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Research article

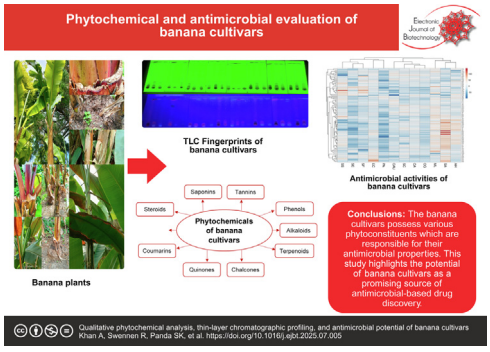
Qualitative phytochemical analysis, thin-layer chromatographic profiling, and antimicrobial potential of banana cultivars ☆

Ajmal Khan [a,b,*], Rony Swennen [c,d,*], Sujogya Kumar Panda [a,e], Liliane Schoofs [a], Walter Luyten [a]

[a] Department of Biology, Animal Physiology and Neurobiology Section, Katholieke Universiteit Leuven, Leuven, Belgium
[b] Center for Animal Sciences and Fisheries, University of Swat, Main Campus Charbagh, Swat, Khyber Pakhtunkhwa, Pakistan
[c] Laboratory of Tropical Crop Improvement, Division of Crop Biotechnics, Katholieke Universiteit Leuven, Leuven, Belgium
[d] International Institute of Tropical Agriculture, Namulonge-Sendusu, Kampala, Uganda
[e] Centre for Biotechnology, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan, Kalinga Nagar, Bhubaneswar, India

GRAPHICAL ABSTRACT

Qualitative phytochemical analysis, thin-layer chromatographic profiling, and antimicrobial potential of banana cultivars



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ABSTRACT

Background: Banana plants possess numerous medicinal properties due to the presence of various phytochemicals. This study aimed to assess the phytochemical profile of the crude extracts of leaf, pseudostem, and corm parts of selected banana cultivars via standard techniques and thin-layer chromatography (TLC) and to evaluate their antimicrobial activities against several food-borne and clinically important human pathogens, including two Gram-positive bacteria, six Gram-negative bacteria, and four yeasts.

Results: The results demonstrated that the Cachaco (41 %), Tereza (38 %), Fougamou (30 %), Pelipita (28 %), Giant Cavendish (26 %), and Kluai Teparot (26 %) cultivars presented significant antimicrobial activity against pathogens compared with Dole (24 %), Namwah Khom (20 %), and Mbwazirume (16 %) cultivars. Moreover, the leaves (40 %) of cultivars extracted in water (61 %) and acetone (55 %) yielded the most active antimicrobial extracts compared with the pseudostem (33 %) and corm (26 %) extracts prepared in ethanol (38 %) or hexane (28 %). Overall, the antimicrobial activities with the lowest 50 % inhibitory concentration (IC_{50}) values, especially those with values less than 200 µg/mL for bacteria and 100 µg/mL for yeasts, were reported in the leaves of Cachaco and Giant Cavendish, followed by

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* Corresponding authors.
E-mail addresses: ajmal.khan@kuleuven.be (A. Khan), rony.swennen@kuleuven.be (R. Swennen).

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different parts of Tereza, Pelipita, and other banana cultivars. Phytochemical analysis and TLC profiling confirmed the presence of various groups of phytochemicals in the extracts of the selected banana cultivars.

Conclusions: This study revealed that the Cachaco, Giant Cavendish, Pelipita, and Tereza cultivars possess significant antimicrobial activity, warranting further bioassay-guided antimicrobial studies for the isolation and identification of bioactive compounds, which could be useful as novel drug candidates with the highest potency.

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1. Introduction

Natural products play a significant role in drug development and the treatment of human diseases [1]. Many currently marketed drugs are directly or indirectly derived from natural sources, and medicinal plants have been used for thousands of years for human health [2,3,4]. Since ancient times, eco-friendly and safe medicines have been derived from a wide range of plants and are in use throughout the world for disease treatment [5,6]. Plants contain phytochemicals and secondary metabolites, many of which have medicinal properties [7,8]. Plant-derived medicines are still used as primary healthcare sources in Asia, Africa, and some parts of America, whereas in other parts of the world, the standard healthcare system has been combined with the plant-based ethnomedicine system. Researchers have characterized many phytochemicals, such as alkaloids, flavonoids, terpenoids, and phenols, and investigated their pharmacological properties and mechanisms of action [1,9,10].

Bananas (*Musa* spp.) are economically important fruit crops, especially in tropical and subtropical regions of the world. Currently, they are cultivated in more than 130 countries, with an approximate global production of 145 million tons. Bananas belong to the family Musaceae and order Zingiberales, which contains three genera, namely, *Musa*, *Musella* and *Ensete* [9,11]. According to recent classification, bananas are classified into two groups: Eumusa and Callimusa. The Eumusa section includes most edible bananas [12]. There are more than 1000 genotypes of banana derived from intra- or interspecific hybridization of the wild diploid ancestral species *Musa acuminata* Colla (A genome) and *Musa balbisiana* Colla (B genome). The domesticated edible bananas are parthenocarpy and are classified as diploids (AA, AB), triploids (AAA, AAB, ABB), or tetraploids (AAAA, AAAB, AABB, ABBB). Sweet bananas belong to the AA, AAA, and AAB groups and are widely cultivated on all continents of the world except Antarctica, whereas cooking bananas belong to the AAA, AAB, ABB or BBB group. However, molecular biology studies have shown that the different banana cultivars are derived from many different genotypes. The important cooking banana cultivars include Cachaco, Saba, Pisang Awak, Pelipita, and Cardaba [13,14,15,16,17]. India is the leading banana producer in the world with an annual production of 30,460,000 tons, followed by China (23,654,000 tons) and Indonesia (7,280,600 tons), according to reports of the Food and Agriculture Organization Statistics [18].

Banana plants not only act as food crops but also possess various medicinal properties. The fruits, peels, leaves, roots, and pseudostems of banana plants have different pharmacological effects. Various parts of banana plants contain phytoconstituents, phytonutrients, phenolic compounds, vitamins and other bioactive chemicals, which have a variety of pharmacological effects [19,20]. The leaves and peels of banana plants possess antioxidant and other biological activities, including antidiabetic, antitumor, antiulcerogenic, antidiarrheal, and antimutagenic properties [21]. Banana plants are a rich source of antimicrobial agents that inhibit the growth of bacteria or fungi. Microbial infections are a prime source of disease among humans and animals and lead to crucial economic losses. The lack of effective vaccines, bacterial resistance to currently available antibiotics, and demand for potent and less expensive antimicrobial products have stimulated the identification and isolation of new and effective antimicrobial compounds from natural resources. Various studies have reported the medicinal uses and bioactivities of the extracts of some banana plant parts against some pathogens [18].

Phytochemical characterization of different plant parts involves detecting and identifying phytoconstituents with potential pharmacological effects. The qualitative and quantitative detection of phytochemicals is performed with different advanced techniques such as high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LCMS), gas chromatography-mass spectrometry (GCMS), and nuclear mass resonance (NMR) spectroscopy. However, such advanced techniques are largely unavailable, unaffordable and cost-effective, impeding drug discovery by many researchers. Alternatively, phytochemical analysis can also be performed via different conventional methods and thin-layer chromatography (TLC), which are considered good choices for preliminary phytochemical screening because of their affordability, fast results and economic feasibility [22,23]. Phytochemical analysis via standard techniques is considered the most convenient method for target-based identification of bioactive compounds in pharmaceutical circles because of its crucial role in drug discovery [24]. Similarly, TLC fingerprinting is a reliable technique approved by the World Health Organization (WHO) and is considered a good tool for separating various phytochemicals on a planar surface on the basis of polarity [25,26]. On the basis of the advantages and disadvantages of different analytical techniques, this study used the conventional phytochemical analysis methods and TLC profiling to demonstrate the potential richness of the bioactive compounds in different parts of banana cultivars to provide valuable insights for future preliminary screening studies.

Most of the previous studies [15,18,27] related to banana cultivars were confined to testing a limited number of banana extracts against a few microorganisms with no consideration of extensive phytochemical analyses. For example, Jouneghani et al. [27] studied the antimicrobial activity of selected banana cultivars against a limited range of human pathogens and one yeast *Candida albicans*. This study screened a more extensive range of microorganisms by targeting not only human pathogens but also foodborne pathogens by specifically adding two important pathogenic bacteria and three yeast species. Researchers previously studied the total phenolic content only, while we also performed qualitative

phytochemical analyses to investigate the presence of secondary metabolites such as alkaloids, steroids, saponins, tannins, phenols, and flavonoids. We also performed TLC to confirm the presence of compounds using R_f values which were lacking in previous studies. Thus, our study validates the work of other researchers while broadening it by adding new insights. Hence, our work is more comprehensive than previous studies and strengthens the scientific foundation on a number of phytochemicals and compounds in bananas with antimicrobial activity.

The present study was designed to detect more phytoconstituents with antimicrobial activities in crude extracts of different plant parts of selected banana cultivars against important human and food-borne pathogens as a foundation for extensive in vitro and in vivo studies for the management of various microbial infections and for antimicrobial drug discovery.

2. Materials and methods

2.1. Plant material and reagents

The leaves, pseudostems and corms of nine adult banana cultivars were collected from the Laboratory of Tropical Crop Improvement greenhouse, KU Leuven, Heverlee Campus, Leuven, Belgium [27]. The KU Leuven's International Transit Centre (ITC) holds the world's largest collection of bananas, with a mission of collaboration to conserve and distribute banana germplasm for research, breeding and food security purposes to various countries of the world. A total of 100 extracts of nine banana cultivars, viz. Giant Cavendish, Tereza, Mbwazirume, Cachaco/Bluggoe, Dole, Pelipita, Namwah Khom, Fougamou/Pisang Awak, and Kluai Teparot were used (Table 1). HPLC-grade n-hexane and acetone were purchased from Sigma-Aldrich Co. (USA), and absolute ethanol was purchased from Fischer Chemicals (UK). Deionized sterile water was produced with a Milli-Q Reagent Water System (MA, USA). Bacto™ peptone and yeast extract were purchased from Lab M Ltd. (Lancashire, UK). DMSO, dextrose, ciprofloxacin, and miconazole were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Extract preparation

The collected parts of nine banana cultivars (i.e., leaves, pseudostems, and corms) were cut into small slices, dried at 70°C in an oven and then powdered with an electric grinder as described by Panda et al. [28] with some modifications. The powdered plant materials (1 g) were extracted with 10 mL of each solvent (acetone, hexane, ethanol or water) in 15 mL sterile Falcon tubes. The tubes were repeatedly vortexed and sonicated four times for 15 min with an interval of 6 h. Afterwards, the tubes were centrifuged for 10 min at 3500 rpm. After centrifugation, the supernatants were transferred to 1.5 mL Eppendorf tubes in 1 mL aliquots, and the solvent was evaporated in a SpeedVac concentrator. The dried extracts (1 mL) were then dissolved at 20 mg/mL in DMSO or water for nonaqueous or aqueous extracts, respectively. The banana extract samples were stored in a 4°C refrigerator until further use.

2.3. Qualitative phytochemical analysis

Qualitative phytochemical analysis was performed to detect the presence or absence of secondary metabolites or phytochemicals in the different plant parts. The plant parts were extracted in the respective solvents, and 10 mg/mL plant extracts were used for phytochemical analysis via conventional methods. In this study, we analyzed a number of phytochemical constituents, such as alkaloids, saponins, steroids, flavonoids, phenols, terpenoids, coumarins, chalcones, tannins, and quinones, of the acetone crude extracts of banana cultivars using conventional assay methods as described in previous studies [22,29,30,31]. The results were denoted as (+++) for very strong positive tests, (++) for strong positive tests, (+) for weak positive tests, and (–) for negative tests of the analyzed phytochemicals.

2.3.1. Detection of alkaloids

The presence of alkaloids was confirmed via Wagner's reagent test. In this test, first, 1 mL of the extract was mixed with 2 mL of 1 % aqueous hydrochloric acid in a steam bath, and the mixture was then treated with a few drops of Wagner's reagent. A positive test was indicated by a cream or reddish-brown color precipitate.

2.3.2. Detection of flavonoids

The alkaline reagent test was employed to detect the presence of flavonoids. One milliliter of the extract was mixed with 2 mL of a 2 % NaOH solution. The mixture was then treated with a few drops of 1 % aqueous HCl. The test was regarded as positive if the solution turned colorless from yellow.

2.3.3. Detection of phenols

The ferric chloride test was employed to detect the presence of phenols. In this test, 1 mL of extract was mixed with 2 mL of 5 % ferric chloride ($FeCl_3$). The test was considered positive upon the development of a blue-green color.

2.3.4. Detection of steroids

The Salkowski test was used to detect the presence of steroids. In this test, 2 mL of the extract was mixed with 2 mL of chloroform, followed by the addition of 100 % concentrated sulphuric acid (H_2SO_4). The test was regarded as positive upon the appearance of a red color in the chloroform layer.

2.3.5. Detection of terpenoids

The Salkowski test was employed to detect the presence of terpenoids. For this test, 5 mL of the extract was mixed with 2 mL of chloroform. This was followed by the addition of 3 mL of 100 % concentrated sulphuric acid (H_2SO_4). The test was considered positive because of the development of a reddish-brown color.

Table 1
Banana cultivars studied.

S. No.	International Transit Centre Code	Cultivar name	Genome group	Subgroup
1	ITC0346	Giant Cavendish	AAA	Cavendish
2	––––––––––	Tereza	AAA	Mutika/Lujugira
3	ITC1356	Mbwazirume	AAA	Mutika/Lujugira
4	ITC0643	Cachaco/Bluggoe	ABB	Bluggoe
5	ITC0767	Dole	ABB	Bluggoe
6	ITC0472	Pelipita	ABB	Unknown
7	ITC0659	Namwah Khom	ABB	Pisang Awak
8	ITC0101	Fougamou/Pisang Awak	ABB	Pisang Awak
9	ITC0652	Kluai Teparot	ABB	Unknown

2.3.6. Detection of coumarins

The NaOH test was employed to detect the presence of coumarins. In this test, 2 mL of the extracts was added to 3 mL of 10 % sodium hydroxide (NaOH) solution. The test was considered positive when a yellow color was observed.

2.3.7. Detection of tannins

The presence of tannins was tested by stirring 2 mL of plant extract with 3 mL of distilled water, followed by the addition of 5 drops of 10 % $FeCl_3$ solution per the protocol of Braymer's test. The test was considered positive upon the formation of a dark blue precipitate.

2.3.8. Detection of saponins

A foaming test was employed to detect the presence of saponins. In this test, 3 mL of the extract was vigorously mixed with 3 mL of distilled water. The test was considered positive after the formation of a foam or froth upon shaking.

2.3.9. Detection of chalcones

The ammonia test was employed to detect the presence or absence of chalcones in the plant extracts by adding 1 mL of the extracts to 2 mL of a 5 % ammonia (NH_3) solution. The test was considered positive upon the development of a reddish color.

2.3.10. Detection of quinones

The concentrated HCL test was employed to detect the presence of quinones in the plant extracts. In this test, 1 mL of the extract was mixed with 2 mL of 98 % concentrated hydrochloric acid (HCl). The test was considered positive upon the development of a green color.

2.4. Thin-layer chromatographic (TLC) profiling

TLC profiling of the selected banana cultivars was performed according to standard methods [25,32,33] with some modifications. The crude extracts (10 mg/mL) of the selected banana cultivars were prepared and dissolved fully through vortexing and sonication. The extract solution (5 μL) was spotted on a TLC plate (2.5 × 7.5 cm) coated with silica gel through a fine-bore glass capillary tube over a marked pencil line on the lower side of the TLC plate. The prepared spotted glass plates were dried at ambient temperature. The sample-loaded plates were then placed in a glass chamber containing a mixture of solvents of the chosen mobile phases, i.e., hexane and ethyl acetate (6:4) or hexane and ethyl acetate (3:7), to optimize the separation of various components of the mixture. The developed plates were dried in a fume hood. The dried plates were visualized under ultraviolet (UV) light at a wavelength of 254 nm. The R_f value of each spot was calculated as indicated in [**Equation 1**]:

$$R_f = \frac{distance\ travelled\ by\ the\ solute}{distance\ travelled\ by\ the\ solvent} \quad (1)$$

2.5. Antimicrobial tests

For antimicrobial tests, hexane (nonpolar), acetone (aprotic polar), ethanol (protic polar) and aqueous (polar) crude extracts of the leaves, pseudostems and corms of all nine banana cultivars were used.

2.5.1. Tested microorganisms

Two Gram-positive bacteria, namely, *Staphylococcus aureus* (ATCC 65385) and *Micrococcus luteus* (DPMB 3), six Gram-negative bacteria, namely, *Escherichia coli* (ATCC 47076), *Aeromonas hydrophila* (ATCC 7966), *Pseudomonas aeruginosa* (PA 01), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (LMG 10472), *Shigella sonnei* (LMG 10473), and four yeast species, namely *C. albicans* (SC 5314), *Candida auris* (OS 299), *Candida glabrata* (ATCC 2001), and *Saccharomyces cerevisiae* (ATCC 7754) were used for their antibacterial and antifungal activities, respectively.

2.5.2. Antimicrobial assay

The antimicrobial assay was performed as described previously [34,35]. In brief, the frozen bacterial and fungal strains were inoculated on LB agar plates and YPD agar plates at 37°C and 35°C, respectively, for overnight incubation. After the growth of colonies, a single colony of the corresponding microorganism was inoculated into aseptic tubes containing 5 mL of LB medium or 5 mL of YPD medium for bacteria or fungi, respectively. The inoculum-containing tubes were then placed in a shaker incubator for 16–24 h. A broth microdilution method was used for the antimicrobial activity test. Each well of a microtiter plate was inoculated with 190 μL of diluted standardized bacterial inoculum (OD = 0.003) and 10 μL of test sample for antibacterial activity or 196 μL of the diluted yeast suspension and 4 μL of extract for antifungal activity. The positive control was ciprofloxacin (200 μg/mL) for bacterial strains and miconazole (250 μg/mL) for yeast species, while 5 % or 2 % DMSO was used as a solvent control for bacteria or fungi, respectively. The plates were incubated for 22–24 h at 37°C in a shaker incubator and then read on a multimode microplate reader at 620 nm (lamp energy: 13,000) via the MikroWin 2000 software package. The relative inhibition (%) was calculated via [**Equation 2**].

$$Relative\ inhibition(\%) = \left(\frac{OD\ of\ test\ sample - OD\ of\ non-inoculated\ sample\ control}{average\ OD\ of\ solvent\ control}\right) \times 100 \quad (2)$$

The IC_{50} (concentration yielding 50 % inhibition) was determined by using GraphPad Prism software. For this purpose, the selected active extracts (20 mg/mL) were serially diluted and tested against the respective bacteria and fungi.

2.6. Data analysis

All the tests were conducted twice, and the average values were calculated. The data from the antimicrobial experiments are presented as % inhibition and were further analyzed via the web tool ClustVis (https://biit.cs.ut.ee/clustvis) to obtain hierarchical clustering heatmaps. The graphs and tables were designed via the Microsoft Excel 97-2003 Worksheet. The IC_{50} values were calculated via nonlinear regression with GraphPad Prism 10.1.1 (323) software (San Diego, CA, USA). Post-hoc analysis was employed to determine the associations between plant parts (leaf, pseudostem, and corm) and significant antimicrobial activity (inhibition values > 50 %). Chi-square tests were used to evaluate whether the type of solvent influenced the proportion of extracts showing significant antimicrobial activity. A p-value of less than 0.05 was considered statistically significant.

3. Results

The present study reports the qualitative phytochemical analysis, TLC profiling, and antimicrobial properties of the leaves, pseudostems, and corms of nine widely cultivated banana cultivars.

3.1. Qualitative phytochemical analysis

Phytochemical analysis of the acetone extracts (25) of nine banana cultivars revealed the presence of various secondary

metabolites, including alkaloids, steroids, saponins, tannins, phenols, terpenoids, flavonoids, coumarins, quinones, and chalcones (Table 2). All the tested extracts exhibited positive results for the majority of these secondary metabolites. The extracts of Cavendish-Pseudostem, Tereza-Leaf, Tereza-Corm, Mbwazirume-Pseudostem, Cachaco-Pseudostem, Dole-Pseudostem, Fougamou-Pseudostem, and Kluai Teparot-Pseudostem contained all the tested types of secondary metabolites. Similarly, Cavendish-Leaf, Cachaco-Leaf, Cachaco-Corm, Dole-Leaf, Dole-Corm, Pelipita-Corm, Namwah Khom-Pseudostem, Fougamou-Corm, and Kluai Teparot-Corm also contained the majority (9/10) of the secondary metabolites tested. Alkaloids were detected at high concentrations in the majority of the extracts, followed by tannins and phenols, whereas chalcones were the least detected secondary metabolites among the tested banana extracts. The presence of a large number of secondary metabolites in the extracts of banana cultivars indicates the presence of a diverse range of compounds potentially responsible for their bioactivities. Thus, further work is needed for the isolation of bioactive compounds from these banana extracts for the development of more effective antimicrobial agents.

3.2. TLC profiling

TLC profiling of the acetone extracts of the test banana cultivars was carried out to separate compounds such as alkaloids, steroids, flavonoids, phenols, terpenoids, and other secondary metabolites. The test extracts were subjected to TLC to develop fingerprints of various phytochemicals. The calculated retention factor (R_f) values of all the test extracts, along with the distance traveled by each spotted extract, are shown in **Table S1**. The different R_f values and observed colors indicate the presence of various groups of secondary metabolites in the test banana cultivars, which could be responsible for the reported bioactivities. We performed TLC with two mobile phases, but some banana extracts still presented highly similar fingerprints and identical R_f values, suggesting the presence of similar chemical classes.

3.3. Antimicrobial properties

The antimicrobial activity of the crude extracts of different parts of banana cultivars is shown in the form of a heatmap in Fig. 1 and in **Table S2** as average percent inhibition values. Several extracts of the nine banana cultivars are significantly active (≥50 % inhibition) against different microbes.

Overall, the Cachaco cultivar showed the highest inhibitory activity (41 %), followed by Tereza (38 %), Fougamou (30 %), Pelipita (28 %), Giant Cavendish (26 %), Kluai Teparot (26 %), Dole (24 %), and Namwah Khom (20 %), while the lowest activity (16 %) was shown by Mbwazirume (Fig. 2).

The most potent extracts with prominent activity against the test microbes varied between 69 % (Cachaco leaf) and 8–17 % (Kluai Teparot corm, Dole leaf, Cachaco corm, Namwah Khom pseudostem), with most plant parts showing inhibitions ranging from 23 to 42 % (Fig. 3). The strong inhibitory activity of various banana cultivars against various microbes indicates the presence of active antimicrobial compounds specifically in the leaves (Cachaco, Giant Cavendish, Fougamou, Tereza and Kluai Teparot), pseudostems (Cachaco and Tereza), and corms (Cachaco and Tereza).

The extracts showing more than 50 % inhibition were selected to determine their IC_{50} values. The majority of the selected banana extracts showed moderate activities, with IC_{50} values between 200 and 1000 μg/mL for bacteria and 100 to 400 μg/mL for fungi, whereas some extracts showed stronger inhibition, with IC_{50} values below 200 μg/mL for bacteria and below 100 μg/mL for fungi. The most potent extracts with the lowest IC_{50} values include Cachaco leaf with IC_{50} values (μg/mL) of 74, 35 [SA], 92, 63, 143 [ML], 68 [SLE], 164 [PA], 46, 104, 69 [AH], 95, 88 [SF], 75 [SS], 59, 54 [CA], 53 [CG], 56, 96 [SC], followed by Cavendish leaf with IC_{50} values of 115, 194, 179 [SA], 90, 191 [ML], 178 [SLE], 151 [AH], 139 [SF], and 190, 178 [SS], as well as Cavendish pseudostem, Tereza corm, Pelipita pseudostem, and Mbwazirum pseudostem (Table 3). This needs to be studied further via bioassay-guided fractionation and isolation methods.

Table 2
Qualitative phytochemical analysis of banana cultivar extracts from leaves, pseudostems, and corms.

S. No.	Banana cultivar Part	Alkaloids	Steroids	Saponins	Tannins	Phenols	Terpenoids	Flavonoids	Coumarins	Quinones	Chalcones
1	Cavendish-Leaf	+	+++	++	+++	+++	++	–	+	+	+
2	Cavendish-Pseudostem	++	+	+	+	++	+	++	++	+++	+
3	Cavendish-Corm	++	+	–	++	+	++	+++	–	+++	–
4	Tereza-Leaf	+++	+++	++	+++	++	+++	+	++	+	+
5	Tereza-Pseudostem	+	+	+	+	++	++	+++	–	–	–
6	Tereza-Corm	++	+++	+	++	+	++	++	+	++	+++
7	Mbwazirume-Pseudostem	++	+	++	+	+	++	++	++	+	++
8	Cachaco-Leaf	++	+	+	+	+	++	+++	++	++	–
9	Cachaco-Pseudostem	+++	+	++	+	+	+	+++	+	+	+
10	Cachaco-Corm	+++	++	++	++	+++	+++	+++	+++	++	–
11	Dole-Leaf	+	+++	+++	+++	++	++	–	++	++	+
12	Dole-Pseudostem	+++	++	++	++	++	++	+	++	+++	+
13	Dole-Corm	+	+	+++	+	+	++	+++	++	+++	–
14	Pelipita-Leaf	+++	+++	–	++	++	++	–	+++	++	+
15	Pelipita-Pseudostem	++	–	+	+	+	–	+++	+	++	–
16	Pelipita-Corm	+	+	–	+	+	++	++	+	+	+
17	Namwah Khom-Leaf	++	+++	+	++	++	++	–	++	–	+
18	Namwah Khom-Pseudostem	++	+	–	+	++	+	++	+	+	+
19	Namwah Khom-Corm	++	–	++	++	++	–	+++	+	+++	–
20	Fougamou-Leaf	+++	+++	+	+++	+++	+++	–	++	++	–
21	Fougamou-Pseudostem	+	++	+	+	+	+	++	++	++	+
22	Fougamou-Corm	+	+	++	++	+	+	+++	+	++	–
23	Kluai Teparot-Leaf	+++	+++	+	+++	+++	+++	–	++	–	–
24	Kluai Teparot-Pseudostem	++	+++	+	+	++	++	+	++	+++	+
25	Kluai Teparot-Corm	+	+	++	+	+	+	++	+	+++	–

Note: +++ = very strong positive test, ++ = strong positive test, + = weak positive test, – = negative test.

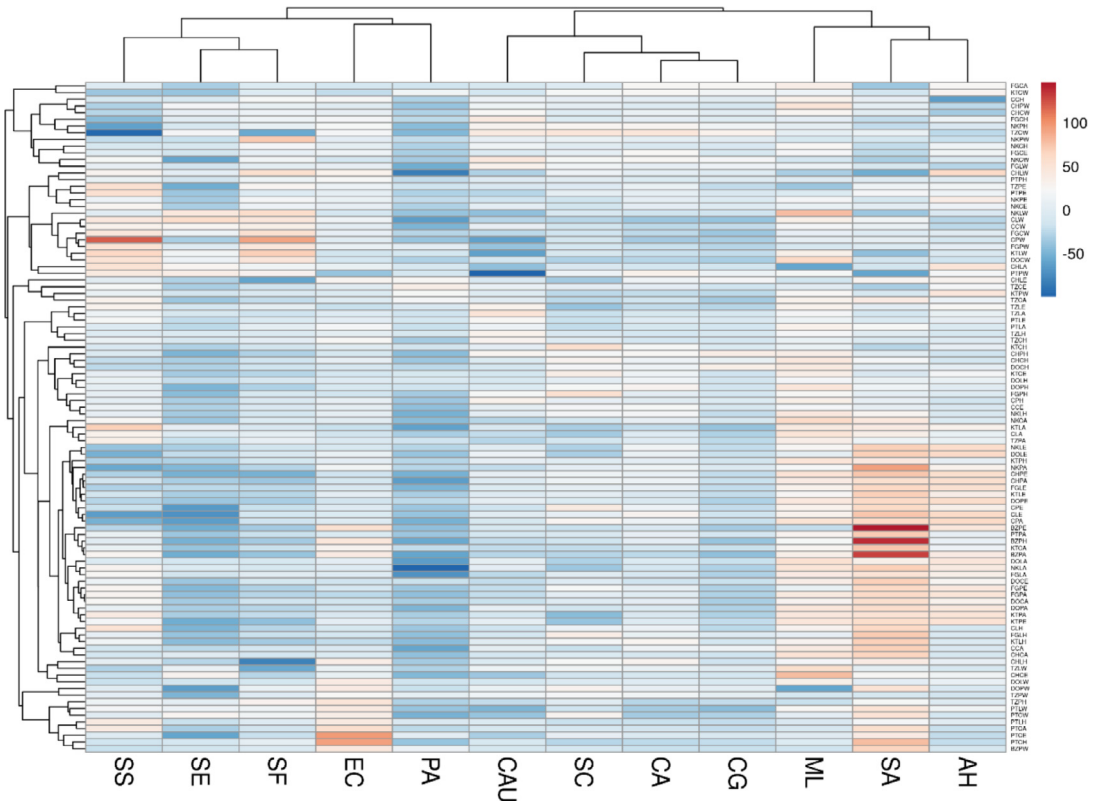

Fig. 1. Heatmap of the antimicrobial activity of extracts from leaves, pseudostems and corms of various banana cultivars. Legends on the right: C-Cavendish; TZ-Tereza; BZ-Mbwazirume; CH-Cachaco; Do-Dole; PT-Pelipita; NK-Namwah Khom; FG-Fougamou; KT- Kluai Teparot; L-Leaf; P-Pseudostem; C-Corm; H-Hexane; A-Acetone; E-Ethanol; W-Water. Legends on the X-axis: SS-*Shigella sonnei*; SE-*Salmonella enteritidis*; SF-*Shigella flexneri*; EC-*Escherichia coli*; PA-*Pseudomonas aeruginosa*; CAU-*Candida auris*; SC-*Saccharomyces cerevisiae;* CA-*Candida albicans*; CG-*Candida glabrata*; ML-*Micrococcus luteus;* SA-*Staphylococcus aureus*; AH-*Aeromonas hydrophila.*

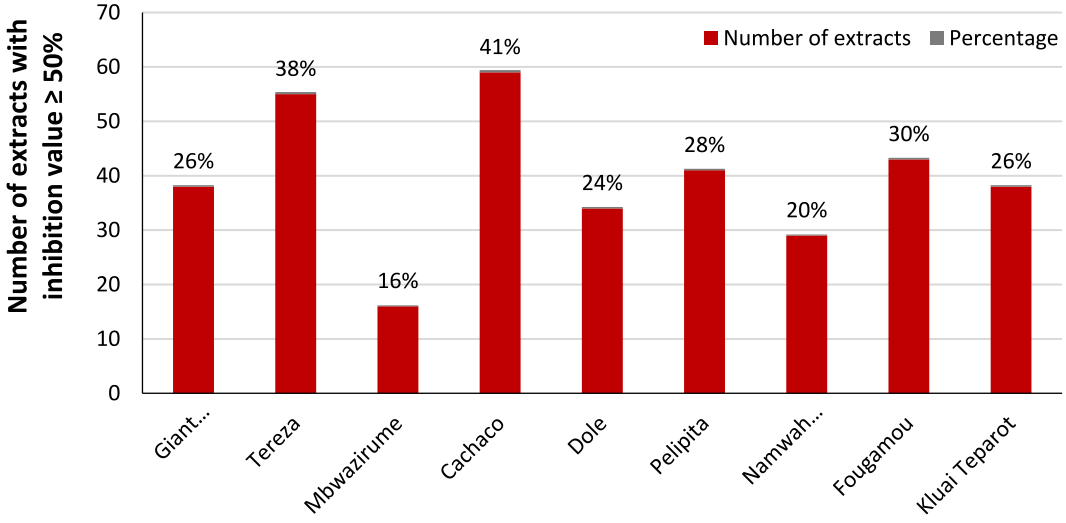

Fig. 2. Antimicrobial activity of extracts from any plant part (leaf, pseudostem or corm) of each banana cultivar. Note: The results from all plant parts are pooled per banana cultivar, and the % represents the percentage of the number of extracts with an inhibition value ≥ 50 % divided by the total number of extracts of the respective banana cultivar tested against the panel of test microbes.

On the basis of the susceptibility of microbes to the test extracts, the bacterium *M. luteus* was the most susceptible (inhibited by 63 % of the extracts), followed by *S. aureus* (59 %), whereas *P. aeruginosa* was the least susceptible (6 %). Among the yeasts, *S. cerevisiae* was the most susceptible (28 %), and *C. glabrata* was the least susceptible (12 %) to the different test extracts (Fig. 4). The susceptibility of methicillin-resistant *S. aureus* (WHO high-priority pathogen) to extracts of different banana cultivars, especially those from Cachaco and Giant Cavendish, highlights the potential to isolate bioactive compounds from these banana cultivars as potential sources of novel drugs against *S. aureus* and other pathogens.

Extracts from different plant parts of several banana varieties exhibited potent antibacterial activity, with an inhibition

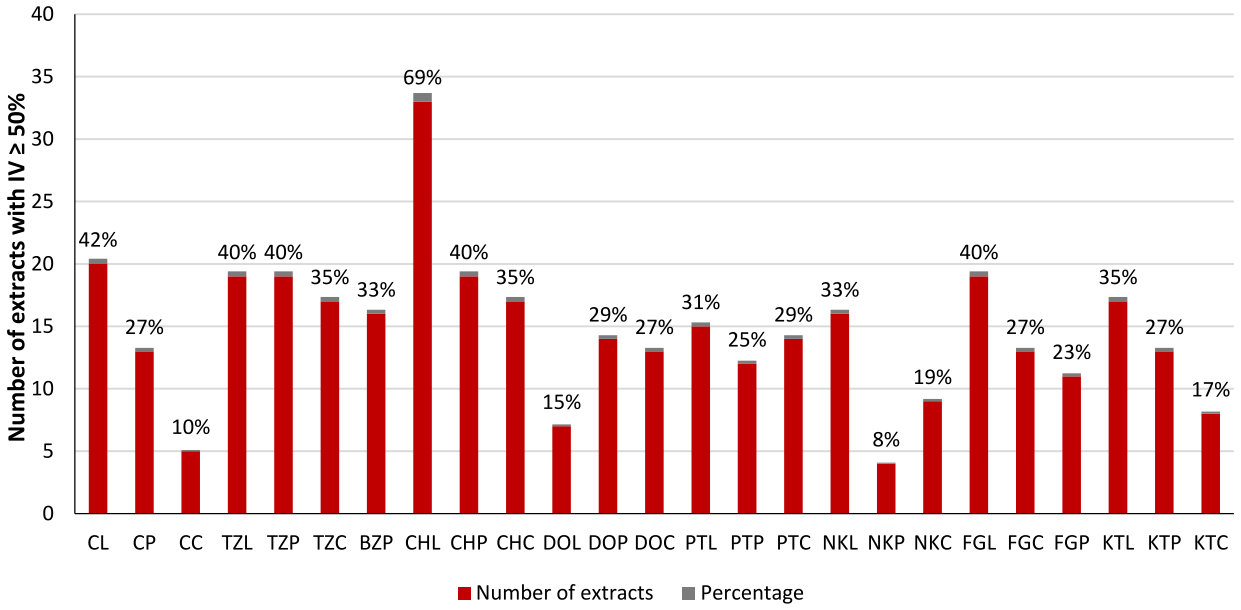

Fig. 3. Antimicrobial activity of extracts from specific plant parts (leaf, pseudostem or corm) of different banana cultivars. Legends on the X-axis: C-Cavendish; TZ-Tereza; BZ-Mbw.azirume; CH-Cachaco; Do-Dole; PT-Pelipita; NK-Namwah Khom; FG-Fougamou; KT- Kluai Teparot; L-Leaf; P-Pseudostem; C-Corm. Note: The results for each cultivar plant part are pooled, and the % represents the percentage of the number of extracts with an inhibition value ≥ 50 % divided by the total number of extracts of the respective banana cultivar part tested against the test microbes.

value ≥ 50 % (Fig. 5). Most of the leaf extracts were active (40 %), with higher growth inhibition rates against multiple microbes than the pseudostem extracts (33 %) and corm extracts (26 %). Post-hoc analysis via pairwise comparisons with Z-tests for proportions revealed significant differences between leaves and corms (p-value = 0.002), whereas no significant differences were found between leaves (p-value = 0.109) and pseudostems or between pseudostems and corms (p-value = 0.150). These findings suggest that the leaves of the tested banana cultivars contain more (potent) antimicrobial compounds than do their pseudostems and corms. Thus, the leaf extracts of these banana cultivars are recommended for further bioassay-guided fractionation and isolation of potent compounds.

The antimicrobial activity clearly varies with the solvent used for extraction (Fig. 6). Sixty-one percent of the water extracts and 55 % of the acetone extracts showed good antimicrobial activity, with inhibition values ≥ 50 %, whereas 38 % for ethanol and 28 % for hexane were effective. The results of the chi-square test revealed a significant association (X^2 = 34.41, df = 3, p-value < 0.001) between the solvents used and the proportion of extracts showing significant antimicrobial activity.

The prominent activity of water extracts predicts the presence of water-soluble chemicals in these banana cultivars, but further investigation is needed to determine the nature and type of these chemicals and their suitability as effective antimicrobial agents. However, water extracts often face contamination issues, so acetone extracts could be a better alternative for further studies. Hexane appears to be the least effective solvent in these extracts, although many cultivars yield effective hexane extracts. This suggests that hexane does not extract as many potent active compounds as water or acetone does.

4. Discussion

4.1. Qualitative phytochemical analysis

Phytochemical analysis of the banana cultivar extracts confirmed the presence of various secondary metabolites, which may possess diverse bioactivities. Our findings are in line with those of previous studies [36,37,38,39], which reported various groups of secondary metabolites from different parts of banana cultivars. Phytochemical analysis of herbal extracts aids in the search for bioactive constituents, which may be useful in the development of novel therapeutic agents [40]. Phytochemicals provide a basis for the targeted isolation of bioactive compounds and play an important role in drug discovery [24]. The biological activities of plant materials are mainly due to the presence of secondary metabolites such as alkaloids, flavonoids, steroids, phenols, coumarins, tannins, terpenoids, etc., which are scattered throughout several parts of the flora [41]. The secondary metabolites play many roles, including self-defense, response to environmental stresses and activities against disease-causing organisms. Many modern medicines and medicinal herbs rely on secondary plant metabolites for their action [42]. Alkaloids, flavonoids, phenols, and tannins are the most common types of secondary metabolites and have been reported to have medicinal importance in various pathological conditions [43]. The secondary metabolites present in the test banana cultivars may play a significant role in drug development; hence, their isolation and identification are highly important for the development of novel and effective therapeutics. The phytochemical constituents reported in our study from various banana cultivar extracts reflect their reported preliminary biological properties. However, it is very difficult to identify individual compounds via this method, as the extracts are complex mixtures. More advanced bioassay-guided isolation and purification techniques, such as LC-MS, GC–MS, or NMR spectroscopy, must be used for detailed chemical profiling and structural elucidation of bioactive compounds.

4.2. Thin-layer chromatographic (TLC) profiling

The TLC fingerprints confirmed the presence of various groups of phytochemicals in the extracts of the tested banana cultivars. TLC separation is an easy way of separating many compounds on a planar surface, and this separation is based on polarity [25]. Hence, both polar (R_f values above 0.5) and nonpolar (R_f values

Table 3
IC_{50} values of the most active banana cultivar extracts from leaves, pseudostems and corms against different microorganisms from the test panel (μg/mL).

No.	Voucher	Cultivar Name-Part	SS	SE	SF	EC	PA	CAU	SC	CA	CG	ML	SA	AH
1.1	CLH	Cavendish-Leaf	280		_	_	_	_	_	_	_	716	**115**	_
1.2	CLA		**190**	886	672	_	_	_	_	344	_	**90**	**194**	637
1.3	CLE		_	_	_	_	_	_	_	339	_	661	**179**	**151**
1.4	CLW		**178**	**178**	**139**	420	_	_	_	_	_	**191**	365	_
2.1	CPH	Cavendish-Pseudostem	_	_	_	_	_	363	_	_	_	913	_	_
2.2	CPA		_	_	_	_	_	_	196	361	_	216	**119**	**170**
2.3	CPE		_	_	_	_	_	_	136	_	_	663	**157**	531
2.4	CPW		**91**	_	**110**	_	_	_	_	_	_	_	_	_
3.1	CCH	Cavendish-Corm	_	_	_	_	_	_	_	_	_	_	_	_
3.2	CCA		_	_	_	_	_	_	_	_	_	486	**168**	_
3.3	CCE		_	_	_	_	_	_	_	_	_	_	_	_
3.4	CCW		632	867	586	_	_	_	_	_	_	_	_	_
4.1	TZLH	Tereza-Leaf	_	_	_	_	_	235	_	_	_	924	_	_
4.2	TZLA		908	_	_	_	_	**76**	_	_	_	799	_	_
4.3	TZLE		370	_	916	_	_	174	_	_	_	421	_	456
4.4	TZLW		_	509	_	256	_	136	138	139	261	**100**	731	867
5.1	TZPH	Tereza-Pseudostem	_	_	603	**177**	_	_	_	_	_	_	_	_
5.2	TZPA		292	_	494	_	_	_	_	287	_	**191**	732	676
5.3	TZPE		225	_	578	750	_	_	_	346	_	_	750	750
5.4	TZPW		_	_	_	450	_	314	192	209	320	_	_	_
6.1	TZCH	Tereza-Corm	_	_	_	_	_	_	_	_	_	_	_	_
6.2	TZCA		**199**	_	_	_	515	363	_	_	_	275	347	662
6.3	TZCE		850	_	_	_	403	_	_		_	_	_	838
6.4	TZCW		_	314	_	270	_	**44**	**47**	**51**	**66**	599	587	_
7.1	BZPH	Mbwazirume-Pseudostem	938	_	_	231	_	_	_	362	_	**148**	**193**	684
7.2	BZPA		**66**	_	_	**166**	_	_	_	_	_	**76**	211	343
7.3	BZPE		_	_	_	**151**	_	_	_	_	_	_	**115**	277
7.4	BZPW		_	_	_	399	_	_	_	_	_	_	238	_
8.1	CHLH	Cachaco-Leaf	453	_	_	216	_	314	**56**	**59**	_	**92**	**74**	493
8.2	CHLA		**75**	**68**	**95**	_	266	_	**96**	133	153	**63**	489	**46**
8.3	CHLE		356	_	_	813	164	190	_	**54**	**53**	**143**	**35**	**104**
8.4	CHLW		270	_	**88**	_	_	_	295	_	_	387	_	**69**
9.1	CHPH	Cachaco-Pseudostem	_	_	_	_	_	344	_	387	187	709	_	_
9.2	CHPA		_	_	_	_	_	_	330	367	_	449	254	**175**
9.3	CHPE		_	_	_	_	_	_	_	_	_	347	230	318
9.4	CHPW		_	397	692	872	_	200	286	_	_	**152**	796	_
10.1	CHCH	Cachaco-Corm	_	_	_	_	_	_	371	_	_	576	_	_
10.2	CHCA		_	_	_	_	_	_	_	_	_	367	**113**	_
10.3	CHCE		_	691	_	_	_	_	_	_	_	**177**	757	876
10.4	CHCW		_	840	651	666	_	114	305	241	320	306	735	_
11.1	DOLH	Dole-Leaf	_	_	_	_	_	_	_	_	_	_	_	_
11.2	DOLA		_	_	_	_	_	_	_	_	_	**116**	483	369
11.3	DOLE		_	_	_	_	_	_	_	_	_	_	205	266
11.4	DOLW		_	_	_	_	_	_	_	_	_	906	_	_
12.1	DOPH	Dole-Pseudostem	_	_	_	_	_	_	340	316	_	408	_	_
12.2	DOPA		680	_	_	_	_	359	_	_	_	**115**	209	**197**
12.3	DOPE		_	_	_	_	_	_	_	_	_	329	258	241
12.4	DOPW		_	_	_	325	_	_	251	_	_	_	396	_
13.1	DOCH	Dole-Corm	_	_	_	_	_	_	_	_	211	590	_	_
13.2	DOCA		259	_	_	_	_	_	_	_	_	468	226	422
13.3	DOCE		_	_	_	_	_	_	_	_	_	743	237	798
13.4	DOCW		146	357	49	_	_	_	_	_	_	**138**	_	_
14.1	PTLH	Pelipita-Leaf	277	_	_	205	_	_	_	_	_	_	662	_
14.2	PTLA		_	_	_	_	_	_	_	_	_	702	_	_
14.3	PTLE		810	_	_	_	_	305	_	_	_	903	_	_
14.4	PTLW		**158**	207	279	**125**	_	_	311	_	_	**185**	200	232
15.1	PTPH	Pelipita-Pseudostem	_	_	_	_	_	_	_	_	_	_	_	_
15.2	PTPA		_	_	_	_	_	_	_	_	_	375	**187**	_
15.3	PTPE		435	_	_	_	_	_	_	_	_	_	_	_
15.4	PTPW		**171**	**190**	**110**	_	876	_	106	**63**	104	320	_	336
16.1	PTCH	Pelipita-Corm	_	_	_	**154**	_	_	_	_	_	_	300	_
16.2	PTCA		252	_	_	**149**	_	_	_	_	_	_	355	_
16.3	PTCE		_	_	_	**177**	_	_	_	_	_	_	836	_
16.4	PTCW		846	287	435	238	_	_	**76**	_	_	770	251	_
17.1	NKLH	Namwah Khom-Leaf	_	_	_	_	_	_	300	373	_	360	674	_
17.2	NKLA		402	_	_	_	_	331	_	_	_	**61**	**116**	304
17.3	NKLE		_	_	_	_	_	_	_	394	_	836	204	204
17.4	NKLW		_	433	217	_	_	_	_	_	_	**64**	_	_
18.1	NKPH	Namwah Khom- Pseudostem	_	_	_	_	_	_	_	_	_	_	_	_
18.2	NKPA		_	_	_	_	_	_	_	_	_	_	235	760
18.3	NKPE		_	_	_	_	_	_	_	_	_	_	_	847
18.4	NKPW		_	_	**166**	_	_	_	_	_	_	_	_	_
19.1	NKCH	Namwah Khom-Corm		_	_	_	_	_	_	_	_	_	_	_
19.2	NKCA		713	_	_	_	_	_	_	_	_	**80**	469	_

(*continued on next page*)

Table 3 (*continued*)

No.	Voucher	Cultivar Name-Part	SS	SE	SF	EC	PA	CAU	SC	CA	CG	ML	SA	AH
19.3	NKCE		_	_	_	_	_	_		_	_	_	_	_
19.4	NKCW		409	_	618	_	_	**74**	121	164	_	_	_	_
20.1	FGLH	Fougamou-Leaf	_	_	_	_	_	_	214	_	_	_	264	_
20.2	FGLA		**125**	_	567	884	_	_	312	_	_	306	273	214
20.3	FGLE		_	_	_	_	_	_	_	_	_	496	237	_
20.4	FGLW		505	_	708	546	_	**52**	151	225	123	367	909	_
21.1	FGPH	Fougamou-Pseudostem	_	_	_	_	_	_	129	_	_	_	_	_
21.2	FGPA		**148**	_	_	_	_	_	_	_	_	426	**182**	**156**
21.3	FGPE		715	_	_	_	_	_	239	_	_	_	227	234
21.4	FGPW		269	_	391	_	_	_	_	_	_	_	_	_
22.1	FGCH	Fougamou-Corm	_	_	_	_	_	302	_	_	_	_	_	_
22.2	FGCA		_	_	_	_	_	_	_	224	219	540	_	616
22.3	FGCE		_	_	_	_	_	_	_	_	_	_	_	_
22.4	FGCW		223	330	**82**	318	_	_	326	_	_	300	607	419
23.1	KTLH	Kluai Teparot-Leaf	_	_	_	_	_	_	285	285	_	626	214	_
23.2	KTLA		**147**	359	422	_	_	260	_	_	_	**46**	229	436
23.3	KTLE		_	_	_	_	_	_	_	_	_	835	291	601
23.4	KTLW		243	_	**124**	_	_	_	_	_	_	697	_	_
24.1	KTPH	Kluai Teparot-Pseudostem	_	_	_	_	_	_	_	_	_	449	457	_
24.2	KTPA		273	_	_	_	_	_	_	_	_	**63**	334	292
24.3	KTPE		783	_	_	_	_	_	_	_	_	256	289	294
24.4	KTPW		_	_	_	_	846	_	_	_	_	817	_	416
25.1	KTCH	Kluai Teparot-Corm	_	_	_	_	_	_	135	_	_	_	_	_
25.2	KTCA		904	_	_	_	_	_	_	_	_	751	**191**	788
25.3	KTCE		_	_	_	_	_	_	239	_	_	462	_	_
15.4	KTCW		_	_	_	_	_	_	_	_	_	_	_	_
*P**			0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.12	2.11	0.28	0.02

*P** (positive control): ciprofloxacin for bacteria, and miconazole for fungi. IC_{50} values less than 200 µg/mL for bacteria and less than 100 µg/mL for yeasts are marked in bold. The number (No.) consists of two parts: the first part (1–25) designates the banana cultivar studied; after the period, the number designates the solvent used for extraction: 1: hexane, 2: acetone, 3: ethanol, and 4: water. Voucher codes: C-Cavendish; TZ-Tereza; BZ-Mbwazirume; CH-Cachaco; Do-Dole; PT-Pelipita; NK-Namwah Khom; FG-Fougamou; KT- Kluai Teparot; L-Leaf; P-Pseudostem; C-Corm; H-Hexane; A-Acetone; E-Ethanol; W-Water. SS-*Shigella sonnei*; SE-*Salmonella enteritidis*; SF-*Shigella flexneri*; EC-*Escherichia coli*; PA-*Pseudomonas aeruginosa*; CAU-*Candida auris*; SC-*Saccharomyces cerevisiae;* CA-*Candida albicans*; CG-*Candida glabrata*; ML-*Micrococcus luteus;* SA-*Staphylococcus aureus*; AH-*Aeromonas hydrophila*.

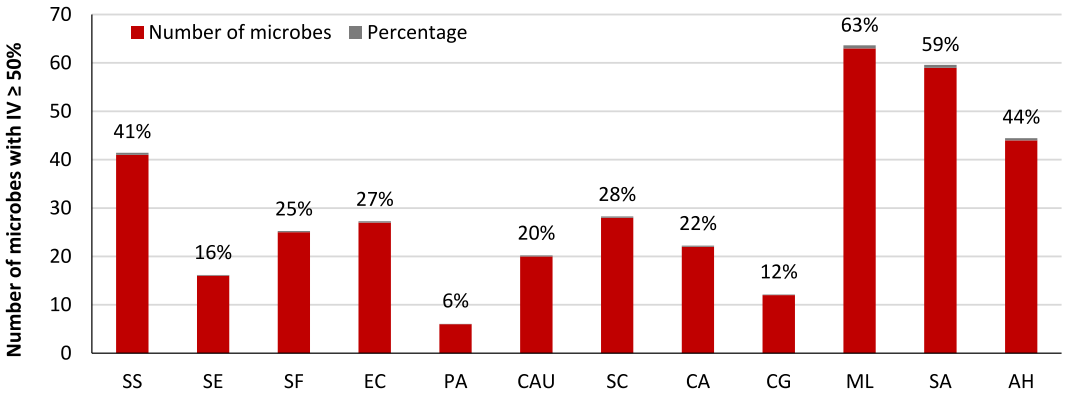

Fig. 4. Susceptibility of microbial species tested to extracts from different banana cultivar plant parts (leaf, pseudostem and corm). Note: The results from all plant parts of all banana cultivars are pooled, and the tested microbes are combined so that the % represents the percentage of the number of microbes that showed an inhibition value ≥ 50 % to the test extracts, divided by the total number of microbes exposed to the test extracts. SS-*Shigella sonnei*; SE-*Salmonella enteritidis*; SF-*Shigella flexneri*; EC-*Escherichia coli*; PA-*Pseudomonas aeruginosa*; CAU-*Candida auris*; SC-*Saccharomyces cerevisiae;* CA-*Candida albicans*; CG-*Candida glabrata*; ML-*Micrococcus luteus;* SA-*Staphylococcus aureus*; AH-*Aeromonas hydrophila*.

below 0.5) compounds could be reported. Since herbal formulations are becoming more popular, chromatographic fingerprinting has gained more significance because of its approval by the WHO as a tool for ensuring herbal quality [26]. Compared with other chromatographic techniques, TLC is an easy way to characterize the plant extracts in the form of fingerprints, which provides a better direction for advanced techniques to isolate specific bioactive compounds [33]. TLC profiling can accurately authenticate and identify the active marker compounds of plant materials, and multiple samples can be run simultaneously with a small amount of mobile phase, less analysis time and low cost per sample [26]. TLC can also accelerate the identification of the active compounds from plant materials by exposing test microorganisms to TLC plates through contact bioautography [44]. Our TLC analyses resulted in a range of R_f values that provide fingerprint profiles of extracts for each cultivar. This method has also been used by other researchers, which highlights the significance of TLC in preliminary phytochemical analyses. However, this method is not

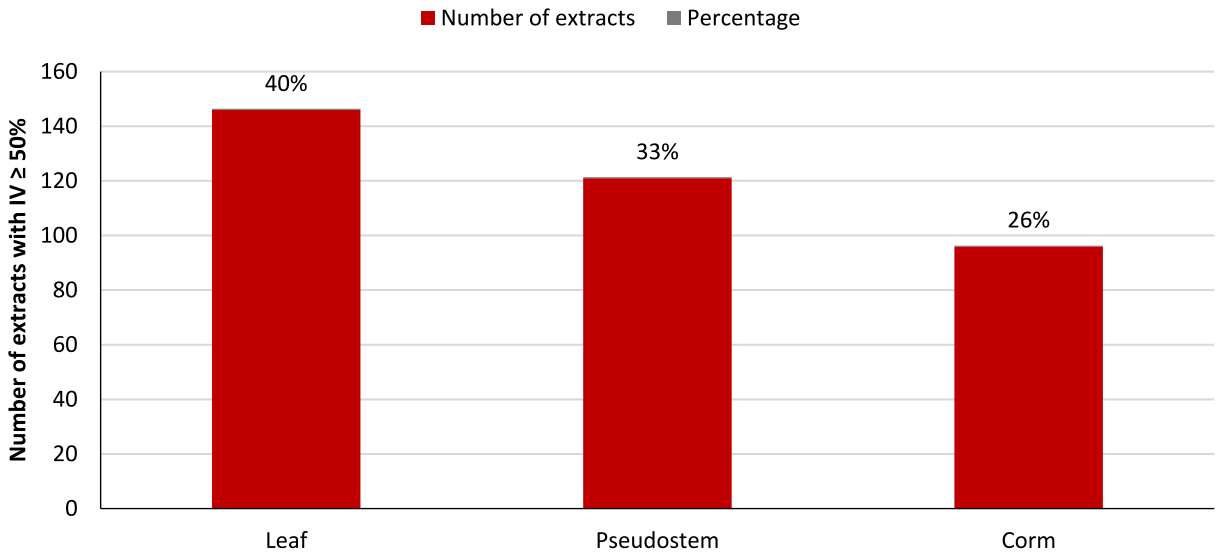

Fig. 5. Antimicrobial activity of extracts from various plant parts (leaf, pseudostem and corm) of selected banana cultivars. Note: The results of the respective banana cultivar extracts are pooled by plant part, and the % represents the percentage of the number of extracts of the respective banana parts with an inhibition value ≥ 50 % divided by the total number of extracts tested.

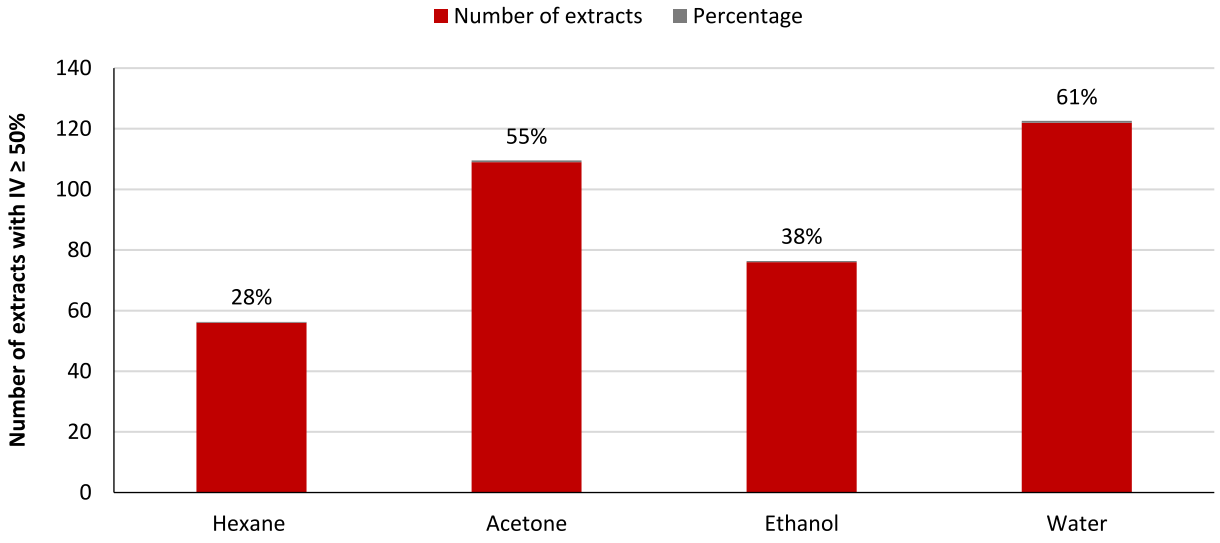

Fig. 6. Effect of solvent on the activity of banana extracts from leaves, pseudostems and corms. Note: The results of all the banana cultivar extracts are pooled per solvent, and the % represents the percentage of the number of extracts of the respective solvent with an inhibition value ≥ 50 % divided by the total number of extracts prepared in that solvent.

helpful in providing information regarding the identification and quantification of compounds. More advanced techniques, such as LC-MS or GC–MS, are needed to obtain a more complete overview of the chemicals that have been visualized through TLC fingerprints and to identify the bioactive compounds of the plant extracts.

4.3. Antimicrobial properties

In Asia, Africa and (South-)America, various banana parts, viz. leaves, fruits, pseudostems, flowers, or peels are used in traditional medicine [45]. Traditionally, different banana parts, such as the juice of stems, fruits, and flowers, are commonly used for diarrhea and dysentery treatment [15]. The use of various parts of banana plants in traditional medicine has recently been reviewed [19,46,47], but most studies on the antimicrobial effects of banana plants have been based on a single cultivar, with a single plant part [48,49,50,51,52,53]. Unlike Siddique et al. [54], who reported the antimicrobial activity of the peels of *Musa sapientum*, we considered only plant parts that are easy to harvest and available at any time during the year in the field. Jouneghani et al. [27] evaluated the antimicrobial effects of various banana cultivars against mostly food-borne pathogens and one yeast, *C. albicans*. Most of the previous studies were limited to the antimicrobial effects of a narrow range of banana cultivars against a limited range of microorganisms. We extended the range of samples covering a wide range of cultivars and tissues by testing a more extensive range of microorganisms. Our study largely confirms earlier research but also extends and expands it, thus making our work more comprehensive than other previously performed studies.

On the basis of our results, the Cachaco cultivar appears to be the most active, followed by Tereza, Fougamou, Pelipita, and Giant

Cavendish. The Kluai Teparot, Namwah Khom, and Mbwazirume cultivars were less active. Overall, the leaf parts of the banana cultivars extracted in water and acetone were better at inhibiting multiple test pathogens, especially *M. luteus* and *S. aureus.*

The findings of Jouneghani et al. [27] are in line with our findings, which also indicate that compared with corms, pseudostems and leaves have greater antibacterial effects. The variations in activity of the same plant parts are presumably due to differences in the concentrations of bioactive metabolites. The antimicrobial activity patterns also vary when different solvents are used, which suggests the presence of multiple bioactive compounds. Compared with the hexane and ethanol extracts, the water and acetone extracts resulted in greater activity. In an earlier study [27], hexane extracts of leaves from all banana cultivars exhibited similar activity against *S. faecalis* and *B. cereus*, which suggests that similar lipophilic compounds are responsible for the antibacterial activity of various banana cultivars. The antimicrobial properties have been correlated with the genetic relatedness of different banana varieties [15], with banana cultivars whose ABB genome has relatively high antimicrobial activity against food-borne and clinically important pathogens. They also reported stronger antimicrobial activity in leaves and pseudostems than in corms.

The initial screening results of the nine banana cultivars revealed that the most active banana extracts against the test microbes were the water and acetone extracts of the leaves and pseudostems of the Cachaco, Giant Cavendish, Tereza, Pelipita, Fougamou, and Kluai Teparot cultivars. These findings suggest that these extracts can be considered as potential candidates for antimicrobial drug discovery. Our findings also support the ethnomedicinal uses of banana cultivars for various microbial infections in different parts of the world and support some of the previously performed studies regarding the therapeutic potential of some banana cultivars and the importance of their secondary metabolites. The promising results of these banana cultivars warrant further investigation for the purification and identification of the active compounds via bioassay-guided fractionation and isolation techniques. More advanced analytical techniques, such as LC-MS, GC–MS, or NMR spectroscopy coupled with bioassays, will be used for chemical profiling and structural elucidation of the bioactive compounds of banana cultivars. This will help to explore the possible uses of banana cultivars in the pharmaceutical market in the near future.

5. Conclusions

The present study reports the qualitative phytochemical analysis, TLC profiling, and antimicrobial assessment of nine widely cultivated banana cultivars against different food-borne and clinically important human pathogens. The effects of the leaves, pseudostems and corms extracted in the four solvents were assessed against Gram-positive bacteria, Gram-negative bacteria and yeasts. The water and acetone extracts of the leaves of the tested banana cultivars showed good antimicrobial activities. The acetone extracts contained a number of phytoconstituents with potential therapeutic uses. On the basis of our results and good inhibition activities with lower IC_{50} values, the Cachaco, Giant Cavendish, Pelipita, and Tereza cultivars are recommended for further studies. This research establishes a framework for further investigation and research in the field by bridging phytochemical analysis and the biological effects of plant materials, thus laying a more convenient and novel scientific foundation for extensive preclinical studies. Potential banana cultivar extracts warrant further investigation involving the isolation and identification of biologically active compounds through advanced bioassay-guided purification techniques, such as LC-MS, GC–MS, or NMR spectroscopy for the establishment of banana cultivars as potential antimicrobial candidates in the pharmaceutical market in the near future.

CRediT authorship contribution statement

Ajmal Khan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Rony Swennen:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Funding acquisition. **Sujogya Kumar Panda:** Writing – review & editing, Supervision. **Liliane Schoofs:** Writing – review & editing, Supervision, Resources, Methodology. **Walter Luyten:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization.

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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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Supplementary material

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Data availability

The data will be made available upon request.

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