Evaluation Of The Antimicrobial Potential Of Phenolic Compounds Extracted From Banana Peels (Musa paradisiaca L.) Against S. aureus and S. typhimurium In Meat and Fish

Mohammad J. Al-Jassani
Department of Forensic Sciences, College of Science, Al-Karkh University of Science, Iraq, pcr2000@kus.edu.iq

Abstract

Salmonella typhimurium and Staphylococcus aureus are two examples of pathogenic bacteria that contaminate food and release enterotoxins. One way to get around this is to use biological agents as antibacterials. Including phenolic chemicals, banana peels are a promising source of natural bioactive substances. The extraction with ethanol gave a higher yield of extraction with a total phenol content of 2.98mg followed by methanol. The chromatogram of HPLC revealed that the ethanol extract of banana peel had substantial levels of phenolic compounds such as caffeic acid, catechin, cinnamic acid, and ferulic acid. The most prevalent foodborne pathogens in hamburger meat and fish were S. aureus and S. typhimurium. S. aureus isolates were more resistant to ciprofloxacin than S. typhimurium. On the other hand, the phenolic compounds showed a high level of activity against all foodborne pathogens. So that these active phenolic compounds have promising applications as antibacterial agents against the increasing infections caused by foodborne S. aureus and S. typhimurium.

Keywords: *foodborne pathogens, phenolic compounds, banana peels.*

INTRODUCTION

A foodborne disease outbreak is characterized by an instance in which two or more people contract the same ailment after consuming a common meal (1). These illnesses include a wide variety of agents, and as new agents are discovered each year, their number keeps growing (2). Diseases that are spread through food are thought to be caused by pathogens including, Staphylococcus aureus, Salmonella sp., Escherichia coli, Bacillus cereus, Clostridium perfringens, Campylobacter, Vibrio parahaemolyticus, and Listeria monocytogenes, are just a few examples (2).

Numerous diseases have recently been in charge of ensuring food safety. One of the most common foodborne pathogens in fresh and ready-to-eat foods and the cause of numerous diseases all over the world is Staphylococcus aureus. It may grow at temperatures between 15 and 45 degrees Celsius and at up to 15% NaCl concentrations (4). At room temperature, this bacterium grows rapidly and produces harmful toxins Escherichia coli, Staphylococcus aureus, and Salmonella sp. isolated from commonly consumed items (raw chicken, raw beef, and fish) in Lebanon have been tested for heat resistance (5). Salmonella spp. and S. aureus, which can cause severe foodborne infections in humans, are highly prevalent in chicken, beef, pork, and fish samples (2, 6).

Accessible, simple to extract or synthesize, and effective new antimicrobial medicines are required, but they must also be long-lasting by preventing the spread of horizontal gene transfer and antibiotic resistance in S. aureus or other bacteria (7).

Plants and their byproducts are major potential sources of novel antimicrobials due to their availability, chemical variety, and complicated chemical makeup (8, 9). Extracts derived from diverse plant sources have been shown to include a wide range of bioactive organic small molecules with antibacterial properties, particularly polyphenols. These polyphenols are frequently ingested along with foods including cereals, vegetables, herbs, and fruits that contain them (10). Therefore, the purpose of this study is to identify and isolate foodborne pathogens while also extracting, identifying, and employing phenolic chemicals as antibacterial agents from banana peels.

MATERIALS AND METHODS

Preparation of banana peel powder

Ripe bananas were brought in from nearby marketplaces. In a hot air oven, the banana peels were divided into small pieces and dried for 48 hours at 50°C. The dried materials were ground into a powder using a blender and stored for later use in a vacuum aluminum bag at 4°C.

Detection of extraction yield

Five gm of the powdered materials were extracted using a microwave and acetone, methanol, ethanol, and hexane. There were 10, 20, and 30-minute extraction times. The filter paper was used to filter the solutions, which contained extracts and solvents. At 40, 50, and 60°C, a rotary evaporator was used to evaporate the solvents. The dried extract was precisely weighed, and the extract yield was calculated as (10): Extraction yield (%)=(gm of extract/gm of the dried sample)*100

Determination of Total Phenolic

By using the Folin-Ciocalteu micro-method, the total phenolic content of each extract was determined (10). A portion of the extract solution (20 liters) was mixed with 1.16 milliliters of distilled water, 100 milliliters of the Folin-Ciocalteu reagent, and 300 milliliters of 20 % Na2CO3 solution. After 30 minutes of shaking incubation at 40°C, the mixture's absorbance at 760 nm was measured. The calibration curve's standard was gallic acid. Based on the calibration curve, as gallic acid equivalent (GAE), the total phenolic content was calculated using the following linear equation: A=0.98C+9.925x 10-3, where A is the absorbance and C is the concentration.

Identification of phenolic compounds by HPLC

HPLC was used to identify phenolic chemicals in the acetone extract of banana peels. The ZORBAX-EclipseXDB-C18 column (4.6250 mm, particle size 5 m) was used for the chromatographic separations. The mobile phase, which was 0.5% acetic acid in distilled water with a pH of 2.65, was utilized at a constant flow rate of 1 ml/min. Utilizing a UV detector with a wavelength set at 280 nm, the elution gradient was linear with the elution buffer. By comparing the relative retention periods of the phenolic compounds in banana peel extract to those in the standard mixture chromatogram, the compounds' identities were determined.

Food poisoning bacteria Isolation

From supermarkets, 10 samples each of hamburger meat and fish were gathered. From these types, small sections were homogenized, suspended in ordinary saline, and then grown on MacConkey's agar and blood agar. Following the incubation period, the colonies were identified based on their morphological and cultural traits. The diagnosis was verified using the VITEK2 compact.

The efficiency of phenolic compounds as an antibacterial agent against food-borne pathogens

The microdilution assay in microplates was used to investigate the effect of phenolic compounds and ciprofloxacin on food-borne pathogens by calculating the MIC for phenolic compounds and ciprofloxacin as follows (11): Using the 0.5 McFarland turbidity standard, a bacterial inoculum of an overnight-growing culture was created. 80 ml of various quantities of phenolic compounds (1-1024 µg/ml) or ciprofloxacin (1-1024 µg/ml) were added to each well along with 100 ml of Mueller-Hinton broth, 20ml of microbial inoculum, and 80 ml of Mueller-Hinton broth. The microtiter plates were incubated for 24 hours at 37°C. The minimum inhibitory concentration (MIC) was determined at which the organism's visible growth was inhibited.

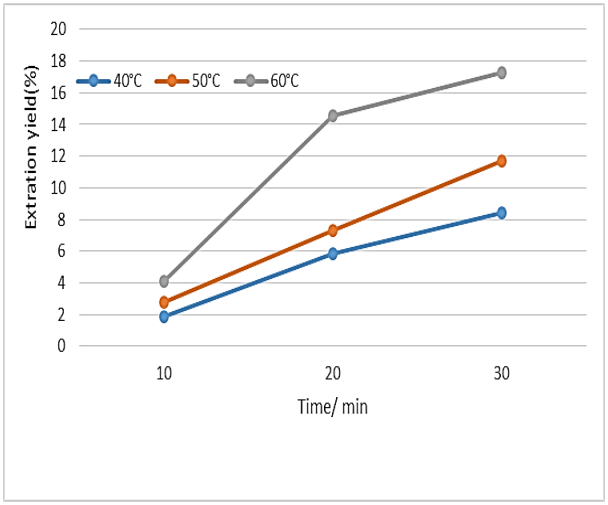
RESULTS AND DISCUSSION

Detection of extraction yield

The impacts of the extraction settings for the microwave extraction yield are shown in Figure (1). As the temperature and time increased, the yield percentage rose. The maximum yield for the vacuum microwave method for extracting banana peels was 17.30% when the conditions were at 60°C for 30 minutes, and the lowest yield was 4.12% when the conditions were at 40°C for 10 minutes.

When using vacuum microwave and ultrasonic-assisted extraction techniques, the circumstances that used the greatest temperature and the longest duration produced the maximum yields of banana peels and cinnamon barks (10). Shorter extraction times caused by higher extraction temperatures are advantageous for extraction and result in increased phenolic contents (12).

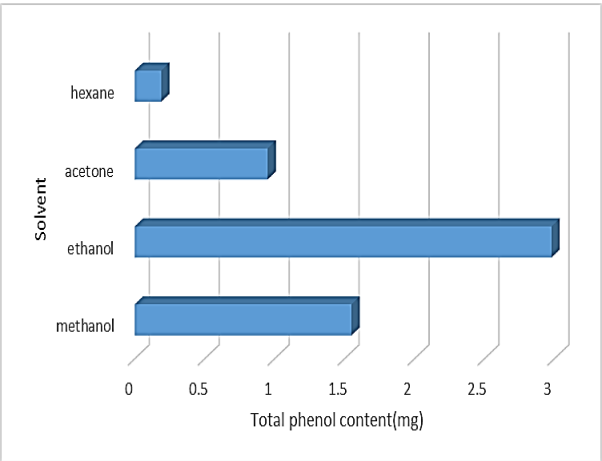
Figure 1: Effect of temperature and time on extraction yield



Determination of total phenolic

Because of its higher polarity and superior phenolic component solubility, ethanol outperformed the other solvents in the extraction of phenolic compounds according to figure (2), ethanol, followed by methanol and acetone with respective values of 2.98 and 1.55 mg, and hexane with a lower extraction level of 0.19 mg, was the best solvent for extracting phenolic chemicals (2). In comparison to the higher-polarity solvents, the lower-polarity solvents, in particular hexane, petroleum ether, and diethyl ether, had a significantly lesser ability to extract phenolic chemicals (13). The phenolic content of banana peel varied from 18.21 mg gallic acid/g extract to 35.06 mg gallic acid/g extract with the ultrasonic technique at 60°C and 30 min. yielding the highest result (10).

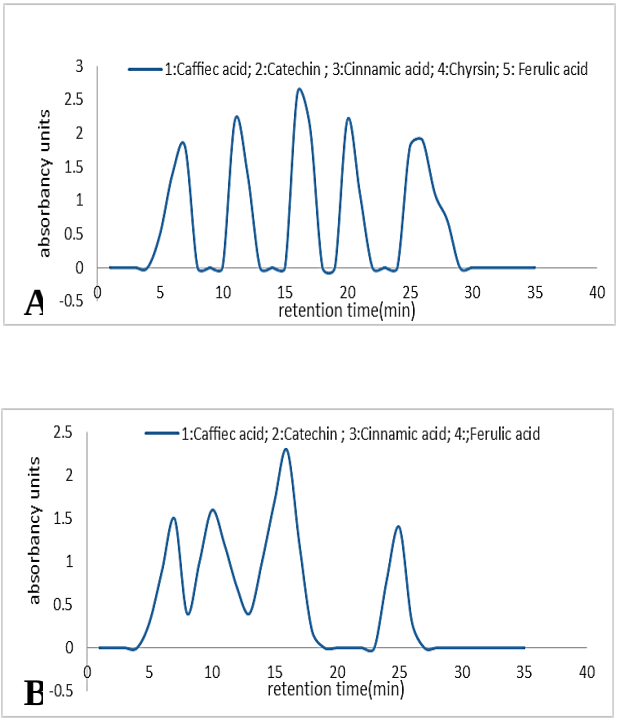
Figure 2: Detection of total phenolic content by using different extraction solvents



Identification of phenolic compounds by HPLC

In comparison to the commonly used standard phenolic chemicals such as caffeic acid, catechin, cinnamic acid, chrysin, and ferulic acid, data in figure (3- a and b) showed that the ethanol extract of banana peel had substantial levels of caffeic acid, catechin, cinnamic acid, and ferulic acid. The type of solvent and the various amounts utilized had a significant impact on the phenolic and flavonoid levels. (14). other authors observed that the solvent polarity had a significant impact on the extraction yield of phenols. Additionally, the HPLC-UV approach identified ferulic acid as the main insoluble phenolic acid in banana extracts (15). As opposed to this, the HPLC/DAD analysis of the phenolic composition revealed that gallic acid, caffeic acid, ellagic acid, rutin, isoquercitrin, and quercetin in unripe plantain (16).

Figure 3: HPLC analysis for (A) standard phenolic compounds, (B) phenolic compounds extracted from banana peels



Food poisoning bacteria Isolation

There were 6 isolates of S. aureus after cultured hamburger meat on adequate culture media, including 2 isolates from hamburger meat and 4 from fish. In contrast, 2 isolates from hamburger meat and 3 isolates from fish made up the 5 isolates of S. typhimurium.

Salmonella spp. is one of the most common foodborne pathogens in fresh meat in Southeast Asia (17). Because of the probability of increased moisture and wetness on handling equipment, Salmonella spp. infection of meat was more common during the rainy season (18).

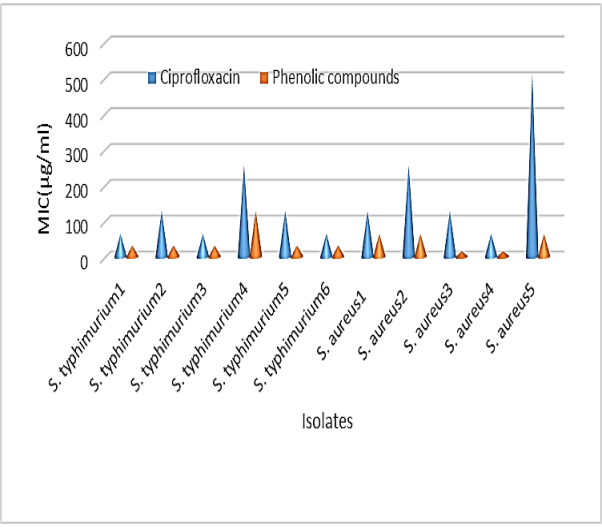
The greater frequency of S. aureus contamination during the dry season might be attributable to the high levels of moisture and temperature in wet markets, which promote pathogen development in meat (19). S. aureus

was blamed for causing gastroenteritis. These occurrences were caused by poor handwashing and personal hygiene habits, as well as contamination from other raw animal products (20).

The efficiency of phenolic compounds as an antibacterial agent against food-borne pathogens

The minimum inhibitory concentration for phenolic compounds and ciprofloxacin against S. aureus and S. typhimurium food-borne pathogens in microtiter plates revealed that S. aureus isolates were more resistant to ciprofloxacin than S. typhimurium. On the other hand, the phenolic compounds showed high levels of activity against all foodborne pathogens, especially towards S. aureus isolates with MICs reached to 16-64µg/ml followed by 32-128 µg/ml for S. typhimurium isolates (figure 4).

Figure 4: Minimum inhibitory concentrations for phenolic compounds and chosen antibiotic against foodborne pathogens



MDR S. aureus is being isolated from food products and implicated in food poisoning outbreaks more frequently these days. In this study, S. aureus had a higher level of resistance than other food pathogens like Salmonella (21). Further in-depth investigations of phenolic acid exposure have been linked to decreased metabolic activity, suppression of enzyme function, and cell membrane damage in Gram-negative bacteria (22). Even though they could all be factors in cell death, there has been a pattern of outer membrane damage that calls for additional research because Gram-negative bacteria's outer membrane is critical for survival in severe environments and drug resistance (23).

CONCLUSION

Ethanol extract gave a higher yield with a total phenol content of 2.98mg followed by methanol. The ethanol extract of banana peel had substantial levels of phenolic compounds such as caffeic acid, catechin, cinnamic acid, and ferulic acid. The phenolic compounds showed a high level of activity against all foodborne pathogens. So that these active phenolic compounds have promising applications as antibacterial agents against the increasing infections caused by foodborne S. aureus and S. typhimurium.

Reference

Akbar, A. Kumar, A. (2013). Prevalence and antibiogram study of Salmonella and Staphylococcus aureus in poultry meat. Asian Pac J Trop Biomed.; 3(2): 163-168.

Pires, S.M.; Vieira, A.R.; Perez, E.; Wong, D. and Hald, T. (2012). Attributing human foodborne illness to food sources and water in Latin America and the Caribbean using data from outbreak investigations. Int J Food Microbiol.;152: 129–138.

Costa, L.F.; Paixão, T.A.; Tsolis, R.M.; Bäumler, A.J.; Santos, R.L.(2012). Salmonellosis in cattle: Advantages of

being an experimental model. Res Vet Sci.; 93: 1–6.

Aydin, A.; Sudagidan, M. and Muratoglu, K.(2019). Prevalence of Staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne S. aureus strains isolated in the Marmara Region of Turkey. Int J Food Microbiol.;148: 99–106.

Hassan, H.; Iskandar, C.F.; Hamzeh, R.; Malek, N.J.; El Khoury, A. & Abiad, M.G.(2022). Heat resistance of Staphylococcus aureus, Salmonella sp., and Escherichia coli isolated from frequently consumed foods in the Lebanese market. International Journal of Food Properties; 25(1): 2435–2444.

Minami, A.; Chaicumpa, W.; Chongsa-Nguan, M.; Samosornsuk, S.; Monden, S.; Takeshi, K.; Makino, S.I.; Kawamoto, K. (2010). Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control 2010, 21, 221–226.

Fluit, A.C. (2015). Towards More Virulent and Antibiotic-Resistant Salmonella? FEMS Immunol. Med. Microbiol.;43:1–11.

Bythwood, T.N.; Soni, V.; Lyons, K.; Hurley-Bacon, A.; Lee, M.D.; Hofacre C., Sanchez S., Maurer J.J.(2019). Antimicrobial Resistant Salmonella Enterica Typhimurium Colonizing Chickens: The Impact of Plasmids, Genotype, Bacterial Communities, and Antibiotic Administration on Resistance. Front. Sustain. Food Syst.;3:20.

Cowan, M.M. (2010). Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev.;12: 564–582.

Anal, A.K.; Jaisanti, S. & Noomhorm, A.(2014). Enhanced yield of phenolic extracts from banana peels (Musa acuminata Colla AAA) and cinnamon barks (Cinnamomum varum) and their antioxidative potentials in fish oil. J Food Sci Technol. 51(10):2632–2639.

Gohil, N.; Bhattachariee, G. and Singh, V. (2020). Synergistic bactericidal profiling of prodigiosin extracted from Serratia marcescens in combination with antibiotics against pathogenic bacteria. Microb Pathog.; 149: 104508

Prasad, K.N.; Yang, B.; Dong, X.; Jiang, G.; Zhang, H.; Xie, H. and Jiang, Y.(2009). Flavonoid contents and antioxidant activities from Cinnamomum species. Innov Food Sci Emerg Technol.; 10:627–632.

Mohdaly, A.A.; Hassanien, M.F. Mahmoud, A.; Sarhan, M.A. and Iryna Smetanska, I.(2020). Phenolic extracted from potato, sugar beet, and sesame processing by-products. International Journal of Food Properties, 16:1148–1168.

Ahmed M. Aboul-Enein1,A.M.; Salama, Z.A.; Gaafar, A.A.; Aly, H.F.; bou-Elella,F.A. and Ahmed, H.A.(2016). Identification of phenolic compounds from banana peel (Musa paradaisica L.) as antioxidant and antimicrobial agents. J. Chem. Pharm. Res., 8(4):46-55.

Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J. and Nacoulma, O.G. (2005). Determination of total phenolics, flavonoid and proline contents in Burkian Fasan honey as well as their radical scavenging activity. Food Chem; 91:571–577.

González-Montelongo, R.; Gloria, L. M and González, M (2010) Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. Food Chem.; 119:1030–1039.

Ta, Y.T.; Nguyen, T.T.; To, P.B.; Pham. D.X.; Le, H.H.; Alali, W.Q.; Walls, I.; Lo, F.;

Wong, D. and Doyle, M.P.(2018). Prevalence of Salmonella on Chicken Carcasses from Retail Markets in Vietnam. J. Food Prot.;75: 1851–1854.

Wang, Y.; Chen, Q.; Cui, S.; Xu, X.; Zhu, J.; Luo, H.; Wang, D.; Li, F.(2015). Enumeration and characterization of Salmonella isolates from retail chicken carcasses in Beijing, China. Foodborne Pathog. Dis.;11:126–132.

Grace, D. and Fetsch, A.(2018). Staphylococcus aureus. Elsevier; Amsterdam, The Netherlands: Staphylococcus aureus—A Foodborne Pathogen; pp. 3–10.

Kimsean, P.; Sreng, K.; Has, P.; Ly, S.; Sim, S.; Chhay, S.; Prak, D. and Bun, S.(2017). An Outbreak of gastrointestinal illness associated with Khmer noodles: A multipronged investigative approach, Kandal Province, Cambodia in 2014. OSIR J.; 9:1–6.

Wa,S.;Huang,J.; Wu, O.; Zhang, J.; Zhang, F.; Yang, X.; Zeng, H.; Chen, M.; Ding, Y.; Wang, J.; Lei, T.; Zhang, S.; Xue, L.(2018). Staphylococcus aureus Isolated from Retail Meat and Meat Products in China: Incidence, Antibiotic Resistance and Genetic Diversity. Font Microbiol.; 9: 2767.

Liu, J.; Du, C.; Beaman, H.T.and Monroe, M.B.B. (2020). Characterization of Phenolic Acid Antimicrobial and Antioxidant Structure–Property Relationships. Pharmaceutics.; 12:419.

Gootz, T.D. (2006). The Forgotten Gram-Negative Bacilli: What Genetic Determinants Are Telling Us about the Spread of Antibiotic Resistance. Biochem. Pharm.;71: 1073–1084.