Pharmaciana
Vol.14, No.3, Nov 2024, Page. 434-442
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v14i3.29919

Phytochemical constituent, α-amylase and α-glucosidase inhibitory activities of Black Soybean (*Glycine soja* (L.) Merr.) ethanol extract

Afifah Bambang Sutjiatmo[1], Suci Narvikasari[1], Ananda Khairunisa Solihat[1], Wahyu Widowati[2]*, Hanna Sari Widya Kusuma[3], Fadhilah Haifa Zahiroh[3]

[1]Faculty of Pharmacy, Universitas Jenderal Achmad Yani,
Jl. Ters Jend Sudirman, Cibeber, Cimahi *Sel., Cimahi 40531, West Java, Indonesia*
[2]Faculty of Medicine, Maranatha Christian University,
Jl. Surya Sumantri no 65, Bandung 40164, West Java, Indonesia
[3]Biomolecular and Biomedical Research Center, Aretha Medika Utama
Jl. Babakan Jeruk II No. 9, Bandung 40163, West Java, Indonesia,

Submitted: 02-09-2024 ***Reviewed: 10-09-2024*** ***Accepted: 06-10-2024***

ABSTRACT

Diabetes is characterized as a hyperglycemic condition impacted by β-cell dysfunction and insulin deficiency. Black soybean (*Glycine soja* (L.) Merr.) is widely known as an origin of nutritious food that has shown activities in preventing cardiovascular disease and reducing hyperglycemia. This research aimed to evaluate the potential of black soybeans ethanol extract (BSEE) as an α-amylase and α-glucosidase activity inhibitor. Black soybean seeds were extracted using the Soxhlet method with 50% ethanol as a solvent. The BSEE were screened for the presence of phytochemicals content. Inhibitory activity of α-amylase and α-glucosidase enzymes was tested in vitro with acarbose as a control. The absorbance measurement was conducted at 565 nm and 400 nm, respectively. BSEE contained alkaloids, flavonoids, polyphenols, saponins, quinones, tannins, and terpenoids. The results indicated that BSEE exhibited a weak inhibitory effect of α-amylase enzyme activity, with an IC_{50} value of 360.37 ± 20.80 µg/mL, in contrast to acarbose, which showed a significantly lower IC_{50} of 4.02 ± 0.56 µg/mL. Meanwhile, BSEE was classified as an active inhibitor of α-glucosidase enzyme activity, presenting 25.67 ± 0.27 µg/mL IC_{50} value, while acarbose demonstrated 10.85 ± 0.5 µg/mL IC_{50} value. In conclusion, BSEE inhibits α-amylase and α-glucosidase.

Keywords: α-amylase, α-glucosidase, acarbose, antidiabetic, black soybean (*Glycine soja* (L.) Merr.)

Corresponding author:
Wahyu Widowati
Faculty of Medicine, Maranatha Christian University,
Jl. Surya Sumantri no 65, Bandung 40164, Indonesia
Email: wahyu_w60@yahoo.com

INTRODUCTION

Diabetes Mellitus (DM) known as condition marked by disturbances in the metabolism of fat, proteins, and carbohydrates as a consequences of inadequate insulin secretion and Hyperglycemia. Both of which can cause damage, abnormalities, and failure in tissues and organs such as the kidneys, heart, nerves, and blood vessels even cause death (Alam et al., 2014). Several varieties of diabetes exist, including gestational, type I, type II (Baynest, 2015). Type II DM is common and accounts for between 90% of people with diabetes. Type II DM is a chronic disease caused by cell dysfunction and insulin deficiency (World Health Organization, 2019).

One of the pharmacological therapies used to treat DM is the use of oral antidiabetic which has a mechanism of inhibiting α-amylase and α-glucosidase enzyme (Gondokesumo et al., 2017; Hamid et al., 2015; Widowati et al., 2022). α-amylase can hydrolyze α-(1,4)-glucosidic bonds to form glucose and maltose, while α-glucosidase releases glucose from sucrose and maltose thereby increasing the level of glucose in the blood. By blocking the activity of these two enzymes, it is possible to postpone glucose absorption into the bloodstream, thereby helping reduce the symptoms of diabetes mellitus (Hamid et al., 2015).

Chemical drugs are widely used for the treatment of DM such as miglitol and acarbose, but some of these drugs are pricey and have some complications, such as diarrhea and abdominal distention (Dipiro et al., 2020). So many studies have been carried out to find active compounds that have antidiabetic activity derived from plants as an alternative diabetes treatment with minimum side effects. It is assumed that traditional herbal therapy has hypoglycemic properties. More than 800 plant species have been found to have anti-diabetic effects (Rosemar et al., 2014).

Many plants contain phytochemical compounds with various biological activities. These biological activities use plants or herbal medicines for treatment, including diabetes mellitus. An important group of phytochemical compounds, including phenols, flavonoids, tannins, and saponins, is responsible for most plant extracts' beneficial activities. Many phytochemical compounds are also high in antioxidants because they can scavenge ROS (Si & Liu, 2014). With this, phytochemical screening in plants is an important step toward their empirical medicinal utilization.

Soybean (*Glycine soja* L) refers to legume species originating from East Asia that is commonly cultivated for its seeds. Soybeans are widely consumed as a nutrient-rich food consisting of protein, oil, carbohydrates, and dietary fiber, as well as a large number of vitamins and minerals. Soybean seed coats have a variety of colors, including brown, black, yellow, and green. The black soybean seed coat improves insulin sensitivity and reduces hyperglycemia (Kurimoto et al., 2013). Black soybeans are also beneficial in improving blood vessel function and preventing cardiovascular disease (Yamashita et al., 2020). This study investigates the black soybean (*G. soja* (L.) Merr.)'s extract anti-diabetic activities, namely inhibitor of α-amylase and α-glucosidase, that are carried out in vitro.

MATERIALS AND METHOD

Materials

Materials used are ethanol (Merck KGaA, 10.099.831.000), Magnesium powder (Mg) powder (Merck, 1.05815.1000), HCl, Mayer's reagent, Dragendroff's reagent, Stiasny reagent, Liebermann Burchard reagent, NaOH, $FeCl_3$ reagent, α-amylase enzyme (Sigma Aldrich, A75955-50ML), ddH_2O, Lugol, DMSO, phosphate buffer (pH 7.4), p-nitrophenyl-α-D-glucopyranoside (PNPG) (N1377, Sigma Aldrich, St. Louis, USA), α-glucosidase enzyme, Na_2CO_3 (Merck, 1.063.921.000).
Equipments employed in this study are microplate reader (Thermo Fisher Logical, Multiskan GO Microplate Spectrophotometer).

Preparation of black soybean extract

The production of black soybean extract (BSEE) was carried out using the Soxhlet method of extraction. Black soybean seeds are obtained from PT Lingkar Organik, Sleman Regency, Special Region of Yogyakarta, Indonesia. Plant identification was performed at Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Padjadjaran University. The dried black soybean seeds were ground and then weighed as much as 200 g, wrapped in a filter paper sleeve, and then put into a Soxhlet tube, then as much as 2000 mL of 50% ethanol was put into a round bottom flask and

extracted until the solvent liquid dripping on the material became clear. The filtrate was concentrated utilizing a rotary vacuum evaporator set to 20 Psi and 70°C. After that, a freeze-drying process was carried out to obtain a solid extract. The dried black soybean extract was then immersed in 50% distilled ethanol, the filtrate then filtered for every 24 hours until it became colorless. Black soybean ethanol extract (BSEE) was obtained after the filtrate was evaporated, subsequently stored at 20°C (Widowati et al., 2022).

Phytochemical screening of Soybean ethanol extract

Phytochemical screening of BSEE included examination of flavonoid compounds, alkaloids, tannins, saponins, steroids/triterpenoids, quinones, and polyphenols.

Flavonoids identification

BSEE 1 g was diluted in aquadest, then heated in a water bath, and then filtered. Fill a test tube with 2 mL of filtrate, 1 mL Magnesium powder (Mg) powder, and 1 mL 2N HCl, then heat for 5 minutes and filter. To the filtrate, up to amyl alcohol 5 mL was added, shaken, and allowed to separate. The formation of a reddish-orange to purplish-red color indicates a positive reaction for the flavonoid compounds (Safrina et al., 2022).

Alkaloids identification

Alkaloids identification was initiated with subjecting 0.5 g of BSEE into a mortar and crushing it. Then 5 mL of dilute ammonia and chloroform 5 mL were added and crushed. Then the solution was filtered, and 5 mL of 2N HCl was subjected to the filtrate. The mixture then shaken and left for some time to form two layers. The top layer is splited into two test tubes. To the first tube, Mayer's reagent 3 drops were added. If a white or yellow precipitate forms, the reaction is positive for alkaloids. Add 3 drops of Dragendorff's reagent to the second tube. If an orange precipitate forms, there is a positive reaction for alkaloids (Prahastuti et al., 2019).

Tannins identification

BSEE, as much as 1 g, was dissolved in distilled water, heated in water bath, then filtered. After that, five drops Stiasny reagent were added to the filtrate. The positive reaction of tannin compounds is indicated by the formation of a pink precipitate (Benzidia et al., 2019).

Saponins identification

The test tube contained SSE 10 mg was filled with a small amount of water, and it was then brought to a boil for five minutes. The presence of foam on the surface after shaking indicates a positive reaction to the presence of saponins (Prahastuti et al., 2019).

Steroids/triterpenoids identification

BSEE was subjected as much as 10 mg to dropping plate, subsequently added with acetic acid. Sulfuric acid (H_2SO_4) (Merck, 109073) after 10-15 minutes. Green or blue color demonstrated the steroid presence. Meanwhile, red/orange sediment formation demonstrated the triterpenoid presence (Pavani & Shasthree, 2022).

Quinones identification

One gram of BSEE was dissolved in aquadest, then filtered above on an air bath. The filtrate was added with 3 drops of 1N NaOH. The positive reaction of quinone compounds is indicated by the yellow-to-red color formation (Prahastuti et al., 2019).

Polyphenols identification

One gram of BSEE was dissolved in distilled water, then heated in a water bath, and then filtered. Added 2 drops of $FeCl_3$ reagent (Merck 1.03861.0250). Green, purple, blue, red, and black are indications of positive polyphenol reactions (Rao, 2016).

α-amylase inhibitory activity assay

A modified method was utilized to evaluate the activity of α-amylase inhibition (Gondokesumo et al., 2017; Widowati et al., 2018). Subsequently, 25 µL of starch added to positive control well and sample well, then 25 µL of ddH_2O added to negative and blank control well. The 50 µL samples were added to the wells of smple and the blank wells. Each positive control well and sample received 50 µL of the α-amylase enzyme. Ten minutes at 37°C were spent incubating the plate. Each well was filled with 50 µL of HCl and 25 µL of Lugol to halt the enzymatic reaction. The microplate reader was utilized to measure the absorbance at λ = 565 nm. Mapping of the assay is shown in Table 1. Equation 1 was utilized to calculate the α-glucosidase inhibition percentage.

Table 1. Mapping of the α-amylase inhibitory assay

	Positive control well	Negative control well	Sample well	Blank well
Starch	25 µL	-	25 µL	-
ddH_2O		25 µL	-	25 µL
Sample	-	-	50 µL	50 µL
α-amylase enzyme	50 µL		50 µL	
HCl	50 µL	50 µL	50 µL	50 µL
Lugol	25 µL	25 µL	25 µL	25 µL

$$Inhibition\ (\%) = \frac{(C-S)\times 100}{C} \quad \text{.....(1)}$$

C: control absorbance
S: sample absorbance

α-glucosidase inhibitory activity assay

A modified procedure was utilized to evaluate α-glucosidase inhibitory activity (Gondokesumo et al., 2017; Widowati et al., 2021). Samples and comparisons were inserted into the wells, DMSO in the control wells, and blanks. Then, phosphate buffer (pH 7.4) and p-nitrophenyl-α-D-glucopyranoside (PNPG) (N1377, Sigma Aldrich, St. Louis, USA) were added to all wells. The α-glucosidase enzyme added to control well, sample, and comparison, incubated 30 minutes in 37°C, then sodium carbonate (Na_2CO_3) was added. The microplate reader was utilized to measure the absorbance at λ = 400 nm. Equation 1 was utilized to calculate the α-glucosidase inhibition percentage.

Statistical analysis

Statistical analysis was conducted in SPSS ver 20.0. while the GraphPad Prism 9 was utilized to analyze research data. Differences in the mean between the sample and analysis were analyzed utilizing ANOVA followed Tukey HSD post hoc test ($P < 0.05$). α-amylase and α-glucosidase inhibition activity was ascertained using linear regression based on the mean inhibition (IC_{50}).

RESULT AND DISCUSSION

Black soybean (*Glycine soja* (L.) Merr.) seed extract contains various chemical compounds. In this study, alkaloids, flavonoids, polyphenols, saponins, quinones, tannins, and terpenoids were found as secondary metabolites in BSEE. The results of which were strongly influenced by cultivars (Table 2). BSEE in other research contains tannins, saponins, alkaloids, and steroids/triterpenoids (Prahastuti et al., 2019). These polyphenols and flavonoids have been shown to contribute to treating DM by increasing cellular antioxidant activity, increasing ROS scavengers, reducing glucotoxicity, decreasing α-glucosidase activity, and increasing cellular viability (Li et al., 2017). The phytochemical content in black soybean seeds has been widely studied for use as an anti-inflammatory, anti-obesity, anti-apoptotic, and anti-dyslipidemia (Hidayat et al., 2015; Prahastuti et al., 2016; Widowati et al., 2018).

Table 2. Phytochemical screening of black seed soy extract

Phytochemical Test	Results (+/-)
Alkaloid	+
Flavonoid	+
Polyphenol	+
Saponin	+
Quinone	+
Tannin	+
Steroid Triterpenoid	+
Monoterpenoid-Sesquiterpenoid	+

+ = detected, - = not detected

α-amylase is an enzyme that contributes to the starch degradation process by hydrolyzing starch glycosidic bonds of starch. The α-amylase inhibitory activity causes inhibition of starch hydrolysis, which reduces the speed of carbohydrate digestion and absorption, resulting in less post-prandial hyperglycemia (Soeng et al., 2015). In this research, BSEE inhibited the α-amylase activity (Figure 1, Table 3). α-amylase inhibition increased when the concentration of BSEE increased. The most effective inhibitory activity of BSEE was the highest concentration (750 µg/mL) which could inhibit α-amylase activity with a percentage of BSEE inhibition of 63.88% with an IC_{50} of 360.37 ± 20.80 µg/mL, even though BSEE has less inhibitory activity than acarbose which had an 4.02 ± 0.56 µg/mL IC_{50}. Inhibition of α-amylase is caused by the presence of flavonoids in BSEE which inhibit α-amylase in two ways, specifically by directly interacting with amino acid residues on the enzyme active site and displacing the binding substrate, so that glucose cannot hydrolyze starch into maltose compounds (Zhu et al., 2020). (Jia et al. 2024) reported that genistein, one of predominant black soybean compound, binds to α-amylase and α-glucosidase through hydrophobic interactions and hydrogen bonds, forming complexes that inhibit both enzymes.

The presence of α-glucosidase inhibitory activity in Figure 2 and Table 3 indicates that BSEE can act as an antidiabetic. α-glucosidase is an important enzyme in carbohydrate metabolism because it has an impact on the breakdown of carbohydrates into glucose (Chen & Guo, 2017). According to research, inhibition of α-glucosidase can help reduce DM symptoms by delaying glucose absorption into the bloodstream (Hamid et al., 2015). α-glucosidase inhibition increased significantly with increasing BSEE concentration ($p<0.05$) (Figure 2). The most effective BSEE inhibitory activity was the highest concentration (23.81 µg/mL) which might inhibit α-glucosidase activity at IC_{50} of 25.67 ± 0.27 µg/mL (Table 4). Based on the results, the IC_{50} value is in the IC_{50} range = 25–50 g/mL which means the inhibitor is active, while the inhibition of Acarbose is in the IC_{50} range = <10 g/mL which is classified as very active (Marjoni & Zulfisa, 2017). Acarbose is an inhibitor that works competitively with the p-nitrophenyl-D-glucopyranoside substrate to bind to the active enzyme site, preventing the breakdown of the substrate into p-nitrophenol and glucose. This leads to high inhitory activity.

BSEE has inhibitory activity due to its secondary metabolite content on black soybeans. Flavonoid compounds in BSEE can competitively inhibit α-glucosidase enzyme activity by binding to the active enzyme site (Yang et al., 2021). The potential of flavonoids to inhibit α-glucosidase activity is influenced by the structure of the flavonoid, its position, and several OH groups (Proença et al., 2017). The anthocyanins contained in BSEE are also thought to provide α-glucosidase enzyme inhibitory activity. Moreover, the addition of hydroxyl groups at positions C3' and C4' on ring b can increase the strength of anthocyanins in inhibiting α-glucosidase enzyme activity (Promyos et al., 2020). Therefore, BSEE has the potential as the treatment of DM by inhibit α-glucosidase and α-amylase.

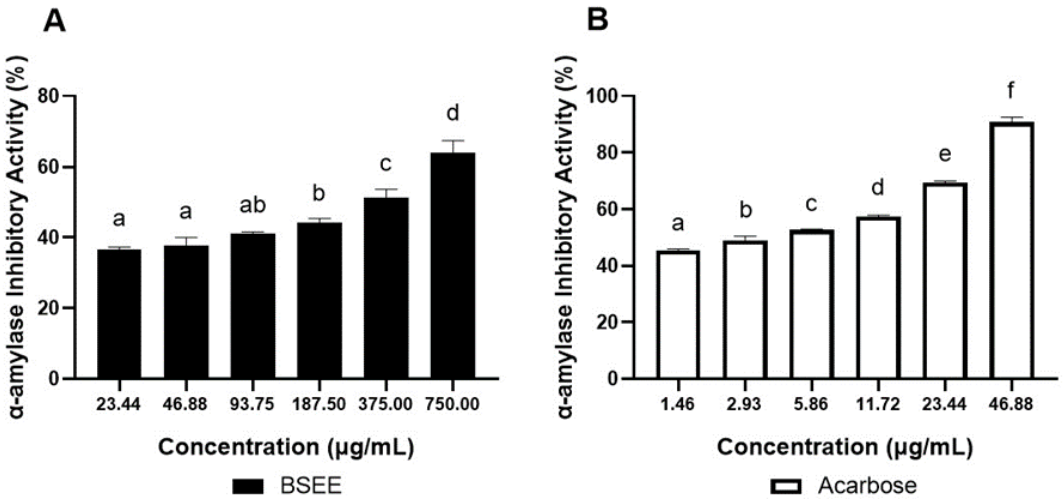

Figure 1. Histogram of black soybeans etanol extract (A) and acarbose (B) on alfa amylase inhibition

**The data is showed as mean ± SD. A: BSEE; B: Acarbose. Based on Tukey HSD post hoc test, the various superscript marks (a, b, ab, c, d) in Figure 1A and (a, b, c, d, e, f) in Figure 1B revealed significant differences between concentrations ($p < 0.05$)

Table 3. The IC_{50} (µg/mL) values of α-amylase inhibition by BSEE Acarbose

Sample	Equation	R^2	IC_{50}
BSEE	y = 0.037x + 36.722	0.992	360.37 ± 20.80
Acarbose	y = 0.968x + 45.922	0.996	4.02 ± 0.56

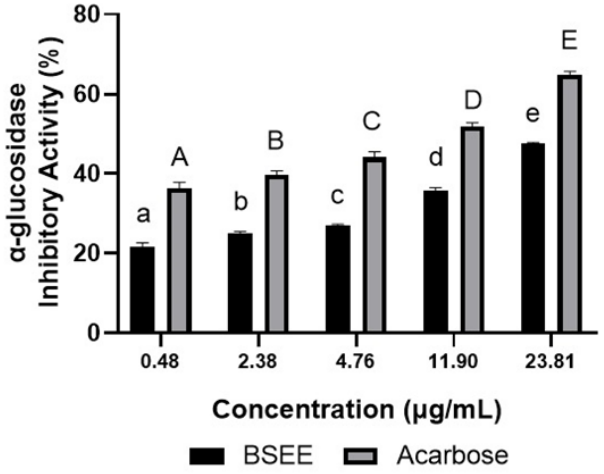

Figure 2. Histogram of black soybeans ethanol extract (A) and acarbose (B) on alfa glucosidase inhibition

**The data is presented as mean ± SD. According to Tukey HSD post hoc test, various superscript marks (a, b, c, d, e and A, B, C, D, E) revealed significant differences between concentrations ($p < 0.05$)

Table 4. The IC_{50} of α-glucosidase inhibitio by BSEE and acarbose

Sample	Equation	R^2	IC_{50} (µg/mL)
BSEE	y = 1.0939x + 21.916	0.996	25.67 ± 0.27
Acarbose	y = 1.1919x + 37.068	0.991	10.85 ± 0.5

CONCLUSION

Based on phytochemical identification, it was revealed that BSEE comprises alkaloid, flavonoid, polyphenol, saponin, quinone, tannin, steroid triterpenoid, and monoterpenoid-sesquiterpenoid. BSEE exhibited weak α-amylase inhibition activity and strong α-glucosidase inhibition activity with IC_{50} of 360.37 ± 20.80 µg/mL and 25.67 ± 0.27 µg/mL, respectively. Further research on the antidiabetic activity of BSEE in animal models of DM is recommended.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support by research grant 2019 from the Research and Community Service Center-University of Jenderal Achmad Yani, Cimahi, Indonesia. The research methodology along with laboratory facilities were provided by Aretha Medika Utama, Bandung, West Java, Indonesia. We gratefully acknowledge the contributions of Adilah Hafizha Nur Sabrina, Fadhilah Haifa Zahiroh, Annisa Firdaus Sutendi, Vini Ayuni, Dwi Nur Triharsiwi, and Faradhina Salfa Nindya from Aretha Medika Utama, Bandung, West Java, Indonesia.

REFERENCES

Alam, U., Asghar, O., Azmi, S., & Malik, R. A. (2014). *General aspects of diabetes mellitus* (pp. 211–222). https://doi.org/10.1016/B978-0-444-53480-4.00015-1

Baynest, H. W. (2015). Classification, pathophysiology, diagnosis and management of Diabetes Mellitus. *Journal of Diabetes & Metabolism*, *06*(05). https://doi.org/10.4172/2155-6156.1000541

Benzidia, B., Barbouchi, M., Hammouch, H., Belahbib, N., Zouarhi, M., Erramli, H., Ait Daoud, N., Badrane, N., & Hajjaji, N. (2019). Chemical composition and antioxidant activity of tannins extract from green rind of Aloe vera (L.) Burm. F. *Journal of King Saud University - Science*, *31*(4), 1175–1181. https://doi.org/10.1016/j.jksus.2018.05.022

Chen, G., & Guo, M. (2017). Rapid screening for α-glucosidase inhibitors from gymnema sylvestre by affinity ultrafiltration–HPLC-MS. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00228

Dipiro, J. T., Posey, L. M., Yee, G. C., Haines, S. T., Nolin, T. D., & Ellingrod, V. (2020). *Pharmacotherapy: a pathophysiologic approach* (Eleventh E). McGraw-Hill Companies

Gondokesumo, M. E., Kusuma, H. S. W., & Widowati, W. (2017). α-/β-Glucosidase and α-Amylase Inhibitory activities of Roselle (Hibiscus sabdariffa L.) ethanol extract. *Molecular and Cellular Biomedical Sciences*, *1*(1), 34. https://doi.org/10.21705/mcbs.v1i1.3

Hamid, H. A., Yusoff, M. M., Liu, M., & Karim, M. R. (2015). α-Glucosidase and α-amylase inhibitory constituents of Tinospora crispa: Isolation and chemical profile confirmation by ultra-high performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. *Journal of Functional Foods*, *16*, 74–80. https://doi.org/10.1016/j.jff.2015.04.011

Hidayat, M., Soeng, S., Prahastuti, S., Erawijantari, P. P., & Widowati, W. (2015). Inhibitory potential of ethanol extract of Detam 1 soybean (Glycine max) seed and Jati belanda (Guazuma ulmifolia) leaves on adipogenesis and obesity models in 3T3-L1 cell line. *Journal of Scientific Research & Reports*, *6*(4), 304–312. https://doi.org/10.9734/JSRR/2015/16273

Jia, J., Dou, B., Gao, M., Zhang, C., Liu, Y., & Zhang, N. (2024). Effect of genistein on starch digestion in vitro and its mechanism of action. *Foods*, *13*(17), 2809. https://doi.org/10.3390/foods13172809

Kurimoto, Y., Shibayama, Y., Inoue, S., Soga, M., Takikawa, M., Ito, C., Nanba, F., Yoshida, T., Yamashita, Y., Ashida, H., & Tsuda, T. (2013). Black soybean seed coat extract ameliorates hyperglycemia and insulin sensitivity via the activation of AMP-Activated protein kinase in diabetic mice. *Journal of Agricultural and Food Chemistry*, *61*(23), 5558–5564. https://doi.org/10.1021/jf401190y

Li, F., Zhang, B., Chen, G., & Fu, X. (2017). The novel contributors of anti-diabetic potential in mulberry polyphenols revealed by UHPLC-HR-ESI-TOF-MS/MS. *Food Research International*, *100*, 873-884. https://doi.org/10.1016/j.foodres.2017.06.052

Marjoni, & Zulfisa. (2017). Antioxidant activity of methanol extract/fractions of Senggani leaves

(Melastoma candidum D. Don). *Pharmaceutica Analytica Acta*, *08*(08), 1–6. https://doi.org/10.4172/2153-2435.1000557

Pavani, C., & Shasthree, T. (2022). Qualitative screening and quantitative determination of secondary metabolites from different plant extracts of Solanum khasianum Clarke. *Research Journal of Chemistry and Environment*, *26*(12), 113–123. https://doi.org/10.25303/2612rjce1130123

Prahastuti, S, Hidayat, M., Hasianna, S. T., Widowati, W., Amalia, A., Yusepany, D. T., Rizal, R., & Kusuma, H. S. W. (2019). Antioxidant potential ethanolic extract of Glycine max (l.) Merr. Var. Detam and daidzein. *Journal of Physics: Conference Series*, *1374*(1), 012020. https://doi.org/10.1088/1742-6596/1374/1/012020

Prahastuti, Sijani, Hidayat, M., Kurniadi, M. W., & Christiany, S. (2016). Potency of Black Soybean (Glycine max L. Merr) and Jati Belanda Leaves (Guazuma ulmifolia Lamk) for Dyslipidemia Treatment In Vivo. *Journal Of Medicine & Health*, *1*(3). https://doi.org/10.28932/jmh.v1i3.515

Proença, C., Freitas, M., Ribeiro, D., Oliveira, E. F. T., Sousa, J. L. C., Tomé, S. M., Ramos, M. J., Silva, A. M. S., Fernandes, P. A., & Fernandes, E. (2017). α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 1216–1228. https://doi.org/10.1080/14756366.2017.1368503

Promyos, N., Temviriyanukul, P., & Suttisansanee, U. (2020). Investigation of anthocyanidins and anthocyanins for targeting α-glucosidase in Diabetes Mellitus. *Preventive Nutrition and Food Science*, *25*(3), 263–271. https://doi.org/10.3746/pnf.2020.25.3.263

Rao, U. M. (2016). Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of Musa paradisiaca. *Malaysian Journal of Analytical Science*, *20*(5), 1181–1190. https://doi.org/10.17576/mjas-2016-2005-25

Rosemar, Rosidah, & Haro, G. (2014). Antidiabetic effect of roselle calyces extracts (Hibiscus sabdariffa L.) in streptozotocin-induced mice. *International Journal of PharmTech Research*, *6*(5), 1703–1711

Safrina, U., Wardiyah, W., & Cartika, H. (2022). Evaluation of total flavonoid, total phenolic, and antioxidant activity of etlingera elatior (Jack) R.M.Sm Flower, Fruit, and Leaf. *Majalah Obat Tradisional*, *27*(1), 50. https://doi.org/10.22146/mot.72210

Si, H., & Liu, D. (2014). Dietary antiaging phytochemicals and mechanisms associated with prolonged survival. *The Journal of Nutritional Biochemistry*, *25*(6), 581–591. https://doi.org/10.1016/j.jnutbio.2014.02.001

Soeng, S., Evacuasiany, E., Widowati, W., & Fauziah, N. (2015). Antioxidant and hypoglycemic activities of extract and fractions of Rambutan seeds (Nephelium lappaceum L.). *Biomedical Enginering*, *1*(1), 13–18

Widowati, W., Prahastuti, S., Hidayat, M., Hasianna, S. T., Wahyudianingsih, R., Eltania, T. F., Azizah, A. M., Aviani, J. K., Subangkit, M., Handayani, R. A. S., & Kusuma, H. S. W. (2022). Detam 1 black soybean against cisplatin-induced acute ren failure on rat model via antioxidant, antiinflammatory and antiapoptosis potential. *Journal of Traditional and Complementary Medicine*, *12*(4), 426–435. https://doi.org/10.1016/j.jtcme.2022.01.004

Widowati, W., Tjokropranoto, R., Wahyudianingsih, R., Tih, F., Sadeli, L., Kusuma, H. S. W., Fuad, N. A., Girsang, E., & Agatha, F. A. (2021). Antidiabetic potential Yacon (Smallanthus sonchifolius (Poepp.) H. Rob.) leaf extract via antioxidant activities, inhibition of α-glucosidase, α-amylase, G-6-Pase by in vitro assay. *Journal of Reports in Pharmaceutical Sciences*, *10*(2), 247–255. https://doi.org/10.4103/jrptps.JRPTPS_3_21

Widowati, W., Wargasetia, T. L., Afifah, E., Mozef, T., Kusuma, H. S. W., Nufus, H., Arumwardana, S., Amalia, A., & Rizal, R. (2018). Antioxidant and antidiabetic potential of Curcuma longa and its compounds. *Asian Journal of Agriculture and Biology*, *6*(2), 149–161

World Health Organization. (2019). *Classification of diabetes mellitus*

Yamashita, Y., Nakamura, A., Nanba, F., Saito, S., Toda, T., Nakagawa, J., & Ashida, H. (2020). Black soybean improves vascular function and blood pressure: a randomized, placebo controlled, crossover trial in humans. *Nutrients*, *12*(9), 2755. https://doi.org/10.3390/nu12092755

Yang, J., Wang, X., Zhang, C., Ma, L., Wei, T., Zhao, Y., & Peng, X. (2021). Comparative study of inhibition mechanisms of structurally different flavonoid compounds on α-glucosidase and

synergistic effect with acarbose. *Food Chemistry*, *347*, 129056. https://doi.org/10.1016/j.foodchem.2021.129056

Zhu, J., Chen, C., Zhang, B., & Huang, Q. (2020). The inhibitory effects of flavonoids on α-amylase and α-glucosidase. *Critical Reviews in Food Science and Nutrition*, *60*(4), 695–708. https://doi.org/10.1080/10408398.2018.1548428