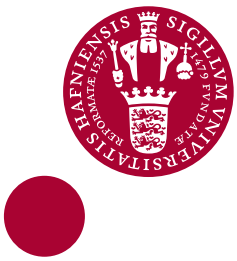

High-resolution assays combined with HPLC for identification of antidiabetic constituents in Vietnamese plants

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Publication date:
2015

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

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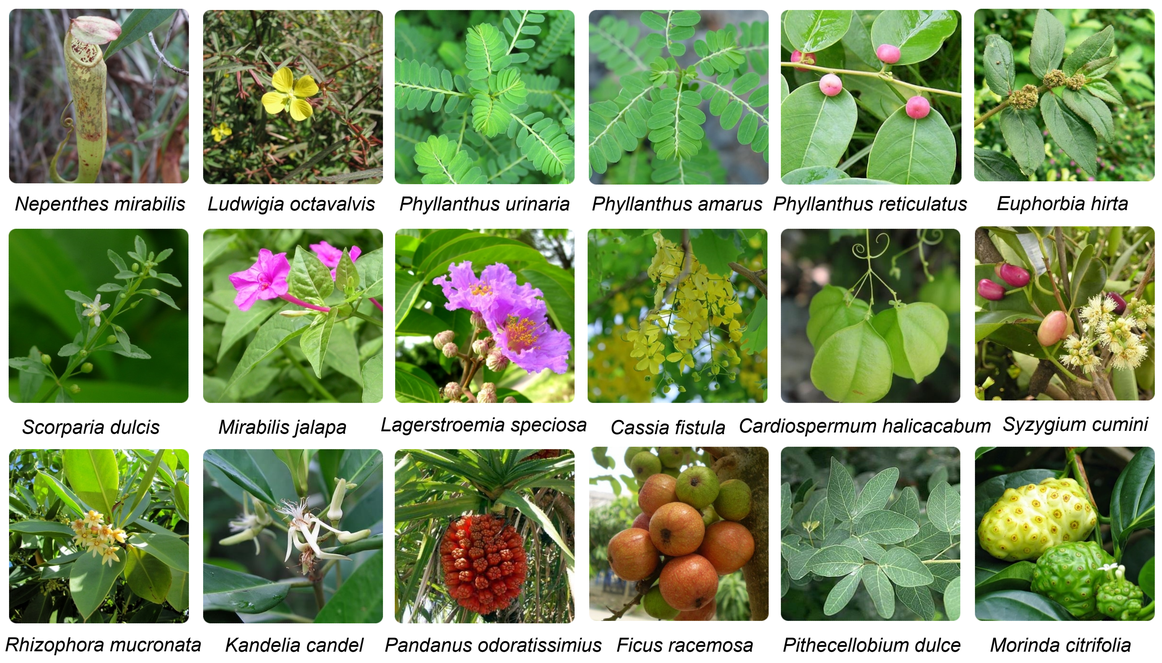
BACKGROUND

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.



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[1] and α-amylase[2] 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.

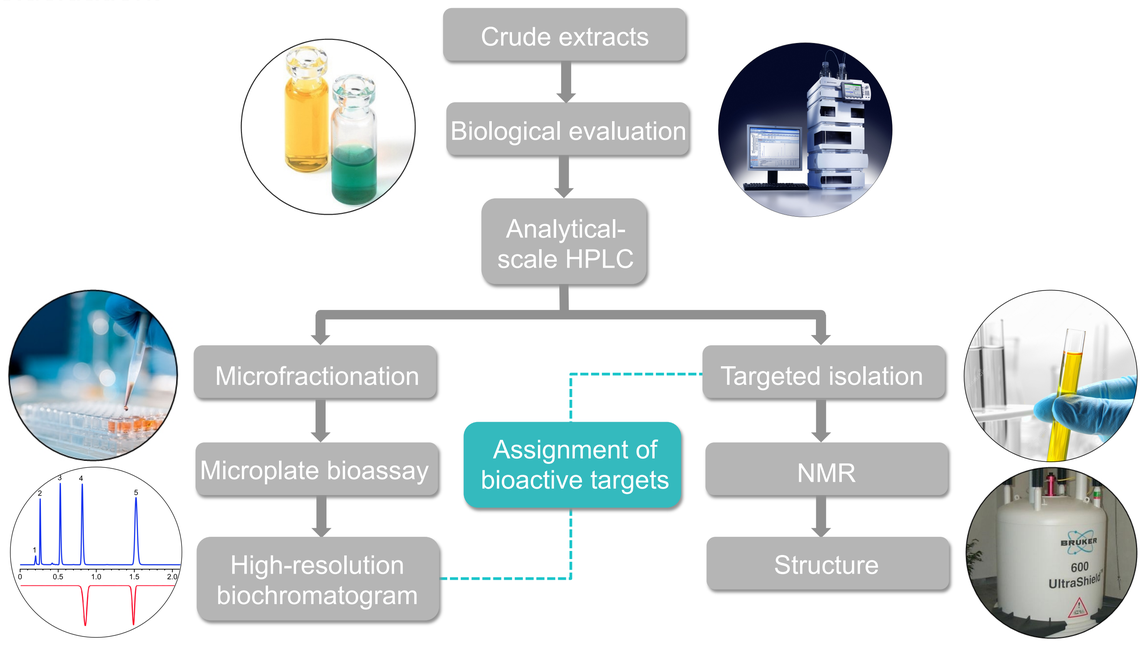


Figure 1. Flowchart of the procedure used in this work

BIOLOGICAL EVALUATION

Ethanol and water extracts of *P. amarus*, *P. urinaria*, *L. speciosa*, *N. mirabilis, S. cumini*, *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.

α-Glucosidase inhibition assay

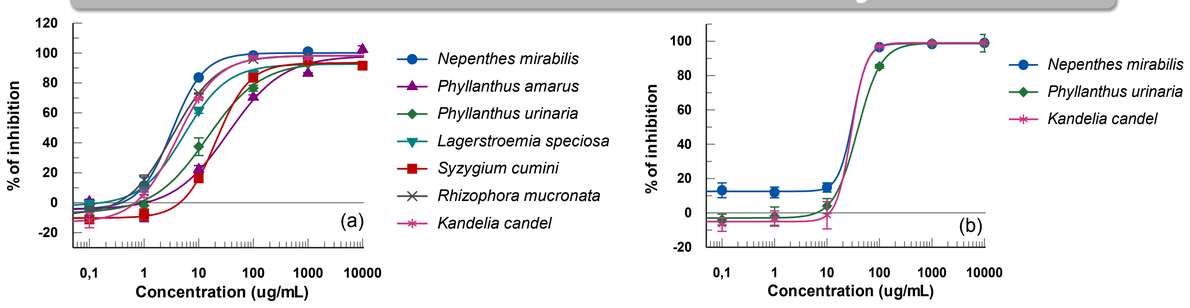


Figure 2. IC_{50} curves of extracts. (a) Water extracts, IC_{50} values in μg/mL: *N. mirabilis* = 3.31 ± 0.77, *P. amarus* = 34.92 ± 1.52, *P. urinaria* = 14.64 ± 4.56, *L. speciosa* = 5.39 ± 0.54, *S. cumini* = 20.93 ± 1.77, *R. mucronata* = 3.32 ± 0.55, *K. candel* = 3.99 ± 0.75 (b) Ethanol extracts, IC_{50} values in μg/mL: *N. mirabilis* = 32.70 ± 6.33; *P. urinaria* = 39.72 ± 9.73; *K. candel* = 35.38 ± 13.93

α-Amylase inhibition assay

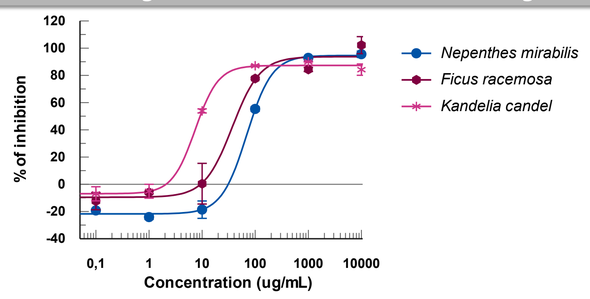


Figure 3. IC_{50} curves of ethanol extracts. IC_{50} values in μg/mL: *N. mirabilis* = 73.66 ± 10.18; *F. racemosa* = 46.70 ± 23.60; *K. candel* = 7.66 ± 0.90

PERSPECTIVE AND FUTURE WORK

Biochromtograms 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

[1] Schmidt JS et al. *Food Chem* **2012**; *135*: 1692-99; [2] Okutan L et al. *J Agric Food Chem* **2014**; *62*: 11465-71; [3] Sudjaroen Y et al. *Phytochemistry* **2012**; *77*: 226-237

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

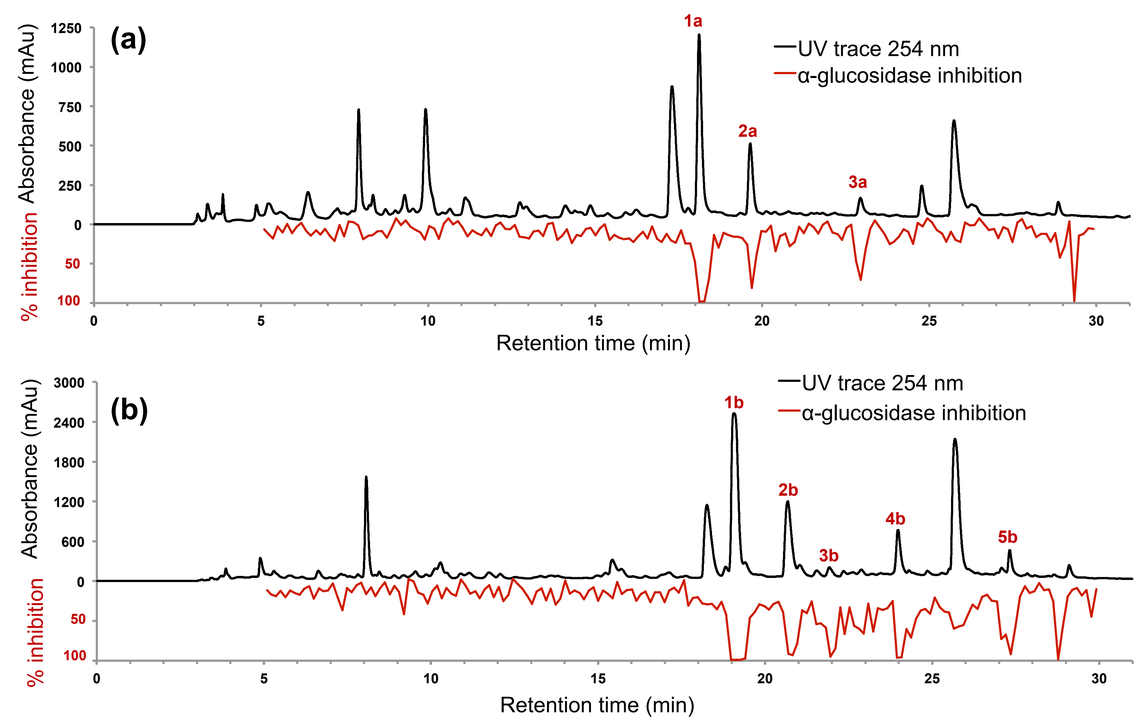


Figure 4. High-resolution α-glucosidase biochromatogram of water extract of *P. amarus* (a) and *P. urinaria* (b)

ISOLATION AND STRUCTURE ELUCIDATION

Peak **1a-3a** and **1b-5b** in chromatograms of *P. amarus* and *P. urinaria* were isolated and purified. The structures of **1a** and **1b** were identified as corilagin[3].

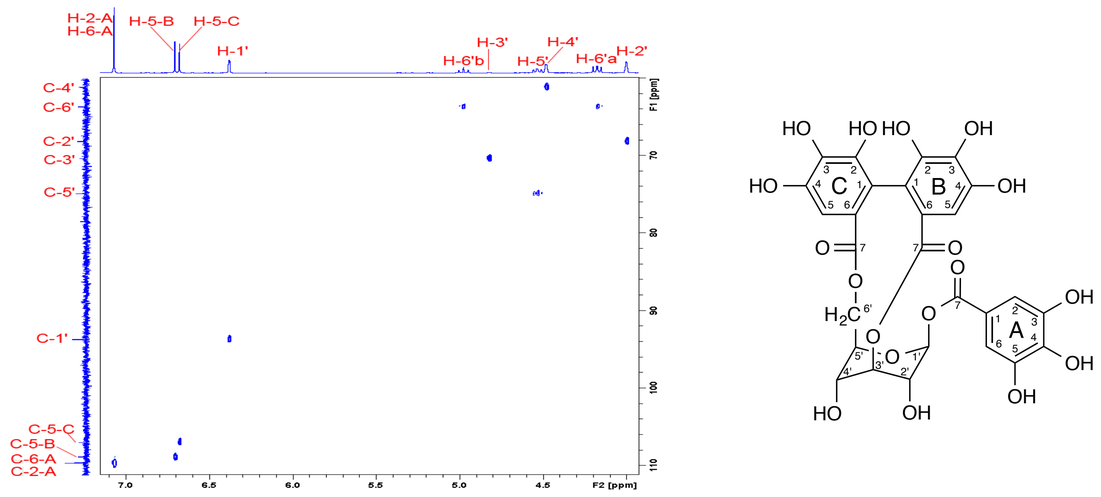


Figure 5. HSQC spectrum of **1a** and structure of corilagin