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Research Article
Influence of Harvest Age, Extraction Method and Solvent Polarity on the Enzyme Inhibitory Activity of *Orthosiphon aristatus* Extracts

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Abstract

Background and Objective: The rising prevalence of diabetes mellitus in Indonesia highlights the urgent need for effective, natural therapeutic options. This study evaluates the antidiabetic potential of *Orthosiphon aristatus* (Blume) Miq. (cat's whiskers) by analyzing the effects of harvest age, extraction method and solvent polarity on its ability to inhibit α-amylase and α-glucosidase enzymes-key targets in diabetes management. **Materials and Methods:** Tissue-cultured *O. aristatus* plants harvested at 6 and 9 months were subjected to three extraction methods: Ultrasonic-assisted, maceration and infusion, using solvents of varying polarity (water, 30 and 70% ethanol). Extract yields and inhibitory activities were assessed and IC values were determined using standard *in vitro* enzyme inhibition assays. Data were analyzed statistically to compare extraction efficiency and biological activity. **Results:** Ultrasonic extraction with 30% ethanol from 9-month-old plants produced the most effective extract, yielding 10% extract with strong inhibitory activity: IC values of 64.35 ppm (α-amylase) and 64.68 ppm (α-glucosidase). Both harvest age and extraction parameters significantly influenced extract potency. **Conclusion:** The study demonstrates that harvest age and extraction strategy critically affect the antidiabetic efficacy of *O. aristatus* extracts. These findings support its potential as a complementary therapy in diabetes management and provide direction for optimized extraction protocols in future pharmacological applications.

Key words: *Orthosiphon aristatus* (Blume) Miq., antidiabetic activity, extraction methods, solvent polarity, tissue-cultured plants

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rising prevalence of diabetes mellitus is a pressing public health challenge in Indonesia, with recent statistics indicating an increase in the prevalence rate from 6.9 to 8.5% between 2018 and 2019, highlighting the urgent need for effective therapeutic interventions[1]. The management of diabetes imposes a significant burden on healthcare systems and necessitates the exploration of novel treatment modalities, particularly those leveraging natural products that have been integral to traditional medicine for centuries. Among the numerous medicinal plants utilized in Indonesia *Orthosiphon aristatus* (Blume) Miq., commonly known as cat's whiskers, has emerged as a prominent candidate due to its potential antidiabetic properties and historical use in treating various ailments such as hypertension, rheumatism and kidney disorders[2,3].

Research into *Orthosiphon aristatus* has revealed an array of bioactive compounds, including flavonoids and polyphenols[4], which are believed to contribute to its pharmacological activities[5,6]. The plant's efficacy in diabetes management is attributed to multiple mechanisms, such as enhancing insulin secretion and promoting glucose utilization in peripheral tissues[7]. Several studies highlight its effectiveness in lowering blood glucose levels in experimental models, indicating its potential as a complementary therapeutic option[8]. However, the pharmacological efficacy of *Orthosiphon aristatus* is influenced by several factors, including the age of the plant at harvest, the methods used for extraction and the solvent employed, which can significantly affect both the yield and biological activity of its extracts[9,10].

Given the crucial need for effective diabetes management strategies in Indonesia, this study aims to evaluate the antidiabetic potential of *Orthosiphon aristatus* extracts derived from tissue-cultured plants harvested at different ages. Specifically, the study investigates the impact of various extraction methods, including ultrasonic extraction, maceration and infusion, using solvents of differing polarities (70, 30% ethanol and water). Previous works have indicated significant variations in the extraction efficiency and biological activity of herbal extracts due to these factors, underlining the importance of standardization in herbal medicine[11-13]. This comprehensive approach not only seeks to quantify the inhibitory effects of the extracts on important enzymes such as α-amylase and α-glucosidase but also aims to establish a clear understanding of the optimal conditions for harnessing the plant's antidiabetic potential, ultimately positioning *Orthosiphon aristatus* (Blume) Miq. as a viable complementary therapy in the management of diabetes mellitus within Indonesia's healthcare landscape.

The study has produced promising results, particularly with extracts obtained using 30% ethanol through ultrasonic extraction, which outperformed others in both yield and enzyme inhibitory activity[9]. These findings are pivotal in elucidating the mechanisms by which *Orthosiphon aristatus* exhibits its antidiabetic effects, suggesting that it may play a crucial role as an adjunctive treatment option. Continued exploration into the pharmacological applications of *Orthosiphon aristatus* not only reflects its significance in traditional medicine but also enhances the scientific understanding of its potential in modern therapeutic paradigms.

This research, therefore, contributes valuable insights into the multifaceted roles that natural products can play in contemporary medicine, further emphasizing the importance of retrospective validation and the usage of herbal remedies. This process can be instrumental in addressing the rapid rise of diabetes in Indonesia and potentially other regions facing similar health challenges.

MATERIALS AND METHODS

Study area and duration instrument: Cat's whisker leaves are taken from Purwakarta, West Java, Indonesia and have been planted since March, 2024. Ultrasonic LC 30 H Elma (Elma Schmidbauer Gmbh, Gottlieb-Daimler Straße 17 78224, Singen, Germany), infusion pot, oven, analytical scales, micropipette, Eppendorf pipette, Microplate Reader Elx800, freeze dryer, rotary vacuum evaporator (Heidolph, Germany).

Materials: Cat's whiskers leaves, ethanol 30%, 70%, enzymes α-glucosidase (Sigma Aldrich, United States), substrate p-nitrophenyl-D-glucopyranoside (*p*-NPG) (Sigma Aldrich, United States), α-amylase enzyme, Dimethyl Sulfoxide (DMSO) (Merck, Germany), acarbose, sodium carbonate (Merck, Germany), α-amylase enzyme, substrate starch (soluble starch), Na_2HPO_4, NaH_2PO_4, 3,5-dinitrosalicylate (DNS), potassium sodium tartrate, potassium dihydrogenphosphate (Merck, Germany), distilled water, demineralized water.

Extraction sample: Cat's whiskers leaf simplicia from tissue culture were extracted by infusion, maceration (30% ethanol and 70% ethanol) and UAE (water, 30% ethanol and 70% ethanol). The extraction results were filtered and evaporated

with a rotary vacuum evaporator until a thick extract was obtained. All extracts obtained. The yield was calculated and the inhibitory activity of α-glucosidase and α-amylase enzymes was tested *in vitro*.

Test activity inhibition enzyme α-glucosidase and α- amylase

Preparation of extract solution: Several extracts dissolved with Dimethyl Sulfoxide (DMSO), then enough volume with buffer for sfat pH 6.8, until a stock solution with a concentration of 1000 µg/mL. Then dilution was carried out to obtain concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg /mL.

Preparation of acarbose solution (positive control): Acarbose solution was made by weighing 10 mg of acarbose powder, then dissolved in 10 mL of phosphate buffer pH 6.9 until homogeneous and became a stock solution with a concentration of 1000 ppm. Then dilution was carried out to obtain concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Inhibition of α -amylase enzyme activity.

Preparation of blank solution (α-glucosidase): The blank solution consists of a Dimethyl Sulfoxide (DMSO) solution plus phosphate buffer pH 6.8 and substrate p-nitrophenyl-α-D-glucopyranoside (p-NPG) 10 mM, pre-incubated for 5 min at a temperature of 37°C. Then the α-glucosidase enzyme solution was added 0.049 U/mL and re-incubated at 37°C for 30 min. After the incubation period is complete, 100 µL of Na_2CO_3 is added. 200 mM. The absorbance of the solution was measured using a microplate reader at λ 405 nm.

Testing control blank (α-amylase): A total of 1 µL of DMSO was added with 49 µL of phosphate buffer with pH 6.9 and 25 µL of 1% starch solution. Then incubated for 10 min at a temperature of 25°C. Furthermore, 25 µL of 0.15 U/mL enzyme solution and incubated again at a temperature of 25°C for 10 min. The reaction was then stopped by adding 100 µL of DNS reagent. Then incubated in boiling water for 5 min and cooled to room temperature. Furthermore, the reaction mixture was diluted with 1 mL of distilled water and its absorbance was measured at λ 540 nm.

α-glucosidase enzyme inhibition assay: The extract sample solution consists of 25 µL of extract solution, added with 40 µL of buffer. phosphate pH 6.8 and 10 µL solution substrate p-nitrophenyl-α-D-glucopyranoside (p-NPG) 5 mM, pre-incubated for 5 min at a temperature of 37°C. Then 25 µL of α-glucosidase enzyme solution was added at 0, 15 U/mL and re-incubated at 37°C for 30 min. After the incubation time finished, I added 100 µL Na_2CO_3 200 mM. The absorbance of the solution was measured using a microplate reader at λ 405 nm.

Inhibition test of α-amylase enzyme activity: Testing using the dinitrosalicylic acid (DNS) method by determining the level of starch hydrolysis. A total of 1 µL of each sample was added with 49 µL of phosphate buffer with a pH of 6.9 and 25 µL of 1% starch solution. Then incubated for 10 min at a temperature of 25°C. Furthermore, 25 µL of 0.15 U/mL enzyme solution and incubated again at a temperature of 25°C for 10 min. The reaction was then stopped by adding 100 µL of DNS reagent. Then incubated in boiling water for 5 min and cooled to room temperature. Furthermore, the reaction mixture was diluted with 1 mL of distilled water and its absorbance was measured at λ 540 nm.

All testing was repeated as many as three times. Percentage inhibition α-glucosidase activity can be calculated using the equation[14]:

$$\text{Inhibition } (\%) = \frac{[A_0 - A_1]}{[A_0]}$$

Information:

A_0 = Blank absorbance (DMSO)
A_1 = Sample absorbance (comparator/extract)

All inhibition calculation results are made into a linear regression equation y = a+bx where x is the extract concentration in µg/mL and y is the percentage of inhibition. Through this linear regression equation, the mark IC_{50} can be calculated using the formula:

$$IC_{50}\ (\%) = \frac{50 - a}{b}$$

The cat's whisker leaves used in this study have the Latin name *Orthosiphon aristatus* (Blume) Miq., according to the determination results carried out in the Biology Laboratory of FMIPA (Faculty of Mathematics and Natural Sciences) Unpad (Padjadjaran University) Bandung.

RESULTS AND DISCUSSION

Cat's whiskers plant seeds were obtained from UNJANI as a result of tissue culture and were planted in the plantation area at PT. Holistic Bio Medicine in Purwakarta, the plants were harvested at the ages of 6 months and 9 months, until the

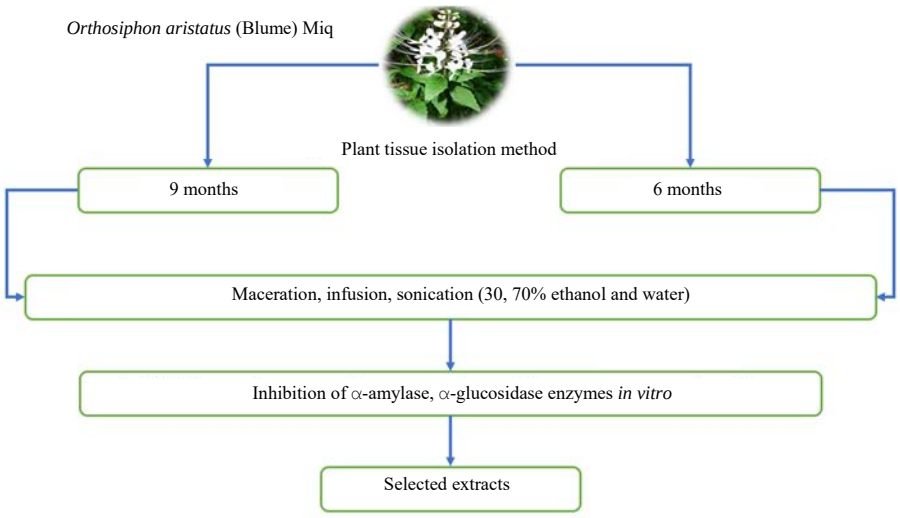

Fig. 1: Flowchart of the research

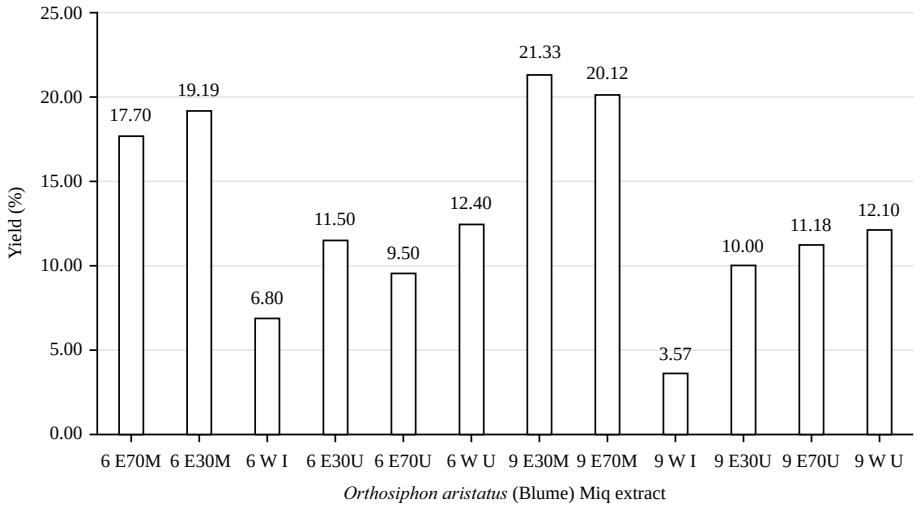

Fig. 2: Yield *Orthosiphon aristatus* (Blume) Miq extract with difference age, method extraction and solvents
M: Maceration, U: Ultrasonic, I: Infused, 6: 6 month, 9: 9 month and W: Water

simplicia with a water content of 4.06% and 4.13% were obtained. As much as 100 g of simple extract, with a number of methods and different concentrations of ethanol and water, the results can be seen in Fig. 1.

Based on Fig. 2, it can be seen that cat's whisker leaves with a harvest age of 6 months have the highest yield in 70% ethanol extract with the maceration method, while the age of 9 months has the highest yield in 30% ethanol extract with the ultrasonic method. This shows that even though using the same plant seeds, if the extraction method and solvent are different, it will affect the yield. The difference in yield percentage indicates the number of compound components extracted through the extraction method and the polarity of the solvent. Ethanol 30% has a greater polarity than ethanol 70%, so the possible compounds that are attracted are also different. Likewise, the effect on pharmacological activity.

Based on the findings regarding the extraction yields of cat's whiskers (*Orthosiphon aristatus*) leaves through different methodologies, it is evident that both the age of the leaves at harvest and the extraction method significantly influence the efficiency of extracting bioactive compounds. Specifically, the findings indicate that leaves harvested at 6 months yield higher results with 70% ethanol extract

through the maceration method, while leaves harvested at 9 months yield greater extracts when using a 30% ethanol solution via ultrasonic extraction. This highlights the importance of processing conditions and methods in optimizing extract quality and quantity.

The selection of extraction methods plays a fundamental role in determining the yield of plant bioactive compounds. Previous studies emphasize that the choice of solvent and extraction conditions can lead to varying profiles of phytochemicals based on the chemical properties of the compounds in the plant material. For instance, Nawaz *et al*.[13] highlighted that the solvent polarity significantly impacts the quantity and type of phytochemicals extracted from corn silk, showcasing similar trends across various plant materials. Different extraction conditions can optimize yields and bioactivity, reinforcing the critical role of solvent selection in extraction outcomes[14].

The polarity of the solvents used during extraction processes is a vital determinant in extracting desired compounds. Ethanol, especially at a lower concentration like 30%, exhibits certain benefits for extracting specific bioactive compounds compared to more concentrated ethanol solutions. Research has indicated that using polar solvents, including aqueous mixtures, enhances extraction yields of important phytochemicals[15]. Compounds containing hydroxyl groups tend to be more effectively extracted in polar solvents, reinforcing the significance of solvent characteristics in extraction efficiency[16].

Variations in extraction methodologies, such as maceration and ultrasonic methods, further accentuate the influence of operational parameters on yields and phytochemical profiles. Ultrasonic extraction utilizes ultrasonic waves to promote cell wall disruption and enhance mass transfer, leading to improved extraction efficacy[17]. Ultrasonic-assisted extraction significantly boosts yields in both polar and non-polar solvent scenarios by enhancing the solvent's access to the target analytes[18].

Besides solvent polarity, factors such as extraction time, temperature and solid-to-solvent ratio must be meticulously optimized to maximize extraction efficiency. Research by Melo *et al*.[19] and by Martínez Ramos *et al*.[20] illustrates how optimizing these parameters can yield substantially higher quantities of valuable bioactive compounds. For example, adjusting ultrasonic power or treatment time can drastically enhance flavonoids and phenolic content, both of which are integral in health research due to their antioxidant properties[21].

The harvesting period is also crucial for the extraction process. The differences in extraction yields between the 6 month and 9 month age groups for cat's whisker leaves may stem from the leaves' physiological maturity, affecting both phytochemical concentration and diversity[22]. As a plant matures, certain phytochemicals can accumulate or diminish based on environmental conditions, thereby influencing extraction efficacy based on their composition. This finding is consistent with Chu *et al*.[23], who indicated that chemical profiles in plant materials evolve, affecting extraction methodologies and yields.

Furthermore, the application of ultrasonic-assisted methods is known to enhance the bioactivity of extracts through a more effective release of bioactive compounds, as suggested by various studies. For instance, Shaterabadi *et al*.[24] report significant enhancements in antioxidant activities in ultrasonic extracts compared to conventional extraction methods. This underscores that not only do extraction processes determine the quantity of bioactive compounds, but they can also enhance their efficacy.

The maceration method, while simpler and commonly used because of its ease, may not achieve the same extract potency as ultrasonic techniques, which utilize targeted mechanistic benefits[25]. Investigating these differences highlights the need for comprehensive evaluations comparing various extraction techniques to determine their operational efficiencies across different plant species and biological outputs.

While this analysis is focused on cat's whisker leaves, contextualizing these findings within the broader spectrum of plant biotechnology is essential. Understanding the principles of phytochemical extraction, such as the effects of solvent polarity and the characteristics of extraction methodologies, strengthens the foundation for future innovations in natural product research. Studies across diverse plant materials verify that optimizing extraction conditions can lead to enhanced bioactivity, support traditional medicinal practices and open avenues for industrial applications[26].

The number of chemical compound components extracted does not necessarily increase activity, because each compound has a different activity. For this reason, in this study, the extract activity test was carried out on the inhibition of α-glucosidase and α-amylase enzymes and the results can be seen in Table 1. Table 1 shows that the 9-month-old *Orthosiphon aristatus* extract obtained by ultrasonic extraction with 30% ethanol had the strongest inhibitory activity, with IC values of 64.35 µg /mL (α-glucosidase) and 64.68 µg/mL (α-amylase). This activity was lower than that of the standard drug Acarbose but better than other tested extracts. Both harvest age and extraction method influenced enzyme inhibition.

Table 1: Inhibitory activity of the β-glucosidase and α-amylase enzymes of the cat's whisker extract

No.	Sample	IC_{50} enzyme (µg/mL)	
		α-glucosidase	α-amylase
1.	OS6m 70% ethanol maceration	70.66±0.46	64.83±0.13
2.	OS6m 30% ethanol maceration	65.10±0.10	69.50±0.51
3.	OS6m ultrasonic ethanol 70%	67.09±0.21	57.68±0.34
4.	OS6m ultrasonic ethanol 30%	66.72±0.39	59.30±0.35
5.	OS6m ultrasonic water	67.83±0.048	58.48±0.28
6.	OS6m water infusion	65.95±0.24	59.68±0.47
7.	OS9m 70% ethanol maceration	70.54±0.61	60.68±1.60
8.	OS9m 30% ethanol maceration	66.49±0.07	63.70±0.21
9.	OS9m ultrasonic ethanol 70%	67.53±0.27	67.46±0.27
10.	OS9m ultrasonic ethanol 30%	64.35±0.67	64.68±0.67
11.	OS9m ultrasonic water	68.20±0.20	69.83±0.32
12.	OS9m water infusion	64.26±0.17	66.50±0.42
13.	Acarbose (positive control)	12.56±0.043	11.23±0.06

OS: *Orthosiphon aristatus*, 6m: 6-month-old plant and 9m: 9-month-old plant

Table 1 provides information that the activity of cat's whiskers extract with different ages, extraction methods and solvents showed results that were not significantly different, however for the 9-month-old extract, the ultrasonic method using 30% ethanol solvent had the same inhibitory activity of glucosidase and amylase enzymes with an IC_{50} 64.35 and 64.68 µg/mL and active category also.

The intensive investigation of the inhibitory effects of different extracts of cat's whiskers (*Orthosiphon aristatus*) on β-glucosidase and α-amylase enzymes showcases a promising avenue in the search for natural antidiabetic agents. While the extracts exhibited inhibitory characteristics across a range of ages, the 9-month-old extract, particularly when processed through ultrasonic extraction with a 30% ethanol solvent, demonstrated significant inhibitory activity approaching an IC50 of 64.61±0.67 µg/mL. This effectiveness positions it within the active category of enzyme inhibitors, indicating a substantial potential for managing conditions associated with hyperglycemia, such as Type 2 diabetes mellitus.

Understanding the extraction methods and their efficacy is crucial in delineating the optimal conditions for enhancing the bioactive properties of phytochemicals. Traditional methods often yield less concentrated extracts compared to modern techniques like ultrasonic extraction. This has been supported by studies demonstrating that ultrasound-assisted extraction significantly improves the release of bioactive compounds from plant materials, thereby resulting in extracts with enhanced pharmacological activities[27]. Moreover, ultrasonic extraction can facilitate the extraction of saponins and other phytochemicals that have robust antioxidant and enzyme-inhibitory properties beneficial in diabetic management[28].

The solvent used in the extraction process, in this case, 30% ethanol, is particularly interesting because the polarity of the solvent affects the extract's composition and consequently its biological activity. Ethanol is known to effectively solubilize both polar and non-polar compounds, making it ideal for attracting diverse phytochemicals including flavonoids, phenolics and triterpenes, which are known for their potential inhibitory effects on carbohydrate-hydrolyzing enzymes[29,30]. Studies have shown that the presence of hydroxyl groups and modifiable chemical structures within these bioactive compounds significantly influences their ability to inhibit glycosidases[31]. The extracted compounds from Cat's Whiskers have been indicated as possessing a robust capacity to interact with the active sites of β-glucosidase and α-amylase enzymes. Several mechanisms have been identified for β-glucosidase inhibition, ranging from reversible binding to competitive inhibition[32]. The effectiveness of inhibitors is generally related to their structural characteristics, such as the configuration and number of hydroxyl groups, which facilitate binding affinity towards enzyme active sites[33]. Notably, compounds such as quercetin and catechins found in various plant extracts have shown promising results as α-glucosidase inhibitors across various studies[34].

In aging, the activity of plant extracts can vary depending on plant maturation and environmental factors influencing phytochemical accumulation[35]. For example, micro-environmental factors can affect the biosynthesis of secondary metabolites, which in turn alter the bioactivity of herbal extracts. The selection of the 9-month extraction timeline for analysis may reflect a peak maturity where the perceptible concentrations of potent phytochemicals align optimally for bioactivity. This hypothesis aligns with similar findings across other plant studies, where optimal harvesting times correlate to maximal extract efficacy for biological activities[36].

It also warrants mentioning that while the extracts under scrutiny revealed promising inhibitory activity, variations in IC_{50} values across different extracts indicate a complex interaction between extraction methods, solvent systems and specific age stages of the plant[37]. The implication is that the regulatory framework encompassed within the extraction methodologies directly influences the bioactive constituents that contribute to desired pharmacological outcomes in the extracts. This leads to questions surrounding the standardization of extraction processes and age considerations in potential therapeutic applications. Studies on polysaccharides from various plant sources have elucidated similar patterns of inhibition of β-glucosidase through mechanisms involving structural transformations in the enzyme itself, rendering it less accessible for substrate binding[38]. Furthermore, the presence of proanthocyanidins or other complex polyphenols in the extracts likely contributes to the modulation of enzyme activity through mechanisms analogous to competitive inhibition, affecting not just the enzyme but potentially broader metabolic pathways involved in carbohydrate metabolism[39].

The pharmacokinetic profiles of such extracts when applied *in vivo* could elucidate further interactions with glucose transport mechanisms, potentially leading to a multifaceted approach for glycemic control. While extracting bioactive components is crucial, understanding their interaction with biological systems provides an additional layer of essential knowledge aimed at advancing therapeutic applications around diabetes management. Recent advancements conducted on novel extraction processes, bundling both *in vitro* and *in silico* analyses, facilitate this understanding[40]. Given that diabetes is increasingly prevalent, there is a significant impetus for research focusing on naturally sourced enzyme inhibitors free from adverse effects common in synthetic drugs[41]. The inclination towards biocompatible and less toxic options presents herbal extracts like those of Cat's Whiskers as promising candidates for future diabetic therapies. By rigorously assessing extraction methods and solvent efficacy, researchers can maximize possible yields of these inhibitors, developing evidence-based strategies to treat hyperglycemia[42]. Empirical investigations yet need to chart the precise biochemical pathways these extracts utilize to impart their effects, potentially utilizing metabolomic analyses to profile active compounds following extraction and subsequent biological testing[43]. Enhanced insight into these mechanisms would not only provide clarity on their specific actions but may also facilitate the identification of biomarkers relevant to the consumption of such phytochemicals[44]. Therefore, integrative approaches that include novel extraction methods, detailed structural analyses of active compounds and comprehensive bioactivity assessments must be leveraged to fully understand and utilize these natural products effectively in diabetes management strategies.

The enzymatic activity of β-glucosidase and α-amylase is central to carbohydrate metabolism, particularly in the context of glucose release. Factors such as substrate concentration, enzyme concentration and incubation time considerably influence these enzymatic activities. The concentration of substrates is particularly critical because it can enhance or inhibit enzyme activity, depending on the specific kinetics involved. For instance, substrate inhibition occurs for certain β-glucosidases when there is a high concentration of substrate, suggesting that there exists an optimal substrate concentration for maximal enzyme activity before inhibition[45]. This substrate inhibition is commonly observed in enzymatic systems, where excessive substrate can lead to competition between the enzyme and the substrate for active sites, thereby affecting the overall reaction dynamics[45].

α-amylase and α-glucosidase enzymatic reactions are contingent upon their concentration, which directly affects the rate of hydrolysis of complex carbohydrates into simpler sugars[46]. In their *in vitro* analyses, varying enzyme concentrations demonstrated significant changes in hydrolytic efficacy, emphasizing the need for optimization when designing therapeutic interventions aimed at inhibiting these enzymes in diabetic management.

Incubation time is another critical component in enzyme kinetics. Studies have shown that an increase in incubation time allows for greater enzyme-substrate interactions, thus enhancing catalytic activity up to a point[47]. Understanding the optimal incubation time can significantly improve the extraction yields and bioactivity of enzyme inhibitors from natural sources. Research exploring the inhibitory effects of various plant extracts has evidenced this relationship[47]. The aforementioned inhibitory activities can be quantified by determining their IC_{50} values, which are a comprehensive measure of inhibitor potency that presents an essential factor for developing new biochemical drugs. The effects of different extracts on β-glucosidase activity, revealing variations in inhibitory potency influenced by extraction methodologies and conditions[48]. This research illustrated the significant impact of extraction duration and temperature on the efficacy of inhibitors, suggesting that these factors are vital in maximizing β-glucosidase activity inhibition.

The importance of chemical composition in plant extracts should not be overlooked, as bioactive compounds within these extracts significantly influence their efficacy against β-glucosidases and amylases. The inhibition mechanism can

involve competitive inhibition, in which the inhibitor binds to the active site of the enzyme, thus blocking the substrate from accessing its active site[49]. Studies involving the identification of specific compounds as potent inhibitors have gained traction, wherein molecules like flavonoid derivatives demonstrate substantial inhibition of both β-glucosidase and α-amylase activities. Such investigations are critical as they highlight potential formulations for developing natural-based anti-diabetic agents.

Recent advancements in understanding the stability of these enzymes under various conditions also provide crucial insights. Studies indicated that β-glucosidases demonstrate varied levels of resistance to substrate inhibition and can retain their activity better when immobilized or subjected to specific environmental conditions[50]. Moreover, the kinetic properties of these enzymes, including temperature and pH dependence, underscore the need to investigate varying conditions under which these enzymes maintain optimal activity levels while functioning as effective therapeutic targets[51].

Research on the stability and effectiveness of β-glucosidase inhibitors has evolved, where factors such as temperature, ionic strength and the presence of specific additives like biosurfactants have been studied. For example, the activity and binding characteristics of β-glucosidase may be enhanced when interacting with surface-active agents, which can modify hydrophobic interactions and potentially lead to improved catalytic efficiencies[51]. Such findings suggest that a comprehensive understanding of the biochemical interactions of these enzymes holds value not only in therapeutics but also in biotechnological applications.

Furthermore, the health implications of managing β-glucosidase activity extend into chronic diseases, such as Type 2 diabetes, emphasizing the importance of dietary regulation towards maintaining optimal glucose levels post-consumption. This necessitates ongoing research into natural product chemistry for the identification of potent enzyme inhibitors across various plant species[46]. The pharmacological potential and applications of these natural extracts present promising avenues for developing new classes of drugs aimed at effectively controlling glycemic levels, while also maintaining a profile that mitigates side effects associated with synthetic alternatives[52]. Overall, a synthesis of these insights underscores the multifaceted influences of substrate and enzyme concentrations, incubation times and extraction methods on the activities of glucosidase and amylase enzymes. As further examination of these factors is undertaken, the potential for harnessing plant extracts as effective therapeutic agents for glycemic control appears increasingly promising. The substantial variety of screening methodologies that elucidate enzyme interactions, stability and inhibition mechanisms paves the way for innovative treatments capable of addressing the global burden of hyperglycemia.

The ultrasonic method has a number of advantages over other methods maceration and infusion, that is, among others, more efficiency in use time, no need for high temperature, a more friendly environment and costs more cheap, but still can extract the same number of compounds. In addition, the ultrasonic mechanism is more efficient because it needs more solvent for the same amount of the same compound. The ultrasonic method uses ultrasonic waves around 20-40 kHz, the output wave causes heating of materials so that the compound can break apart, break apart the wall cells and release the Contents cell to the extraction medium.

CONCLUSION

Orthosiphon aristatus greatly impacts its biological activity. By investigating both 6-month- and 9-month-old tissue-cultured plants, it has been demonstrated that the age of the plant plays a crucial role in determining the yield of active compounds and their subsequent efficacy against diabetes-related enzymes. The tendency for older plants to provide superior extracts suggests an accumulation of pharmacologically beneficial constituents over time, which is an important consideration for agricultural and therapeutic practices when cultivating this species for medicinal use. This research sheds light on the broader implications for the pharmacological application of *Orthosiphon aristatus* not only as a stand-alone treatment but also as a complementary therapeutic option for diabetes management. Integrating this plant into diabetes care protocols could serve to enhance glycemic control and reduce reliance on synthetic medications, which can have significant side effects. This aligns with a growing emphasis in global healthcare towards integrative approaches that prioritize natural products and their role in treatment paradigms.

SIGNIFICANCE STATEMENT

The significance of this research lies in its systematic approach to evaluating the pharmacological potential of cat's whiskers through rigorously designed experiments. By comparing the biological activities garnered from 6- and 9-month-old tissue-cultured plants, the study provides essential insights into how the harvest age can considerably alter the phytochemical profile and, consequently, the

antidiabetic efficacy of the extracts. This aspect is particularly relevant in Indonesia, where plant material is often harvested without stringent consideration of age and maturity, which may inadvertently affect the extraction yield and the therapeutic outcomes derived from such botanicals.

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