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Quantification of Bioactives and Bioactivities in Different Parts of *Abelmoschus esculentus*

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Abstract. The presence of natural antioxidants in medicinal plants plays a crucial role in inhibiting the detrimental effects of oxidative stress. The aim of this research is to explore more deeply all parts of A. esculenta L from flowers, fruits, seeds, leaves, and stems for the levels of compounds and antioxidant and enzyme inhibitor activities. The flowers demonstrated the highest TPC with 173.15942 ± 6.5083 mg GAE/g. The stems exhibited the lowest TPC value at 69.1967 ± 2.8408 mg GAE/g. The flowers also showed TFC value of 83.157 ± 2.021 mg QE/g while the stems displayed the lowest with 36.7240 ± 1.337 mg QE/g. IC_{50} value that the flowers possessed the highest antioxidant activity with 22.6539 ± 1.6452 μg/mL, whereas the stems displayed a slightly lower. In terms of the inhibitor of α-amylase activity, the flowers had an IC_{50} value of 102.4885 ± 11.4370 μg/mL whereas the stems had a lower. The highest IC_{50} value of the α-glucosidase inhibitor was 76.95 ± 12.0888 μg/mL in the flowers, and the lowest was in the stems. The highest IC_{50} of pancreatic lipase inhibitor was 109.5943 ± 9.7391 μg/mL in the flowers, and the lowest was in the stems. This study show that there is a relationship between the high content of total phenolic and total flavonoids on antioxidants, antidiabetic and antilipase activities.

Keywords: *Abelmoschus esculentus* L.; Antioxidant, α-Amylase; α-Glucosidase; Pancreatic Lipase Inhibitor

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INTRODUCTION

Since ancient times, medicinal plants have been utilized to cure a range of illnesses in a variety of traditional herbal remedies. According to the World Health Organization (WHO), herbal therapy is the main therapeutic approach utilized by about 80% of the global population to address health issues (World Health Organization 2019). Furthermore, in wealthy countries, 25% of prescription drugs have active ingredients derived from plants (Khan and Ahmad 2019). Many plant extracts are used in natural pesticides, food additives, medications and nutraceuticals (Lourenço et al. 2019). The primary source of the health benefits of plants is their secondary metabolites or bioactive compounds (Wawrosch and Zotchev 2021).

Many phytochemicals are naturally present in plants and products derived from plants, including phenols, flavonoids, alkaloids, glycosides, lignins, and tannins. Phenols and flavonoids are the most common phytoconstituents found in a wide range of fruits, vegetables, aromatic plants, and medicinal plants, and they offer the largest potential for use in pharmaceutical applications (Pinto et al. 2021). Due to possible toxicological effects or synthetic antioxidants, natural antioxidants derived from plants, such as phenols and flavonoids, are gaining popularity these days

(Kiani et al. 2022). An antioxidant is a substance that scavenges such free radicals as peroxide or hydroperoxide, thereby preventing or delaying the risk of oxidative damage to an organism's cells.

Abelmoschus esculentus L. is a plant with excellent nutritional content and antioxidant activity that is often eaten as a vegetable. It is a member of the genus Okra and the family Malvaceae. *A. esculentus* is known to contain high levels of polyphenol and flavonoid, which are important bioactive compounds in plants (Elkhalifa et al. 2021). Research on *A. esculentus* fruit has been carried out on extracts, mucilage or flour and the results were obtained that *A. esculentus* fruit has antioxidant and antidiabetic activities (Dantas et al., 2021; Fatima et al., 2024; Romdhane et al., 2020; Panighel et al., 2022; Reine et al., 2018). The metabolism of fat and carbohydrates, insulin resistance, dyslipidemia, hyperglycemia, and oxidative stress in an inflammatory process are all influenced by polyphenol compounds. They also improve the metabolism of adipose tissue. Inhibition of α-amylase and α-glucosidase enzymes can be utilized in antiobesity and antidiabetic drugs to reduce glucose and excess fat deposition in the body by blocking the breakdown of polysaccharides (Shahwan et al. 2022).

Such polyphenols as flavonoids have the ability to inhibit the α-amylase and α-glucosidase enzymes (Ćorković et al. 2022). Flavonoids have conjugated bonds and specific hydroxyl groups at the C7 and/or R4' positions of the enzyme catalytic residue that help to form hydrogen bonds and stabilize the interaction between the inhibitors (flavonoids) and the residue. Polyphenols, which are also known to have enzyme inhibitory activity against amylase, prevent the hydrolysis of α-1,4-glicoside in carbohydrates by forming quinone or lactone structures or compounds containing α-oxo-pyrane structures (Jakaria et al. 2019).

In other conditions, if excess sugar is hydrolyzed but not immediately utilized, the body will store it in the adipose tissue. Obesity due to increased intake of fats and carbohydrates causes an increase in triglyceride levels which is the main cause of hyperlipidemia (Wang et al. 2017). Hyperlipidemia is a condition that indicates increasing lipids in the blood plasma, either cholesterol or triglycerides or both. Abnormally increased fat in the bloodstream allows Low Density Lipoprotein (LDL) to be deposited in artery walls and develop into atherosclerotic plaques. The presence of atherosclerotic plaques will then cause blockage of arteries, thus leading to hypertension, insulin resistance, dyslipidemia, reduction in the amount of oxygenated blood reaching the heart, and increased risk of coronary heart disease (CHD) and atherosclerosis (Roy et al. 2023). One of the developed and circulating mechanisms of action of antihyperlipidemic medication is inhibiting fat absorption by blocking the activity of the lipase enzyme.

All parts of the *Abelmoschus esculentus* plant are thought to be rich in active compounds that have certain pharmacological activities. The benefits in this study is to explore more deeply all parts of *A. esculenta* L from flowers, fruits, seeds, leaves, and stems for the levels of compounds and activities. Further study is conducted to test the activity of the extracts of *Abelmoschus esculentus* parts as an antioxidant and inhibitor of α-amylase and α-glucosidase as well as anti-pancreatic lipase. Hence, the goal of this work was to evaluate the influence of total phenolic and total flavonoid on antioxidant capacity and inhibitor enzymatic. Compounds with high antioxidant activity are expected to be able to provide good activity against enzymatic inhibitor activity which can be used as one of the mechanism for treatment of diabetes and obesity. In this study, active compounds in all parts of plant will be examined and wether the amount of polyphenol or flavonoid content affects the activity of antioxidants, α-amylase, α-glucosidase and pancreatic lipase inhibitors.

METHODS

The sample used was the Okra fruit (*Abelmoschus esculentus* L.) collected in June 2024 from Purwodadi Regency, Central Java Province, Indonesia (Figure 1). This plant material were authentically identified by the Department of Biological Sciences of Semarang Pharmaceutical College, Semarang, Indonesia with number 041/EL-AFM/IV/2025

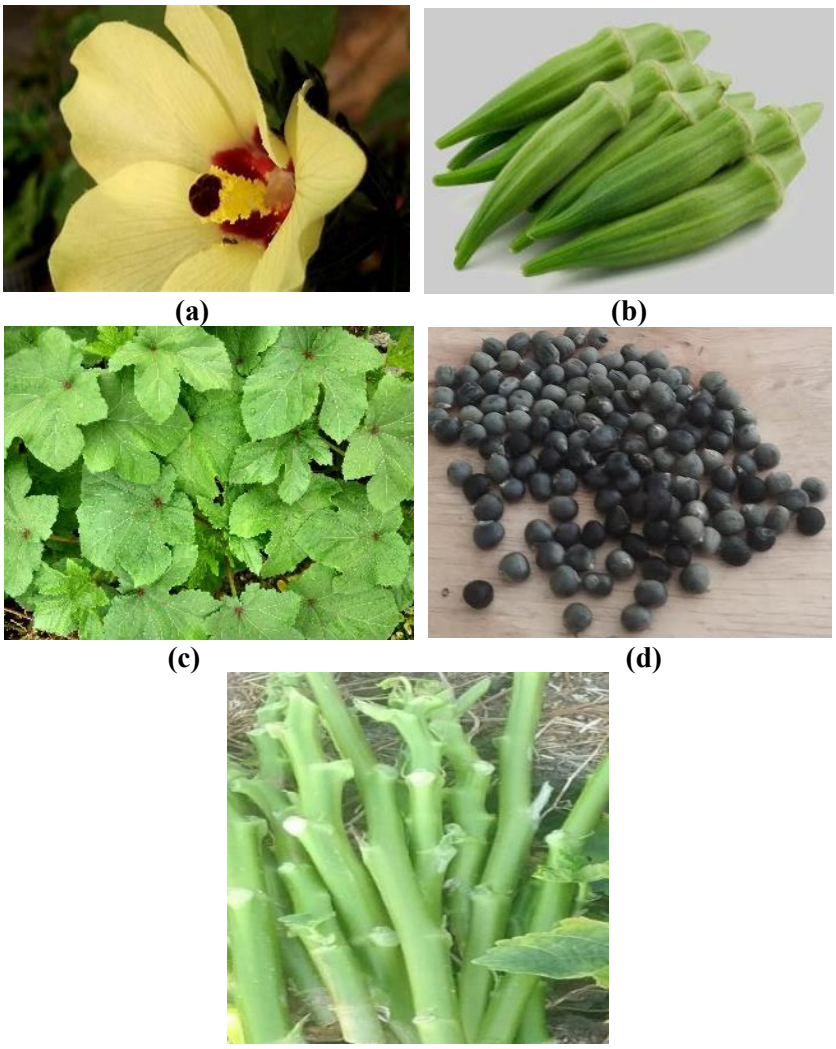

Figure 1. Different part of Okra (*Abelmoscus esculentus*) (a) flowers; (b) fruits; (c) Leaves; (d) Seeds; and (e) Stems

Extraction

Different parts of the plant were first cleaned to remove dust and other unwanted elements then dried to reduce water content and stop microbial growth. After that, the fruit was ground into a fine powder. One liter of 80% ethanol was added to an Erlenmeyer flask containing about 200 g of the powder. The sample was macerated for five days. This process was carried out repeatedly until about 150 g of viscous extract was produced. The extract was stored at 4°C for further analysis (Astutiningsih and Anggraeny, 2023).

Qualitative Testing

Flavonoid. The sample was mixed with powder Mg, 1 mL $HCl_{(P)}$, and 1 mL amyl alcohol. Positive flavonoids in the amyl alcohol layer would show a yellow, orange to red color.

Phenolic. The sample was mixed with 1% $FeCl_3$, and positive phenolics would show a black green or black blue color.

Saponin. The sample was mixed with boiling water and shaken until foam was formed. If the foam dripped with 1 drop of 2N HCl, then it would remain stable.

Alkaloid. The sample mixed with 1 mL HCl 2N, 9 mL boiling aquadest, and Dragendorff's reagent would form an orange precipitate when it was alkaloid positive. With Mayer's reagent, a white precipitate would form, and with Wagner's reagent a brown precipitate would form.

Steroid. The sample added with 2 mL chloroform, 0.5 mL anhydrous acetic acid, and concentrated sulfuric acid would turn green if it contained steroids and blue or red if it had triterpene compounds.

(Sonam et al., 2017; Roghini and Vijayalakshmi 2018; Shaikh and Patil, 2020)

Total Phenolic Content

The total Phenolic Content (TPC) in different extracts of the flowers, fruits, leaves, seeds, and stems of *Abelmoschus esculentus* was determined by using the Folin–Ciocalteu (F–C) method with gallic acid as the standard and the principle of reduction and oxidation reaction (Kim, 2020). The standard curve measurement used the gallic acid concentration series of 20, 40, 60, 80, and 100 µg/mL at a wavelength of 738nm. One mL sample was added with 1.5 mL (1:10) of Folin-Ciocâlteu reagent. Next, 10 mL of distilled water and 1.2 mL of 7.5% Na_2CO_3 were added (Ghazi et al. 2012). For 90 minutes, the solution was incubated at a room temperature. A UV-Vis spectrophotometer was used to measure the wavelength of the solution. The absorbance measurement used an incubation period of 90 minutes. The total phenolic content in the extracts of various parts of *Abelmoschus esculentus* was calculated by incorporating the extract absorbance into the standard curve equation of gallic acid.

Total Flavonoid Content

A total of 50 mg quercetin standard was dissolved in 50 mL ethanol. A series of concentrations (20, 40, 60, 80, and 100 µg/mL) was prepared from the 100 µg/mL quercetin solution standard. Next, 0.1 mL of 2% $AlCl_3$, 2.8 mL of distilled water, and 0.1 mL of 1 M sodium acetate were added (Imtara et al. 2021). The samples were then incubated at a room temperature for thirty minutes. At a maximum wavelength of 436.5nm, the absorbance was measured using the UV-Vis spectrophotometry method.

DPPH Method for Antioxidant Activity Test

All the extract samples were dissolved in methanol p.a. and made in various concentration series (20, 40, 60, 80, and 100 µg/mL). The antioxidant activity was determined by means of 1.0 mL solution. All the extract samples were put in test tubes and added with 4.0 mL of 0.1mM DPPH for each concentration. The mixture was then homogenized for 1 minute using a vortex and left to stand for an operating time of 30 minutes. At the maximum wavelength, the absorbance of the solution was found. The quercetin standard concentration series in the absorbance reading was determined using the same procedure (Kyene et al., 2023).

Alpha Amylase Enzyme Inhibition Test

With a few minor adjustments, the standard procedure was followed when testing the activity of the extract for α-amylase enzyme inhibition. A 96-well plate was filled with 50 µL phosphate buffer (100mM), 10 µL α-amylase enzyme (2U/ml), and 20 µL sample at different concentrations (acarbose and extract sample for the positive control) and then incubated at 37^0C for 20 minutes. After that, 20 µL 1% starch dissolved in phosphate buffer (100mM) was added as the substrate, and the mixture was incubated for 30 minutes at 37^0C. Next, 100 µL DNS was added and put in an oven at 100^0C for 10 minutes. Utilizing a multiplate reader, the absorbance was determined at a wavelength of 540 nm (Muñoz et al. 2022; Aroua et al. 2023).

Alpha Glucosidase Enzyme Inhibition Test

To conduct the α-glucosidase inhibition test, 50 µL extract and 1250 µL of 67 mM KH_2PO_4 with 50 µL α-glucosidase were combined in a test tube and incubated for 5 minutes at 37°C. After 20 minutes, 2 mL of Na_2CO_3 (100 mM) solution was added to start and stop the reaction, which was the followed by the addition of 125 µL p-Nitrophenyl-β-D-glucopyranoside (10 mM) solution. At 400 nm, the absorbance of the mixture was measured (Lourenço et al. 2019; Cam et al. 2020). A spectrophotometer (Shimadzu UV 1700, Tokyo, Japan) was utilized to record absorbances to ascertain the inhibitory activity of both enzymes.

Pancreatic lipase inhibition assay

The samples underwent an anti-lipase activity test in a 96-well plate using an Elisa reader. The enzyme stock concentration was adjusted to be thoroughly 0.1 mg/L for every 1 mg of solid PPL powder. Each of the 96-well plate contained 50 µL enzyme PPL, 50 µL sample in different concentrations (Orlistat and extract sample for the positive control), and p-NPB in 1% DMSO, and it was incubated at 37^0C for 10 minutes (Trendafilova et al., 2018; Alnukari, 2020).

Data analysis

The values were expressed as a mean ± SD. The statistical difference between the sample and the control group was determined using the GraphPad Prism one-way analysis of variance (ANOVA). The sig* $p<0.05$, sig** ($p<0.01$), sig***($p<0.001$), sig****($p<0.0001$) was considered significant.

RESULTS AND DISCUSSION

Extraction of the different parts of *Abelmoschus esculentus*

Samples with various parts of the plant that have been dried and extracted until a thick extract is obtained. The extraction was carried out by weighing 200 g of different parts of *Abelmoschus esculentus* (flowers, fruits, leaves, seeds, and stems). The resulting extract is entirely thick, brown in color, has a fragrant odor and tastes bitter. The extract yield shown in Table 1.

In table 1 it can be seen that the results of extraction with the same solvent produced the highest yield percentage in the leaves followed by fruit, flowers and seeds, while the lowest yield was in the stems. The resulting yield is around 30%.

Profiling of the Chemical Compounds in the Extract of Different Parts of *Abelmoschus esculentus*

The phytochemical screening of the extract showed that the secondary metabolites were more abundant with almost all the tests showing positive results. The first step used to profile the compounds in the parts of *Abelmoschus esculentus* was the phytochemical screening. This identification revealed the chemical compounds contained in the extract. The secondary metabolites in the different parts of *Abelmoschus esculentus* had an important role in their activity. The extract of the different parts of *Abelmoschus esculentus* contained flavonoids, alkaloids, saponins, phenolics, and steroids as shown in Table 2.

Table 1. Yield Extract (%) of Different part of plant *Abelmoschus esculentus*

Sample	Replication	Sample weight (gram)	Extract Weight (gram)	Yield (%)
Flowers of *A. esculentus*	1	198.9821	64.4523	32.3910
	2	203.9865	65.0897	31.9088
	3	205.9876	63.0054	30.5870
Average ± SD				31.6289 ± 0.9340
Fruits of *A. esculentus*	1	207.9857	66.2354	31.8461
	2	202.6754	67.9066	31.5051
	3	203.4562	65.0865	31.9904
Average ± SD				32.4472 ± 0.9190
Leas of *A. esculentus*	1	201.9890	69.9892	34.6500
	2	202.5421	70.8021	34.9567
	3	210.8965	68.4323	32.4483
Average ± SD				34.0183 ± 1.3683
Seeds of *A. esculentus*	1	196.5463	60.7654	30.9166
	2	207.5432	63.5431	30.6168
	3	210.8765z	65.8753	31.2388
Average ± SD				30.9241 ± 0.3111
Stems of *A. esculentus*	1	208.8765	61.2365	29.3171
	2	200.4328	59.8928	29.8817
	3	211.7863	60.6573	28.6408
Average ± SD				29.2799 ± 0.6213

Table 2. Phytochemical Screening Test of Different of Plant *Abelmoschus esculentus*

Test	Extract Flowers	Extract Fruits	Extract Leaves	Extract Seeds	Extract Stems
Alkaloids :					
a. Mayer	(-)	(+)	(-)	(+)	(-)
b. Dragendorf	(-)	(+)	(-)	(+)	(-)
c. Wagner	(-)	(+)	(-)	(+)	(-)
Flavonoids	(+)	(+)	(+)	(+)	(+)
Saponins	(+)	(+)	(+)	(+)	(+)
Phenolics	(+)	(+)	(+)	(+)	(+)
Steroids	(-)	(+)	(+)	(+)	(+)

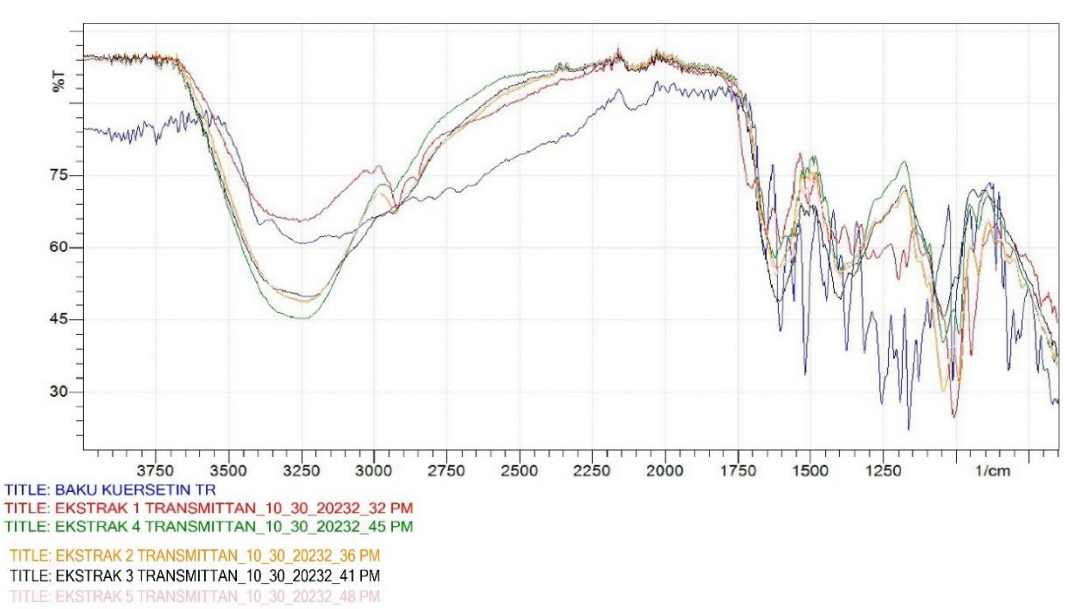

Figure 2. FTIR spectra of different part of *A. esculentus.* Overlay spectra shows the presence of lines : --- quercetin standard,--- flowers,--- fruits, --- leaves, --- seeds, and --- stems extract

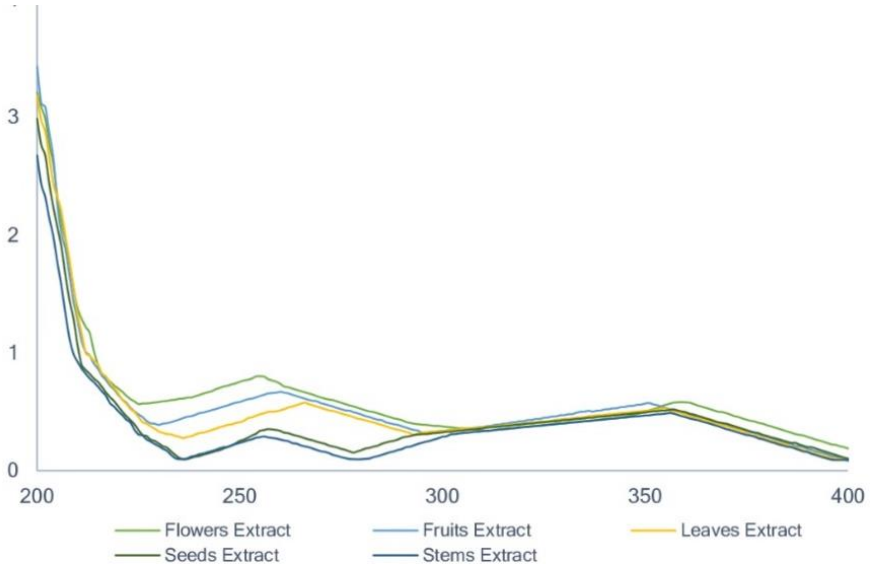

Figure 3. Spectrofotometer UV-Vis Spectra of Different Part from *A. esculentus.* Overlay Spectra Shows The Presence of Lines : --- Flowers,--- Fruits, --- Leaves, --- Seeds, and --- Stems Extract

Extracts from various parts of the plant were identified using FTIR and UV to see the spectrum pattern. Based on Figure 2, the analysis of the functional groups of quercetin standard and extract of different parts of *Abelmoschus esculentus* indicated that the quercetin standard contained several functional groups that were similar to those in the extract. The spectrum of pure quercetin presented characteristic peaks at 3266 cm^{-1} (O-H stretching vibration) and 1611 cm^{-1} (carbonyl C=O stretching vibration). There were shifts of C=O band from 1611 cm^{-1} (pure quercetin) to 1641 cm^{-1} (SD) and of O-H band from 3266 cm^{-1} (pure quercetin) to 3384 cm^{-1} (Febriyenti et al. 2020; Krysa et al. 2022).

Based on figure 3, the spectrum of the extracts of the flowers, fruits, leaves, seeds, and stems of *Abelmoschus esculentus* analyzed with a UV-Vis spectrophotometer showed the presence of two peaks of the flower extract at 255nm and 359nm, fruit extract at 255nm and 351nm, leaf extract at 266nm and 355nm, seed extract at 257nm and 357nm, and stem extract

at 256nm and 356nm wavelengths. The results of these spectrum readings can be associated with the results of the secondary metabolite compound tests that show positive flavonoid group compounds. The spectrum reading results are in the wavelength range of 230-295nm and 300-250nm (Syarifah and Retnowati 2019; Kim 2020). Based on the phytochemical screening, the spectra of FTIR and spectra of UV-Vis spectrophotometer showed positive phenolic and flavonoid levels in all extract samples.

Determination of the Total Phenolic Content and Total Flavonoid Content

The Total Flavonoid Contents (TFC) in different extracts of the flowers, fruits, leaves, seeds, and stems of *A. esculentus* was determined based on the formation of $AlCl_3$ complex with an ortho-dihydroxy group and a ketone hydroxy group in the flavonoid compounds. The quercetin standard curve equation of the 20, 40, 60, 80 and 100 µg/mL concentration series was analyzed at a wavelength of 445nm. The absorbance measurement used an incubation period of 90 minutes. The total phenolic content in the extract of various parts of *A. esculentus* was determined by incorporating the absorbance of the extract into the standard curve equation of quercetin. The results of the determination of total phenolic and total flavonoids can be seen in the following table 3. The highest TPC and TFC value was observed in the flowers followed by the fruits, leaves, and seeds, and the lowest was in the stem extract.

The results of the determination of the phenolic and flavonoid levels in various parts of *Abelmoschus esculentus* provide important information about the use of 80% ethanol as an extraction solvent that is highly effective for binding flavonoids because ethanol has high polarity to bind phenolic groups, especially flavonoid compounds. This shows that flavonoid components in plant extracts, especially in the flowers, can be considered quite high and have the potential to be further developed and explored in terms of the biological activity because such activity is not only produced from the concentrations contained but also influenced by the chemical structure of the compounds contained. The statistical analysis of TPC and TFC data in this study used the GraphPad Prism which obtained a significance value of > 0.05.

Flowers, fruits, leaves, seeds, and stems contain different amounts of phenolics and flavonoids in an ethanol extract. The greatest phenolic and flavonoid content is found in the flower extract followed by the fruit, leaf, seed, and stem extracts. The high content of TPC and TFC in flowers is due to the contribution of anthocyanin compounds that are abundant in flowers. The polyphenolic compounds in extracts are known to have multifunctional properties, such as a reducing agent, donating hydrogen atoms as an antioxidant, and reducing the formation of singlet oxygen. Flavonoids and their derivatives are a group of polyphenols that are numerous and very important. The antioxidant activity of polyphenolic compounds is characterized by the relatively high activity as hydrogen or electron donors as well as the ability to chelate transition metals (Gorecka et al., 2014). The high correlations between TPC, TFC value, and antioxidative values indicated that phenolic contents, such as phenolic acids and anthocyanins, contributed towards the strong antioxidant capacities of these flowers.

Table 3. The Determination of Total Phenolic and Total Flavonoid of Different Parts of Plant *A. esculentus*

Sample	The Total Phenolic (TPC) mg GAE/g	The Total Flavonoid (TFC) mg QE/g
The Flower Extract	171.1594 ± 83.1573	83.1573 ± 2.0299
The Fruit Extract	116.1573 ± 5.005	64.136 ± 2.9547
The Leaf Extract	103.2942 ± 2.2276	51.0657 ± 2.815
The Seed Extract	91.8283 ± 2.6429	44.1234 ± 1.9297
The Stem Extract	69.1967 ± 2.8408	36.7240 ± 1.337

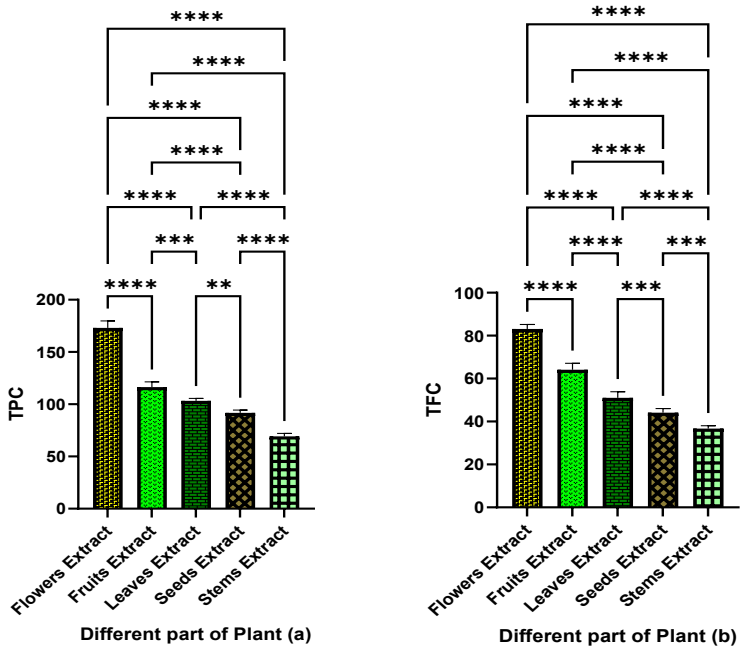

Figure 4. Determination of Total Phenolic Content (TPC) **(a)** and Total Flavonoid Content (TFC) **(b)**, there is a significant difference in the groups with sig** (p<0.01), sig***(p<0.001), sig****(p<0.0001), n=3

Antioxidant Test

In this study, the antioxidant activity of the ethanol extracts of *Abelmoschus esculentus* has been tested by using DPPH. The effectiveness of flavonoids in counteracting DPPH radicals largely depends on the structure, hydrophobicity, biological activity, and oxidative activity (Parcheta et al. 2021). The ability and break of radical chain reaction by flavonoids depends mainly on the presence of at least two o-hydroxyl groups on the B ring. Among the most widely used procedures for measurement of antioxidant activity capacity is the DPPH radical scavenging (Makarewicz et al., 2021). In this study, the DPPH radical scavenging activities of the extracts increased gradually in a concentration-dependent dose (20-100 µg/mL). All the extracts showed a 50% inhibitory concentration (IC_{50}) of less than 50 ppm. The IC_{50} of *Abelmoschus esculentus* ethanol extract is influenced by the phenolic and flavonoid content in the extract, with a high compound content in the extract providing high antioxidant activity characterized by a small IC_{50} value. Based on the results of IC_{50} calculations, the highest IC_{50} value of the antioxidant was 22.6539 ± 1.6452 µg/mL in the flowers, and the lowest was 44.11962 ± 3.1586 µg/mL in the seeds. While the IC_{50} of quercetin standard at concentration of 15.3608 ± 1.0124 µg/mL. The smaller the IC_{50} value means the higher the antioxidant activity. The antioxidant activity of a compound can be classified based on the IC_{50} value obtained. If the IC_{50} value of an extract of the plant part of *A. esculentus* is still in the very strong category (Ullah et al., 2024). Figure 5 illustrates the inhibitory activity of the flower extract, fruit extract, leaf extract, seed extract, stem extract, and quercetin standard.

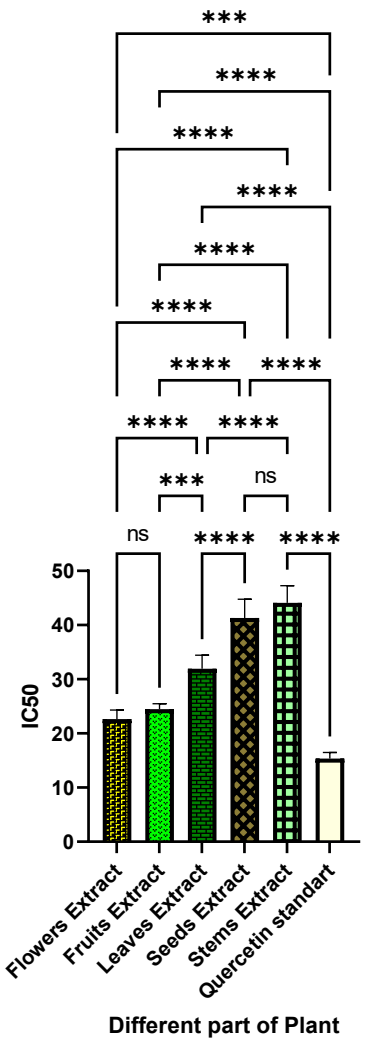

Figure 5. The Antioxidant Activity (IC_{50} µg/mL) of Different Parts From *A. esculentus,* ns = not significant, there is a significant difference in the groups with sig***(p<0.001), sig****(p<0.0001), n=3

α-Amylase Inhibition Test

Alpha-amylase and α-glucosidase are carbohydrate digestion enzymes that can significantly decrease the postprandial elevation of blood glucose after a carbohydrate diet. Therefore, inhibition of these enzymes is considered an essential strategy in blood glucose management. In this study, the in vitro α-amylase inhibitory activity of the ethanol extracts of different parts of *Abelmoschus esculentus* was examined. The in vitro α-amylase inhibitory study demonstrated that all the extracts of the flowers, fruits, leaves, seeds, and stems had α-glucosidase inhibitory activity. The percentage inhibition at 20, 40, 60, 80, and 100 µg/mL extract concentrations showed a concentration-dependent reduction in the percentage of inhibition. The highest IC_{50} value of α-amylase inhibitor was 102.4885 ± 11.4370 µg/mL in the flowers, and the lowest was 174.2729 ± 12.0987 µg/mL in the stems a compared to the positive control Acarbose being 43.99 ± 3.0759 µg/mL. Figure 6 illustrates the inhibitory activity of the flower extract, fruit extract, leaf extract, seed extract, stem extract, and Acarbose against α-glucosidase. The statistical analysis of the α-amylase inhibition data using GraphPad Prism obtained a significance value of > 0.05. Based on the post-ANOVA test of the standard α-amylase inhibition of acarbose and the extract, there were significant differences of < 0.05. However, between the flower extract and the fruit extract, the fruit extract and the leaf extract, and the leaf extract and the seed extract, as well as the seed extract and the stem extract, no significant differences were found, while there were significant differences among other extracts.

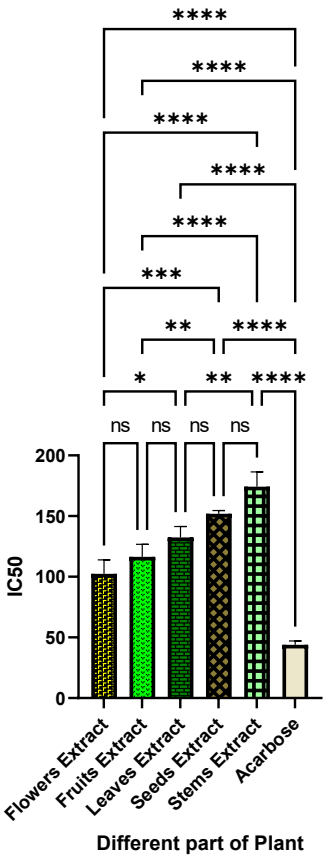

Figure 6. The α-Amylase Inhibition (IC_{50} µg/mL) of Different Part From *A. esculentus*, ns = not significant, there is a significant difference in the groups with sig*(p<0.05), sig**(p<0.01), sig***(p<0.001), sig****(p<0.0001), n=3

The inhibitory activity shows how strongly the sample inhibits/stops the performance of the enzyme α-amylase or α-glucosidase in breaking down starch substrates. The higher the activity, the more potential it has to be developed as a hypoglycemic therapy. The higher the concentration, the greater the ability of the extract and acarbose to inhibit the α-amylase enzyme. The IC_{50} value is the concentration of the extract that is able to inhibit enzyme activity by 50 percent, the smaller the IC50 value indicates the higher the inhibition activity is higher. When compared to the IC50, the positive control of acarbose is much smaller than that of the sample extract, meaning that the activity of the sample extract is still far below the activity of the alpha amylase inhibitor acarbose.

α-Glucosidase Inhibition Test

The in vitro α-glucosidase inhibitory study demonstrated that all the extracts of the flowers, fruits, leaves, seeds, and stems had α-glucosidase inhibitory activity. The percentage inhibition at 20, 40, 60, 80, and 100 µg/mL extract concentrations showed a concentration-dependent reduction in the percentage of inhibition. The highest IC_{50} value of the inhibitor of α-glucosidase was 76.95 ± 12.0888 µg/mL in the flowers, and the lowest was 185.2531 ± 6.3671 µg/mL in the stems, with a comparison to the positive control Acarbose being 33.8145 ± 0.4033 µg/mL. Figure 8 illustrates the inhibitory activity of the flower extract, fruit extract, leaf extract, seed extract, stem extract, and Acarbose against α-glucosidase. The statistical analysis of the α-glucosidase inhibitory data using GraphPad Prism in this study resulted in a significance value of > 0.05. Based on the post-ANOVA test of the standard α-glucosidase inhibitory activity of acarbose and the extracts, there were significant differences of < 0.05, but between the flower extract and the fruit extract, as well as between the seed extract and the stem extract, there were no significant differences. Meanwhile, among the other extract samples, significant differences were found.

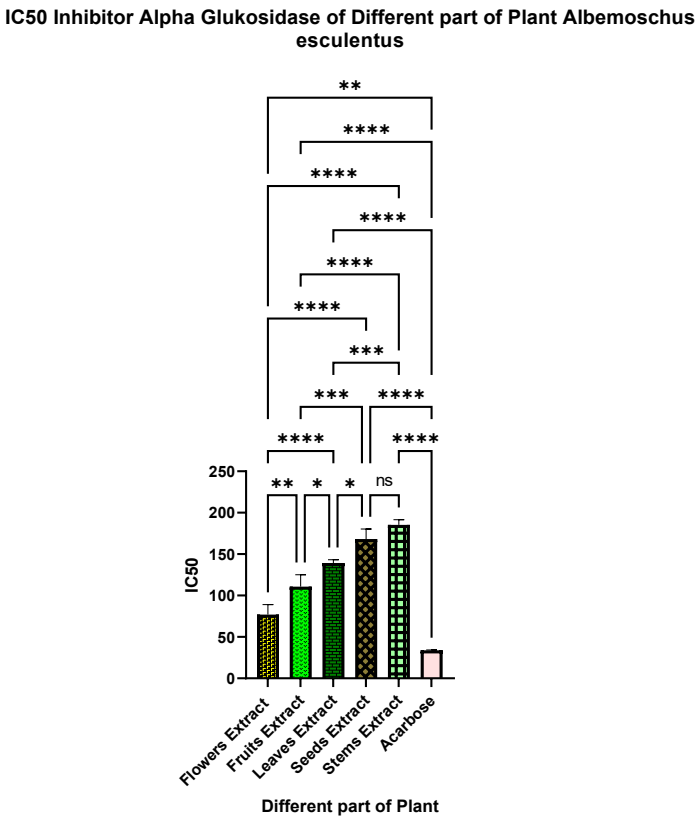

Figure 7. The α-Glukosidase Inhibition (IC_{50} μg/mL) of Different Part From *A. esculentus*, ns = not significant, there is a significant difference in the groups with sig*(p<0.05), sig**(p<0.01), sig***(p<0.001), sig****(p<0.0001), n=3

In the condition of diabetes mellitus, there is an increasing production of Radical Oxygen Species (ROS), resulting in the body experiencing oxidative stress (Bhatti et al. 2022). Oxidative stress is a condition where the content of oxidants or free radicals in the body is higher than the antioxidants, making it necessary to have antioxidants from outside, and one of which is polyphenolic compounds. In addition to functioning as an antioxidant, it is highly possible that polyphenolic compounds also have the ability to inhibit α-amylase and α-glucosidase which are the key enzymes involved in sugar metabolism. This is considered a good model for studying the effect of nutraceuticals on type II diabetes. Referring to the classification of IC_{50} strength levels used by Lodato et al. (2023), the strength level of the acarbose inhibition was very active (< 50 ppm) while the strength level of the extract inhibition effect was 50-200 μg/mL.

The strength level of the acarbose inhibitory effect was better than that of the extract because acarbose is a tetrasaccharide. Tetrasaccharides have a hexose structure similar to polysaccharides, α-glucosidase enzyme substrate, and α- amylase enzyme. Meanwhile, acarbose is a drug that inhibits the activity of α-glucosidase and α-amylase enzymes, which has been standardized for use not only in Indonesia but throughout the countries in the world (Seetaloo et al. 2019). The inhibition of α-glucosidase enzyme and α-amylase by the extracts of various parts of *Abelmoschus esculentus* is enabled by the presence of secondary metabolites in the form of phenolic compounds in the extracts that are able to bind α-glucosidase and α-amylase competitively or noncompetitively. Other flavonoid metabolites, such as polyphenols, have the ability to maintain blood glucose stability through various mechanisms, such as inhibiting the work of α-glucosidase and α-amylase enzymes on the mucosa of the small intestine wall, inhibiting glucose absorption by occupying its transporter, protecting pancreatic β-cells from glucotoxicity, inhibiting liver glycogen from releasing glucose into blood circulation, and improving the work of peripheral fat tissue in glucose uptake and absorption (Hanhineva et al. 2010).

Some tests conducted in vitro and in vivo demonstrated that flavonoids may have antidiabetic effects. Epicatechin stimulates insulin

synthesis and increases the level of cAMP in the β cells of Langerhans islets, thus increasing insulin secretion. EGCG (epigallocatechin 3-gallate) has a hypoglycemic effect by inhibiting the production of glucose in the liver. Flavonoids may also influence glucose absorption in the intestine. Daidzein, luteolin, and the 7-O-glucoside of luteolin inhibit the activity of α-amylase and β-glucosidase, and quercetin glycosides influence SGLT-1 glucose transporters in enterocytes. Flavonoids seem like precious natural compounds not only because they prevent rapid blood sugar rises in the serum but also because they can protect diabetics from the complications of this metabolic disorder (Oteiza et al. 2018). Natural polyphenols, particularly flavonoids, can inhibit the activity of carbohydrate-hydrolyzing enzymes, such as *α*-amylase and *α*-glucosidase (Barber et al. 2021). Two binding modes between enzymes and flavonoids have been detected: (i) flavonoids bind directly to amino acid residues in the active sites of enzymes and exclude the binding of substrate, and (ii) flavonoids can interact with amino acid residues near the active site and close the channel to the active center (Zhu et al. 2020).

Pancreatic Lipase inhibiton Test

In this study, an *in vitro* analysis of the anti-lipase activity of *Abelmoschus esculentus* extracts was conducted to confirm its traditional use as an anti-obesity plant. The highest IC_{50} value of the anti-pancreatic lipase was 109.5943 ± 9.7391 µg/mL in the flower extract, and the lowest was 205.6726 ± 9.4542 µg/mL in the seed extract. In comparison, the positive control Orlistat had 35.181 ± 4.9884 µg/mL. Orlistat, as a lipase inhibitor, was used in this study as a positive control. The lipase inhibitors that are used to reduce the activity of lipases found in the intestine prevent the hydrolysis of dietary triglycerides to monoglycerides and fatty acids, thus preventing absorption. This mechanism can be used for the treatment of obesity. The results of this study showed that the flower extract of the crude ethanol extract of the plant was found to be the most active followed by the extracts of the fruits, leaves, seeds, and stems. The results of the test can be seen in Figure 9. The statistical analysis of the anti-lipase data using the GraphPad Prism in this study resulted in a significance value of > 0.05. Based on the post-ANOVA test of the standard anti-lipase power of Orlistat and the extracts, there were no significant differences of < 0.05.

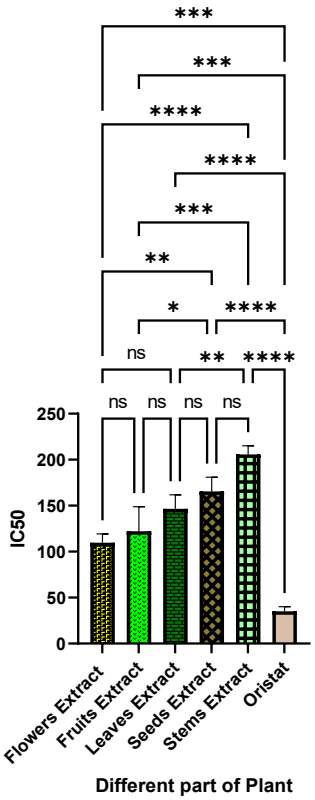

Figure 8. The Pancreatic Lipase Inhibition (IC_{50} µg/mL) of Different Part From *A. esculentus*, ns = not significant, there is a significant difference in the groups with sig*(p<0.05), sig**(p<0.01), sig***(p<0.001), sig****(p<0.0001), n=3

An anti-lipase activity test is carried out using a chromogenic substrate named p-nitrophenyl butyrate (p-NPB) through spectrophotometry. This test results in p-NPB hydrolysis which will release the p-nitrophenolate chromophore, leading to a yellow color change that can be measured at a wavelength of 405. Lipid digestion in the body is strongly influenced by the presence of pancreatic lipase enzyme. Pancreatic lipase (EC 3.1.1.3; triacylglycerol acyl hydrolase) produced by the pancreatic cells hydrolyzes triglycerides mainly into 2-monoacylglycerols and free fatty acids, and it is responsible for the hydrolysis of 50%-70% of total dietary fats in the intestinal lumen (Jaradat et al. 2017). Inhibition of pancreatic lipase, which splits triacylglycerols into absorbable monoglycerides and fatty acids, is an important strategy in the treatment of obesity. The extracts of *Abelmoschus esculentus* parts were found to inhibit the activity of pancreatic lipase in an in vitro assay system. As a comparison, the IC_{50} of Orlistat was 35.181 ± 4.9884 μg/mL. The extracts of *Abelmoschus esculentus* parts showed much lower activity than Orlistat. Orlistat is a specific, potent gastric and pancreatic lipase inhibitor, which acts to reduce the absorption of dietary fat by forming covalent bonds with active serine sites of the digestive tract so as to disable the hydrolysis of dietary fats into fatty acids and glycerol (Shenoy et al. 2022).

The results of this study showed that the flower extract had a higher total phenolic content, total flavonoid content, antioxidant capacity, α-amylase inhibition, α-glucosidase inhibition, and pancreatic lipase inhibition than the other extracts. There was a positive correlation among polyphenol content, flavonoid content, DPPH radical scavenging activity, α-amylase inhibitor, α-glucosidase inhibitor, and anti-pancreatic lipase. This indicates that polyphenol and flavonoid compositions may affect the strength of inhibitory activity against different digestive enzymes. Some studies also demonstrated that lipase activity can be effectively inhibited by phenolic-rich extracts (Tan et al. 2017; Zhang et al. 2018; Wang et al. 2023). Polyphenolic extracts are able to lower body weight, fat weight, plasma free fatty acid levels, and hepatic lipid accumulation (Peng et al. 2020). Flavonoids represent the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants with kaempferol and quercetin as the main aglycon representatives (Barreca et al. 2021; Jan et al. 2022). Flavonoids have also been identified as an α-amylase inhibitor in other studies (Barreca et al. 2021). Additionally, flavonoids are considered as a natural alternative in diabetes treatment as they can regulate insulin secretions through different mechanisms. For example, taxifolin-3-O-rhamnoside acts similarly to sulfonylureas by blocking KATP channel letting Ca^{2+} channels cell surface signal (Soares et al. 2017).

The TPC and TFC of ethanolic and aqueous extract of a flower part of similar plant. They found 155.5 mgGAE/100 g extract and 113.5 mg GAE/100 g extract for TPC and 23.9 mg QE/100 g and 17.2 mg QE/100 g for TFC of ethanolic and aqueous extracts respectively. A significantly high amount of phenolics can contribute to the antioxidant properties in fruit extract of A. esculentus. Study by Jiang et al. (2011) the given antioxidant activity of the *A. esculentus* fruit extract could be connected to their high amount of phenolic contents which functions as metal chelators, singlet oxygen quenchers, reduction agents, hydrogen donors and free radical scavenger (Nagaoka et al. 2017). Diabetes mellitus is a metabolic disorder may be due to enhanced cellular oxidative stress and reduced antioxidant activity. Polyphenols and flavonoids of *A. esculentus* fruit extract are natural antidiabetic agents, which interferes the production of free radicals, reduce oxidative stress and inhibit digestive enzyme, thus lowering postprandial glucose. The extract shows as α-amylase, α-glucosidase, and pancreatic lipase inhibitory activity, this suggests that these extracts, rich in flavonoids and phenolics, have the potential to contribute to the management of diabetes.

CONCLUSION

The *A. esculentus* plant has many parts, such as flowers, fruits, leaves, seeds, and stems, which are rich in polyphenols and flavonoids. These components have antioxidant properties and act as α-amylase, α-glucosidase, and pancreatic lipase inhibitors. The flowers have the highest levels of phenolic and flavonoid compounds, which contribute to their significant antioxidant activity. Additionally, the flowers possess potent inhibitory activity against α-amylase, α-glucosidase, and pancreatic lipase, surpassing the inhibitory effects of the fruits, leaves, seeds, and stems. Future research could proceed by isolating the specific compound responsible for the observed activity or conducting in vivo studies to confirm bioactivity.

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AUTHOR CONTRIBUTION STATEMENT

CA and RS collect materials, extract, and perform qualitative and quantitative analysis on all extracts and antioxidant tests, MIS and SF perform in vitro enzymatic tests, and FNM perform data analysis on in vitro results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript.

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