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Identification of phenolic compounds from banana peel (*Musa paradaisica* L.) as antioxidant and antimicrobial agents

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ABSTRACT

This study was carried out to investigate the chemical composition and biological activity of banana peel extracts; the efficiency of the different solvent systems: aqueous, 80 % methanol, 80% ethanol and 80% acetone was used for extraction of phenolic, flavonoid and tannin compounds. Banana peel relative antioxidants potential by four assays DPPH˙, Fe^{2+}-chelating, Reducing power and $ABTS^{\cdot+}$ inhibitor activities was evaluated. Analysis showed that the percentage of moisture, protein, crude fat and total carbohydrates were 88.10, 13.42, 7.57, 10.44 and 68.31 g/100g DW respectively. For mineral content, potassium is the major element found in banana peel was (9.39 % of DW) followed by magnesium, calcium, sodium and phosphorus were (0.71, 0.44, 0.18 and 0.09 % of DW), respectively. Also, the content of microelement including iron, manganese, zinc and copper were 96.50, 35.01, 27.95 and 3.37 ppm, respectively. Methanolic extract (80%) had the highest content of total phenolic, flavonoid and tannin were 17.89, 21.04 and 24.21 mg /g DW respectively. Most of acetone banana peel extracts (80%) was found to be highest antioxidant and antimicrobial activity at 600 ppm against gram positive and negative bacteria, fungi and yeast. The phenolic profiles of banana peel acetone extract was identified by HPLC. The main phenolic compounds was chyrsin, qurectein and catchin. These results clearly encourage the application of banana peel as a potent natural source of antioxidant and antimicrobial sources.

Key words: Antioxidant, Antimicrobial, banana peel, HPLC

INTRODUCTION

Fruits and vegetables are considered as an important part of a good diet. They are known to reduce risk of several chronic diseases. Fruits and vegetables contain significant amounts of bioactive constituents which are negatively associated with the morbidity and mortality from cardiovascular and certain types of cancer. Fruits and vegetables wastes and their by-product are formed in great amounts during industrial processing and hence represent a serious problem, as they exert harmful impact on environment [1].

Fruits and vegetables peels are the major by-products obtained during the processing of various fruits and some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health. Different components having activities like antimicrobial, antioxidant and anti-cancers [2, 3].

Bananas belonging to the family *Musaceae* are one of the most important tropical fruits in the world market. Significant quantities of banana peels, equivalent to 40% of the total weight of fresh banana, are generated as a

waste. Recently, researchers have found Musa sapientum (*Musaceae*), drugs and the burden of healthcare costs are the main possess a prominent antidiabetic [4], anti-ulcer agent [5] obstacles for the cancer patients, additional approach antioxidant and anti-inflammatory effects [6,7].

Therefore, the present work was carried out to investigate the chemical composition and bioactive compounds in banana peels extract, and evaluate their antioxidant and antimicrobial activities.

EXPERIMENTAL SECTION

Chemicals and reagents

ABTS+ (2, 2'-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid)), Folin–Ciocalteau reagents, Gallic acid, Quercetin, DPPH· (2, 2-diphenyl-1- picrylhydrazyl), Ferrozine: (3- (2 - pyridyl) - 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine, BHT: Butyl Hydroxy toluene and, potassium ferricyanide, were purchased from Sigma Chemical Co.(St. Louis, MO, USA).

Proximate analysis

The moisture, ash, total nitrogen, crude protein, total lipid and total carbohydrates were determined according to [8, 9].

Preparation of banana peel extract

Ten (10) grams of dried powder of banana peel was dispensed in 100ml of distilled water, 80% ethanol, 80% methanol and 80% acetone, overnight at room temperature using shaker. The mixture was filtered through whatman No 1 filter paper and the extraction step was repeated twice. The filtrate was then concentrated to dryness at 40 °C in a rotary evaporator. The crude extracts were stored in a refrigerator until further analysis.

Total phenolic content

The total phenolic (TP) banana peel extracts were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid as standard according to [10]. The absorbance was determined at 750 nm using spectrophotometer (Unicum UV 300). The total phenolic content in the samples was expressed as mg gallic acid equivalents (GAE)/g dry weight sample. All samples were analyzed in triplicates.

Total flavonoid content

Total flavonoid (TF) of banana peel extracts were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard [11]. The absorbance was measured against blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/ g dry weight.All samples were analyzed in triplicates.

Total tannins content

Total tannins (TT) of banana peel extracts were measured using the Folin-Ciocalteu reagent according to [12]. Absorbance was measured against prepared reagent blank at 775 nm by using spectrophotometer (Unicom UV 300). Total tannins in sample were expressed as mg tannic acid equivalent (TE)/g dry weight sample. All samples were analyzed in triplicates.

Antioxidant activity

DPPH· Free radical scavenging assay

Determination of DPPH· free radical scavenging activity was measured according to [13]. The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxy toluene (BHT, Sigma) was used as positive control while the negative control is contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank.

The capacity to scavenge the DPPH· radical was calculated using the following equation:

DPPH· scavenging effect (Inhibition %) = $[(A_c - A_s / A_c) \times 100]$

Where: A_c is the absorbance of the control reaction.

A_s is the absorbance in the presence of the plant extracts.

Metal chelating activity
Metal chelating effects on ferrous ions was carried out calorimetrically according to [14]. The absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation:

Iron chelating activity (Inhibition %) = $[(A_c - A_s / A_c) \times 100]$

Where: A_c is the absorbance of the control reaction.
A_s the absorbance in the presence of the plant extracts.

Determination of scavenging activities on $ABTS^{\cdot+}$ radicals
Scavenging activity of $ABTS^{\cdot+}$ assay was determined according to [15]. The absorbance was measured at 734 nm using the spectrophotometer (Unicom UV 300). Results were expressed as in comparison with standard BHT. A bigger antioxidant capacity of the sample exhibited a smaller production of free radicals.

Percent activity was calculated using the following equation:

% Inhibition = $[(A_0 - A_1) /A_0] \times 100$ %

Where: A_0 is the $ABTS^{\cdot+}$ absorbance of the control reaction.
A_1 is the $ABTS^{\cdot+}$ absorbance in the presence of the sample.

Reducing power
The reducing power was assayed spectrophotometrically according to [16]. Increased absorbance values indicate a higher reducing power.

Antimicrobial activity
Different bacteria, including gram positive (*Bacillus subtilis* NRRL B-94, *Staphylococcus aureus* NRRL B-313), gram negative (*Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL B-32), fungus *Aspergillis fluves* NRC, yeast *Candida albicans* NRRL477 and *Saccharomyces cerviciea* strains, were used for measurement the growth inhibition according to [17].

Identification of phenolic compounds by HPLC
Phenolic compounds in banana peels acetone extract were identified using HPLC according to [18]. All chemicals and solvents used were HPLC spectral grade, and obtained from Sigma (St. louis, USA (from Merck – Shcuchrdt) Munich, Germany).

The HPLC system is Agilent 1100 series coupled with UV-Vis detector (G1315B) and (G1322A) DEGASSER. Sample injections of 5 µl were made from an Agilent 1100 series auto-sampler; the chromatographic separations were performed on ZORBAX-EclipseXDB-C18 column (4.6×250 mm, particle size 5 µm). A constant flow rate of 1 ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 280 nm. Phenolic compounds of banana peel extract were identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg phenolic/ g dry weight.

Statistical analysis
Data were statistically analyzed using Costat statistical package data according to [19].

RESULTS AND DISCUSSION

Proximate composition
The proximate composition of banana peels is shown in Table (1). The moisture content was 88.10 % in the peel is comparable with other varieties of banana fruit peels: *Yelakkibale* and *Nendranbale*, were 88.2 and 88.90 % respectively [20]. In addition, [21] reported that the moisture content of foods or its processed products gives an indication of its freshness and shelf life as well as the high moisture content in subject's food stuff is responsible for microbial spoilage, deterioration and short shelf life.

On the other hand the quality of the product can be provided by ash content. The ash content in banana peel (13.42%) was significantly higher than that obtained by [20] who found 8.90, 12.90 and 12.96 % respectivelly based on dry weight for three varieties of banana peels *Pachabale'*,*'Yelakkibale'* and *'Nendranbale'* respectively and more than that obtained by [21] 8.50%.

Table 1. Proximate analysis of banana peel

Compound	(g/100 g dry weight basis)
Moisture content	$88.10^{a} \pm 0.18$
Carbohydrate content	$68.31^{b} \pm 0.83$
Crude fat	$10.44^{d} \pm 0.89$
Crude protein	$7.57^{e} \pm 0.30$
Ash content	$13.42^{c} \pm 0.35$
L.S.D	1.58

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at $p \leq 0.05$

Protein is an essential component of diet for survival of animals and human being to supply adequate amounts of essential amino acids. The crude protein content in banana peel (7.57%) was significantly higher than was obtained by [21] which was recorded 0.90%. [20] Reported that the ash content was 7.77% for *Yelakkibale* banana peels.

The fat content of banana peel was recorded to 10.44% which is higher than was obtained by [21] and [7] as they recorded 1.70 and 0.90 % respectively.

Considering total carbohydrate in banana peel it reveal 68.31%which was higher than found by [21] and [22] who demonstrated 59.00 and 32.64 % respectively. So, banana peel can be considered as a good source of energy for the animals. Table (2) shown the results of mineral content of banana peel. Macro element, potassium is the major element found in banana peel was (9.39 % of DW) followed by magnesium, calcium, sodium, phosphorus were (0.71, 0.44, 0.18 and 0.09 % of DW) respectively and microelement iron, manganese, zinc and copper, (96.50, 35.01, 27.95 and 3.37 ppm) respectively, and were less than was obtained by [7] who declared that the mineral content of *Musa paradaisica* L. peel based on mg/g DW was rich in potassium (78.10 mg), calcium (19.20 mg) and sodium (24.30 mg).

Table 2. Nutrients content of banana peel

Macronutrients	Concentration (g/100gDW)	Micronutrients	Concentration (mg/Kg)
K	$9.39^{a} \pm 1.74$	Fe	$96.50^{a} \pm 19.73$
Ca	$0.44^{b} \pm 0.08$	Cu	$3.73^{c} \pm 1.14$
Na	$0.18^{b} \pm 0.02$	Zn	$27.95^{bc} \pm 3.31$
P	$0.09^{b} \pm 0.01$	Mn	$35.01^{b} \pm 4.25$
Mg	$0.71^{b} \pm 0.05$	----------	-------
LSD at 0.05	2.10	LSD at 0.05	26.33

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at $p \leq 0.05$

Furthermore, the iron level in banana peel was higher (96.50 mg/Kg) than that obtained by the values recorded by [21] who found 0.61mg/100g dry weight.

The differences between the recent results of proximate analysis may be attributed to plant species and environmental conditions which were also mentioned by several investigators [23].

Total phenolic, total flavonoids and total tannins of banana peel extract

Diets that are rich in fruits and vegetables are associated with decreased risk of cancer and heart diseases. The protective effect of fruits and vegetables has been attributed to their antioxidants constituents including; polyphenol, flavonoid and tannin which have biological and pharmacological properties [24]. The levels of phenolic compounds in different extracts (aqueous, methanol, ethanol and acetone) of banana peels are shown in Table (3). With increasing the solvent polarity used in extraction the total phenol (TP), total flavonoid (TF) and total tannin (TT) were increased. Data showed that TP, TF and TT were 17.89, 21.04 and 24.21 mg/g DW respectively in methanolic extract of banana peels. Significant differences between solvents ($p \leq 0.05$) were observed for TP, TF and TT contents. The total phenolic content in banana peel was higher than that previously reported by [23] who found that 585.29 and 685.57 mg GAE/100 g dry matter in two varieties (Cavendish and Dream) and 91.90 and 160.77 mg GAE/100 g dry matter in two stages of ripeness (ripe and green) of banana peel . [23] found that TP 1.31 to 3.99 g

GAE/100 g, with ethyl acetate peels extract of *muli* banana had the lower content than our results. [25] reported that solvent with high polarity gave high content of polyphenol and antioxidant activity [26, 27]. The Research conducted by [28] confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitis vinifera*). Whereas, [29] showed that the 80% ethanol or 80% acetone extract were better solvents compared to pure ethanol or acetone. Also, the same authors confirmed that the methanol was better for flavonoid in special for catechin, epicatechin and epigallocatechin.

Table 3. Total phenolic (TP), total flavonoids (TF) and total tannins (TT) in banana peel extract

Sample	Extracts	TP (mg*/g DW)	TF (mg**/g DW)	TT (mg***/g DW)
Banana Peels	Aqueous	9.89 ^{c} ± 0.16	8.56 ^{d} ± 0.22	14.69 ^{d} ± 0.34
	Methanol 80%	17.89 ^{a} ± 0.16	21.04 ^{a} ± 0.28	24.21 ^{a} ± 0.17
	Ethanol 80%	15.21 ^{b} ± 0.09	18.52 ^{b} ± 0.06	17.66 ^{b} ± 0.34
	Acetone 80%	15.44 ^{b} ± 0.19	16.15 ^{c} ± 0.28	15.90 ^{c} ± 0.28
L.S.D		0.41	0.60	0.75

*Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at p≤0.05 * As Gallic acid, ** As Quercetin and *** As Tannic acid*

From Table (3) the total phenolic of banana peels with different solvent systems showed significant changes in TP, TF and TT contents and the values were also dependent on the solvent polarity. From the results obtained in Table (3) it was observed that the methanolic extract contain the most phenolic and flavonoid contents because it can release the cell wall bound polyphenol from the cells and neutralize the activity of poly phenol oxidase (PPO) which degrades the polyphenol in plants as described by [30].

Methanolic extract of banana peel had higher content of TP than that reported previously by [23] who studied the influence of variety (Cavendish and Dream), stage of ripeness (green and ripe) and parts (pulp and peel) on antioxidative compounds and antioxidant activity. The TP and TF ranged widely from 75.01 to 685.57 mg GAE/100 g and 39.01 to 389.33 mg CEQ/100 g of dry matter respectively. Also, [23] estimated the total phenolic compound in two varieties (Cavendish and Dream) and two stages of ripeness (ripe and green) of banana peel which were 585.29 for (Cavendish ripe and 685.57 mg GAE/100 g dry matter for Cavendish green, 91.90 for Dream ripe and160.77 mg GAE/100 g dry matter for Dream green. These results were lower than that obtained in the present results.

The present results reveal that the total flavonoids in banana peel recorded the highest content (21.04mg/g DW) in methanolic extract followed by ethanol (18.52mg/g DW),acetone (16.15 mg/gDW) then aqueous extract (8.56 mg/g DW) were higher than that found by [29] who reported the total flavonoids in ethyl extract 10.22 g QE/100 g DW of *raja bulu* banana as compared to n-hexane which recorded 0.55 g QE/100 g for RB1 peels extract. [31] found that the total flavonoid content in the nine different varieties in 10% banana peel ethanol extract was ranged from 11.91 to 22.83 mg as rutin /g fresh tissue. Despite it was very high in two varieties (Cavendish and Dream) and two stages of ripeness (ripe and green) hence (Cavendish ripe was 225.91 and Cavendish green was 389.33 mg CEQ/100 g of dry matter) while (Dream ripe was 72.46 and Dream green 96.92 mg CEQ/100 g of dry matter). On the other hand, [23] and [32] detected flavonoid in ethanolic extract of banana peels but absent in water extract.

Banana peel may contain tannins, which are important phytochemicals with a wide range of medicinal properties, including anticancer, anti-inflammatory, antioxidant, and antibacterial activities [33].

The change in solvent polarity gave the same trend for the total tannins content. The highest content was observed with 80% methanol (24.21 mg/g) followed by ethanol (17.66), acetone (15.90) and water (14.69 mg /g DW) respectively. [25] found that the TT depends on the type of banana peels green, almost ripe and unripe was 6.84, 4.97 and 4.69% respectively. Also, the same authors found that the percentage to be high in fresh and dry banana peel of methanol extract (202.59 and 1886.32 mg/kg respectively). The total tannins content in banana peel (24.21mg/g DW)was found to be higher than that was found by [20] who demonstrated 517.00 to 1114.00 mg/100g DW in three varieties of Musa *paradaisica* L. peels *Pachabale Yelakkibale* and *Nendranbale*, respectively. Also, [34] found that the TTC was 67.594% for the stem of banana.

The differences between the present results in total phenolics, flavonoids and tannins contents and other investigators may be attributed to plant species, environmental condition and sample preparation. Also, the differences in phenolic content could be related to the part of fruit used for making the extract and type of solvent. The data obtained indicate that phenolic, flavonoids and tannins compounds of banana peels were extracted with 80% methanol better than all the other solvents tested.

Antioxidant activity

Reactive Oxygen Species (ROS), superoxide anion, hydrogen peroxide play an important role in oxidative stress related to pathogenesis of various important diseases. The production of free radicals is balanced by the antioxidative defense system [35].

The antioxidant properties of extracts of banana peels were determined using four different methods namely $DPPH^{\cdot}$, Reducing power, Fe^{2+}-chelating and $ABTS^{\cdot+}$ assays. The results obtained in Table (4) showed significant differences in the antioxidant activity between the solvents used.

$DPPH^{\cdot}$ scavenging activity

The acetone and methanol extracts however, showed greater $DPPH^{\cdot}$ scavenging activity than the ethanol and aqueous extracts. However, at a concentration 50 µg/ml, more or less similar results were obtained for banana peel with acetone and methanol extract (46.63% and 45.76%) respectively followed by ethanol (40.45 %) and aqueous (37.85%) with IC_{50} values 55.45, 56.03 and 75.34 µg/ml) respectively compared to BHT (4.73 µg/ml) . It is better to mention that a lower IC_{50} value represents more potent free radical inhibitory activity. Thus, the present results indicated that acetone and methanol extracts have powerful antioxidant activity as compared to ethanolic and aqueous extracts. The strong antioxidative properties of banana extracts could be attributed to the presence of different antioxidant components [36].

Table 4. $DPPH^{\cdot}$ scavenging activity of banana peel extract at different concentrations

Sample	Solvent type	Scavenging activity %				IC_{50} µg/ml
		5 µg/ml	20 µg/ml	35 µg/ml	50 µg/ml	
Banana Peels	Aqueous	29.13 [b] ± 0.49	30.67 [b] ± 0.57	32.84 [d] ± 0.67	37.85 [c] ± 0.67	120.03 [a] ± 8.61
	Methanol 80%	24.18[c] ± 0.60	31.29 [b] ±0.21	41.43 [b] ± 0.88	45.76 [b] ± 0.93	56.22 [c] ± 1.25
	Ethanol 80%	21.71[c] ± 0.67	28.01 [c] ± 0.37	32.65 [ed] ± 0.32	40.45 [c] ± 1.48	75.34 [b] ± 4.77
	Acetone 80%	17.50[d] ± 1.05	27.03 [c] ± 0.47	36.61[c] ± 0.21	46.63 [b] ± 0.60	55.45 [c] ± 0.86
BHT as standard		48.80[a] ± 1.82	70.42 [a] ± 0.52	71.47[a] ± 0.34	73.12 [a] ± 1.04	4.73 [d] ± 0.72
L.S.D at 0.05		2.81	1.20	1.47	2.68	12.01

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at $p \leq 0.05$

[37, 38] reported that the higher activity of $DPPH^{\cdot}$ radical scavenging activity may be attributed to the presence of higher levels of total phenolic and flavonoids as they play a key role as proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Fe^{2+}-chelating Activity

The ferrous chelating capacity is expressed by the percentage of inhibition of ferrozine–Fe^{2+} complex formation by different extracts. In this assay all extracts of banana peel are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion. As shown in Table (5), the ethanolic extract exhibit the highest percentage of metal chelating capacity (19.40 % ± 0.94) at concentration 200 µg/ml, as compared to the other three extracts at the same concentration, 15.45% ± 0.67 , 12.20+0.94 and 12.34% ± 0.43for aqueous, methanolic and acetone extracts respectively ($p \leq 0.05$).

Table 5. Fe chelating activity of banana peel extract at different concentrations

Sample	Solvent type	Chelating activity%			
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
Banana Peels	Aqueous	7.07 [b] ± 0.83	9.77 [b] ± 0.42	12.06 [b] ± 0.83	15.45 [c] ± 0.67
	Methanol 80%	2.29 [c] ± 1.36	6.24 [c] ± 0.42	8.80 [c] ± 0.64	12.20 [d] ± 0.94
	Ethanol 80%	5.13 [b] ± 0.60	8.87 [b] ± 0.73	11.50 [b] ± 0.73	19.40 [b] ± 0.94
	Acetone 80%	2.36 [c] ± 0.64	3.74 [d] ± 0.36	7.97 [c] ± 0.84	12.34 [d] ± 0.43
EDTA as standard		79.55 [a] ± 0.49	85.01 [a] ± 0.62	87.59 [a] ± 0.41	90.24 [a] ± 0.72
L.S.D 0.05		2.27	1.42	1.90	2.05

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at $p \leq 0.05$

Power reducing assay

It was observed that acetone extract of banana peel had higher reducing power EC_{50} = 399 ± 5.13, while the aqueous extract of banana peel showed the lowest potential activity EC_{50} = 774 ± 5.29 Table (6). The reducing power activity may be due to the presence of reductans as electron donors and are capable of converting them into a more stable product and terminating the free radical reaction. The reducing power ability of BHT was EC_{50} = 29 ± 0.72 µg/ml. The reducing power of banana peel extracts is probably due to the action of hydroxyl group of the phenolic compounds which might act as electron donors.

Table 6. Power reducing activity of banana peel extract at different concentrations

Sample	Solvent type	Absorption at 700nm				EC_{50} µg/ml
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
Musa Peels	Aqueous	0.124 [d] ± 0.004	0.149 [d] ± 0.002	0.156 [d] ± 0.001	0.170 [c] ± 0.002	774 [a] ± 5.29
	Methanol 80%	0.187 [b] ± 0.004	0.209 [b] ± 0.005	0.223 [b] ± 0.006	0.241 [b] ± 0.004	470 [b] ± 5.02
	Ethanol 80 %	0.180 [b] ± 0.007	0.190 [c] ± 0.001	0.208 [c] ± 0.005	0.232 [b] ± 0.003	487 [b] ± 11.43
	Acetone 80%	0.164 [c] ± 0.003	0.186 [c] ± 0.005	0.216b [c] ± 0.003	0.233 [b] ± 0.003	399 [c] ± 5.13
BHT as standard		0.478 [a] ± 0.007	0.698 [a] ± 0.004	0.886 [a] ± 0.006	1.168 [a] ± 0.005	29 [d] ± 0.72
L.S.D 0.05		0.014	0.010	0.012	0.009	17.46

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at p≤0.05

This may be attributed to the low viscosity of the solvent which has low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents [39]. Regarding the reducing power, the amount of phenolic compounds was high in acetone extract of banana peel .These results were previously reported by [40] who indicated that the reducing power of bioactive compounds is associated with antioxidant activity.

$ABTS^{\cdot+}$ scavenging activity of banana peel extracts

The antioxidant capacities of aqueous, methanol, ethanol and acetone extracts of banana peel were evaluated according to the $ABTS^{\cdot+}$ discolorations method. The results were given in Table (7) which showed that at the highest concentration (100 µg/ml), the acetone extract of banana peel gave the highest antioxidant activity (75.39% ± 0.59) followed by ethanol (28.30% ± 0.85), methanol (27.91 ± 0.83) and aqueous extract (25.16 ± 1.42) (Table 7).

Table 7. $ABTS^{\cdot+}$ scavenging activity of banana peel extract at different concentrations

Sample	Solvent types	Scavenging activity %			
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Banana Peels	Aqueous	12.89 [c] ± 0.76	15.64 [d] ± 0.72	22.64 [d] ± 0.62	25.16 [d] ± 1.42
	Methanol 80%	5.42 [d] ± 0.62	15.80 [d] ± 0.47	24.53 [c] ± 0.85	27.91[c] ± 0.83
	Ethanol 80%	14.62 [c] ± 0.41	18.95 [c] ± 0.36	24.76 [c] ± 0.71	28.30 ± 0.85 [c]
	Acetone 80%	55.11[b] ± 0.59	65.57 [b] ± 0.41	72.48 [b] ± 0.59	75.39 [b] ± 0.59
BHT as standard		81.04 [a] ± 0.96	84.12 [a] ± 0.46	86.97 [a] ± 0.44	89.12 [a] ± 0.86
L.S.D at 0.05		1.87	1.35	1.77	2.56

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at p≤0.05

Identification of phenolic compounds by High Performance Liquid Chromatography (HPLC)

Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors [41]. Data in Table (8) reveal that the acetone extract of banana peel had high amounts of chyrsin, quercetin, catchin, cianamic, caffiec and coumarin 460.20, 78.62, 30.21, 3.23, 0.79 mg/100g respectively.

[42] found that phenolic composition of banana peel 19.618, 4.745 and 0.157 µg/g for Gallic, vanillic and cinnamic respectively. In addition to ferulic acid was found to be dominant insoluble phenolic acids of banana extracts which recorded 219.50 µg/g dry weight using HPLC-UV method [43]. Whereas, [44] found that phenolic and flavonoid contents were strongly depend on the type of the solvent as well as on the different percentages used. Many authors established that the extraction yield of phenols was greatly depending on the solvent polarity [25, 44, 45]. Moreover, ethanol and methanol were found to be more effective than water for phenols from peanut skin [45]. Data in Table

(8) showed that the content of phenolic compounds in banana peel ranged from 460.2 to 0.79 mg/100g for chyrsin and coumarine, respectively.

Table 8. Profiling of phenolic compounds of banana peel acetone extract

Phenolic compounds mg/kg crude extract	Acetone extract
Quercetin	78.62
Coumarin	0.79
Catechin	30.21
Caffiec acid	3.23
Cinnamic acid	8.14
Chyrsin	460.24

[46] Identified the polyphenols in the methanol extract of four varieties of banana fresh part: yellow, green, rasthali and karpooravalli as chlorogenic acid it ranged from 34.60 to 782.00, quercetin 32.30 to 125.09 and naringenin 8.90 to 12.20 µg/g. They added that rasthali variety possessed the highest concentration of chlorogenic acid and naringenin whereas, karpooravalli variety possessed the highest concentration of quercetin.

[47] examined the phenolic composition by HPLC/DAD and found that Gallic acid 3.75, caffeic acid 18.90, ellagic acid 20.65, rutin 19.36, isoquercitrin 40.71 and quercetin 51.83 mg/g in unripe plantain. In addition, [48] investigated and quantified the phenolic compounds of nine plantain peel which were performed by means of HPLC–ESI-HR-MS and HPLC-DAD . They found that flavonol glycosides were predominant in plantain peels and rutin was the most abundant 242.2–618.7 µg/g of dry weight.

Antimicrobial activity

Fruit peels have a valuable source for maintaining human health. The use of fruit peels extracts for antimicrobial properties can be of great significance in therapeutic treatments. The antimicrobial properties of the ethanol and acetone extracts of *banana peels* were evaluated by well diffusion assay against different microbial isolates The results presented in Table (9) showed that 80% acetone extract inhibited bacterial species at 600 ppm against gram-positive bacteria including *B. ubtilis* (20.60%), *S. aureus* (19.75mm), *E. coli* (18.15mm) and *P. auegino* (19.57mm). Antimicrobial activity and preservative of banana peel extract are believed to be associated with phytochemical components of the banana peel, like phenolic, tannins as reported by [49]. The presence of tannins showed some antimicrobial activity against three (*E. coli*, *S. aureus*, and *P. aeruginosa*) of the tested microorganisms. By comparing the previously published results with those of the current study, it was appeared that, the antimicrobial activity is strongly affected by the presence of phenolic and tannins compounds acetone 80% extract. It is well known that the concentration of biologically active constituents varies with the plant parts, and the polarity of the solvent used, which directly reflects this activity [50].

Table 9. Antimicrobial activity of banana peel

Extracts	Conc. µg/ml (ppm)	Zone Inhibition (mm)						
		Bacteria				Fungus	Yeast	
		B. subtilis	*St. aureus*	*E. coli*	*P. aueginosa*	*A. fluves*	*S. cervisiae*	*C. albicans*
Ethanol 80%	200	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	400	10.66	10.53	09.67	10.61	10.33	11.47	11.47
	600	15.32	14.17	15.23	15.86	11.76	15.60	15.60
Acetone 80%	200	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	400	13.73	14.70	13.73	12.62	12.30	11.56	11.83
	600	20.60	19.57	18.15	19.57	17.81	16.87	17.61

CONCLUSION

The obtained results concluded that banana fruit peel has a wide range of phenolic compounds with different polarities. Acetone extracts of fresh yellow banana peels could be considered as a good antioxidant and antimicrobial material against both gram positive and negative bacteria which encourage replacing the synthetic medicines in treatment of diseases caused by these bacteria. Further studies are needed for isolation of the other active ingredients, and evaluation of cytotoxicity effects. These observations let to suggest that the extracts can also be as potent source of pharmaceutical ingredient.

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REFERENCES

[1] PM, Dewick; Medicinal Natural Products, Wiley, Chichester, England, **2002**; p. 121.
[2] SK, Sharma; M, Le Maguer, *Italian Journal of Food Science*, **1996**; 2, 107–113.
[3] Y, Lario; E, Sendra; J, Garcıa-Perez; C, Fuentes; E, Sayas-Barbera; J, Fernandez-Lopez, *Innovative Food Science and Emerging Technologies*, **2004**; 5,113–117.
[4] P, Dikshit; K, Shukla; MK, Tyagi; P, Garg; JK, Gambhir; R, Shukla, *Journal of Diabetes*, **2012**; 4(4), 378-385.
[5] RK, Goel; K, Sairam, *Indian Journal of Pharmacology*, **2002**; 34(2), 100-110.
[6] P, Phuaklee; S, Ruangnoo; A, Itharat, *Journal of the Medical Association of Thailand= Chotmaihet thangphaet*, **2012**; 95: S142-6
[7] OT, Okareh; AT Adeolu; OT, Adepoju, *African Journal of Food Science and Technology*, **2015**; 6(2), 53-57.
[8] AOAC. Official methods of analysis of the Association of Official Analytical Chemists.17th Ed. AOAC, Gaithersburg, MD., USA, (**2005**); p 18.
[9] M, Dubois; KM, Gilles; JK, Hamilton; PA, Rebers; F, Smith, *Analytical Chemistry*, **1956**; 28, 350-356.
[10] VL, Singleton; JA, Rossi, *American Journal of Enology and Viticulture*, **1965**; 16, 144 –158.
[11] J, Zhishen; T, Mengcheng; W, Jianming, *Food Chemistry*, (**1999**); 64, 555–559.
[12] SA, Polshettiwar; RO, Ganjiwale; SJ, Wadher; PG, Yeole, *Indian Journal of Pharmaceutical Sciences*, **2007**; 69: 574-576.
[13] YH, Chu; CL, Chang; HF, Hsu, *Journal of the Science of Food and Agricultural*, **2000**; 80: 561–566.
[14] C, Su; W, Chen; Y, Weng; C, Tseng, *Food Chemistry*, **2003**; 83: 85–92.
[15] MB, Arnao; A, Cano; M, Acosta, *Food Chemistry*, **2001**; 73, 239–244.
[16] T, Kuda; M, Tsunekawa; H, Goto; Y, Araki, *Journal of Food Composition and Analysis*, **2005**; 18, 625–633.
[17] D, Greenwood; Antimicrobial chemotherapy, Part II, In Laboratory Aspects of Antimicrobial Therapy. (Greenwood, D Ed.) Bailliere, Tindall, London, **1983**; 71, p 323.
[18] M, Ben-Hammouda; RJ, Kremer; HC, minor; MA, Sarwar, *Journal of Chemical Ecology*, **1995**; 21, 775–786.
[19] F, Silva; de A, Se, The ASSISTAT Software: statistical assistance, in: international conference on computers, 6 Cancun, 1996. Anais... Cancun: American Society of Agricultural Engineers, **1996**; p. 294-298.
[20] BN, Shyamala; P, Jamuna, *Asian Journal of Food and Agro-Industry*, **2011**; 4(01), 31-46.
[21] BA, Anhwange; TJ, Ugye; TD, Nyiaatagher, *Electronic Journal of Environmental Agricultural and Food Chemistry*, **2009**; 8 (6), 437-442.
[22] A, Maniyan; j, Reshma; M,Anu, *Emergent Life Sciences Research*, **2015**; 1(2), 13–19.
[23] SR, Fatemeh; R, Saifullah; FMA, Abbas; ME, Azhar, *International Food Research Journal*, **2012**; 19 (3), 1041-1046.
[24] MZ, Imam; S, Akter, *Journal of Applied Pharmaceutical Science*, **2011**; 1 (5), 14-20.
[25] N, Turkmen; F, Sari; YS, Velioglu, *Food Chemistry*, **2006**; 99, 838-841.
[26] P, Siddhuraju; K, Becker, *Journal of Agricultural Food Chemistry*, **2003**; 51, 2144-2155.
[27] B, Sultana; F, Anwar; R, Przybylski, *Food Chemistry*, **2007**; 104, 1106-1114.
[28] GK, Jayaprakasha; RP, Signh; KK, Sakariah, *Food Chemistry*, **2001**; 73: 285– 290.
[29] I, Fidrianny; RR, Kiki; M, Insanu, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2014**; 6 (8), 299-303.
[30] B, Lapornik; M, Prosek; AG, Wondra, *Journal of Food Engineering*, **2005**; 71(2), 214-222.
[31] JJ, Bhaskar; MS, Shobha; K, Sambaiah; PV, Salimath, *Journal of Physiological Biochemistry*, **2011**; 67, 415-425.
[32] G, Ehiowemwenguan; AO, Emoghene; JE, Inetianbor, *IOSR Journal of Pharmacy*, **2014**; 4 (8), 18–25.
[33] K, Lee; Y, Kim; H, Lee; C, Lee, *Journal of Agricultural and Food Chemistry*, **2003**; 51, 7292-7295,
[34] ML, Apriasari; Iskandar; E, Suhartono, *International Journal of Bioscience, Biochemistry and Bioinformatics*, **2014**; 4 (2), 110–115.
[35] W, Huang; H, Zhang; W, Liu; C, Li, *Journal of Zhejiang University SCIENCE B*, **2012**; 13, 94-102.
[36] J, Mrvcic; S, Posavec; S, Kazazic; D, Stanzer; A, Pesa; V, Stehlik-Tomas, *Croatian Journal of Food Science and Technology*, **2012**, 4, 102–111.
[37] MLDL, Fransisco; AVA, Resurrection, *Journal of Food Composition and Analysis*, **2009**; 22, 16-24.
[38] A, Ghasemzaeh; HZE, Jaafar; A, Rahmat, *Molecules*, **2010**; 15, 4324- 4333.
[39] R, Amarowicz; F, Shahidi, *Journal of Food Lipids*, **1995**; 2, 47-56.
[40] N, Hounsome; B, Hounsome; D, Tomos; G, Edwards-Jones, *Postharvest Biology and Technology*, **2009**; 52, 173-179.
[41] A, Rodtjer; LH, Skibsted; ML, Andersen, *Food Chemistry*, **2006**; 99, 6-14.
[42] UP, Singh; A, Singh; R, Srivastava. (2012). *World Journal of Agricultural Sciences*, **2012**; 8 (2): 150–155.
[43] N, Ongphimai; S, Lilitchan; K, Aryusuk; A, Bumrungpert; K, Krisnangkura, *Chiang Mai Journal of Sciencie*, **2013**; 40 (4): 636–642.
[44] J, Yu; M, Ahmedna; I, Goktepe, *Food Chemistry*, **2015**; 90, 199-206.

[45] CH, Jung; HM, Seog; IW, Choi; MW, Park; HY, Cho, *Food Science and Technology*, **2006**; 39, 266–274.
[46] MA, Babu; MA, Suriyakala; KM, Gothandam, *Journal of Pharmaceutical Sciences and Research*, **2012**; 4 (10), 1950–1955.
[47] S, Salawu; AA, Boligon; ML, Athayde, *International Journal of Applied Research in Natural Products*, **2014**; 7 (2), 11–20.
[48] CVP, Tsamo; MF, Herent; K, Tomekpe; TH, Emaga; JQ, Leclercq; H, Rogez; Y, Larondelle; C, Andre, *Food Chemistry*, **2015**; 167, 197–204.
[49] GO, Adegoke; BA, Odesola, *International Biodeterioration and Biodegradation*, **1996**; 6, 81–84.
[50] M, Govindappa; S, Naga Sravya; MN, Poojashri; TS, Sadananda; CP, Chandrappa, *Journal of Pharmacognosy and Phytotherapy*, **2011**; 1*(*3), 51-63.