



# *In vitro* antioxidant, hypoglycemic and oral glucose tolerance test of banana peels



V.V. Navghare, S.C. Dhawale\*

Department of Pharmacology, School of Pharmacy, S.R.T.M. University, Nanded 431606, Maharashtra, India

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 Oral glucose tolerance test

**Abstract** Banana fruit is claimed to have antidiabetic effects despite its high calorie content, and its peels also contain vital phytoconstituents including galocatechin. Previously banana pulp has been studied for antihyperglycemic effects, and in the present investigation antihyperglycemic effect of ethanolic extract of inner peels of *Musa sapientum* (EMS), *Musa paradisiaca* (EMP), *Musa cavendish* (EMC) and *Musa acuminata* (EMA) fruit was evaluated using oral glucose tolerance test in normoglycemic rats. *In vitro* antioxidant study was conducted using DPPH, H<sub>2</sub>O<sub>2</sub> radical scavenging assay and ferric reducing power assay. Wistar rats were divided into fourteen groups and twelve groups received different doses of aforementioned extracts, while control group received gum acacia solution and remaining group received standard drug, glimepiride. All the rats received glucose load at a dose of 2 g/kg body weight. Groups treated with EMC and EMA showed significant decrease in glucose level ( $p < 0.01$ ) at 150 min as compared to control group. In hypoglycemic study, only EMP 500 mg/kg, p.o. treated group revealed a significant decrease ( $p < 0.05$ ) in glucose level at 120 min, while other groups did not show any sign of hypoglycemia. In glucose tolerance test, animals treated with EMC and EMA depicted dose dependent antihyperglycemic effect at 150 min while EMS and EMP showed significant reduction in plasma glucose at higher doses. In a similar fashion, EMA i.e. *M. acuminata* demonstrated highest antioxidant activity followed by EMC against DPPH radical. In ferric reducing power and H<sub>2</sub>O<sub>2</sub> scavenging assay, EMA demonstrated maximal antioxidant activity when compared with other extracts.

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## 1. Introduction

Diabetes mellitus (DM) ranks highly with the top ten disorders which cause mortality throughout the world and is affecting approximately 30% of the population worldwide.<sup>1–3</sup> It is not

a single disease entity, but a set of metabolic disorders with a common underlying feature of high blood glucose level. It is a systemic metabolic disease characterized by increased blood glucose, triglyceride and hypo insulinemia that may lead to decrease in both insulin action and insulin secretion.<sup>4,5</sup> The increased blood glucose is associated with reduced quality of life and high risk factors for mortality and morbidity.

Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin is released from the pancreas to normalize the glucose level.<sup>6</sup> It

\* Correspondent author. Tel.: +91 2164 263030.

E-mail address: [shashiprathmesh@gmail.com](mailto:shashiprathmesh@gmail.com) (S.C. Dhawale).

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lowers the post-prandial blood glucose level when it is raised (e.g. after eating food). Hyperglycemia in DM, results from defects in insulin secretion, insulin signaling pathway or most commonly both.<sup>7</sup>

Uncontrolled DM is often associated with complications which pose challenges to clinicians. These complications include development of micro- and macro-vascular complications such as neuropathy, nephropathy, retinopathy, cardiovascular and cerebrovascular diseases.<sup>8,9</sup>

Currently available antidiabetic agents possess potential side effects such as risk of hypoglycemia, anemia, cholestatic jaundice.<sup>10</sup> Many herbal constituents have varying degree of hypoglycemic and antihyperglycemic activity and until now no case of adverse effect is counted with herbal constituents. Among these are alkaloids, glycosides, galactomannan, gallo-catechin, hypoglycans, guanidine, steroids, carbohydrates, terpenoids, glycopeptides, amino acids and inorganic ions.<sup>11,12</sup> *Musa* commonly known as banana, reported to possess therapeutic potential in treatment of hyperglycemia.<sup>13,14</sup>

In Indian system of folk medicine, the peel of banana is also used to treat various diseases and disorders such as for treatment of wound, hyperglycemia, ulcer, dysentery.<sup>15</sup> Some people have a habit of eating inner peel, in addition to the pulp of banana fruit. Several flavonoids and related compounds (leucocyanidin, quercetin and its 3-O-glucoside, 3-O-galactoside, and 3-O-rhamnosyl glucoside, gallo-catechin) were isolated from the unripe pulp and peel of plantain.<sup>14,16,17</sup> The various antioxidant components identified in bananas includes tocopherol, ascorbic acid, beta carotene, dopamine, phenolic groups and gallo-catechin.<sup>18</sup> Banana is also reported to be a rich source of calcium, vitamins A, B1, B2, B3, B6, C and minerals such as potassium and phosphorus.<sup>19</sup> These phytochemicals have shown protective action against diseases which involve oxidative stress. On the basis of traditional claim, reported activities and chemical constituents, the present study was aimed to evaluate antihyperglycemic, hypoglycemic and *in vitro* antioxidant potential of peels of *Musa* species.

## 2. Materials and methods

### 2.1. Experimental animals

Albino rats of Wistar strain weighing 160–200 g were obtained from National Institute of Nutrition (NIN), Hyderabad. Animals of either sex were housed under standard laboratory conditions of  $22 \pm 3$  °C temperature and relative humidity 30% and 12 h light and dark cycle maintained, free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (1613/PO/a/12/CPCSEA).

### 2.2. Acute toxicity study

The LD50 of the peel extract was tested to determine the safety of the agent according to the guidelines set by the OECD (Organization for Economic Cooperation and development) No. 423.<sup>20</sup> The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per group and administered 100, 600 and 1000 mg/kg of the *Musa* species extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality.

The results of this phase informed the choice of doses for the second phase, in which 2000, 3000 and 5000 mg/kg were administered to another set of three mice per group and these animals were observed for signs of toxicity and mortality for 72 h.

### 2.3. Collection and authentication of the plant material

Fresh unripe fruits of *Musa sapientum*, *Musa paradisiaca*, *Musa cavendish*, and *Musa acuminata* (*Musa* species) Linn. (Musaceae) were collected from Nanded, Satara of Maharashtra, Goa and Kerala states of India. The specimens were authenticated at Botanical Survey of India, Pune (MUP-NAV2), and Science College Nanded (25-9/12).

### 2.4. Preparation of extract

Banana pulp was removed and from the remaining peel inner fibrous part was removed by knife (we termed it as 'inner peel') and it was shed dried at room temperature and latter powdered using grinder. This powder was then defatted with petroleum ether. *M. sapientum*, *M. paradisiaca*, *M. cavendish*, and *M. acuminata* peel powder was extracted with ethanol using soxhlet extraction. It was then removed and the solvent was evaporated under vacuum and the residue was stored for further use.

The extract was not freely soluble in distilled water so the suspension of extract was prepared using 1% gum acacia (as a suspending agent).

### 2.5. Experimental design

The Wistar albino rats weighing 160–200 g were used. The overnight fasted animals were divided into fourteen groups ( $n = 6$ ). Group 1 served as Control: The animals of this group received 1% gum acacia (1 ml/kg, p.o.). Group 2 served as Glim (Standard): The animals of this group received glimepiride (0.09 mg/kg, p.o.). Groups 3 to 5: Received ethanolic extract of *M. sapientum* (EMS) 50, 100 and 200 mg/kg, p.o. Groups 6 to 8: Received ethanolic extract of *M. paradisiaca* (EMP) 125, 250 and 500 mg/kg, p.o. Groups 9 to 11: Received ethanolic extract of *M. cavendish* (EMC) 250, 500 and 1000 mg/kg, p.o. Groups 12 to 14: Received ethanolic extract of *M. acuminata* (EMA) 100, 200 and 400 mg/kg, p.o.

#### 2.5.1. Hypoglycemic study in non-diabetic rats<sup>21</sup>

The effect of various extracts of *Musa* species was studied in non-diabetic rats for the assessment of hypoglycemic effect if any. Animals were treated with ethanolic extract of different species of *Musa* and glimepiride was used as standard drug as per the experimental design. Blood samples were collected by puncturing retro-orbital plexus at the 0, 60, 120 and 180 min after drug administration.

#### 2.5.2. Oral glucose tolerance test (OGTT) in non-diabetic rats<sup>21,22</sup>

Oral glucose tolerance test of different extracts of *Musa* species was conducted in non-diabetic rats. In this study, glucose solution (2 g/kg, p.o.) was administered 30 min after vehicle/drug administration. Blood samples were collected at the 0, 30, 90

and 150 min after glucose load. The blood glucose level was estimated by using catalyst diagnostic kits.<sup>22</sup>

## 2.6. In vitro antioxidant activity

### 2.6.1. DPPH<sup>•</sup> free radical scavenging activity<sup>23,24</sup>

The free radical scavenging activity of *M. cavendish* and *M. acuminata* was measured by DPPH<sup>•</sup> assay, wherein the bleaching rate of the stable free radical, (1, 1-Diphenyl-2-picrylhydrazyl) DPPH<sup>•</sup> is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH<sup>•</sup> absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorbance decreases. Briefly, 0.1 mM solution of DPPH<sup>•</sup> in ethanol was prepared and 1 ml of this solution was added to 3 ml of *M. cavendish* and *M. acuminata* solution in water at various concentrations (25–250 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.<sup>23,25</sup> IC<sub>50</sub> value (concentration required to scavenge 50% free radicals) of the test samples was also determined.

The DPPH<sup>•</sup> radical scavenging activity was calculated according to the following equation:

DPPH radical scavenging activity (%)

$$= \left[ \frac{A_0 - A_1}{A_0} \right] \times 100.$$

where

A<sub>0</sub> is the absorbance of DPPH<sup>•</sup>,

A<sub>1</sub> is the absorbance of DPPH<sup>•</sup> solution in the presence of the extract.

### 2.6.2. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging assay<sup>26–28</sup>

The hydrogen peroxide scavenging ability of *M. cavendish* and *M. acuminata* was determined according to the method of Ruch et al.<sup>26</sup> A solution of H<sub>2</sub>O<sub>2</sub> (40 mM) was prepared in phosphate buffer (pH 7.4). The different concentrations of all *M. cavendish* and *M. acuminata* (10–50 µg/ml) in phosphate buffer were added to a H<sub>2</sub>O<sub>2</sub> solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm and phosphate buffer without H<sub>2</sub>O<sub>2</sub> was used as a blank.<sup>23</sup> The percentage of H<sub>2</sub>O<sub>2</sub> scavenging of *M. cavendish*, *M. acuminata* and standard compound (Ascorbic acid) was calculated as

H<sub>2</sub>O<sub>2</sub> radical scavenging activity (%)

$$= \left[ \frac{A_0 - A_1}{A_0} \right] \times 100.$$

where A<sub>0</sub> is the absorbance of H<sub>2</sub>O<sub>2</sub> and A<sub>1</sub> is the absorbance of H<sub>2</sub>O<sub>2</sub> solution in the presence of the extract.

### 2.6.3. Ferric reducing antioxidant power (FRAP) assay

Depending upon the reducing power (changes in brown color of peel extract) of each antioxidant sample of *M. cavendish* and *M. acuminata*, the reduction potential was determined. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity. The presence of reductant such as antioxidant substance causes the reduction in the Fe<sup>3+</sup>/ferricyanide complex to the ferrous form. Therefore, the Fe<sup>2+</sup> can be monitored by measuring the formation of Prussian

blue at 700 nm.<sup>26,29,30</sup> Different concentrations of *M. cavendish* and *M. acuminata* (25–250 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Reducing power assay (%) =  $\left[ \frac{A_0 - A_1}{A_0} \right] \times 100.$

where

A<sub>0</sub> is the absorbance of FeCl<sub>3</sub>,

A<sub>1</sub> is the absorbance of FeCl<sub>3</sub> solution in the presence of the extract.

## 2.7. Statistical analysis

The data obtained were treated statistically by using analysis of variance (ANOVA) followed by Dunnett's test to detect any significant difference among different means, with level of significance set at  $p < 0.05$ . The results were expressed as mean ± S.E.M.

## 3. Results

### 3.1. Acute toxicity

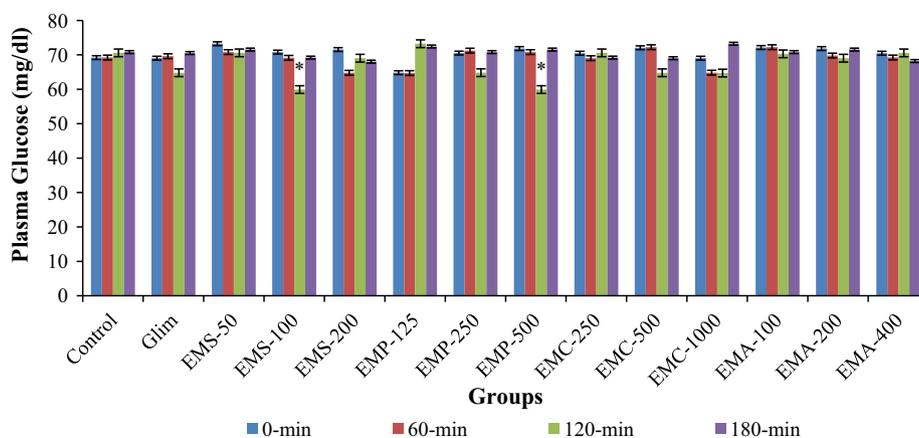
The acute oral toxicity test showed normal behavior of the treated animals. There was no mortality observed at a high dose of 2000 and 5000 mg/kg. Hence the 1/10th of the safer dose was selected as a therapeutic dose.

### 3.2. Effect of various extracts of *Musa* species on blood glucose in normoglycemic rats

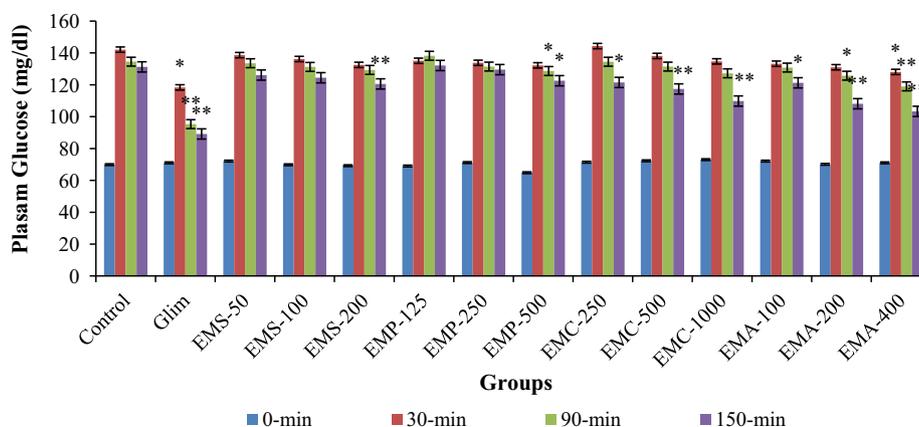
As like glimepiride-treated group, groups treated with different extracts of *Musa* species did not show any significant change in blood glucose level, when compared with control group (Fig. 1). However, marginal reduction in blood glucose level below normal was observed at higher dose of EMS-100 mg/kg, p.o. at 120 min. But, this effect of smaller decrease in blood glucose disappeared at 180 min. Group treated with EMP-500 mg/kg, p.o. showed minor reduction ( $p < 0.05$ ) in blood glucose level at 120 min when compared with the control group. Apart from above mentioned groups, no other treated groups depicted any sign of hypoglycemia.

### 3.3. Effect of various extracts of *Musa* species on blood glucose in glucose loaded rats

Effect of *Musa* species on glucose loaded normoglycemic rats is presented in Fig. 2. From these studies it was evident that out of four species, *M. acuminata* (EMA-200 and 400) depicted greater decrease ( $p < 0.01$ ) in blood glucose level, followed by *M. cavendish* (EMC 500 and 1000) i.e. ( $p < 0.01$ ) at 150 min. Hyperglycemic animals treated with EMS-200 mg/kg, p.o. showed significant reduction in blood glucose level at



**Figure 1** Hypoglycemic effect of *Musa* species on normoglycemic rats. Values are statistically significant at  $p < 0.05$ . Significance determined by ANOVA followed by Dunnett's test.



**Figure 2** Effect of *Musa* species on glucose loaded normoglycemic rats. Values are statistically significant at  $p < 0.05$ . Significance determined by ANOVA followed by Dunnett's test.

150 min ( $p < 0.01$ ). EMP at the dose of 500 mg/kg also demonstrated noteworthy anti-hyperglycemic effect from 90 min onward. All the groups treated with various extracts of *Musa* species showed dose dependant reduction in the blood glucose level.

#### 3.4. *In vitro* antioxidant study

As depicted in Table 1 *M. acuminata* and *M. cavendish* demonstrated significant DPPH· radical scavenging activity in a dose-dependent manner in a range of 25–250  $\mu\text{g/ml}$ . Ethanolic extract of *M. acuminata* revealed highest inhibition considering the IC50 value (139.50  $\mu\text{g/ml}$ ). Similarly, in ferric reducing power activity, dose-dependent iron chelating activity of both extracts was observed and *M. acuminata* demonstrated greater reducing power than *M. cavendish* with IC50 value (198.80  $\mu\text{g/ml}$ ). Extracts were also screened for  $\text{H}_2\text{O}_2$  scavenging activity (Table 2). In conformity with the earlier results, both extracts revealed potential  $\text{H}_2\text{O}_2$  scavenging activity. In comparison with *M. cavendish*, the ethanolic extract of *M. acuminata* demonstrated greater  $\text{H}_2\text{O}_2$  scavenging activity.

#### 4. Discussion

Earlier studies have demonstrated anti-hyperglycemic activity of various parts of different *Musa* species.<sup>13,31,32</sup> Mostly its pulp has been explored for antidiabetic potential. The inner peel that is consumed by some local population for their belief has not been fully explored for antidiabetic activity. Moreover, comparative evaluation of different *Musa* species is yet to be explored. In the present study evaluation of *Musa* species for possible antidiabetic effect was performed by glucose tolerance test and hypoglycemic study. Significant anti-hyperglycemic effect was seen with all four *Musa* species viz. *M. cavendish*, *M. acuminata*, *M. sapientum*, and *M. paradisiaca*. Anti-hyperglycemic effect was more prominent with *M. acuminata* and *M. cavendish* species. However, extract of other two species also demonstrated significant effect at higher doses. The result confirmed earlier findings of *Musa* species.<sup>33,34</sup> Anti-hyperglycemic effect of *Musa* species may be due to the presence of phyto-constituents such as tannins, saponins and flavonoids.<sup>16,17</sup> The phytochemical investigation of various extracts of *Musa* species showed the presence of

**Table 1** *In vitro* antioxidant activity of ethanolic extracts of *Musa cavendish* and *Musa acuminata* fruit peel by DPPH and ferric reducing power assay.

Concentration ( $\mu\text{g/ml}$ )	DPPH <sup>*</sup> radical scavenging activity			Ferric reducing power activity		
	EMC	EMA	Ascorbic acid	EMC	EMA	Ascorbic acid
25	6.05 $\pm$ 0.21	12.06 $\pm$ 0.25	26.1 $\pm$ 0.23	9.25 $\pm$ 0.23	12.02 $\pm$ 0.22	32.45 $\pm$ 0.32
50	16.12 $\pm$ 0.14	22.17 $\pm$ 0.18	38.3 $\pm$ 0.46	14.84 $\pm$ 0.28	24.57 $\pm$ 0.14	46.78 $\pm$ 0.17
100	24.72 $\pm$ 0.35	40.19 $\pm$ 0.35	59.7 $\pm$ 0.19	19.06 $\pm$ 0.15	32.08 $\pm$ 0.23	59.45 $\pm$ 0.45
150	37.08 $\pm$ 0.18	53.74 $\pm$ 0.21	79.1 $\pm$ 0.14	28.06 $\pm$ 0.21	45.65 $\pm$ 0.28	78.95 $\pm$ 0.19
200	48.06 $\pm$ 0.19	76.48 $\pm$ 0.19	93.5 $\pm$ 0.89	37.05 $\pm$ 0.14	50.27 $\pm$ 0.31	86.82 $\pm$ 0.46
250	54.63 $\pm$ 0.25	89.14 $\pm$ 0.20	97.9 $\pm$ 0.45	51.03 $\pm$ 0.19	62.14 $\pm$ 0.19	98.45 $\pm$ 0.17
IC <sub>50</sub>	232.08	139.50	82.50	247.80	198.80	63.00

Results are Mean  $\pm$  S.E.M. of three parallel measurements. EMC-Ethanolic extract of *Musa cavendish* fruit peel. EMA-Ethanolic extract of *Musa acuminata* fruit peel.

**Table 2** *In vitro* antioxidant activity of ethanolic extracts of *Musa cavendish* (EMC) and *Musa acuminata* (EMA) fruit peel using H<sub>2</sub>O<sub>2</sub> scavenging activity.

Concentration ( $\mu\text{g/ml}$ )	Hydrogen peroxide scavenging activity		
	EMC	EMA	Ascorbic acid
10	15.26 $\pm$ 0.23	14.34 $\pm$ 0.32	21.27 $\pm$ 0.22
20	16.03 $\pm$ 0.28	31.45 $\pm$ 0.34	32.45 $\pm$ 0.16
30	18.09 $\pm$ 0.15	47.12 $\pm$ 0.28	46.12 $\pm$ 0.21
40	31.09 $\pm$ 0.24	56.02 $\pm$ 0.19	59.12 $\pm$ 0.18
50	52.03 $\pm$ 0.23	71.01 $\pm$ 0.26	78.49 $\pm$ 0.31
IC <sub>50</sub>	47.60	35.70	34.60

Results are Mean  $\pm$  S.E.M. of three parallel measurements.

tannins, alkaloids and flavonoids (e.g. gallic acid, gallic acid, gallic acid).<sup>14,35</sup> These substances are frequently implicated for its anti-hyperglycemic effects.<sup>36,37</sup> Furthermore, banana peels are rich in phytochemical composites, principally antioxidants. Developed banana peels, which hold the anthocyanins, delphinidin and cyanidin, and catecholamine may also contribute to this effect.<sup>38</sup> Inhibition of intestinal absorption of glucose or increased secretion of insulin by pancreatic beta cells may be one of the probable mechanisms for this anti-hyperglycemic effect as gallic acid is one of the active constituents of banana peel which increases peripheral glucose utilization.<sup>14,39</sup>

Marginal hypoglycemia observed with the administration of extract of *M. sapientum* could be due to increased utilization of glucose in the liver for glycogen synthesis and decreased degradation of glycogen and due to reduced gluconeogenesis.<sup>40</sup>

On the basis of hypoglycemic and anti-hyperglycemic activity, most active *Musa* species i.e. *Musa cavendish* and *M. acuminata* were further evaluated for its *in vitro* antioxidant activity, using DPPH radical scavenging assay, Ferric reducing power assay and H<sub>2</sub>O<sub>2</sub> scavenging activity. The present work has shown that the extracts of both *Musa* species exhibited a marked DPPH scavenging activity (Table 1). As a result, the new findings showed dose dependent decrease in the concentration of DPPH due to the free radical scavenging effect of *M. acuminata*. In the presence of hydrogen donors, DPPH gets oxidized and a stable free radical is formed from the scavenger.<sup>41</sup> Since the hydrogen donating ability of *Musa* species

was comparable to ascorbic acid, it was evident that the *M. acuminata* could serve as hydrogen donors, and thus consequently terminating the radical chain reaction.

Phenolic compounds are good electron donors, and they reveal reducing power and have ability to reduce ferric ion to ferrous ion by donating electron.<sup>42</sup> This may explain a current interest in the applicability of the reducing power assay in determining the antioxidant capacity of plant extracts. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The present study indicates the reducing power of *M. acuminata* as a potential source of antioxidants. This study, therefore, suggests that the reducing power of *M. acuminata* might be due to the presence of its phenolic content.<sup>38</sup>

Hydrogen peroxide itself is not very reactive because of its weak oxidizing and reducing capabilities but it can inactivate few enzymes by oxidizing essential thiol (-SH) groups and it also can cross the cell membrane rapidly and once inside the cell, in the presence of metal ions it is converted into highly toxic hydroxyl radical which may originate from many of its toxic effects.<sup>43</sup> Therefore it is biologically important for cells to scavenge hydrogen peroxide that gets entered into the cell. Hence *in vitro* testing for H<sub>2</sub>O<sub>2</sub> scavenging activity of extracts is one of the valuable tools to discover new natural antioxidants to control diseases induced by H<sub>2</sub>O<sub>2</sub> and its products. From the result (Table 2), it appeared that *M. acuminata* possesses marked H<sub>2</sub>O<sub>2</sub> scavenging ability.

## 5. Conclusion

In the present study, inner peels of various *Musa* species demonstrated significant antihyperglycemic and *in vitro* antioxidant activity. In particular ethanolic extracts of *M. acuminata* demonstrated marked antihyperglycemic activity in Wistar rats. Similarly antioxidant assays *viz.*, DPPH, ferric reducing power and H<sub>2</sub>O<sub>2</sub> scavenging assay also confirmed noteworthy free radical scavenging activity of *M. acuminata* peels. Hence, it can be concluded that eating of inner peel of banana fruit would be beneficial considering its potential antioxidant and antihyperglycemic property.

## Conflict of interest

We have no conflict of interest to declare.

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