BIOLOGICAL CHARACTERIZATION AND TOXICITY ANALYSIS OF ETHANOLIC BANANA (*Musa paradisiaca* L.) FLOWER EXTRACT AS ANTIMICROBIAL FOR CHERRY TOMATO

By

KHADIJA AHMED MOHAMED MOUSA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is wholeheartedly dedicated to my late father and my beloved mother and siblings, who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual and emotional support.

To my supervisor who always guided and motivated me to do my best and to my friends who stood beside me, for their assistance and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Yaya Rukayadi, PhD
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Food products can be subjected to contamination by bacteria and fungi. The growth of pathogenic bacteria in food products caused foodborne illnesses resulting consumption of contaminated food. Food spoilage bacteria, yeast, and mold may contribute to the deterioration of the color**,** texture, and flavor of food**.** To overcome this problem, prevention should be done at the early stage of food processing such as sanitizing. Commonly, chemical sanitizers had been applied in the food industry. However, applications of these chemicals in long term will affect human health. Therefore, developments of natural sanitizers derived from plant sources are gaining more attention nowadays. In this study, the antimicrobial activity of the ethanolic extract of banana flowers against foodborne pathogens and food spoilage microorganisms was conducted. The antimicrobial analysis includes Disc Diffusion Assay (DDA), Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC), and Time kill curve. Other tests were phytochemicals analysis Gas Chromatograph Mass Spectra (GC-MS) and Liquid Chromatograph Mass Spectra (LC-MS), toxicity test using brine shrimp lethality assays, and application of an ethanolic extract of banana flower on microbial population in cherry tomato. The susceptibility test showed that all tested pathogenic bacteria were inhibited by ethanolic extract of banana flower, with the range of inhibition zone between 8.83 ± 0.29 to 10.67 ± 0.29 mm. The ethanolic extract was significantly effective against *Bacillus subtilus* ATCC6633 with an inhibition zone of 10.67 ± 0.29 mm. The MICs values of the extract against all tested bacteria strains ranged between 3.13 to 6.25 mg/mL. Ethanolic extract was highly effective against *Escherichia coli* ATCC43895, *B. subtilis* ATCC6633, *B. pumilus* ATCC14884, and *Proteus mirabilis* ATCC21100 with a MIC of 3.13mg/ml. On the other hand, the MBC values ranged between 6.25 to 25 mg/mL. *E. coli* ATCC43895 was the most susceptible bacteria with an MBC value of 6.25 mg/mL. The time-kill curve study showed that *E. coli* was found to be completely killed after exposure to the ethanolic extract of banana flower at 4× MIC after 2 h of incubation time. However, the population of *Klebsiella pneumoniae* ATCC13773, *B. pumilus* ATCC14884, *B. subtilus*

ATCC6633, *B. megaterium* ATCC14581, and *P. mirabilis* ATCC21100 were reduced to less than 3 log CFU/mL once treated with the ethanolic extract of banana flower at 4× MIC for 4 h. Moreover, the antifungal activity of the ethanolic extract of banana flower in terms of inhibition zone against *Aspergillus niger* ATCC9029, *Rhizopus oligosporus* ATCC22959, *Rhizopus oryzae* ATCC22580, and *Candida* spp. (*Candida albicans* ATCC10231, *Candida krusei* ATCC32196, and *Candida parapsilosis* ATCC22019) ranged between 6.13 ± 0.06 to 9.67 ± 0.62 mm. The MIC values were 6.5 to 12.5 mg/mL while the MFC values were 12.5 to 25 mg/mL. The time-kill curve result for *C. albicans* was found to be killed completely at 4× MIC for 4 hr of exposure time, while *C. krusei* and *C. parapsilosis were* found to be reduced to less than 3 $\log_{10}$ CFU/mL after exposure to the extract at 4× MIC for 4 h. In inhibition of conidia germination, qualitative analysis of all the tested fungi species showed no growth after being treated with extract started at 2× MIC and 4 × MIC for 14 days. Whereas the quantitative analysis using 4× MIC values for 48 h showed that the percentage of conidia germinations were completely inhibited for *Rh. oligosporus* at 2× MIC and 1× MIC for *Asp. niger* and *Rh. oryzae*. Cell constituents release analysis; crystal violet assay showed altering in cell wall linearity, cells ruptured, and leakage of the cytoplasm. Generally, the antimicrobial activity of the ethanolic extract of the banana flower was not affected by different pHs and temperatures. The identified bioactive compounds in the ethanolic extract of the banana flower by using GC-MS were hexadecenoic acid, 1- heptacosanol, 1-heneicosanol, 17-Pentatriacontene, diacetone alcohol, diisooctyl phthalate, fucosterol, heptadecanol, octadecane, octadecanoic acid, methyl ester, phenol, 2,4-bis(1,1-dimethylethyl)- (CAS) 2,4-Di-tert-butylphenol, squalene, and triacontane. The identified bioactive compounds by using LC-MS included hippeastrine, L-(-)-carvone, 4-hydroxybenzaldehyde, and vanillin. The toxicity study demonstrated that the ethanolic extract of the banana flower was not toxic with LC_{50} = 4.1993 mg/mL. Ethanolic extract of banana flower had been used in the washing treatment of cherry tomato at different concentrations of 0.05 %, 0.50%, 0.25%, and 5.00% with an exposure time of 5 min and 15 min. For the storage study, the treated samples were kept at 4°C and 25 ± 2°C for 21 days. TPC, yeast, and mold start to reduce when treated with 0.05% and 0.25% concentrations of the extract with an exposure time of 5 and 15 min as compared to tap water treatment. Significant reduction of the microorganism was observed at 5.00% concentration with 5 min and 15 min exposure time. In the storage study, *E. coli* was not detected at 4˚C and 25 ± 2˚C in control and treated samples starting from day 0 until the 21 days. This means that *E. coli* was not detected on the cherry tomato samples before starting with washing treatment. TPC and yeast and mold populations from the samples kept at 4˚C and 25 ± 2˚C showed greater reduction up to less than 3 Log CFU/mL in all treated samples. There is no previous study of ethanolic *M. paradisiaca* L. flower extract on food spoilage microorganisms, it is best to assess the antifungal action of the extract against more food spoilage fungi, including yeast and filamentous fungi. This is first study of Four bioactive compounds identified by LC-MS and detected of *M. paradisiaca* L. flower extract was hippeastrine, L-(-)-Carvone, 4-Hydroxybenzaldehyde, and Vanillin. In conclusion, the ethanolic extract of banana flowers exhibited antimicrobial activity, thus it can be developed as a natural sanitizer for washing raw food materials and preventing food spoilage during storage.

Keywords: Antimicrobial activity, ethanolic extract of banana flower, cherry tomato, phytochemicals, toxicity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

ANALISIS ANTIMIKROB, FITOKIMIA DAN KETOKSIKAN EKSTRAK ETANOL BUNGA PISANG (*Musa paradisiaca* L.*)* DAN APLIKASINYA DALAM TOMATO CERI

Oleh

KHADIJA AHMED MOHAMED MOUSA

Disember 2022

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Produk makanan boleh dicemari oleh bakteria dan kulat. Pertumbuhan bakteria patogenik dalam produk makanan menyebabkan penyakit bawaan makanan akibat memakan makanan tercemar. Untuk mengatasi masalah ini, pencegahan harus dilakukan pada peringkat awal pemprosesan makanan seperti sanitasi. Biasanya, agen sanitasi kimia telah digunakan dalam industri makanan. Walau bagaimanapun, aplikasi bahan kimia ini untuk jangka masa panjang akan menjejaskan kesihatan manusia. Oleh itu, pembangunan bahan sanitasi semula jadi yang berasal daripada sumber tumbuhan semakin mendapat perhatian pada masa kini. Dalam kajian ini, aktiviti anti-mikrob ekstrak etanol bunga pisang terhadap patogen bawaan makanan dan mikroorganisma perosak makanan telah dijalankan. Analisis anti-mikrob termauk esei resapan cakera (Disc Diffusion Assay (DDA), kepekatan perencatan minimum (Minmum Inhibitory Concentration (MIC), kepekatan bactericidal minimum (Minimum Bactericidal Concentration (MBC), kepekatan fungisidal minimum (Minimum Fungicidal Concentration (MFC) dan keluk masa membunuh (Time kill curve). Ujian lain ialah analisis fitokimia spektrum jisim kromatografi gas (gas chromatography mass spec (GC-MS) dan spektrum jisim kromatografi cecair (Liquid chromatograph mass spec (LC-MS), ujian ketoksikan menggunakan esei kematian udang air garam dan aplikasi ektarak etanol bunga pisang terhadap populasi mikrob dalam tomato ceri. Ujian kerentanan menunjukkan bahawa semua bakteria patogen yang diuji telah direncatkan oleh ekstrak etanol bunga pisang dengan julat zon perencatan di antara 8.83 ± 0.29 mm hingga 10.67 ± 0.29 mm. Ekstrak etanol bunga pisang sangat berkesan terkesan terhadap *Bacillus subtilus* ATCC6633 dengan zon perencatan 10.67 ± 0.29 mm. Nilai MIC ekstrak terhadap kesemua strain bakteria yang diuji di antara 3.13 hingga 6.25mg/ml. Ekstrak etanol sangat berkesan terhadap *Escherichia coli* ATCC43895, *B. subtilus* ATCC6633, *B. pumilus* ATCC14884 and *Proteus mirabilis* ATTCC 21100 dengan MIC 3. 13 mg/ml. Selain itu, nilai MBC di antara julat 6.25 hingga 25 mg / mL. *E. coli* ATCC43895 sangat mudah dibunuh dengan nilai MBC sebanyak 6.25 mg/ml. Kajian lengkuk masa membunuh menunjukkan *E. coli* di dapati dibunuh sepenuhnya setelah didedahkan kepada ekstrak bunga pisang pada 4 ×

MIC setelah 2 jam masa pengeraman. Walau bagaimanapun, populasi *Klebsiella pneumoniae* ATCC13773, *B. pumilus* ATCC14884, *B. subtilus* ATCC6633, *B. megaterium* ATCC14581, und *P. mirabilis* ATCC21100 berkurangan kepada 3 log CFU / mL setelah dirawat dengan ekstrak etanol bunga pisang pada 4 × MIC selama 4 jam. Aktiviti anti-kulat ekstrak etanol bunga pisang. dari segi zon perencatan terhadap *Aspergillus niger* ATCC9029, *Rhizopus oligosporus* ATCC22959, *Rhizopus oryzae* ATCC22580, dan *Candida spp.* (*Candida albicans* ATCC10231, *Candida krusei* ATCC32196, dan *Candida parapsilosis* ATCC22019) di antara julat 6.13 ± 0.06 hingga 9.67 ± 0.62 mm. Nilai MIC di antara 6.5 hingga 12.5 mg / mL sementara nilai MFC berada di antara 12.5 hingga 25 mg / mL. Lengkuk masa membunuh mendapati *C. albicans* dibunuh sepenuhnya pada 4 × MIC selama 4 jam masa pendedahan sementara. *C. krusei* dan *C. parapsilosis* di dapati dikurangkan kepada kurang daripada 3 log CFU / mL setelah pendedahan kepada ekstrak pada 4 × MIC selama 4jam. Dalam merencatkan pertumbuhan konidia, analisis kualitatif kesemua spesis kulat yang diuji tidak menunjukkan pertumbuhan setelah dirawat dengan ekstrak bermula pada 2 × MIC dan 4 × MIC selama 14 hari. Bagaimanapun analisis kuantitatif menggunakan nilai 4 × MIC selama 48 jam menunjukkan peratusan percambahan konidia direncatkan sepenuhnya untuk *Rh. oligosporus* pada 2 × MIC dan 1 × MIC untuk *Asp. niger* dan *Rh. oryzae*. Analisis pembebasan sel konstituen, esei kristal violet menunjukkan perubahan dalam dinding sell mendatar, sel pecah dan pembocoran sitoplasma. Secara amnya, aktiviti antimikrob ekstrak etanol bunga pisang tidak dipengaruhi oleh perbezaan pH dan suhu. Sebatian bioaktif dalam ekstrak etanol bunga pisang telah dikenalpasti dengan menggunakan GC-MS ialah termasuk hexadecanoic acid, 1-heptacosanol, 1-heneicosanol, 17-Pentatriacontene, diacetone alcohol, diisooctyl phthalate, fucosterol, heptadecanol, octadecane, octadecanoic acid, methyl ester, phenol, 2,4- bis(1,1-dimethylethyl)- (CAS) 2,4-Di-tert-butylphenol, squalene dan triacontane, sebatian bioaktif telah dikenalpasti menggunakan LC-MS termasuk hippeastrine, L - (-) - Carvone, 4-Hydroxybenzaldehyde dan vanillin. Kajian ketoksikan menunjukkan bahawa ekstrak etanol bunga pisang tidak toksik dengan LC50 = 4.1993 mg / mL. Ekstrak ethanol bunga pisang. telah digunakan dalam kajian rawatan pencucian tomato ceri pada kepekatan 0.05%,0.50%,0.25% dan 5.0% dengan masa pendedahan 5min dan 15min. Untuk kajian penyim panan, sapel yang dirawat disimpan pada 4˚C dan 25 ± 2˚C selamas 21 hari. TPC, yis dan kulat mula berkurangan 0.05% dan 0.25% kepekatan ekstrak dengan masa pendedahan 5 dan 15 minit berbanding rawatan air paip. Penurunan yang signifikan populasi mikroorganisms dilihat pada kepekatan ekstrak 5.00% dengan masa pendehan 5min dan 15min. Dalam kajian penyimpanan, *E. coli* tidak dikesan pada suhu 4˚C dan 25 ± 2˚C sampel kawalan dan sampel yang dirawat bermula daripada hari 0 hingga hari ke 21. Ini bermaksud *E. coli* tidak wujud pada sampel tomato ceri sebelum memulakan rawatan pencucian. Populasi TPC, yis dan kulat daripad sampel yang disimpan pada suhu 4˚C dan 25 ± 2˚C menunjukkan penurunan kepada kurang daripada 3 Log CFU/mL pada semua sampel yang dirawat. Kesimpulannya, ekstrak etanol bunga pisang. menunjukkan aktiviti antimikrob, oleh itu dapat dibangunkan sebagai agen sanitasi semulajadi untuk mencuci bahan makanan mentah dan mencegah kerosakan makanan semasa penyimpanan.

Kata kunci: Aktiviti anti-mikrob, ekstrak etanol bunga pisang, tomato ceri, fitokimia, ketoksikan.

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"In the name of Allah, the most Gracious and the most Merciful"

In the beginning, all praise due to ALLAH (SWT), lord of the world, and peace be upon His messenger, prophet Mohammad (PBUH). Only by ALLAH's Grace and Mercy, this work has been completed and Insha Allah, it will be successful.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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ATCC	American Type Culture Collection
ANOVA	Analysis of variance
CFU	Colony forming unit
CHX	Chlorhexidine
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl sulfoxide
GC-MS	Gas Chromatography-Mass Spectrometry
GRAS	Generally Regarded as Safe
LC_{50}	Lethality concentration
LC-MS	Liquid Chromatography – Mass Spectrometry
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
MIC	Minimum Inhibitory Concentration
MOH	Ministry of Health
m/z	Mass/charge ratio
NIST	National Institute of Standards and Technology
OD	Optical density
PBS	Phosphate buffered saline
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
Rpm	Revolutions per minute
SDA	Sabouraud Agar

SDB	Sabouraud Broth
spp.	Species
TCBS	Thiosulfate citrate bile salts sucrose
TPC	Total Plate Count
UPM	Universiti Putra Malaysia
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

The foodborne pathogens issue is an important communal problem both in developed and emerging nations of the world. They are threatening food safety, particularly in third-world countries with unhygienic conditions and poor public health infrastructure. Each year, the upsurge of foodborne pathogens causes thousands of cases of infectious diseases those raise medical care expenses and cause production losses (Willmott *et al*., 2016). Foodborne diseases' symptoms include nausea, vomiting, fever, diarrhea, and even death. Around 1210 cases of foodborne infection per 100,000 people are reported in France, while 2600 and 25000 cases per 100,000 people are reported in the UK and US, respectively. Additionally, approximately 5.4 million infections are registered in Australia, with 15,000 patients and 120 fatalities yearly (Abdul- Mutalib *et al*., 2015).

In the US, the registered cases of foodborne contamination have spiked to almost 48 million, with 128,000 patients hospitalized and 3,000 fatalities annually (Law *et al.*, 2015). In Malaysia, the prevalence of confirmed foodborne infections in 2013 was 47.79 for every 100,000 people, with a 0.04% fatality rate (Rahim *et al.*, 2019). The incidence of food poisoning in school pupils has surged by 57% in only four months of 2016 compared to 2015 (A-Rahim *et al*., 2019).

Improper food handling and unhygienic practices, particularly food processing and storage (Scott, 2003), typically cause Foodborne diseases. The most common instances of frequently detected foodborne pathogenic bacteria are *B. cereus*, *Campylobacter*, *Clostridium*, *E. coli*, *L. monocytogenes*, and *S. aureus* (Bhargava *et al.*, 2015). Additionally, the degradation of food and food products by foodborne fungi at various phases of food production influences the agro-based food supply chain to consumers. It was reported that around 5-10% of food wastage was induced by spoilage fungi (Rawat, 2015). Several studies stated that *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Rhizopus species* are linked to food spoilage (Raak *et al.*, 2017; Ramli, 2018; Rawat, 2015). The contamination of food and food products could be occurred at both pre-and post-harvesting stages because of unfavorable field environmental conditions, deprived irrigation systems, and the scarcity of manure (Tabah *et al.*, 2016).

Due to cross-contamination, these pathogenic bacteria and fungi will continue to grow without appropriate sanitization methods, particularly during food processing and storage phases (Zanin *et al.*, 2017). The sanitization process reduces microbes' number to a level that minimizes the potential for infection. Surfaces and objects should be cleaned before sanitization. If cleaning is not done well to sanitizing, the sanitizer might not be effective. This is because dirt, dust, and other material can interrupt the sanitizers' contact with the object. Although sanitizers do not kill all microbes on surfaces and objects compared to disinfectants, it is safe when they come into contact with the skin (Minnesota Department of Health, 2017). It has been critical to completely eradicate or

minimize the microorganisms at the initial phase of food processing. Food washing materials using certain food sanitizers were, therefore, essential, particularly for ready-to-eat foods, including vegetables and fruits.

Chlorine, iodine, and quaternary ammonium are sanitizing agents for food contact surfaces—factor such as concentration, temperature, and contact time, influence the effectiveness of the sanitizers. Therefore, food handlers must ensure these factors are followed; otherwise, the destruction of equipment may result in poor sanitizing ability (Shahbaz *et al*., 2020). Nevertheless, these chemicals can be detrimental to humans and the climate (Prado-Silva *et al.*, 2015). Furthermore, cleansing via tap or chlorinated water is a common sanitization practice utilized in homes. Nonetheless, such activity is becoming debated because of the accumulation of cancer-causing by-products like trihalomethanes once chlorine combines with carbon-based materials, including decomposed vegetation. Moreover, chlorine can oxidize organic compounds in food, while carcinogenic and mutagenic compounds (halo forms and acetic acids) can be produced in water (Bhargava *et al.*, 2015).

Owing to the shortcomings of synthetic food sanitizers, the alternative option is to investigate plant-based active antimicrobial compounds for food sanitization. Although commercial plant-based food sanitizers are still uncommon, they are getting broader attention since most of them are considered generally recognized as safe (GRAS) status and highly effective for food safety (Mostafa *et al.*, 2018). Several kinds of research have stated that most medicinal plants, such as herbs and spices, possess a substantial quantity of antimicrobial substances (Dethoup *et al.*, 2018). *M. paradisiaca* L. flower comprises 22.84 g carbohydrate, 1.09g protein, 0.33 mg fat, 260 mg dietary fiber, calcium, magnesium, manganese, phosphorus, and vitamins such as A, E, C, and E (Rajesh *et al.*, 2017). This plant's flower is effective in the treatment of bronchitis, diarrhea, ulcers, and diabetics (Shruthi, 2019), and bioactive compounds that possess antimicrobial activity such as tannins, saponin, phenols, flavonoids, and alkaloids (Ajijolakewu *et al.*, 2021). Due to limited research on this project and the abundance of *M. paradisiaca* L. flower available in Malaysia, therefore need to explore. Hence, this study aimed to evaluate the antimicrobial activity of ethanolic banana (*M. paradisiaca* L.) flower extract against specific foodborne pathogens and spoilage microorganisms and its stability at different pHs and temperatures, identify the phytochemical compounds, toxicity analysis, and to evaluate its effectiveness. Cherry tomato is one of the most essential horticultural as it has been used as a salad. Additionally, it can be consumed as fresh fruit or processed in tomato ketchup, juices, paste as well as other products. Cherry tomato has a pleasant flavor and is an excellent source of several vitamins, minerals, and bioactive compounds, including vitamin A, vitamin C, potassium, folate, carotenoids as well as flavonoids, which are suitable for human health. The presence of sugar content contributes to the sweet and delicious taste, and this encourages fungal growth (Kasih, 2022).

Buendía *et al.* (2019) reported that cherry tomato is a fragile product with a limited shelf life at ambient temperature and is very limited mainly due to softening with high relative humidity (RH), modified atmosphere packaging, and storage at improper temperatures. In contrast, cherry tomatoes require an improved strategy in post-harvest processing and preservation owing to their excellent economic viability. It is required to devise a technology that allows the preservation of cherry tomatoes with minimum processing

and optimal storage life. Washing cherry tomatoes using disinfectant may prolong their shelf and reduce the number of spoilage microorganisms that have a significant impact on the quality of cherry tomatoes. Chlorination is the most standard method for sanitizing newly harvested crops. Chlorine concentrations of 100 - 200 ppm are used for washing fresh vegetables and fruits. The use of chlorine may cause harm to human health due to the residue levels (Santosa, 2018).

1.2 Problem Statements

The contamination of food poses a significant risk to the public's health around the globe. Excellent farming practices and efficient industrial techniques are the first defensive line against food contamination (Stefano & Avellone, 2014). Considering worldwide ramifications, the food industry and customers must comprehend the essence of such contaminants, their origins, consumer risks, and techniques to eradicate or limit them; specific consideration ought to be paid to food processing, handling, and servicing.

Food handlers must always be educated in food safety, hygiene practices, and contamination management (Sadiku, 2020). A foodborne epidemic may result in health issues as well as financial losses. The expected annual cost of food safety issues to the United States economy is about $7 billion. Similarly, economies of almost all other nations have suffered from economic losses. The majority of these losses are attributable to lost markets, declining consumer demand, litigation, and business closures (Hussain & Dawson, 2013). Fresh food like fresh cherry tomatoes that can easily be spoiled is highly prone to foodborne pathogenic bacteria and fungi such as *Escherichia coli*, *Salmonella* spp., *Rhizopus* spp., and *Aspergillus* spp. (Aloui *et al*., 2021; Kong *et al*., 2019; Won *et al*., 2018; Cui *et al*., 2017; Tian *et al*., 2015). For instance, CDC (2017) reported that fresh produce was associated with 163 of 1779 foodborne outbreaks in the US from 2004 to 2010, causing 4949 illnesses,895 hospitalizations, and nine deaths.

Microbes easily contaminate tomatoes as they are consumed fresh, and these have been identified as potential factors of a foodborne outbreak. Out of 38 outbreaks linked to fresh tomato consumption in the United States since 1990, 4,028 illnesses were reported (Cabrera-Díaz *et al*., 2018). Without suitable decontamination measures, microorganisms will continue to develop, particularly during food storage and processing (Scott, 2003). Even though there are many food decontamination treatments and food sanitizers used to remove or significantly reduce microbial populations (Leistner, 2000). However, it was essential to eliminate or reduce the microbial population at the early stage of food processing. Therefore, washing the food materials with any food sanitizer was crucial, especially for foods that are ready to eat, like fruits and vegetables.

However, these chemicals can harm human health and the environment (Neo *et al*., 2013). Additionally, rinsing fruits and vegetables with tap or chlorinated water is the most popular cleansing method used in homes. However, this approach has been called into question because of the production of carcinogens such as trihalomethanes once chlorine combines with organic substances, including decaying leaves (Chang & Fang, 2007). Besides, organic substances in foods can be oxidized by chlorine, whereas in

water, carcinogenic and mutagenic by-products such as haloforms and haloacetic acids can be formed (Perez *et al*., 2011).

Due to the disadvantage of chemical food sanitizer, a study on natural antimicrobial agents from plants is the alternative way. Furthermore, commercial natural food sanitizer still needs to be made available. Plants' antimicrobials are getting extensive interest from the public since they have been designated as Generally Recognized as Safe (GRAS) and proven to be safer for food (Alzarokey & Nakahara, 2003). Many studies have reported that most medicinal plants, including spices and herbs, have solid antimicrobial compounds (Limsuwan *et al*., 2009).

The banana (*Musa paradisiaca* L.) is an herbaceous plant that originated in India and Southeast Asia. The plant is cultivated for its fruit. Its parts, blossoms, peels, roots, and seeds, are classified as agricultural industry by-products and are always considered valuable. However, these by-products may encompass phytochemicals and other biologically active compounds. According to Padam *et al.* (2014), banana flowers have been found to contain antioxidants and epigallocatechin. Epigallocatechin exhibits antibacterial (Nikoo *et al*., 2018), chemotherapeutic, (Chen *et al*., 2020), neuroprotective (Singh *et al*., 2015), and chemopreventive activities (Du *et al.*, 2012; Hussain & Ashafaq, 2018). Unfortunately, limited study has been performed regarding the biological and chemical analysis of *Musa* spp., since many preceding works have concentrated on peels and pulps.

Hence, a study has been conducted to determine the antimicrobial activities of ethanolic banana (*M. paradisiaca* L.) flower extract against several types of pathogenic microorganisms and to measure the ability of this plant extract as natural food sanitizer.

1.3 Objectives

The specific objectives are:

1. To determine the antimicrobial activity of ethanolic *M. paradisiaca* L. flower extract on foodborne pathogens and spoilage microorganisms.
2. To analyze the antimicrobial action modes and effect of different pHs and temperatures of ethanolic *M. paradisiaca* L. flower extract on foodborne pathogens and spoilage microorganisms.
3. To identify the bioactive compounds in ethanolic *M. paradisiaca* L. flower extract will be examined for antimicrobial activity using Gas Chromatography-Mass-Spectrometry (GC-MS) and Liquid Chromatography- Mass-Spectrometry (LC-MS).
4. To evaluate the toxicity of ethanolic *M. paradisiaca* L. flower extract by using brine shrimp (*Artemia salina* spp.) assay and to determine its effect on natural.

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