

***In Vitro* α -Glucosidase Enzyme Inhibition Activity Test of Water Extract From Sintok (*Cinnamomum sintoc* Blume) Bark**

Astellra Amanda Putri Saksono¹, Inana Ratu Soripada Simanjuntak¹, Mariana Wahjudi², Marisca Evalina Gondokesumo^{1*}

¹Faculty of Pharmacy, University of Surabaya, Surabaya, East Java, Indonesia

²Faculty of Technobiology, University of Surabaya, Surabaya, East Java, Indonesia

ABSTRACT

Diabetes mellitus is a condition characterized by elevated blood glucose levels beyond the normal limits. One therapeutic approach for managing diabetes mellitus involves inhibiting the α -glucosidase enzyme, which plays a role in glucose absorption in the body. However, the use of oral antidiabetic drugs from the α -glucosidase inhibitor class often causes gastrointestinal side effects. Therefore, exploring natural materials as alternative treatments is a promising option. In Indonesia, one plant with potential as an alternative treatment is sintok (*Cinnamomum sintoc* Blume), a member of the *Cinnamomum* genus, which is widely utilized in traditional medicine for chronic diseases such as diabetes mellitus. This study aimed to evaluate the α -glucosidase inhibitory activity of a water extract of sintok bark. Preliminary tests were conducted to determine the optimal conditions for measuring α -glucosidase enzyme inhibition activity *in vitro* using a UV-vis spectrophotometer. The results showed optimal enzyme activity at a wavelength of 405 nm, an incubation time of 40 minutes, and a substrate concentration of 12.5 mM. The water extract of sintok bark exhibited the highest inhibitory activity at a concentration of 5 ppm, with an inhibition value of 28.66%, while acarbose, used as a positive control, achieved the highest inhibition value of 96.36% at a concentration of 4.5 ppm. In conclusion, the aqueous extract of sintok bark demonstrates inhibitory activity against the α -glucosidase enzyme, indicating its potential as a natural antidiabetic agent.

Keywords: acarbose; α -glucosidase; *in vitro*; sintok; stem bark

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*corresponding author
Email: marisca@staff.ubaya.ac.id

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels above normal due to abnormalities in insulin secretion, insulin action, or both (PERKENI, 2021). DM is classified into four categories: type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, and other types of diabetes (ADA, 2020). According to the World Health Organization (WHO), the global prevalence of diabetes mellitus increased from 4.5% to 8.5% among adults (WHO, 2019). Indonesia ranks fifth worldwide in terms of diabetes mellitus cases, with 19.5 million sufferers recorded in 2021 (IDF, 2021). DM can result from several factors, including insufficient physical activity, a family history of the disease, and unhealthy eating patterns (Alam et al., 2021).

One therapeutic approach for treating DM involves inhibiting the enzymes responsible for glucose absorption in the body (Syachriyani & Firmansyah, 2022).

Two key enzymes involved in carbohydrate digestion are α -amylase and α -glucosidase (Deveci et al., 2021). The α -amylase enzyme initiates carbohydrate digestion by hydrolyzing the 1,4-glycosidic bonds of polysaccharides such as starch and glycogen into disaccharides. Inhibition of this enzyme results in incomplete carbohydrate breakdown. Subsequently, disaccharides are catalyzed by the α -glucosidase enzyme into monosaccharides, increasing the postprandial blood sugar levels. Therefore, inhibiting the α -glucosidase enzyme is crucial for delaying glucose release and improving blood sugar control (Narumalla et al., 2023).

Oral antidiabetic drugs that inhibit α -glucosidase activity, such as acarbose, magnitol, and voglibose, are commonly used, with acarbose being the most frequently prescribed in Indonesia (Margono & Sumiati, 2019). However, prolonged use of these medications can lead to side effects, including gastrointestinal disturbances (Altay, 2022).

The use of plants as alternative treatments offers numerous advantages, such as fewer side effects, ease of use, and lower costs (Patra et al., 2018). One plant in Indonesia with potential for alternative treatment is sintok (*Cinnamomum sintoc* Blume). The bark of the sintok plant is commonly used for medicinal purposes. Belonging to the genus *Cinnamomum* in the Lauraceae family, the bark of this plant is widely utilized as both a spice and a traditional remedy for chronic diseases, including diabetes mellitus (Kumalasari et al., 2019; Ilham et al., 2023).

The secondary metabolite compounds found in sintok bark powder include polyphenols, monoterpenoid, sesquiterpenoids, and steroids (Sumiwi et al., 2016). Among these, polyphenols and terpenoids exhibit α -glucosidase inhibitory activity (Assefa et al., 2020). According to the second edition of the Indonesian Herbal Pharmacopoeia (2017), Sintok bark contains eugenol as a key compound. Eugenol demonstrates antidiabetic activity through mechanisms such as the inhibition of α -glucosidase activity (Khalil et al., 2017).

Although research on the genus *Cinnamomum* has highlighted its medicinal potential, studies specifically investigating the α -glucosidase inhibitory activity of sintok bark (*Cinnamomum sintoc* Blume) remain unexplored. This study aimed to identify the activity of the α -glucosidase inhibitory enzyme from the aerial extract of sintok bark by optimizing several parameters, namely maximum wavelength, incubation time, substrate concentration, and sintok extract concentration.

METHODS

Extraction of Sintok Bark (*Cinnamomum sintoc* Blume)

Sintok bark powder was weighed and placed into a beaker. The solvent (water) was added at a ratio of 1:10 (w/v), and the mixture was subjected to microwave-assisted extraction (MAE) using a microwave set at 600 watts for 1 minute per cycle. The extraction was performed in three cycles to ensure the maximal recovery of polar compounds, including carbohydrates, cellulose, lignin, and mucilage. After each cycle, the extract was filtered through the filter paper to remove the solid particles, resulting in a clear liquid extract. The combined filtrates were concentrated using a two-step evaporation process. Initially, evaporation was conducted using a water bath at a controlled temperature of 60°C to minimize the degradation of thermolabile compounds while reducing the water content. This process was carefully monitored, and the endpoint was identified by a reduction in the liquid volume and a visible thickening of the extract. However, as the complete removal of water is challenging at this temperature, a secondary drying step was employed.

To achieve a consistent and semi-viscous extract suitable for accurate weighing, the semi-concentrated product was subjected to low-temperature vacuum drying at 40°C. This step further reduced the residual moisture without compromising the integrity of the bioactive compounds. The final viscosity and moisture content of the extract were qualitatively assessed to ensure a paste-like consistency, which was stable for subsequent testing.

Preliminary Test for Wavelength

The preliminary wavelength test was performed using a modified method. The test mixture consisted of 0.1 M phosphate buffer at pH 6.8 and 0.1 U/mL α -glucosidase enzyme. The α -glucosidase enzyme used in this study was derived from *Saccharomyces cerevisiae*, a microorganism source commonly used in *in vitro* enzyme inhibition studies. Although this enzyme shares functional similarities with the α -glucosidase enzyme found in humans, it may exhibit slight differences in activity or specificity. The substrate used was p-nitrophenyl- α -D-glucopyranoside (PNPG), a synthetic analog designed to mimic the natural substrates of α -glucosidase, widely used for enzymatic studies due to its ability to produce a measurable chromogenic product.

Pre-incubation was conducted for 15 minutes at 37°C, followed by the addition of 5 mM PNPG substrate solution and further incubation for 30 minutes at 37°C in a water bath. Subsequently, 0.2 M sodium carbonate was added, and the absorbance was measured using a microplate reader at wavelengths ranging from 390 to 500 nm. The same procedure was applied to both the blank solution and the control blank solution, with the control blank solution excluding the α -glucosidase enzyme solution (Lee et al., 2014).

Preliminary Test for Incubation Time

The preliminary test for incubation time utilized a mixture of 0.1 M phosphate buffer at pH 6.8 and 0.1 U/mL α -glucosidase enzyme. Pre-incubation was conducted for 15 minutes at 37°C, followed by the addition of 5 mM PNPG substrate solution. Incubation times ranged from 0 to 130 minutes at 37°C in a water bath. Following the incubation, 0.2 M sodium carbonate was added. Absorbance readings were taken every 5 minutes using a UV-Vis spectrophotometer at a wavelength of 405 nm. The procedure was also applied to the blank and control blank solutions (Lee et al., 2014).

Preliminary Test for the Substrate Concentration Variation

In the preliminary test for substrate concentration variation, the test mixture consisted of 0.1 M phosphate buffer at pH 6.8 and 0.1 U/mL α -glucosidase enzyme. Pre-incubation was conducted for 15 minutes at 37°C, followed by the addition of PNPG substrate solutions

at varying concentrations: 0.1 mM, 0.5 mM, 1 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 12.5 mM, and 15 mM. The mixture was incubated for 40 minutes at 37°C in a water bath, and 0.2 M sodium carbonate was added afterward. Absorbance readings were taken using a UV-Vis spectrophotometer at a wavelength of 405 nm. The same procedure was applied to the blank and control blank solutions (Lee et al., 2014).

α -Glucosidase Enzyme Inhibition Activity Test

The α -glucosidase enzyme inhibition activity test was conducted under the optimized conditions determined from preliminary tests. The α -glucosidase enzyme from *Saccharomyces cerevisiae* was used at a concentration of 0.1 U/mL, and the substrate was p-nitrophenyl- α -D-glucopyranoside (PNPG) at a final concentration of 12.5 mM. Six types of solutions were prepared for testing: sintok bark water extract sample solution, sintok bark water extract sample control solution, blank solution, blank control solution, acarbose solution, and acarbose control solution. Each test mixture had a total volume of 200 μ L, consisting of 50 μ L of 0.1 M phosphate buffer (pH 6.8), 50 μ L of α -glucosidase enzyme solution (0.1 U/mL), 50 μ L of the test sample solution (either sintok extract or acarbose at varying concentrations), and 50 μ L of 12.5 mM PNPG substrate solution. The mixture was pre-incubated at 37°C for 15 minutes, after which the substrate solution was added, followed by an additional incubation period of 40 minutes at the same temperature. The enzymatic reaction was terminated by adding 50 μ L of 0.2 M sodium carbonate solution, and the absorbance of the resulting mixture was measured using a UV-Vis spectrophotometer at a wavelength of 405 nm.

The percentage of α -glucosidase inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{B-S}{B} \times 100\% \quad (1)$$

Explanation:

B = difference between the absorbance of the blank solution and the control blank solution; S = difference between the absorbance of the sample solution and the sample control solution

RESULTS

Preliminary Screening of Wavelength

The wavelength screening was conducted before the incubation time and substrate concentration variation screenings. The purpose of the wavelength screening was to determine the optimal wavelength for measuring the α -glucosidase enzyme inhibition activity of the sintok bark water extract as a sample and acarbose as a positive control. Based on the results presented in Figure 1, the absorbance increased steadily from 390 to

405 nm, reaching a peak of 0.1870 at 405 nm. After 405 nm, the absorbance decreased, reaching 0.0025 at 500 nm. Therefore, subsequent enzymatic reaction tests were conducted at a wavelength of 405 nm, corresponding to the absorbance peak.

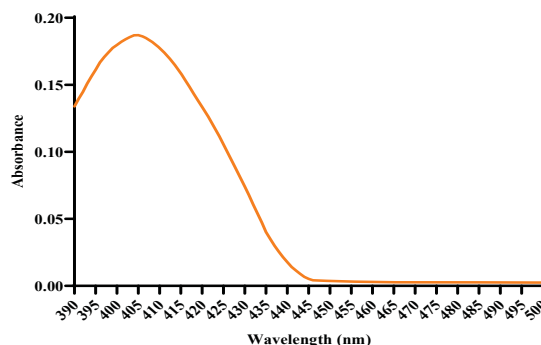


Figure 1. Wavelength screening results

Incubation Time Screening

The incubation time screening was performed following the wavelength screening. Incubation was monitored over a range of 0 to 130 minutes, with absorbance readings taken every 5 minutes using a UV-Vis spectrophotometer at 405 nm. As shown in Figure 2, the absorbance increased progressively with longer incubation times. The effective incubation time chosen for subsequent tests was 40 minutes, as this duration corresponded to a significant increase in absorbance, despite a lower steepness in the graph compared to the first 20 minutes.

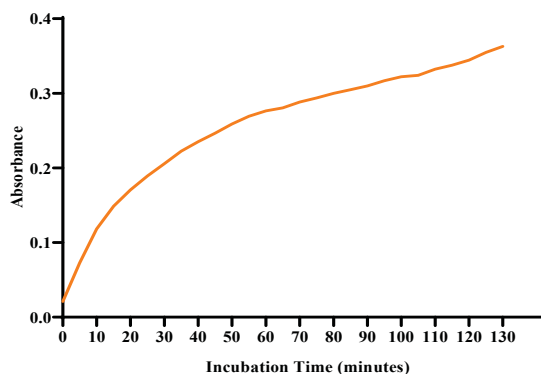


Figure 2. Incubation time screening results

Substrate Concentration Variation Screening

The substrate concentration variation screening was conducted after the wavelength and incubation time screenings. The PNPG substrate concentrations tested were 0.1 mM, 0.5 mM, 1 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 12.5 mM, and 15 mM. The absorbance was measured using a UV-Vis spectrophotometer at 405 nm. The results (Figure 3) demonstrated an increasing in absorbance with rising substrate concentrations up to 12.5 mM. At 15 mM, the absorbance decreased,

indicating that the enzyme activity had reached its maximum rate. Consequently, a substrate concentration of 12.5 mM was selected for subsequent tests.

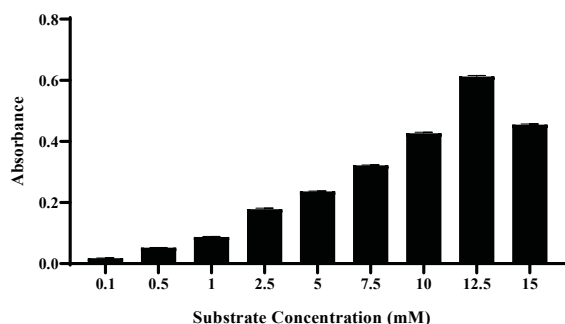


Figure 3. Substrate concentration variation screening results

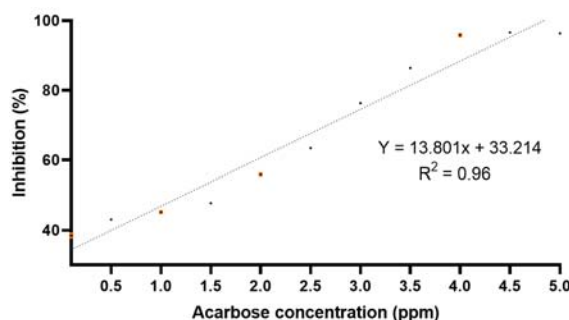


Figure 4. Percentage inhibition results of acarbose on the α-glucosidase enzyme

α-Glucosidase Enzyme Inhibition Activity of Acarbose

The inhibition activity of acarbose as a positive control was assessed at concentrations of 0.10 ppm, 0.50 ppm, 1.00 ppm, 1.50 ppm, 2.00 ppm, 2.50 ppm, 3.00 ppm, 3.50 ppm, 4.00 ppm, and 5.00 ppm. Table 1 indicates that the highest absorbance (0.3777) occurred at 0.10 ppm, while the lowest absorbance (0.0207) was observed at 4.50 ppm. The lower the absorbance, the greater the percentage inhibition of the α-glucosidase enzyme. As shown in Figure 4, the inhibition activity increased significantly with higher acarbose concentrations. The lowest inhibition percentage was 38.49% at 0.10 ppm, while the highest was 96.63% at 4.50 ppm. These findings confirm the potent inhibitory effect of acarbose on the α-glucosidase enzyme.

α-Glucosidase Enzyme Inhibition Activity of Sintok Bark Water Extract (*Cinnamomum sintoc* Blume)

The inhibition activity of sintok bark water extract was tested at concentrations of 0.10 ppm, 0.50 ppm, 1.07 ppm, 1.50 ppm, 2.00 ppm, 2.50 ppm, 3.04 ppm, 3.50 ppm, 4.00 ppm, 4.51 ppm, and 5.00 ppm. Table 2 shows that the highest absorbance (0.5617) occurred at 0.10 ppm, while the lowest (0.4380) was at 5.00 ppm. As with acarbose, lower absorbance values correspond to higher enzyme inhibition. The percentage inhibition values (Figure 5) indicate that the lowest inhibition (8.52%) occurred at 0.10 ppm, while the highest inhibition (28.66%) was at 5.00 ppm. These results demonstrate that sintok bark water extract inhibits the α-glucosidase enzyme, with higher concentrations yielding greater inhibition.

Tabel 1. Results of α-glucosidase enzyme inhibition activity of acarbose

Acarbose concentration (ppm)	Average absorbance	% Inhibition ± SD	CV (%)
0.10	0.3777	38.49 ± 0.62	1.60
0.50	0.3497	43.05 ± 0.19	0.44
1.00	0.3367	45.17 ± 0.34	0.75
1.50	0.3147	48.75 ± 0.25	0.51
2.00	0.2710	55.86 ± 0.33	0.58
2.50	0.2247	63.41 ± 0.25	0.39
3.00	0.1453	76.33 ± 0.09	0.12
3.50	0.0837	86.37 ± 0.19	0.22
4.00	0.0257	95.82 ± 0.34	0.35
4.50	0.0207	96.63 ± 0.19	0.19
5.00	0.0223	96.36 ± 0.09	0.10

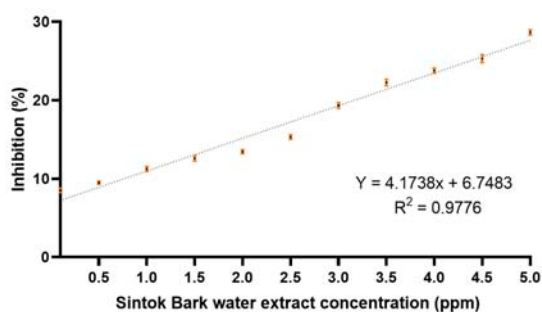


Figure 5. Percentage inhibition results of sintok bark water extract on the α -glucosidase enzyme

DISCUSSION

This study demonstrates the potential of water extract from sintok bark (*Cinnamomum sintoc* Blume) to inhibit α -glucosidase activity *in vitro*, a mechanism relevant to managing diabetes mellitus by reducing postprandial glucose absorption. The extraction process used microwave-assisted extraction (MAE), chosen for its efficiency in reducing the extraction time and solvent usage. Water was selected as the solvent because of its high dielectric constant, which enhances microwave absorption, improves heating efficiency, and increases penetration into the sample (Daou et al., 2022). This method facilitated uniform and rapid heating, contributing to better extraction yields and preserving the quality of bioactive compounds such as polyphenols, monoterpenoid, sesquiterpenoids, and eugenol, which are known inhibitors of α -glucosidase (Sumiwi et al., 2016; Kaskoos, 2019).

Optimization tests identified a wavelength of 405 nm, an incubation time of 40 minutes, and a substrate concentration of 12.5 mM as the optimal parameters

for α -glucosidase inhibition assays. The wavelength selection was consistent with previous studies using water extracts of *Cinnamomum burmannii* and *Cinnamomum zeylanicum* (Ervina et al., 2019; Niroshani Wariyapperuma et al., 2020). Although the absorbance increased up to 30 minutes during incubation, statistical analysis indicated that changes beyond this point were not significant, validating 40 minutes as the ideal incubation time. The reaction was effectively terminated using 0.2 M sodium carbonate, ensuring accurate measurements of the enzymatic activity. However, inconsistencies in the substrate concentrations used during different optimization tests may have influenced the results, warranting future studies that standardize these parameters to strengthen reliability.

The water extract of sintok bark achieved a maximum inhibition of 28.66% at a concentration of 5 ppm, while acarbose, the positive control, exhibited a significantly higher inhibition (96.63% at 4.5 ppm) (Nurcholis et al., 2019). These findings suggest that although the extract demonstrates notable inhibitory activity, it may benefit from further refinement or combination with other therapies to enhance its efficacy. The presence of eugenol and polyphenols in the extract likely contributes to its inhibitory effect, consistent with studies on other species within the genus *Cinnamomum*, such as *C. zeylanicum* (Kaskoos, 2019). The determination of IC_{50} values is crucial in evaluating the potency of enzyme inhibitors. In this study, the IC_{50} value for acarbose was not calculated, which limits the ability to directly compare its inhibitory effect to that of the extract. Future studies should focus on the systematic determination of IC_{50} for both standard inhibitors and test extracts to provide a more robust comparison.

Tabel 2. Results of α -glucosidase enzyme inhibition activity of sintok stem bark water extract

Sintok stem bark water extract concentration (ppm)	Average absorbance	% Inhibition $\pm$ SD	CV (%)
0.10	0.5617	8.52 $\pm$ 0.25	2.92
0.50	0.5557	9.50 $\pm$ 0.19	1.98
1.07	0.5450	11.24 $\pm$ 0.32	2.90
1.50	0.5367	12.60 $\pm$ 0.34	2.69
2.00	0.5313	13.46 $\pm$ 0.25	1.85
2.50	0.5200	15.31 $\pm$ 0.28	1.84
3.04	0.4953	19.33 $\pm$ 0.34	1.75
3.50	0.4773	22.26 $\pm$ 0.41	1.84
4.00	0.4680	23.78 $\pm$ 0.32	1.37
4.51	0.4587	25.30 $\pm$ 0.52	2.07
5.00	0.4380	28.66 $\pm$ 0.32	1.14

It is crucial to acknowledge that the *in vitro* model employed, using α -glucosidase derived from *Saccharomyces cerevisiae*, may not fully replicate the human enzymatic activity. Although this model is widely accepted for screening, additional studies using human-derived enzymes are necessary to confirm its clinical applicability. Despite these limitations, the findings highlight the potential of sintok bark as a natural antidiabetic agent, supporting the exploration of local plants for safe and effective herbal therapies (Olaokun et al., 2022; Hidayat, 2021). By focusing on the relevance and interpretation of these results, this study contributes valuable insights to the ongoing development of alternative treatments for diabetes mellitus.

CONCLUSION

The optimal conditions for the *in vitro* α -glucosidase enzyme inhibition activity test, based on preliminary tests, were determined to be a wavelength of 405 nm, an incubation time of 40 minutes, and a substrate concentration of 12.5 mM. Under these conditions, the water extract of sintok bark (*Cinnamomum sintoc* Blume) exhibited α -glucosidase inhibition, with the highest inhibition percentage of 28.66% observed at a concentration of 5 ppm. These findings establish the key parameters for α -glucosidase inhibition testing and provide valuable insights for further investigation into the antidiabetic potential of sintok bark.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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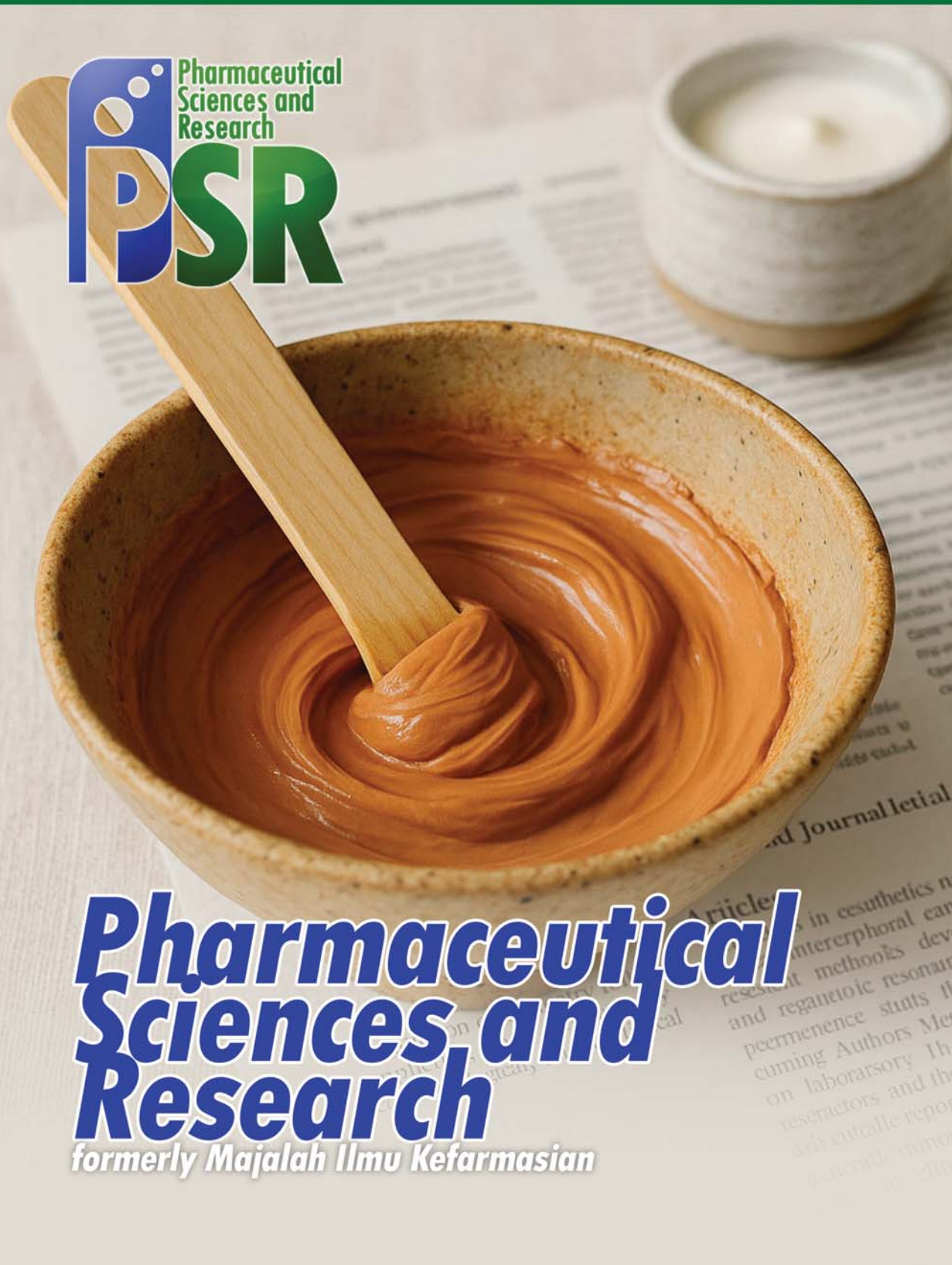
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All articles in this issue (4 original research articles, 1 Review Article, and 1 mini review article) include authors from countries/regions of origin (Thailand and Indonesia)

Original Articles



Gastroprotective Potential of Medicinal Plant From Central Kalimantan: Sungkai Leaves (*Peronema canescens* Jack)-Ultrasonic Assisted Extraction in Rats Induced with Absolute Ethanol (<https://scholarhub.ui.ac.id/psr/vol12/iss1/6>)
Diasari Savitri, Elsa Hihovita, Ratna Widayati, Septi Handayani, and Sintha Nugrahini
<https://doi.org/10.7454/psr.v12i1.1433> (<https://doi.org/10.7454/psr.v12i1.1433>)

Authentication of Kaffir Lime Leaf Oil (*Citrus hystrix* D.C.) in Products Using FTIR Combined with Chemometrics (<https://scholarhub.ui.ac.id/psr/vol12/iss1/5>)
Jeffrey Jeffry, Any Guntarti, and Nina Salamah
<https://doi.org/10.7454/psr.v12i1.1408> (<https://doi.org/10.7454/psr.v12i1.1408>)

In Vitro α -Glucosidase Enzyme Inhibition Activity Test of Water Extract From Sintok (*Cinnamomum sintoc* Blume) Bark (<https://scholarhub.ui.ac.id/psr/vol12/iss1/4>)
Astrella Amanda Putri Saksono, Inana Ratu Soripada Simanjuntak, Mariana Wahjudi, and Marisca Evalina Gondokesumo
<https://doi.org/10.7454/psr.v12i1.1402> (<https://doi.org/10.7454/psr.v12i1.1402>)

Optimization of Formula, Stability Testing, and Antioxidant Activity of Clay Masks as Skin Moisturizers (<https://scholarhub.ui.ac.id/psr/vol12/iss1/3>)
Naniek Widyaningrum, Agung Dwi Anggoro Bhakti, Alghafiq Harya Evan Maulana, Amalia Safitri, Adinda Fajrah Ramadhani, Anisa Febriani, Anggit Naufal Hanafi, and Thendi Abdul Arief
<https://doi.org/10.7454/psr.v12i1.1410> (<https://doi.org/10.7454/psr.v12i1.1410>)

Review Article

Potential of Legume Seeds from the Leguminosae Family as Material Source of Food and Medicine : A Literature Review (<https://scholarhub.ui.ac.id/psr/vol12/iss1/2>)
Ni Made Ayu Gangga Dewi, Nina Dewi Oktaviyanti, Aurapa Sakulpanich, and Kartini Kartini
<https://doi.org/10.7454/psr.v12i1.1391> (<https://doi.org/10.7454/psr.v12i1.1391>)

Mini Review Article

Artificial Intelligence for Detecting Non-alcoholic Steatohepatitis (NASH) (<https://scholarhub.ui.ac.id/psr/vol12/iss1/1>)
Muhammad Wildan Gifari, Yogi Ramadhani, and Dhadhang Wahyu Kurniawan
<https://doi.org/10.7454/psr.v12i1.1268> (<https://doi.org/10.7454/psr.v12i1.1268>)



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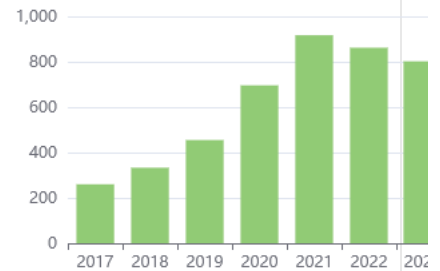
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