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RESEARCH ARTICLE
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Antibacterial and Antifungal Abilities of Tacca (*Tacca leontopetaloides* L. Kuntze) Leaf Extraction and its Application in Fresh Mango Preservation

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ABSTRACT

Tacca *(Tacca leontopetaloides (L.) Kuntze)* is a perennial herb, distributed from West Africa through South Asia to Northern Australia. Tacca leaves, which is 35-40% of the plant's weight are considered a waste product. In this study, the anti-microbial properties of Tacca leaf extract were investigated for their potential use in antimicrobials and fungicides. Tacca leaf extract can be obtained using different solvents including water, diethyl ether, ethyl acetate, and N-butanol. The anti-bacterial ability of the extract was evaluated by the inhibition zone diameter and the minimum inhibitory concentration (MIC) against *Pantoea stewartii subsp. Steartii* (P. steartii; bacteria) and *Aspergillus niger* (mold) on the mango skin. The results showed that the n-butanol extract of Tacca leaves had the best antibacterial activity against *P. steartii* at a concentration of 80%, with an inhibition zone diameter of 0.67cm. The MIC value of the Tacca leaf extract with N-butanol as a solvent against *A. niger* was 60%.

Keywords: Anti-bacterial properties, Mango diseases, Preservation, Spot damage, Tacca leaf.

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INTRODUCTION

Tacca (*Tacca leontopetaloides (L.) Kuntze*) is a native plant in Malaysia and the Pacific islands, distributed from West Africa through South Asia to Northern Australia (Drenth, 1972). Tacca plants are found in mountainous areas of Vietnam, including Tinh Bien ward in An Giang province (Vu et al., 2017). Tacca (called "Huyen" in Vietnamese) normally grows in moist, humus-rich places, such as under the forest canopy or edge of mountains, and is cultivated for its tubers. This plant is a perennial herb with no stem and its root has round brown tubers that mostly contain starch (Vu et al., 2017).

Tacca tubers have been used as a source of starch (Omojola, 2013). Alkaloids, flavonoids, saponins, and tannins have been found in high concentrations in Tacca leaves (Syafi et al., 2023) which can act as antimicrobial agents. Borokini (2011) reported that in Ivory Coast, Tacca leaf extract has been used as a mouthwash or consumed to treat gingivitis and stomach swelling. It is proposed that the antibacterial properties of Tacca leaves could be exploited to prevent bacterial spoilage of fresh fruit. However, there is limited research on using Tacca leaf extract as an antimicrobial agent on the surface of fresh mangoes. Tacca leaf extract can be obtained using different solvents and may can result in varying antibacterial abilities/potentials of the Tacca leaf extract (Syafi et al., 2023). Anthracnose infection occurs pre-harvest in the fruit, leaves, and stems of numerous fruit crops (Asrey and Das, 2021). This fungus produces enzymes (polygalacturonase and pectatelyase) that break down plant cell walls, resulting in substantial economic losses (Siddiqui and Ali, 2014). Disease symptoms on fruit are characterized by small, sunken, water-soaked, circular or angular lesions with a translucent, light brown margin. As the infection progresses within these sunken, circular lesions, orange to pink, circular spore masses develop and often appear in concentric rings (Siddiqui and Ali, 2014).

This research aimed to evaluate the potential of Tacca leaf extract as a natural antibacterial agent for preserving fresh mangoes. The study had focus on analysing the influence of leaf maturity on the concentration of bioactive compounds (flavonoids, tannins, alkaloids, saponins) in Tacca leaves; Identifying the optimal solvent and extraction conditions to maximize the yield of bioactive compounds from Tacca leaves; Isolating and identifying the

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microorganisms responsible for spoilage on fresh mango skins; and evaluating the antibacterial activity of Tacca leaf extracts against the identified spoilage microorganisms.

MATERIALS & METHODS

Materials

Fresh Tacca leaves were harvested from Tịinh Bien district of An Giang province. Hoa Loc mangoes were purchased from local fruit market of An Giang province. After harvesting, the produce was individually wrapped in paper to minimize moisture loss and immediately transported to the laboratory for processing.

Methods

Flavonoid Concentration: The flavonoid was determined using aluminium chloride colorimetric method with minor modification. Pipette 1mL of the extract filled into a test tube, then 3mL of ethanol, 0.2mL of 10% aluminum chloride solution, 0.2mL of 1 M sodium acetate solution, and 5.8mL of distilled water were added. Incubate the mixture at room temperature for 30min was done, and the absorbance of the reaction mixture at 415nm was measured. Then, a quercetin standard curve by making quercetin solutions at concentrations of 0, 16, 32, 64 to 128 and 320mg/L in ethanol was prepared. This method is described by El-Sayed et al. (2017).

Tannin Concentration: 0.5mL of the extract and 0.5mL of distilled water transferred into a test tube. Subsequently, 0.5mL of Folin-Denis reagent and 2mL of 20% Na2CO3 solution were add. Then mixed well and heated in a boiling water bath for 60s, and allowed to cool (room temperature).

The absorbance at a wavelength of 700nm was measured. Tannin content is calculated based on a tannic acid standard curve. Construct a tannic acid standard curve was at concentrations ranging from 0, 16, 32, 64 to 128mg/L (Laitonjam et al., 2013).

Saponin Concentration: 100mL of the extraction solution into a beaker containing 20mL of 40% MgCO3 solution was transferred. The resulting mixture is then filtered through Whatman No. 1 filter paper used to obtain a clear and colourless solution. 1mL of the filtrate taken and transferred it into a 50mL volumetric flask. 2mL of 5% iron (III) chloride (FeCl3) solution added and make up to the mark with distilled water. Kept to stand for 30min for color development. The absorbance was read against a blank at 380nm. The saponin concentration is calculated based on the following equations:

- The concentration of standard saponin (mgSE/L) y = (200*OD)/0.389.
- The saponin content Saponin (mgSE/g material) = ((OD*200)/0.389)/V/W

Where, OD is the absorbance of the sample at 380nm; V is the volume of the extraction solution (L) and W is the weight of the sample (g) (Adewole et al., 2013)

Colorimeter: The color of the product was determined using a Minolta Spectro Colorimeter. Measurements were taken at three different locations on each sample. Color values were expressed in terms of L, a, and b values.

L value indicates lightness: L = 0 (black); L = 100 (white).

Positive a value indicates red color, while negative a value indicates green color.

Positive b value indicates yellow color, while negative b value indicates blue color.

Microbial Isolation and Identification

Bacterial Isolation on the Mango Skin: Mango which its skin exhibiting circular, brown or dark brown spots was selected as samples (Siddiqui and Ali, 2014). The spoiled surface point was located and submerged in 1mL of sterile distilled water for 30min. The sample solution was spread on nutrient agar (NA) medium, incubated at 28°C, and monitored for 2 to 5 days. The emerged colony morphology, size, and color were recorded, and cell morphology and Gram staining properties were assessed (Abussaud et al., 2013). Bacterial strains then were identified by PCR and 16S rRNA gene sequencing with primer pair 27F:5'AGAGTTTGATCMTGGCTCAG-3' and 1492R:5'-ACGGYTACCTTGTTACGACTT-3' (Weisburg et al., 1991). Bacterial identification was performed according to Sanger and Coulson (1975) method. PCR products will be sent for sequencing at Loci Institute for Molecular Biology Research, HCM city.

Fungal Isolation on the Mango Skin: Mango skin exhibiting circular, brown or dark brown spots was selected as samples (Siddiqui and Ali, 2014). The spoiled surface point was located and submerged in 1mL of sterile distilled water for 30min. The sample solution was spread on Potato Dextrose Agar (PDA) medium, incubated at 28°C, and monitored for 2 to 5 days for the emergence of fungal colonies. Suspected colonies of *Colletotrichum gloeosporioides* (white) and *A. niger* (black or dark coffee brown) were noted. Spores were then picked and point-inoculated onto PDA medium, followed by incubation at 28±1°C for 2-5 days.

After isolation, morphological characteristics were observed and described, including colony morphology, size, color, as well as microscopic features such as hyphal structure, asexual reproductive structures, and the shape of spore-bearing structures. Mango fruits with signs of anthracnose, such as small, light brown spots or large, dark brown spots (Jeevanantham et al., 2024), these signs were used for the isolation of *Colletotrichum spp*.

Fungi were isolated according to the modified and supplemented procedure of Araújo et al. (2001). Mango fruits were surface sterilized with 70° alcohol. Cut diseased samples (choose the location, including both diseased and healthy tissues) to a size of 5x5mm, put them in 2% Javel, leave for 2–3min, wash the samples with sterile distilled water 3 times (1 minute each time), and place the samples on sterile paper to dry. Then, place the sample on PDA agar (Potato Dextrose Agar, potato extract (200g/L water), 20g/L glucose, and 20g agar/L, pH 7.0), and store the sample at 25-30°C. Colletotrichum spp. were preliminarily identified based on the morphological characteristics of mycelium and spores according to Sutton (1980).

Mango fruit was cut into small pieces (10g sample) placed in 250mL Erlenmeyer flasks, diluted to 100mL with

double distilled water, and then serially diluted tenfold. The medium used for fungal isolation was Czapek-Dox agar (Iqbal and Utara, 2015). After 96 h of incubation, the plates were visually inspected for Aspergillus-like mycelia, then aseptically harvested and transferred to Czapek-Dox Agar plates. The plates were then inoculated for up to 96 h before further examination for culture consistency and contamination. After isolation, fungal samples were examined by naked eye and microscopic observation for uniformity of color and surface of fungal colonies and shape of mycelium (Klich, 2002). The plates were incubated at 28 °C for 7 days, and conidia production was determined microscopically with cotton blue staining with lactophenol (Živković et al., 2018).

The isolated fungal strains were identified by molecular biology techniques (PCR) and gene sequencing with primer pairs ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ (White et al., 1990). PCR products were sent for sequencing at the Loci Institute for Molecular Biology Research, Ho Chi Minh City.

Antibacterial activity was determined using the agar well diffusion method (Pham and Pham, 2013). The diameter of the inhibition zone was calculated using the following formula:

DIZ (cm) = D – d. Where:

DIZ: Diameter of the inhibition zone (cm)

D: Diameter of the bacterial growth inhibition zone, including the diameter of the well (cm)

d: Diameter of the paper disc (d = 0.6cm)

The antifungal activity of the extract was determined using the agar disc diffusion method (Klich, 2002; Adjou et al., 2012; Le et al., 2020). The percentage of mycelial growth inhibition by the essential oil was calculated using the following formula:

Mycelial growth inhibition (%) = [(dc - dt) / dc] x 100

Where:

dc: diameter of the control colony

dt: diameter of the colony in the sample containing the extract.

Statistical Analysis

The experiments were conducted in a completely randomized design with the specified factors and three replications. Optimal parameters from the previous experiment were used as the basis for subsequent experiments. The collected analytical and evaluation data were processed using Microsoft Excel and statistically analysed using Statgraphics Centurion XV software.

RESULTS & DISCUSSION

Tacca Leaves Components

The colour values of Tacca leaves at different stages of development, from young to mature, are represented by L*****, a*****, and b***** indices and presented in Table 1.

The results indicate a statistically significant difference in leaf color across growth stages for all three L*, a*, and b* values. Specifically, mature leaves exhibited the highest lightness (44.80±2.83), while senescent leaves displayed the lowest (37.79±3.02), as reflected in the L* values. Similarly, the a* values of mature leaves were the lowest (-15.56±2.17), indicating the most intense green color, whereas the b* values of senescent leaves were the highest, corresponding to the most intense yellow color. This change is attributed to the growth process, during which chlorophyll in senescent leaves is degraded, resulting in a lower color intensity compared to the other two leaf samples.

Table 1: Colorimetric of tacca leaves at different matured stage

Leave	Color		
	L*	a*	b*
Young leaves	41.23±3.19ab	-10.18±2.13a	20.91±2.82ab
Matured leaves	44.80±2.83a	-15.56±2.17b	17.31±2.23c
Old leaves	37.79±3.02bc	-11.18±1.71ab	25.54±2.84a
P	0.0777	0.0370	0.0250

Values (mean±SD) with different letters in a column indicate significant (P≤0.05).

The results indicated that the moisture content of tacca leaves varied between 79.75% and 86.07% across different growth stages. Specifically, young leaves exhibited the highest moisture content, while mature leaves displayed the lowest. This observation can be attributed to the decrease in moisture content as tacca leaves progress through their growth stages, with moisture loss occurring after the leaves have fully developed. This result is similar to the study of Nguyen et al., (2022) on Sauropus and rogynous leaves with moisture content ranging from 69.9-89.9%.

In addition to color values and moisture content, bioactive compounds are also crucial due to the numerous benefits associated with the bioactive compounds present in tacca leaves. However, because tacca leaves are often considered a byproduct, there is limited research available on them. Statistical analysis results indicate that tacca leaves at different growth stages significantly influence the bioactive compound content (flavonoids, tannins, alkaloids, and saponins). The results of the bioactive compound analysis are presented in Table 2.

Table 2: Bioactive compounds of tacca leaves at different matured stages

Samples	Bioactive compounds			
	Flavonoid (mgQE/100g)	Tannin (mgTAE/100g)	Alkaloid (mgCE/100g)	Saponin (mgSE/100g)
Young leaves	2.85±0.06b	2.05±0.04c	0.11±0.07c	12.07±0.04c
Matured leaves	3.51±0.05a	2.45±0.02a	0.12±0.05b	12.56±0.05a
Old leaves	2.82±0.08c	2.26±0.04b	0.13±0.09a	12.51±0.01b
P	0.0000	0.0000	0.0000	0.0000

Values (mean±SD) with different letters in a column indicate significant (P≤0.05).

Statistical analysis revealed that the developmental stages of Tacca leaves significantly influenced the levels of bioactive compounds, including flavonoids, tannins, alkaloids, and saponins, at a 5% significance level. Specifically, the content of flavonoids, tannins, and saponins tended to increase from young leaves to mature leaves but showed a decreasing trend from mature leaves to old leaves, ranging from 3.51 to 2.82 (mgQE/100g), 2.45 to 2.26 (mgTAE/100g), and 12.56 to 12.51 (mgSE/100g), respectively. In contrast, the alkaloid content exhibited a gradual increasing trend from young leaves to mature leaves and then to old leaves, ranging from 0.11 to 0.13 (mgCE/100g). This observation might be attributed to the presence of different types of alkaloids at various

developmental stages of Tacca leaves. Ndouyang and Abdelsalam (2024) discovered that alkaloids and phenolic are present in large amount in the tacca leaves. However, almost all leaf alkaloids differed across different growth stages. Therefore, the moisture content and color values of tacca leaves vary depending on the leaf's developmental stage. Based on the obtained results, mature leaves, which exhibited high levels of flavonoids (3.51±0.05), tannins (2.45±0.02), and saponins (12.56±0.05), were selected for further investigation.

The Effects of Solvents and Extraction Ratio on the Compositions of the Tacca Leaf Extract

The analysis of results (Table 3) indicated that the n-butanol extract of Tacca leaves exhibited the highest content of bioactive compounds (flavonoids and alkaloids) compared to the other extracts. Additionally, the water extract of tacca leaves yielded higher levels of bioactive compounds (saponins and tannins) than the other solvents at a 1/50 ratio. Specifically, with diethyl ether as the solvent, the flavonoid and alkaloid contents reached 8.57mgQE/100g and 0.5mgCE/100g, respectively. However, when the solvent was changed to n-butanol, the highest values of 12.58mgQE/100g and 0.8mgCE/100g were achieved.

Table 3: Flavonoid content (mgQE/100g) in different solvents

Solvents	Ratio leaves /solvent (w/w)				Average	P
	1/20	1/30	1/40	1/50		
Diethyl ether	2.50±0.15	7.48±0.08	8.48±0.02	15.81±0.01	8.57^{b}	0.0000
Ethyl acetate	3.09±0.08	4.25±0.12	4.68±0.23	4.17±0.32	4.05^{c}	
N – butanol	1.35±0.06	5.57±0.09	6.63±0.07	36.77±0.04	12.58^{a}	
Water	1.29±0.01	2.18±0.10	2.77±0.04	2.48±0.01	2.18^{d}	
Average	2.05^{d}	4.87^{c}	5.64^{b}	14.81^{a}	-	
P	0.0000					

Mean±SD values (n=3) are presented; Treatments with different letters following them within the same column or row indicate significant differences according to the LSD test at a significance level of P≤0.05.

Research by Nguyen et al. (2023) demonstrated that the flavonoid and phenolic content in betel leaf extracts decreased with the use of ethanol, hexane, and ethyl acetate solvents, in that order. In contrast, the tannin (Table 4) and saponin (Table 5) contents increased with the change in solvent. Specifically, with diethyl ether as the solvent, the tannin and saponin content reached 0.93mgTAE/100g and 31.30mgSE/100g, respectively. When the solvent was changed to water, the tannin and saponin content reached their highest values of 1.11mgTAE/100g and 36.02mgSE/100g, respectively. For saponins, n-butanol has the ability to dissolve many components in the raw material; however, its ability to dissolve saponins is less than that of water, as water is the best extraction solvent. This result is consistent with the report by Nguyen et al. (2020), which suggests that due to the high polarity of saponin molecules, they dissolve well in strong polar solvents. As for tannins, the use of water as the extraction solvent may limit the contact of tannins with oxygen, thereby preventing their degradation.

The choice of solvent and the material-to-solvent ratio are crucial factors that not only affect the extraction efficiency but also influence subsequent research. During the extraction process, the raw material dissolves into the solvent, leading to the dissolution of bioactive compounds as well. The dissolution of bioactive compounds into the solvent is a physical process. Increasing the amount of solvent provides more opportunities for bioactive compounds to come into contact with the solvent, resulting in higher permeability (Wong et al., 2024). When the material-to-solvent ratio increases, the concentration difference between the solvent and the solutes becomes larger. Therefore, more bioactive compounds can dissolve if a larger amount of solvent is used (Cacace and Mazza, 2003). Another research by Syafi et al. (2023) used 95% ethanol solution to soak the tacca leaves harvested in the North Maluku region (Indonesia) for 72 hours, then extracted the leaves at 70 °C, the results showed a number of alkaloid compounds around 2,496.35mg/kg and saponins (4,203.32mg/kg), followed by flavonoids (7.20), tannins (12.74) and phenol (4.08). These bioactive compounds had quite high results, but the soaking time of 72 hours was quite long, and not convenient for the production process.

Table 4: Tannin (mgTAE/100g) in different solvents

Solvents	Ratio leaves /solvent (w/w)				Average	P
	1/20	1/30	1/40	1/50		
Diethyl ether	0.52±0.04	0.72±0.01	0.90±0.01	1.57±0.09	0.93^{c}	0.0000
Ethyl acetate	0.12±0.03	0.47±0.13	0.91±0.03	1.19±0.18	0.67^{d}	
N – butanol	0.36±0.03	0.61±0.04	0.86±0.13	2.00±0.17	0.96^{b}	
Water	0.72±0.02	1.64±0.13	1.35±0.14	0.71±0.22	1.11^{a}	
Average	0.43^{d}	0.86^{c}	1.01^{b}	1.37^{a}	-	
P	0.0000					

Mean±SD values (n=3) are presented; Treatments with different letters following them within the same column or row indicate significant differences according to the LSD test at a significance level of P≤0.05.

Table 5: Saponin content (mgSE/100g) in different solvents

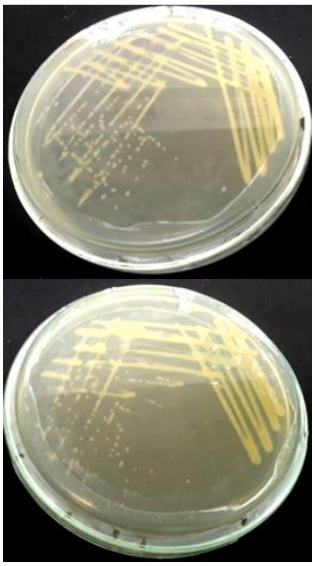
Solvents	Ratio leaves /solvent (w/w)				Average	P
	1/20	1/30	1/40	1/50		
Diethyl ether	46.38±0.19	37.45±0.09	22.06±0.05	19.30±0.03	31.30^{d}	0.0000
Ethyl acetate	50.15±0.02	31.97±0.07	24.46±0.06	21±0.03	31.89^{c}	
N – butanol	44.38±0.10	40.71±0.02	30.47±0.04	24.2±0.01	34.94^{b}	
Water	56.85±0.02	36.05±0.07	28.61±0.01	22.58±0.07	36.02^{a}	
Average	49.44^{a}	36.54^{b}	26.40^{c}	21.77^{d}		
P	0.0000					

Mean±SD values (n=3) are presented; Treatments with different letters following them within the same column or row indicate significant differences according to the LSD test at a significance level of P≤0.05.

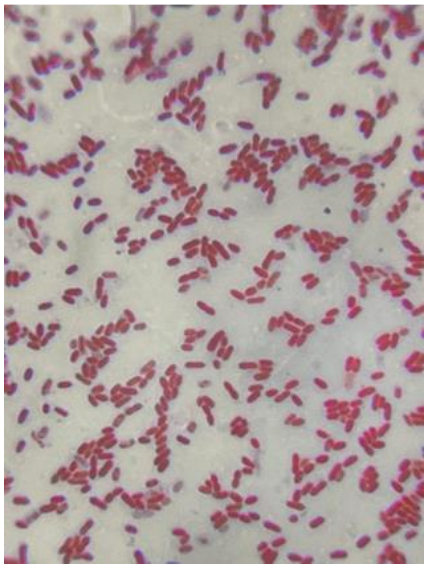
In conclusion, the extraction of tacca leaves with different solvents affects the bioactive compound content. The results show that the n-butanol extract of tacca leaves has high levels of flavonoids (12.58) and alkaloids (0.8), while the water extract has high levels of saponins (36.02) and tannins (1.11). Therefore, these two extracts were selected for further research.

Bacterial Isolation on the Mango Skin Dark Spots

Bacterial colonies present on the blackened spot of mango skin have the following characteristics: a size of 3mm, yellow coloration, smooth margins, circular shape, create mucoid substance. The bacterial cells were rod-shaped, with dimensions of 0.3-0.7x0.8-2.1µm (Fig. 1). They were catalase-positive (effervescence observed compared to the bacteria-free control), Gram-negative, and non-motile. The results of bacterial identification revealed that the identified bacterium exhibited a 99.86% sequence similarity to *Pantoea stewartia* bacterial (Fig. 2), this agree with the research of Ha et al. (2024) on Thai's jackfruit.

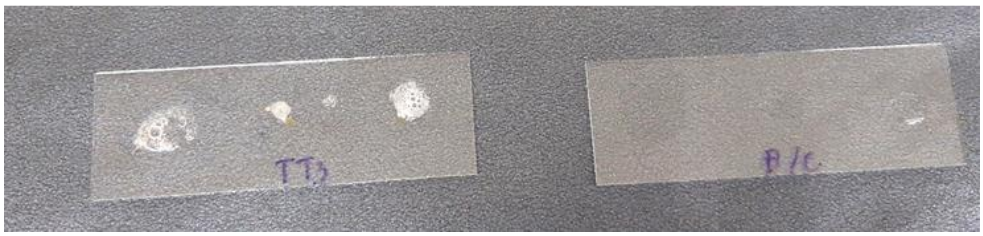


Colonies



Gram stain (negative)

Fig. 1: Identified bacterial characteristic (*Pantoea stewartia*); The identification results confirmed that the identified bacteria on the dark spots of mango skin is *Pantoea stewartii*. This bacterium is commonly associated with jackfruit bronzing disease and bacterial leaf blight in corn. *Pantoea steartii subsp. steartii* is a Gram-negative, non-motile, non-spore-forming bacterium belonging to the family *Erwiniaceae* (Kini et al., 2021).



Catalase-positive (*The identified bacterial on the left and control sample on the right)*

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pantoea stewartii strain DZA1-4 16S ribosomal RNA gene, partial sequence	Pantoea stewartii	2564	2564	100%	0.0	99.86%	1407	ON203057.1
Uncultured Pantoea sp. clone p1 16S ribosomal RNA gene, partial sequence	uncultured Pant...	2560	2560	99%	0.0	99.86%	1548	MT106843.1
Pantoea stewartii strain SC3430KR 16S ribosomal RNA gene, partial sequence	Pantoea stewartii	2560	2560	99%	0.0	99.86%	1549	MG517424.1
Pantoea sp. strain RPST-1 16S ribosomal RNA gene, partial sequence	Pantoea sp.	2560	2560	99%	0.0	99.86%	1422	KY818305.1
Pantoea stewartii subsp. stewartii DC283 chromosome, complete genome	Pantoea stewart...	2556	17857	100%	0.0	99.78%	4528215	CP017581.1
Pantoea stewartii strain HR3-48 chromosome, complete genome	Pantoea stewartii	2556	17781	100%	0.0	99.78%	4439845	CP099540.1

Fig. 2: Bacterial sequences result (tested by Loci Institute for Molecular Biology Research, HCM city).

Antibacterial Activity of Tacca Leaf Extract against *Pantoea stewartii* Bacteria

The antibacterial activity of the tacca leaf extract against *Pantoea stewartii* was assessed through the formation of a clear zone around the wells containing different concentrations of the extract on LB agar plates inoculated with the pathogenic *Pantoea stewartii*. The formation of the clear zone was observed within 12-48 hours after inoculation. The *Pantoea stewartii* strain grew without inhibition and did not form a zone of inhibition around the well containing the negative control, 5% DMSO. In contrast, the antibiotic Imipenem at 10mg/mL exhibited the highest growth inhibition against the bacteria, with a zone of inhibition diameter of 2cm.

Tacca leaf extracts prepared with diethyl ether and water did not form any zones of inhibition, indicating that these extracts did not affect the growth of *Pantoea stewartii*. Extracts prepared with ethyl acetate and N-butanol demonstrated antibacterial activity against *Pantoea stewartii*, with statistically significant differences compared to the other two extracts. The N-butanol extract exhibited the highest antibacterial activity (zone of inhibition: 0.67cm (Fig. 3)), followed by the ethyl acetate extract (zone of inhibition: 0.05cm), and all differences were statistically significant at the 5% level. Statistical analysis revealed that increasing the extract concentration led to a larger zone of inhibition against *Pantoea stewartii*, and these differences were statistically significant ($P<0.05$).

According to Faikoh et al. (2014) protocol, the antibacterial activity of an extract can be classified into four levels: D≥15mm: strong antagonism, 15mm>D≥ 7.5mm: moderate antagonism, D<7.5mm: weak antagonism, D=0mm: no antagonism. Where D represents the diameter of the zone of inhibition formed around the paper discs (excluding the diameter of the paper disc itself). The results obtained indicate that the tacca leaf extract exhibits moderate antibacterial activity against *Pantoea stewartii*.

The determination of the minimum inhibitory concentration (MIC) of the tacca leaf extract against *Pantoea stewartii* revealed that the N-butanol extract had the best antibacterial activity at a concentration of 80%. At this concentration, bacterial growth was completely inhibited.

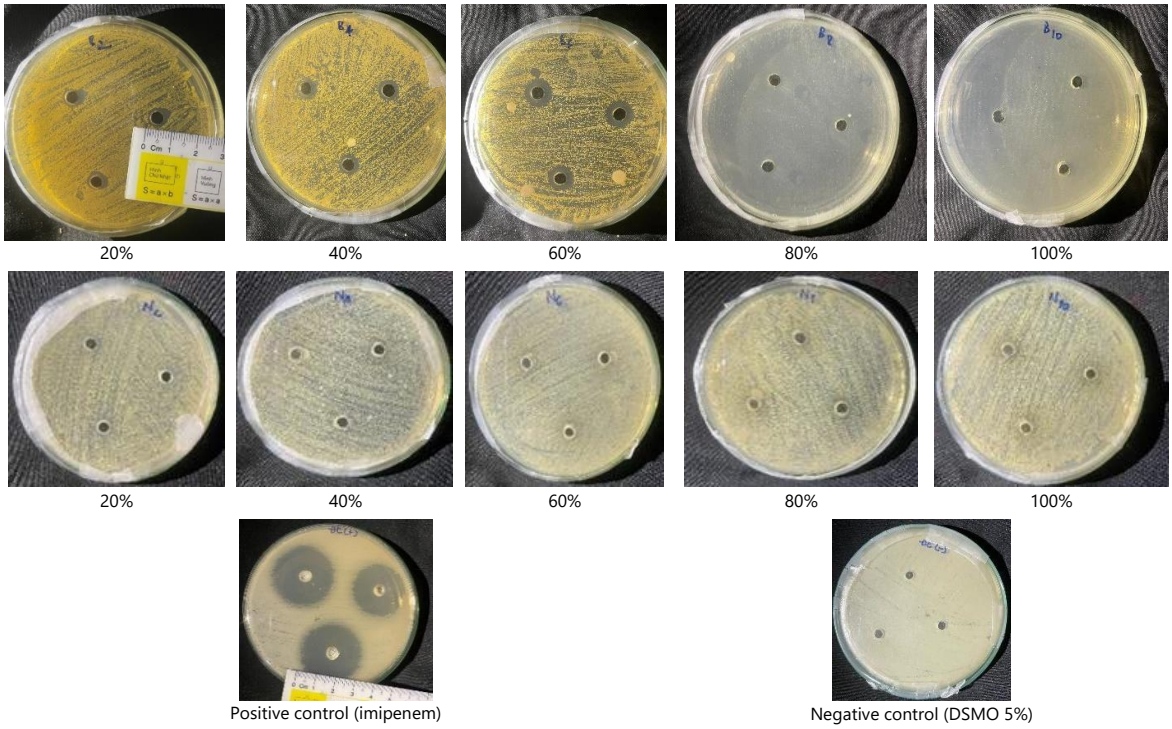


Fig. 3: The antibacterial activity of tacca leaf extract with N-butanol (top), water (middle) solvents and control samples (bottom) to *Pantoea stewartii* at different treatment concentrations.

Natural compounds such as alkaloids and flavonoids possess various biological activities, particularly antibacterial properties (Kumar et al., 2011; Courts and Williamson, 2015; Mishra, 2024). Although the antibacterial activity of the tacca leaf extract is significantly lower than that of the commercially available antibiotic Imipenem, the presence of bioactive compounds (flavonoids, tannins, alkaloids, and saponins) in the extract suggests its potential use as a safe, natural antibiotic.

The antimicrobial activity of essential oils is due to their effects on the structure and function of cells. In fact, the antimicrobial activity of essential oils includes the degradation of cell walls, phospholipid bilayers (Gill and Holley, 2006), and the destruction of membranes and membrane components, which are important components in cells. Alkaloids have many different physical and biological properties and can be found in many pharmacological methods (Yu, 2022) which are the basis for promoting the production of many antibiotics with potent activity (Othman, 2019). Besides, many flavonoids have antibacterial activity against many pathogens, which can be effectively applied against pathogens (Pandey and Kumar, 2013).

Fungal Isolation on the Mango Skin Dark Spots

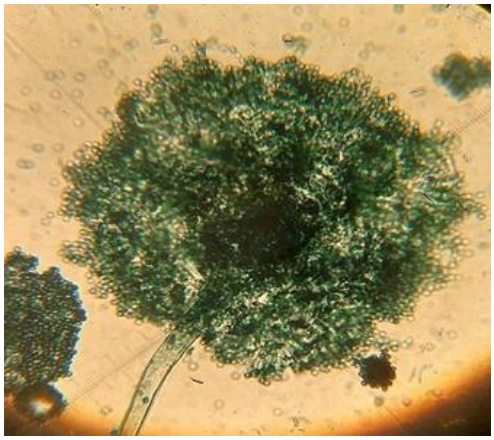
The mango skin sample with disease symptoms was analyse to isolate fungal specie. The morphology of identified mold was obtained: moderate growth rate, 5-day-old colonies with a diameter of 3.8-4.5cm, powdery and granular in appearance, concentric rings, convex colony center, obverse side black with a brownish tint, opaque white margin, white mycelia, black spores, white mycelial fringe at the margin (Fig. 4). Reproductive structures: numerous conidial heads arising from the substrate, spherical, radiate, black in color, with spherical vesicles and phialides biseriate. Conidia globose, black, with a roughened surface, measuring approximately 2.5-4.5µm. These findings are consistent with the preliminary description of *Aspergillus Niger* by Raper and Fennell (1965) and Buah et al. (2024). The identification results (Fig. 5) indicated that identified fungus exhibited 100% sequence similarity to *Aspergillus Niger*.

Antifungal Activity of Tacca Leaf Extract against *Aspergillus Niger*

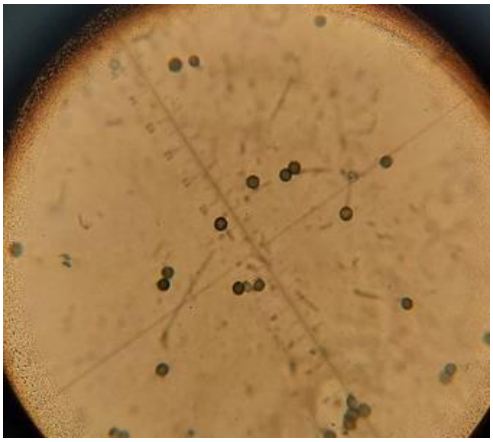
The findings on the antifungal activity against *Aspergillus niger* indicate: Aqueous extracts of tacca leaves and all extracts at a 0% concentration did not produce any zones of inhibition, suggesting no antifungal activity against *Aspergillus niger*. Extracts using Ethyl acetate and Diethyl ether as solvents showed antifungal activity against *Aspergillus niger* at a 100% concentration, with an inhibition zone of 0.23cm. Extracts at concentrations of 0-20-40-60-80% with ethyl acetate and Diethyl ether did not exhibit antifungal activity. Extracts using N-butanol as a solvent demonstrated strong inhibition zones, with 0.8cm at a 40% concentration and 0.3cm at a 20% concentration (Fig. 6). The antifungal activity increased with increasing extract concentration. At concentrations of 60, 80, and 100%, *Aspergillus niger* mycelial growth was completely inhibited, suggesting that the minimum inhibitory concentration (MIC) of the tacca leaf extract with N-butanol as a solvent is 60%. Present data agreed with early reports about antimicrobial activity of alkaloids of plant origin (El Kamari et al., 2024; Schneider et al., 2024; Hendel et al., 2024). They can inhibit the growth of fungi, bacteria and viruses through various mechanisms (Mabhiza et al., 2016).

Top and bottom surface of identified fungus

Fig. 4: Identified fungal characteristic (*Aspergillus niger*); The identification results confirmed that identified fungus is *Aspergillus Niger*. *Aspergillus Niger* is commonly associated with spoilage in fruits and food products. Mold and fungi cause decay in agricultural products, especially fruits with typical examples including *Aspergillus spp., Penicillium spp., and Monilinia spp* (Peng et al., 2025; Luciano-Rosario et al., 2025; Li et al., 2025).



Microscopic examination of the fungi



Microscopic examination of the fungi

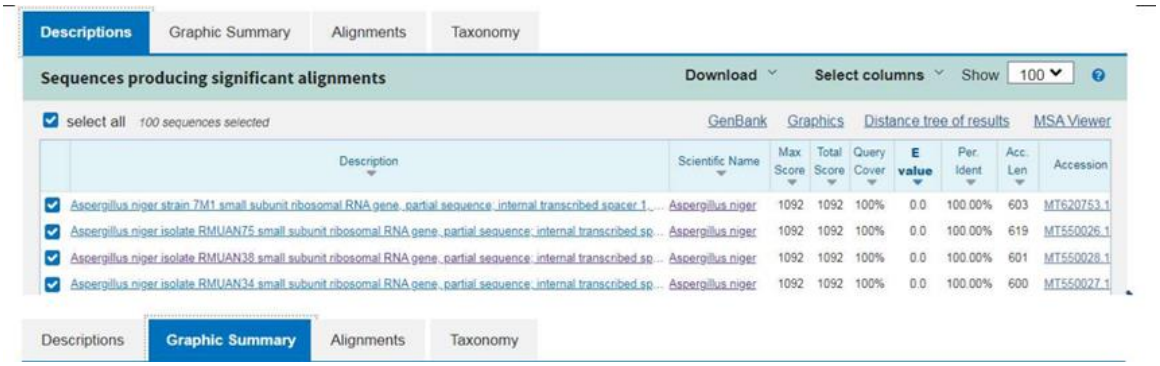
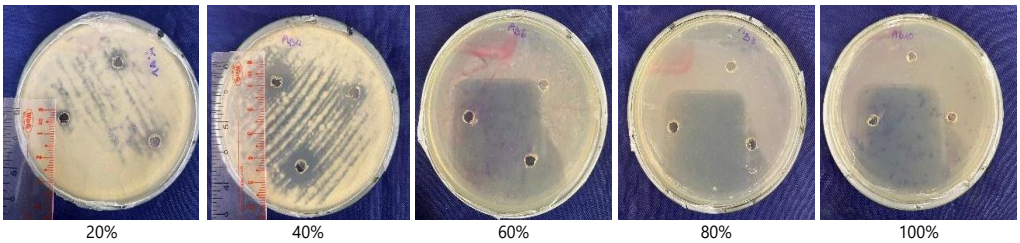
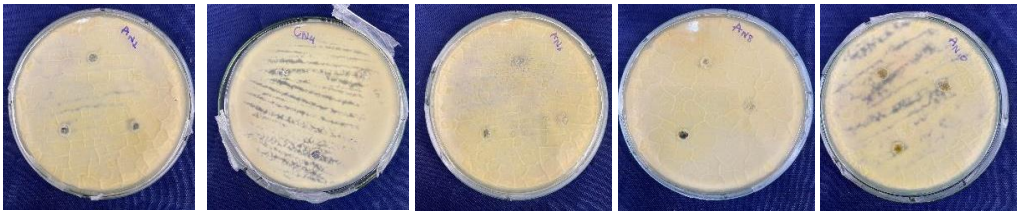


Fig. 5: Fungal sequences result (tested by Loci Institute for Molecular Biology Research, HCM city).



20% 40% 60% 80% 100%



20% 40% 60% 80% 100%

Fig. 6: The antibacterial activity of tacca leaf extract with N-butanol (top), water (middle) solvents to *Aspergillus Niger* at different treatment concentration.

Conclusion

Based on the results obtained throughout this research, it can be concluded that: Mature leaves exhibit higher levels of these compounds compared to the other two leaf samples: flavonoids at 3.514mgQE/100g; tannins at 2.54mgTAE/100g; alkaloids at 0.121mgCE/100g, and saponins at 12.56mgSE/100g. Extracts from tacca leaves contain bioactive compounds (flavonoids, tannins, alkaloids, and saponins). The results indicated that n-butanol extracts possess higher levels of flavonoids and alkaloids compared to other solvents, with a 1/50 ratio demonstrating superior extraction efficiency. Additionally, water extracts contain higher levels of tannins and saponins, when compared to other solvents. The determination of the minimum inhibitory concentration (MIC) of tacca leaf extracts against the bacterium *Pantoea stewartii* revealed that the n-butanol extract exhibited the best antibacterial activity at an 80% concentration, with an inhibition zone diameter of 0.67cm at a 60% concentration. The n-butanol extract demonstrated strong antifungal activity against *Aspergillus niger* and with inhibition zones of 0.57cm at 40% concentration. At concentrations of 60, 80, and 100%, *Aspergillus niger* growth was completely inhibited. These results suggest that the MIC value of the n-butanol extract of hoary tacca leaves against the two tested fungal strains is 60%.

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REFERENCES

Abussaud, M.J., Alanagreh, L., and Abu-Elteen, K. (2013). Isolation, characterization and antimicrobial activity of *Streptomyces* strains from hot spring areas in the northern part of Jordan. *African Journal of Biotechnology*, 2, 7124-7132.

Adewole, E., Ajiboye, B., Idris, O., Ojo, O., Onikan, A., Ogunmodede, O., and Adewumi, D.J.P. (2013). Phytochemical, Antimicrobial and Gc-Ms of African Nutmeg *(Monodora Myristica). International Journal of Pharmaceutical Science Invention*, 2, 25-32.

Adjou, E.S., Sandrine, K., Edwige, D.A., Sohounhloue, D.C., and Soumanou, M.M. (2012). Antifungal activity of Ocimum canum essential oil against toxinogenic fungi isolated from peanut seeds in post-harvest in Benin. *International Research Journal of Biological Sciences*, 1, 20-26.

Araújo, W.L., Maccheroni, W., Aguilar-Vildoso, Jr.C.I., Barroso, P.A., Saridakis, H.O., and Azevedo, J.L. (2001). Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Canadian Journal of Microbiology*, 47, 229-236. https://doi.org/10.1139/w00-146

Asrey, R., and Das, A. (2021). Effect of pre-harvest practices on postharvest quality and diseases of fruits. In postharvest handling and diseases of horticultural produce, CRC Press, pp. 25-32. https://doi.org/10.1201/9781003045502

Borokini, T.I. (2011). Invasive alien plant species in Nigeria and their effects on biodiversity conservation. *Tropical Conservation Science*, 4(1), 103-110. https://doi.org/10.1177/194008291100400110

Buah, J.D., Eberemu, N.C., & Okeseni, O. (2024). Identification of fungal species associated with the spoilage of fruit in dutsin-ma metropolis, katsina state. *Fudma Journal of Sciences*, *8*(1), 6-10. https://doi.org/10.33003/fjs-2024-0801-2245

Cacace, J. and Mazza, G. (2003). Optimization of Extraction of Anthocyanins from Black Currants with Aqueous Ethanol. *Journal of Food Science*, 68, 240-248. https://doi.org/10.1111/j.1365-2621.2003.tb14146.x

Courts, F.L., and Williamson, G. (2015). The occurrence, fate and biological activities of C-glycosyl flavonoids in the human diet. *Critical Reviews in Food Science and Nutrition*, 55(10), 1352-1367. https://doi.org/10.1080/10408398.2012.694497

Drenth, E. (1972). A revision of the family Taccaceae. *Blumea: Biodiversity, Evolution and Biogeography of Plants*, *20*(2), 367-406.

El Kamari, F., El Omari, H., El-Mouhdi, K., Chlouchi, A., Harmouzi, A., Lhilali, I., El Amrani, J., Zahouani, C., Hajji, Z., & Ousaaid, D. (2024). Effects of Different Solvents on the Total Phenol Content, Total Flavonoid Content, Antioxidant, and Antifungal Activities of Micromeria graeca L. from Middle Atlas of Morocco. *Biochemistry Research International*, 2024(1), 9027997. https://doi.org/10.1155/2024/9027997

El-Sayed, M.A., Al-Gendy, A.A., Hamdan, D.I., and El-Shazly, A.M. (2017). Phytoconstituents, LC-ESI-MS profile, antioxidant and antimicrobial activities of *Citrus x limon L. Burm. f.* cultivar variegated pink lemon. *Journal of Pharmaceutical Sciences Research*, 9(4), 375.

Faikoh, E.N., Hong, Y.H., and Hu, S.Y. (2014). Liposome-encapsulated cinnamaldehyde enhances zebrafish (Danio rerio) immunity and survival when challenged with *Vibrio vulnificus* and *Streptococcus agalactiae*. *Fish and Shellfish Immunology*, 38(1), 15-24. https://doi.org/10.1016/j.fsi.2014.02.024m

Gill, A.O. and Holley, A.R. (2006). Discruption of *E. coli, Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Journal of Food Microbiology*, 108(1), 1-9. https://doi.org/10.1016/j.ijfoodmicro.2005.10.009

Ha, V.T., Hoang, L.K., & Huyen, P.K. (2024). Pantoea stewartii subsp. stewartii, the causative agent of Thai jackfruit's bronzing disease and its possible host range in Vietnam. *Journal of Plant Protection Research*, 64(2), 149-157. https://doi.org/10.24425/jppr.2024.150249

Hendel, N., Sarri, D., Sarri, M., Napoli, E., Palumbo Piccionello, A., & Ruberto, G. (2024). Phytochemical Analysis and Antioxidant and Antifungal Activities of Powders, Methanol Extracts, and Essential Oils from Rosmarinus officinalis L. and Thymus ciliatus Desf. Benth. *International Journal of Molecular Sciences*, 25(14), 7989. https://doi.org/10.3390/ijms25147989

Iqbal, J., and Utara, U. (2015). Isolation of *Aspergillus niger* strains from soil and their screening and optimization for enhanced citric acid production using cane molasses as carbon source. *Journal of Applied Environmental and Biological Sciences*, 5(4), 128-137.

Jeevanantham, S., Praveen, A., Livitha, R., & Balamurugan, K. (2024). Post Harvest Anthracnose of Mango Caused by Colletotrichum gloeosporioides: A Review. *Archives of Current Research International*, 24(2), 106-115. https://doi.org/10.9734/acri/2024/v24i2637

Kini, K., Agnimonhan, R., Dossa, R., Silué, D., and Koebnik, R. (2021). Genomics-informed multiplex PCR scheme for rapid identification of rice-associated bacteria of the genus Pantoea. *Plant Disease*, 105(9), 2389-2394. https://doi.org/10.1094/PDIS-07-20-1474-RE

Klich, M.A. (2002). Biogeography of *Aspergillus* species in soil and litter. *Mycologia,* 94, 21-27. https://doi.org/10.2307/3761842

Klich, M.A. (2002). Identification of common *Aspergillus* species. Centraal bureau voor Schim, Utrecht.

Kumar, S., Narwal, S., Kumar, V., and Prakash, O. (2011). [alpha]-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews*, 5, 19-29. https://doi.org/10.4103/0973-7847.79096

Laitonjam, W.S., Yumnam, R., Asem, S.D., and Wangkheirakpam, S.D. (2013). Evaluative and comparative study of biochemical, trace elements and antioxidant activity of *Phlogacanthus pubinervius T. Anderson* and *Phlogacanthus jenkinsii CB Clarke* leaves. *Indian Journal of Natural Products and Resources,* 4(1), 67-72.

Le, A.D.N., Phu, T.M., Douny, C., Quetin-Leclercq, J., Hue, B.T.B., Bach, L.T., Quynh Nhu, T., Bui, T.B.H., Duong, T.T.H., and Thanh Phuong, N. (2020). Screening and comparative study of in vitro antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. *International Journal of Food Properties*, 23(1), 481-496. https://doi.org/10.1080/10942912.2020.1737541

Li, X., Liao, Q., Zeng, S., Wang, Y., & Liu, J. (2025). The use of Trichoderma species for the biocontrol of postharvest fungal decay in fruits and vegetables: Challenges and opportunities. *Postharvest Biology and Technology*, 219, 113236. https://doi.org/10.1016/j.postharvbio.2024.113236

Luciano-Rosario, D., Castro, J., Peter, K.A., Cox, K.D., Fonseca, J.M., Gaskins, V.L., & Jurick II, W.M. (2025). Mold in, mold out: Harvest bins harbor viable inoculum that can be reduced using novel sanitation methods

to manage blue mold decay of apples. *Postharvest Biology and Technology*, 221, 113323. https://doi.org/10.1016/j.postharvbio.2024.113323

Mabhiza, D., Chitemerere, T., and Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*, 2016, 6304163. https://doi.org/10.1155/2016/6304163

Mishra, M.K. (2024). Phytochemical analysis and evaluation of antibacterial activity of various extracts from leaves, stems and roots of Thalictrum foliolosum. *Future Journal of Pharmaceutical Sciences*, 10(1), 70. https://doi.org/10.1186/s43094-024-00643-z

Ndouyang, C.J., & Abdelsalam, T. (2024). Significance of Mineral Bioavailability in Food Containing Antinutrients, Tacca leontopetaloides L. (Taccaceae) versus Cochlospermum tinctorium A. Rich (Bixaceae). *European Journal of Medical and Health Research*, *2*(6), 32-38. https://doi.org/10.59324/ejmhr.2024.2(6).04

Nguyen, T.M.N., Nguyen, N.T., and Le, P.T.Q. (2022). Effects of storage conditions, preparation and heat treatment on some group B vitamins in *Sauropus androgynous*. *Current Research in Nutrition and Food Science*, 59, 685-697. http://dx.doi.org/10.12944/CRNFSJ.10.2.23

Nguyen, T.P., Nguyen, Q.D., and Nguyen, N.N. (2023). Comparison of the total phenolic content and antioxidant and antibacterial activities of different fractions obtained from selected plant leaves native to Vietnam. *Science & Technology Development Journal*, 26(4),1-8.

Nguyen, V.T., Tran, T.G., Huynh, Q.T., Phan, T.B.T, Pham, C.A., and Tran, T.M.H. (2020). Effects of solvents and extraction methods on the ability to extract phenolics, saponins and alkaloids from cocoa pods *(Theobroma cacao L.). Can Tho University Journal of Science*, 56(4), 71-78. https://doi.org/10.22144/ctu.jvn.2020.084

Omojola, M.O. (2013). Tacca starch: a review of its production, physicochemical properties, modification and industrial uses. *African Journal of Food, Agriculture, Nutrition and Development*, 13(4), 7972-7984. https://doi.org/10.18697/ajfand.59.12930

Othman, L., Sleiman, A., and Abdel-Massih, R.M. (2019). Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Frontiers in Microbiology*, 10, 911. https://doi.org/10.3389/fmicb.2019.00911

Pandey, A.K., and Kumar, S. (2013). Perspective on plant products as antimicrobials agents: a review. *Pharmacologia*, 4, 469-480.

Pham, H.V., and Pham, T.H.B. (2013). Antibiotics - Antibiotic resistance - Antibiogram techniques. Common basic problems. Medical Publiser, pp. 61-63.

Peng, S., Duan, C., Liu, Q., Wang, Q., Dai, Y., Hao, L., & Li, K. (2025). Biocontrol potential of Streptomyces sp. N2 against green and blue mold disease in postharvest navel orange and the action mechanism. *Food Microbiology*, 125, 104658. https://doi.org/10.1016/j.fm.2024.104658

Raper, K.B. and Fennell, D.I. (1965). The genus of Aspergillus. The Williams and Wilkins Co., Baltimore.

Sanger, F., and Coulson, A.R. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology*, 94(3), 441-448. https://doi.org/10.1016/0022-2836(75)90213-2

Schneider, C., González-Reyes, M., Vergara, C., Fuica-Carrasco, C., & Zapata, P. (2024). Antioxidant and Antifungal Activities and Characterization of Phenolic Compounds Using High-Performance Liquid Chromatography and Mass Spectrometry (HPLC-MS) in Empetrum rubrum Vahl ex Willd. *Plants*, 13(4), 497. https://doi.org/10.3390/plants13040497

Siddiqui, Y., and Ali, A. (2014). Colletotrichum gloeosporioides (Anthracnose). In Postharvest Decay. Academic Press, pp. 337-371. https://doi.org/10.1016/B978-0-12-411552-1.00011-9

Sutton, B.C., (1980). The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, pp. 696.

Syafi, S., Pujiasmanto, B., Purwanto, E., and Suryanti, V. (2023). Phytochemical Screening of Tacca Plant (Tacca leontopetaloides L.) Ethanol Extract Using Spectrophotometry UV-VIS Method. *International Journal on Advanced Science, Engineering and Information Technology*, *13*(4), 1316. https://doi.org/10.18517/ijaseit.13.4.18251

Vu, Q.T.H., Le, P.T.K., Vo, H.P.H., Nguyen, T.T., and Nguyen, T.K.M. (2017). Characteristics of Tacca leontopetaloides L. Kuntze collected from An Giang in Vietnam. In *AIP Conference Proceedings*, 1878, 020022. https://doi.org/10.1063/1.5000190

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991). 16S Ribosomal DNA Amplification for Phylogenetic Study. *Journal of Bacteriology*, 173, 2, 697-703. https://doi.org/10.1128/jb.173.2.697-703.1991

White, T.J., Bruns, T., Lee, S.J.W.T., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, 18, 1, 315-322.

Wong, Y.S., Yusoff, R., & Ngoh, G.C. (2024). Phenolic compounds extraction by assistive technologies and natural deep eutectic solvents. *Reviews in Chemical Engineering*, *40*(2), 229-246. https://doi.org/10.1515/revce-2022-0043

Yu, X. (2022). An innovative extraction strategy for herbal medicine by adopting psulphonatocalixarenes. *Phytochemical Analysis*, 33(7), 1068-1085. https://doi.org/10.4103/0973-7847.79096

Živković, S., Stevanović, M., Đurović, S., Ristić, D., and Stošić, S. (2018). Antifungal activity of chitosan against *Alternaria alternata* and *Colletotrichum gloeosporioides*. *Pesticides and Phytomedicine / Pesticidi i fitomedicina*, 33, 3-4.