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Antioxidant activities of different parts of *Musa sapientum* L. ssp. *sylvestris* fruit

Mohammad Zafar Imam, Saleha Akter, Md. Ehsanul Hoque Mazumder and Md. Sohel Rana

Mohammad Zafar Imam
 Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh.

Saleha Akter
 Department of Pharmacy, Primeasia University, 9, Banani C/A, Dhaka-1213, Bangladesh.

Md. Ehsanul Hoque Mazumder, Md. Sohel Rana
 Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

For Correspondence
Mohammad Zafar Imam,
 Lecturer, Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh.
 Tel: +08801816684184
 Email: zafarimam@gmail.com

ABSTRACT

Musa sapientum L. ssp. *sylvestris* (Family: Musaceae) is a popular edible fruit. It is used by the traditional healers in the treatment of diarrhoea and dysentery. In the present study, methanolic extracts of peel (MSPE), pulp (MSPU) and seed (MSSE) of the fruit were investigated for *in vitro* antioxidant activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, reducing power, CUPRAC (Cupric Reducing Antioxidant Capacity) and total antioxidant capacity. The phenolic content of the extracts were also determined. The plant extracts showed a direct concentration dependent increase in scavenging DPPH radical. MSSE showed better scavenging activity than MSPE and MSPU with an IC₅₀ value of 54.92 µg/ml, while the standard antioxidant, ascorbic acid, showed an IC₅₀ value of 13.75 µg/ml. The Fe³⁺/ferricyanide to ferrous and cupric ion reduction capacity of MSSE were better than the reference agent ascorbic acid. All three extracts showed good results in total antioxidant assay. MSSE was also found to contain good amount of phenols (244.38 mg/g of plant extract in GAE). The results of this study indicate that MSSE has strong *in vitro* antioxidant activity.

Keywords: Musaceae, antioxidant, free radical scavenging, banana.

INTRODUCTION

Reactive oxygen species (ROS) are produced physiologically in normal biochemical and metabolic processes in human body. These ROS increase the oxidative stress in the body which is tolerated when it exists in a mild degree by the physiological antioxidant defense systems (Babior, 1984). However, the antioxidant producing capacity of the animal cells are limited (Halvorsen et al., 2006). The excess oxidative stress has been correlated with different human diseases such as cancer, AIDS, inflammation, hypertension, neurodegenerative disorders, rheumatoid arthritis, atherosclerosis, lung disease, and reperfusion injury (Babior, 1984; Hegde et al., 2005; Papaharalambus and Griendling, 2007; Stangeland et al., 2009). This has enforced the research in finding antioxidants in foods and medicinal plants and as a result approximately 4,000 antioxidants have been identified (Zadák et al., 2009). However, the best antioxidants found in nature so far are vitamin C, vitamin E and the carotenoids. Synthetic antioxidants have been found very effective but there are safety concerns about them (Okonogi et al., 2007) as they may cause carcinogenesis (Ito et al., 1983). Great numbers of fruits, seeds, vegetables and plants have been tested for their antioxidant activity. They are found to be a good source of natural antioxidants including ascorbic acid, carotenoids, polyphenols, such as flavonoids, tannins, catechins, eugenol, quercetin, gallic acid derivatives etc. (Okonogi et al., 2007; Stangeland et al., 2009). *Musa sapientum* L. ssp. *sylvestris*, is locally known as 'Bichi kola' or 'Aitta kola'. *M. sapientum* ssp. *sylvestris* fruit is used in the treatment of diarrhoea, dysentery and in excess menstruation (Partha and Hossain, 2007).

Traditionally different bananas are used in diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes, sprue, uremia, nephritis, gout, hypertension, cardiac disease (Ghani, 2003; Khare, 2007). The fruits of *M. sapientum* possess significant antiulcerant (Goel and Sairam, 2002), antibacterial (Imam et al., 2011), wound healing (Agarwal et al., 2009) and anti-allergic activity (Tewtrakul et al., 2008). Carbohydrates, norepinephrine, serotonin, dopamine (Waalkes et al., 1958), tryptophan, indole compounds (Shanmugavelu and Rangaswami, 1962), alkaloids, tannin, ascorbic acid, several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) have been isolated from the pulp of different bananas (Lewis et al., 1999; Lewis and Shaw 2001). Sterols such as β -sitosterol, campesterol, stigmasterol were isolated from the fruit, peel and plant (Ghoshal, 1985; Mokbel and Hashinaga, 2005). The presence of flavonoids and other phytochemicals raises the antioxidant potential of different parts of banana fruits. The present study therefore evaluated the antioxidant potential of peel (MSPE), pulp (MSPU) and seed (MSSE) of *M. sapientum* ssp. *sylvestris*.

MATERIALS AND METHODS

Chemicals

The following chemicals were used in this study: 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Chemicals Co., USA), Trichloroacetic acid, Ferric chloride, Copper (II) chloride, Sodium phosphate, Follin-ciocalteu's phenol reagent, Neocaproine (Merck, Mumbai, India), Ammonium molybdate (Merck DGaA, Germany), Potassium ferricyanide (Loba Chemie Pvt. Ltd, Mumbai, India), Sodium nitroprusside (BDH Chemicals Ltd., Poole, England), Ascorbic acid (SD Fine Chemicals Ltd., Biosar, India).

Plant material and extraction

Mature and unripe fruits of *M. sapientum* ssp. *sylvestris* were collected from Roypur village in Jibannagar Upazila, Chuadanga, Bangladesh in January 2009. The botanical identification was done by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB: 33833). The separated peel, pulp and seeds were dried, powdered and then extracted by Soxhlet apparatus using methanol. The dried methanolic extract of the peel, pulp and seed were used for the experiments.

Assay of DPPH radical scavenging activity

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 μ l plant extract of different strength (5, 25, 50, 100, 500 μ g/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 reducing power

The assay is based on the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form by antioxidant in the sample (Oyaizu, 1986). In the experiment, different concentrations of extract solutions (5-200 μ g/ml) were mixed with 2.5ml (1%) of potassium ferricyanide and incubated for 10 min at 50°C. Then trichloroacetic acid (2.5 ml, 10%) was added and the mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml (0.1%) ferric chloride. Then the absorbance of the solution was measured at 700 nm against blank. The reaction mixture without the extract was used as blank. The percentage reducing capacity was calculated from the formula: % reducing capacity = $\{(A_m - A_b) / A_b\} \times 100$; where A_m is the absorbance of the reaction mixture and A_b is the absorbance of the blank.

Assay of cupric ion reducing capacity

The cupric ion reducing capacities of the plant extracts were performed by the method described by Resat et al. (2004). 1.0 ml of 0.01 M $CuCl_2 \cdot 2H_2O$ solution was mixed with 500 μ l of plant extract or standard of different concentrations (5, 25, 50, 100, 200 μ g/ml). Then 1.0 ml of ammonium acetate buffer (pH 7.0) was mixed followed by the addition of 1.0 ml of 0.0075 M of neocaproine solution. Finally, 600 μ l of distilled water was added and the final volume of the mixture adjusted to 4.1 ml. The total mixture was incubated for 1 h at room temperature. Then the absorbance of the solution was measured at 450 nm using UV spectrophotometer against blank.

Determination of total antioxidant capacity

Total antioxidant capacities of the extracts were determined by the phosphomolybdenum method described by Prieto et al. (1999). 0.3 ml extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and were incubated at 95°C for 90 min. The reduction of Mo(VI)-Mo(V) by the extracts followed by the formation of a green phosphate/Mo(V) complex at acid pH forms the basis of the method. After cooling the reaction solution to room temperature, the absorbance was measured at 695 nm against blank. The antioxidant activity of the extract was expressed as the number of equivalent of ascorbic acid.

Determination of total phenolic compounds

The content of total phenolic compounds in the extracts was determined by Folin-Ciocalteu reagent (Singelton et al., 1999). 1 ml of extract (200 μ g/ml) was mixed with 500 μ l of Folin-Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution. The mixture was then incubated for 1 h at 20°C. The absorbance of the solution was measured at 765 nm against blank. The total content of phenolic compounds was calculated in gallic acid equivalents (GAE) using the formula: $A = (C \times V) / m$; where A is the total content of phenolic compounds, mg/g plant extract in GAE; C is the concentration of gallic acid established from the calibration curve, mg/ml; V is the volume of extract in ml and m is the weight of plant extract in g.

Statistical analysis

All the experimental results are mean \pm SD of duplicate parallel measurements. IC₅₀ values of were calculated using SPSS 11.5.

RESULTS

DPPH radical scavenging activity

DPPH was used to evaluate the possible antioxidant principles present in the extracts by its radical scavenging capacity measurement (Braca et al., 2001). The percent DPPH radical scavenging by the plant extracts was found concentration dependent (Figure 1). Among the samples, MSSE showed better scavenging activity than MSPE and MSPU with an IC₅₀ value of 54.92 μ g/ml while the standard, ascorbic acid, showed an IC₅₀ value of 13.75 μ g/ml. MSPE and MSPU showed IC₅₀ value of > 5000 μ g/ml.

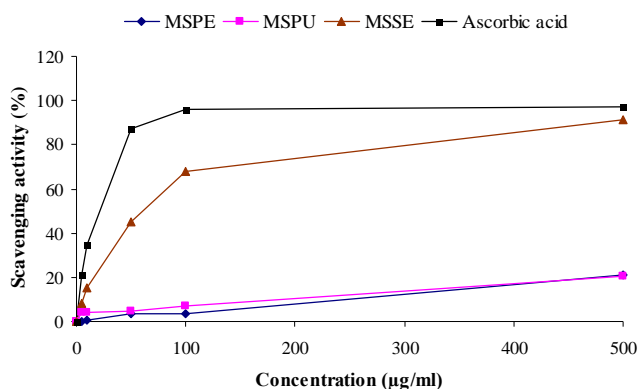


Fig 1: DPPH radical scavenging activities of peel, pulp and seed extracts of *M. sapientum* ssp. *sylvestris*. Values are the mean of duplicate experiments and represented as mean \pm SD. MSPE = *M. sapientum* ssp. *sylvestris* peel extract, MSPU = *M. sapientum* ssp. *sylvestris* pulp extract, MSSE = *M. sapientum* ssp. *sylvestris* seed extract.

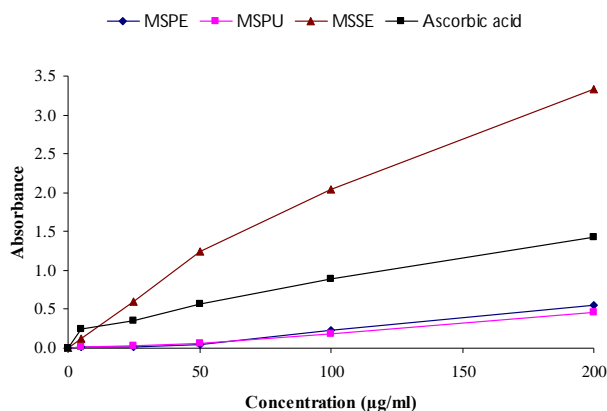


Fig 2: Reducing power of peel, pulp and seed extracts of *M. sapientum* ssp. *sylvestris*. Values are the mean of duplicate experiments and represented as mean \pm SD. MSPE = *M. sapientum* ssp. *sylvestris* peel extract, MSPU = *M. sapientum* ssp. *sylvestris* pulp extract, MSSE = *M. sapientum* ssp. *sylvestris* seed extract.

Reducing power

The reducing power of the plant extracts were measured based on the Fe³⁺/ferricyanide to ferrous reduction at 700 nm

(Oyaizu, 1986). MSSE has been found to show better reducing capacity than the standard ascorbic acid. The reducing power of the extracts and standard are in the order: MSSE > Ascorbic acid > MSPE > MSPU (Figure 2). The Fe³⁺ to Fe²⁺ reducing capacity of the plant extracts showed a dose dependent increment.

Cupric ion reducing capacity

All the extracts and the standard showed a concentration dependent increase in cupric ion reduction. MSSE showed stronger activity than the standard ascorbic acid (Figure 3). The cupric ion reducing capacity of the extracts and standard are in the order: MSSE > Ascorbic acid > MSPU > MSPE.

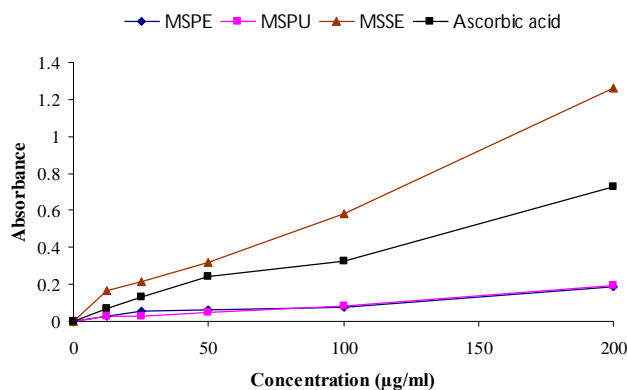


Fig 3: Cupric ion reducing power of peel, pulp and seed extracts of *M. sapientum* ssp. *sylvestris*. Values are the mean of duplicate experiments and represented as mean \pm SD. MSPE = *M. sapientum* ssp. *sylvestris* peel extract, MSPU = *M. sapientum* ssp. *sylvestris* pulp extract, MSSE = *M. sapientum* ssp. *sylvestris* seed extract.

Total antioxidant activity

The total antioxidant capacity of the extracts was quantitatively determined by the phosphomolybdenum method. Total antioxidant capacities of MSPE, MSPU and MSSE were expressed as ascorbic acid equivalent (AAE) per gram of plant extract. The total antioxidant capacities of MSPE, MSPU and MSSE 675.56 \pm 5.23, 543.70 \pm 3.14 and 416.85 \pm 5.50 mg/g plant extract in AAE.

Total phenolic content

Total phenol contents of MSPE, MSPU and MSSE are expressed as gallic acid equivalent (GAE) per gram of plant extract. The total phenol content of MSPE, MSPU and MSSE are 0.18, 0.91 and 244.38 mg/g plant extract in GAE. Only the seed extract contained good amount of phenolic compounds.

DISCUSSION

The antioxidant activity of the extracts found in different methods indicates the involvement of multiple mechanisms. DPPH radicals are usually scavenged by the phenol, amino or thiophenol group. The scavenging of DPPH radical occurs either by a direct H-atom-abstraction process or a proton concerted electron-transfer process (Wang and Zhang, 2003). Fe³⁺/ferricyanide to ferrous and cupric ion reduction assay measures the reducing power of the compounds present in the extracts. Reducing power is usually

correlated with the presence of reductones in a sample (Duh, 1998). Reductones donate a hydrogen atom to break the free radical (Gordon, 1990) and give the antioxidant effect. They also prevent the formation of peroxide by reacting with some of its precursors. These effects of reductones in the sample indicate their contribution in the antioxidant effect (Kumaran and Karunakaran, 2007). The stronger activity of the MSSE may indicate the presence of strong reductones. Different phenolic compounds in the extracts may play a good role in showing this activity.

The major types of plant phenolic compounds are the hydroxy-cinnamic acids (HCAs), flavonoids, anthocyanins and tannins. Consumption of dietary phenolics is thought to exert different health benefits, especially in coronary heart disease, cancer, diabetes, etc., possibly by acting as antioxidants, anticarcinogens and cardioprotective agents (Smirnoff, 2005). The antioxidant activity of phenolics is believed to contribute a part in these activities (Rietveld and Wiseman, 2003). These effects of the phenolic compounds are due to the electron donating activity of the 'acidic' phenolic hydroxyl group. The free radical scavenging activity of flavonoids are well established and a major area of dietary antioxidant research. Flavonol quercetin, the anthocyanidins cyanidin and delphinidin, and the green tea flavan-3-ols epicatechin gallate and epigallocatechin gallate are well studied flavonoids that possess strong radical scavenging activity (Rice-Evans et al., 1996; Rice-Evans et al., 1997). However, nonflavonoid phenolic compounds have also been found to possess significant antioxidant activity. The strong antioxidant activity of condensed and hydrolyzable tannins, a group of high molecular weight phenolics, has also been reported (Hagerman et al., 1998). So, strong antioxidant activity can be correlated with a good phenolic content in plant extract (Yang et al., 2002).

On the basis of the findings of the present study it can be assumed that the seed of *M. sapientum* L. ssp. *sylvestris* (MSSE) has strong antioxidant activity. The phytochemicals present, particularly the phenolic compounds, may be attributed to the antioxidant activity. Isolation of bioactive constituent(s) from the crude extracts and subsequent tests both *in vitro* and *in vivo* models may help to reach a conclusion about the current findings.

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