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Research Article

Comparative assessment of human salivary α-amylase inhibitor from Indonesian herbs

Khomaini Hasan[1], Angga Himas Setyawan[1], Muhamad Abidin[2], Nurul Aida Fathya[1], Dewi Ratih Handayani[1], Desy Linasari[1], Saronom Silaban[3], Toto Subroto[2], and Muhammad Yusuf[2]

[1]Faculty of Medicine, Universitas Jenderal Achmad Yani, Jalan Terusan Jenderal Sudirman, PO Box 148, Cimahi, West Java, Indonesia
[2]Pusat Riset Bioteknologi Molekular dan Bioinformatika, Universitas Padjadjaran, Jalan Singaperbangsa 2, Bandung, West Java, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan 20221, Indonesia

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Corresponding author:
E-mail: k.hasan@lecture.unjani.ac.id
(Khomaini Hasan)

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Abstract

Diabetes mellitus (DM) is a multifactorial disease defined by persistent hyperglycemia and impaired carbohydrate, lipid, and protein metabolism due to a lack of insulin secretion. The objective of this study was to assess the Indonesian herbs which have the ability to act as human salivary α-amylase inhibitors, thus, that they can be implemented for medical purposes. In this study, fifteen Indonesian herbs were assessed for their capability as α-amylase inhibitor. The water-reflux method was used to extract all potential water-soluble active components. The Fuwa technique was used to test α-amylase activity. There were significant variations in the effects of herbs on salivary α-amylase activity, according to the statistical using one-way ANOVA and Post Hoc Tukey between the with and without inhibitors. According to the findings, Turmeric (*Curcuma longa* L.) and pandan leaf (*Pandanus amaryllifolius* Roxb) had the highest inhibitory power (80%).

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism due to insufficient insulin secretion (Frederico et al. 2016). Type 1 DM is caused by autoimmune destruction of β-pancreatic cells, while type 2 DM Type 2 is caused by impaired insulin secretion due to the dysfunction of insulin receptors on Glucose Transporter 4 (GLUT 4), which leads to disturbances in glucose uptake into the cells (Deka et al. 2022).

The highest prevalence of diabetes is found in type 2 due to insulin resistance in pancreatic β-cells, resulting in hyperinsulinemia (Guillén and Benito, 2018). The high levels of insulin are closely related to obesity. Increased blood glucose levels also contribute to insulin resistance (Wang et al. 2021). Decreasing blood glucose levels can be controlled by increasing glucose entry into the bloodstream or by inhibiting the action of carbohydrate-hydrolyzing enzymes, thereby reducing glucose absorption, such as with acarbose (Bhatia et al. 2019).

The management of DM involves increasing glucose entry into the bloodstream or inhibiting enzymes involved in carbohydrate hydrolysis (Sahiner et al. 2018). The enzyme α-amylase plays a crucial role in carbohydrate digestion (Marghich et al. 2022). Inhibiting α-amylase enzyme slows down carbohydrate digestion, while inhibiting α-glucosidase enzyme reduces glucose absorption (Pérez-Nájera et al. 2018).

Flavonoids, found in various herb extracts, have been found to inhibit a-amylase activity and have anti-diabetic properties. These properties include increasing insulin sensitivity, reducing glucose absorption, stimulating insulin secretion, improving glucose tolerance, and decreasing glucose uptake in carbohydrate metabolism. Indonesian herbs, such as Green Betel Leaf, Bay Leaf, Pandan Leaf, Kaffir Lime Leaf, Cinnamon

Bark, Turmeric, fresh ginger, Tamarind, Dragon Scale Fern, Red Beans, Guava Leaf, Papaya Leaf, Mexican Sunflower Leaf, Jackfruit Leaf, and Avocado Leaf, contain bioactive compounds that contribute to their medicinal properties. These findings highlight the diverse bioactive compounds present in Indonesian plant leaves, underscoring their potential for various medicinal applications (Frederico et al. 2016; Omuketi, 2020; Agustina et al. 2023).

Some of the major active components in fresh ginger include zingerone, gingerols, shogaols, paradols, and gingerdione. The diverse bioactive compounds present in Indonesian plant leaves underline their potential for various medicinal applications.In this study, fifteen herbs (*P. betle* linn, *S. polyanthum*, *P. amaryllifolius* Roxb, *C. hystrix* D.C, *C. hurmannii*, *C. Longa* L, *Z. officinale* Rosc, *T. indica* L, *P. piloselloid*, *P. vulgaris* L., *P. guajava* L., *C. papaya* L., *T. diversifolia*, *A. integra* Merr., *P. americana* Mill) containing flavonoids and other active compounds were assessed for their characteristic as human salivary α-amylase inhibitor. The goal of this study was to evaluate Indonesian herbs that can act as human salivary α-amylase inhibitors, allowing them to be used for medical purposes. All these herbs are readily available and commonly used by Indonesians.

Method

The ethical clearance of this study was approved by Ethical Commission for Research, Padjadjaran University, with ethical approval number 1342/UN6.KEP/EC/2018.

Materials

The materials used in this study included α-amylase obtained from human saliva, extract *P. betle* linn, *S. polyanthum*, *P. amaryllifolius* Roxb, *C. hystrix* D.C, *C. hurmannii*, *C. Longa* L, *Z. officinale* Rosc, T. indica L, *P. piloselloid*, *P. vulgaris* L., *P. guajava* L., *C. papaya* L., *T. diversifolia*, *A. integra* Merr., *P. americana* Mill, soluble starch, iodine solution, HCl, distilled water, Bradford reagent, BSA (Bovine Serum Albumin), alcohol, and ethylene diamine tetraacetate.

Saliva Collection

Saliva sampling was conducted using specially prepared sample tubes, with each participant providing 10 mL of saliva. The spitting method, renowned for its simplicity and ability to yield a substantial saliva volume, was employed for collection purposes. Participants were instructed to observe an approximate one-hour fasting period prior to collection, followed by a comfortable five-minute sitting period and mouth rinsing using distilled water. After a one-minute period of collecting saliva with closed mouths and open eyes, participants were instructed to expectorate it into a designated glass or collection tube.

The Preparation of the Herbal Extract

The extraction of fifteen herbs was carried out using the reflux method. Hundred grams of finely chopped leaves or rhizomes were added to a round-bottom flask and 100 ml of water. The reflux apparatus was assembled, and the samples were subjected to extraction at 75°C for 2 hours. The solution was filtered using sterilized gauze and pre-sterilized filter paper before being transferred into an Erlenmeyer flask.

Enzyme Activity

The activity of α-amylase enzyme was determined using the Fuwa method (Fuwa, 1954) as follows: 50 µL of saliva was added to 50 µL of distilled water, and 100 µL of 0.1% starch solution, and incubated at 37°C for 30 minutes. The reaction was stopped by adding 100 µL of 1M HCl, followed by the addition of 100 µL of iodine solution and 1.6 mL of distilled water into a cuvette. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm. For inhibition test, the 50 µL of distilled water was replaced by herb extract. All samples were tested triplicates.

Protein Concentration Assay

Protein concentration of human salivary α-amylase was determined by Bradford assay using bovine serum albumin (BSA) as a standard. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 595 nm.

Data Analysis

The specific activity of human salivary α-amylase in the presence of the herb extract was analyzed using univariable analysis. Human salivary α-amylase activity data underwent a normality test, and if the p-value was >0.05, the data were in the normal distribution. An ANOVA test, Kruskall-Wallis, Post hoc, and Mann Whitney tests were employed to investigate the effect of herb extracts on the specific activity of human salivary α-amylase. The SPSS program (Windows version 18.0) was used to analyze the data with a 95% confidence level and a 0.05 significance level.

Results and Discussion

The participants in this study were Jenderal Achmad Yani University students whose saliva was collected and who complied with the inclusion and exclusion standards. They had already completed the preliminary screening step a history of a systemic condition, specifically diabetes mellitus, and the subsequent screening step, which identified damage to the morphology of the salivary gland because it reflected on the salivary flow rate. The effect of fifteen herb extracts on human saliva α-amylase is presented in Fig.-1.

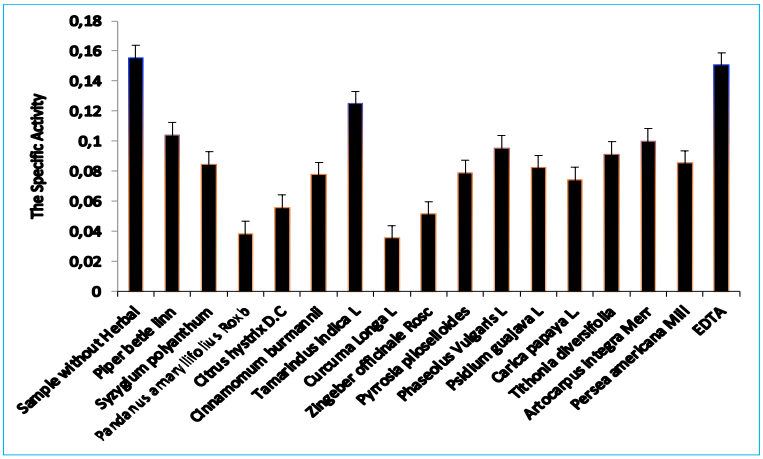

Fig.-1. Specific activity comparison of human salivary α-amylase in the presence and the absence of herb extract.

As shown in Fig.-1, human salivary α-amylase activity was 0.15 U.mg-1 in the absence of herbs, corresponding to 100% α-amylase activity without inhibition. All the herbs examined demonstrated inhibitory action against human salivary α-amylase. The lowest specific activity of α-amylase was observed in the presence of *C. longa* L. and *P. amaryllifolius* Roxb., with a specific activity of 0.04 U.mg-1. The α-amylase activity in the presence of *C. hystrix* D.C., *Z. officinale* Rosc., *P. piloselloides*, and *C. papaya* L. was retained at 0.05-0.07 U.mg-1.

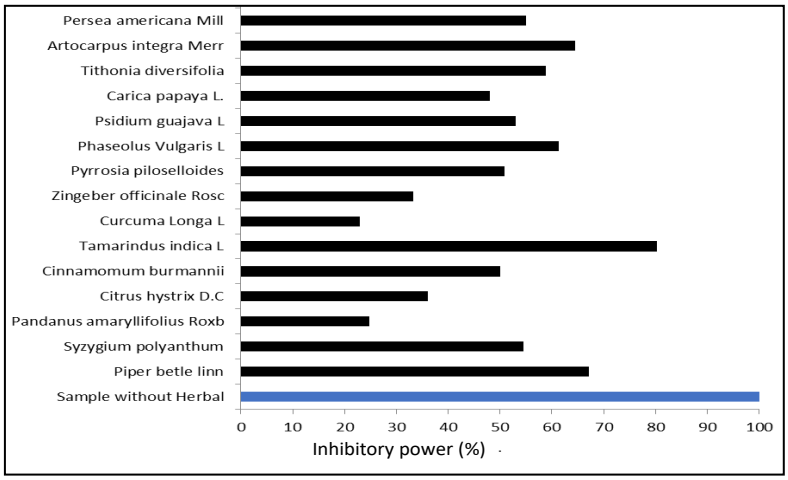

Fig.-2. Inhibitory comparison of fifteen herbs against human salivary α-amylase. One hundred percent α-amylase activity (blue bar) was 0.15 U.mg-1.

To determine the indicator of inhibition, the group of extracts without herbal additives was used, which represents the α-amylase activity without any herbs (Fig.-2). The percentage of remaining α-amylase activity ranges from 22% to 80%, indicating the inhibitory power of each herb against α-amylase activity. The data distribution of the specific activity of human salivary α-amylase was normalized using the Shapiro-Wilk test. The sample that did not contain any herbs got the highest average, 0.155, which was considered a 100% remaining activity. The highest α-amylase inhibitor of herbs was *C. longa* and *P. amaryllifolius*, with a residual activator of approximately 22% and a p-value of 0.001 ($p<0.05$). The one-way ANOVA findings demonstrated that two herbs significantly affected human salivary α-amylase inhibitors (Table 1). The Post-hoc Tukey testing supports the One-Way ANOVA (Table 2).

Tabel 1. ANOVA analysis of treatment samples

Treatment	Human Salivary α-amylase ± Standard deviation	*p*
Without herbal	0.155±0.026	
Piper betle L.	0.104±0.018	
Syzygium polyanthum	0.084±0.017	
Pandanus amaryllifolius	0.038±0.024	
Citrus hystrix	0.056±0.026	
Cinnamomum verum	0.077±0.024	
Tamarindus indica	0.124±0.023	
Curcuma longa	0.035±0.017	<0.001*
Zingiber officinale	0.051±0.016	
Pyrosia piloselloides L.	0.079±0.022	
Paseolus vulgaris L.	0.095±0.024	
Psidium guajava L.	0.082±0.024	
Carica papaya L.	0.074±0.015	
Tithonia diversifolia	0.091±0.026	
Artocarpus integra Merr.	0.100±0.015	
Persea americana Mill.	0.085±0.030	

**) if p<0.05 is significance difference*

According to finding, several herbs have the potential as a significant blocker for α-amylase inhibitors. Among all herbs tested, *C. longa* L. and *P. amaryllifolius* Roxb. demonstrated as the strongest α-amylase inhibitor. Nonproteinaceous inhibitors include a variety of chemical substances, including acarbose, isoacarbose, acarviosine-glucose, hibiscus acid, and cyclodextrins. There are several natural compounds that have been reported to have alpha-amylase inhibitory activity. These include Curcumin, Berberine, Docosanol, 16-H, Actinodaphnine/Tetracosanol, Catechin, and Quercetin (Jhong et al. 2015). Common beans also have 3 isoforms of alpha-amylase inhibitor (alpha-A1, alpha-A12, alpha-AIL) with the alpha-AI isoform having anti-amylase activity in humans (Barrett and Udani, 2011). In this study, all herbs tested are demonstrated human salivary a-amylase inhibitory. Water-extract of *C. longa* L. and *P. amaryllifolius* Roxb are potent against human salivary -amylase. These compounds' inhibitory effect against a-amylases occurs due to their cyclic structures, which resemble α-amylase substrates, and thus bind to α-amylase catalytic sites.

Curcumin is the yellow-colored bioactive constituent of the perennial plant, *C. longa* L., which possesses a wide range of physiological and pharmacological properties such as antioxidant, anti-inflammatory, anticancer, neuroprotective and anti-diabetic activities (Nabavi et al. 2015). A systematic review of the effects of *C. longa* L. or curcumin on diabetes mellitus found that curcumin's anti-diabetic activity might be due to its capacity to suppress oxidative stress and inflammatory process. It also significantly reduces fasting blood glucose, glycated hemoglobin, and body mass index (Marton et al. 2021). C. longa also contains a compound called Bis-demethoxycurcumin (BDMC) which has been reported to inhibit human pancreatic α-amylase, a therapeutic target for oral hypoglycemic agents in type-2 diabetes (Ponnusamy et al. 2013). The mode of inhibition as determined kinetically is uncompetitive. BDMC inactivates HPA with a stoichiometry of 1:1 and an apparent Ki of 3.0 μM. Bisdemethoxycurcumin (BDMC) is a curcuminoid found in turmeric (*Curcuma longa*), along with other curcuminoids such as curcumin and demethoxycurcumin (Ponnusamy et al. 2012). BDMC is used as a

pigment and nutraceutical with antimutagenic properties. It also possesses anti-inflammatory properties similar to demethoxycurcumin (Aquila et al. 2019; Choi et al. 2019; Li et al. 2019; Gouthamchandra et al. 2021).

Table 2. Comparison analysis between herbs-treatment and without herb samples by Post-hoc Tukey

Treatment (I)	Treatment (J)	*p*
	Piper betle L.	0.338
	Syzygium polyanthum	0.036*
	Pandanus amaryllifolius	<0.001*
	Citrus hystrix	<0.001*
	Cinnamomum verum	0.013*
	Tamarindus indica	0.951
	Curcuma longa	<0.001*
	Zingiber officinale	<0.001*
	Pyrosia piloselloides L.	0.016*
	Paseolus vulgaris L.	0.136
	Psidium guajava L.	0.026*
	Carica papaya L.	0.008*
	Tithonia diversifolia	0.084
	Artocarpus integra Merr.	0.229
	Persea americana Mill.	0.039*

**) if p<0.05 is significance difference compare with sample without herbs*

P. amaryllifolius is a tropical plant in the Pandanus (screwpine) genus, commonly known as pandan. The characteristic aroma of pandan is caused by the aroma compound 2-acetyl-1-pyrroline. P. amaryllifolius is a tropical plant in the Pandanus (screwpine) genus, commonly known as pandan (Setyaningsih et al. 2019; Diyana et al. 2021). *P. amaryllifolius* leaves are used in traditional medicine for the treatment of diabetes (Liaotrakoon et al. 2021). A study evaluated the effect of crude extract from *P. amaryllifolius* leaves on blood glucose level and found that it had antihyperglycemic effects (Reshidan et al. 2019).

The effectiveness of herbs as α-amylase inhibitors can vary depending on the specific compound and the individual. Some natural compounds have been shown to have alpha-amylase inhibitory activity in laboratory studies, but their effectiveness in humans has not been fully established. It is important to note that while natural remedies may have potential health benefits, they should not be used as a substitute for conventional medical treatment.

Conclusion

Taken together, it can be concluded that *C. longa* L. and *P. amaryllifolius* Roxb. are potential α-amylase inhibitors. These findings also highlight the potential of herbs as inhibitors for α-amylase and their potential role in preventing diabetes mellitus.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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