



Anti-microbial and cytotoxic activities of green and ripe banana peels

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ABSTRACT

Banana peels (BPs) have an excellent nutrient content due to presence of many bioactive compounds. However, an enormous amount of them is wasted daily, leading to environmental pollution. This study aimed to evaluate the anti-microbial and *in vitro* cytotoxic activities of green and ripe peel extracts (GPE and RPE, respectively) of banana. Total phenolic (TPC), total flavonoid (TFC), saponin contents, DPPH, and total antioxidant capacity (TAC), total protein (T.P), lipid (T.L), carbohydrate (T.C), were detected in GPE and RPE. Gas-chromatography mass spectrophotometry (GC-MS) analysis was determined in BPs extracts. Anti-fungal, anti-bacterial and cytotoxic activities were determined. The results showed that the TPC, TFC, saponin, T.P, T.L T.C and TAC contents in GPE were much higher than those in RPE. According to the results, only hexadecanoic acid was found in both extracts. Both GPE and RPE did not show anti-fungal activity, however, the GPE showed superior anti-bacterial and cytotoxic activities than RPE. Overall, the GPE had higher potential anti-microbial and cytotoxic effects than RPE.

Keywords:

Anti-bacterial, Anti-fungal, Cytotoxic, Green, Phytochemical, Ripe peel.

1. Introduction

The genus *Musa* includes the herbaceous plant known as the banana (Sidhu et al., 2018). It is typically consumed uncooked or made on a large or small scale into a wide range of products, such as bread, wheat, winemaking, dried fruit, snacks, smoothies, ice cream, and beneficial food additives (Vu et al., 2018). Banana peels (BPs) make up roughly 40% of the fruit's overall weight. Since BPs were typically disposed of as waste, enormous volumes of organic waste were dumped in landfills (Ali et al., 2021). Most people believe that only fruits and not their peels have nutritional value. Astoundingly, the BPs are rich in nutrients and bioactive substances, including antioxidant enzymes, carotenoids, biogenic amines, polyphenols, tannins, flavonoids, alkaloids, saponins, glycosides,

terpenoids, and phytosterols. It also contains minerals like iron, calcium, sodium, phosphorus, magnesium, and good levels of dietary fiber (Puraikalan and Yamunadevi, 2018).

The food processing, pharmaceutical, cosmetic, beverage, textile, energy resources, paper, bio-absorbent, pesticide, biofuel, and agricultural sectors are just a few of the industries that use banana peels (Khan et al., 2015; Munfarida et al., 2021; Bhavani et al., 2023). BPs can be used to make a wide variety of culinary and beverage items. Tea brewed from BPs hinders the loss of weight. In the food sector, ferulic acid that has been isolated from BPs is typically utilized as a flavoring and aroma-enhancing agent (Alzate Acevedo et al., 2021). As a result, it can be utilized to make ice cream, cakes, cookies, bread, and

bio-vanillin goods (Hikal et al., 2021). BPs are used in the production of enzymes like amylase and cellulase which play a crucial role in the hydrolyzing of complex compounds like starch into simpler units as glucose in the food, beverage, and baking industries, while cellulase help in hydrolysis (Bhavani et al., 2023). Eco-friendly, biodegradable films for food packaging are made from banana peels. Because BPs are high in antioxidant enzymes, which can help lower the risk of diseases like cancer, and because they have numerous anti-inflammatory and antibacterial properties against both Gram-positive and Gram-negative bacteria, they are beneficial to health and wellbeing (Dmochowska et al., 2020). According to Ranjha et al. (2022), banana peels are used in traditional medicine to cure a variety of conditions including fevers, burns, diarrhoea, intestinal sores, constipation, ulcerative colitis, nephritis, gout, coronary heart infections, high blood pressure, diabetes, aches, and snake bites. They work well as teeth-whitening agents (Salman et al., 2022). Because flavonoids stimulate and repair damaged hair structures, they are used as shampoos to prevent hair loss (Broto et al., 2022). They were used to produce wax that could shine other substances like wood, leather, and stainless steel. Recent studies used BPs in antibacterial wax production, which could clean surfaces and remove bacteria (Naeem et al., 2022). Banana peels contain a large amount of potassium, so they are used in the garden to provide nutrients that plants need to thrive, and they also act as a pest repellent (Hussein et al., 2019). Their extracts were used as biosorbents because they are an excellent source of functional groups like hydroxyl groups that are used for the removal of pollutants like heavy metals from polluted water bodies (Negroiu et al., 2021). According to previous studies, green banana peel extract (GPE) has higher levels of dietary fiber, total starch, and resistant starch than ripe peel extract (RPE). RPE has a higher sugar content than GPE, according to Abbas et al. (2016). Compared to RPE, GPE has the highest levels of antioxidant activity, antioxidant enzymes, phenolic compounds, and flavonoid compounds (Khacharat et al., 2022). Therefore, the objective of this study was to

explore the antifungal, antibacterial and cytotoxic activities of GPE and RPE.

2. Materials and Methods

Preparation of GPE and RPE

Green and ripe banana fruits were purchased from the El-Gharbiah governorate's market in Egypt. The green and ripe peels were then cut into extremely thin pieces and allowed to dry in the shade after being washed twice to remove any chemicals or dust. Grinding before extraction, not after it, and the 3 days is the period of the extraction not of the grinding. Ultimately, the hydro-alcohol extract was obtained by filtering the supernatants and allowing them to dry.

Determination of the phytochemical constituents

Total phenolic content (TPC) from extracts was determined according to Miliauskas et al. (2004). Total flavonoids content (TFC) was determined according to Zhishen et al. (1999). Total antioxidant activity (TAC) was estimated according to Prior et al. (2005). The 2,2 diphenyl-1-picrylhydrazyl (DPPH), free radical scavenging activity was determined according to the method by Asnaashari et al. (2011). Saponin content was estimated by the method described by Hiai et al. (1976).

Determination of the total protein, lipid, and carbohydrate contents

Total protein (T.P) content was calculated according to the method of Waterborg (1984). Total lipid (T.L) assay principle of unsaturated fatty acids was used to measure the lipid content of samples according to Minnoti and Aust (1987). Total carbohydrate (T.C) was estimated according to Plummer (2006).

Gas chromatograph–mass spectrometry (GC-MS) analysis

GPE and RPE were prepared to determine the different chemical profile in each condition. The chemical composition of each sample was performed using a Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 μm film thickness). The components were identified by comparison of their retention times and mass spectra with those of the WILEY 09 and NIST

11 mass spectral database (Abdallah et al., 2021).

Antifungal activity of green and yellow peel extracts

The antifungal activity of BPs was tested against two pathogenic strains: the mold *Aspergillus fumigatus* (RCMB 002008), and the yeast *Candida albicans* (R9CMB 005003). The pathogenic strains were obtained from the regional center of mycology, and biotechnology (RCMB), Al-Azhar University, Egypt. Ketoconazole was purchased from commercial markets. GPE and RPE were dissolved and/or suspended in distilled water in a final concentration of 50 mg/mL. Ketoconazole was used as a positive control or a reference drug at concentration of 100 µg/mL (Esmadi, 2013).

Determination of anti-bacterial activity

For antibacterial screening, a stock solution of GPE and RPE was dissolved in 1 mL of dimethyl sulfoxide (DMSO), and graded quantities of the test polymers were incorporated in a specified quantity of molten sterile agar (nutrient agar, 28 g/L, pH = 7.4) (Padam et al., 2012).

Cancer cell lines

The American Type Culture Collection (ATCC), located in Manassas, Virginia, USA, provided the human breast cancer cell line (MCF-7). The cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS, BioWest, Nuaille, France), 100 U/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL glutamine. The culture was maintained at 37 °C in a humidified environment with 5% CO₂. Sub-culturing of cells occurred every two days.

In vitro cytotoxic effect of GPE and RPE.

MCF-7 cells were employed to assess the cytotoxic activities of GPE and RPE. Using the MTT experiment, the inhibitory concentration (IC₅₀) that killed 50% of cells was found. In summary, the MCF-7 cells were treated in triplicate with varying doses of GPE and RPE when they were 70–80% confluent. The wells were then incubated, and 10 µL of a 12 Mm MTT stock solution [5 mg/mL MTT in sterile phosphate buffered saline (PBS)] was added to each well. After that, the mixture was

incubated at 37 °C for 4 hours. After removing the MTT solution, 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the purple formazan crystal that had formed at the bottom of the wells for a duration of 20 minutes. Cis served as a benchmark for positivity. The absorption at 570 nm was read on an enzyme-linked immunosorbent assay reader (StatFax-2100, Awareness Technology, Inc.). The IC₅₀ was calculated with the sigmoidal curve (Horiuchi et al., 1998).

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the significant differences. The criterion for statistical significance was set at $p \leq 0.05$. All data were presented as mean \pm Standard deviation (SD).

3. Results

Phytochemical analysis of GPE and RPE.

The phytochemical analysis showed that the TPC of GPE was 4.7 ± 0.13 , while this value represented 4.1 ± 0.07 mg GAE/g DW in RPE. The TFC of GPE and RPE were 0.9 ± 0.05 and 0.6 ± 0.03 mg RE/g DW, respectively. The TAC of GPE was 0.086 ± 0.03 while, that of RPE was 0.042 ± 0.02 mg AAE/g DW. DPPH scavenging activity and the IC₅₀ in GPE were 18.32 % and 27.29 mg/ml while, in RPE were 16.76 % and 29.83 mg/ml, respectively. The saponin concentration in GPE was 124 ± 1.95 mg/g DW, while in RPE was 102 ± 1.34 mg/g DW (Table 1).

Total protein, lipid, and carbohydrate contents of GPE and RPE.

The results showed that the TP, TL and TC contents of GPE were 14.2 ± 1.02 , 3.9 ± 0.28 and 476.9 ± 8.98 mg/g D.wt., respectively, while in RPE were represented as 9.7 ± 1.13 , 2.7 ± 0.14 and 394 ± 4.21 mg/g D.wt., respectively (Table 2).

GC-MS analysis of green and yellow banana peel extracts.

As shown in fig 1A, the GC-MS analysis of GPE showed that there are several phytochemical compounds that were reported at retention times (RTs) from 8.41 to 29.23. The results showed that at RTs 13.57, 21.62 and 24.16, compounds 4-Penten-1-one, 2-

[bis(methylethio)methylene E]-4-methylphenyl, n-hexadecanoic acid and 9,12-Octadecadienoyl chloride, (Z, Z)- showed peak areas of 15.47%, 15.63% and 14.58%, respectively (Table 3a). As shown in fig 1B, GC-MS analysis of RPE showed that there are several phytochemical compounds that were reported at RTs from 4.13 to 31.64. The results showed that at RTs 21.54, 23.08 and 24.16, compounds hexadecanoic acid, 9-octadecenoic acid (z) methyl ester and 13-heptadecyn-1-ol showed peak areas of 8.95%, 13.15% and 8.87%, respectively (Table 3b).

Antifungal and antibacterial activities of GPE and RPE

As shown in table 4, the results showed that there is no antifungal activity against *A. fumigatus* and *C. albicans*, either for GPE or RPE extracts. The data showed that the GPE and RPE exhibited antibacterial activity against gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*.

In vitro cytotoxic effect of GPE and RPE

The results showed that the GPE has a potential cytotoxic effect against MCF-7 cells after 24 hours of *in vitro* treatment. In contrast,

the RPE did not show any cytotoxic effect against the MCF-7 cells when treated under some conditions (Fig. 2).

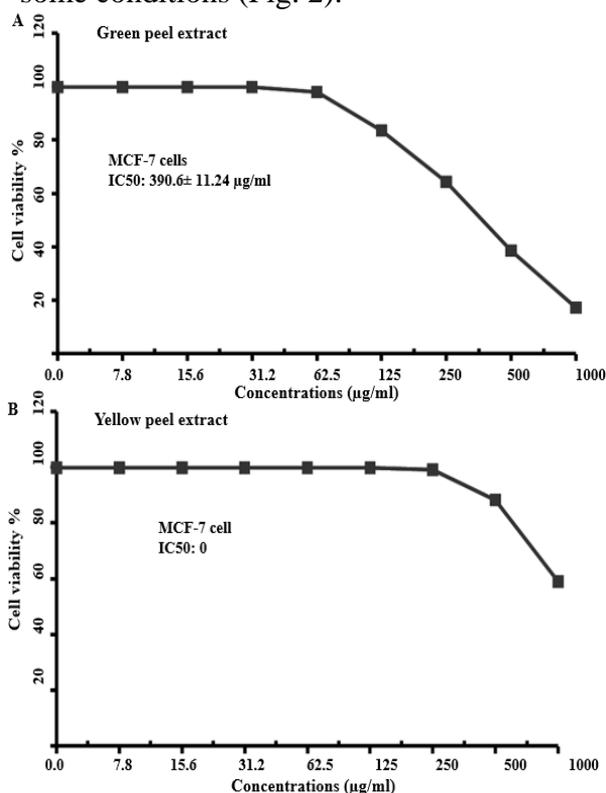


Fig. 2. Median inhibition concentrations (IC₅₀) of green and ripe banana peel extracts

Table 1. Quantitative analysis of phytochemicals in GPE and RPE

Phytochemicals	GPE	RPE
TPC (mg GAE/g DW)	4.7 ± 0.13	4.1 ± 0.07
TFC (mg RE/g DW)	0.9 ± 0.05	0.6 ± 0.03
Saponin (mg/g DW)	124 ± 1.95	102 ± 1.34
DPPH scavenging activity (%)	18.32 %	16.76 %

The values represented mean ± SD; GPE: Green banana peel extract, RPE: Ripe banana peel extract. TPC: Total phenolic content, TFC: Total flavonoid content, TAC: Total antioxidant capacity.

Table 2. Total protein, lipids, and carbohydrate contents in GPE and RPE.

Macromolecules	GPE	RPE
TP (mg/g D.W)	14.2 ± 1.02	9.7 ± 1.13
TL (mg/g D.W)	3.9 ± 0.28	2.7 ± 0.14
TC (mg/g D.W)	476.9 ± 8.98	394 ± 4.21

The values represented means ± S.D; GPE: Green banana peel extract, RPE: Ripe banana peel extract. TP: Total protein, TL: Total lipid, TC: Total carbohydrate.

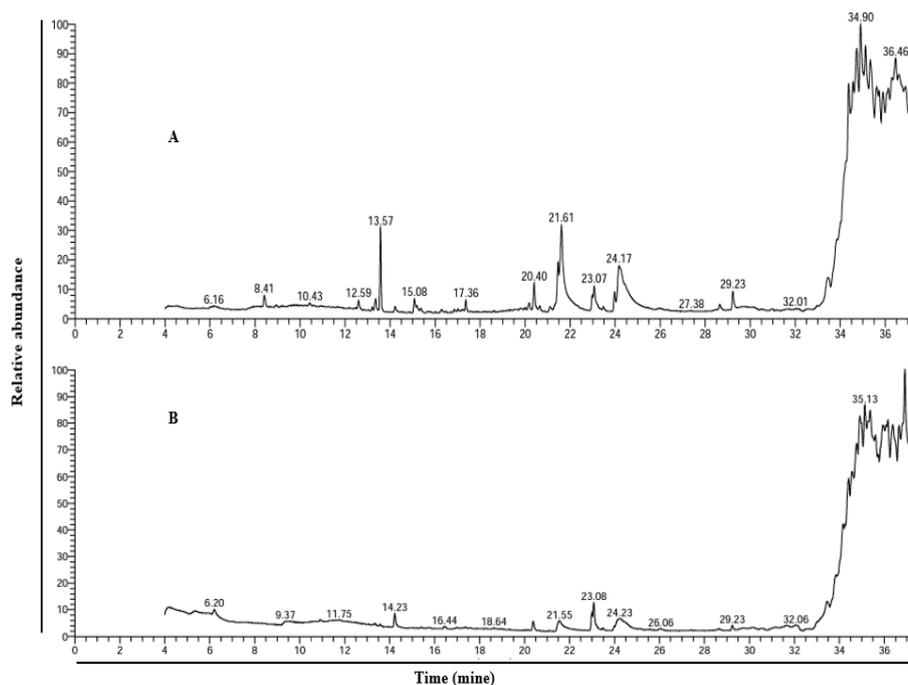


Fig. 1. GC-MS chromatograms of green and ripe banana peel extracts

Table 3a. GC-MS analysis of green peel extract.

RT	Compound name	M.F.	M.Wt.	P.A. %
8.41	Glycerol, 3TMS derivative	C ₁₂ H ₃₂ O ₃ Si ₃	308	2.77
13.36	L-Threitol, 4TMS derivative	C ₁₆ H ₄₂ O ₄ Si ₄	410	2.83
13.57	4-PENTEN-1-ONE,2-[BIS(METHYLTHIO)METHYLEN E]-4-METHYL-1-PHENYL	C ₁₅ H ₁₈ OS ₂	278	15.47
15.07	3,5,8a-trimethyl-4,4a,8a,9-tetrahydro naphtho[2,3-b]furan	C ₁₅ H ₁₈ O	214	3.27
20.40	d-Galactose,2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)-	C ₂₂ H ₅₅ NO ₆ Si ₅	569	5.99
21.45	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	4.71
21.62	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	15.63
22.97	9,12-OCTADECADIENOIC ACID, METHYL ESTER, (E, E)-	C ₁₉ H ₃₄ O ₂	294	2.61
23.07	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	5.35
23.97	Butyl 9,12-octadecadienoate	C ₂₂ H ₄₀ O ₂	336	2.94
24.16	9,12-Octadecadienoyl chloride, (Z, Z)-	C ₁₈ H ₃₁ ClO	298	14.58
28.64	13-Docosenoic acid, methyl ester	C ₂₃ H ₄₄ O ₂	352	1.29
29.23	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	4.20

R.T.: Retention time, M. F: Molecular formula, M.Wt: Molecular weight, P.A.: Peak area, GPE: Green banana peel extract.

Table 3b. GC-MS analysis of ripe peel extract.

RT	Compound name	M.F.	M.Wt.	P.A. %
4.13	Melezitose	C ₁₈ H ₃₂ O ₁₆	504	6.55
4.23	d-Gala-l-ido-octonic amide	C ₈ H ₁₇ NO ₈	255	4.98
6.20	D-Fructose, diethyl mercaptal, pentaacetate	C ₂₀ H ₃₂ O ₁₀ S ₂	496	3.67
9.35	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	279	3.90

14.23	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methyl-, [1ar-(1aà,4aà,7á,7aá,7bà)]	C ₁₅ H ₂₄ O	220	7.85
16.44	1-HEXADECANOL, 2-METHYL	C ₁₇ H ₃₆ O	256	1.85
20.39	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	5.20
21.54	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	8.95
22.99	9,12-oTadecadienoic acid, methyl ester, (e, e)	C ₁₉ H ₃₄ O ₂	294	7.30
23.08	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	13.15
24.16	13-Heptadecyn-1-ol	C ₁₇ H ₃₂ O	252	8.87
26.07	9-Otadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	1.23
29.23	4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8 di-á-D-glucopyranosyl-5,7 dihydroxy	C ₂₇ H ₃₀ O ₁₆	610	2.65
31.64	ISOCHIAPIN B %2<	C ₁₉ H ₂₆ O ₆	350	1.49

R.T.: Retention time, M. F: Molecular formula, M.Wt: Molecular weight, P.A.: Peak area, RPE: Ripe banana peel extract.

Table 4. The anti-fungal and anti-bacterial of GPE and RPE.

Tested microorganisms	Anti-fungal activity		
	GPE	RPE	Ketoconazole (100 µg/ml)
<i>Aspergillus fumigatus</i> (RCMB 002008)	NA	NA	19
<i>Candida albicans</i> (ATCC 10231)	NA	NA	20
	Anti-bacterial activity		
	GPE	RPE	Gentamycin (40 µg/ml)
Gram positive bacteria: <i>Staphylococcus aureus</i> (ATCC 25923)	16	10	24
Gram negative bacteria: <i>Escherichia coli</i> (ATCC 25922)	13	12	30

GPE: Green banana peel, RPE: Ripe banana peel.

4. Discussion

This study was conducted to evaluate the phytochemical constituents, the anti-microbial and cytotoxic effects of GPE and RPE. The results of the study revealed that TPC in GPE was higher than its content in RPE. These findings agreed with Fatemeh et al. (2012) and Oduje et al. (2015), who reported that total phenolic content decrease during the ripening process accompanied by a decrease in antioxidant enzymes. These events may lead to increased oxidative stress and cause many

metabolic changes associated with ripening and maturation. Zhang et al. (2022) reported that green banana peel can be used as a high-quality raw material for extracting phenolic compounds.

Furthermore, green banana peels could be exploited in food and pharmaceutical industries for their high content of phenolic compounds. Phenolics can scavenge free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α -tocopherol radicals, and inhibiting oxidases (Sundaram et al., 2011). The results of the study revealed that TFC was higher in GPE than in RPE. This result agreed with Khacharat et al. (2022). Saponin content and DPPH were higher in GPE than in RPE. Sundaram et al. (2011) reported that the extracts of the unripe banana peels have higher saponin and DPPH than those of the overripe peels. Also, Oduje et al. (2015) reported the same results. TAC in GPE was higher than its content in RPE. These findings agreed with Fatemeh et al. (2012) and Vu et al. (2018) who reported that banana peel antioxidant activity decreased when it became overripe. The high level of polyphenol in unripe banana peel makes it more effective than ripe BP

extract at inhibiting human red blood cells oxidative hemolysis. The obtained results showed that the T.P content in GPE was higher than its content in RPE. This finding agreed with a previous study that reported that the content of T.P in green banana peel was higher than this content in ripe peel (Agama-Acevedo et al., 2016). The results showed that T.L content in GPE was higher than content in RPE. This result agreed with a previous study by Eshak (2016) who reported that T.L in green peel was higher than its content in ripe banana peel. The T.C content in GPE was higher than its content in RPE. This finding agreed with a previous study that showed the same results (Khoozani et al., 2019). Castelo-Branco et al. (2017) reported that green banana peel presented the highest moisture, ash, and carbohydrate contents than ripe banana peel. GC-MS analysis showed that there were many phytochemical compounds recorded in the GPE, with the reading was recorded at 8.41 and ended at 29.23. The highest boat was at Rt 21.62 and peak area 15.63, while RPE recorded at 4.13 and ending at 31.64. The highest boat was at Rt 23.08 and peak area 13.15. Mordi et al. (2016) reported the isolation of several triterpenes such as cyclo-musalenol, cyclomusalenone, 24-methylenecycloartanol, stigmast-7-methyl-encycloartanol, stigmast-7-en-3-ol, lanosterol and amyirin. An antihypertensive principle, 7, 8-dihydroxy-3-methylisochroman-4-one, was isolated from the peel of *M. sapientum* Cycloartane triterpenes such as 3-epicyclo-eucalenol, 3-epicyclomusalenol, 24 methylenepollinastanone and 28-norcyclo-musalenone, 24-oxo-29-norcycloartanone have been isolated from the fruit peel of *M. sapientum* (Bediako et al., 2019).

The results showed that both the green and ripe peels have no anti-fungal activities against *A. fumigatus* and *C. albicans*. This finding is not in agreement with a previous study by Puraikalan and Yamunadevi, (2018) who reported the antifungal activity of banana peel. The GPE showed higher antibacterial activities against *S. aureus* and *E. coli* than RPE. This result agreed with Mokbel and Hashinaga (2005) and Susanah

et al. (2018) who reported that green peel extract showed higher antimicrobial and antioxidant activity than ripe banana peel. Several studies reported that GPE is richer in phytochemical compounds like flavonoids and phenolic contents than RPE. It also contains antimicrobial activities, which benefit human health (Karasawa et al., 2018). The cytotoxicity of the green and ripe extracts was measured using the MTT assay. The cytotoxicity assay showed that the extract of GPE had higher cytotoxic activity than the extract of RPE against MCF-7 cell line. The cytotoxic effect could be due to the presence of secondary metabolites such as phenolic, flavonoid and saponin contents which inhibit reactive oxygen species (ROS), hindering the cell cycle, and destroying tumor cell production. Bioactive compounds have long been shown to play a significant role in preventing the development of terminal illnesses such as cancer (Zaini et al., 2022).

In conclusion, the results demonstrated that GPE has potential biological activities as anti-bacterial and cytotoxic agents.

Conflicts of interest

There are no conflicts of interest to declare.

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