**Flavonoids as dual-target inhibitors against alpha-glucosidase and alpha-amylase: a systematic review of *in vitro* research protocol**

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**INTRODUCTION**

Diabetes mellitus is a chronic metabolic disorder associated with the persistent elevation of blood glucose caused by endocrine system impairments. Type 2 diabetes mellitus (T2DM) is notorious for its threatening complications including microvascular and macrovascular conditions, leading to retinopathy, nephropathy, neuropathy, coronary heart disease, cerebrovascular disease, and peripheral vascular diseases. According to the 2021 International Diabetes Federation report, the number of fatalities caused by diabetes is estimated up to 6.7 million globally, placing a significant burden on society.1 Over the past 15 years, the cost of treating diabetes has increased by 316%, to at least 966 billion USD.1,2 Therefore, managing diabetes while preventing complications has become a pressing concern for medical professionals.

Over decades, evidence has accumulated supporting the principal therapy strategy of maintaining blood glucose levels within normal range.3,4 Since T2DM considered a complicated disease, diverse pharmacological agents (sulfonylureas, meglitinides, thiazolidinediones, and metformin) acting on several targets are available in conjunction with lifestyle changes. Alpha-glucosidase inhibitor agents (acarbose, miglitol, voglibose) became available in clinics as a prime method for suppressing starch digestion by alpha-amylase and alpha-glucosidase enzymes. Consequently, the amount of glucose entering blood vessels decreased, reducing postprandial glucose levels. These medications show substantial inhibition against intestinal alpha-glucosidases and modest inhibition against pancreatic alpha-amylase. Unmetabolized starch is subsequently transported to the large intestine, where it is digested by bacterial enzymes, producing gases and bloating, causing adverse effects such as flatulence, abdominal distention, and diarrhea.5 Therefore, compounds with strong inhibitory activity against alpha-glucosidase but limited inhibitory activity against alpha-amylase may improve clinical outcomes by reducing the side effects of carbohydrates catabolism in the colon.5

Flavonoids are naturally phenolic compounds composed of a fifteen-carbon skeleton (C6-C3-C6) consisting of two benzene rings (A and B rings) linked through a heterocyclic pyran ring (C ring). Numerous studies have demonstrated that flavonoids have alpha-amylase or alpha-glucosidase inhibitory properties.6-8 Additionally, some flavonoids with stronger inhibitory effects against alpha-glucosidase than alpha-amylase (such as daidzein) could be of significant relevance to attenuating gastrointestinal adverse effects.9,10

The overview of flavonoids having the ability to inhibit both enzymes, however, is not supported by sufficient data. For this purpose, this systematic review provides a small appraisal on T2D and the available flavonoids with a focus on their simultaneous inhibition of alpha-amylase and alpha-glucosidase activity.

**METHODS**

**Eligibility criteria**

This review will include original *in vitro* studies involving the inhibitory effects of purified flavonoids on alpha-amylase or alpha-glucosidase. The language will be restricted to Vietnamese and English only. Editorials, case reports, reviews, systematic reviews, abstract-only articles, and unpublished papers will be excluded. We will also eliminate pure *in silico*, *in vivo*, *ex vivo,* or clinical studies. Studies without concurrent positive and negative samples, those without a clear method description that could produce unreliable extracted data, will not meet our criteria. With regard to outcome, we will include articles that provided IC50 results.

**Information sources**

The following electronic databases will be used in the systematic review:

* PubMed <https://pubmed.ncbi.nlm.nih.gov/>
* Virtual health library (VHL) <https://bvsalud.org/en/>
* EMBASE <https://www.embase.com>
* SCOPUS <https://www.scopus.com/>
* Web Of Science (WOS) <https://www.webofknowledge.com>
* WHO Global Index Medicus (GIM) <https://www.globalindexmedicus.net/>

**Search strategy:**

In this systematic review, the following terms will be used and customized to fit on the corresponding electronic databases: (alpha-glucosidase OR maltase OR sucrase OR alpha-amylase) AND (flavonoid OR flavonoids).

**Data management, screening and extraction**

***Data selection process:***

Our study will be conducted following The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.11 In this study, we will use the online systematic review software Rayyan (https://rayyan.ai)12 to screen the titles, abstracts and full texts. Our study selection process consists of three main parts, including duplicate removal, title and abstract screen, and full-text screening.

Due to the great amount of articles retrieved from six different sources, the initial database will be split into three parts. After duplicate removal utilizing the Zotero tool, six review authors, who work in pairs of two, will screen the title and abstract for fitting articles. Further full-text screening will be conducted by three pairs of reviewers to decide whether the article would be included or excluded. Prior mentioned reviewers will work independently throughout the screening process. On the condition that disagreement occurred, discussion and consensus would be conducted, and even a third reviewer opinion will be consulted to arrive at a final conclusion. The summary of the screening results will be plot graphically in a PRISMA flow chart. In addition, the reasons for full-text exclusion will also be mentioned in the flow chart.

***Data collection process***

Following information will be collected from each study, of which, the extracted data will include 3 parts:

* The first part: Study characteristics, including corresponding journal, title, authors, country of research conduction and publication year.
* The second part: Study design (assay protocol, types of enzymes), sample size and inclusion and exclusion criteria.
* The final part: Outcome of the study. Main outcomes were sought including the structures of amylase/glucosidase inhibitors with their inhibitory efficiency.

**Outcomes and prioritization**

Flavonoid compounds and derivatives that have concurrently inhibitory activity against human alpha-glucosidase and alpha-amylase will be categorized based on their respective flavonoid subclasses. The structures of those compounds will be visualized using ChemDraw/BIOVIA draw software. Results for alpha-amylase and alpha-glucosidase inhibitory activity will be presented as half maximal inhibitory concentration (IC50).

**Meta-biased (Quality assessment)**

A modified version of CONSORT checklist developed by Faggion13 will be used in the quality assessment process. The checklist consists of 14 items regarding the assessment of abstract, introduction, method, results, discussion, and other information to interpret the quality of the research studies.

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