High-resolution assays combined with HPLC for identification of antidiabetic constituents in Vietnamese plants

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BACKGROUND
In recent years, diabetes has become a common disease. Accounting for roughly 90% to 95% of all diabetes cases worldwide, type-2 diabetes is affecting 246 million worldwide and its incidence and serious complications continue to grow rapidly. Patients with type 2 diabetes suffer from different serious complications such as high blood pressure, blindness, kidney failure, heart disease and stroke.

AIM OF THE STUDY
Vietnam is a tropical country with more than 10,000 plant species, many of which have been long used as folk remedies for the treatment of diseases. 18 medicinal plants traditionally used for the management of diabetes were collected for the investigation of the non-tannin compounds able to cure type 2 diabetes.

BIOLOGICAL EVALUATION
Ethanol and water extracts of P. amarus, P. urinaria, L. speciosa, N. mirabilis, S. cuminii, R. mucronata and K. candel show IC$_{50}$ below 40 µg/mL in the α-glucosidase inhibition assay, and ethanol extracts of N. mirabilis, K. candel and F. racemosa show IC$_{50}$ below 75 µg/mL in the α-amylase inhibition assay.

METHOD
- Chloroform, ethanol and water extracts were evaluated for α-glucosidase and α-amylase inhibitory activity.
- The most active extracts were investigated on analytical-scale HPLC.
- Samples were fractionated into 96-well microplates, followed by α-glucosidase and α-amylase inhibition assaying of each well.
- High-resolution biochromatograms constructed from these assays allowed fast identification of active compounds.
- Subsequent HPLC and NMR experiments will allow isolation and structural elucidation.

HIGH-RESOLUTION BIOCHROMATOGRAMS
P. amarus, P. urinaria, and L. speciosa water extracts and F. racemosa ethanol extract were chosen for microfractionation followed by α-glucosidase and α-amylase inhibition assays. High-resolution biochromatograms of P. amarus and P. urinaria extracts showed several active peaks against α-glucosidase.

PERSPECTIVE AND FUTURE WORK
Biochromatograms of P. amarus and P. urinaria water extracts have many promising peaks with more than 90% inhibitory activity. Further work could include structure determination of the remaining active peaks and bioactivity tests of all isolated compounds.

REFERENCES

Figure 1. Flowchart of the procedure used in this work.

Figure 2. IC$_{50}$ curves of ethanol extracts. (a) Water extracts. IC$_{50}$ values in µg/mL. N. mirabilis = 3.31 ± 0.77; P. amarus = 34.92 ± 1.92; P. urinaria = 14.64 ± 4.56; L. speciosa = 5.39 ± 0.64; S. cuminii = 20.93 ± 1.77; R. mucronata = 3.31 ± 0.55; K. candel = 3.99 ± 0.75. (b) Ethanol extracts. IC$_{50}$ values in µg/mL. N. mirabilis = 32.70 ± 6.33; P. urinaria = 39.72 ± 9.73; K. candel = 35.38 ± 13.93

Figure 3. IC$_{50}$ curves of ethanol extracts. IC$_{50}$ values in µg/mL. N. mirabilis = 73.66 ± 10.18; P. racemosa = 46.70 ± 23.66; K. candel = 7.66 ± 0.90

Figure 4. High-resolution α-glucosidase biochromatogram of water extract of P. amarus (a) and P. urinaria (b)

Figure 5. HSQC spectrum of 1a and structure of corilagin.