Antioxidant, cholesterol lowering activity, and analysis of Caesalpinia bonducella seeds extract

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Research Article

Antioxidant, cholesterol lowering activity, and analysis of *Caesalpinia bonducella* seeds extract

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Abstract

Tombili plant (*Caesalpinia bonducella*) belongs to the family of *Fabaceae*. The seed extract of tombili has been empirically used as a traditional medicine. The purpose of this research was to fractionate tombili seed extract and test their antioxidant and cholesterol lowering activities. Extraction was made into fractions using *n*-hexane, ethyl acetate, and methanol as a solvent. The chemical compound of the ethyl acetate fraction was analyzed using liquid chromatography-mass spectrometry (LC-MS/MS). Antioxidant activity was tested using the DPPH method. The highest antioxidant activity was shown in ethyl acetate fraction with an IC_{50} value of 86.153 µg/mL. The second was the methanol fraction with an IC_{50} value of 94.053 µg/mL, and the third was the *n*-hexane fraction with an IC_{50} value of 100.933 µg/mL. The cholesterol lowering activity analysis showed that all fractions could inhibit cholesterol. The highest anti-cholesterol activity shown in ethyl acetate fraction with the concentration of 600 µg/mL can inhibit 81.5% of the cholesterol activity. The LC-MS/MS analysis showed that the ethyl acetate fraction contained glucoside, homoplantaginin, and vernolic acid compounds.

Keywords

antioxidant, cholesterol lowering activity, *Caesalpinia bonducella*, LC-MS/MS analysis

Introduction

Cardiovascular diseases (CVDs) are the diseases of heart and blood vessels which considered one of the leading causes of death worldwide. Hypercholesterolemia is a key risk factor for cardiovascular disease and monitors the primary mortality of developing heart disease. The release of toxic free radicals by endothelial cells and vascular smooth muscle cells is the primary pathogenic factor for CVDs (Taleb et al. 2018). The cholesterol present in these matrices also can oxidize in favorable conditions such as the presence of water, temperature, pH, and the form of substrate (de Oliveira et al. 2018). Cholesterol oxidation susceptible to attack by reactive oxygen species (ROS) which are formed of molecules hydroxy (OH), peroxy (OOH) groups, $^1O_2$, $^3O_2$ and hydrogen peroxide (H_2O_2). The use of natural antioxidants from herbs represents an efficient alternative to control the formation of cholesterol oxidation product.

One common plant that has been used in traditional medicine is *Caesalpinia bonducella*, belongs to the family of *Fabaceae* (Aswar and Bhanudas 2011). This plant originated in Gorontalo, Indonesia (Sembiring et al. 2018). In Indonesia, *C. bonducella* is known as Tombili, Bagore, Kalici, Tinglur, and Areuy (Kakade et al. 2017). Tombili plants are also distributed in other tropical and subtropical parts of Asia, such as India, Sri Lanka, Vietnam, China, Myanmar, and Bangladesh (Musa et al. 2020).

In an earlier study, the phytochemical screening of Tombili seed showed that tombili seed contains saponins, phytosterols, flavonoids, and phenolics group compounds (Singh and Raghav 2012). This plant has proven pharmacological activity including the reduction of glucose levels (Esmaeili et al. 2015), reduce postprandial blood glucose levels and prevent the reduction of degraded plasma insulin levels in diabetic male albino rats (Larasati et al. 2016). Tombili seed methanol extract was also reported to have antihyperglycemic and hypolipidemic activity in induced diabetic rats (Modak et al. 2007), but limited report that studied its advantages to lowering cholesterol activity. Since the process of cholesterol oxidation is similar to the process of other unsaturated lipids, we try to prove the effectiveness antioxidants activity of tombili to disrupt the radical chain reactions by donating hydrogen atoms to these molecules. The active hydrogen atom of the antioxidant abstracted by the reactive radicals; thus, inactive species are formed. In this research, antioxidant, cholesterol lowering activity, and chemical content analyses from fractions of tombili were evaluated. Antioxidant activity *in vitro* method was done by DPPH scavenging (Rafi et al. 2020).

Materials and methods

Materials

The tombili seeds collected from Bobohu villages, Gorontalo province, Indonesia. Tombili was identified at the Laboratory of Biology Department (Plant Taxonomy), Faculty of Mathematics and Natural Science, Gorontalo State University, Indonesia with the specimen being identified by Weny JA Musa and verified by the relevant department under specimen number of 021/UN47.B4./LL/2019 as *Caesalpinia Bonducella* (L.) *Fleming*. The chemicals used were methanol, ethyl acetate, and *n*-hexane solvent (Pro Analysis Grade) used for extraction and fractionation. 2,2- diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The cholesterol solution Sigma-Aldrich (St. Louis, MO, USA), sulfate acid (H_2SO_4), and acetic anhydride were purchased from Merck (Darmstadt, Germany).

Extraction and partition

The extract was made with 1.2 kg of Tombili seed powder and macerated for three days. To obtain n-hexane and ethyl acetate fractions, 200 of the extract was partitioned by liquid-liquid extraction with n- n-hexane and ethyl acetate at a 1:1 ratio. The rotary evaporator was used to concentrate all fractions. The yield of each solvent was then determined. The fractions were created by combining n-hexane (29.5 g) and ethyl acetate (63.4 g) Antioxidant and cholesterol-lowering activity was assessed in all fractions. The fraction with the highest antioxidant activity and the lowest cholesterol activity was injected into an LC-MS/MS for chemical compound analysis. The external appearance and necessary procedure for the extraction are shown on Fig. 1.

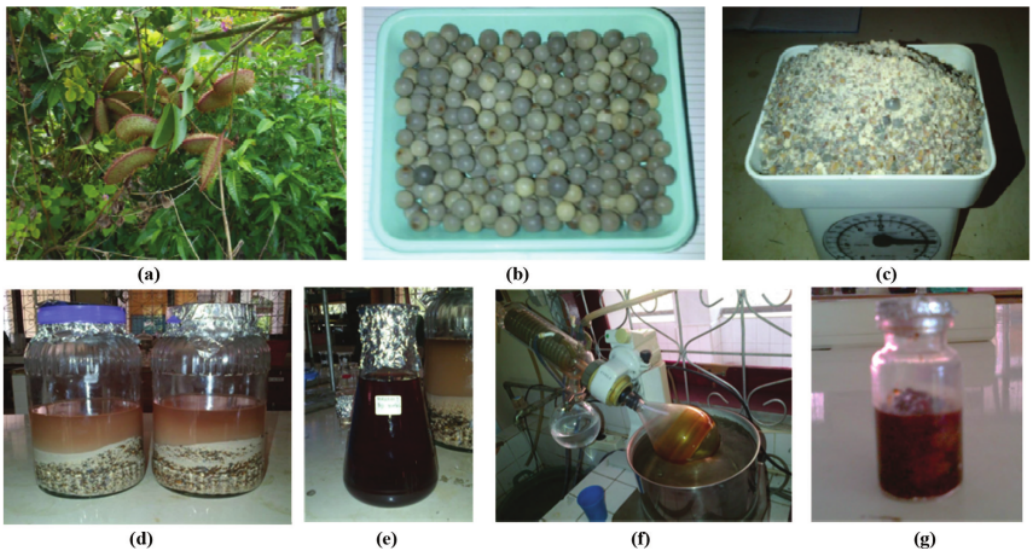

Figure 1. Tombili plant (**a**), Tombili fruit (**b**), Tombili powder (**c**), Extraction of tombili (**d**), Extract of tombili seed (**e**), Tombili extract evaporation, (**f**) Tombili thick extract (**g**).

Antioxidant activity test procedure

The antioxidant activity of each fraction was tested using the DPPH technique. According to Fareza et al. 2021, the test process was followed. A total of 2400 L stock solution of fractions was made in series concentration (200, 250, 300, 350, and 400 ppm). 600 L of DPPH solution with a concentration of 160 g/mL was added to each tube. The tube was incubated at room temperature for 30 minutes. The absorbance was measured at 517 nm using UV-Vis spectrophotometry (Rafi et al. 2020). As a control, methanol and DPPH were employed. The experiments were carried out in triplicate (n=3). The inhibition percentage (%) was computed as follows:

$$\%\text{inhibition} = [(\text{absorbance control} - \text{absorbance sample}) / \text{absorbance control}] \times 100\%$$

Determination of cholesterol-lowering potential

The fractions' reduced cholesterol levels were determined by comparing them to the blank absorbance (cholesterol solution). The cholesterol solution stock was created by combining 100 mg of cholesterol in 100 mL of ethanol (1000 ppm). H_2SO_4 (0.1 mL) and acetic anhydride (2 mL) were added to 0.025, 0.05, 0.1, 0.2, 0.3, and 0.4 mL of cholesterol solution in a cuvette (BRAN UV cuvette) and diluted with ethanol solvent until 5 mL, respectively (blank solutions). To 0.5 mL of cholesterol solutions, 0.025, 0.05, 0.1, 0.2, 0.3, and 0.4 mL of solution (1000 ppm) to concentrations of 100, 200, 400, and 600 ppm sample, H_2SO_4 (0.1 mL), acetic anhydride (2 mL), and ethanol until 5 mL were added, respectively. After being incubated for 30 minutes at room temperature, the absorbance of the solutions was measured at λ 423 nm (Musa et al. 2019).

Results and discussion

Extraction and partition

The extraction efficiency from plant materials is influenced by several factors, including the chemical screening of phytochemicals, the partition method used, the size of sample particles, and the presence of interfering substances (Stalikas 2007). The extractions yield depends on the temperature, extraction time, solvent polarity, and sample content. In this work, the extracts of tombili seed were partitioned by using various range polarity of solvent from nonpolar (*n*- hexane) to polar (methanol). The final methanol fraction obtained 107.1 g (55.35%), *n*-hexane (14.7%) while ethyl acetate (31.7%). It can also be seen that the fraction yield of methanol is higher than ethyl acetate. These results show that the extraction yield.

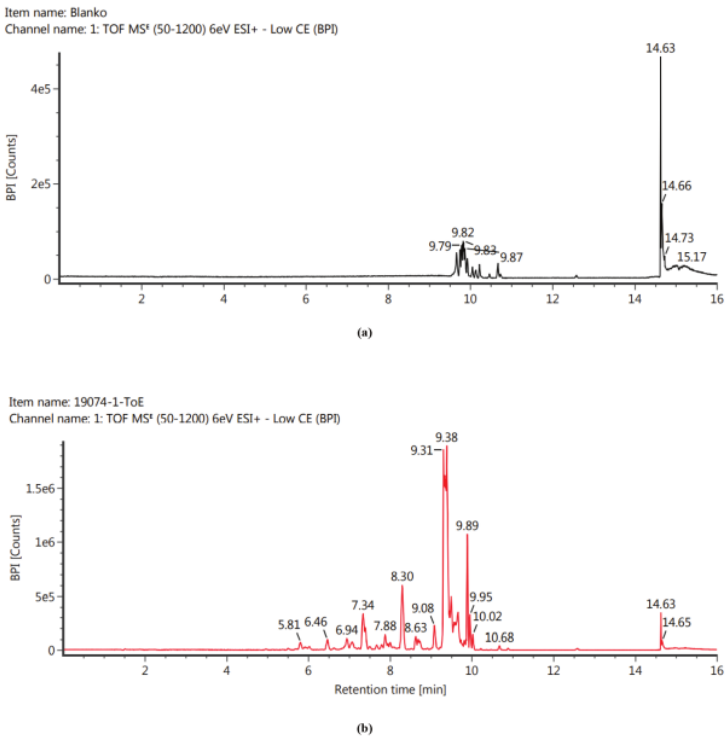

Figure 2. LC/MS profiles of (**a**) Blank, (**b**) Ethyl acetate fraction.

Antioxidant activity of tombili seed extracts

DPPH was used to determine the antioxidant activity of tombili seed extracts. With the highest absorption band at 517 nm, DPPH was used as a stable organic free radical reagent. The absorption of this radical species was lost when the polarity of the solvent used in the extraction process of tombili seed was increased. Primary metabolites (proteins and carbohydrates) have been extracted in water and ethanol, resulting in a higher yield than ethyl acetate and *n*-hexane.

Accepted, resulting in a purple-to-yellow visual discoloration This radical reacts quickly with a wide range of samples and is sensitive enough to distinguish active ingredients at low concentrations (Esmaeili et al. 2015). The results of the antioxidant activity test of fractions shown in Table 1.

Table 2 shows the in vitro DPPH radical scavenging ability of tomboli seed extract in different solvents. The n-hexane extract had the lowest DPPH radical inhibition, with an IC50 value of 100.933, while the ethyl acetate extract had the highest activity (86.153 g/mL). Although the DPPH radical scavenging activities of different solvent extracts were lower than those of ascorbic acid as a standard reference compound, this study shows that tombili seed extract has free radical scavenging properties as a primary antioxidant. Some free radical reactions are inhibited by phenolic and flavonoid compounds. Some phenolic compounds in ethyl acetate extracts, such as flavonoid and phenolic acids, demonstrated strong antioxidant activity via their reductive capacity (Rafi et al. 2020). The hydroxyl group of homoplantaginin and vernolic acid compounds was thought to be involved in free radical reduction. Considering the abundance of hydroxyl group of homoplantaginin it can be considered the biologically active from the fraction of ethyl acetate, thus allowing the antioxidant activity of the fraction toward the DPPH. Homoplantaginin contains several aromatic rings and hydroxyl group to implement the antioxidant mechanism. In comparison with the previous research done by Badami et al. (2003) using *Caesalpinia Sappan* yield a very strong antioxidant activity from the ethyl acetate extract while no further metabolites were explained from the literature. The secondary metabolites consisted of glucoside, homoplantaginin, and vernolic acid contributed to the fraction ability as antioxidant for the DPPH method analysis due to the hydroxyl group from the homoplantaginin to prevent free radicals from DPPH to oxidize the supposed protected molecule in practice as illustrated in Fig. 4 which illustrate the reaction of the molecule.

Table 1. Antioxidant activity test of fractions of *Caesalpinia bonducella* seed.

Fractions	Concentration (ppm)	% inhibition[1]	% inhibition[2]	% inhibition[3]	IC_{50} µg/mL	Average (µg/mL)
n-Hexane	20	6.308	5.900	6.088	100.798 99.505 102.495	100,933±1.4995
	25	9.227	10.041	9.803		
	30	12.409	12.008	11.764		
	35	14.994	15.010	14.860		
	40	16.856	17.080	16.718		
Ethyl acetate	20	4.251	5.678	6.832	88.499 81.286 88.675	86.153±4.2161
	25	8.299	10.972	9.420		
	30	12.348	13.474	14.078		
	35	14.372	16.554	17.184		
	40	17.712	20.692	18.840		
Methanol	20	11.334	6.308	6.617	93.551 95.890 92.718	94.053±1.6445
	25	12.938	9.227	10.084		
	30	16.248	12.409	11.974		
	35	18.115	14.994	15.336		
	40	22.066	17.786	18.907		

Table 2. Cholesterol lowering activity test of fractions *Caesalpinia bonducella* seed.

Sample	Concentration (ppm)	% exhibited[1]	% exihibited[2]	% exihibited[3]	Average (%)
n-hexane	100	7.9	6.1	7	7
	200	2	6.1	4.05	4.05
	400	22.8	21.2	22	22
	600	25.7	31.3	28.5	28.5
Positive Control	100	57.4	49.5	53.5	53.5
Ethyl acetate	100	15.9	13.8	14.9	14.9
	200	43	40.6	41.8	41.8
	400	67.9	67.5	67.7	67.7
	600	80.9	82	81.5	81.5
Positive Control	100	90.9	91.6	91.4	91.3
Methanol	100	15.5	7.7	11.6	11.6
	200	47.1	39.5	43.3	43.3
	400	68.2	65.5	66.9	66.9
	600	76.2	77.3	76.8	76.8
Positive Control	100	83.7	83.4	83.6	83.6

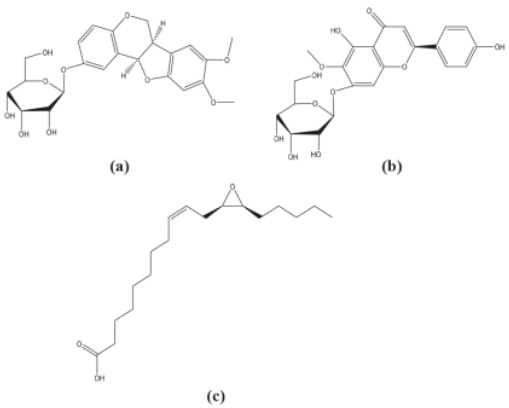

Figure 3. Chemical structures of compounds: (**a**) 9,10-Dimethoxy-pterocarpan-3β-glucoside, (**b**) homoplantaginin, (**c**) vernolic acid.

Cholesterol lowering activity of tombili seed extracts

The amount of cholesterol-lowering salt was reduced dose-dependently. The cholesterol level in the untreated fractions, also known as the negative control, was calculated to be 100%. All experiments were repeated three times (n = 3) and compared to negative and positive controls. Table 2 shows the outcome of the cholesterol-lowering activity.

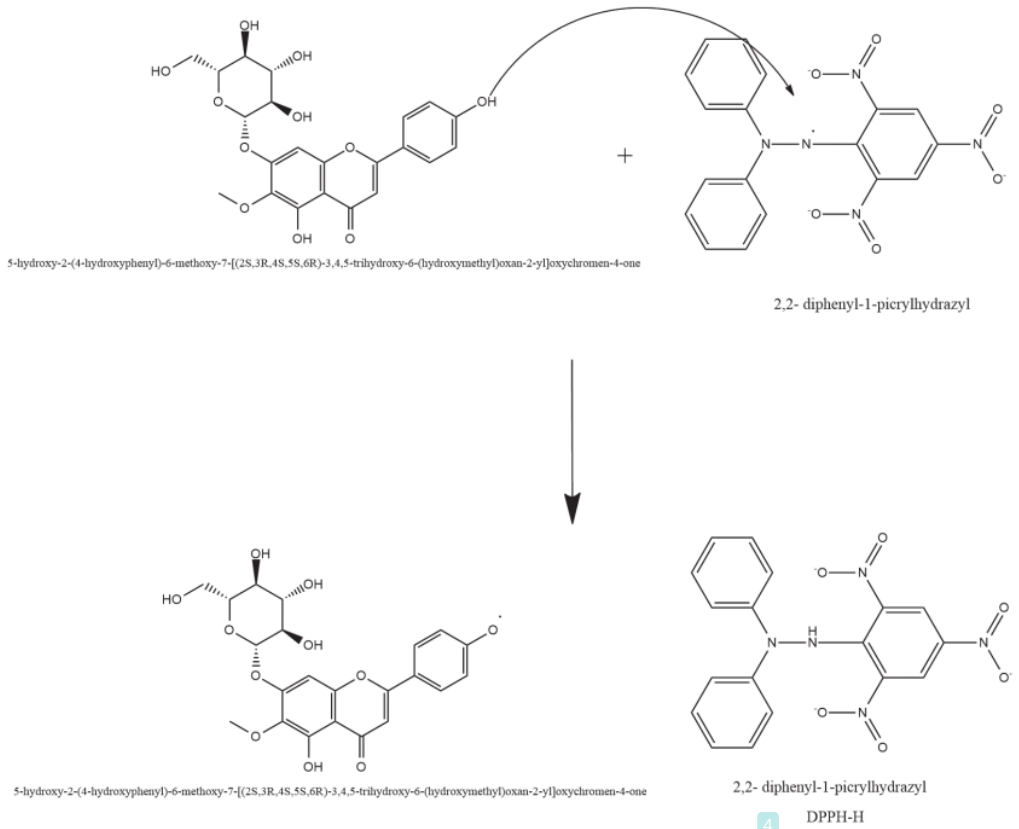

Figure 4. Antioxidant chemical reaction of 2,2-diphenyl-1-picrylhyrazyl and homoplantaginin (5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one).

Item name: 19074-1-ToE, Sample position: 2:A,5, Replicate number: 1

.	Component name	Identification status	Observed m/z	Neutral mass (Da)	Observed RT (min)
1	9,10-Dimethoxy-pterocarpan-3-O-β-D-glucoside	Identified	485.1405	462.15260	8.63
2	Homoplantaginin	Identified	463.1223	462.11621	7.16
3	Vernolic acid	Identified	319.2252	296.23514	9.35
4	Candidate Mass C17H16N2O2	Identified	281.1275	280.12118	9.89
5	Candidate Mass C26H38O7	Identified	463.2671	462.26175	9.35

.	Component name	Identification status	Detector counts	Response	Adducts
1	9,10-Dimethoxy-pterocarpan-3-O-β-D-glucoside	Identified	34844	24981	+Na
2	Homoplantaginin	Identified	72640	49657	+H
3	Vernolic acid	Identified	3466951	2607711	+Na
4	Candidate Mass C17H16N2O2	Identified	515704		+H
5	Candidate Mass C26H38O7	Identified	69954		+H

.	Component name	Identification status	Formula	Mass error (mDa)
1	9,10-Dimethoxy-pterocarpan-3-O-β-D-glucoside	Identified	C23H26O10	-1.4
2	Homoplantaginin	Identified	C22H22O11	-1.2
3	Vernolic acid	Identified	C18H32O3	0.8
4	Candidate Mass C17H16N2O2	Identified	C17H16N2O2	-0.9
5	Candidate Mass C26H38O7	Identified	C26H38O7	-2.0

Figure 5. The profiles of the identified five major compounds measured with LC/MS apparatus.

It was first reported that tombili fractions have cholesterol-lowering properties. All fractions were carried out at concentrations of 100, 200, 400, and 600 ppm, respectively. The colorimetric experiment revealed an anti-cholesterol effect for n-hexane, ethyl acetate, and methanol fractions. The ethyl acetate component has the highest percentage. More than 50% of cholesterol can be detected in a 600ppm ethyl acetate fraction. Ethyl acetate has a greater effect than n-hexane and methanol.

Caused by the concentration of the chemical compound in fraction with the hydroxyl group that interacted with another functional group through hydrogen bonding. Unlike ethyl acetate, the intramolecular reaction of *n*-hexane and methanol fractions does not occur.

Identification of chemical content

The ethyl acetate fraction TEA3 was analyzed for its chemical composition using LC-MS/MS (Fig. 2). Further MS analysis of fraction was conducted on three major compounds 9,10-Dimethoxy-pterocarpan-3-O-β- D-glucoside, homoplantaginin, and vernolic acid are shown in Fig. 3. The profiles for the compounds are shown on Fig. 5.

Role in its higher activity as an anticancer therapy. These outcomes Liquid chromatography-mass spectrophotometry (LC/MS) analysis of ethyl acetate fraction showed five obvious peaks at retention times of 7.34, 8.63, 9.31, 9.38, and 9.89 min as shown on Fig. 6. Based on the LC/MS/MS spectra database finding; the compounds 9,10-dimethoxy-pterocarpan-3β glucoside, homoplantaginin, and vernolic acid were identified at retention times 7.34, 8.63, and 9.31 min, respectively. The fragmentation resulted in five candidate masses with spectra (m/z) of 462.11621, 485.1406, 39.2252, 463.2671, and 281.127 are shown in Table 3.

Conclusion

The tested cholesterol lowering property of *n*-hexane, ethyl acetate, and methanol fraction from tombili seed was first reported. The highest antioxidant activity was shown in the ethyl acetate fraction. All fractions showed cholesterol lowering activity. Most importantly, the raised concentration of fractions exhibited a dose-dependent manner. The analysis of chemical content showed that the ethyl acetate fraction contains 9,10-Dimethoxy-pterocarpan-3β-glucoside, homoplantaginin, and vernolic acid compounds as lowering cholesterol agent.

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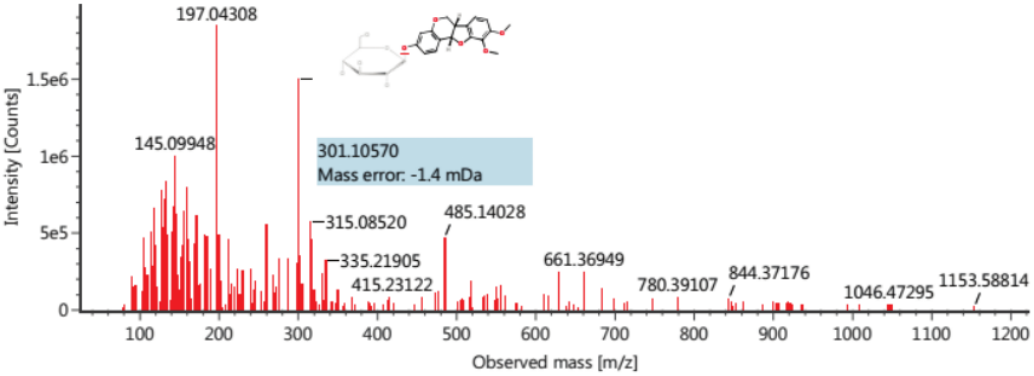

Figure 6. The mass spectrometer analysis on 9,10-dimethoxy- pterocarpan-3β- glucoside.

Table 3. The profiles of the identified five major compounds.

Peak	tR (min)	Formula	Observed actual mass	Neutral mass (Da)	Adduct	Mass error (mDa)	Identification
1	8.63	C23H26O10	485.1405	462.15260	+Na	-1.4	9,10-dimethoxy-pterocarpan-3β-glucoside
2	7.34	C22H22O11	463.1223	462.11621	+H	-1.2	Homoplantaginin
3	9.31	C18H32O3	319.2252	296.23514	+Na	0.8	Vernolic acid
4	9.89	C17H16N2O2	281.127	280.12118	+H	-0.9	–
5	9.38	C26H38O7	463.2671	462.26175	+H	-2.0	–

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