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The Effect of Ethanolic Extract of Bangle Rhizome (*Zingiber purpureum* Roxb.) on Reducing Blood Sugar Levels in Hyperglycemic Mice: In vivo and In silico Studies

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Abstract: Diabetes mellitus (DM) is a metabolic disease in which blood sugar levels are uncontrolled. Type 2 diabetes mellitus is uncontrolled blood sugar levels that occur due to insulin resistance or reduced body sensitivity to insulin. Treatment of type 2 diabetes mellitus, pharmacologically, uses oral antihyperglycemic drugs and injectable antihyperglycemic drugs. Although effective, antidiabetic drugs often cause adverse side effects. Indonesian people are concerned about synthetic drugs because of the potential side effects caused so that people are encouraged to look for more natural or herbal alternative treatments that are believed to have antidiabetic activity, one of which is ginger.

Bangle (*Zingiber purpureum* Roxb.) is one of the plants that belongs to the zingiberaceae or ginger family. Bangle rhizome has a major compound of curcumin of 2,633% w/w and phenylbutanoid which has antioxidant, anti-inflammatory, and AMPK (AMP-activated protein kinase) agonist activity. With this activity, the antidiabetic potential of bangle rhizome was tested in vivo using alloxan-induced hyperglycemic mice, observed in 14 days with *bangle rhizome* extract doses of 100mg/kgBW, 200mg/kgBW, 400mg/kgBW, and in silico to determine compounds that have antidiabetic activity.

The results obtained from the in vivo test for reducing blood sugar on days 7 and 14 had a significance value of 0.383 ($p>0.05$) and 0.253 ($p>0.05$) which means that the administration of bangle rhizome extract did not have a significant effect on reducing blood sugar in hyperglycemic male mice. The in silico test conducted was in line with the in vivo results where bangle rhizome extract had greater potential for anticancer, anti-inflammatory, and fat metabolism activities. The PASS Online results did not show any activity related to insulin sensitivity or alpha glucosidase inhibitor activity.

Keywords: bangle rhizome, antidiabetic, *in vivo*, *in silico*

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both (Sapra & Bhandari, 2021). This disease is a global public health problem with increasing prevalence, especially in developing countries. Data from the International Diabetes Federation (IDF, 2024) shows that Indonesia is ranked fifth in the world for the number of diabetes sufferers. In addition, the Institute for Health Metrics and Evaluation (IHME, 2019) report states that diabetes is the third highest cause of death in Indonesia, with a death rate of 57.42 per 100,000 population.

Type 2 diabetes mellitus (T2DM) is the most common form and accounts for approximately 90–95% of all diabetes cases. T2DM is characterized by insulin resistance and pancreatic β-cell dysfunction, leading to progressive impairment of blood glucose regulation (Banday *et al*., 2020). Uncontrolled chronic hyperglycemia can lead to microvascular complications such as nephropathy, retinopathy, and neuropathy, as well as macrovascular complications such as coronary heart disease and stroke (Leahy, 2005; Muoio & Newgard, 2008).

Management of T2DM generally includes lifestyle modification and pharmacological therapy. Antihyperglycemic drugs such as metformin, sulfonylureas, glinides, and insulin are often used to control blood glucose levels. However, long-term use of these drugs is not free from side effects such as hypoglycemia, gastrointestinal disorders, and increased risk of liver and kidney toxicity (Nakhleh & Shehadeh, 2021; Aldhaleei *et al*., 2024; Yao *et al*., 2024). The imbalance between the benefits and risks of pharmacological therapy encourages the community and researchers to look for safer and more natural-based alternative treatments.

Indonesia is a megabiodiversity country with an abundance of traditional medicinal flora. One of the local plants that has the potential as an antidiabetic agent is bangle *(Zingiber purpureum* Roxb.), a member of the Zingiberaceae family. This plant is widely known in traditional medicine to treat digestive disorders, inflammation, pain, and even as a health tonic (Noviyanto *et al*., 2020). Bangle has a complex composition of bioactive compounds such as curcumin, phenylbutanoids (cassumunarin and cassumunin), terpinen-4-ol, flavonoids, saponins, and alkaloids (Devkota *et al*., 2021; Yazid Yusuf *et al*., 2023).

Curcumin compound in bangle is known to have activity as an AMPK (AMP-activated protein kinase) agonist, an energy sensor enzyme that plays an important role in regulating glucose and lipid homeostasis (Liu *et al*., 2017). AMPK activation has been associated with increased insulin sensitivity, increased glucose uptake by muscle and adipose, and inhibition of hepatic glucose production. Therefore, compounds that can activate AMPK have great

potential as antidiabetic agents (Zhang *et al*., 2016).

Research on similar plants such as *Zingiber officinale* (ginger) has shown that active compounds such as [6]-gingerol and [6]-shogaol can lower blood glucose levels through AMPK activation and increased GLUT4 expression (Noipha & Ninla-Aesong, 2018; Deng *et al*., 2019). In addition, [6]-shogaol and [6]-paradol can also increase glucose consumption by muscle cells and adipocytes and stimulate GLP-1 secretion (Wei *et al*., 2017). However, scientific evidence regarding the antidiabetic effects of Zingiber purpureum is still very limited, although the content of its bioactive compounds is similar to ginger. In vitro research by Yuniarto and Selifiana (2018) showed that Zingiber cassumunar extract has inhibitory activity against the α-glucosidase enzyme with an IC_{50} value of 98.31 µg/mL. Although lower than acarbose (IC_{50} 36.17 µg/mL), these results indicate initial potential as an inhibitor of enzymes that break down complex carbohydrates into glucose. In addition, the hexane fraction of bangle also showed quite significant inhibitory activity (IC_{50} 61.02 µg/mL) (Indrianingsih & Prihantini, 2018).

In vivo studies in animal models are also an important approach to assess the pharmacological activity of a medicinal plant. The use of alloxan as a diabetogenic agent is common in experimental studies due to its ability to damage pancreatic β cells through the formation of free radicals (Ighodaro *et al*., 2017; Ghasemi & Jeddi, 2023). Using an alloxan-induced hyperglycemic mouse model, the effectiveness of plant extracts can be assessed through longitudinal changes in blood glucose levels. In addition to the in vivo approach, in silico methods or computer simulations are efficient and economical tools in predicting the activity of active compounds. Platforms such as PASS Online are used to evaluate the biological potential of compounds based on their chemical structure in SMILES format. This method allows the identification of potential protein targets such as JAK2, HIF1A, caspase-3, as well as prediction of possible activity pathways for active compounds from *bangle rhizomes* (Pagadala *et al*., 2017; Susanti *et al*., 2021). Several compounds such as cassumunin A, B, and C are known to have high Pa values as JAK2 and HIF1A inhibitors, which play an important role in the inflammatory process and abnormal cell growth (Devkota *et al*., 2021).

Previous studies have shown that bangle rhizome extract has other pharmacological activities such as anti-inflammatory and anticancer, but there has been no comprehensive study that systematically combines in vivo and in silico approaches to evaluate the antidiabetic potential of this plant (Rohmah Aulia & Rahmah, 2021). Thus, further research is needed to examine the hypoglycemic effects of ethanol extract of bangle rhizome in vivo, as well as to verify the predicted biological activity of its constituent compounds through in silico methods.

Based on this background, this study aims to evaluate the potential of ethanol extract of bangle rhizome (Zingiber purpureum Roxb.) in reducing blood glucose levels in hyperglycemic male mice induced by alloxan, and to analyze the possible mechanism of action through an in silico approach. The results of this study are expected to provide a strong scientific basis for the development of bangle as a safe, effective, and sustainable natural-based antidiabetic agent.

METHODS

Material

The main material used in this study was Zingiber purpureum rhizome (bangle), which was used in the form of ethanol extract. In addition, 96% ethanol was also used as a solvent, 0.5% CMC-Na solution as a suspending agent, alloxan monohydrate solution with a dose of 175 mg/kg body weight (BW) for hyperglycemia induction, glibenclamide with a dose of 0.65 mg/kgBW as a positive control, distilled water as an additional solvent, and test animals in the form of healthy male Swiss Webster mice, aged 8-12 weeks and weighing between 25-30 grams.

Tool

This study uses various laboratory equipment that supports the extraction process to the analysis of the results. The tools used include oral sonde, rotary evaporator, pH meter, analytical balance, glucose, cholesterol, and uric acid meters (GCU meters), test tubes, stands, droppers, injection syringes, drying ovens, centrifuges, maceration tools, blenders, laboratory animal cages, alcohol cotton, insulin needles, and other laboratory stationery that support experimental activities.

Research Procedurs

In Vivo

The bangle rhizome is first washed until clean, then thinly sliced and dried using an oven at 45°C until it reaches a dry and brittle condition. The dried rhizome is then ground using a blender to become a simple powder. A total of 100 grams of powder is macerated with 1 liter of 96% ethanol for 48 hours at room temperature with periodic stirring and solvent replacement every 24 hours. The filtrate obtained is filtered, then evaporated using a rotary evaporator to obtain a thick extract.

The test solution was prepared by dissolving glibenclamide at a dose of 0.65 mg/kgBW and alloxan at a dose of 175 mg/kgBW. The bangle extract was dissolved in 0.5% CMC-Na to produce three test doses, namely 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. Pure 0.5% CMC-Na solution was used as a negative control.

A total of 30 mice were acclimatized for seven days in plastic cages with wood shavings as a base, at room temperature with natural lighting of 12 hours of light and 12 hours of darkness. Mice were given standard feed and drinking water ad libitum. After the acclimatization period, mice were randomly divided into five treatment groups of six mice each, using the sequential randomization method.

Before the induction process, mice were fasted for 12 hours. Furthermore, alloxan was injected intraperitoneally according to the specified dose. Three days after induction, mice were fasted again for 12 hours, then blood glucose levels were measured by taking blood from the tail vein using a GCU meter. Mice that showed fasting blood glucose levels of more than 126 mg/dL were categorized as hyperglycemic mice and were used as subjects in this study.

The treatment was given orally to each group of mice for 14 consecutive days at 15.00 WIB every day. The treatment group consisted of a positive control group given glibenclamide, a negative control group given 0.5% CMC-Na, and three test groups each given bangle extract at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. Blood glucose levels were measured on the 7th and 14th days to monitor the effectiveness of the treatment.

Blood glucose level data were analyzed using IBM SPSS Statistics software version 29.0. Data normality test was performed using the Shapiro-Wilk method, while data homogeneity was tested using the Levene Test. If the data is proven to be normally distributed and homogeneous, then the One-Way ANOVA test is performed with a significance level of $p>0.05$ and continued with the LSD post hoc test to determine the differences between groups. However, if the data is not normally distributed or not homogeneous, then the Kruskal-Wallis non-parametric test is used with the same significance.

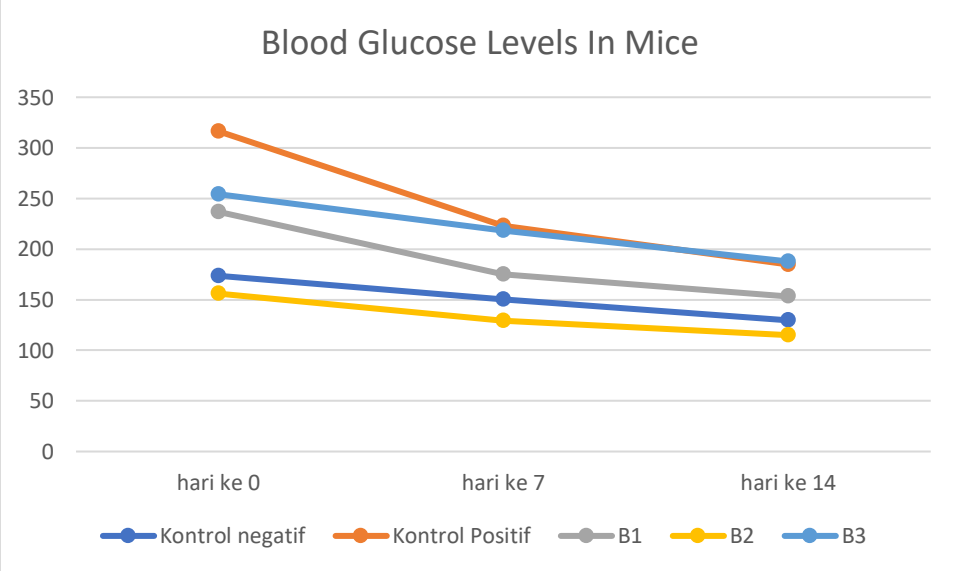
In Silico

In silico analysis was conducted to predict the biological activity of active compounds in bangle rhizomes. The chemical structure of the active compound was obtained from the PubChem database in SMILES format. The structure was then analyzed using the PASS Online (Prediction of Activity Spectra for Substances) platform which can be accessed through the page https://www.way2drug.com/passonline/predict.php. Predictions are made based on the activity probability value (Pa), focusing on compounds that have a Pa value of more than 0.7 because they are considered to have significant biological activity potential.

RESULT AND DISCUSSION

The Effect of Giving Bangle Rhizome Extract on Blood Sugar in Hyperglycemic Mice

Bangle rhizome extract was given to hyperglycemic male mice for 14 days with doses of 100 mg/kg BW, 200 mg/kg BW, 400 mg/kg BW, aimed to determine the effect of giving bangle extract on reducing blood sugar levels in hyperglycemic male mice. The results of blood sugar level measurements are presented in the graphic below.



Graphic 1. Blood Glucose Levels in Hyperglycemic Mice Treated with Bangle Extract

The effect of the extract on reducing blood sugar levels was carried out by calculating the difference in blood sugar levels on days 7 and 14 compared to day 0. The normality and homogeneity tests of the data showed that the data did not meet the parametric assumptions ($p < 0.05$). Statistical analysis was performed using the non-parametric Kruskal-Wallis test. The Kruskal–Wallis test showed that there was no significant difference in reducing blood sugar

levels between groups on day 7 (p = 0.383) and day 14 (p = 0.253). These results indicate that bangle extract has not shown significant effectiveness in reducing blood sugar levels in mice with hyperglycemia.

This study provides preliminary data on the in vivo antidiabetic potential of Zingiber purpureum. Studies on the antidiabetic effects of bangle are still limited, in contrast to *Zingiber officinale* (ginger) which has been widely studied and shows in vitro potential in increasing GLUT1 expression through stimulation of the PI3-Kinase and AMPK pathways (Noipha & Ninla-Aesong, 2018). Administration of ginger extract (2 g/kg BW) is also known to increase mitochondrial function through activation of AMPK–PGC1α (Deng *et al*., 2019). In addition, n-hexane extract from red ginger (80, 200, 500 mg/kg BW) has been shown to significantly reduce blood sugar levels (Cahyaning *et al*., 2023).

The difference in effects between bangle and ginger is thought to be due to differences in active compound content. Ginger is known to be rich in [6]-gingerol, [6]-shogaol, and paradol (Habtemariam, 2019). In vitro, [6]-shogaol and [6]-gingerol compounds are able to prevent diabetes complications by inhibiting the formation of AGEs (Zhu *et al*., 2015). In adipocyte and myotubule cells, [6]-shogaol and [6]-paradol also increase glucose consumption (Wei *et al*., 2017). In addition, [6]-gingerol increases GLP-1 levels in hyperglycemic mice through regulation of the cAMP, PKA, and CREB pathways (Wei *et al*., 2017). The main active compounds in ginger show stronger antidiabetic activity compared to bangle.

In vitro Zingiber cassumunar extract is known to have inhibitory activity against the alpha-glucosidase enzyme (IC_{50} 98.31 µg/ml) although it is still weaker than acarbose (IC_{50} 36.17 µg/ml) (Yuniarto & Selifiana, 2018). However, the hexane fraction of bangle extract showed better inhibitory activity (IC_{50} 61.02 µg/ml), although it was still lower than quercetin (IC_{50} 4.20 µg/ml) (Indrianingsih & Prihantini, 2018). This variation in results is likely due to differences in solvents and fractionation methods that affect the composition of bioactive compounds. The hexane fraction of bangle is known to be rich in compounds such as triquanecene, 1,4-bis(methoxy), and β-sesquiphellandrene (Indrianingsih & Prihantini, 2018).

It is likely that increasing the dose of bangle extract is needed to produce a significant antidiabetic effect, considering that in vitro tests show that high concentrations are needed to inhibit alpha-glucosidase activity. The main compounds in bangle, such as curcumin and terpinen-4-ol, are different from those found in ginger (Devkota *et al*., 2021). This difference is thought to be the main factor in the difference in antidiabetic activity between the two. Curcumin itself is better known as an anti-inflammatory and antioxidant agent (Yazid Yusuf *et al*., 2023), so bangle is likely to have more potential as an anti-inflammatory or antioxidant agent than as an antidiabetic agent.

***In silico* Test of Active Compounds of Bangle Rhizome**

In silico analysis was conducted to evaluate the potential of active compounds from bangle rhizome (Zingiber purpureum) against protein targets that play a role in glucose regulation and insulin sensitivity. The compounds used were derived from the literature on Zingiber montanum and Zingiber cassumunar, which are known as synonyms of bangle. The molecular structure of each compound was identified through the SMILES (Simplified Molecular Input Line Entry System) format, either from the PubChem database or drawn manually using Marvin JS if not available.
The canonical SMILES structure was further analyzed using PASS Online software to predict the potential biological activity of each compound. The prediction results are presented in the form of Pa (probability of activity) values, which indicate the possibility of a compound showing a certain biological activity under experimental conditions. The higher the Pa value, the greater the potential biological activity.

Table 1. Prediction of Bangle Compound Activity According to PASS Online

Activity	PASS Prediction	Compound
Anticancer	JAK 2 expression inhibitor	Cassumunarin A, B and C, Cassumunin A, B, C, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′-methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	HIF1A expression inhibitor	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one,
	Preneoplastic conditions treatment	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	Apoptosis agonist	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one
	MMP9 expression inhibitor	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one,
	Ubiquinol-cytochrome-c reductase inhibitor	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	AR expression inhibitor	Cassumunin A, B, C

	Free radical scavenger	Cassumunin A, B, C
	Caspase 3 stimulant	cis-3-(2′,4′,5′-Trimethoxyphenyl)-4-[(E)-2‴,4‴,5‴-trimethoxystyryl] cyclohex-1-ene, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-2‴,4‴,5‴-trimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′-methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene.
	MAP kinase stimulant	cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′-methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene
	Antineoplastic	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	Prostate cancer treatment	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	Antileukemic	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
Anti-inflammatory	Mucositis treatment	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	TNF expression inhibitor	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one
	HMOX1 expression enhancer	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one
	MAP kinase stimulant	cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′-methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene
	Antiseborrheic	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	Anti-inflammatory	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
	Anti-eczematic	(E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
Fat metabolism	1-Acylglycerol-3-phosphate O-acyltransferase inhibitor	Cassumunin A, B, C
	Antihypercholesterolemic	Cassumunin A, B, C
	Fatty-acyl-CoA synthase inhibitor	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one,
	APOA1 expression enhancer	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-2‴,4‴,5‴-trimethoxystyryl]cyclohex-1-ene
	Lipid metabolism regulator	(E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
Digestion	Carminative	(±)-trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, Cassumunin A, B, C
	Mucomembranous protector	(E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate, Cassumunin A, B, C
Detoxification agent	UDP-glucuronosyltransferase substrate	Cassumunin A, B, C
	GST P substrate	Cassumunin A, B, C
	GST A Substrate	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	GST M substrate	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one
Vitamin A synthesis	Beta-carotene 15,15'-monooxygenase inhibitor	Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	Phosphatidylcholine-retinol O-acyltransferase inhibitor	(1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatriene-3-one, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
	All-trans-retinyl-palmitate hydrolase inhibitor	(E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate
Blood clotting	Fibrinolytic	Cassumunin A, B, C, (E)-4-(3',4'- dimethoxyphenyl)but 3-enyl acetate

In its effect as an anticancer, several compound components such as Cassumunarin A, B and C, Cassumunin A,

B, C, cis-3-(3′,4′-Dimethoxyphenyl)-4- [(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′-methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4- Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene, have high activity against JAK 2 inhibitors, namely inhibiting the performance/signaling of JAK-STAT, as a producer of pro-inflammatory cytokines and cell growth, thereby preventing inflammation, cancer cell growth, and even several autoimmune diseases (Bose & Verstovsek, 2017; Nielsen *et al*., 2023). In addition, bangle compounds have very high activity against HIF1A inhibitors which play an important role in tumor growth and leukemia. HIF1A (Hypoxia-Inducible Factor 1-alpha) is a transcription factor that is activated in hypoxic conditions. Inhibiting HIF1A has the potential to disrupt the metabolic adaptation of cancer cells to a hypoxic environment, namely the state of the cell environment when there is a lack of oxygen (Xu *et al*., 2022).

Some compounds such as cis-3-(2′,4′,5′-Trimethoxyphenyl)-4-[(E)- 2‴,4‴,5‴-trimethoxystyryl] cyclohex-1-ene, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)- 3‴,4‴-dimethoxystyryl]cyclohex-1-ene, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)- 2‴,4‴,5‴-trimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(4′-Hydroxy-3′- methoxyphenyl)-4-[(E)-3‴,4‴-dimethoxystyryl]cyclohex-1-ene, (±)-trans-3-(3,4- Dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene has caspase 3 stimulant activation. Caspase 3 stimulant is a proteolytic enzyme that functions in protein breakdown, is not in active form in cells and zymogen, which means it requires stimulation to be active. Activation is carried out after the removal of the large and small subunits. The large subunit contains the active site

Cys285 in the catalytic dyad residue, which is part of the conservative pentapeptide sequence 'QACXG', and His237 (caspase-1 numbering). Activation of this enzyme plays a role in apoptosis of tumor or cancer cells (Huang *et al*., 2011; Naseer, 2022). Apoptosis is the process of eliminating damaged or unwanted cells. This process is very important for normal development, tissue homeostasis, and prevention of diseases such as cancer (Fristiohady & Agustina, 2020). In addition to the caspase 3 stimulant pathway, compounds in bangle rhizomes have MMP 9 expression inhibitor activity. Matrix Metalloproteinase-9 (MMP-9) is an enzyme that can activate VEGF (Vascular Endothelial Growth Factor) which plays a role in tumor angiogenesis, degrading collagen-IV which is a structure of extracellular cells so that it can help tumor cells to metastasize. Bangle compounds that have MMP9 expression inhibitor activity can prevent tumor cell growth and tumor angiogenesis (Augoff *et al*., 2022; Kalali, 2023).

Other anticancer potential of active bangle compounds is shown through the activity of ubiquinol-cytochrome c reductase (UQCRB) inhibitors which can suppress cancer cell growth by targeting UQCRB which reduces the activity of the mitochondrial ROS/HIF-1α/c-Met pathway, thereby inhibiting proliferation, self-renewal, and migration of cancer cells (Jung *et al*., 2018). Several bangle compounds also have potential in prostate cancer therapy, which have biological activity pathways as androgen receptor inhibitors and Testosterone 17beta-dehydrogenase (NADP+) inhibitors which suppress the development of prostate cancer cells (Jamroze *et al*., 2021; Ning *et al*., 2017). With various potential activities of bangle rhizome compounds such as Cassumunarin A, B, C which are heavily involved in cell apoptosis pathways and prevent cell proliferation, these compounds have great potential for research into their activity as anticancer.

In addition, compounds in bangle such as Cassumunin A, B, C, (1E,4E,6E)- 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and (E)-4-(3',4'-dimethoxyphenyl)but 3-enyl acetate has great potential in the anti-inflammatory activity pathway. Tumor Necrosis Factor (TNF) is an agent that produces pro-inflammatory cytokines such as IL-6 and IL-1. TNF can cause acute inflammation and chronic inflammation which if left untreated will cause neurodegenerative diseases and cancer. With the activity of the compound against TNF expression inhibitors, it will reduce TNF production thereby preventing pro-inflammatory diseases such as asthma, allergies, Parkinson's, Alzheimer's, and sclerosis (Nobari *et al*., 2024; Peng *et al*., 2021; Tomeh *et al*., 2019). In addition to the TNF pathway, bangle compounds can prevent inflammation through the Mitogen-activated protein kinases (MAPK) pathway which is an agent that produces pro-inflammatory cytokines IL-1β and IL-6 because MAPK is connected to the TNF pathway, especially TNF-α (Chowdhury *et al*., 2019; Peng *et al*., 2021).

Not only through the cytokine inhibition pathway, another pathway of anti-inflammatory activity possessed by bangle rhizome is the HMOX1 expression enhancer. HMOX1 expression enhancer is an enzyme that catalyzes the degradation of heme into biliverdin, carbon monoxide (CO), and iron ions (Fe^{2+}) which have antioxidant effects so that anti-inflammatory performance leads to reducing oxidative stress (Ryter, 2022). With various potential anti-inflammatory mechanism pathways possessed by bangle rhizome, either through inhibition of TNF expression, inhibition of the MAPK pathway, or increasing HMOX1 expression, compounds such as (E)-4-(3',4'-dimethoxyphenyl)but 3-enyl acetate, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and Cassumunin A, B, C can be potential therapies for eczema, seborrhea, and mucositis as predicted by PASS in these compounds.

In addition to the anticancer and anti-inflammatory properties of bangle rhizomes, bangle rhizome compounds have the potential to regulate fat metabolism. Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-2‴,4‴,5‴-trimethoxystyryl]cyclohex-1-ene has the potential to have activity in lipid metabolism and as an antihypercholesterolemia. Its potential as a Fatty-acyl-CoA Inhibitor plays a role in suppressing VLDL (Very Low Density Lipoprotein Lipid) production thereby reducing triglyceride production

(Jensen-Urstad & Semenkovich, 2012) and with the presence of activities such as APOA1 expression enhancer which is a component of HDL (High Density Lipoprotein), it will help reduce LDL (Low Density Lipoprotein) levels in blood vessels (Georgila *et al*., 2019). Another pathway that has the potential to suppress lipid synthesis is 1-Acylglycerol-3-phosphate O-acyltransferase inhibitor, suppressing Acyl-CoA production which is a TG synthesis agent (Yamashita *et al*., 2014). According to PASS predictions, bangle compounds have potential activity in lipid synthesis or regulating lipid metabolism.

Other potentials of compounds such as (±)-trans-3-(3,4-Dimethoxyphenyl)-4- [(E)-3,4-dimethoxystyryl]cyclohex-1-ene, (E)-4-(3',4'- dimethoxyphenyl)but 3- enyl acetate, cis-3-(3′,4′-Dimethoxyphenyl)-4-[(E)-3‴,4‴- dimethoxystyryl]cyclohex-1-ene, Cassumunin A, B, C in bangle have carminative activity that prevents the formation of gas in the intestines, thereby preventing bloating (Trie Buana *et al*., 2020) and have activity as a mucomembranous protector that works by protecting the gastric mucosa from stomach acid (Krawczyk-łebek *et al*., 2022). Cassumunin A, B, C, (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and (E)-4-(3',4'-dimethoxyphenyl)but 3-enyl acetate compounds have activity as detox agents such as Glutathione S-transferases (GST) which are detox enzymes that catalyze the conjugation of glutathione reduction which helps xenobiotic detoxification (Sainas *et al*., 2018; Valenzuela-Chavira *et al*., 2017) and UDP-glucuronosyltransferase (UGT) which catalyzes the glucuronidation reaction by conjugating the same glucuronide and making the toxin compound more polar and can be excreted in the urine (Sri Laasya *et al*., 2020). In addition, these compounds have activity in vitamin A synthesis such as Beta-carotene 15,15'-monooxygenase inhibitor which functions as an inhibitor in breaking down beta carotene into vitamin A (Kim *et al*., 2019), which has the potential to play an important role in vitamin A poisoning and has a role as a fibrinolytic, namely an agent that breaks down blood clots that block blood vessels (Novrianti *et al*., 2021; Syahbanu & Pawestri, 2023).

The results of the PASS prediction showed no direct interaction with AMPK signaling pathways, insulin sensitivity, and alpha glucosidase inhibitors, so the next method in the bioinformatics approach process was not carried out. The next method can be carried out if there is activity related to AMPK signaling pathways, insulin sensitivity, and alpha glucosidase inhibitors. Drug likeness, ADMET, and molecular analysis tests. Docking was not performed because there was no mechanism of activity related to AMPK signaling pathways, insulin sensitivity, and alpha glucosidase inhibitors and no target protein directly related to AMPK signaling pathways, insulin sensitivity, and alpha glucosidase inhibitors.

CONCLUSION

Bangle rhizome extract did not show a significant effect on reducing blood sugar levels in male mice experiencing hyperglycemia, and there were no compounds that had the potential to be α glucosidase inhibitor or able to influence the insulin regulation pathway in the context of antidiabetic activity. However, the results of in silico analysis showed a strong potential of the compounds in the extract for anticancer, anti-inflammatory, and lipid metabolism modulation activities. Therefore, it is recommended that further research be focused on evaluating the anticancer potential, investigating anti-inflammatory properties, and examining the ability of the extract to influence lipid metabolism.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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