Trop J Nat Prod Res, August 2023; 7(8):3666-3670 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical and Screening of α-Glucosidase and α-Amylase Inhibitory Activities of Five Litsea Plants from East Borneo, Indonesia

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ARTICLE INFO

Article history:
Received 16 June 2023
Revised 09 July 2023
Accepted 10 August 2023
Published online 01 September 2023

ABSTRACT

The Lauraceae genus Litsea includes many tree species with pharmacological capabilities, including anti-diabetic benefits. Five Litsea plant species were tested for their anti-diabetic activity on α-amylase and α-glucosidase. This study evaluated phytochemical composition, phenolic and flavonoid levels, and α-amylase and α-glucosidase inhibitor activity of *Litsea elliptica*, *Litsea ferruginea*, *Litsea firma*, *Litsea garciae*, and *Litsea* sp. Phytochemical screening was performed using specific reagents in reaction tubes, and UV-Vis spectrophotometry evaluate extract phenolic and flavonoid levels. The *α*-amylase and *α*-glucosidase inhibitor activities were determined spectrophotometrically, with acarbose utilized as a positive control. All extracts had rich phytochemical profiles, as evidence by the occurence of alkaloids, flavonoids, phenols, tannins, saponins, and steroid/triterpenoid, according to the findings. *L. firma* extract was found to have the greatest phenolic levels, measuring 31.96 ± 1.2 g GAE/100 g, as determined by the Folin-Ciocalteu technique. Additionally, the *Litsea* sp. extract exhibited the highest content of flavonoid compounds, measuring 1.88 ± 0.04 g QE/100 g. Furthermore, the *L. firma* extract showed the strongest inhibition activity on *α*-amylase and *α*-glucosidase compared to the other four Litsea species, with IC_{50} values of 52.1 ± 1.01 and 55.21 ± 1.46 µg/mL, respectively. These findings establish a positive association between the concentration of phenolic compounds and *α*-amylase and *α*-glucosidase inhibitor activity. In conclusion, these study highlights the potential by Litsea genus plants as an alternative source of biologically active compounds with antidiabetic properties. The observed noteworthy inhibitor activities of *α*-amylase and *α*-glucosidase implies their potential as viable therapeutic options for the diabetes management.

Keywords: *α*-Amylase, *α*-Glucosidase, Flavonoid, Litsea, Phenolic

Introduction

Chronic metabolic diseases known as Diabetes Mellitus (DM) was defined by an elevated levels of blood glucose (≥200 mg/dL). Diabetes was classified into two varieties: type 1 diabetes (DMT1) and type 2 diabetes (DMT2). DMT1 was attributed to a conginetal anomaly that hindered the body's ability to synthesise adequate amounts of insulin when DMT2 was characterized by a diminished responsiveness of the body's cells to insulin, resulting in insulin resistance. 90% of cases of DM are DMT2 and are often brought on by an unhealty lifestyle, such as consuming too many foods and drinks that are high in energy and not moving around enough. The International Diabetes Federation (IDF) reports that diabetes diagnoses are increasing globally on an annual basis.[1]

DMT2 treatment option had been thoroughly investigated. The *α*-glucosidase inhibitors that are commonly prescribed for treatment type 2 DM encompass acarbose, miglitol, and voglibose. These medications help patients with DM in managing their postprandial glucose levels in their blood by preventing hydrolysis of disaccharides into glucose. However, there are complications associated with using these kinds of drugs.

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Citation: Rijai AJ, Fidrianny I, S Sukrasno. Phytochemical and Screening of *α*-Glucosidase and *α*-Amylase Inhibitory Activities of Five Litsea Plants from East Borneo, Indonesia. Trop J Nat Prod Res. 2023; 7(8):3666-3670 http://www.doi.org/10.26538/tjnpr/v7i8.15

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

There have been reported asssociations between these medications and gastrointestinal symptoms such as diarrhoea and flatulence, as well as more serious adverse effects including cholestasis and metabolic hypoglycemia.[2] In conclusion, with the increasing number of diabetes cases worldwide and the potential risks associated with current DMT treatment options, there has been ongoing investigation into alternative pharmaceuticals with fewer adverse effects. One promising option being explores was the use of natural sources, such as Litsea genus plants, which had been studied for their antidiabetic effects.[3]

Litsea is a genus of evergreen shrubs and trees in the Lauraceae family, and is known for its remarkable diversity, comprising approximately 200-400 tree species. These species can be found in large numbers across Asia's tropical and subtropical climates, as well as in North and South America. The Litsea genus displays a broad range of biological and pharmacological activities, including its potential as an anti-diabetic agent. Previous studies have extensively examined and evaluated the chemical constituents of Litsea plants.[4] Sun *et al* (2018) observed that *Litsea coreana*'s total flavonoid content lowered blood glucose in DMT2-treated rats.[5] In another investigation by Chakraborty and Mandal (2018), it was discovered that methanolic extract derived from *Litsea cubeba* demonstrated inhibitory abilities on *α*-amylase and *α*-glucosidase.[6] Furthermore, Kuspradini *et al* (2018) documented the presence of approximately 38 species of Litsea plants in East Borneo, with 26 of them being identified in Wanariset Samboja.[7] These plants have a long history of traditional uses, making it intriguing to explore whether Litsea plants, similar to other members of the genus, possess antidiabetic properties.

Litsea elliptica, *Litsea ferruginea*, *Litsea firma*, *Litsea garciae*, and *Litsea* sp. were among the Litsea plants that garnered particular interest for research due the limited reporting their phytochemical profiles and pharmacological activities, specifically their antidiabetic potential.

Previous research on other Litsea plants has indicated that phenolic and flavonoid compounds have potential as antidiabetic agents. In their study, Yang *et al* (2023) identified a total of sixteen phenolic compounds from the pericarp of *Sterculia nobilis*. These compounds were found to exhibit uncompetitive inhibition on *α*-amylase and *α*-glucosidase. The IC_{50} concentration for inhibiting *α*-amylase and *α*-glucosidase by the ethyl acetat fraction of *S. nobilis* (EAF) were determined to be 2.151 ± 0.044 and 69.390 ± 1.410 µg/mL, respectively.[8] Out of the 16 phenolic compounds mentioned, three compounds had been previously found in Litsea plants, namely catechin from *L. coreana*, isoquercitrin from *Litsea acuminata*, *Litsea acutivena*, *L coreana*, *Litsea hypophaea*, *Litsaea lii*, and *Litsea morrisonensis*, and vanillin from *L. hypophaea*.[4]

Considering the information presented, it was imperative to further explore the association between antidiabetic activity in other Litsea plants and their phenolic and flavonoid contents. The focus of this investigation was to assess the potential inhibitor of Litsea plant extracts on *α*-amylase and *α*-glucosidase. Additionally, we aimed to investigate any potential correlation between the inhibitory activity and phytochemical of these extract. This investigation contributes to expanding our understanding of the potential therapeutic applications of Litsea plants in diabetes management and provides valuable insights into the relationship between their bioactive compounds and antidiabetic properties.

Materials and Methods

Chemicals and reagents

The study utilized *α*-glucosidase (G5003) from *Saccharomyces cerevisiae*, *α*-amylase (A1031) from human saliva, starch from potato (S2004), 4-nitrophenyl-*α*-D-glucopryranoside (*p*-NPG, N1377), 3,5-dinitrosalicylic acid (D0550), quercetin, gallic acid, and acarbose. These substances were all purchased from Sigma Aldrich (Saint Louis, USA). All other reagents, including the Folin-Ciocalteu reagent, were analytical grade and came from Milipore Co. (Merck, Darmstadt, Germany).

Plants material and sample preparation

Litsea plants were obtained from Kawasan Hutan dengan Tujuan Tertentu (KHDTK) Samboja in March 2021. The collected plants were authenticated by a qualified botanist affiliated with Balai Penelitian Teknologi Konservasi Sumber Daya Alam (Balitek KSDA) Samboja, East Borneo with specimen voucher number FF3.21 for *Litsea firma*, FF4.21 for *Litsea garciae*, FF5.21 for *Litsea elliptica*, FF6.21 for *Litsae ferruginea*, and FF7.21 for *Litsea* sp. The barks were immediately washed, then sliced and dried for 72 h in an oven at 50° C. The plant is then ground to produced powdered bark.

Sample extraction

The extraction was performed using the reflux method with ethanol 96% at a powder-to-solvent ratio of 1:10. About 100 g of bark powder was extracted for 1 h, and then the extract was filtered. Extraction was performed three times employing solvent exchange. A rotary evaporator was used to concentrate the ethanolic extract at 40 ºC to produce a crude extract, was then used for experiments. The phytochemical test on extracts was carried out using the procedures specified in Farnsworth and Sarker's method.[9,10] Extract density determination was conducted for all extract. First, a 1% extract was prepared using 96% ethanol. Next, the extract was poured carefully into a 5 mL pycnometer until it reached the mark. The weight of both the empty pycnometer and the pycnometer filled with the extract were recorded. For reference, the specific gravity of water was assumed to be 1 g/mL. finally, the extract density was calculated using formula:

$$extract\ density = \frac{weight\ of\ pycnometer\ and\ extract - weight\ of\ empty\ pycnometer}{volume\ of\ pycnometer}$$

Total phenolic content (TPC)

The assessment of TPC was conducted using Pourmorad's method with slight modification, employing the Folin-Ciocalteu reagent.[11] The experimental procedure involved the combination of 50 µL Folin-Ciocalteu reagent and 400 µL aqueous Na_2CO_3 solution. The mixture was allowed to rest for fifteen minutes. The measurement of absorbance at a wavelength of 765 nm was conducted. The results were quantified by employing a calibration curve of gallic acid spanning a concentration range of 60-130 g/mL. The measurement of gallic acid equivalent (GAE) per 100 g extract was utilised.

Total Flavonoid Content (TFC)

Chang's technique was modified to measure flavonoid content.[12] A mixture was prepared by combining 100 µL of plant extract in methanol with 300 µL methanol, 20 µL of 10% aluminium chloride solution, 20 µL potassium acetate, and 560 µL distilled water. Absorbance was recorded at 415 nm after 30 minutes at room temperature. The analysis of each extract was conducted on three separate occasions. The outcome was reported as grammes quercetin equivalents (QE) per 100 grammes extract (g QE/100 g), utilising the calibration curve with a range of 40-110 g/mL as the standard reference.

α-Amylase inhibitory activity

The measurement of *α*-amylase inhibitory activity occurred using Odhav's modified method.[13] Following dissolution in dimethyl sulfoxide (DMSO), the extract underwent dilution with pH 6.9 phosphate buffer. The concentration of DMSO in the solution was less than 0.5%. 500 µL extract solution was mixed with 500 µL of *α*-amylase solution (1 U/mL in phosphate buffer). The mixture was incubated at of 37 ºC for 30 minutes. Next, 500 µL of 1% w/v starch solution was added to the mixture and incubated at 37 ºC for 10 minutes. After adding 500 µL DNS reagent and heating for five minutes in scalding water, the reaction was terminated. Absorbance was measured at 540 nm. The acarbose and extract evaluation were conducted in triplicate. The percent inhibition of *α*-amylase inhibitor activity quantified using the equation provided.

$$percent\ inhibition\ [\%] = \left[\frac{A - B}{A}\right] x\ 100$$

The percent inhibition from measurement of *α*-amylase absorbance without the test extract (A) and with the test extract (B) calculated the IC_{50} value. Acarbose was a positive control in this investigation.

α-Glucosidase inhibitory activity

α-glucosidase inhibitory activity was evaluated with spectrophotometer in 96-well plates (Biologix®), following the method described by Kim *et al*. (2005) with modest modification.[14] DMSO dissolved and diluted the extract in a 6.8 pH phosphate buffer. DMSO did not surpass a concentration of 0.5%. The experimental procedure consisted of combining 30 µL extract solution, 50 µL phosphate buffer, and 20 µL of freshly prepared *α*-glucosidase solution (0.5 U/mL in phosphate buffer). The solution was subsequently to a pre-incubation step at a temperature of 37 ºC for 5 minutes. Following the pre-incubation step, a volume of 20 µL *p*-NPG was introduced to experimental setup, with a concentration of 5 mM. The incubation of the mixture took place at a temperature of 37 ºC for 15 minutes. At last, 100 µL of 0.1 M sodium carbonate was added. The absorbance was recorded at 405 nm using microplate reader. The *α*-glucosidase inhibitory was examined with the appropriate formula:

$$percent\ inhibition\ [\%] = \left[\frac{A - B}{A}\right] x\ 100$$

The IC_{50} value was determined by calculating the absorbance from *α*-glucosidase in the absence of the extract (A) and comparing it to the absorbance of extract (B). In this experiment, acarbose was the positive control.

Statistical analysis

The analyses were performed in triplicate, and outcomes were presented as the mean ± standard deviation (SD).

Results and Discussion

In the present investigation, total phenolic and flavonoid content, *α*-amylase and *α*-glucosidase inhibitory activities of five Litsea plants from East Borneo, *L. elliptica* (LE), *L. ferruginea* (LFE), *L. firma* (LFI), *L. garciae* (LG), and *Litsea* sp (LSP) were evaluated. The samples were

extracted by reflux using 96% ethanol in this study. The extraction yields for LE, LFE, LFI, LG, and LSP were 9.28, 10.43, 13.74, 12.27 and 10.48%, respectively. The yield analysis in this study is significant since it may be used to measure the extraction process's success rate, with the higher the yield value, the higher the compound components produced. According to the result of the phytochemical screening in Table 1, all extracts contained alkaloids, flavonoids, phenol, tannin, saponin, and steroid/triterpenoid. Meanwhile, no steroid/triterpenoid was found in LE, LFE and LG, although saponin was found in LFE.

The extract density was measured as an essential assay for comparing its activity and phytochemical component levels. The density of each extract in this investigation ranged from 0.78 to 0.81 g/mL, measured using a pycnometer with a 1 % extract solution. The assessment of phenolic compounds of the five Litsea plants was conducted using the Folin-Ciocalteu assay. TPC in all five ethanolic Litsea extracts ranged from 6.35 to 31.96 g GAE/100 g, with LFI ethanolic extract exhibiting the greatest TPC at 31.96 ± 1.2 g GAE/100 g. as shown in Table 2. Prior research by Aleixandre *et al.* (2022) demonstrated that phenolic compounds, such as phenolic acids, could reduce the *α*-amylase and *α*-glucosidase activities, with some compounds showing similar IC_{50} values to acarbose.[15] TFC leves in all ethanolic Litsea extracts was started from 0.61 to 1.88 g QE/100 g, with LSP revealing the highest TFC at 1.88 ± 0.04 g QE/100 g. Many studies have shown that flavonoids, including their various subclasses, possess significant inhibitory properties towards *α*-amylase and *α*-glucosidase.[16,17] Kaempferol-3-(*p*-coumaroyl-diglucoside)-7-glucoside obtained from *Persicaria hydropiper* L. exhibited strong affinity to *α*-glucosidase.[18] Additionally, phenolic compounds that have been isolated from *Glycine max* and *Phaseolus vulgaris*, such as myricetin, exhibited potent inhibitory activity on *α*-amylase and *α*-glucosidase.[19] The present study's results align with previous reports, indicating a close relationship between phenolic and flavonoid content and the inhibitory effects on *α*-amylase and *α*-glucosidase.

Five Litsea plants were evaluated for their *α*-amylase inhibitory (AAIs) activities. Acarbose was utilised as the standard. AAIs activities in all ethanolic Litsea extracts presented various results from 52.1 to 194.68 µg/mL. Ethanolic extract of LFI gave the highest AAIs activity (52.1 ± 1.01 µg/mL) where the IC_{50} value was only 2 times lower than acarbose. The *α* – glucosidase inhibitory (AGIs) activities were evaluated with acarbose as a standard. The IC_{50} values of AGIs activities of all extracts presented different results from 55.21 to 189.4 µg/mL. The ethanolic extract of LFI (55.21 ± 1.46 µg/mL) showed the highest AGIs activity from all extracts. It's IC_{50} was almost the same as standard acarbose.

Table 3 shows extracts with lower IC_{50} had stronger AAIs and AGIs activity. The most active LFI ethanolic extract exhibit AAIs and AGIs IC_{50} of 52.1 and 55.21 µg/ mL, respectively. Meanwhile, the value for standard acarbose was 24.1 µg/mL for AAIs and 55.95 µg/mL for AGIs. According to prior research, phenolics and flavonoids has AAIs and AGIs activity. Wang *et al.* reported that some quercetin, isobavachalcone, morachalcone and jabitocabin that were isolated from mulberry fruit had AGIs activity with a IC_{50} value of 8.57, 67.3, 49.96, and 31.7 µM, respectively.[16]

Based on the obtained results, screening phytochemicals, total phenolic, and flavonoid revealed that that all ethanolic extract of the plants are rich in flavonoids and phenolics compounds. Previous studies have demonstrated that phenolic and flavonoids compounds possess inhibitory effect on the enzymatic activity of *α*-amylase and *α*-glucosidase.[20,21] AAIs and AGIs activities in some plants showed a correlation with their total phenolic content.[22] Furthermore, it has been observed that structure-activity relationship of flavonoids play a crucial role in influencing their inhibitory activity on *α*-amylase and *α*-glucosidase. Specifically, glycosylation from hydroxyl groups at position 3 and/or 7 has been found to significantly reduce the inhibitory activity, while glycosylation at both positions leads to a notable decrease in inhibitory activity. However, ring B with more hydroxyl groups inhibits these enzymes adequately. The findings highlight the importance of specific structural features in modulating the inhibitory potential of flavonoids.[16] The ethanolic extract of LFI had the greatest AAIs and AGIs activity. This activity may be correlated to the elevated TPC and TFC levels in LFI relative to other extracts. These findings support previous studies that highlight the inhibitory properties of phenolic and flavonoid compounds about the *α*-amylase and *α*-glucosidase.[8,23,24] The results clearly demonstrate the AAIs and AGIs potential of the selected Litsea extracts. The relationship between phenolics, flavonoids, and the activities of AAIs and AGIs is crucial for controlling postprandial glucose levels and managing diabetes. Limiting the metabolism of carbohydrate-digesting enzymes especially *α*-amylase and *α*-glucosidase can reduce the breakdown and absorption of carbohydrates in the gastrointestinal tract. AAIs act as carbohydrate blockers, while AGIs delay carbohydrate degradation and intestinal absorption, leading to hypoglycemic effects. Phenolic compounds and flavonoids, including iminosugars, thiosugars, flavonoids, alkaloids, and terpenes have been identified as natural AGIs. These inhibitors work by directly blocking the active centres of the enzyme, reducing polysaccharide degradation and delaying carbohydrate absorption. The inhibition of these enzyme is significant for preventing and treating diabetes, hyperglycaemia, hyperlipidemia and obesity. Further research and exploration of phenolic compounds and flavonoids as potential inhibitors of carbohydrate digestive enzymes can contribute to the development of effective strategies for diabetes management.[25] Egharevba *et al* (2019) found that phenolic and flavonoid levels affect *in vivo* antidiabetic performance. They reported that the phenolic and flavonoid rich methanolic extract of *Stachytarpheta jamaicensis* leaves had the ability to reduce blood sugars levels in rodents induced with streptozotocin (STZ).[26] Moreover, it is worth noting that extracts abundant in phenolic and flavonoid compounds often possess high antioxidant activity, which not only contributes to their anti-diabetic effects but also impacts various other pharmacological activities.[27]

Conclusion

Alkaloids, flavonoids, phenols, tannins, saponins, and steroid/triterpenoids were present in the ethanolic extracts of five Litsea plants in the present investigation. These extracts exhibited *in vitro* activity as AAIs and AGIs with varying IC_{50} values compared to acarbose. The ethanolic extract of LFI demonstrated the most significant AAIs and AGIs activity with IC_{50} values of 52.1 and 55.21 µg/mL, respectively. The heightened activity can be attributed to the elevated quantities of TPC and TFC in LFI relative to other extracts.

Table 1: Phytochemical screening of five Litsea extracts

Sample	Phytochemical screening					
	Alkaloid	**Flavonoid**	**Steroid/ Triterpenoid**	**Saponin**	**Tannin**	**Phenols**
LE	+	+	-	-	+	+
LFE	+	+	-	+	+	+
LFI	+	+	+	+	+	+
LG	+	+	-	-	+	+
LSP	+	+	+	+	+	+

(-) not detected; (+) detected. LE = *L. elliptica,* LFE = *L. ferruginea,* LFI = *L. firma,* LG = *L. garciae,* LSP = *Litsea* sp

Table 2: The yield, density of extract, TPC, and TFC of Litsea extracts

Sample	Yield (%)	The density of extract 1% (g mL^{-1})	TPC (g GAE/100 g)	TFC g QE/100 g
LE	9.28	0.816	22.5 ± 0.17	1.23 ± 0.26
LFE	1.43	0.787	6.35 ± 0.25	0.61 ± 0.03
LFI	13.74	0.801	31.96 ± 1.2	1.73 ± 0.13
LG	12.27	0.787	14.46 ± 0.57	1.52 ± 0.03
LSP	10.48	0.793	31.22 ± 0.96	1.88 ± 0.04

LE = *L. elliptica*, LFE = *L. ferruginea*, LFI = *L. firma*, LG = *L. garciae*, LSP = *Litsea* sp

Table 3: AAIs and AGIs of five ethanolic Litsea extracts

Sample	IC_{50} (µg/mL)	
	AAIs	AGIs
LE	62.41 ± 2.12	60.43 ± 2.16
LFE	194.68 ± 3.74	189.4 ± 3.83
LFI	52.1 ± 1.01	55.21 ± 1.46
LG	60.71 ± 1.05	59.96 ± 3.00
LSP	55.31 ± 2.21	55.95 ± 3.85
Acarbose	24.1 ± 1.88	55.95 ± 3.84

Values are expressed as mean ± standard deviation of replicate experiments (n=3)

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors respect the facilities and support of the Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Indonesia.

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