddiqui *et al*., 2023). Banana (*M. sapientum*) is a popular fruit widely consumed by people and have a long history of being used for medicinal purposes. Recently, there has been growing interest in exploring the antimicrobial properties of natural ingredients found in banana plants (Mostafa, 2021; Gupta *et al*., 2022). Medicinal plants, including bananas, have been traditionally used for treating infections caused by microorganisms, and their antifungal activities have been recognized (Harith *et al.,* 2018; Jouneghani *et al*., 2020). In this study, the antifungal activities of banana leaf extracts against fungi isolated from spoilt bakery products were evaluated. The findings from the results provide valuable insights into the phytochemical composition and antifungal activities of ethanol and aqueous extracts of banana leaves. Qualitative phytochemical screening provides insights into the presence of bioactive secondary metabolites with therapeutic importance. The phytochemical screening of ethanol and aqueous extracts of banana leaves revealed the presence of various compounds. The ethanol extract contained high quantities of saponins, steroids, terpenoids, and glycosides, with lower quantities of alkaloids, tannins, and flavonoids. Similarly, the aqueous extract showed high quantities of alkaloids, tannins, and flavonoids, with lower quantities of saponins, glycosides, steroids, and terpenoids. The absence of phenols in both extracts highlights the differences in bioactive compound composition between the two solvents used for extraction. The qualitative differences in phytochemical compositions indicate the variation in the bioactive compound composition between the two extraction methods, with ethanol being more effective in extracting certain phytochemicals. This current study's findings is in line with previous reports (Onyema *et al.,* 2016; Elayabalan *et al.,* 2017; Harith *et al*., 2018), confirming the presence of similar phytochemical compounds in banana plants. This agreement between these studies strengthens the scientific understanding of the qualitative phytochemical composition of bananas and provides a reliable foundation for future research in this area.

In terms of antifungal activities, the crude ethanol leaf extract demonstrated inhibitory effects against the tested fungal isolates, but only at the highest concentration (800mg/mL). At lower concentrations (200

mg/mL and 400 mg/mL), the extract did not exhibit significant antifungal activities. The positive control, ketoconazole, showed inhibition of fungal growth for both the ethanol and aqueous leaf extracts. In contrast, the aqueous banana leaves extract (ABLE) did not show any inhibitory activity against the test microorganisms, except at 800 mg/mL, where insignificant bioactivity was noted against *A. niger* and *R. stolonifer*. The findings indicate that the ethanol banana leaf extract (EBLE) possesses moderate antifungal activity against the tested microorganisms, while the aqueous banana leaf extract (ABLE) shows limited inhibitory effects. These findings suggest that higher concentrations or different extraction methods may be required to achieve stronger antifungal effects with the aqueous extract. The observed dose-dependent antifungal activities could be influenced by the choice of solvent for extraction and probably the types of fungal species. The choice of solvent plays a crucial role in the extraction process. Ethanol has proven to be more effective in extracting bioactive compounds with nutritive and antimicrobial potential (Plaskova and MLcek, 2023). It has a higher capacity to extract a wide range of phytochemicals, including polar and non-polar compounds, due to its ability to dissolve both hydrophilic and hydrophobic molecules (Altemimi *et al.,* 2017). On the other hand, water-based extraction methods may have limitations in extracting certain bioactive compounds, leading to lower antifungal activities. Additionally, the presence of different phytochemicals in the ethanol and aqueous extracts may contribute to the differences in antifungal activities. The phytochemical screening results indicated that the ethanol extract contained higher quantities of saponins, steroids, terpenoids, and glycosides, whereas the aqueous extract exhibited higher quantities of alkaloids, tannins, and flavonoids. These bioactive compounds have been reported to possess antimicrobial properties and can potentially disrupt fungal cell membranes, interfere with enzymatic activities, or inhibit vital cellular processes (Sitarek et al., 2020; Vaou *et al.,* 2021). It is important to consider that the sensitivity of different fungal species to the bioactive compounds present in the extracts can also influence the observed antifungal activities. In this study, the ethanol extract showed inhibitory effects against all the tested fungal isolates, while the aqueous extract had limited activity, particularly against *A. niger* and *R. stolonifer*. This suggests that the susceptibility of fungal species to the bioactive compounds may vary, highlighting the need for further investigations on the specific mechanisms of action and target fungi. It is worth noting that the absence of antifungal activity at certain concentrations does not necessarily imply the absence of bioactive compounds or antifungal potential in the plant extracts. Factors such as the age and part of the plant, harvest time, drying and processing methods, extraction techniques, and solvents used can significantly influence the activity and composition of plant extracts (Ogu *et al*., 2012; Truong *et al*., 2019; Abubakar and Haque, 2020; Cor-Andrejc *et al.*, 2022). Therefore, further optimization of extraction methods, identification of specific mechanisms of action, and assessment of safety and efficacy are required before considering the use of banana leaf extracts as potential antifungal therapies. The determination of the minimum inhibitory concentration provided valuable information about the potency of the ethanol (EBLE) and the aqueous banana leaf extract (ABLE) on the test fungi. These results further support the weaker antifungal activity of ABLE compared to EBLE. The results of this study highlight the potential of banana leaf extracts as natural antifungal agents for food preservation. The use of plant-derived compounds as alternatives to synthetic preservatives aligns with the growing demand for safer and more sustainable food preservation methods. Incorporating banana leaf extracts into food packaging materials or developing edible coatings enriched with these extracts may offer effective protection against spoilage fungi, thereby extending the shelf life of perishable food products.

V. Conclusion

In conclusion, the findings from the study highlight the potential of ethanol and aqueous banana leaf extracts as antifungal agent against three spoilage fungi, including *A. niger*, *F. oxysporium* and *R. stolonifer*. The presence of bioactive compounds, including saponins, terpenoids, steroids, and glycosides, in the ethanol extract contributes to its moderate antifungal activities. However, the aqueous banana leaf extract (ABLE) showed limited inhibitory effects, indicating further research is needed to optimize the extraction methods, elucidate the specific mechanisms of action, and evaluate the safety/efficacy of banana leaf extracts in inhibiting fungal growth and spoilage in bakery products.

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