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Full Length Research Paper

Antibacterial and antioxidant activities of local seeded banana fruits

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The antibacterial and antioxidant activities of different parts of local seeded banana fruit were investigated *in vitro*. Dried peels, pulps and seeds of the fruit were extracted with hexane, ethyl acetate and ethanol. Antibacterial property of the extracts was evaluated against four Gram positive and four Gram negative bacteria using disc diffusion technique. Ethyl acetate and ethanol extract of both pulp and peel exhibited antibacterial activity with zone of inhibition ranging from 9 to 24 mm. On the other hand, only ethyl acetate extract of seeds showed antibacterial activity and the zone of inhibition ranged from 8.5 to 10 mm; interestingly, none of the hexane extracts of the three banana parts exhibited zone of inhibition. Antioxidant activity of the extracts was evaluated by total phenolic content determination and 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging assay. Total phenolic content expressed as gallic acid equivalents (GAE) was found to be highest in ethyl acetate extract of banana seed (19.46 mg GAE/g extract) followed by the same extract of banana pulp (15.78 mg GAE/g extract) and peel (11.23 mg GAE/g extract). High free radical scavenging activity was observed with ethyl acetate extracts of banana seed, peel and pulp with an ascorbic acid equivalent antioxidant capacity (AEAC) value of 1238.33, 1011.43 and 588.03 mg AA/ 100 g extract, respectively.

Key words: Seedy banana, antibacterial, antioxidant, phenolic content.

INTRODUCTION

The emergence of antibiotic resistance by pathogenic organisms to conventional drugs has necessitated the search for novel therapeutics. Previous studies have shown that medicinal plants are one of the best resources for the isolation and development of novel bioactive molecules (Mohan et al., 2008). Moreover, plant-derived preparations have drawn attention of people worldwide because of their fewer side effects and lesser toxicity in comparison to synthetic drugs.

To date, many medicinal plants have been screened for their therapeutic potential and their pharmacological properties like antimicrobial and antioxidant effects evaluated (Lai et al., 2010). Still, extensive search for natural antioxidants that could minimize free radical induced damage to biomolecules like lipids, proteins and nucleic acids is going on world over. These natural antioxidants might play an important role in combating oxidative stress associated degenerative diseases such as cancer, cardiovascular diseases, diabetes, atherosclerosis, Alzheimer's disease and aging (Turkoglu et al., 2010). Among various plants, fruits are considered rich in antioxidants due to presence of compounds like polyphenolics and vitamins, which play an important role in scavenging of free radicals (Okonogi et al., 2007). Peels and seeds are often the waste part of various fruits. Interestingly, the peel and seed fractions of some fruits were found to show higher antimicrobial and antioxidant activity than the pulp fractions (Jayaprakasha et al., 2001; Mokbel and Hashinaga, 2005; Okonogi et al., 2007; Sulaiman et al., 2011) . However, there is only little information about the antibacterial and antioxidant activities of the different parts of such fruits (Okonogi et al., 2007). Banana is one of the most popular fruits and several studies have indicated that both banana pulp and peel contain antibacterial and antioxidant principles (Mokbel and Hashinaga, 2005; Sulaiman et al., 2011). Again, various cultivars of edible bananas have evolved that are derived from two wild species, namely: *Musa acuminate* and *Musa balbisiana* (Stover and Simmonds,

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1987; Sulaiman et al., 2011).

Most of the previous studies with banana have focused on popular cultivars. However, the antioxidant activity in fruit varies among species and cultivars (Award et al., 2001; Kondo et al., 2004; Okonogi et al., 2007). In addition, differences in growth environment may also result in variation of active constituents in a cultivar. Bangladesh, being a tropical country has diversity of edible fruits and more than fifteen cultivars of banana are reported to be grown here (Chowdhury, 1996). *Musa sapientum* L. subsp. *sylvestris*, which is locally called seeded banana or bichi kola is grown in most parts of Bangladesh. This tree like herbaceous plant belongs to the family *Musaceae* and grows up to 5 to 9 m in height. The fruits are yellowish, sweet and juicy when ripe are 12 to 15 cm long, tapering to the base and apex and are full of seeds. This fruit is traditionally used in diarrohea, dysentery, enteric infections and diabetes (Yusuf et al., 2009). The main objective of the present investigation was to evaluate the antibacterial and antioxidant activities of various extracts of peel, pulp and seed portions of local seeded banana fruit *in vitro*.

MATERIALS AND METHODS

Sample collection

Yellowish fruits of *M*. *sapientum* L. subsp. *sylvestris* (seedy banana) were collected from the local market at Dhaka, Bangladesh. Authentication was done at Bangladesh National Herbarium where voucher specimen (Accession No. 35349) was deposited.

Extract preparation

The fruits were washed thoroughly with water and various parts of the fruit (peel, pulp and seed) were separated carefully. Peel and pulp parts of the fruit were cut into small pieces and dried separately under shade for several days. In case of pulp, after several days of air drying at about 35°C, the sample was oven dried at 45°C to constant weight. Dried samples were then powdered using a laboratory scale mill and blender. Ground material (100 g) was extracted independently with 500 ml of hexane, ethyl acetate and ethanol at room temperature. Extraction was carried out for 7 days with occasional shaking. The resulting extracts were filtered using filter paper (Whatman No. 1) and each filtrate was concentrated with a rotary evaporator (Eyela, Japan). All the extracts were kept in a desiccator at 4°C until use.

Test organisms

Four Gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus and Sarcina lutea*) and four Gram- negative bacteria (*Salmonella paratyphi*, *Pseudomonas aeruginosa, Shigella boydii and Vibrio mimicus*) were used for the evaluation of antibacterial activity. The strains were maintained on agar slant at 4°C and activated at 37°C for 24 h on nutrient agar prior to any screening.

Antibacterial assay

Antibacterial activity of the extracts was determined by paper disc diffusion (Kirby- Bauer) method (Lai et al., 2010). For all the bacterial strains, overnight cultures grown in broth were adjusted to an inoculum size of approximately 10^6 CFU/ml for inoculation of the agar plates. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates and plates were allowed to dry for 5 min. Sterile filter paper discs (6 mm in diameter) impregnated with different test extracts (1 mg/disc) were then placed on the surface of seeded agar plate. The plates were then incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zones of inhibition in millimeter. 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 MIC

The minimum inhibitory concentration (MIC) considered as the lowest concentration of the sample which inhibits the visible growth of a microbe was determined by the broth dilution method. Various dilutions were prepared from the stock solution of crude extracts to give concentrations ranging from 10 to 0.5 mg/ml. Each tube was inoculated with an overnight culture of strains diluted to give a final concentration of 10^6 cells/ml. The culture tubes were then incubated aerobically at 37°C for 24 h and the MIC values were recorded as the lowest concentration that inhibits the visible bacterial growth (Murthy et al., 2006; Kuta, 2008).

Determination of total phenolic content

Total phenolic content (TPC) in the extracts was determined spectrophotometrically according to the Folin–Ciocalteu procedure (Kahkonen et al., 1999). Briefly, 1.5 ml Folin–Ciocalteu's reagent (diluted 1:10) and 1.2 ml 7.5% (w/v) Na_2CO_3 were added to 0.3 ml of the extracts and the mixtures were incubated for 1 h in dark at room temperature. This was followed by measuring absorbance of the samples at 765 nm against blank. The total phenolic content was calculated from the calibration curve using gallic acid as a standard and the results were expressed as gallic acid equivalents (GAE) in mg/g extract.

1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of different extracts of seedy banana fruit was measured in terms of hydrogen donating or radical scavenging ability of the stable (DPPH) free radical (Braca et al., 2001). Briefly, 0.1 ml of plant extract at various concentrations was added to 3 ml of a 0.002% methanolic solution of DPPH. The reaction mixtures were incubated for 30 min at room temperature and the absorbance at 517 nm was read against a blank. Ascorbic acid was used as standard in the experiment and the scavenging ability was expressed as AEAC (ascorbic acid equivalent antioxidant capacity) value in mg ascorbic acid (AA) equivalents per 100 g extract.

RESULTS

Antibacterial activity

Antibacterial activity of all the extracts of seeded banana has been evaluated *in vitro* against Gram positive and Gram negative bacteria by disc diffusion method. The results obtained are presented in Table 1. Ethyl acetate and ethanol extracts of seeded banana peel and pulp exhibited significant antibacterial activity while hexane

Table 1. Antibacterial activity of the various extracts of seedy banana fruit using paper disc diffusion method.

Bacteria	Zone of inhibition (mm)									
	Peel			Pulp			Seed			Standard Kanamycin
	Hexane extract	EtOAc extract	EtOH extract	Hexane extract	EtOAc extract	EtOH extract	Hexane extract	EtOAc extract	EtOH extract	
Gram-positive										
Bacillus subtilis	-	15.5	-	-	19.0	9.5	-	8.5	-	23.5
Bacillus megaterium	-	21.5	9.5	-	22.5	20.5	-	-	-	25.5
Staphylococcus aureus	-	19.5	15.0	-	24.0	21.5	-	10.0	-	27.5
Sarcina lutea	-	21.5	9.5	-	17.5	14.5	-	8.5	-	28.5
Gram-negative										
Salmonella paratyphi	-	15.0	9.0	-	12.5	9.5	-	-	-	29.0
Pseudomonas aeruginosa	-	16.5	12.0	-	22.0	19.0	-	8.5	-	26.0
Shigella boydi	-	17.5	10.0	-	16.0	13.5	-	9.0	-	21.5
Vibrio mimicus	-	16.5	9.0	-	14.0	11.5	-	-	-	26.5

- indicates no inhibition, EtOAc (ethyl acetate) and EtOH (ethanol).

extracts of both peel and pulp displayed no antibacterial activity (Table 1). None of the extract from seeds except ethyl acetate extract recorded any significant antibacterial activity (Table 1). Ethyl acetate extract showed higher activity than the ethanolic extract both in case of peel as well as pulp. In fact, high antibacterial activity against all test organisms was observed with ethyl acetate extract of peel and pulp and zone diameters ranged from 12.5 to 24 mm (Table 1). The standard drug, kanamycin showed a zone of inhibition ranging from 21 to 28 mm (Table 1) against all test organisms while the negative controls did not show any antibacterial activity. MIC values of various extracts expressed as mg/ml were determined against organisms for which highest antimicrobial activity was observed in zone inhibition assay. In case of peel, ethyl acetate extract showed MIC value of 4.0 against both *B. megaterium* and *S. lutea* while ethanol extract exhibited MIC value of 4.5 against *S. aureus*.

In case of pulp, both ethyl acetate and ethanol extract showed MIC value of 3.5 against *S. aureus*. MIC value of ethyl acetate extract obtained from seed was found to be 6.5 mg/ml against *S. aureus.*

Antioxidant activity

Antioxidant activity of all the extracts obtained from local seedy banana fruit was determined by two complementary test systems, namely: total phenolic content and DPPH free radical scavenging activity. The amount of phenolics present in each extract was expressed in terms of gallic acid equivalents (GAE) as calculated from the regression equation of gallic acid calibration curve. Total phenolic content present in the extracts ranged from 1.26 to 19.46 mg GAE/g extract (Figure 1). Ethyl acetate extract of banana seed showed highest phenolic content (19.46 mg GAE/g extract) followed by the same extract of banana pulp (18.23 mg GAE/g extract). The radical-scavenging effects of all the extracts on DPPH were determined and expressed as AEAC (mg AA/100 g extract) values. Results for AEAC values of all the extracts are presented in Table 2. High scavenging activity was observed with ethyl acetate extracts of banana seed and peel with an AEAC value of 1238.33 and 1011.43 mg AA/ 100 g extract respectively. This was followed by the hexane extract of banana peel (800.33 mg AA/ 100 g extract) and seed (741.46 mg AA/ 100 g extract). Weakest antioxidant activity was displayed by ethanol extract of banana pulp with

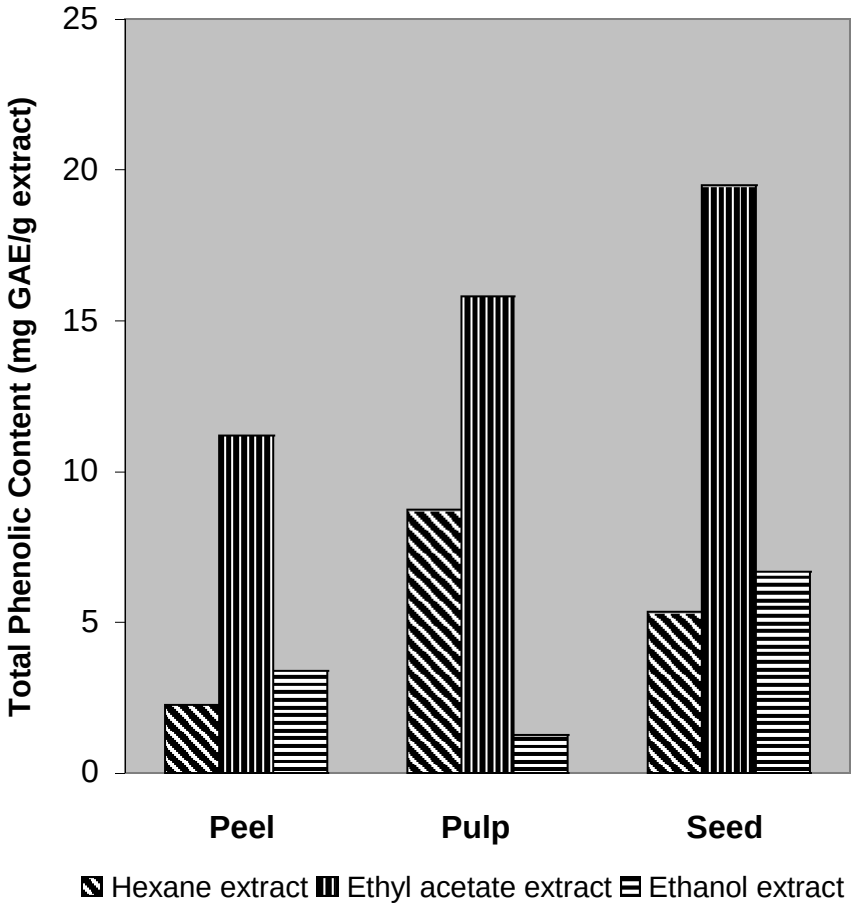

Figure 1. Total phenolic content in different parts of local seedy banana fruit.

Table 2. Ascorbic acid equivalent antioxidant capacity (AEAC) of various extracts (mg AA/100 g extract).

Extracts	Peel	Pulp	Seed
Hexane	800.33	300.40	741.46
Ethyl acetate	1011.43	588.03	1238.33
Ethanol	510.6	190.36	724

AEAC value of only 190.36 mg AA/ 100 g extract.

DISCUSSION

In the present study, antibacterial activity of various extracts was assayed in *vitro* by disc diffusion method against eight different bacterial strains. Among the parts screened, pulp and peel showed considerable antibacterial activity. Antibacterial activity obtained in this study varied with solvents used for extraction. Ethyl acetate extracts showed best antibacterial activity against both Gram positive and Gram negative bacteria with Gram positive slightly more susceptible to the extracts than Gram negative bacteria. Some studies have reported similar findings (Chan et al., 2007) and this difference in sensitivity could be attributed to different morphological constitutions of Gram positive and Gram negative bacteria (Chan et al., 2007). In our study, maximum antibacterial activity was recorded by ethyl acetate extract from pulp with 24 mm of clear inhibition zone against Gram positive bacteria *S. aureus* (Table 1). Hexane extract of either part did not show any antibacterial activity against any of the bacteria used in this study (Table 1). Based on our results, ethyl acetate appeared to be the optimal extracting solvent for anti-bacterial compounds from banana fruit. The MIC values

more than 3.5 mg/ml was observed for various extracts. All the extracts were found to be less effective than the standard antiobiotic kanamycin. Plant phenolics constitute one of the major groups of compounds acting as a primary antioxidant or free radical terminators. Antioxidant activity of phenolic compounds is attributed to their ability to donate hydrogen atoms to free radicals (Zin et al., 2006) . In addition, they possess the structural properties of free radical scavengers which enable them to serve as potential antioxidants (Jayathilakan et al., 2007; Norshazila et al., 2010). Total phenolic content of plants was evaluated using Folin-Ciocalteu method which measured the redox properties of polyphenols (Okonogi et al., 2007). The amount of phenolics in various extracts ranged from 1.26 to 19.46 mg GAE/g extract (Figure 1). Solvents used for extraction significantly affected the amount of phenolics obtained in the extracts.

Our results suggested that ethyl acetate extract of banana seed contained highest amount of phenolic compounds (19.46 mg GAE/g extract). Previous studies have also reported that seeds of most fruits contain higher amount of phenolics compared to other parts like peel and pulp (Soong and Barlow, 2004). The antioxidant activity of the extracts obtained in this study was determined using DPPH method because it is one of the most effective methods for evaluating radical-scavengers. Ascorbic acid was chosen as the reference antioxidant in this study. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for a visible deep purple color. The decolourisation of the purple reaction solution is stoichiometric with respect to number of electrons donated by antioxidant compounds (Norshazila et al., 2010). The extracts obtained in this study showed a varied level of scavenging activities, highest activity being indicated by ethyl acetate extract of seed. This was followed by the same extract from peel (Table 2). Similar to the total phenolic content, antioxidant activity of the extracts also varied with polarities of solvents. Similar findings were reported by Sulaiman et al. (2011) that found that the antioxidant activity exhibited by banana extracts is significantly affected by the solvents used for extraction. Our results indicated that ethyl acetate could be a solvent of choice for extracting antioxidant compounds from banana fruit. Phenolic compounds are although considered as most important antioxidants of plant materials, no significant relationship could be established between antioxidant activity and phenolic content in this study. For example, ethyl acetate extract of seed showed both the highest phenolic content as well as highest scavenging activity but when hexane extract of peel and pulp were compared, the pulp showed approximately 2.5 times lower scavenging activity although it contained almost 4 times higher phenolic content. This suggests that phenolics are not the only contributor to the antioxidant activities found in the various extracts.

Our results are in accordance with some earlier studies (Babbar et al., 2011; Sulaiman et al., 2011) that suggested that several non-phenolic constituents like ascorbates, reducing carbohydrates, tocopherols, carotenoids, terpenes, and pigments as well as the synergistic effect among them might also account for the total antioxidant activity.

Conclusion

Based on our results, it can be concluded that peels, pulps and seeds of local seedy banana fruits possess significant antibacterial and antioxidant activity. The results also suggest that parts of fruit like peels and seeds could serve as potential source of bioactive compounds and can be utilized effectively without being wasted. Further research is needed towards isolation and identification of active principles present in the extracts especially in the ethyl acetate extract which could possibly be exploited for pharmaceutical use.

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REFERENCES

Award M, de Jager A, van der Plas LHW, van der Krol AR (2001). Flavonoid and chlorogenic acid changes in skin of 'Elstar' and 'Jonagold' apples during development and ripening. Scientia Horticulturae (Amsterdam, Netherlands), 90: 69–83.

Braca AT, Nunziatina DB, Lorenzo Di, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod., 64: 892-895.

Babbar N, Oberoi HS, Uppal DS, Patil RT (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int., 44: 391–396.

Chowdhury MSU (1996). Bangladesh: Country report to the FAO International Technical conference on Plant Genetic resources, Leipzig, Germany.

Chan EWC, Lim YY, Omar M (2007). Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. Food Chem., 104: 1586–1593.

Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73: 285–290.

Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmedover- flavour in different species of meat. Food Chem., 105: 908–916.

Kondo S, Jitratham A, Kittikorn M, Kanlayanarat S (2004). Relationships between jasmonates and chilling injury in mangosteens, are effected by spermine. Hort. Sci., 39: 1346–1348.

Kuta FA (2008). Antifungal effect of *Calotropis procera* stem bark on *Epidermophyton flocosum* and *Trichophyton gypseum.* Afr. J. Biotechnol., 7(13): 2116-2118.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala ST, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Lai HY, Lim YY, Kim KH (2010). *Blechnum Orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Comple. Alt. Med., 10: 15.
Mohan S, Abdul AB, Wahab SIA, Al-Zubairi AS, Elhassan MM, Yousif M (2008). Antibacterial and antioxidant activities of *Typhonium Flagelliforme* (Lodd.) Blume Tuber. Am. J. Biochem. Biotechnol., 4(4): 402-407.
Mokbel MS, Hashinaga F (2005). Antibacterial and Antioxidant Activities of Banana (*Musa*, AAA cv. Cavendish) Fruits Peel. Am. J. Biochem. Biotechnol., 1(3): 125-131.
Murthy MM, Subramanyam M, Giridhar KV, Jetty A (2006). Antimicrobial activities of bharangin from *Premna herbaceae* Roxb. and bharangin monoacetate. J. Ethnopharmacol., 104(1-2): 290-292.
Norshazila S, Syed ZI, Mustapha SK, Aisyah MR, Kamarul RK (2010). Antioxidant Levels and Activities of Selected Seeds of Malaysian Tropical Fruits. Mal. J. Nutr., 16(1): 149-159.
Okonogi S, Duangrat C, Anuchpreeda S, Tachakittirungrod S, Chowwanapoonpohn S (2007). Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem., 103: 839–846.
Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Supriatno OKL (2011). Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). J. Food Comp. Ana., 24: 1-10.
Stover RH, Simmonds NW (1987). Bananas (3rd Ed). England: Longman Scientific and Technical.
Soong YY, Barlow PJ (2004). Antioxidant activity and phenolic content of selected fruit seeds. Food Chem., 88: 411–417.
Turkoglu I, Turkoglu S, Celik S, Kahyaoglu M (2010). Antioxidant and antimicrobial activities of Turkish endemic *Achillea* species. Afr. J. Microbiol. Res., 4(19): 2034-2042.
Yusuf M, Begum J, Hoque MN, Chowdhury JU (2009). Medicinal plants of Bangladesh, Bangladesh Council Sci. Ind. Res., pp. 462-463.
Zin ZM, Hamid AA, Osman A, Saari N (2006). Antioxidative activities of chromatographic fractions obtained from root, fruit, and leaf of Mengkudu (*Morinda citrifolia* L.). Food Chem., 94: 169-178.