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# Antidiabetic Compounds in Stem Juice from Banana

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**Abstract**

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The stem juices from *Musa* × *paradisiaca* L. banana plants cultivated in their original natural habitat in Vietnam and those cultivated in a greenhouse in the Czech Republic were investigated for the presence of phytochemicals with antidiabetic potency. Respective bioactivities of these phytochemicals were also determined. Sample screening using ultra-high performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UHPL-HRMS/MS) method showed some differences in the pattern of bioactive compounds, both in terms of their number and concentration. *p*-Hydroxybenzoic and gallic acids were the predominant analytes found in stem juice from plants grown in Vietnam, while ferulic acid was the major compound found in juice obtained from greenhouse bananas. Despite differences in the occurrence of potentially antidiabetic compounds, both extracts exhibited comparable inhibitory activity against α-glucosidase and α-amylase.

**Keywords**: amylase; antidiabetic phytochemicals; plantan; *Musa* × *paradisiaca*; glucosidase inhibitor; inhibitor; extract cocktail

The well-known representative of the *Musaceae* family, *Musa* × *paradisiaca* L., commonly known as the banana, is an herbaceous plant extensively cultivated in tropical and subtropical regions. Due to its worldwide consumption, the banana is of great economic importance. It should be noted, that for banana harvested green (i.e. when contain high levels of starch and little sugar) and widely used in tropics as a cooked vegetable, the name 'plantain' is used. Until now, more than 100 cultivars belonging to the *Musa* genus have been described (Häkkinen 2013). In addition to the consumption of the fruit, all parts of the banana plant, including leaves, peels, root and stems have been used in folk medicine to treat various disorders such as diarrhoea, dysentery, intestinal colitis, inflammation, pain and snakebite. Furthermore, a number of pharmacological activities have been reported not only for *Musa* × *paradisiaca* L., but also for wild species, represented mainly by *Musa acuminate* (Mathew *et al.* 2017). To design potent therapeutic agents derived from these plants, great effort has been devoted to an elucidation of the active principles of extracts obtained from their various parts. A number of low-molecular-weight bioactive phytochemicals such as alkaloids, saponins, flavonoids, tannins, phlobatannins, phenolics, glycosides, terpenoids, and steroids have been identified (Ramu *et al.* 2014). In the last

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decade, special attention has been paid to the potential of extracts derived from different parts of the banana plant in supporting the management of both type I (da Silva *et al.* 2016) and type II (Famakin *et al.* 2016) diabetes mellitus, a metabolic disease whose incidence has been growing significantly in the Western world. Since diabetes has a multifactorial aetiology, various underlying mechanisms have been investigated. In addition to the ability to inhibit carbohydrate-digesting enzymes (α-glucosidase and α-amylase), the potential of banana phytochemicals to affect, e.g., glucose adsorption (Jaber *et al.* 2013) and/or antioxidant activity (Ayoola *et al.* 2017) have been most frequently described. However, a great deal of further research on the development of phytomedicines as well as edible products with functional properties is needed.

It is worth noting that the concentrations of secondary metabolites contained in banana plants, and, consequently, the bioactivities of obtained extracts, may vary depending not only on respective species, but also due to multiple environmental factors. In this study, we aimed at a comparison of the profiles of bioactive phytochemicals and their respective bioactivities in juices obtained from stems of plantains grown in their natural environment in the north of Vietnam, and in a greenhouse in the Czech Republic, i.e., under very different conditions.

## MATERIAL AND METHODS

***Chemicals***. Deionised water for the LC mobile phase was obtained from a Milli-Q® Integral system (Merck, Germany). HPLC-grade methanol and ammonium formate (purity > 99%) were from Sigma-Aldrich (USA).

***Plant material and isolation of phytochemicals***. The stems of *M.* × *paradisiaca* were obtained from (I) plants grown for eight months in Bacninh province, north Vietnam with an alluvium type of soil, temperature ranging between 20–35°C, humidity 70–80%, and (II) plants grown in a greenhouse with ploughland of the botanical garden of the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague; the growing conditions here were 18–23°C, humidity 70–80%. The cultivar of the Vietnamese sample was fully characterised. It is a hybrid between *Musa acuminata* and *Musa balbisiana*, a triploid cultivar of the AAB group. The genotype and age of the Czech banana plant is unknown.

Freshly cut stems were processed to prepare juice as in countries where the practice is common. The juice represented a pooled sample of two stems. The pseudostem layers were peeled off to uncover the central white stem. In the next step, the plant material was crushed using a kitchen juicer (Twin Gear Juicer, Green power) and the slurry was filtered through a 2-mm plastic sieve. To remove fine particles, the crude juice was centrifuged (14 000 *g*, 10 min) and the supernatant was freeze-dried (FreeZone 2.5 l, Labconco). One gram of dry matrix was obtained from 62.5 g of Vietnamese or 62.8 g of Czech pseudostem. One gram of dry matrix was equivalent to 58 ml of banana stem juice. The dry matrix was stored at room temperature in a desiccator prior to further processing.

To isolate phytochemicals, 0.5 g of freeze-dried juice were extracted with 2.5 ml of methanol/water (80/20, v/v) using continuous shaking for 30 min at 20°C. The insoluble fraction was removed by centrifugation for 5 min at 10 000 *g*.

***Analysis of M.* × *paradisiaca* extracts***. For screening of selected metabolome components, ultra-high performance liquid chromatography (UHPLC) coupled with tandem high-resolution mass spectrometry (HRMS/MS) was used. A Dionex UltiMate 3000 RS UHPLC system (Thermo Scientific, USA), equipped with a Acquity UPLC HSS T3 (100 mm × 2.1 mm i.d., 1.8 µm), coupled with TripleTOF® 6600 quadrupole-time-of-flight mass spectrometer (SCIEX, Canada) was employed. The conditions of separation and detection were as in a previously published method (Rubert *et al.* 2016).

***Inhibition of α-amylase***. Inhibition of human saliva α-amylase was determined according to Ashok Kumar *et al.* (2011). Ten microlitres of 10 µg/ml α-amylase (Sigma-Aldrich) were added to 80 µl of banana stem juice samples (0.1 mg/ml to 0.2 µg/ml) in phosphate buffer (40 mM, pH 6.9). The mixture was preincubated for 10 min at 37°C. The reaction was started by the addition of 10 µl of 2-chloro-4-nitrophenyl-α-D-maltotrioside (6.6 mg/ml). The absorbance was measured at 400 nm. Percentage inhibition was calculated as 100 × (average slope of banana – average slope of negative control)/(average slope of positive control – average slope of negative control).

***Inhibition of α-glucosidase***. The effect of the juice on α-glucosidase activity was determined according to Kazeem *et al.* (2013). Fifty microlitres of the α-glucosidase solution (1 U/ml in 20 mM phosphate

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buffer; pH 6.9) was preincubated with 25 µl of dry banana matrix (concentrations 0.1 mg/ml to 0.2 µg/ml) at 37°C for 10 minutes. Then, 25 µl of 3 mM *p*-nitrophenyl glucopyranoside (*p*NPG, Sigma-Aldrich) in the same buffer were added to start the reaction. The released yellow-coloured product *p*-nitrophenol was measured at 405 nm. Percentage inhibition was calculated as described in the previous paragraph.

***Inhibition of preadipocyte differentiation***. Differentiation of the mouse 3T3-L1 preadipocyte cell line was performed according to Skop *et al.* (2014). Briefly, the cells were grown to confluence in DMEM (Sigma-Aldrich) supplemented with 10% FBS (Sigma Aldrich) in 5% $CO_2$ atmosphere at 37°C. Then the medium was exchanged and after two days, differentiation medium I (DMEM with 10% FBS, 1.7 µM insulin, 1 µM dexamethasone and 0.5 mM 1-methyl-3-isobutylxanthine) containing the banana sample (0.1 mg/ml) was added. Two days later, the medium was replaced with differentiation medium II (DMEM supplemented with 10% FBS, 1.7 µM insulin) containing the same concentration of the banana powder. After ten days of incubation (medium was exchanged every two days), the adipocytes were fixed with 3.7% formaldehyde, washed with 60% isopropanol and stained using Oil Red O (2.1 mg/ml; Sigma-Aldrich). Then, the Oil Red O was extracted with isopropanol and quantified photometrically at a wavelength of 500 nm.

## RESULTS AND DISCUSSION

As mentioned in the introduction, all parts of the banana plant contain a number of bioactive secondary metabolites, including those with antidiabetic activity. Considering that metabolome components are inherently very dynamic and their pattern might be influenced, among other factors, by the external environment, it can be assumed that natural raw material derived from different plants of the identical species, will not necessarily exhibit identical biological potency when grown under different conditions. In the paragraphs below, the investigations performed to verify/reject this assumption are presented.

***Screening of antidiabetic compounds***. In the first part of our study, the UHPLC-HRMS/MS method was employed for comparative analyses of samples of dry matrix from stems of *Musa* × *paradisiaca* grown in their natural habitat in northern Vietnam and in a greenhouse. Currently known compounds (Table 1) with antidiabetic activity were selected for targeted screening. The use of tandem high-resolution mass spectrometry enabled acquisition of both highly accurate MS, and MS/MS mass spectra, on the basis of which a number of compounds listed in Table 1 were detected. For their tentative identification (standards of pure compounds needed for unequivocal confirmation were not available), powerful informatics tools

Table 1. Plant antidiabetic compounds identified in freeze-dried samples of banana stems juice

| Screened antidiabetic compounds | Type of antidiabetic activity | Elemental formula | Type of detected ion | *m/z* of detected ion respective extract | |
|---|---|---|---|---|---|
| | | | | banana | |
| | | | | grown in natural habitat | from greenhouse |
| Lupeol[1] | G, A | $C_{30}H_{50}O$ | $[M+H]^+$ | 427.3934 | 427.3916 |
| Ferulic acid[2,3] | G[4] | $C_{10}H_{10}O_4$ | $[M-H]^-$ | 193.0509 | 193.0510 |
| Vanillic acid[2,5] | G, A[6] | $C_8H_8O_4$ | $[M-H]^-$ | 167.0349 | 167.0344 |
| *Trans*-cinnamic acid[2,3] | | $C_9H_8O_2$ | $[M-H]^-$ | 147.0451 | 147.0452 |
| *p*-Hydroxybenzoic acid[2,5] | G, A[6] | $C_7H_6O_3$ | $[M-H]^-$ | 137.0244 | 137.0245 |
| *p*-Coumaric acid[2,5] | G | $C_9H_8O_3$ | $[M-H]^-$ | 163.0400 | 163.0396 |
| Rutin[7,8] | G, A[9] | $C_{27}H_{30}O_{16}$ | $[M+H]^{+-}$ | 611.1598 | – |
| Catechin/epicatechin[2,7] | | $C_{15}H_{14}O_6$ | $[M+H]^+$ | 291.0861 | – |
| Chlorogenic acid[2,10] | G, A[11] | $C_{16}H_{18}O_9$ | $[M-H]^-$ | 353.0882 | 353.0862 |
| Gallic acid[2,12] | G, A[13] | $C_7H_6O_5$ | $[M-H]^-$ | 169.0142 | – |
| Caffeic acid[2,3] | G, A | $C_9H_8O_4$ | $[M-H]^-$ | 179.0351 | 179.0345 |
| Nicotiflorin[2,7] | G, A[14] | $C_{27}H_{30}O_{15}$ | $[M+H]^+$ | – | 595.1640 |

G – α-glucosidase inhibitor; A – α-amylase inhibitor; *the mass detecting errors ranged from –1.5 to 2.8 ppm; [1]Ramu *et al.* (2014); [2]Coman *et al.* (2012); [3]Padam *et al.* (2014); [4]Adisakwattana *et al.* (2008); [5]Oliveira *et al.* (2006); [6]Saltan *et al.* (2008); [7]Rebello *et al.* (2014); [8]Kappel *et al.* (2013); [9]Oboh *et al.* (2014); [10]Kandasamy *et al.* (2014); [11]Oboh *et al.* (2015); [12]Borges *et al.* (2014); [13]Oboh *et al.* (2016); [14]Habtemariam (2011)

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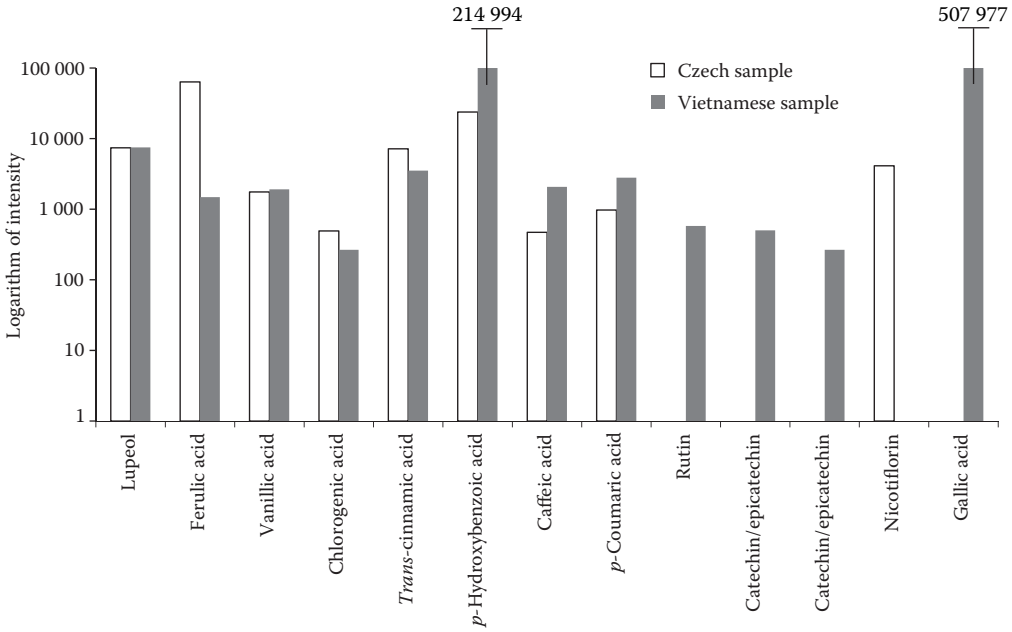


Figure 1. The profiles of major antidiabetic compounds (catechin and epicatechin) identified in freeze-dried samples of banana stem juice; expressed as average signal; $n = 3$ have identical mass spectra; therefore, they could not be distinguished

were used, especially for calculation of the exact *m/z* value of the compounds, assessment of isotopic profiles of the MS data as well as assessment of the MS/MS fragmentation pattern.

We wish to emphasize that the purpose of this study was not the quantification of antidiabetic compounds, but rather a comparison of their levels in plant extracts prepared in the same way. A lower number of these bioactive compounds was found in the greenhouse-grown bananas. From the 17 compounds screened by our method, nine were found in the juice from greenhouse bananas, and 12 in samples from their natural habitat in Vietnam. In Figure 1, the intensities of ions derived from major antidiabetic compounds are shown; in this way, relative concentrations in both tested samples are illustrated.

Gallic acid was the phytochemical with the highest signal intensity (correlates with concentration), and was found exclusively in the Vietnamese sample. The other compounds found only in the Vietnamese sample were rutin, catechin and epicatechin; however, their signals were considerably lower. On the other hand, nicotiflorin (kaempferol-3-rutinoside) was a compound detected exclusively in the stem juice from bananas grown in the greenhouse. Most of the other screened antidiabetic phytochemicals was found in both samples, although, as shown in Figure 1, concentrations of some of them differed significantly. For instance, the signal intensity of *p*-hydroxybenzoic acid in the Vietnamese sample was approx. 9-times higher compared to the sample from the greenhouse. On the other hand, compounds such as lupeol and vanillic acid, detected in both samples, were present at comparable concentrations. As regards phenolic acids widely occurring in plants, i.e., ferulic, chlorogenic and trans-cinnamic acids, these were also found in both stem juices; however, with substantially different signal intensities. Specifically, the concentration of ferulic acid was approx. 40-times higher in the greenhouse sample. As regards the intensity of the trans-cinnamic acid signal, this was approx. 2-times higher in the Czech sample, in comparison to the Vietnamese one. Figure 1 also shows that caffeic and *p*-coumaric acids, other representatives of the hydroxy-cinnamic acids family, were found at relatively low levels, with their concentrations higher in the Vietnamese sample.

***Antidiabetic activity in vitro***. *In vitro* α-amylase and α-glucosidase assays were used for determination of potential diabetes-related inhibitors. Starch and glycogen 1,4-glycosidic linkages are digested by α-amylase to disaccharides which serve as substrates for α-glucosidase. Thus, inhibitors of both enzymes may delay carbohydrate digestion and thus reduce hyperglycaemia (Telagari *et al.* 2015). Both banana stem juices inhibited α-glucosidase (Figure 2) with almost the same efficiency. For total inhibition of $1 \times 10^{-3}$ U of α-glucosidase in the reaction, the addi-

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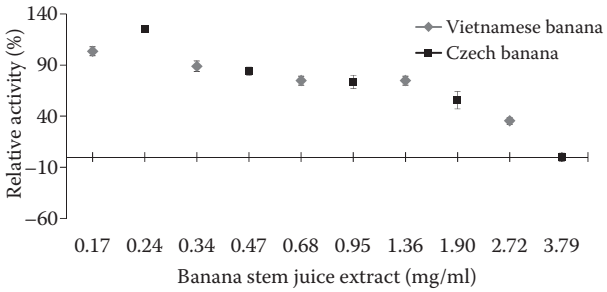


Figure 2. Inhibition of α-glucosidase (Standard error; $n = 3$)

tion of 22 µl of fresh banana stem juice is necessary. To provide some context, human serum contains 7 U of α-glucosidase per litre (Porter *et al.* 1986). However, such data cannot be applied for calculation of α-glucosidase in the body, as bioavailability aspects must be considered.

The previously described inhibitors of α-glucosidase mentioned in Table 1 were found in both samples. Gallic acid and rutin were present only in the Vietnamese sample, while nicotiflorin and high levels of ferulic acid were found only in the Czech sample. As indicated, many compounds are able to inhibit α-glucosidase and therefore their different amounts in such a complex mixture as stem juice had no impact on the total inhibitory activity, which was according to our results almost the same.

Although detectable, the inhibition of α-amylase was less significant than that of α-glucosidase; a slightly higher inhibition potential was exhibited by the Vietnamese sample. Human saliva usually contains 10–160 U of α-amylase per millilitre (Butterworth *et al.* 2011). According to our results, 7.9 µl of Vietnamese or 11 µl of Czech fresh stem juice were required to inhibit 1 U of human saliva α-amylase by 20%. As

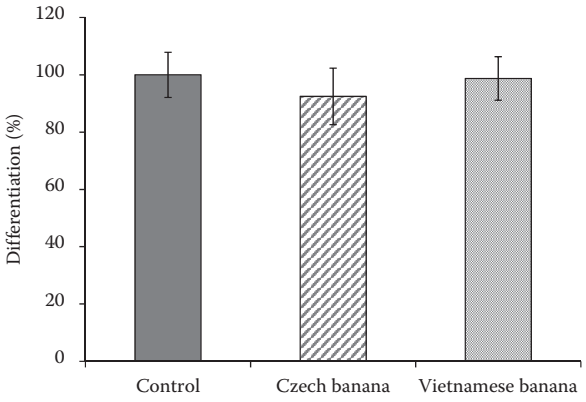


Figure 3. Inhibition of 3T3-L1 differentiation

No statistically significant differences were found using one-way ANOVA ($P = 0.5$) standard deviation; $n = 8$

mentioned above, several α-amylase inhibitors were identified (Table 1). The levels of lupeol, vanillic acid and chlorogenic acid were almost the same in both samples; however, caffeic acid, *p*-hydroxybenzoic acid, and rutin were more concentrated in the Vietnamese sample. In contrast, nicotiflorin was present only in the Czech banana sample and a high level of gallic acid was observed only for the Vietnamese sample. The potential of gallic acid in reducing the side effects of the antidiabetic drug acarbose has been described (Oboh *et al.* 2016).

***Cell-based analysis of antidiabetic activity***. We also tested the putative ability of the banana stem juice extracts to inhibit the differentiation of 3T3-L1 cells into adipocytes. Our results showed that neither the Vietnamese nor the Czech banana stem juice inhibited differentiation (Figure 3).

This is in agreement with published data which showed that banana lectin (mannose-binding protein) activates insulin-like signalling in mesenchymal cells leading to their adipocytic differentiation (Bajaj *et al.* 2011).

## CONCLUSIONS

This pilot study inspired by traditional oriental medicine was performed to investigate the possibility of exploiting natural resources such as banana stems, i.e., waste material, as a source of bioactive substances. The results obtained in this study can be summarised as follows.

Although the genotype and age of the plants were not characterised, we demonstrated differing compositions of metabolome components with antidiabetic potency in stem juices from two plants of *Musa* × *paradisiaca* L. grown in different environments. Significant differences in concentrations of some phenolic acids were observed as well. This indicates that the data collected from one plant cannot be simply generalised.

Both extracts efficiently inhibited α-glucosidase in a dose-dependent manner. They also inhibited α-amylase, although considerably less efficiently.

Both samples failed to inhibit the differentiation of 3T3-L1 cells into adipocytes.

In spite of the differing profiles of compounds known to possess antidiabetic potency, no differences in the aforementioned biological activities were observed between the two samples of banana stem juice, traditionally used in Vietnam as blood sugar-lowering medicine.

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Based on these preliminary results, follow-up investigations are planned to better understand the above observations and to identify and quantify the most potent antidiabetic compounds. Special attention will be paid to cocktail effects of these bioactive phytochemicals, their list will be extended and we will attempt to identify new ones.

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