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Research article

Phytochemical fingerprint revealing antibacterial and antioxidant activities of endemic banana cultivars in Southeast Asia

Liang Ying Ee [a,b,1], Bao Hui Ng [a,1], Bao Yi Ng [a], Anna Karen Carrasco Laserna [c], Hui Ting Chu [a,d], Heng Li Chee [e], Sam Fong Yau Li [a,f,*]

[a] Department of Chemistry, College of Humanities and Sciences, National University of Singapore, 3 Science Drive 3, Singapore, 117543, Singapore
[b] Chemical Engineering Program, Physical Science and Engineering Division, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia
[c] Central Instrumentation Facility (Laguna Campus), Office of the Vice Chancellor for Research and Innovation, De La Salle University, 2041 Taft Avenue, Manila, 1004, Philippines
[d] Singapore Institute of Food and Biotechnology Innovation, Agency for Science, Technology and Research, 31 Biopolis Drive, Singapore, 138669, Singapore
[e] Institute of Materials Research and Engineering, Agency for Science, Technology and Research, 2 Fusionopolis Way, Innovis, Singapore, 138634, Singapore
[f] NUS Environmental Research Institute, National University of Singapore, T-Lab Building, 5A Engineering Drive 1, Singapore, 117411, Singapore

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ABSTRACT

This study explores the bioactive secondary metabolite profiles of the peels of three major cultivars of bananas (*Musa acuminata* and *Musa balbisiana*). These cultivars are primarily grown in Southeast Asia and are widely consumed due to their rich nutritional and fiber content. The research utilizes advanced analytical techniques, specifically HPLC-DAD-q-TOF-MS/MS, in conjunction with both univariate and multivariate statistical analyses, to analyze the ethanolic extracts of the banana peels. This study identifies phenolic acids, flavonoids, and proanthocyanidins as significant contributors to the differentiation of the cultivars. The secondary metabolites rutin, chlorogenic acid, and gentisic acid are pinpointed as the key discriminants. Moreover, the research demonstrates a synergistic contribution of certain phytochemicals to the antioxidant and antibacterial properties of the banana peel extracts. The fingerprint profiling tools introduced in this study offer a reliable method for identifying metabolite biomarkers for the discrimination of banana cultivars.

1. Introduction

The pervasive issue of foodborne illnesses, predominantly resulting from the ingestion of food contaminated with microorganisms or toxins, continues to pose significant threats to human health. This contamination can occur at any stage of food processing, rendering it a persistent challenge [1]. Among the various forms of foodborne illnesses, bacterial infections are particularly prevalent due to the ability of bacteria to thrive and multiply in nutrient-rich environments such as food [2]. Antibiotic-resistant pathogenic

* Corresponding author. Department of Chemistry, College of Humanities and Sciences, National University of Singapore, 3 Science Drive 3, Singapore, 117543, Singapore.
E-mail address: chmlifys@nus.edu.sg (S.F.Y. Li).
[1] These authors contributed equally to this work.

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bacteria, including gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Staphylococcus aureus* (*S. aureus*), are of particular concern. The global impact of foodborne illnesses is staggering, with an estimated 600 million individuals affected annually. In Southeast Asia alone, the highest incidence of foodborne contamination results in 175,000 deaths each year. Alarmingly, several *E. coli* isolates have demonstrated resistance to multiple antibiotics, including ampicillin, tetracycline, kanamycin, and chloramphenicol [3]. Similarly, *S. aureus* has been reported to produce a broad spectrum of emetic and non-emetic toxins, including staphylococcal enterotoxins, which have caused large-scale outbreaks in less developed Asian countries [4].

There has been a burgeoning emphasis and extensive literature on waste valorization [5,6], which has led to a substantial corpus of research exploring the utilization of food waste as a natural antimicrobial agent [7]. Citrus fruits, such as oranges and lemons, which are available in abundance, have exhibited exceptional antimicrobial activity, potentially due to high levels of phenolic acids, zinc, and magnesium, which inhibit bacterial growth [8,9]. In the context of Southeast Asia, waste from durian seeds and husks (*Durio zibethinus*) has been effectively employed as a meat preservative, retarding browning and decay [10]. However, processing durian seeds and husks, despite being native to Southeast Asia, remains a complex task that could escalate the cost of antimicrobial extract production.

Bananas, one of the most widely consumed fruits globally, provide numerous health benefits, including improvements in blood sugar, digestive, and cardiovascular health [11]. This highly antioxidant fruit also exhibits promising antibacterial activity due to the high levels of flavonoids, polyphenols, tannins, and secondary metabolites, in addition to approximately 3 % potassium content [12]. A study by Rita et al. (2020) examined the antibacterial and antioxidant activities of the banana peel waste from Bali, Indonesia and found over 20 mg of flavonoids (quercetin equivalents) in a gram of dry extract, which could have bonded hydrogen to bacterial proteins, leading to cell lysis [13]. This finding has been supported by other researchers conducting similar studies on locally grown bananas in various Southeast Asian regions [14,15]. Southeast Asia, particularly the Philippines, Indonesia, and Malaysia, remains the largest exporter of bananas, with over 25,000 varieties of two main diploid cultivars, namely *Musa acuminata* (*AA* genome) and *Musa balbisiana* (*BB* genome).

Bananas are taxonomically classified under the *Eumusa* section of the *Musa* genus, with the most prevalent edible clones being (*AA*), (*AAA*), (*AAB*), (*ABB*), (*AAAA*) and (*ABBB*) [16]. Notably, triploid hybrid cultivars, primarily cultivated in Southeast Asia, produce larger fruits, thereby attracting increased consumer demand. Further, *Musa balbisiana* exhibits superior disease resistance and abiotic stress tolerance compared to *Musa acuminata* [17]. The digestibility of bananas is influenced by their maturation stages [18]. In a separate study, transcriptome data derived from tissue samples of diverse cultivars revealed 9857 and 4424 sequences from *Musa balbisiana* and *Musa acuminata,* respectively, each displaying genic microsatellites [19]. Numerous researchers have conducted genetic studies on various banana cultivars, contributing to a database that aids in the breeding of cultivars with enhanced tolerance to both biotic and abiotic stresses [20,21]. However, no direct study has yet identified phytochemical markers that differentiate between cultivars in terms of their antibacterial activity.

In light of the potential range of bioactivities and the utility of distinguishing between different banana cultivars, the present study initially developed a straightforward extraction method to optimize the phytochemical content derived from the banana peels. Subsequently, phytochemical fingerprints of three distinct banana species endemic to Southeast Asia were obtained using high-performance liquid chromatography with diode-array detection (HPLC-DAD) and high-performance liquid chromatography with diode-array detection coupled with quadrupole time-of-flight tandem mass spectrometry (HPLC-DAD-q-TOF-MS/MS) analytical techniques. Finally, a chemometric analysis was performed to statistically differentiate banana cultivar species based on the bioactive compounds contributing to their antibacterial and antioxidant properties.

2. Experimental

2.1. Materials

Apple Banana (*Musa AAB*), Cavendish Banana (*Musa AAA*) and Lady Finger Banana (*Musa AA*), all grown in the Philippines, were purchased from *NTUC FairPrice* supermarket in Singapore. The banana samples were selected based on similar banana peel colouration through visual inspection. Folin & Ciocalteu's phenol reagent (FC, 2N), sodium carbonate (ACS, 99.5 %), sodium nitrite (ACS, 97.0 %), aluminum chloride (ReagentPlus®, 99 %), sodium hydroxide pellets (ACS, 97.0 %), 2,2-di(4-tertoctylphenyl)-1-picrylhydrazyl (DPPH, free radical), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, 98 %), ferric reducing antioxidant power (FRAP) assay kit containing 2,3,4-tris(2-pyridyl)-s-triazine (TPTZ) reagent, butylated hydroxytoluene (BHT, 99 %), resazurin sodium salt (BioReagent), sterile phosphate-buffered saline (PBS), Mueller Hinton (MH) broth, absolute ethanol (anhydrous), acetonitrile (CAN, HPLC), methanol (HPLC), formic acid (FA, MS) and hydrochloric acid (ACS, 37 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sterile dimethyl sulfoxide (DMSO) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). All other HPLC standards of analytical standard grade were purchased from Sigma-Aldrich. *E. Coli* and *S. Aureus* bacterial strains were cultivated and supplied by A*STAR Singapore Institute of Materials Research and Engineering (IMRE). Ultrapure water (UPW) used was produced by the Milli-Q® water purification system (EMD Millipore Co., Billerica, MA, USA).

2.2. Sample preparation and extraction

The washed and chopped banana peels were placed in a 900 W R888F double grill/convection microwave oven (Sharp Co., Osaka, Japan) for 15 min until completely dried. The dried peels were first blended in a 200 W Cornell® Personal Blender to disintegrate the fibers and obtain banana peel powder [22]. The powder was then vortexed in 80 % ethanol before adjusting its pH to 2.0, 4.0 and 6.0, respectively, using 10 M HCl and placed in the sonicator bath for 45 min at 60 °C. Centrifugation at 5000 rpm was performed thrice

before subsequent filtration and rotary evaporation in dark to obtain nine different banana peel extracts. The optimal sonication duration and temperature was adapted from previously reported literature [23–26]. These extracts are labeled A2, A4, A6, C2, C4, C6, L2, L4, L6; A, L and C are abbreviated for the Apple Banana, Cavendish Banana, and Lady Finger Banana, respectively, while 2, 4, 6 refer to the pH at which the banana peels were extracted. Banana peel extracts contained in glass vials were wrapped in aluminum foil and stored in a −20 °C freezer before further analysis.

2.3. Determination of total phenolic acids, flavonoids and proanthocyanidins

Spectrophotometric microplate analyses were performed with Synergy™ H4 (BioTek Instruments Inc., Winooski, VT, USA) to quantify total phenolic acids, flavonoids and proanthocyanidins using gallic acid, quercetin and catechin as standards, respectively. These standards were first prepared in a 500 mg L^{-1} stock solution and then serially diluted with 80 % methanol to 10, 20, 40, 60, 80, 100, 150, 200, and 300 mg L^{-1} for linear calibration, according to standard protocols reported elsewhere [27,28]. Analyses were conducted using 350 mg L^{-1} banana peel extracts. The total phenolics assay involved introducing 20 μL of the diluted extract/standard/control into a 96-well plate, followed by the addition of 50 μL of 2N FC reagent. The mixture was left in the dark for 6 min before adding 80 μL of 7.5 % w/v Na_2CO_3 solution. After a 2 h incubation period in the dark, absorbance was measured at 765 nm after a 60 s shake. The total flavonoids assay required 100 μL of UPW, 10 μL of 50 g L^{-1} $NaNO_2$, and 25 μL of the diluted extract/standard/control. After a 5 min dark incubation, 15 μL of 100 g L^{-1} $AlCl_3$ was added. Following another 6 min incubation in the dark, 50 μL of 1 M NaOH and 50 μL UPW were added. Absorbance was measured at 510 nm after a 30 s shake. Lastly, the total proanthocyanidins assay involved mixing 25 μL of the diluted extract/standard/control with a 4 % vanillin-methanol solution. After adding 75 μL of 32 % HCl and mixing, the mixture was left in the dark for 15 min. Absorbance was then measured at 500 nm after a 30 s shake.

2.4. Antioxidant activity assay

DPPH radical scavenging assay, ABTS free radical assay and FRAP were conducted on the banana peel extracts at 10, 30, 50, 100, 250, 500 and 1000 mg L^{-1} for both kinetic studies and equivalence quantification. BHT (abbreviated as S) was used as positive control while 80 % methanol was used as the negative control with the reagents. Finally, the analyses were similarly performed in the BioTek Synergy™ H4 microplate reader measuring absorbance at 517 nm (DPPH assay), 734 nm (ABTS assay) and 593 nm (FRAP assay).

2.5. Antibacterial activity assay

The microtiter plate-based resazurin antibacterial assay was used to evaluate the antibacterial activity of extracts. In brief, a 1000 mg L^{-1} resazurin solution was prepared and kept in dark in the fridge at 4 °C, to be used within a week. The A2, C2 and L2 extracts were dissolved in 10 % sterile DMSO to make working stock solutions of 50 mg mL^{-1}. Then, 50 μL of stock solution was mixed with 50 μL of sterile PBS. Appropriate dilution was done before the addition of 30 μL MH broth, 10 μL resazurin solution, and 10 μL bacteria suspension (10^5–10^7 CFU mL^{-1} of *E. Coli* and *S. Aureus*). In this assay, BHT was used as positive control while absence of the extracts were used as the negative control. Triplicate plates were prepared for each bacterial type and incubated in CelCulture® CO_2 incubator (Esco Micro Pte. Ltd., Singapore) at 37 °C overnight before measuring fluorescence in a microplate reader with excitation and emission wavelengths at 550 nm and 590 nm, respectively. The bacterial inhibition efficiency (%) was calculated using Eq. S4.1.

2.6. HPLC-DAD-q-TOF-MS/MS analysis

The standards used for targeted HPLC-DAD-q-TOF-MS/MS analysis were as follows: 2,5-dihydroxybenzoic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, isovanillin, vanillin, benzoic acid, isoferulic acid, coumarin, naringin, cinnamic acid, quercetin, gallic acid, catechol, syringic acid, *p*-coumaric acid, *m*-coumaric acid, ferulic acid, rutin trihydrate, salicylic acid, myricetin, luteolin, naringenin, apigenin and chrysin (chemical structures drawn in Fig. S1.1 and molecular descriptors summarized in Table S1.1). The banana peel extracts were prepared at 1000 mg L^{-1} and reconstituted in 50 % methanol.

HPLC-DAD-q-TOF-MS/MS analysis was performed using a TripleTOF 5600+ q-TOF-MS/MS (AB SCIEX, Foster City, CA, USA) coupled with an Ultimate3000 LC system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The column used was a Gemini® NX C18 column (Phenomenex, USA) with dimensions of 2.1 × 150 mm and a particle size of 5 μm, with the temperature maintained at 40 °C. Mobile phases A and B were 0.1 % FA in UPW (A) and 0.1 % FA in ACN (B). The time gradient for the HPLC-DAD-q-TOF-MS/MS analysis can be found in Table S2.1. These optimized parameters were adapted from previously reported analytical methods with slight modifications [29,30]. The q-TOF-MS/MS analyses were done in both positive and negative ionization modes.

For the q-TOF-MS/MS scan, the mass range was set to 100 - 2000 Da, with an accumulation time of 0.2 s. The ion source parameters were as follows: 50 psi for nebulizer gas (GS1) and 50 psi for heater gas (GS2), 25 psi for curtain gas (CUR), ion source heater temperature (TEM) at 500 °C, and ion spray voltage (ISVF) at 5400 V for positive mode and −4500 V for negative mode. The declustering potential (DP) was set at 100 V and the collision energy (CE) to 10 V. Tandem MS (MS/MS) data were acquired using Information Dependent Acquisition (IDA), with the 5 highest intensity ions per cycle subjected to a product ion scan with DP of 80 V, CE of 30 V, collision energy spread (CES) of 10 V and accumulation time of 0.1 s.

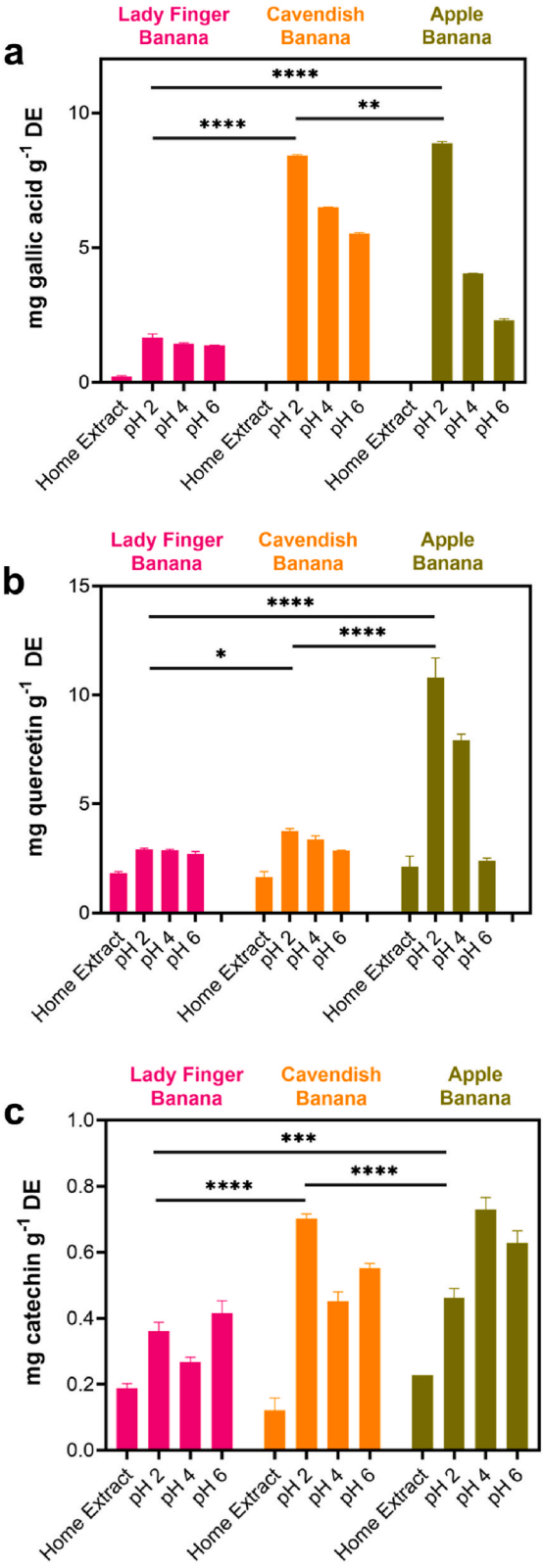

Fig. 1. Content of **a.** phenolic acids, **b.** flavonoids and **c.** proanthocyanidins in the dried banana peel extracts (DE). Mean values of the banana peel extracts from pH 2 extraction were statistically compared for significant difference ($p < 0.05$).

2.7. HPLC-DAD analysis

Using 50 % methanol as reconstitution and dilution solvent, 1, 5, 10, 25, 50, and 100 mg L^{-1} of the standards and 25,000 mg L^{-1} of the extracts were prepared. In the 1200 Series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA), gradient elution was employed via the use of degassed mobile phase A (UPW + 0.1 % FA) and mobile phase B (ACN + 0.1 % FA), according to Table S2.1 at a flow rate of 0.8 mL min^{-1} 10 μL of the respective extracts and standards were injected into the 150 × 4.6 mm Gemini® NX 5-μm C18 110A HPLC column at 40 °C and initial pressure was maintained at 43 bar before analysis. The DAD wavelengths were set at 254, 280 and 365 nm, respectively, to detect the different compounds identified from HPLC-DAD-q-TOF-MS/MS.

2.8. Computational simulation - molecular docking on bacteria proteins

3D structures of *β-Lactamase* (PDB ID 1XPB) from *E. Coli* and Methicillin resistance (*Mec*I) regulatory protein (PDB ID 1OKR) from *S. Aureus* were retrieved from the RSCB protein database (https://www.rcsb.org) and SWISS-MODEL (https://swissmodel.expasy.org/). The pre-existing solvent and ligand molecules were removed before structure minimization was conducted using UCSF chimera. The unbounded structures of proteins with the lowest energy were then stored in PDB format.

Computational docking was performed in PyRx (The Scripps Research Institute, CA, USA), a virtual screening application, and the AutoDock Vina docking software (The Scripps Research Institute, CA, USA) using the previously prepared protein and ligand structures. Throughout the virtual screening, the ligand molecules were flexible while the proteins were kept rigid. Using the binding sites in the protein complexes originally stored from PDB as an estimate, the grid centre for docking was set as X = −12.27, Y = 17.36, Z = 68.27 with the grid box dimensions of 61 x 89 x 84 Å for *β-Lactamase* and X = −24.44, Y = 18.71, Z = −9.63 with the grid box dimensions of 62 x 73 x 40 Å for *Mec*I. Along with the table of binding affinities, and lower and upper bound RMSD values, ligands were saved in their possible orientations and conformations for further visualization.

2D and 3D interactions between the proteins and ligands were then performed using Discovery Studio software (version 3.5, Accelrys Software Inc., San Diego, CA, USA) to identify the interactions between the proteins' amino acids and the ligands. The best conformation of the ligands with favorable interactions with the proteins accounting for lowest non-zero lower bound RMSD value was chosen.

2.9. Statistical analysis

Statistical analyses were performed in GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA) and open-source MetaboAnalyst 4.0 (http://www.metaboanalyst.ca) software packages. ANOVA with post hoc Tukey's test was used to examine the difference in phytochemical contents of the banana peel extracts. Heatmap with Hierarchal Cluster Analysis (HCA) and Principal Component Analysis (PCA) was employed with Pareto scaling for the discrimination work and identification of key phytochemicals that are responsible for the variance in the experimental data set. Furthermore, a supervised partial least squares discriminant analysis (PLS-DA) was performed for discriminative variable selection and predictive model for future works.

3. Results & discussion

3.1. Total bioactive phytochemicals in the banana peel extracts

The banana peel is known to contain a diverse array of phytochemicals, which are typically classified into three major categories: phenolic acids, flavonoids, and proanthocyanidins [31,32], for plant functions such as protection from ultraviolet radiation, defense against pest infestation, and attracting pollinators [33]. Furthermore, a broad spectrum of these phytochemicals are involved in the plant's oxidative reduction process [34]. In this study, the total phytochemicals in the banana peel extracts were quantified using standard calibration curves shown in Fig. S2.1, which confirm the low detection limit and high linearity of the assays.

Fig. 1 and Table S2.2 provide a comprehensive summary of the total phytochemical contents found in the banana peel extracts. The Apple Banana peel extracts exhibit high concentrations of phenolic acids and flavonoids when extracted at pH 2. In contrast, the Lady Finger Banana peel extracts contain the least phytochemicals across all extraction pHs. Generally, it is observed that a more acidic solvent is capable of extracting a larger quantity of phytochemicals. For instance, the total quantity of phenolic acids in the Apple Banana peel extracts decreases from pH 2 to pH 6 extraction (Fig. 1a), as demonstrated by the following sequence: A2 (8.9 mg GAA g^{-1} DE) > A4 (4.0 mg GAA g^{-1} DE) > A6 (2.3 mg GAA g^{-1} DE). This phenomenon can be attributed to the increased polarity of the solvent when more acid is added to the 80 % ethanol extractant. Besides, the high acidity can better break down the lignocellulose and pectin fibers to extract the phytochemicals [35]. A similar trend is also observed in the total flavonoids of the banana peel extracts (Fig. 1b). In contrast, the total proanthocyanidins contents shown in Fig. 1c exhibit fluctuating results, which could be due to their relatively low concentrations (i.e., <0.8 mg CA g^{-1} DE for all extracts). Based on these findings, it can be inferred that the total phytochemical contents follow this descending order: Apple Banana > Cavendish Banana > Lady Finger Banana.

A home extraction of banana peels was performed with 80 % ethanol, dried under the sun, excluding pH adjustment, sonication, centrifugation, filtration, and rotary evaporation. The phytochemical contents were found to be lowest in these extracts, particularly phenolic acids, highlighting the importance of pH adjustment and sonication for effective extraction.

3.2. Evaluation of antioxidant activity of the banana peel extracts

The phytochemical constituents in banana peel extracts were analyzed for their antioxidant potential. Their radical scavenging abilities were assessed using ABTS and DPPH assays, while FRAP assay was used to evaluate their antioxidant effects. BHT, a known antioxidant, was used as a positive control. The spectrophotometric evaluation involved observing the color change of ABTS radical from blue-green to colorless, and DPPH radical from violet to yellow.

The radical scavenging and kinetic results at a fixed extract concentration of 500 mg mL^{-1} are presented in Fig. 2a–d, while kinetic results for varied concentrations of each extract are shown in Fig. S3.1 and Fig. S3.2. Interestingly, the radical scavenging effect demonstrated by the banana peel extracts does not align with the total phytochemical content determined for each extract. For

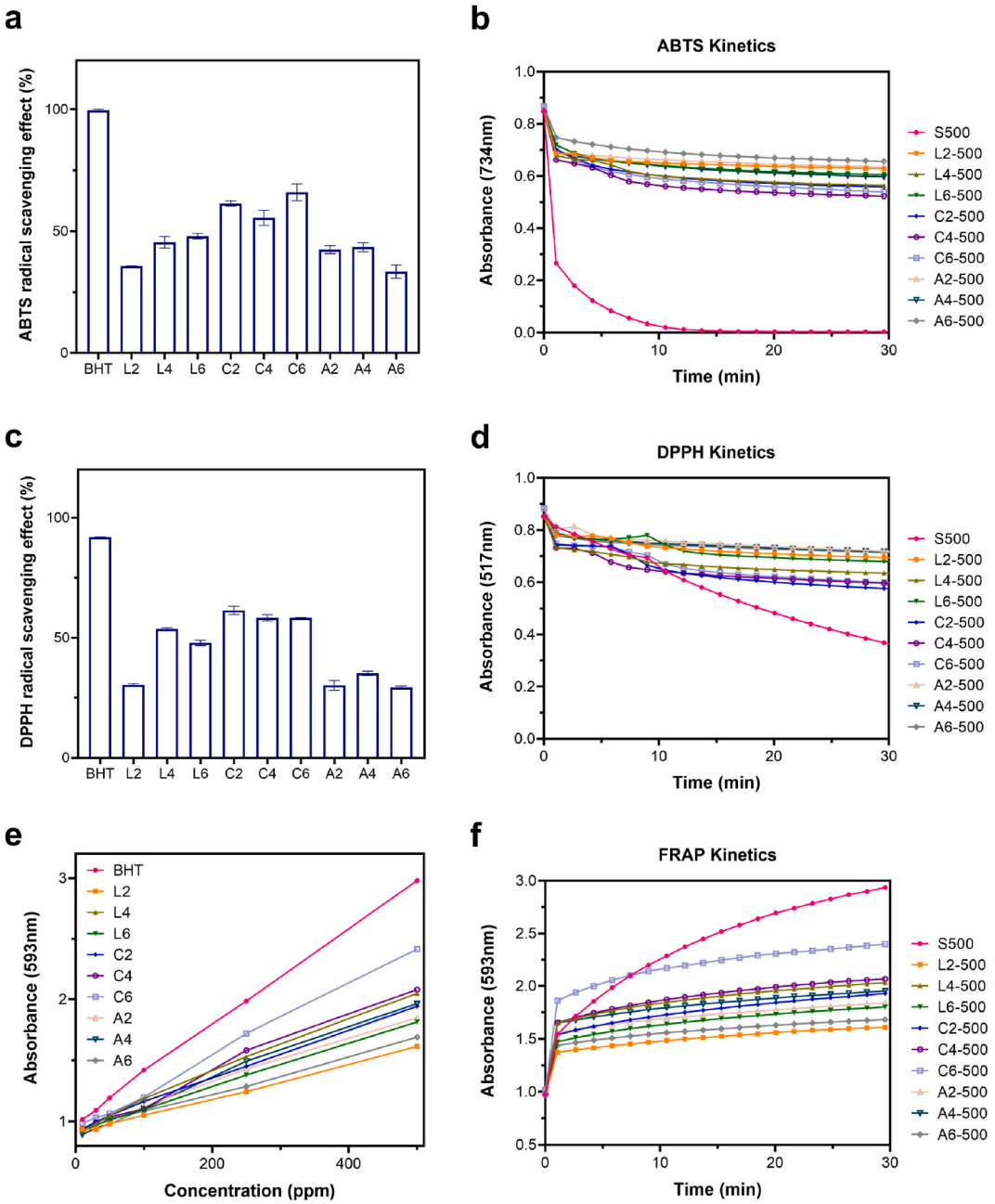

Fig. 2. a. ABTS radical scavenging effect, **b.** ABTS radical scavenging kinetics, **c.** DPPH free radical scavenging effect, **d.** DPPH free radical scavenging kinetics, **e.** FRAP antioxidant effect and **f.** FRAP kinetics of BHT positive control (abbreviated as S) and the banana peel extracts (concentration = 500 mg L^{-1}).

instance, the Apple Banana peel extracts, despite possessing the highest overall phytochemical content, are seen to exhibit the lowest radical scavenging effects of 33.4 ± 3 % (ABTS) and 29.2 ± 0.7 % (DPPH). In stark contrast, the Cavendish Banana peel extracts, at a concentration of 500 mg L^{-1}, exhibit significantly higher radical scavenging effects, as high as 66.0 ± 3 % (ABTS) and 61.4 ± 2 % (DPPH). Furthermore, the ABTS and DPPH radical scavenging kinetics of the Cavendish Banana peel extracts are found to be the most superior. However, no discernible correlation is observed between the antioxidant activity and the pH at which the banana peels were extracted.

The FRAP assay results, depicted in Fig. 2e–f and Fig. S3.3, suggest that all the banana peel extracts have the potential to reduce the ferric-TPTZ complex (colorless) into the ferrous-TPTZ complex (intense blue). Remarkably, at various concentrations, the C6 extract demonstrates the highest reducing power. Moreover, it is found that the initial antioxidant kinetics of the Apple Banana and Cavendish Banana peel extracts are faster than BHT. These observations correspond well with the results from both the ABTS and DPPH radical scavenging assays and could potentially be attributed to the presence of higher concentration of electron-donating polyphenols, phenolic acids, and flavonoids. Based on these findings, it can be inferred that specific compounds could have contributed more significantly to the antioxidant activity of the banana peel extracts, thereby explaining the observed deviation from total phytochemical contents.

Biologically active phenolic acids and flavonoids, mainly polyphenols, in banana peel extract have been linked to changes in gene expression in response to environmental stressors like photo-oxidation [36]. The inherent capacity of these phytochemicals to capture and trap superoxide radicals is attributed to the existence of hydroxyl functional groups [37]. Septembre-Malaterre et al.'s (2016) study found quercetin, resveratrol, and epicatechin as the key antioxidants in bananas [38]. These findings highlight the crucial role of bioactive compounds in giving bananas antioxidant properties. However, the link between phytochemical content variation in

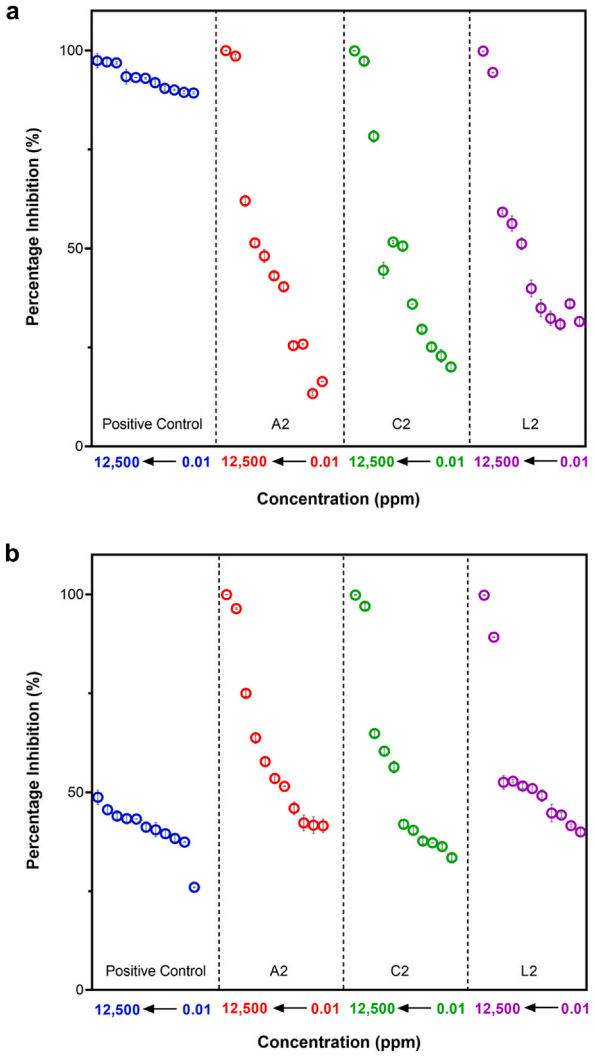

Fig. 3. Antibacterial efficiency of the A2, C2, and L2 extracts at concentrations from 0.01 to 12,500 mg L^{-1} against **a.** *E. Coli* and **b.** *S. Aureus*, and BHT was used as positive control.

different banana cultivars and their antioxidant and antibacterial activity is still not fully understood. Investigating the link between genetic diversity and banana peel extract properties could offer key insights and advance strategies for enhancing antioxidant and antibacterial potentials of banana cultivars. This could have significant implications for agriculture, particularly in improving nutritional value and health benefits of genetically modified bananas.

3.3. Evaluation of antibacterial activity of the banana peel extracts

Banana peel extracts' antibacterial compounds offer a potential natural solution for enhancing microbial safety and food quality. These extracts can inhibit common pathogenic and spoilage microorganisms. This research focuses on their inhibitory and antimicrobial activity against *E. Coli* and *S. Aureus*, with BHT as a positive control. Literature suggests that fruit peel extracts' antibacterial activity is due to secondary metabolites like phenolic acids, flavonoids, tannins, and terpenoids [39,40]. Therefore, for the purpose of this study, the banana peel extracts from pH 2 extraction (specifically labeled as A2, C2, and L2) were selected for the antimicrobial test, given their higher phytochemical content.

Observations from Fig. 3a indicate that BHT maintains a relatively high inhibitory activity, well above 89.0 %, against *E. Coli* at concentrations between 0.01 and 12,500 mg L^{-1}. However, in contrast, all the banana peel extracts fail to reach an 80 % inhibitory threshold when their concentrations are at 780 mg L^{-1}. The inhibitory activity of the extracts increases to around 95 % when their concentrations are elevated above 3125 mg L^{-1}. A key parameter, the minimum inhibitory concentration at which 50 % of the isolates are inhibited (MIC_{50}), for the C2 extract is observed around 12 mg L^{-1}. This is notably lower than the MIC_{50} of the A2 and L2 extracts,

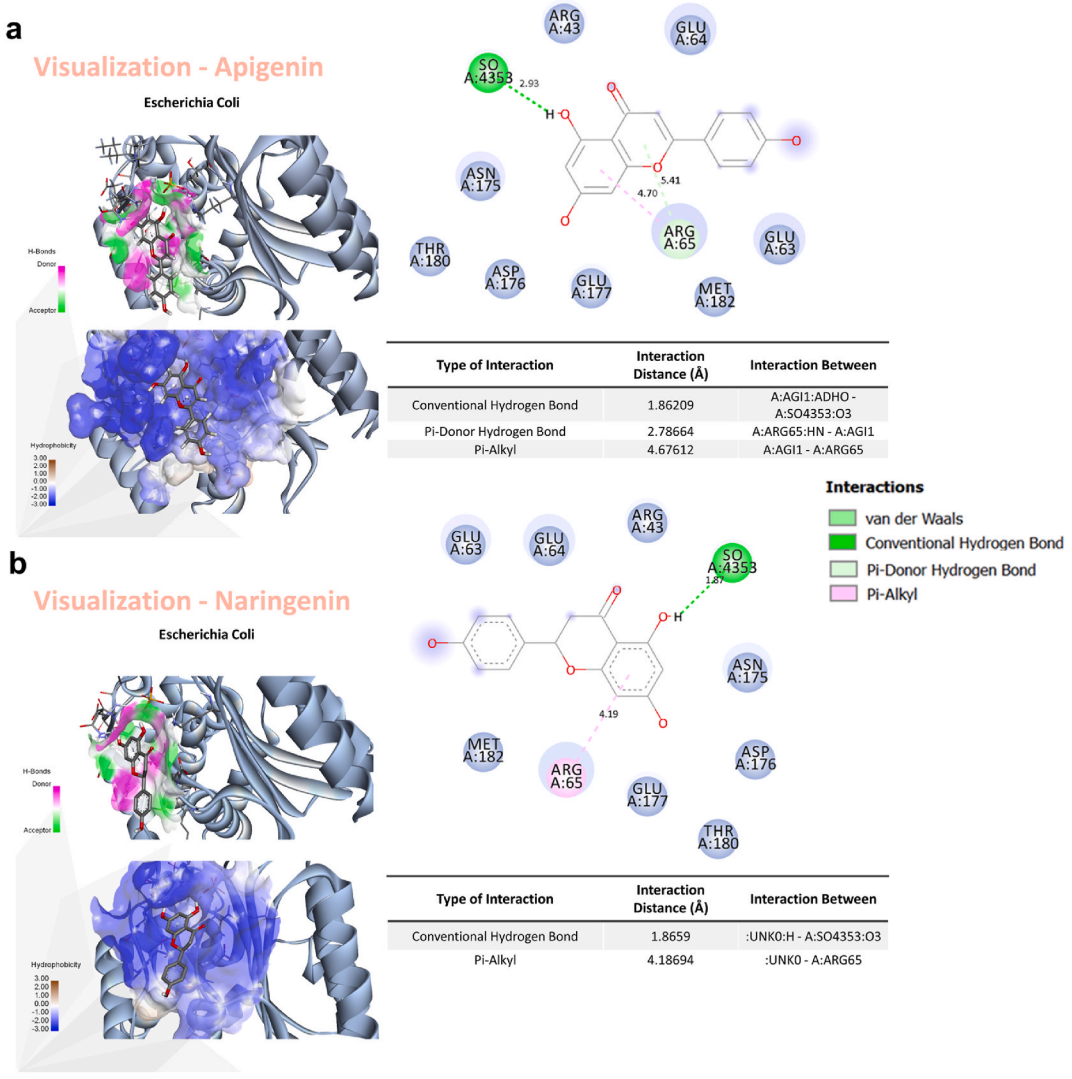

Fig. 4. Molecular docking and visualization (both 2D and 3D) of **a.** apigenin and **b.** naringenin on *E. Coli* β-lactamase protein. The type of interactions between the bioactive phytochemicals and protein are tabulated in the figure.

which are approximately 58 mg L^{-1} and 45 mg L^{-1}, respectively. This may be attributed to the difference in the concentrations of certain phytochemicals. Previous studies have confirmed that the C2 extract contains significantly higher concentrations of syringic acid (330.5 μg g^{-1}) and caffeic acid (209.9 μg g^{-1}), both of which are widely recognized as antibacterial bioactive molecules.

In the case of *S. Aureus*, it is observed that BHT exhibits an unsatisfactory inhibitory effect against this Gram-positive bacterial strain (Fig. 3b). In contrast, the banana peel extracts demonstrate a superior antibacterial effect against *S. Aureus*, particularly at higher concentrations. For instance, all extracts exhibit an inhibitory activity above 90 % at a concentration of 3125 mg L^{-1}, compared to a mere 45.6 % inhibitory activity exhibited by BHT at the same concentration. The MIC_{50} of the A2 extract is determined to be at 2.7 mg L^{-1}, while that of the C2 and L2 extracts are 36 mg L^{-1} and 12 mg L^{-1}, respectively. This disparity may be attributed to the significantly higher concentrations of rutin (119.8 μg g^{-1}), luteolin (303.3 μg g^{-1}), gentisic acid (4445.7 μg g^{-1}), chlorogenic acid (178.5 μg g^{-1}), catechin (711.7 μg g^{-1}), naringenin (384.8 μg g^{-1}), and vanillin (353.9 μg g^{-1}) in the A2 extracts, which are generally much lower in concentrations in the C2 and L2 extracts. Previous studies suggest bioactive molecules demonstrate strong antibacterial activity against Gram-positive bacteria [41,42]. The individual contribution of each bioactive molecule to the overall antibacterial activity requires further investigation, possibly through molecular docking studies.

Banana peel extracts' antimicrobial efficacy is not due to one phytochemical, but a synergistic effect of various compounds. This agrees with Oroojalian et al.'s (2010) findings of differential sensitivity between Gram-positive and Gram-negative bacteria to these extracts [43]. Gram-negative bacteria's unique structure, with an outer membrane around the cell wall, may hinder the diffusion of hydrophobic compounds, explaining their reduced sensitivity to bioactive extracts. In contrast, Gram-positive bacteria's simpler

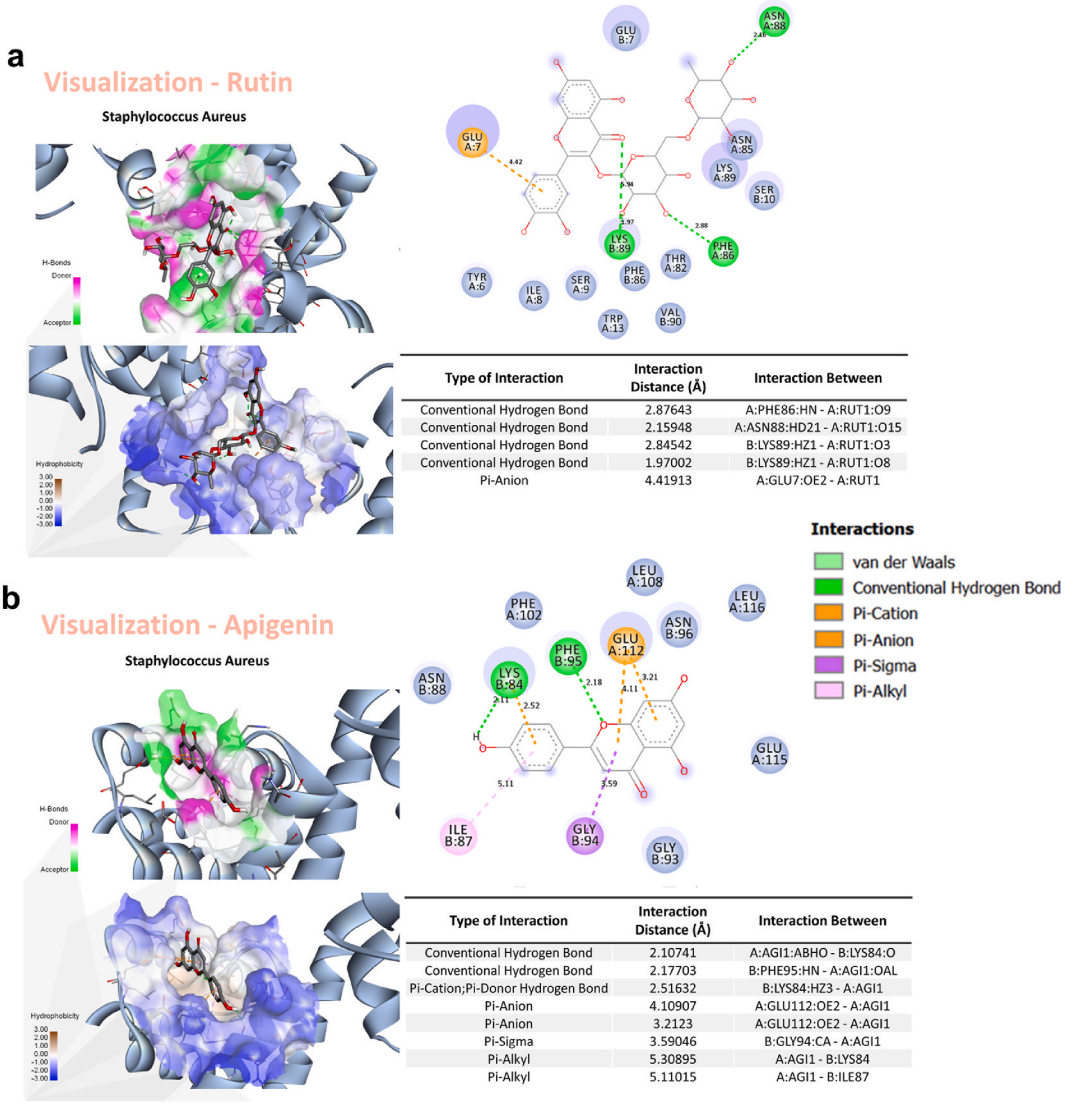

Fig. 5. Molecular docking and visualization (both 2D and 3D) of **a.** rutin and **b.** apigenin on *S. Aureus MecI* protein. The type of interactions between the bioactive phytochemicals and protein are tabulated in the figure.

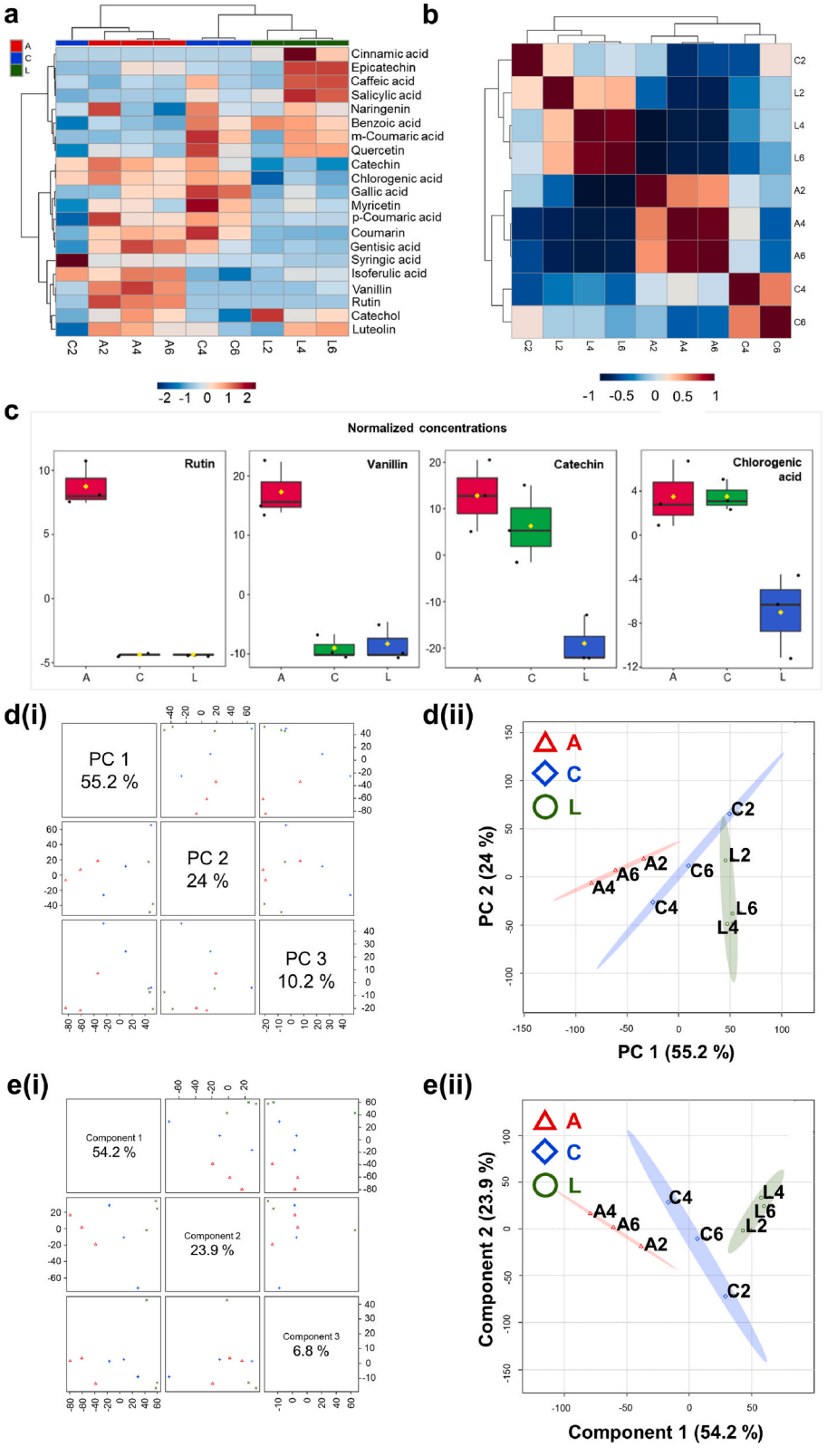

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Fig. 6. Heatmap combined with Euclidean clustering **a.** between the phytochemical contents and the banana peel extracts and **b.** within banana peel extracts; **c.** Statistical box plots for rutin, vanillin, catechin, and chlorogenic acid contents that are significantly different across the banana peel extracts (pH 2); **d(i) – d(ii).** PCA analysis with score plots, and **e(i) – e(ii).** PLS-DA analysis with score plots derived from HPLC-DAD results for discrimination of banana cultivar groups.

structure, lacking an outer membrane, allows direct interaction between hydrophobic constituents and the cell membrane.

Phenolic compounds have bacterial inhibitory properties, but their action mechanisms are often undefined. This study used theoretical calculations and molecular docking simulations to understand these mechanisms. It also emphasized the need to understand the varying sensitivity of bacterial strains to bioactive extracts, influenced by their unique structures. This knowledge is crucial for developing targeted antibacterial treatments.

3.4. Molecular docking of phytochemical on bacterial proteins

Molecular docking was used in this study to assess the binding potential of identified phytochemicals. This allowed for evaluation of interactions between the molecules and targeted bacterial proteins. The two molecules with the highest binding affinity were further analyzed. The visual representations of these interactions are depicted in Figs. 4 and 5, while the corresponding docking scores are presented in Table S4.1 and Table S4.2.

Upon screening all the molecules, it is observed that only 9 phytochemicals exhibit a favorable binding affinity (≤ -6.0 kcal mol^{-1}) towards the *E. Coli β-lactamase* protein. This protein is chosen for study due to the prevalence of *β-lactam* resistant *E. Coli* bacterial strains and its evident threat to human health, which allows our studied phytochemicals to exhibit potentially synergistic inhibitory effects with antibiotics [44,45]. Of particular interest are apigenin and naringenin, which both demonstrate a binding affinity of -6.9 kcal mol^{-1}. This result is anticipated due to the similar chemical structures of these two phytochemicals. The binding of these molecules to the protein is characterized by hydrogen bonding and pi-alkyl interactions with the SO4353 and ARG65 residues, respectively (Fig. 4a and b). The bond distance for the hydrogen bonding is measured at 1.86 Å, while the pi-alkyl interactions, which are weaker, have a bond distance of above 4 Å. Additionally, the ARG65 amino acid residue is found to have pi-donor hydrogen bonding with apigenin, with a bond distance of 2.79 Å. This data suggests that the interactions between the bioactive molecules and the *E. Coli β-lactamase* protein are predominantly hydrophilic in nature. In addition to the flavonoids, caffeic acid also demonstrates a high binding affinity of -6.6 kcal mol^{-1} towards *β-lactamase* protein. This observation may provide an explanation for the lower MIC_{50} of the C2 extract.

In the case of *S. Aureus Mec*I protein, which takes part in regulating the expression of methicillin resistance [46,47], only 4 phytochemicals are found to have a binding affinity below -6.0 kcal mol^{-1}. These are, in a descending order of affinity, rutin (-7.4 kcal mol^{-1}) > apigenin (-6.6 kcal mol^{-1}) > catechin (-6.2 kcal mol^{-1}) > quercetin (-6.2 kcal mol^{-1}). The A2 extract is found to have notably high concentrations of rutin and catechin, which may account for its relatively low MIC_{50} when compared to the C2 and L2 extracts. In-depth examination of the interactions between rutin and the *Mec*I protein (Fig. 5a) reveals extensive hydrogen bonding with the PHE86, ASN88, and LYS89 amino acid residues, with bond distances ranging from 1.97 Å to 2.88 Å. A weaker pi-anion interaction is also observed between rutin and the GLU7 residue, with a bond distance of 4.42 Å. Apigenin, on the other hand, demonstrates more hydrophobic interactions with the *Mec*I protein (Fig. 5b). Alongside hydrogen bonding with LYS84 and PHE95 residues, weaker pi-anion, and pi-sigma interactions with GLU112 and GLY94 residues are observed. Pi-alkyl interactions with LYS84 and ILE87 residues, with bond distances exceeding 5 Å, further contribute to the hydrophobic docking. The strong docking of the rutin and apigenin flavonoids may, thence, provide an explanation for the superior antimicrobial activity of the A2 extracts.

3.5. Chemometrics on discrimination of the banana peel extracts

The representative chromatograms of the banana peel extracts, as obtained through the HPLC-DAD-q-TOF-MS/MS analysis, are depicted in Fig. S5.1 and Fig. S5.2 for both negative and positive modes, respectively. This targeted analysis approach has facilitated the identification of the phytochemicals that are commonly reported to be present in high concentration in the banana peel extracts [48], with mass spectra of compounds and findings being comprehensively summarized in Fig. S5.3 and Table S5.1, respectively. In total, 21 targeted bioactive compounds have been identified. These compounds belong to a diverse range of phytochemical categories. They include benzoic acid, catechol, cinnamic acid, phenolic acids (caffeic acid, chlorogenic acid, gallic acid, gentisic acid, isoferulic acid, *m*-coumaric acid, *p*-coumaric acid, salicylic acid, and syringic acid), flavonoids (luteolin, myricetin, naringenin, quercetin, and rutin), proanthocyanidins (catechin and epicatechin), and other derivatives (coumarin and vanillin). The complex peel extracts is known to contain many more other compounds that warrant a more systemic investigation [49], the targeted screening in this case is suffice to identify those that are significantly high in concentration notwithstanding.

These identified phytochemicals were subsequently quantified at different wavelengths (254, 280, and 365 nm), which shows good separation of peaks in the chromatograms (Fig. S6.1). Moreover, calibration curves of high correlation coefficients have been established for all phytochemical standards, as depicted in Fig. S6.2-S6.4. Finally, the HPLC-DAD chromatograms at respective wavelengths (Fig. S6.5-S6.7) have enabled us to determine the concentrations of individual phytochemicals in the banana peel extracts, wherein these concentrations are summarized in Table S6.1.

Heat maps and average clustering initially categorize banana peel extract results based on phytochemical quantities. Group similarities are assessed via Euclidean distance [50]. The resulting heat map in Fig. 6a indicates that the distribution of phytochemicals

based on HCA has facilitated the grouping of the banana peel extracts into two main groups. The Euclidean distance shows that all the banana peel extracts at pH 4 and pH 6 are similar and belong to the same subgroups. For example, the L4 and L6 extracts have relatively similar concentrations of phytochemicals such as luteolin, epicatechin, caffeic acid, and quercetin. Interestingly, the C2 extract is distinct from the other extracts and shows great deviation from the C4 and C6 extracts. It is more closely related to the Apple Banana extracts due to their coherence in isoferulic, catechin, and chlorogenic acid contents.

The clustered sample correlation matrix depicted in Fig. 6b illustrates the relationship amongst the various extracts, with those demonstrating a close association (as indicated by a warm map) displaying Pearson correlation coefficients nearing 1, while those that are distinctly differentiated (as indicated by a cool map) display Pearson correlation coefficients approaching −1. This correlation matrix provides a comprehensive overview of the interrelationships among the various extracts under investigation. A more detailed examination of the matrix reveals pronounced disparities in the phytochemical contents between the peel extracts of the Apple Banana (*Musa AAB*) and Lady Finger Banana (*Musa AA*). On the contrary, the phytochemical content of the Cavendish Banana (*Musa AAA*) peel extracts exhibit less differentiation when compared to the other two cultivars.

To further elucidate these relationships, a univariate analysis was conducted on the phytochemicals that exhibit the most significant differentiation. The normalized concentrations of rutin, vanillin, catechin, and chlorogenic acid are visualized using box plots, as shown in Fig. 6c. The Apple Banana peel extracts are found to be markedly differentiation from the Lady Finger Banana and Cavendish Banana peel extracts, primarily due to the significantly lower concentrations of rutin and vanillin in the latter two cultivars. In contrast, the Apple Banana peel extracts, across different pH levels, contain an average of 106.4 ± 12 μg g^{-1} of rutin and 376.5 ± 60 μg g^{-1} of vanillin. Conversely, the Lady Finger Banana peel extracts are discriminated against the other two cultivars based on their catechin and chlorogenic acid contents, with significantly lower average concentrations of both phytochemicals (below 122 μg g^{-1}) across the different extraction pH levels.

Statistical differentiation of the banana cultivars is further supported by ANOVA ($p < 0.05$) and post-hoc Tukey's test, which were conducted based on the concentrations of phenolic acids, flavonoids, and proanthocyanidins (Fig. S6.8a-g). Homemade banana peel extracts fail to differentiate between cultivars due to low phytochemical concentrations. The three banana cultivars are also indistinguishable based on flavonoids content in pH 6 extracts. However, phenolic acids show significant variation and can distinguish cultivars with high confidence. Despite being from the same cultivar, most extracts show statistically significant differences in the concentrations of four selected phytochemicals.

The discrimination of the individual banana peel extracts based on the phytochemical content is further enhanced through multivariate analyses, such as PCA and PLS-DA. In addition to the phytochemicals identified in the univariate analysis, gentisic acid, syringic acid, salicylic acid, and epicatechin are found to be well separated in the PCA biplots (Fig. S6.9). Generally, gentisic acid is found to be higher in concentration in the A4 extract, while syringic acid is found in higher concentration in the C6 extract. All extract groups are well separated by the first two principal components, which explain 79.2 % of the total variability (Fig. 6d(i)). Both PC 1 and PC 2 are able to differentiate the Apple Banana from the Cavendish Banana and the Lady Finger Banana. Similarly, the Cavendish Banana and the Lady Finger Banana can be somewhat differentiated, albeit with slight overlap, as shown in Fig. 6d(ii). The discrimination is further improved by PLS-DA, a supervised method that maximizes the covariance between measured data and response variables. Fig. 6e(i) shows that 78.1 % of total variability is explained by the first two components in the PLS-DA analysis. This time, all three banana cultivars are distinctively discriminated against, as can be seen in Fig. 6e(ii). Furthermore, the weighted sum of absolute regression coefficients from the PLS-DA analysis align with the PCA biplots result, indicating that gentisic acid, salicylic acid, and epicatechin are the top three discriminating phytochemicals to differentiate the extracts from various pH (Fig. S6.10).

The phytochemical composition of banana peel extracts from various cultivars was thoroughly examined using statistical analyses such as HCA, PCA, and PLS-DA. This study aimed to differentiate the phytochemical profiles and cultivars. Numerous studies have previously focused on the genomic characterization of banana cultivars, from diploid to tetraploid [51]. However, these genomic approaches are often associated with significant cost and complexity. As an alternative, many studies also incorporated aroma and odor analysis using gas chromatography as a means of cultivar discrimination [52,53]. However, this method posed its own challenges. Common triploid banana cultivars like *Musa AAA* and *Musa AAB* show high similarity in taste and odor, making cultivar discrimination difficult. Gas chromatograms also face challenges with high-resolution separation. Despite available liquid chromatography data on bananas, it has not been used for cultivar discrimination. This study provides insights into the phytochemical content of different banana cultivars and successfully distinguishes them based on their bioactive compound profiles.

Specifically, this study identifies rutin, chlorogenic acid, and gentisic acid as key discriminatory compounds for several reasons. Firstly, rutin was exclusively found in the Apple Banana (*Musa AAB*), distinguishing it from the Cavendish Banana (*Musa AAA*) and Lady Finger Banana (*Musa AA*). Secondly, the high concentration of chlorogenic acid in the triploid cultivars provided a basis for discrimination against the diploid cultivar. Finally, gentisic acid proved to be a crucial compound for differentiating the banana peel extracts across varying pH levels. This study underscores the suitability of liquid chromatography as a highly sensitive and reproducible analytical technique for the cultivar and extract discrimination. Further investigations were conducted to identify and elucidate the phytochemical contributors to the antioxidant and antibacterial properties of the different banana cultivars.

4. Future perspective and conclusions

In this comprehensive investigation, a combination of sophisticated analytical tools was employed to elucidate the phytochemical constituents, antioxidant, and antibacterial properties of three banana cultivars endemic to the Southeast Asian region. These cultivars encompass the Apple Banana (*Musa AAB*), Cavendish Banana (*Musa AAA*), and Lady Finger Banana (*Musa AA*). Our research findings corroborate that the ethanolic extracts from the banana peels of various cultivars, under an acidic pH, are capable of exerting a potent

inhibitory effect on food-borne pathogenic bacteria. Further mechanistic investigations provided insight into the inhibitory mechanisms that these extracts have on *E. Coli* and *S. Aureus* bacterial strains, thereby enhancing our understanding of their antibacterial potential. Crucially, this study identified three phytochemicals – rutin, chlorogenic acid, and gentisic acid – that can effectively differentiate the three banana cultivars. This discovery not only adds to the existing body of knowledge on the phytochemical composition of these bananas but also has significant implications for nutritional strategies aimed at augmenting antioxidant and antibacterial capacities. The presence of these phenolic compounds in banana peels underscores the potential of these often-discarded food wastes as a valuable source of beneficial compounds. However, it is imperative to note that further research is warranted to assess the *in vivo* synergistic effects of these identified phenolic compounds. Such studies would provide a more comprehensive understanding of their absorption and metabolic fates, and their capacity to target cells and modulate gene expression. In addition, the antimicrobial properties of the banana peel extracts shall be further investigated against several other wild-type bacterial strains. These will be instrumental in fully harnessing the health-promoting potential of these phenolic compounds.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Liang Ying Ee: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bao Hui Ng:** Investigation, Data curation. **Bao Yi Ng:** Investigation, Formal analysis. **Anna Karen Carrasco Laserna:** Investigation, Formal analysis. **Hui Ting Chu:** Investigation, Formal analysis. **Heng Li Chee:** Investigation. **Sam Fong Yau Li:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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