Exploring Weed Extracts for β-Glucosidase Inhibitors: Screening Secondary Metabolites through Soxhlet Extraction and TLC Bioautography

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Background

Weeds are plants that are either considered a nuisance to humans or have a detrimental impact on native plant species. Despite their abundance and adaptability, these plants are often overlooked in phytochemical research, even though they, offer a diverse array of compounds and medicinal potential.[1] Our research aims to use a technique called bioautography to help us identify β-glucosidase inhibitors in weed extracts that may be suitable for therapeutic use.[2] β-glucosidase, which is an enzyme present in organs such as the liver, spleen, small intestine, and kidneys, breaks down complex sugars into simpler sugars, such as glucose, for absorption into the bloodstream.[3] Inhibiting β-glucosidase function could lead to a reduction in high blood sugar levels, potentially offering therapeutic benefits for conditions like diabetes, HIV, cancers, and lysosomal storage disorders.[4] Therefore, identifying new β-glucosidase inhibitors can pave the way for advancing treatments for these disorders.

Key terms: β-glucosidase, Bioautography, TLC, Soxhlet Extraction, phytochemicals

β-glucosidase

Enzymes play a key role in regulating biological processes and pathways in our bodies and thus are prime targets for drug development. Targeting these pathways through enzymes offers specificity, minimizing potential side effects; additionally, these enzymes typically contain well-defined active sites where small molecules interact, making them appealing pharmacological targets.

β-glucosidase enzymes contain glutamate residues in their active site. The glutamate residue acts as a nucleophile that attacks the glycosidic bond (glycosylation) of a carbohydrate, forming an intermediate, which, in the presence of water, undergoes a proton transfer reaction, releasing the glucose residue from the glutamate (deglycosylation).[5] So, this is how it breaks down complex sugars. One way we can help reduce high blood sugar is right here by inhibiting the activity of β-glucosidase and reducing the release of glucose

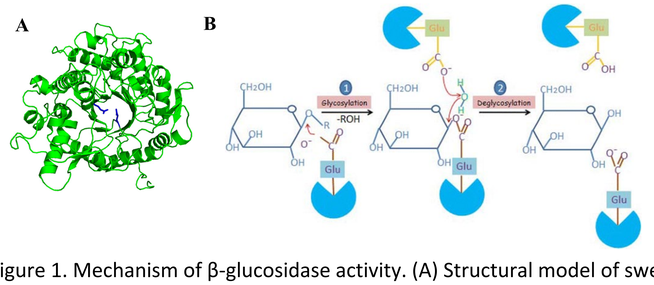


Figure 1. Mechanism of β-glucosidase activity. (A) Structural model of sweet almond β-glucosidase. (B) Schematic of β-glucosidase activity and function.[3]

Central questions:

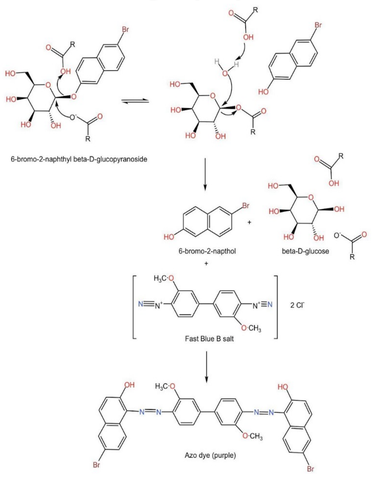
- Do any of the weed extracts in our library contain potential β-glucosidase inhibitors?
- Does polarity-based separation techniques through Soxhlet extraction and bioautography aid in identifying inhibitors in a fast and efficient way?

Methods

Extraction Preparation

The air-dried plants were ground, then sequentially subjected to Soxhlet extraction for 72- hours using organic solvents with varying polarity (methanol, ethyl acetate, and hexanes) to produce the initial crude extracts. Solvents were removed using rotary evaporation and, if not immediately used, the extracts were subject to lyophilization and stored at -20°C.

Bioautography



Bioautography combines thin-layer chromatography (TLC) with a colorimetric enzymatic assay (figure). After separating compounds on the TLC plate, the plate is dried, sprayed sequentially with substrate, fast blue B solution, and then a solution of β-glucosidase enzyme. This is followed by incubation at 37°C for 10-20 mins to observe inhibition as a colorless spot on a purple/ blue background.

Figure 2. Reaction mechanism of β-glucosidase enzyme activity and detection. β-glucosidase breaks down the substrate (6-bromo-naphthyl β-D-glucopyranoside) and the released naphthol reacts with fast B blue to create the purple azo dye.

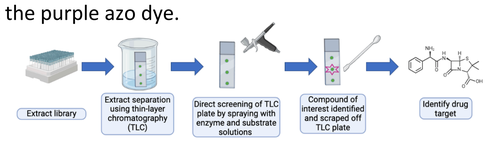


Figure 3. Flowchart illustrating the bioautography process.

Results

Initial Detection

An initial screening of 45 weed extracts identified potential enzyme inhibitors in purple loosestrife (PL) extracts, sow thistle(St), woolly burdock(WB), Japanese knotweed (JK), vetch (V) and yellow iris indicated by colourless regions. Some other colourations, such as yellow, green or brown, may result from compounds in the extract undergoing a side reaction with fast blue b. In these cases, secondary screening is necessary.

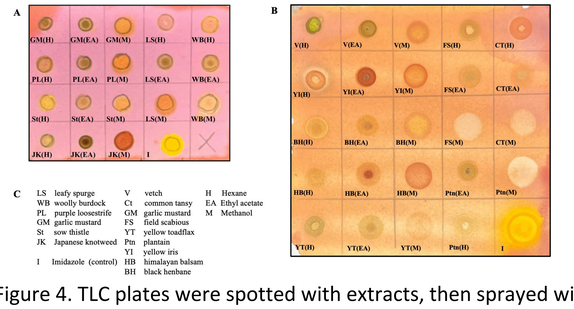
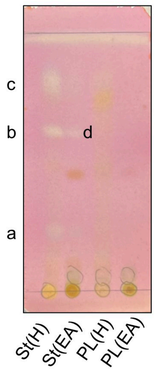


Figure 4. TLC plates were spotted with extracts, then sprayed with solutions of substrate (6-bromo-naphthyl β-D-glucopyranoside), Fast-Blue B, and β-glucosidase, respectively. Inhibition was observed as colourless spots (panels A and B), the key to the 45 weeds and abbreviations (panel c).

Purple Loosestrife and Sow Thistle



The next step involved further separating and characterizing the potential inhibitors based on polarity. This is necessary because these extracts contain numerous compounds, requiring identifying and isolating those compounds responsible for the inhibition.

Figure 5. A TLC plate was spotted with extracts of purple loosestrife(PL) and sow thistle (St) from the ethyl acetate (EA) and hexane (H) extractions, and then eluted using a 9:1; H:EA solvent system. After drying, the plate was sprayed with solutions of substrate (6-bromo-naphthyl β-D-glucopyranoside), Fast Blue B, and β-glucosidase, respectively. Inhibition was observed as colourless spots. β-glucosidase Inhibition was detected in spots a, b, c and d with RF values of 0.24, 0.64, 0.82, and 0.64, respectively.

Woolly burdock and Japanese Knotweed

Bioautography of woolly burdock (WB) and Japanese knotweed (JK) using hexane(H) and ethyl acetate (EA) extracts in a 9:1 hexane to ethyl acetate elution solution reveals five potential β-glucosidase inhibitors

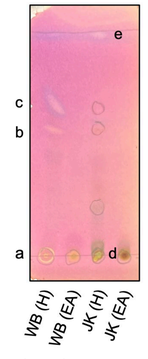


Figure 6. The TLC plate was spotted with extracts of woolly burdock(WB) and Japanese knotweed(JK) from the ethyl acetate (EA) and hexane (H) extractions and then eluted using a 9:1; H: EA solvent system. After drying, the plate was sprayed with solutions of substrate (6-bromo-naphthyl β-D-glucopyranoside), Fast Blue B, and β-glucosidase, respectively. Inhibition was observed as colourless spots. β-glucosidase Inhibition was detected in spots a, b, c, d and e with RF values of 0.0, 0.56, 0.65, 0.0, and 0.95, respectively.

Vetch and Yellow Iris

Bioautography of Vetch (V) and Yellow Iris (YI) using hexane(H) and ethyl acetate(EA) Soxhlet extracts in a 15:1 hexane to ethyl acetate elution solution reveals three potential β-glucosidase inhibitors.

Figure 7. TLC plate was spotted with extracts of Vetch (V) and Yellow Iris (YI) from the ethyl acetate (EA) and hexane (H) extractions, and then eluted using a 15:1; H:EA solvent system. After drying, the plate was sprayed with solutions of substrate (6-bromo-naphthyl β-D-glucopyranoside), Fast Blue B, and β-glucosidase, respectively. β-glucosidase Inhibition was detected as colourless spots labelled a,b and c with RF values of 0.0,0.84, and 0.0 respectively.

Conclusions

Conclusion

All 15 plants, with the exception of garlic mustard, were suspected to have some inhibitory effects towards β-glucosidase following initial detection. However, apart from St, WB , JK ,V , and YI , the number of potential inhibitors in each plant extract and characterization of the suspected compounds remained unexplored. The bioautography process was deemed to be a quick and relatively simple process for the initial screening of extracts in large sample sizes.

Future directions

- Column chromatography allows for scalability, enabling the purification of larger sample volumes and consequently increasing the concentration of the target compounds.
- NMR and MS will be used to determine the structure and purity of the unknown compound(s).
- Enzyme kinetic assays will follow the isolation of compounds to allow for confirmation of enzyme inhibition and analysis of the enzyme activity in real time.

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References

[1]Máximo, P.; Ferreira, L. M.; Branco, P. S.; Lourenço, A. Invasive Plants: Turning Enemies into Value. Molecules 2020, 25 (15), 3529. DOI:10.3390/molecules25153529.
[2] Simões-Pires, C. A.; Hmicha, B.; Marston, A.; Hostettmann, K. A TLC Bioautographic Method for the Detection of α- and β-Glucosidase Inhibitors in Plant Extracts. Phytochemical Analysis 1990, 20 (6), 511–515. DOI:10.1002/pca.1154.
[3]Day, A. J.; DuPont, M. S.; Ridley, S.; Rhodes, M.; Rhodes, M. J. C.; Morgan, M. R. A.; Williamson, G. Deglycosylation of Flavonoid and Isoflavonoid Glycosides by Human Small Intestine and Liver β-Glucosidase Activity. FEBS Letters 1998, 436 (1), 71–75. DOI:10.1016/s0014-5793(98)01101-6. 3.
[4]Panwar, H.; Calderwood, D.; Grant, I. R.; Grover, S.; Green, B. D. Lactobacillus Strains Isolated from Infant Faeces Possess Potent Inhibitory Activity against Intestinal Alpha- and Beta-Glucosidases Suggesting Anti-Diabetic Potential. European Journal of Nutrition 2014, 53 (7), 1465–1474. DOI:10.1007/s00394-013-0649-9.
[5]Singh, G.; Verma, A. K.; Kumar, V. Catalytic Properties, Functional Attributes and Industrial Applications of β-Glucosidases. 3 Biotech 2015, 6 (1). DOI:10.1007/s13205-015-0328-z.