

Phytochemical Screening, LC-MS Studies and Antidiabetic Potential of Methanol Extracts of Seed Shells of *Archidendron bubalinum* (Jack) I.C. Nielson (*Julang Jaling*) from Lampung, Indonesia

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ABSTRACT

Background: Some Malaysia and Indonesia people believed that root and seed shell of *Archidendron bubalinum* can treat diabetes. However, seed shell of *Archidendron bubalinum* has not yet to be scientifically proven and confirmed their ability to treat diabetes. The study of the potential of this seed shell was also scarcely available. **Objective:** The recent work was aimed to investigate the phytochemical screening of methanol extracts of seed shells of *Archidendron bubalinum* and to evaluate their chemical compositions and antidiabetic activities.

Material and Methods: The methods of phytochemical screening were including alkaloids, flavonoids, tannins, polyphenols, saponins, and terpenoids. Their chemical compositions were determined by Liquid Chromatography-Mass Spectrometry (LC-MS) and antidiabetic activities were performed by α -glucosidase inhibitory method. **Results:** The phytochemical screening showed that methanol extracts of seed shells of *Archidendron bubalinum* contain flavonoids, tannins, polyphenols, and terpenoids. This extracts exhibited antidiabetic activity with IC_{50} 7.77 μ g/mL. This result was supported by LC-MS analysis which showed the presence of phlorizin and astilbin, in which these compounds had high inhibitory activity against α -glucosidase or diabetes. **Conclusion:** LC-MS analysis revealed the presence of polyphenol compounds namely phlorizin and astilbin in which had high α -glucosidase inhibitory activity, might largely contribute in the antidiabetic activity.

Key words: Phytochemical screening, Mass spectrometry, Anti-diabetic, *Archidendron bubalinum*.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Remedies made from medicinal plants species are highly sought after these days. This was especially with realization in the scientific and modern medicine world of the unexplored possibilities of medicinal plants species capabilities, used in traditional medicine. Nowadays, the traditional medicines are studied and used as a base in finding the novel compounds to treat various diseases around the world. Most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.¹

According to Indonesia's Ministry of Health, the prevalence of diabetes in 2013 in Indonesia was 2.1 % of the total population aged 15 years and over.² It found that between 1980 and 2014, diabetes has become more common among men than women, and rates of diabetes rose significantly in many low and

middle income countries, including China, India, Indonesia, Pakistan, Egypt and Mexico.³ Diabetes is a well-known metabolic disorder, which is characterized by an abnormal postprandial increase of blood glucose level. The control of postprandial hyperglycemia is believed to be important in the treatment of diabetes. α -glucosidase secreted from intestinal chorionic epithelium is responsible for the degradation of carbohydrates. α -glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking the activity of α -glucosidase. Consequently, the peak concentration of postprandial blood glucose is reduced and the blood sugar level comes under control.⁴ Some of α -glucosidase inhibitors, such as acarbose that obtained from natural sources, can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes.⁵ Only a few α -glucosidase inhibitors are commercially available. All of them contain sugar

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moieties and their synthesis involves tedious multistep procedures. Moreover, clinically they have been associated with serious gastrointestinal side effects. Therefore, it is necessary to search for alternatives that can display α -glucosidase inhibitory activity but without side reactions.⁶ α -glucosidase inhibitors can be used as a new class of antidiabetic drug. 411 compounds exhibiting α -glucosidase inhibitors activity were summarized and isolated them from medical plants. The compound classes isolated include: 61 terpenes, 37 alkaloids, 49 quinines, 103 flavonoids, 37 phenols, 73 phenylpropanoids, 8 steroids, and 43 other types of compounds. Compounds with α -glucosidase inhibitors activities are abundant in nature and can be obtained from several sources.⁷⁻⁸

The species *Archidendron bubalinum* belongs to the family Fabaceae or Leguminosae in the major groups of Angiosperms. It contains about 126 species and is a large and economically important family of flowering plants. Fabaceae is the most common family found in tropical rainforests and in dry forests.⁹ The species is indigenous to Sumatra in Indonesia, Peninsular Malaysia and Thailand. The vernacular name is kabau or *julang jaling* (Indonesia), kerdas (Malaysia) and nieng-nok (Thailand). The strong pungent smelling seeds have an odor like jering (*Archidendron jiringa*) and petai (*Parkia speciosa*). The bark of *Archidendron bubalinum* is used as febrifuge. Young seeds can be eaten in raw as traditional vegetable salad and can be used traditionally as a diuretic but excessive consumption can lead to poisonous and kidney problem.¹⁰ *Archidendron bubalinum* seeds were found to have high moisture content, low fat content, high protein content but being limited in valine, methionine and tyrosine.¹¹ *Archidendron bubalinum* contained of anti-nutrient: tannins, trypsin inhibitors and hemagglutinin. Studies in Thailand have been reported that crude extracts of *Archidendron bubalinum* seeds had superoxide scavenging activities.¹² Malaysia and Indonesia aborigines tribe believed root and seeds of *Archidendron bubalinum* can treat diabetes.¹³⁻¹⁴ The study of the potential of this seed is scarcely available and little attention is given due to limited resources. *Archidendron bubalinum* has not yet to be scientifically proven and confirmed their ability and chemical constituents to treat diabetes.¹⁴

The objective of this study is to identify the types of compounds found in the methanol extracts from seed shells of *Archidendron bubalinum* from Lampung Indonesia and to investigate its potential antidiabetic activity.

MATERIALS AND METHODS

The seeds of *Archidendron bubalinum* were collected from uncultivated farm lands located at Lampung, Sumatera Island, one of province in Indonesia. The seeds were peeled to separate the seed shells and seed (Figure 1). The seed shells were collected, thoroughly washed and then sun dried for 4 h. The dried seed shells were soaked in methanol with ratio 1:10 (w/v) and extracted by maceration methods for 24 h. Then, the supernatant was filtered by filter paper. Soaking process was repeated once again in the same sample and the supernatant was filtered. All the supernatant were collected together and concentrated using rotary evaporator at 50°C.

Alkaloids determination

About 100 mg of methanol extracts was mixed in 5 ml of 10% hydrochloric acid then pH was adjusted to 8 with dilute ammonia solution. The mixture was added with 20 ml chloroform and then dried. The extracts were tested with Dragendorff reagent and Mayer's reagent. The formation of precipitate was indicating the presence of alkaloids.

Flavonoids determination

About 100 mg of methanol extracts was boiled in 100 ml of distilled water for 5 min and filtered. 5 ml of the filtrate was mixed with 0.1 mg magnesium powder, 1 ml concentrated hydrochloric acid and 1 ml amyl

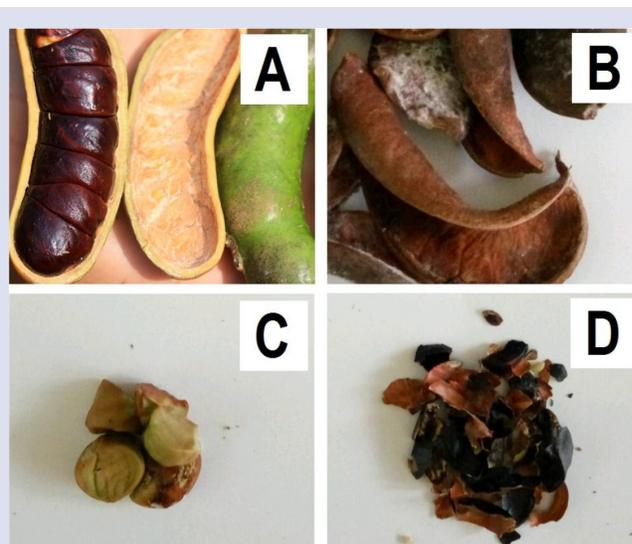


Figure 1: A) Fruits, B) Fruit shells, C) Seeds, D) Seed shells of *Archidendron bubalinum*.¹⁵

alcohol then shaken vigorously. The colour changed to yellow or red or orange, indicating the presence of flavonoid.

Terpenoids and steroids determination

About 100 mg of methanol extracts were mixed with 25 ml diethyl ether and shaken vigorously. The diethyl ether layer was separated then added 2-3 drops of Liebermann-Burchard reagents. A bluish colour of the interface was formed, indicating positive results for the presence of terpenoids while greenish colour, indicating positive results for the presence of steroids.

Saponins determination

About 100 mg of methanol extracts were added with 10 ml of boiled distilled water, cooled and shaken vigorously for 10 min. The foam formation was observed and will be stable if added with a few drops of hydrochloric acid, indicating the presence of saponins.

Tannins and polyphenols determination

About 100 mg of methanol extracts were extracted with 1 ml ethanol and 1 ml distilled water. The filtrate was mixed with a few drops of 1% FeCl₃ and into another filtrate added with gelatin salt. Then observe the colour changing, if a green or blue or purple colour was appeared, indicating the presence of tannins.

Liquid Chromatography-Mass Spectrometry (LC-MS) studies

The chemical constituents of the methanol extracts were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump. The HPLC was interfaced with a Q-TOF mass spectrometer fitted with an ESI source. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. HPLC column Phenomenex 5 μ C8, (150 \times 2 mm i.d.) was used for the analysis. Solvent was methanol with 0.3% formic acid. Solvents were delivered at a total flow rate of 0.1 mL/min. The solvent was run by isocratic elution. The MS spectra were acquired in the positive ion mode. The temperature of the drying gas (N₂) was 350°C, at a gas flow rate of 6 mL/min, and a nebulizing pressure (N₂) of 25 psi. About 0.5 g of sample extracts was diluted with methanol and filtered with 0.22 μ m nylon filter

prior to analysis. A 5 µl volume of the extracts were injected onto the analytical column for analysis. The mass fragmentations were identified by using spectrum database for organic compounds in SDBS application.

α-Glucosidase inhibition test ^{16,17}

A 250 µL solution of p-nitrophenyl-α-D-glucopyranoside 5 mM and 495 µL phosphate buffer 0.1M pH 7 was added to the reaction tube containing 5 µL of the sample solution in DMSO with a concentrations variation of 100, 50, 25, and 10 µg/mL. After homogeneous, the solution was pre incubated for 5 min at 37°C, the reaction was initiated by the addition of 250 µL α-glucosidase solution (0.062 units), incubation was continued for 15 min. The reaction was stopped by the addition of 1 ml of Na₂CO₃ 0.2 M. The activity of the enzyme was measured, based on the reading of p-nitrophenol absorbance at λ 400 nm. Quersetin was used as a reference standard with concentration of 10, 7.5, 5, 2.5, and 1 µg/mL. % inhibition was measured by using the equation:

$$\% \text{ Inhibition} = \frac{(B-S)}{B} \times 100$$

B = Absorbance of blank (DMSO)

S = Absorbance of sample

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of methanol extracts of *Archidendron bubalinum* seed shells revealed the presence of flavonoids, tannins, total phenols, and terpenoids, while alkaloids and saponins were not detected (Table 1). These results are the same as in the husks sample analysis. However, this extract provides a different bioactivity, in which this extract gives positive results against antidiabetes (Table 3). This is interesting so it proceeds with the analysis of phytoconstituents using LC-MS

Liquid Chromatography-Mass Spectrometry (LC-MS) studies

LC-MS analysis of methanol extracts of *Archidendron bubalinum* seed shells had detected five peaks with the retention time 0.41, 1.28, 1.90, 2.63, and 2.80 minutes (Figure 2). Then each peak was fragmented, resulting 5 fragmentation spectrum with candidates mass (m/z) 365, 436, 436, 247 and 450. (Figure 3 and Table 2)

The LC-MS spectrums interpretation was performed using a spectrum database for organic compounds in SDBS application. The results of spectrum interpretation on methanol extracts of *Archidendron bubalinum* seed shells, indicating that there are substance of phlorizin and astilbin with the retention time of 1.90 and 2.80, respectively. These results were confirmed by each of the fragmentation pattern as figure-4 and figure-5

Table 1: Phytochemical screening of methanol extracts of *Archidendron bubalinum* seed shells.

Constituents	Test	Seed shells (This research)	Husks ¹⁵
Alkaloids	Mayer	-	-
	Dragendorff	-	+
Flavonoids	Shinoda	++	++
Tannins	Gelatin	+++	+++
Polyphenol	Ferric chloride	+++	+++
Saponins	Frothing	-	-
Terpenoids	Liebermann-Buchard	+	+

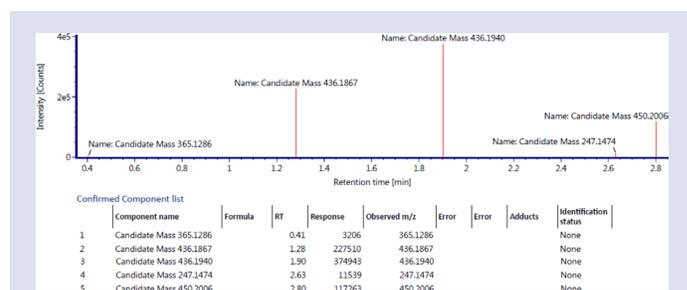


Figure 2: LC-MS chromatogram of methanol extract of *Archidendron bubalinum* seed shells.

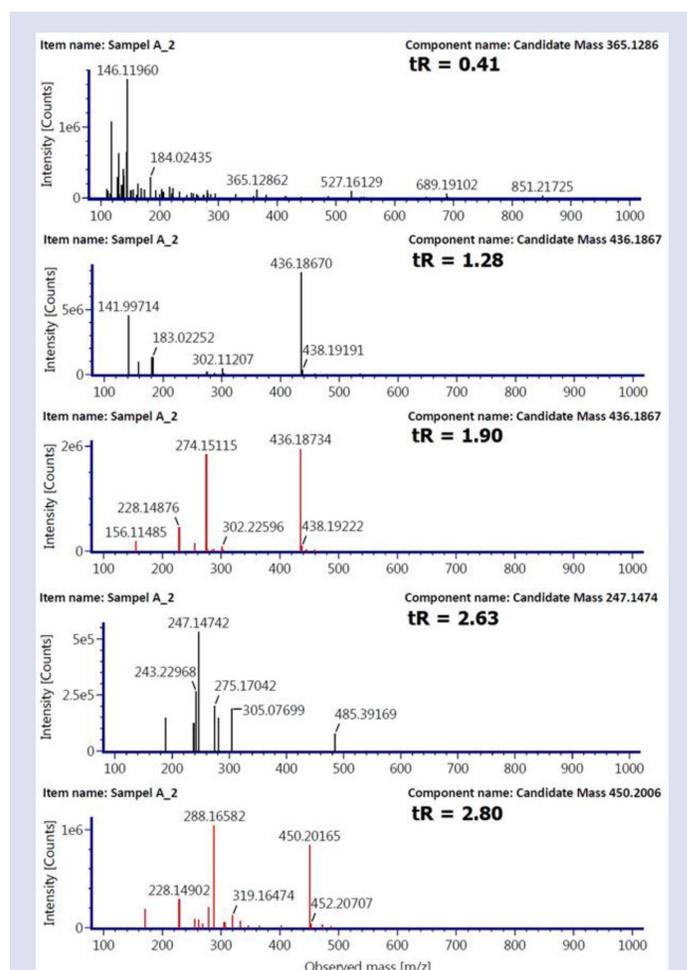
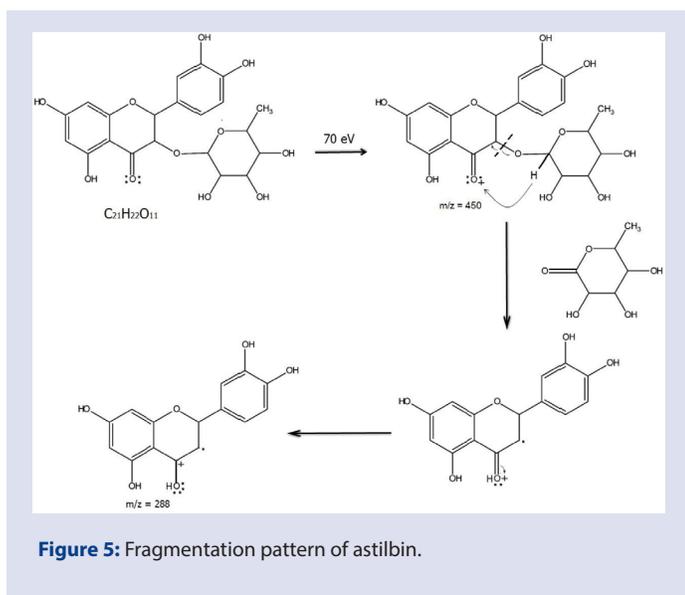
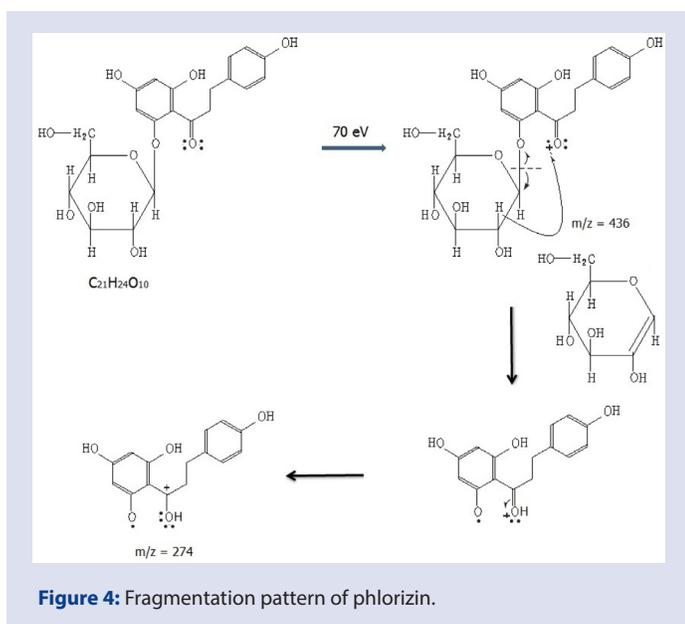


Figure 3: Mass spectrums of 5 components of methanol extracts.

Table 2: LC-MS profile of methanol extracts of *Archidendron bubalinum* seed shells.

Peak no.	RT (min)	Candidate mass	MS ² ions	Proposed compound	Molecular formula
1	0.41	365	184, 146	-	-
2	1.28	436	183, 142	-	-
3	1.90	436	274	Phlorizin	C ₂₁ H ₂₄ O ₁₀
4	2.63	247	-	-	-
5	2.80	450	288	Astilbin	C ₂₁ H ₂₂ O ₁₁



mentioned.¹⁸ The spectrum of fragmentation mass of phlorizin was written by m/z 436 of molecular ion of $C_{21}H_{24}O_{10}$ resulted from phlorizin substance when it was exposed with 70 eV of energy. Molecular ion of $C_{21}H_{24}O_{10}$ was experiencing fragmentation and McLafferty reorganization by releasing $C_6H_{10}O_5$ and producing fragments by m/z 274 originating from $C_{15}H_{14}O_5$. (Figure 4)

The mass spectrum of astilbin was written by m/z 450 originating from the molecular ion of $C_{21}H_{22}O_{11}$ which was formed when it was exposed by 70 eV of electron. Molecular ion of $C_{21}H_{22}O_{11}$ was experiencing fragmentation and McLafferty reorganization by releasing $C_6H_{10}O_5$ and producing fragments by m/z 288 originating from $C_{15}H_{12}O_6$. The pattern of fragmentation of astilbin can be seen in Figure 5.

Antidiabetic activity

Based on the antidiabetic test, quercetin solution at concentrations of 10, 7.5, 5, 2.5, and 1 $\mu\text{g/mL}$ had % inhibition of 77.59%, 61.06%, 45.22%,

Table 3: Results of antidiabetic test.

Standard/ Sample Name	Concentration ($\mu\text{g/mL}$)	% inhibition	IC_{50} ($\mu\text{g/mL}$)
Quercetin	1	7.42+0.85	6.04+0.14
	2.5	27.01+3.23	
	5	45.22+1.33	
	7.5	61.06+0.31	
	10	77.59+1.10	
Methanol extracts	10	50.30+2.1	7.77+0.11
	25	82.51+6.4	
	50	97.19+1.0	
	100	99.89+0.2	

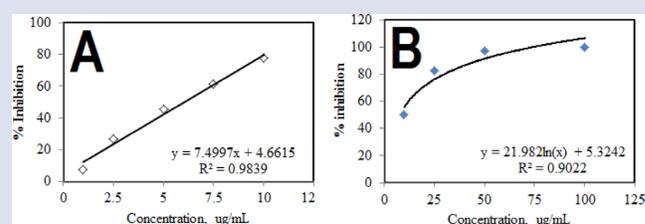


Figure 6: Graph of the relationship between concentration and % inhibition for IC_{50} determination. A) Quercetin B) Methanol extracts.

27.01%, and 7.42% respectively. The methanol extracts of *Archidendron bubalinum* seed shells at concentrations of 100, 75, 50, and 25 $\mu\text{g/mL}$ had a % inhibition of 99.89%, 97.19%, 82.51%, and 50.30% respectively. Based on the results of antidiabetic test in Table 3, Figure 6, it can be seen that the methanol extracts of *Archidendron bubalinum* seed shells had high antidiabetic activity with IC_{50} of 7.77+0.11 $\mu\text{g/mL}$. This value was almost same as IC_{50} of quercetin that was 6.04+0.14 $\mu\text{g/mL}$. Quercetin had high α -glucosidase inhibitor activity and often used as a standard on antidiabetic test.¹⁹⁻²⁰

Phlorizin is a natural product and dietary constituent found in a number of fruit trees. It has been used as a pharmaceutical and tool for physiology research for over 150 years.²¹ Both the phlorizin which was alkaloid compound, as well as astilbin have many phenolic groups in their molecules. Polyphenols were often present in polar glycosides and were easily soluble in polar solvents. The antioxidant and antidiabetic properties of these compounds were related to the presence of phenolic groups that can donate hydrogen atoms to a free radical so that the radicals become less reactive.

The antidiabetic mechanism of methanol extracts of *Archidendron bubalinum* seed shells depended on the content of the compounds therein. Methanol extracts of *Archidendron bubalinum* seed shells was useful as an antidiabetic through several mechanisms:

1. Methanol extracts of *Archidendron bubalinum* seed shells were rich in flavonoids and phenolic compounds such as phlorozin and astilbin. Phenolic compounds can donate their hydrogen atoms and function as free radical inhibitors, where phenolic compounds will protect organs such as the pancreas from free radicals attack. (Figure 7)
2. Another mechanism was by competitively inhibiting α -glucosidase activity, these inhibitors help to prevent the fast breakdown of sugars and thereby control the blood sugar level.

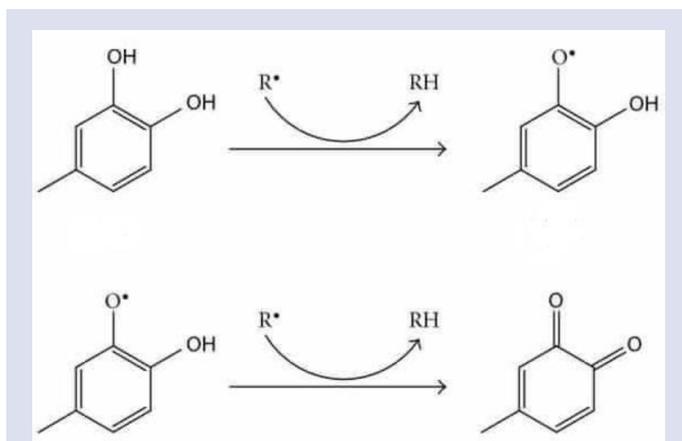


Figure 7: Reaction of phenolic compounds with free radicals.

CONCLUSION

Phytochemical screening showed that methanol extracts from *Archidendron bubalinum* seed shells containing many compounds either flavonoids or phenolic compounds. Based on the LC-MS analysis which was interpreted by SDBS application, it can be identified that some of the compounds in the methanol extracts were phlorizin and astilbin. From the results of antidiabetic test, it was proved that methanol extracts of *Archidendron bubalinum* seed shells had high antidiabetic activity with IC_{50} of $7.77 \pm 0.11 \mu\text{g/mL}$ which was almost same with IC_{50} of quercetin standard, $6.04 \pm 0.11 \mu\text{g/mL}$. High antidiabetic activity of methanol extracts of *Archidendron bubalinum* seed shells were coming from the compounds of phlorizin and astilbin. From these results, it can be concluded that methanol extracts of *Archidendron bubalinum* seed shells had great potential as biomedicine for diabetes diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ABBREVIATIONS

LC-MS: Liquid Chromatography Mass Spectrometry; MS: Mass Spectrometry; Q-TOF: Quadrupole-Time of Flight; SDBS: Spectral Data Base

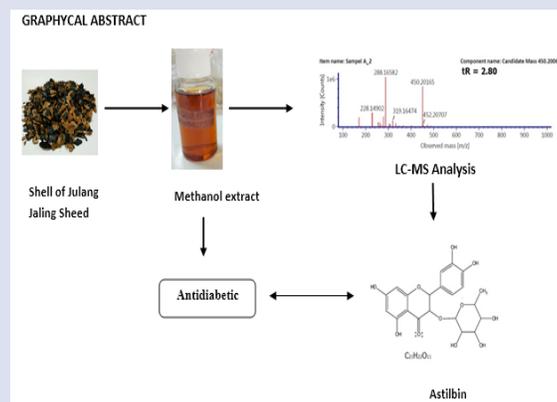
System; DMSO: Dimethyl Sulfoxide; HPLC: High Performance Liquid Chromatography; IC: Inhibitory Concentration.

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GRAPHICAL ABSTRACT



SUMMARY

- Phytochemical screening showed that methanol extracts from *Archidendron Bubalinum* seed shells containing many compounds either flavonoids or phenolic compounds.
- Based on the LC-MS analysis which was interpreted by SDBS application, it can be identified that some of the compounds in the methanol extracts were phlorizin and astilbin.
- From the results of antidiabetic test, it was proved that methanol extracts of *Archidendron Bubalinum* seed shells had high antidiabetic activity with IC50 of 7.77 ± 0.11 g/mL which was almost same with IC50 of quercetin standard, 6.04 ± 0.11 g/mL.
- High antidiabetic activity of methanol extracts of *Archidendron Bubalinum* seed shells were coming from the compounds of phlorizin and astilbin.

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