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The phytochemical composition, anti-microbial and *in vitro* cytotoxic activities of green and ripe banana pulps

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ABSTRACT

To enhance the use of banana fruits and their byproducts in food applications, several trials have been made. In this work, the phytochemical screening, anti-microbial, and *in vitro* cytotoxic properties of green and ripe banana pulps (BPs) were examined. Both the green and the ripe BPs were diced, dried in the shade, then grinded to make pulp flour. To obtain their extracts, the flour was extracted in hydro-alcohol. 2,2-diphenyl-1-picrylhydrazyl (DPPH), total antioxidant capacity (TAC), total protein (T.P), total lipid (T.L), total carbohydrate (T.C), total phenolic (TPC), total flavonoid (TFC), and total saponin concentrations were determined. Gas-chromatography mass spectrometry (GC-MS), the anti-bacterial, anti-fungal, and cytotoxic properties were evaluated. The results demonstrated that green BP had substantially greater T.P, T.L, and T.C levels than ripe pulps. Green pulp had substantially higher levels of TPC, TFC, saponin, and TAC than ripe pulp did. According to GC-MS analysis, green pulps contained high peak areas of three phytochemical substances, including hexadecanoic acid, 9,12-octadecadienoic acid, and 17-oxaandrostan-3-ol (3á, 5à). Hexadecanoic acid, 9,12-octadecadienoic acid (z,z), and 11-octadecenoic acid, methyl ester were present in the pulps of the ripe fruits. Both green and ripe pulp extracts had no anti-fungal activity, while the green pulp extract outperformed the ripe pulp extract in terms of its anti-bacterial and cytotoxic properties. Compared to the ripe pulp, green ones may have potential antioxidant, anti-bacterial, and cytotoxic properties.

Keywords:
Anti-microbial, Banana, Cytotoxicity, Cell line, Green pulp, MCF-7, Phytochemical, Ripe.

1. Introduction

According to Kwon et al. (2020), bananas are the sixth agricultural crop marketed in international markets. It is a member of the Musaceae family and is one of the most significant crops. Because banana fruit contains vitamins and minerals, it can be eaten raw or prepared (Sarma et al., 2021). Banana flour can be made from unripe fruit as part of new economic techniques being used to increase the use of banana fruits. As a result, this flour is used in a variety of cutting-edge food products (Khoozani et al., 2019). The green banana flour's high concentration of bioactive components is anticipated to have positive health effects on people (Oguntibeju, 2019). Additionally, distinct banana fruit pulp portions (unripe and ripe) have been employed for various ethnopharmacological treatments in traditional medicine. The banana fruit pulps include many alkaloids, flavonoids, saponins, tannins, glycosides, and terpenoids (Afzal et al., 2022). Traditional medicine has employed extracts from various plant components to treat fevers, burns, diarrhea, inflammation,

discomfort, and snake bites (Ahmad et al., 2019). According to research, *Musa sapientum* (Musaceae) has strong anti-inflammatory, antioxidant, and anti-ulcer properties (Ashwini and Venkatesh, 2021). The amount of sugar in ripe banana flour is substantial. Banana fruit is divided into two pieces, or peel and pulp (BP). The portion of BP that is edible is rich in nutrients. Depending on their chemical makeup, BP and its flour may have a variety of purposes (Kritsi et al., 2023). Banana peel suggests a possible better value in terms of antioxidant content because a prior study found that the antioxidant chemicals in the peel were higher than in the pulp (Parvez et al., 2023). Ripe BP contains a variety of phytochemicals, including phenolics, flavonoids, anthocyanin, and catecholamines (Pereira and Maraschin, 2015; Sarma et al., 2021). Studies on BP looked into a variety of topics, from its usage as a food enrichment ingredient to the extraction and isolation of numerous health-beneficial components, including various types of starch, cellulose, and bioactive chemicals. BPs have a stronger antioxidant capacity than various berries and vegetables as a result of the presence of these chemicals. Numerous research is being conducted to assess the impact of bioactive compounds in BPs on the qualities of food since they have drawn more attention to functional food products (Sulistyaning et al., 2022). Previous research has shown that BP contains a variety of antioxidants (Kim et al., 2022). The impact of ripeness stage on antioxidant components and antioxidant activity has not yet been thoroughly investigated. In order to assess the phytochemical components, antibacterial, and cytotoxic properties of extracts obtained from *Musa sp.* pulps at two different stages of ripeness and unripeness, this study was carried out.

2. Materials and Methods

Collection and preparation of green and ripe banana pulp extracts

Bananas were harvested from the farms in El-Gharbiah governorate, Egypt both green and ripe. Fruits were cleaned to remove any dust, and the pulp was then diced into extremely small pieces and dried in the shade. These compartments of the unripe and ripe pulps were being ground into fine powders in a motorized mortar. 500 mL of 70% ethanol was used to soak 50 grams of each powder for three days. To obtain extracts, the supernatants were finally filtered and dried.

Determination of the total protein, lipid, carbohydrate contents

The method proposed by Waterborg (2009) was used to determine the total protein (T.P) content. According to Minnoti and Aust, (1987), the total lipid (T.L.) content of the samples was calculated. According to Plummer (2006), a method was used to estimate the amount of total carbohydrates (T.C).

Determination the phytochemical constitutes

According to Miliauskas et al. (2004), the total phenolic content (TPC) was calculated. According to Zhishen et al. (1999) and Prior et al. (2005), respectively, the total flavonoids content (TFC) and the total antioxidant activity (TAC) were evaluated. Asnaashari et al. (2011) and Hiai et al. (1976) were used to assess the DPPH, free radical scavenging activity, and saponin content, respectively.

Gas chromatograph–mass spectrometry (GC-MS) analysis

Extracts of banana green and ripe pulps were prepared to determine different chemical profiles according to the method of Abdallah et al. (2021).

Anti-fungal and anti-bacterial activities of green and ripe pulp extracts

Two pathogenic strains of mold and yeast, *Aspergillus fumigatus* (RCMB 002008) and *Candida albicans* (R9CMB 005003), were used as test subjects for antifungal activity. The regional center of mycology and biotechnology (RCMB), Al-Azhar University, Egypt, provided the pathogenic strains. According to Esmadi (2013), the inhibitory zone was measured in millimeters (mm) for checking for germs. The microorganism was produced as a suspension with a 5 × 10−5 cfu/mL concentration. Test organisms were used to seed the culture plates. Following that, the zones of inhibition were assessed, noted, and contrasted with

positive standard control ciprofloxacin (Padam et al., 2012).

Cancer cell lines and propagation:

Human breast cancer cells (MCF-7) were obtained from the American Type Culture Collection (ATCC) in Manassas, Virginia, and cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS, BioWest, Nuaille, France), 100 U/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL glutamine at 37 ^{o}C in a humid environment containing 5% CO_2. Every two days, the culture of the cells was divided.

***In vitro* cytotoxic effect of green and ripe pulp extracts**

MCF-7 cells were employed to ascertain the cytotoxic activities of green and mature pulp extracts. Using the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) experiment, the inhibitory concentration (IC_{50}) that killed 50% of the cells was identified. In a nutshell, the MCF-7 cells received triplicate additions of various extract doses, and the wells were incubated for 24 hours. Then, each well received 10 L of a 12 Mm MTT stock solution. After that, the sample was incubated at 37 ^{o}C for 4 hours. The purple formazan crystal that had developed at the bottom of the wells was then dissolved in 100 L of dimethyl sulfoxide for 20 minutes after the MTT solution had been withdrawn. The absorbance at 570 nm was read on an enzyme-linked immunosorbent assay reader (StatFax-2100, Awareness Technology, Inc.). The IC_{50} was calculated with the sigmoidal curve.

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 are presented as mean ± SD.

3. Results

Total protein, lipid, and carbohydrate contents of green and ripe

The results showed that the T.P, T.L and T.C of green BP were, 14.2 ± 1.02, 3.9 ± 0.28 and 476.9 ± 8.98 mg/g DW, respectively. While these macromolecules were represented in ripe BP as 9.7 ± 1.13, 2.7 ±0.14 and 394 ± 4.21 mg/g DW, respectively (Table 1).

Phytochemical analysis of green and ripe BP extracts.

The phytochemical analysis showed that the total phenolic contents (TPC) of green pulp was 4.7 ± 0.13 while this value represented as 4.1 ± 0.07 mg GAE/g DW in ripe pulp. The total flavonoid contents (TFC) of green and ripe pulp were 0.9 ± 0.05 and 0.6 ± 0.03 mg RE/g DW, respectively. The total antioxidant activity (TAC) of green pulp was 0.086 ± 0.03 while, in ripe pulp was 0.042 ± 0.02 mg AAE/g DW. DPPH scavenging activity and its IC_{50} in green pulp were 18.32 % and 27.29 % while in ripe pulps were 16.76 and 29.83 mg/ml. The saponin concentration in green pulp was 124 ± 1.95 mg/g DW while, in ripe pulp was 102 ± 1.34 mg/g DW (Table 2).

GC-MS analysis of green and ripe BP extracts.

As shown in Figure 1a, the GC-MS analysis showed that there are several phytochemical compounds that were reported at retention time (RTs) from 8.41 to 29.23. The results showed that at RTs 13.57, 21.62 and 24.16, compounds 17-oxaandrostan-3-ol, (3á,5à)-, hexadecanoic acid and 9,12-octadecadienoic acid showed peak area 15.47%, 15.63% and 14.58%, respectively (Table 3a). Also, as shown in Figure 1b GC-MS analysis 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, 11-octadecenoic acid, methyl ester and 9,12-octadecadienoic acid (z,z)- showed peak area 8.95%, 13.15% and 8.87%, respectively (Table 3b).

Antifungal and antibacterial activities of green and ripe pulps.

As shown in Table 4, the results showed that there is no antifungal activity against *Asperagillus sp.* and *Candida albicans*, either for green or ripe pulp extracts. The data showed that the green and ripe pulp extracts exhibited antibacterial activity against gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*.

***In vitro* cytotoxic effect of green and ripe pulps.**

The results showed that the green pulp extract has a potential cytotoxic effect against MCF-7 cells after 24 hrs of the *in vitro* treatment. In contrast, the ripe pulp extract did not show any cytotoxic effect against the MCF-7 cells that were treated under some conditions (Fig. 2).

Table 1. Total protein, lipid, and carbohydrate contents in green and yellow pulps

Phytochemicals	Green pulp	Yellow pulp
Total protein (mg/g D.W)	14.2 ± 1.02	9.7 ± 1.13
Total lipid (mg/g D.W)	3.9 ± 0.28	2.7 ± 0.14
Total carbohydrate (mg/g D.W)	476.9 ± 8.98	394 ± 4.21

Table 2. Quantitative analysis of phytochemicals in green and yellow pulps

Phytochemicals	Green pulp	Yellow pulp
Total phenols (mg GAE/g DW)	4.7 ± 0.13	4.1 ± 0.07
Total flavonoids (mg RE/g DW)	0.9 ± 0.05	0.6 ± 0.03
Saponins (mg/g DW)	124 ± 1.95	102 ± 1.34
DPPH scavenging activity (%)	18.32 %	16.76 %
IC_{50} of DPPH (mg/ml)	27.29	29.83
TAC (mg AAE/g DW)	0.086 ± 0.03	0.042 ± 0.02

Table 3 a. GC-MS analysis of green banana pulp extract.

RT	Compound name	M.F.	M.Wt.	Area %
8.41	Glycerol, 3tms derivative	$C_{12}H_{32}O_3Si_3$	308	2.77
12.59	Benzofuran,	$C_{15}H_{20}O$	216	2.01
13.36	Meso-erythritol, 4tms derivative	$C_{16}H_{42}O_4Si_4$	749	2.83
13.57	17-oxaandrostan-3-ol, (3á,5à)-	$C_{18}H_{30}O_2$	278	15.47
14.22	1h-cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-	$C_{15}H_{24}O$	220	1.30
15.07	3,5,8a-trimethyl-4,4a,8a,9-tetrahydro naphtho[2,3-b]furan	$C_{15}H_{18}O$	214	3.27
15.18	4as,8as)-3,8a-dimethyl-5-methylene-4,4a,5,6,8a,9-hexahydronaphtho[2,3-b]furan	$C_{15}H_{18}O$	214	1.42
17.36	l-(-)-arabitol, 5tms derivative	$C_{20}H_{52}O_5Si_5$	512	2.22
20.17	d-(+)-xylose, tetrakis(trimethylsilyl) ether, ethyloxime (isomer 1)	$C_{19}H_{47}NO_5Si_4$	481	1.78
20.40	d-galactose, 2,3,4,5,6-pentakis-o-(trimethylsilyl)-, o-methyloxyme, (1z)-	$C_{22}H_{55}NO_6Si_5$	569	5.99
21.10	octanal, (2,4-dinitrophenyl) hydrazone	$C_{14}H_{20}N_4O_4$	308	1.15
21.45	hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.71
21.62	hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.63
22.97	hexadecadienoic acid, methyl ester	$C_{20}H_{36}O_2$	308	2.61
23.07	9-octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	296	5.35
23.97	n-propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322	2.94
24.16	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280	14.58
28.64	erucic acid	$C_{22}H_{42}O_2$	338	1.29
29.23	di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	4.20

R.T.: Retention time; M. F: Molecular formula; M.Wt: Molecular weight

Table 3b. GC-MS analysis of yellow banana pulp extract.

RT	Compound name	M.F.	M.Wt.	Area %
4.13	2,3,4,5-Tetrahydroxypentanal	$C_5H_{10}O_5$	150	6.55
4.23	d-Fructose, diethyl mercaptal,pentaacetate	$C_{20}H_{32}O_{10}S_2$	496	4.98
5.34	l-Glucose	$C_6H_{12}O_6$	180	2.90
6.20	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	3.67
9.35	d-Galactose, diethyl mercaptal, pentaacetate	$C_{20}H_{32}O_{10}S_2$	496	3.90
13.34	Maltose	$C_{12}H_{22}O_{11}$	342	1.50
14.23	(-)-Spathulenol	$C_{15}H_{24}O$	220	7.85
16.44	1-Hexadecanol, 2-methyl	$C_{17}H_{36}O$	256	1.85
20.39	Hexadecanoic acid, 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-Octadecadienoic acid, methyl ester, (e,e)-	$C_{19}H_{34}O_2$	294	7.30
23.08	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	13.15
24.16	9,12-Octadecadienoic acid (z,z)-	$C_{18}H_{32}O_2$	280	8.87
26.07	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282	1.23
29.23	Isochiapin b	$C_{19}H_{22}O_6$	346	2.65
31.14	Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1058	1.02
31.64	Dotriacontane	$C_{32}H_{66}$	450	1.49

R.T.: Retention time; M.F: Molecular formula; M.Wt: Molecular weight

Table 4. The anti-fungal and anti-bacterial of green and yellow banana pulp extract.

Tested microorganisms	Anti-fungal activity		
	Green pulp extract	Yellow pulp extract	Positive control Ketoconazole (100 μg/ml)
Aspergillus fumigatus (RCMB 002008)	NA	NA	19
Candida albicans ATCC 10231	NA	NA	20
	Anti-bacterial activity		
	Green pulp extract	Yellow pulp extract	Gentamycin (40 μg/ml)
Gram Positive Bacteria: *Staphylococcus aureus* ATCC 25923	16	12	24
Gram Negative Bacteria: *Escherichia coli* ATCC 25922	12	13	30

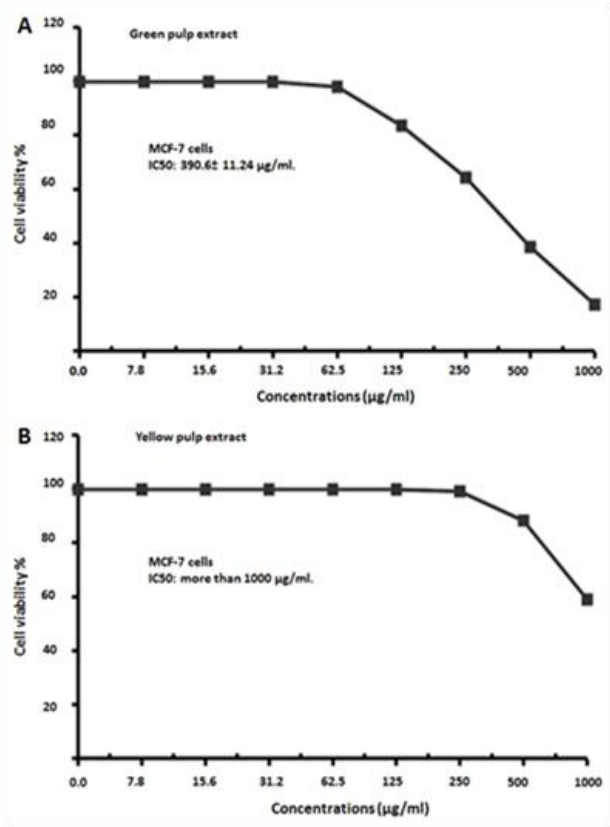

Fig. 2. MCF-7 cell viability after the treatment with green and yellow banana pulp extract.

4. Discussion

Macro and micronutrients such as carbs, protein, unsaturated fatty acids, vitamins (A and C), and minerals are mostly found in bananas (Afzal et al., 2022). This research was done to determine the phytochemical composition, anti-microbial activity, and cytotoxic potential of green and ripe BPs. The results obtained indicated that the T.P. content in green pulp was higher than that in ripe pulp. This result was consistent with a prior study that found that green pulp had a greater T.P concentration than mature pulp (Segundo et al., 2017). The outcomes demonstrated that green pulp had a higher T.L content than ripe pulp.

This outcome was consistent with a prior study by Khoozani et al. (2019), which found that the content of T.L. in green pulp was higher than that in mature pulp. Additionally, the T.C content of green pulp was higher than that of ripe pulp. Additionally, a prior study (Haslinda et al., 2009) that produced the same results supported this conclusion. The findings indicated that green pulp had a higher total phenolic content (TPC) than ripe pulp. This finding was consistent with the other study's finding that banana pulp's TPC reduced as fruits ripened (Kritsi et al., 2023).

The primary bioactive components of banana fruits are phenolic compounds, which provide antioxidant, anti-inflammatory, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and anti-allergic activities (Moo-Huchin et al., 2015; Islam et al., 2018). According to earlier research, BP typically contains fewer phenolic chemicals than banana peels (Khoozani et al., 2019). According to this study, the green pulp had higher levels of TFC, saponin, and TAC than the ripe pulp did. These results corroborated Gedük and Zengin's findings from 2021, which showed that green pulps had higher concentrations of TFC, saponin, and TAC than mature pulps did. These findings are consistent with earlier studies that have been published and found that the presence of flavonoids was higher in the pulp of unripe bananas than ripe bananas (Bashmil et al., 2021). Flavonoid functions as a scavenger of free radicals and is connected to significant antioxidant activity (Wongwaiwech et al., 2022). Quercetin and kaempferol are also said to have a significant impact on cardiovascular protection. According to GC-MS analysis of green pulp, various phytochemical substances were found with retention times (RTs) ranging from 8.41 to 29.23. The findings revealed that the molecules 17-oxaandrostan-3-ol, (3á,5à)-, hexadecanoic acid, and 9,12-octadecadienoic acid, respectively, showed peak areas of 15.47%, 15.63%, and 14.58% at RTs 13.57, 21.62, and 24.16. As opposed to this, GC-MS analysis of ripe pulp revealed the presence of numerous phytochemical substances at RTs ranging from 4.13 to 31.64. Hexadecanoic acid, 11-octadecenoic acid, methyl ester, and 9,12-octadecadienoic acid (z,z)- exhibited peak areas of 8.95%, 13.15%, and 8.87%, respectively, at RTs 21.54, 23.08, and 24.16. The green pulp extract showed higher antibacterial activities against *S. aureus* and *E. coli* than the ripe pulp extract. This finding could be due to its phytochemical constitutes. Several studies have shown that BP and banana peel contain antimicrobial activities (Chaudhry et al., 2022). Likewise, in a previous study, the antibacterial activity of various extracts of peel.

According to reports, GC-MS examination of banana pulp found the presence of substances

with anticancer activity, including palmitic acid, linoleic acid, oleic acid, campesterol, stigmasterol, and α-sitosterol (Kim et al., 2022). According to the findings, neither the green nor the ripe pulps exhibit any anti-fungal properties against *A. fumigatus* or *Candida albicans*.

There are very few studies examining the potential cytotoxic effects of bananas. The present outcomes demonstrated that the MTT test was used to determine the cytotoxicity of the green and ripe extracts. The cytotoxicity assay revealed that the extract of green pulp had greater cytotoxic activity against the MCF-7 cell line than the extract of ripe pulp. The presence of secondary metabolites such as phenolic, flavonoid, and saponin contents, which stimulate the apoptotic effect on malignant cells, may be the cause of the cytotoxic action. The prevention of terminal illnesses like cancer has long been demonstrated to be significantly aided by bioactive chemicals (Muniraj et al., 2019; Kim et al., 2022).

5. Conclusion

The findings of this study conclusively show that the green pulp of banana fruit has the capacity to act biologically as an anti-bacterial and cytotoxic agent. More importantly, the results showed that green pulps, according to their phytochemical composition, can be employed as a good source of antioxidants. To determine the active ingredients in BP extracts and to assess their mode of action, additional research is necessary.

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Conflict of interest

All authors declared that there was no conflict of interest.

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Author contributions

The authors have equal contribution. All authors read and approved the final manuscript.

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