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Comparative antibacterial and antioxidant activities of edible and non-edible parts of unripe banana (*Musa sapientum* L.) Fruit

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Abstract

Comparative antibacterial and antioxidant *in vitro* activities of edible (pulp) and non-edible (peel) parts of unripe banana fruit were investigated. Peels and pulps of fruit were extracted using methanol and acetone separately. Antibacterial property of these extracts was evaluated against four species of multiple drug resistant (MDR) bacteria namely *Escherichia coli*, *Klebsiella* sp, *Salmonella* sp. and *Shigella* sp. using disc diffusion technique. When compared between pulp and peel on the basis of zone of inhibition created by them, pulp and peel exhibited 14.5 mm and 16.2 mm, respectively, whereas the standard drug, Kanamycine (30µg/disc) showed >30mm. In case of MIC, pulp was ranged from 200-300 mg/ml, while peel was from 200-250 mg/ml. As like MIC, MBC also found better in peel (400-550 mg/ml) than pulp (450-600 mg/ml). Results showed that non-edible part was better than edible part for controlling bacteria. Antioxidant activity of the extracts was evaluated by total phenolic content determination and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH scavenging activities of non-edible part (70.62%) was found higher than edible part (54.79%), while 100% was observed by ascorbic acid at same concentration (100µg/ml). In case of phenol content, edible part of banana showed lower phenolic content (71.49 mgL^{-1} GAE / g dry material) than non-edible part (92.76 mgL^{-1} GAE / g dry material). Comparing between pulp and peel, peel found better than pulp in every case, e.g., antibacterial (both MIC and MBC), antioxidant activities and total phenol content. The results suggest that banana peels could serve as potential source of bioactive compounds and can be utilized effectively without being wasted.

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Introduction

Plants have been valuable source of natural products for various human beneficial products and maintaining human health, especially in the last decades, with more intensive studies for natural therapies. The use of plant extracts with known antibacterial and antioxidant properties are effective in the treatment of the infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic compounds. Fruits and vegetables have been used as antibacterial and antioxidant agent, because they synthesized many compounds by secondary metabolism of the plant (Priya *et al.*, 2014). *Secondary metabolism* produces a large number of specialized *compounds,* such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins, fatty acids, gums etc. which are capable of producing definite physiological action on the human body (Bishnu *et al.*, 2009).

Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever, bronchitis, etc. (Srivastava *et al.*, 2013).

Banana is the common name for herbaceous plant of the genus *Musa*. It is a familiar tropical fruit and important source of food in the world. From its native South western Pacific home, the banana plant spread to South Asia by about 600 BC and later on it spread all over the tropical world. It is possibly the world's oldest cultivated crop (Karuppiah and Mustaffa, 2013). It possesses efficient medicinal values such as stem juice is also used in nervous affectations like epilepsy, hysteria and in dysentery and diarrhoea. Several oligosaccharides comprising fructose, xylose, galactose, glucose and mannose occur naturally in banana making it an excellent prebiotic for the selective growth of beneficial bacteria in the intestine (Debabandya *et al.*, 2010). It aids in combating diarrhoea and dysentery and promotes healing of intestinal lesions in ulcerative colitis. Roots of banana are antihelmintic, flowers are astringent and fruits are mild laxative. It is also useful in celiac disease, constipation and peptic ulcer (Mallick *et al.*, 2007).

The objective was comparative *in vitro* studies on antibacterial and antioxidant activities between edible (pulp) and not-edible (peel) solvent extracts of unripe banana fruit available in local market.

Materials and methods

Plant material

Banana (*Musa sapientum*) fruits were collected from Rajshahi local market, Saheb Bazar, at green stage confirm no chemical treatment and the sample was identified and authenticated as *Musa sapientum* (L.) by Dr. AHM Mahbubur Rahman, Professor and Plant Taxonomist, Department of Botany, University of Rajshahi, Bangladesh and a voucher specimen was deposited at the Herbarium of the department. The freshly collected bananas were washed with distilled water to remove dirt, followed by separation of edible (whole fruit –peel = pulp) and non-edible (whole fruit –pulp = peel) parts using autoclaved knife. Then the edible and non-edible parts were chopped separately into pieces and dried at room temperature (32-35°C) for five days until a constant weight was obtained. 250g of each of the parts were coarsely powdered using a mortar and pestle, and were further reduced to powder using an electric blender. Then the powdery materials (edible and non-edible) were stored separately in air tight bottles for further use.

Extraction procedures

Plant extracts were prepared using the methods of Sultana *et al.* (2009) with slight modification. Each of the powdered air-dried plant material was extracted with methanol and acetone solvent separately. 5g of each powdered sample was mixed in a conical flask with 100ml of organic solvent, and then allowed to soak at ambient temperature for 24 h. The extracts were then filtered with tetron cloth into a beaker and then using Whatman no. 1 filter paper. The filtrates concentrated at 40 ^{O}C using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan). Then the concentrated extracts were collected in a screw cap tube and stored at 4 ^{O}C for further use. The yield was found to be 2.45±0.87 and 4.66±1.63% w/w in methanol and acetone, with reference to the air dried powdery plant material. The dried extracts were

dissolved in dimethylsulphoxide and subjected to antibacterial activity.

Test organisms

Four species of multiple drug resistant (MDR) bacteria were used for this investigation namely *Escherichia coli*, *Klebsiella* sp, *Salmonella* sp. and *Shigella* sp. All clinical isolates obtained from the Plant Biotechnology and Microbiology Laboratory of the Department of Botany, Rajshahi University.

The organisms were periodically subcultured and maintained in nutrient agar slant at 4 ^{O}C.

Antibacterial activities

Antibacterial activity of the methanolic and acetonic extracts of the plant sample (edible and non-edible part of unripe banana) was evaluated using disc diffusion method according to Bauer *et al.* (1966) with slight modifications. For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto Nutrient agar (oxoid) plates (diameter: 15cm).

Sterile filter paper discs (diameter 6mm) impregnated with 100µl of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100mg/ml were applied over each of the culture plates previously seeded with the 0.5 McFarland and 106cfu/ml cultures of bacteria. After placing the disc, the plate was inverted and incubated at 37°C for 24 hours.

Then the plate was examined and measured the diameters of the clear zones on the agar surface including the diameter of the disk. The zones were measure to the nearest millimeter using a ruler and values <8 mm were considered as not active against microorganisms (Bhalodia and Shukla, 2011).

The experiment was replicated three times to confirm the reproducible results. Commercially available kanamycin discs (30 µg/disc) were used as positive control while the discs prepared using the appropriate solvents only served as negative control.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to Doughari *et al.* (2007) with slide modification. The MIC of each extracts was determined against each of the test bacteria in varying concentrations of 100, 200, 300, 400, 500, 600 and 700 mg/ml. Then the nutrient broth (9ml) tube was added with 1ml of test organism previously diluted to 0.5 McFarland turbidity standards. A tube containing only nutrient broth was seeded with the test bacteria, as described above, to serve as controls. All the tubes were then incubated at 37°C for 24 h and then examined for growth by observing for turbidity. MIC was measured by the concentration at which there was no change of turbidity (Ajaiyeoba *et al.*, 2003).

Determination of antioxidant activity using free radical scavenging activity (DPPH)

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging by the extracts was determined by the method described by Braca *et al.* (2001). 200 µl plant extract of different strength (5, 25, 50, 100, 500 µg/ml) was mixed with 2 ml of a 0.004% methanol solution of DPPH. After 30 min, the absorbance was determined at 517 nm using a UV spectrophotometer (Shimadzu, UV-1601PC) against a blank. Absorbance of DPPH solution only without the extract or standard agent was used as control. The percentage scavenging activity of the extracts was calculated using the formula: % scavenging activity = $\{(A_0 - A_1) / A_0\} \times 100$; where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard.

Determination of IC_{50} value

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50 percent inhibition was determined and expressed as IC_{50} value. The lower the IC_{50} value indicates high antioxidant capacity. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Determination of total phenolic content

Estimation of total phenol content was conducted using the Folin–Ciocalteu (F–C) reagent spectrophotometric method described by Ainsworth and Gillespie (2007) with slight modification. The F–C assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic or phosphotungstic acid complexes, which are determined spectroscopically at 765nm. Although the electrons transfer reaction is not specific for phenolic compound. For creating the calibration curve, gallic acid was used as a standard material.

Results and discussion

In vitro antibacterial activity

Antibacterial activity of edible and non-edible parts of unripe banana fruit extracts has been evaluated *in vitro* against four species of MDR bacteria eg. *Escherichia coli*, *Klebsiella* sp, *Salmonella* sp. and *Shigella* sp. by disc diffusion method. The results showed varying magnitudes of inhibition patterns with standard drug Kanamycine, a well-known broad-spectrum antibacterial agent. The mean inhibitory zone of used parts (edible and non-edible) and the standard drug Kanamycine against four MDR bacterial species is summarized in Table 1.

Table 1. Antibacterial activities of Edible and Non-edible Parts of *M. sapientum* fruit extracts against selected MDR bacteria.

Used Sample (Part of banana)		Zone of inhibition (mm)							
		Methanol				Acetone			
		Escherichia coli	*Klebsiella*	*Salmonella*	*Shigella*	*Escherichia coli*	*Klebsiella*	*Salmonella*	*Shigella*
Edible Part	200	+	+	+	+	+	+	+	+
Non- Edible Part		+	+	+	+	+	+	+	+
Edible Part	400	8.4±0.74	+	8.3±0.45	9.3±0.27	+	+	+	+
Non- Edible Part		8.6±0.44	8.4±0.58	10.9±0.39	10.2±0.66	+	8.1±0.48	+	+
Edible Part	600	9.8±1.17	9.6±0.63	12.7±0.77	11.9±0.69	8.3±0.55	8.3±0.68	9.2±0.61	9.7±0.64
Non- Edible Part		10.6±0.67	10.9±0.57	15.2±0.88	13.1±0.37	9.3±0.60	9.4±0.55	9.7±0.50	10.9±0.44
Edible Part	800	12.1±0.15	11.8±0.25	14.5±0.75	13.3±0.25	11.8±0.71	10.9±0.34	11.1±0.35	11.6±0.85
Non- Edible Part		14.2±0.65	14.5±0.88	16.2±0.41	15.9±0.59	12.3±0.75	12.4±0.95	12.6±1.20	12.5±0.48
Negative Control		+	+	+	+	+	+	+	+
Positive Control (Kanamycine 30µg/disc)		>30	>30	>30	>30	>30	>30	>30	>30

Data are represented as mean ± SD of triplicate experiments. + = Bacterial growth.

ANOVA for the Table 1.

Item	SS	df	MS	F
Sample	19.53	1	19.53	5.39
Solvent	88.74	1	88.74	24.48
concentration	10218.29	5	2043.66	563.86
Bacteria	8.93	3	2.98	0.82
Error	308.08	85	3.62	
Total	10476.02	95	110.27	

The diameters of growth inhibition zone produced by banana fruit extracts were in the range maximum of 16.2 mm and minimum of 8.1 mm whereas the standard drug, Kanamycine (30µg/disc) showed higher inhibition zone (>30mm). Non-edible part showed higher antibacterial activity than edible part in both methanolic and acetonic extract against most of used bacteria. Negative controls (only solvents) did

not show inhibitory effect against the studied bacteria as expected.

The result showed that the non-edible part showed wider zone of inhibition (16.2mm) than edible part (14.2mm) against *Salmonella* sp. at 800mg/ml concentration. At 600mg/ml and 400mg/ml concentration, non-edible part again showed wider zone (15.2mm and 10.9mm) than edible part (12.7mm and 9.3mm). But both edible and non-edible part showed no activities against any used bacteria at 200mg/ml concentration in both solvents.

Anova analysis confirmed that non-edible part was better than edible part in order to create zone of inhibition, as well as, antibacterial activities.

Table 2. MIC and MBC value of Edible and Non-edible Part of *M. sapientum* fruit extracts (methanolic and acetonic) against selected MDR bacteria.

Used Part	MIC value ($mgml^{-1}$)							
	Escherichia coli		*Klebsiella* sp.		*Salmonella* sp		*Shigella* sp.	
	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
Edible Part	200	300	250	300	200	250	200	250
Non- Edible Part	200	250	200	250	200	200	200	200
	MBC value ($mgml^{-1}$)							
Edible Part	450	550	550	600	450	600	450	550
Non- Edible Part	450	500	450	550	400	550	400	500

ANOVA for MIC and MBC for Table 2.

Item	Minimum inhibitory concentration (MIC)				Minimum bactericidal concentration (MBC)			
	SS	Df	MS	F	SS	df	MS	F
Used Part	3906.25	1	3906.25	9.615	10000	1	10000.00	16.000
Solvent	7656.25	1	7656.25	18.846	40000	1	40000.00	64.000
Bacteria	4218.75	3	1406.25	3.462	8750	3	2916.66	4.667
Error (within)	4062.5	10	406.25		6250	10	625.00	
Total	19843.75	15			65000	15		

Similar observations were reported by Jain *et al.* (2011) where peel extract showed wider zone of inhibition (21.5mm, 17.5mm, 16.5mm and 15.0mm) than pulp extract (17.5mm, 16.0mm, 14.0mm and 12.5mm) against *Sarcina* sp., *Shigella* sp., *Vibrio* sp. and *Salmonella* sp. respectively. Ahmad and Beg (2001) found better positive activity of peel extract than leaf extract of banana against *Staphylococcus* and *Pseudomonas* species in alcoholic extracts of banana.

Table 3. Determination DPPH Radical Inhibition or Scavenging Assay/Activity.

Musa sapientum	Abs (sample) [A_1]	Abs [blank]	Abs (control) [A_0]	DPPH scavenging /inhibition activity [I(%) = 100 x (A_0-A_1)/A_0] Mean ± SD
Edible part	0.1325	0	0.451	54.79 ± 0.27
Non-edible part	0.2039	0	0.451	70.62 ± 0.71
Ascorbic acid (standard)	0.0000	0	0.451	100 ± 0.90

Again, Extracts from bananas peel, showed activity against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella enteritidis, Escherichia coli* reported by Chanda *et al.* (2010).

The present result also indicated that methanolic extracts showed significant antibacterial activity than acetone extracts. This signifies that methanol can dissolve the more active phytochemicals than

acetone. Dhawan and Gupta (2017) reported that using methanol as an extraction solvent works best for the extraction of various active phytochemicals.

MIC and MBC for Edible and Non-edible part of Musa sapientum

The results of screening MIC and MBC values of edible and non-edible part of unripe banana fruit extracts are presented in Table 2. Here the MIC values were ranged from 200 to 300 mg/ml and the MBC were from 450 to 600 mg/ml.

In case of edible part, MIC were ranged from 200-300mg/ml and MBC were from 450-600, while in non-edible part those were 200-250 and 450 -550 mg/ml for MIC and MBC, respectively. Comparing two solvents, methanol gave lower MIC and MBC value than acetone.

Table 4. Total Phenols content of the edible and non-edible part of banana.

Musa sapientum	Total Phenols [mgL^{-1} GAE / g dry material] Mean ± SD
Edible part	71.49 ± 1.78
Non-edible part	92.76 ± 1.74

Microorganisms vary widely in their degree of susceptibility to anti-microbial agents. A high MIC value indicates low activity and vice versa. In this study edible part gave lower MIC and MBC value than non-edible part.

This suggests MDR bacteria showed higher susceptibility to the extract of the peels. On the basis of the result obtained in this investigation it can be concluded that methanol extract of *Musa sapientum* peels had significant *in vitro* broad spectrum antimicrobial activity than pulp extract.

It was reported by many investigators that the peel fractions of some fruits were found to show higher antimicrobial activity than the pulp fractions (Jayaprakasha *et al.*, 2001; Mokbel and Hashinaga, 2005; Jain *et al.*, 2011 and Sulaiman *et al.*, 2011).

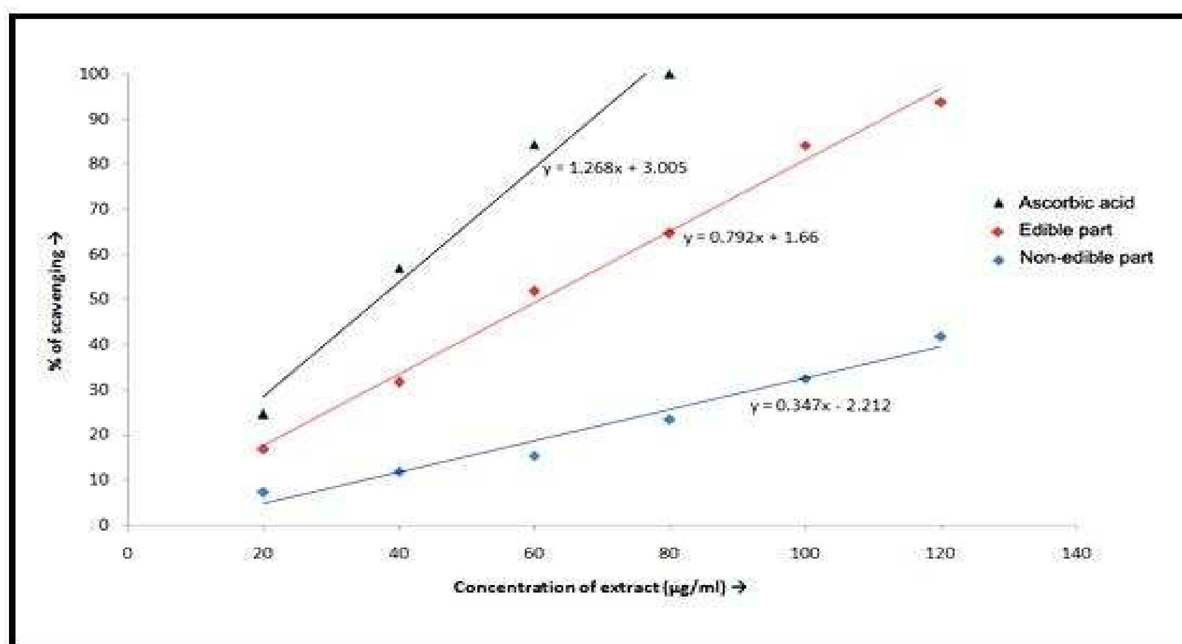

Fig. 1. Calibration curve Determination of IC_{50} value of edible and non-edible part of *M. sapientum* extract along with Ascorbic acid.

Analysis of Antioxidant Activities and IC_{50} Value

The antioxidant activity of the studies extracts was determined by DPPH free radical scavenging assay on the basis of percentage inhibition, because it is one of the most effective methods for evaluating radical-scavengers (Braca *et al*., 2001 and Jain *et al*., 2011). The percent DPPH radical scavenging by the plant extracts was found concentration dependent (Table 3 and Figure 1). Here scavenging activities of non-edible part (70.62%) was found higher than edible part (54.79%), while 100% was observed by ascorbic acid at same concentration (100µg/ml).

The observation was agreed with the findings of Anuj *et al.* (2016) that was 73.51% and 65.27% in unripe banana peel and pulp respectively. Among the samples, non-edible part showed better IC_{50} value of 61.035 µg/ml than edible part (150.466 µg/ml), while the standard, ascorbic acid, showed an IC_{50} value of 37.062 µg/ml.

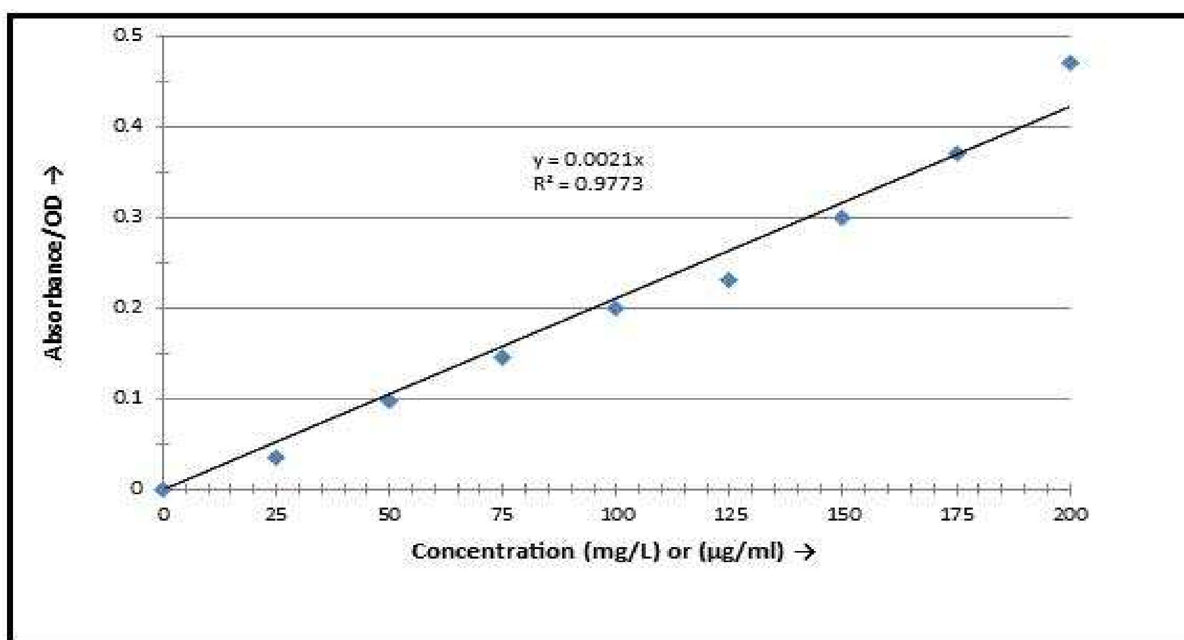

Fig. 2. Calibration curve of gallic acid.

These results were in agreement with Mokbel and Hashinaga (2005), who reported the antioxidant effects of crude extracts of green banana peel more significant than other parts. Jain *et al.* (2011) also reported antioxidant activities of banana peel is better than pulp and seed extract. Banana which is a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant compounds (Kanazawa and Sakakibara, 2000) in peel. For this reason may be non-edible part showed more antioxidant activities than edible part.

Estimation of total phenols content

Estimation of total phenol content (including flavonoid) was conducted using the Folin–Ciocalteu (F-C) reagent spectrophotometric method and the results are presented in Table 4. For creating the calibration curve, gallic acid was used as a standard material.

From the result it was observed that edible part of banana showed lower phenolic content (71.49 mgL^{-1} GAE / g dry material) than non-edible part (92.76 mgL^{-1} GAE / g dry material). As compared to the previous studies Anuj *et al.* (2016) found total phenolic content 892 mg QE/ g of extract in unripe peel, whereas 744 mg QE/ g of extract in unripe pulp. Again, Someya *et al.* (2002) reported that the edible pulp of bananas contains 232 mg/100 g of dry weight phenolic compounds and this amount was about 25% of that present in the peel. Similarly Gorinstein *et al.* (2001) found that the total phenolic compounds in the peels of lemons, oranges, and grapefruits were

15% higher than that of the pulp of these fruits. It is also reported that the total phenolic compounds of non-edible part of several fruits, such as banana, mangos, longans, avocados, and jackfruits, were higher than that of the edible product, and that the byproducts could be a valuable source of phytochemicals (Soong and Barlow, 2004).

Conclusion

In the present study, an attempt has been made to determine the antimicrobial and antioxidant properties of edible (pulp) and non-edible (peel) parts of unripe banana fruit *in vitro*. All the methanolic and acetonic extracts of pulp and peel have been found to show significant antibacterial activities against used MDR bacteria. Thus extracts from the plant can be used to control infections caused by *Escherichia coli, Klebsiella sp., Shigella* sp. *and Salmonella sp.* Results also showed that pulp and peel extracts contain high amount of antioxidants along with phenolic compounds. Comparing between pulp and peel, in case of antibacterial and antioxidant activities, peel found better than pulp. The results suggest that parts of fruit like peels could serve as potential source of bioactive compounds and can be utilized effectively without being wasted. The study also suggests that the pulp and peel of banana may be used for the extraction and preparation of various antioxidant and antibacterial formulations in pharmaceutical industries.

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