

# STUDY ON THE POTENTIAL OF ANTIMICROBIAL COMPOUNDS FROM BANANA/PLANTAIN BY PRODUCTS AGAINST FOODBORNE PATHOGENS

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## OBJECTIVES

The aims of this study were to determine the antimicrobial potential of banana/ plantain by products (leaf and inflorescence) against selected foodborne pathogens and the efficacy of the bioactive components in food model.

## METHODOLOGY

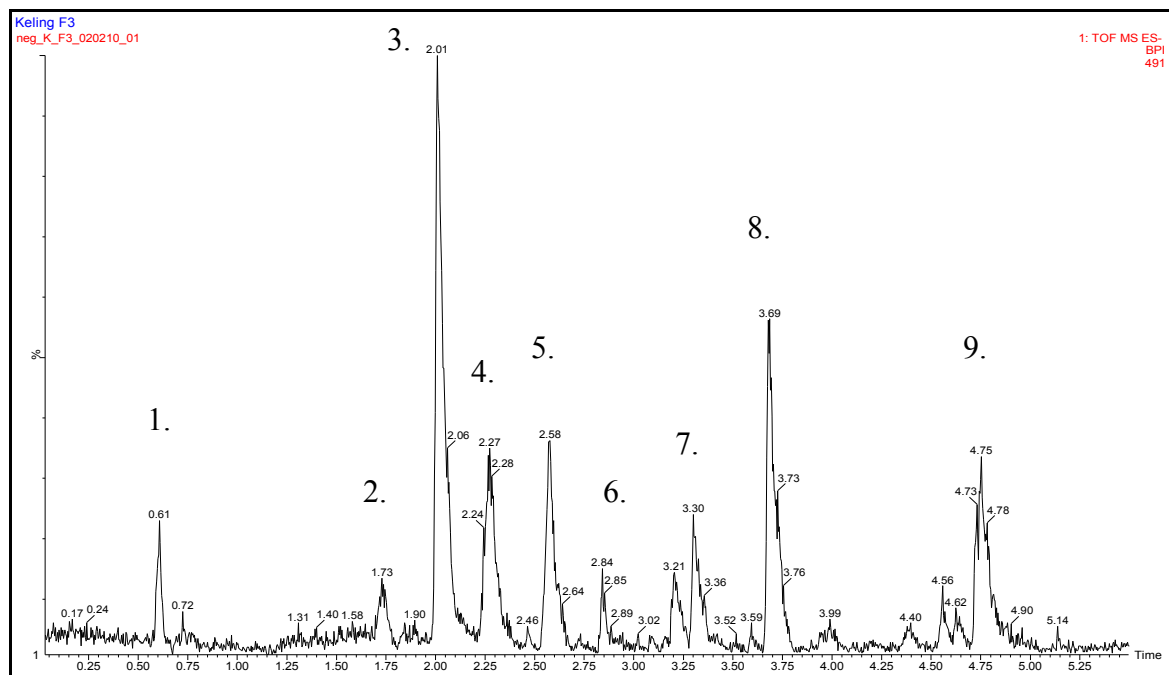
The byproducts of a few varieties of banana (*Musa paradisiaca* cv. Mysore; *Musa x paradisiaca* (AAB) cv. 'Silk'; *Musa x paradisiaca* (AAB) cv. 'Raja'; *Musa x paradisiaca* (AAB) cv. 'Red', *M. acuminata* (AA) cv. 'Sucrier'; *M. x paradisiaca*, (AAB) cv 'Saba') obtained from local plantations were screened for antimicrobial activity. The inflorescences were separated into bract and buds before dried at 50°C until 10.0%±0.5 of moisture level and ground into powder. *Musa paradisiaca* cv. Mysore was selected for individually extraction using hexane, petroleum ether, chloroform, ethyl acetate, isopropanol, acetone, ethanol, methanol, and water by direct infusion. The antimicrobial activity of these extracts were determined using well diffusion against gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*), gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli* O157:H7, *Enterobacter sakazakii*, *Yersinia enterocolitica* and *Vibrio parahaemolyticus*) and fungus (*Candida albicans*).

The effect of drying methods (oven drying, sun drying and freeze-drying) and extraction parameters (solvent to sample ratio, extraction time, and extraction temperature and methanol aqueous mixture) were also evaluated. Extraction parameters were optimized using response surface methodology according to a three levels, three variables central composite face-centered designs (CCFCD). Optimized extract (90% methanol, 40°C for 2 hours) was successively partitioned with chloroform, ethyl acetate and butanol against water and the fractions were tested for antibacterial activity. Selected bioactive water partitions were subjected to SPE (Strata X, Phenomenex) purification prior to LC/MS determination. The third fraction (elution using methanol: acetonitrile 1:1 yield only secondary metabolites) that showed the strongest antibacterial activity was collected and subjected for compounds identification using Waters UPLC-Synapt MS. Meanwhile, the selected butanol and water partition was evaluated for their minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs) based on the kinetic time kill assay against the selected bacterial pathogens (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Vibrio parahaemolyticu*) within 36hrs. It

was also evaluated for the efficacy in food model system using chicken breast meat, loaded with bacterial cultures and dipped in methanolic water partition (2.5% and 5%) as decontaminating solution for *Staphylococcus aureus* and *Listeria monocytogenes* while potassium sorbate (5%) was served as positive control. All the tests were done in triplicate and analysis of variance was performed using the SPSS v.16 for Windows with significant level of ( $p < 0.05$ ).

## RESULTS

Methanolic buds extracts showed the most potent antibacterial activity towards all tested bacterial pathogens (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*) with the inhibition zone ranging from  $7.43 \pm 0.40$  mm to  $13.23 \pm 0.45$  mm. The screened extraction parameters that yielded the highest antibacterial activity and cost effective were justified as oven drying at  $50^\circ\text{C}$ , solvent to sample ratio of 10:1 v/w, 3 hours of extraction time at  $40^\circ\text{C}$ , and with 80% methanol. Results from the CCFCD disclosed that solvent-aqueous mixture ( $X_3$ ) pose significantly ( $p < 0.01$ ) higher effect on antimicrobial activity with the regression coefficient of 2.31-2.7, followed by extraction time ( $X_1$ ) and extraction temperature ( $X_2$ ) at coefficient of -0.30 to -0.42 and -0.16 to -0.32, respectively. The generated response surface plots for all 4 tested bacterial pathogens yielding the combination of 90% methanol by direct solvent infusion at  $40^\circ\text{C}$  for 2 hours with constant shaking. Four partitions (chloroform, ethyl acetate, butanol and water) from the optimized extraction were further screened for antimicrobial activity and water partition shown the highest inhibition diameter of 14.67 – 17.48 mm against *S. aureus*, *B. cereus*, *L. monocytogenes* and *V. parahaemolyticus* which is comparable to kanamycin (13.50 – 21.15mm), followed by butanol partition (14.07 – 16.92 mm). After activity guided fractionation and purification using SPE, the results from UPLC/MS and UPLC/MS/MS revealed that the potential antimicrobial compounds obtained from the fraction were epigallocatechin and its derivatives, benzoic acid derivative, xanthone derivative and 4 tentatively nitrogen containing compounds remain unknown (**Figure 1**). The loading of water partition on the SPE using 5% methanol successively washout primary metabolites especially sugar and protein/peptide while with the subsequent elution of methanol: acetonitrile (1:1) to elute the secondary metabolites. Water partition displayed the highest bactericidal activity against *B. cereus* with the MIC and MBC at 6.25 mg/ml and 9.25mg/ml respectively. However, it took a slightly longer time (18 hours) to achieve MBC (more than 3 log reduction) for *L. monocytogenes* at the concentration of 26.50 mg/ml. Butanol fraction showed MIC value of 8.25 mg/ml and 18.5 mg/ml against *B. cereus* and *S. aureus*, respectively, followed by *L. monocytogenes* (25.00 mg/ml) and *V. parahaemolyticus* (31.50 mg/ml). In food model system, treatment with 5% water partition showed highest inhibition against *S. aureus* on chicken meat that kept at both  $-18^\circ\text{C}$  and  $4^\circ\text{C}$ . Five percent of water partition gave reduction of 1.5 log cfu/g of *S. aureus* as compared to the control, while 2.5% water extract and 5% potassium sorbate only showed one log cfu/g reduction. Nevertheless, it gave slightly higher reduction of *S. aureus* if the meat was kept at higher storage temperature of  $4^\circ\text{C}$ . Meanwhile, *L. monocytogenes* was found much resistant against this natural water extract in both storage temperatures. The overall results suggest the bioactive components obtained from banana inflorescence could serve as potential bio-preservative to control the outgrowth of potential harmful pathogens. However, further toxicological assay and elucidation of the antibacterial mechanisms should be carried out prior to any industrial applications.



Peak No.	Retention Time (min)	Detected Mass (M-H)- Da	Calculated mass (Da)	Molecular formula	i- Fit	Mass Fragments	Identified Compound
1	0.61	305.0700	305.0554	C15 H13 O7	0.3	125, 137, 167, 218	epigallocatechin
2	1.73	609.1318	609.1276	C19 H25 N6 O17	1.9	423, 305, 177	unknown
3	2.01	609.1250	609.1224	C30 H25 O14	0.1	423, 305, 177, 125, 137	prodelphinidin
4	2.27	913.1883	913.1913	C38 H41 O26	1.9	727, 559, 423, 305, 609, 261, 745, 441	epigallocatechin trimer
5	2.58	913.1882	913.1872	C33 H21 N24 O10	1.5	727, 305, 609, 559, 745, 177, 303	unknown
6	2.84	517.1593	517.1602	C10 H13 N24 O3	1.3	175, 193, 134	unknown
7	3.30	273.0431	273.0399	C14 H9 O6	0.6	159, 231, 151, 125	Xanthenes derivatives
8	3.69	570.0683	570.0673	C29 H16 N O12	0.9	172, 216	unknown
9	4.75	417.1558	417.1549	C22 H25 O8	0.3	166, 387, 181, 151	Benzoic acid derivatives

**Figure 1.** Chromatogram of secondary metabolites purified from water partition through SPE Strata-X and tentative compounds identified using LC/MS.