Antioxidant Activities and Anticancer Screening of Extracts from Banana Fruit (*Musa sapientum*)

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**Abstract:** Banana is one of the oldest fruit crops domesticated by humans, delicious test and nutritional value has made it one of the most economically important plants in the globe. In this work, banana peels and pulps were extracted with two organic solvents as well as water. Six extracts were tested for antioxidant properties using two methods (2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH) and ferric reducing antioxidant power (FRAP) assays). They were also screened for antiangiogenic activity using the rat aorta ring assay as well as antineoplastic activity using MTT assay. The correlation between antiangiogenic and antioxidant activities also was evaluated. Of the six extracts tested, 2 exhibited significant antiangiogenic activity. The hexane extracts of banana peels had the highest activity (85.32% inhibition at 100 µg/mL). Similarly, the hexane extract of banana peel had the highest cytotoxic activity and was found to inhibit the growth of colon cancer cell line HCT-116.

**Keywords:** *Musa Sapientum* • Antiangiogenic • Antioxidant • Peel • Banana

**INTRODUCTION**

Fruits are one of the oldest forms of food that has not changed much throughout the history of man. There are many references to fruits are associated with eternal life in ancient civilizations [1]. However, banana fruits are one of the interesting plants which have been regularly cultivated and wide-spread in more than 100 tropical and sub-tropical countries. Although, banana fruits have more commonly been used as source of energy and nutritious diet in humid tropical regions [2, 3]. Bananas are the fourth most important agricultural food product after rice, wheat and maize. It is the most traded fruits in the global markets [4]. Various spices, parts and forms (Unripe/ripe) of banana plant have been used for different ethnopharmacologically applications [5]. Recently, researchers have found *Musa sapientum* (Musaceae), possess a prominent antidiabetic [6] anti ulcer agent [7] antioxidant and anti inflammatory effects [8]. A study have shown that banana's peel and pulp extracts are rich in fatty acids, sterols and steryl ester, in addition to linoleic, linolenic and oleic acids are the dominant components present in banana tissue [4]. However, several findings have provided further insight into the functional and mechanism of the banana products and their active constituents against degenerative diseases. More recently, banana have been proposed to serve as a vector of edible vaccine due to high availability and easy consumption [9]. Although, banana pectin have been extracted and characterized to be used as pharmaceutical excipients in tablet formulation [10]. It is now widely believed that the complex mixture of phytochemical constituents present in the extract of fruits and vegetables are more effective than their individual components in cancer prevention due to both additive and synergistic effects [11]. Moreover, edible phytochemicals offers an available, suitable and approachable basis for cancer control and management, as the side effects of cancer drugs and the burden of healthcare costs are the main obstacles for the cancer patients, additional approaches are needed to establish new cancer prevention programs for general public [12]. Therefore, it is important to investigate biological activities of whole fruit extracts containing all phytochemical components generated by...
different solvent systems. Despite of bananas’ nutritional importance, they did not receive much attention to investigate their role in some biological activities. To the best of our knowledge, there is no report on antiangiogenic effect of Musa sapientum banana extracts. In this study, we generated extracts of banana (Pulp and peel) using two organic solvents as well as water. The aims of the present study were to: (i) determine the antioxidant capacity of banana extracts; (ii) determine their ability to inhibit the proliferation of colon (HCT-116), human breast (MCF-7) and normal cell line (human umbilical vein endothelial cell (HUVEC) line) and (iii) evaluate their antiangiogenic activity using the rat aortic ring assay.

MATERIALS AND METHODS

Extract Preparation: Musa sapientum (Banana) fruits were purchased and collected from well known market (TESCO) in Pinang-Malaysia. Taxonomical authentication was done by senior taxonomist, Mr. Shanmogan School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia. The fruit materials were dried in an oven (35 – 40°C) before being powdered mechanically. The pulverized material (50 g) was subjected to sequential extraction with n-hexane, ethanol and water, in that order. The extracts were prepared in 250 mL of the solvents using hot maceration (40 °C). After filtration, the solvents were evaporated using a rotary evaporator (Buchi, USA) at 75 rpm and 45°C. The concentrated extracts were totally dried overnight at 45°C. Next, stock solutions of the extracts were prepared at 10 mg/mL in 100% dimethyl sulfoxide (DMSO) for further use.

DPPH Radical Scavenging Activity: DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was carried out to evaluate the scavenging activity of the extracts [13]. The stock solution of DPPH was prepared at a concentration of 200 µM in absolute methanol while stock solutions of the extracts were prepared at concentration of 10 mg/mL. DPPH was dispensed into 96-well plate (100 µL/well) and immediately, 100 µL of the tested samples were added at final concentrations of 12.5, 25, 50, 100, 200 µg/mL. Methanol alone and methanol with DPPH were used as blank and negative control, respectively. Ascorbic acid was used as positive control. The mixtures were incubated at 30°C for 30 min in the dark and then the absorbance was measured at 517 nm. The dose response curves were obtained and then used to calculate the median inhibitory concentration (IC₅₀) (n=6).

Ferric Reducing Antioxidant Power (FRAP) Assay: The FRAP assay was conducted according to Dahham et al. [14] protocol, with minor modification. The FRAP reagent consists of 10 mM TPTZ (2,4,6- tripyridyltriazine) in 40 mM HCl, 20 mM FeCl₃ and 300 mM acetate buffer (pH 3.6) in proportions of:1:10 (v/v/v). Various concentrations (3.25 – 100 µM) of banana samples (50 µL) were added to 1.5 ml of freshly prepared FRAP reagent. The absorbance was measured at 593 nm using a TECAN Multi-mode microplate reader Model Infinite® 200 (Mannedorf, Switzerland) after 45 min of incubation. A standard curve was constructed using FeSO₄ solution. The results were expressed as µM Fe²⁺/mg. All measurements were carried out in triplicate and the mean values were calculated.

Cell Lines and Culture Conditions: Human Umbilical Vein Endothelial Cell line HUVEC, catalogue number (C2517A); human colorectal carcinoma cell line HCT-116, catalogue number (CCL-247) and human hormone sensitive and invasive breast cancer cell line MCF-7, catalogue number (HTB-22) were purchased from ScienCell USA. HUVEC were maintained in endothelial cell medium (ECM) (ScienCell, USA) supplemented with endothelial cell growth supplements (ECGS), 5% HIFBS and 1% PS. HCT-116 cells were maintained in RPMI whereas, MCF-7 was maintained in DMEM medium. The media were supplemented with 5% heat inactivated fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured in a humidified incubator at 37°C supplied by 5% CO2. Cell culture work was done in sterile conditions using Class II biosafety cabinet (ESCO USA).

Cytotoxicity Assay: The MTT cytotoxicity assay was performed following protocol describe by Kalla et al. [15], with minor modifications. Cells were seeded at 1.5 × 10⁴ cells in each well of a 96-well plate in 100 µL of fresh culture medium and were allowed to attach overnight. For screening, the cells (70–80% confluency) were treated with the fruit extracts at a concentration of 50 µg/mL. After 48 h of treatment, the medium was aspirated and the cells were exposed to MTT solution prepared at 5mg/mL in sterile PBS. The solution was added to each well at 10% v/v and incubated at 37°C in 5% CO₂ for 3 h. The water-insoluble formazan salts were solubilized with 200 µL DMSO/well. Absorbance was measured using the TECAN microplate reader at the primary wave length of 570 nm and the reference wavelength of 620 nm. Each plate contained the samples, negative control and blank. DMSO (1% v/v) was used as the negative control. The assay was repeated four times and the results were presented as a mean percent inhibition to the negative control ± SEM.
Antiangiogenic Assay: Antiangiogenic effect of banana extracts were investigated by using rat aortic ring assay model according to the protocol described by Hassan et al. Thoracic aortas were removed from euthanized rats, cleaned with serum free medium. Aortic rings were cross sectioned into one millimeter thickness. One ring was placed in the center of each 48 wells plate, then 300 µl serum free M199 media containing 3 mg ml-1 fibrinogen and 5 mg ml-1 aprotinin. 50 NIH U ml-1 thrombin in 0.15 M NaCl were added in each well and incubated at 37°C for 90 min to solidify. Various concentrations of EOs dissolved in 0.3 ml M 199 medium supplemented with 20% HIFBS, 0.1% Ý-aminocaproic acid, 1%L-Glutamine, 2.5 µg/ml amphotericin B and 60 µg/ml gentamicin, were added to each well. Suramin and 0.5% DMSO were used as positive and negative controls respectively. The top layer medium was replaced on the day four with fresh one containing the banana samples and incubated at 37°C in 5% CO2. On day five, aortic rings were photographed using EVOS f1 digital microscope (Advanced Microscopy Group, USA) (40× magnification) and subsequently the length of blood vessels outgrowth from the primary tissue explants was measured using Leica Quin software. The inhibition of blood vessels formation was calculated using the formula; %blood vessels inhibition = [1- (A0/A)] × 100, Where; A0 = distance of blood vessels growth in treated rings in µm, A = distance of blood vessels growth in the control in µm. The results are presented as mean percent inhibition ± SEM, (n =6).

RESULTS

Banana Extracts: Table 1 shows the list of banana fruit parts evaluated in this study. Three exacts were prepared from each part, starting with n-hexane followed by ethanol and then water. The yield of each extract was calculated and is presented as w/w percent yield (Table 1). The ethanol extracts of peel and pulp produced the lowest yield, whereas the water extracts had the highest yield and those of the hexane extracts fell in between. The highest yield was 4.30% for the water extract of pomegranate peel, followed by the water extract of the fig fruit (3.7%) and grape (3.55%), respectively. Only the water extracts of grape and pomegranate seeds exhibited a very low yield.

Antioxidant Activities: Table 1 shows the radical scavenging ability of banana fruit extracts as determined by the DPPH and FRAP assays. In general, the extracts prepared from ethanol had the most potent antioxidant activity, whereas the extracts prepared from the solvent hexane displayed poor or moderate DPPH and FRAP scavenging activity. Among the tested extracts, peels extracted with ethanol exhibited potent antioxidant activity on DPPH, with the lowest IC50 value calculated for the ethanol extract (19.10 µg/mL). Similarly, the ethanol extract of the peels showed significant antioxidant activity on FRAP with IC50 values of (55.10 µg/mL). Moreover, the ethanol extract of banana pulp demonstrated remarkable FRAP radical scavenging effect, with IC50 values of 46.40 µM of Fe2+/mg.

Cytotoxicity Effects: Banana extracts were tested for their ability to inhibit the growth of HCT-116 and MCF-7, tumor cell lines as well as normal HUVEC cells (Table 2). The extracts showing > 60% inhibition of cell proliferation were considered to be active extracts. Hexane extracts of banana peel and pulp exhibited the highest cytotoxicity towards HCT-116 and MCF-7 with inhibition of 62.04 and 61.21 respectively. Whereas the water and ethanol extracts showed low antiproliferative effects against HCT-116 and MCF-7. Most importantly, all banana extracts showed poor cytotoxicity against the normal cell line. The morphological alteration of the treated cancer cells presented clear evidence of significant cytotoxicity of hexane banana extracts. The treated cells with vehicle (0.1% DMSO) displayed a compact monolayer of aggressively growing cancer cells with prominent nuclei and intact cell membrane. Whereas the images taken from the extracts of banana peels and pulps showed a significant reduction in the number of cancer cells (Figure 1).
Fig. 1: Effect of banana peel and pulp extracts on cellular morphology of human cancer and normal cell lines. Photomicrographic images of cell lines, taken under an inverted phase-contrast.

Fig. 2: Photomicrographs showing antiangiogenic effect of extracts of selected fruits (100 µg/mL) against sprouting of microvessels in rat aortic explants. A) Negative control showing extensive growth of microvessels. B) Treatment with the hexane peel extract displaying potent inhibition of growth of microvessels. C) Treatment with the ethanol pulp extract displaying moderate inhibition of growth of microvessels. D) Treatment with standard reference control suramin showing strong inhibitory effect on microvessels growth. E) Graphical representation of antiangiogenic activity of the active extracts of hexane peel extract (HPE) and ethanol pulp extract (EPE). The values are expressed as mean ± SEM (n =10). **p < 0.01 and *p < 0.05 compared to negative control group (0.1% DMSO).
Table 3: Antiangiogenic activity of *Musa sapientum* extracts on rat aortic explants

<table>
<thead>
<tr>
<th>Banana Part used</th>
<th>Solvents</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>n-hexane</td>
<td>85.32</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>50.36</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>12.43</td>
</tr>
<tr>
<td>Pulp</td>
<td>n-hexane</td>
<td>39.90</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>62.71</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>20.55</td>
</tr>
</tbody>
</table>

**Antiangiogenic Activity:** The rat aorta ring assay was used to investigate the antiangiogenic activity of different banana extracts. Figure 2 shows the rat aorta tissue explants for the *ex vivo* angiogenesis screening study. Figure 2A shows sprouting of massive microvessels from untreated explanted tissue. Figures 2B, C and D show the effects of the banana peel n-hexane extract, banana pulp ethanol extract and the standard reference suramin, respectively.

Banana extracts with > 60% inhibition of sprouting of blood vessels were considered to be active extracts. Table 3 shows the antiangiogenic effect of fruit extracts determined by the rat aorta ring assay. Out of 6 extracts, only 2 extracts exhibited potent inhibitory activity (> 60%). The highest inhibition (85.32%) was produced by the hexane extract of banana peel extract. This significant inhibition values were very much comparable with the standard drug (Suramin), which demonstrated potent inhibition of microvessel growth.

**DISCUSSION**

Despite rapid developments in understanding and treating cancer in recent decades, it remains the second leading cause of morbidity and mortality worldwide. According to recent statistics, cancer accounts about 23% of the total deaths in the USA [17]. Although there are many therapeutic approaches to treating cancer relied on the toxic compounds. Unfortunately, the cytotoxicity properties of most chemotherapy drug is nonspecific and therefore do not distinguish between normal healthy cells and tumor cells, these events has lead to inappropriate and toxic therapeutic agents with a wide range of side effects which limits the maximum tolerated doses and the minimum effective doses of chemotherapy [18]. However, several experimental and epidemiological studies have suggested that fruits and vegetables are associated with low risk of various types of cancer. Similarly, the Mediterranean dietary pattern appears to prevent about 25% of colorectal cancers, 15% of breast cancers and 10% of prostate, pancreatic and endometrial cancers [19].

Many fruits have a free sugars, organic acids and amino acid as natural constituents which play a key roles in maintaining fruit quality and nutritional value [20]. On the other hand, some fruit peels are thrown into the environment as agricultural waste which can be utilized as a source of antioxidant and other biological agents. In the present study, peels and pulps of banana fruits were extracted sequentially with n-hexane, ethanol and water to prepare 6 extracts. Two assays were used to assess the antitumorogenesis effects of the banana extracts. Cytotoxicity of the extracts was measured using the MTT assay, which shows cell viability via mitochondrial activity in living cells. The *ex vivo* rat aorta ring assay was used to evaluate the effect of banana extracts on the growth of new blood vessels (Angiogenesis) from explanted tissue. The cytotoxicity assay showed that the highly non-polar solvent extract (i.e., n-hexane extracts) had the highest cytotoxic activity among the extracts tested. Among the six extracts, the hexane extract of banana peel had a strong cytotoxic effect on tested cancer cell lines. Our findings In contrast, all banana extracts were not cytotoxic to the normal cell line (HUVEC). However, the results of the antiangiogenic study showed that out of the six extracts from banana, only two showed a strong inhibitory effect (> 60%). In particular, the hexane extract of banana peel inhibited 85.32% of the aortic microvessels, followed be ethanol extract of banana pulp inhibited 62.71%. previously, Singhal and Ratra [21] have investigated the potential Immunomodulatory activity of hexane extract from *Musa acuminate*. This result is in accordance to results obtained in the present study wherein significant activities were observed by hexane peel extract towards cancer cell lines and angiogenesis.

Moreover, in this study an attempt has been made to evaluate the correlation between antiangiogenic activity and antioxidant activity of banana extracts. The ethanol extracts of banana pulp also exhibited potent antioxidant activity against DPPH with IC₅₀ values of 44.07 μg/mL and 46.40 μM of Fe⁺²/mg on FRAP.

In general, the biological activities of the fruits are partially attributed to their wide range of vital micronutrients and phytochemicals such as, alkaloid, carotenoids, flavonoids, lignans, phenolics and tannins. The mechanistic aspects of fruits bioactive components may prevent cancer through various mode of action including antioxidant activity, Inhibition of cell proliferation, induction of apoptosis, Inhibition of cell invasion and subcellular signaling pathways [22, 23]. However, Cancer cells undergo multistep process,
acquiring specific characteristics in cell physiology for 
growth and survival including six essential hallmarks: 
sustaining proliferative signaling, evading growth 
suppressors, evasion of programmed cell death 
(Apoptosis), enabling replicative immortality, inducing 
angiogenesis and activating invasion and metastasis. 
These distinguishing features are the fundamental 
principle of this malignant alteration [24]. Yet, the ability 
to produce one targeted therapeutic agent act on six 
hallmarks of cancer is still a hypothesis. Besides, the 
actions of individual pure compound alone may lose its 
bioactivity or may not act the same way as the compound 
in crude extracts. 

Therefore, targeting tumor angiogenesis using 
natural antiangiogenic agents that inhibit the growth of 
new blood vessels, which supply the tumor with nutrients 
and oxygen, represents a promising therapeutic approach 
against tumor growth and metastasis.

CONCLUSION

In conclusion, the result obtained in this study 
clearly demonstrates that banana fruit have a broad 
spectrum of biological activities. More importantly, the 
result indicated that banana peels can be used as good 
source of antioxidant and antiangiogenic agent. However, 
further research is needed to identify the active 
components in banana extracts and to evaluate their 
mechanism of action.

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