



A PRELIMINARY STUDY OF INVESTIGATING OF COMPOUND GROUP CONTAINED IN ETHANOLIC EXTRACT OF MAHAGONY (*Swietenia mahagoni* L. Jacq.) SEEDS RELATED TO α -GLUCOSIDASE INHIBITION

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Abstract. A preliminary study to determine the group of compound contained in the ethanolic extract of mahagony (*Swietenia mahagoni* L. Jacq.) seeds and its inhibitory activity to α -glucosidase enzyme has been done. The information from this study will be used in the further investigation about the specific constituents related to the bioactivity. The seed was grounded and then extracted with ethanol by maceration technique. The crude extract was separated with liquid-liquid extraction by using *n*-hexane, ethyl acetate, and methanol as the solvents. The best eluent for isolation, chloroform:ethanol (1:1), was determined by thin layer chromatography while alumina as stationary phase. The isolation step with column chromatography gave two types of isolates, yellow and colorless isolate. In order to get information about the compound, the crude extract was subjected to phytochemical assignment and the isolate with the better activity was analyzed by infrared spectroscopy. The inhibitory activity for the yellow isolate with IC_{50} as 19.345 ppm was better than the colorless isolate. Therefore, the IR spectroscopy assay was subjected to the yellow isolate. Based on the comparison IR spectra with literatures, it has suggested that the yellow isolate contains tetranortriterpenoid or limonoid group.

Keywords: *Swietenia mahagoni* L. Jacq., diabetes type 2, α -glucosidase, tetranortriterpenoid

I INTRODUCTION

Diabetes mellitus (DM) is rising fast enough nowadays [1]. It is divided into two types, type 1 and 2, where the majority of people with diabetes are affected by type 2 DM (T2DM). It is closely related to the lifestyle and diets consumed daily. It includes one of four priority non-communicable diseases (NCDs) in the world after heart disease, cancer, and respiratory syndrome. It also has a greater number of patients which predicted by International Diabetes Federation (IDF) as much as 382 million people in 2013 and will increase to 582 million in 2035 for around the world [2]. DM can lead patients to gain either complications in many parts of the body or overall risk of premature dying (WHO, 2016). WHO has suggested that preventing both of them can be practiced by regular exercising, healthy diets, avoid smoking, and controlling glucose blood via medical treatment. Medical treatment for T2DM has several types of techniques. One of them is oral medicine for increasing the insulin secretion or inhibiting of enzyme dipeptidyl

peptidase IV. Both of them have side effects for body such as hypoglycemia and obesity [3]. However, inhibition of enzyme α -glucosidase through postpone the glycolysis process in the intestines gives interesting option. By decreasing the absorption of monosaccharide in blood, it could be an alternative solution to reduce the side effects of T2DM medical treatment.

For many years, Indonesian people have been performing their local wisdom related to cure some particular diseases by consuming plant's extract from surrounding. Many species of plants could be used to be T2DM medicine and also give lower side effects for body than chemical one [4]. One example, the famous one as a component for traditional medicine called jamu, is *S. mahagoni* L. Jacq. seed [5]. Some experiments have studied it scientifically but none has clearly reported the constituent related to its bioactivity. Li *et al.* (2005) have explained that the ethanolic extract *S. mahagoni* L. Jacq seeds inhibited α -amylase enzyme as much as 70.33% at 200 μ g/mL of concentration and acted as agonist of PPAR- γ [6]. Sathish

et.al. (2010) and Wresdiyati *et al.* (2015) have reported that crude ethanolic extract of the seeds acted as anti-hyperglycemic to diabetic rat significantly [7,8]. The otherhands, Sukardiman *et al.* (2013) has confirmed that 96% ethanolic extract of the seed combined with leaves of *Andrographis paniculata* gave hypoglicaemic activity to diabetic rat [9]. All of them suggested to conduct more deep investigation to the specific compounds related to its bioactivity.

Beside *in vivo* approach researches as mentioned above, there are several investigations which used *in vitro* approach by conducting bioassay to α -glucosidase enzyme inhibition. Masitha (2011) has confirmed that crude extract of *S. mahagoni* has the best inhibition capacity compared with the 14 other plants [10]. The experiment which conducted without purification step has reported the value of IC_{50} as 7.03 ppm [10]. Aliyan (2012) has performed an experiment through fractionation step to determine the group of bioactive compound in the isolate of *S. macrophylla* seeds related to the enzyme inhibition [11]. It said that the best isolate is in petroleum ether fraction with IC_{50} as 15.44 ppm [11]. Based on the literatures review, there was no experiment which deeply studied yet to which kind of the compounds in the *S. mahagoni* L. Jacq seed affects to the inhibition of the enzyme.

Based on the facts above, this experiment is aimed to identify the type of compound group from ethanol extract of *S. mahagoni* L. Jacq. seed and determine its inhibition capacity toward α -glucosidase enzyme. It is an early step of multiple-steps research to investigate the specific chemical constituent in the *S. mahagoni* L. Jacq. seed which responsible to bioactivity of anti-hyperglycemia. The inhibition capacity expressed in inhibition concentration 50 % (IC_{50}) which gained by conducting bioassay of the extract to *p*-nitrophenyl α -D-glucopiranoside in spectrophotometric stop rate determination [12]. Identification step for the type of compound group is performed by using IR spectrophotometry. Through comparing the IR spectra of the experiment with the literatures, the type of the compound group related closely to the bioactivity of the seed could be predicted.

II METHODOLOGY

The experiment was conducted at Chemistry Laboratory of FTK UIN Ar-Raniry, Chemistry Laboratory of FKIP Unsyiah and Laboratory of Pusat Studi Biofarmaka IPB on June to October

2016. The equipment used in preparation of sample were extraction tools (Borosil, Lucknow), scissors, separated funnel (Pyrex), filter tools, blender (Miyako), rotary evaporator (RIIO Buchi, Switzerland), and measuring flask (Pyrex). The chemicals in analytical grade were *n*-hexane, ethyl acetate, and ethanol purchased from Sigma-Aldrich. The sample was *S. Mahagoni* L. Jacq. seeds taken from Gunung kidul Yogyakarta on June 2016. The sample was determined its taxonomy classification by Pusat Konservasi Tumbuhan Kebun Raya-LIPI Bogor. The equipments used in phytochemical screening were hotplate, reaction tube (Pyrex) and the holder, mortar, separated funnel (Pyrex), beaker glass (Pyrex), balance, Erlenmeyer (Pyrex), and measuring flask (Pyrex). The chemicals from Sigma-Aldrich were *n*-hexane, ethyl acetate, methanol, ethanol, ether, chloroform, ammonia, $HgCl_2$, KI, $(BiNO_3)_3$, H_2SO_4 , HCl, and gelatine. The equipment used in purification were column size 50 x 5 cm (Pyrex); titration holder; Erlenmeyer size 100 mL, 250 mL, dan 500 mL (Pyrex); beaker glass 250 mL and 500 mL (Pyrex); rotary evaporator (RIIO Buchi, Switzerland); mixing bar; plate TLC (Merck); and alumina (Merck). The chemicals were *n*-hexane, ethyl acetate, and chloroform. The isolates were identified their functional group by FT-IR (Fourier transform infrared) spectrophotometer Perkin Elmer 100 (Perkin Elmer, Inc., USA).

The isolate was tested for inhibitory activity at Pusat Studi Biofarmaka IPB by spectrofotometric stop rate determination method for determination of IC_{50} . The equipments were pH meter, spectrofotometer UV-Vis (Cary-50-Bio Varian), kuvet, vortex mixer, plate reader, microplate, micro pippet, multichannel pipette, and incubator. The chemical; in analytical grade and provided by the laboratory; were α -glucosidase enzyme which recombinant of *Saccharomyces cerevisiae* (Sigma-Aldrich), substrate of *p*-nitrophenol- α -D-glucopiranoside (PNPG, Sigma-Aldrich), dimethyl sulfoxide (DMSO, Merck), bovine serum albumin (BSA, Sigma-Aldrich), acarbose, sodium carbonate (Merck), potassium dihydrogenfosfate (Merck), sodium hydroxide (Merck), and aquadest.

Procedure of sample preparation adapted from Li *et al.* (2005) with slight modification [6]. The seed of *S. Mahagoni* L. Jacq. was completely washed with water and dried as long as a week faraway from sunlight. The dry seeds were cut in small size about 0.5 - 1 cm for the diameter then granulated by a blender machine.

Wresdiyati *et al.* (2015) described that ethanolic maceration to *S. Mahagoni* L. Jacq. seeds able to keep the better performance of α -glucosidase inhibition than the other methods [8]. As much as 200 g of the powder was mixed with 1 L of ethanol at room temperature as long as 24 h then filtered the mixture in order to the filtrate. The step was repeated 4 times with new solvent in every repeating then collected the filtrate in a beaker glass. Therefore, evaporating the filtrate used rotary evaporator at 30-40°C for 2 h. The sample was stored at refrigerator (below 4°C) for further steps.

Phytochemical Screening

Phytochemical assay is used for qualitative determining to the possibility of presence of secondary metabolite compounds, e.g. alkaloids, phenolic, or terpenoid and the derivatives. The procedures taken from Harborne (1984) with a slight modification [13].

Alkaloid assay

As much as 0.1 g of ethanolic extract dissolved into 5 mL of chloroform and 5 mL ammonia then placed to two tubes. The sample in the first tube added with 10 drops of sulfuric acid 2M. The acidic layer formed is taken off and placed to two tubes then tested to Wagner and Dragendorff reagent. A sample contained alkaloid will give brown precipitate with Wagner reagent and orange precipitate with Dragendorff reagent.

Saponin assay

As much as 0.1 g of crude ethanolic dissolved to 15 mL of hot water then heated for 5 min. The mixture filtered by Whatmann filter paper then 10 mL of the filtrate removed to a tube and shaken. A sample contained saponin will form foam.

Tannin assay

As much as 0.1 g of crude ethanolic extract dissolved into 10 mL of hot water to reach room temperature. Added 5 drops of NaCl 10% then filtered. Filtrate which gained was placed to two tubes, A and B. Filtrate A as stock solution, filtrate B added gelatin salt. White precipitate shows that the sample contains tannin.

Polyphenol assay

As much as 0.1 g of crude ethanolic extract dissolved into 2 mL of ethanol 96%. Then added 5 mL of water and 0.5 mL of Follin-Ciocalteau (50% v/v) reagent, wait for about 5 min. Added 1 mL of sodium carbonate solution (7.5% b/v), incubated at room temperature for 1 h without lightening. The color of the sample

turn to darkish blue indicated that it contains polyphenol compounds.

Flavonoid assay

As much as 0.1 g of crude ethanolic extract dissolved in 10 mL of methanol then placed to four tubes. The first tube as stock solution, second, third, and fourth added by NaOH, H₂SO₄ saturated, and Mg-HCl saturated, respectively. The color changing of the samples in the last third tubes indicated that they contain flavonoid.

Quinone assay

As much as 0.1 g of crude ethanolic extract added by 5 mL KOH 0.5M and 1 mL of hydrogen peroxide 5% then heated for 10 min, filtered, added by acetic acid, and added 5 mL of benzene. Taken off the benzene layer then added by ammonia. The positive result indicated by ammonia layer turns to red and benzene layer remains colorless.

Steroid assay

As much as 0.1 g of crude ethanolic extract added by 2 mL of chloroform and 5 drops of H₂SO₄ 6M. The positive result indicated by the color changing of the sample to brown.

Triterpenoid assay

As much as 1 g of crude ethanolic extract added by 20 mL of ethanol then heated and filtered. The filtrates added 2 mL chloroform and 3 mL of H₂SO₄ saturated. The positive result indicated by the color changing of the sample to red.

Fractionation

In order to separate the sample, the crude extract was needed to be fractionated through liquid-liquid extraction in separating funnel. It was dissolved into a combination of solvent, methanol-water (1:2), then partitioned three times repetition with 50 mL of *n*-hexane, ethyl acetate, and methanol for every repeating. It strongly shakes for about 5 min to ensure a complete dissolving mixture. The sample will separate into two or more fractions based on the type of polarity. The fraction placed in the different erlenmeyer then evaporated at 30-40°C by rotary evaporator for about 2 h. The sample was stored at refrigerator for isolation step.

Isolation

Isolation and purification were conducted with thin layer chromatography (TLC) and column chromatography (CC). These methods have been proven by Kadota *et al.* (1990) who has successfully isolated 30 limonoid compounds

from *S. mahagoni* L. Jacq seeds by using the both methods in purification step [14]. TLC also used in detecting of all fractions resulted from CC [15]. In order to get the best eluent in the purification step, TLC was performed with alumina as static phase and the mobile phase is chloroform:ethanol (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; and 1:9). The best proportion was used as mobile phase in CC with alumina as the absorbent. Through this CC, the fraction will be separated into several sub-fractions based on their similarity of physical properties (phase and color).

The sub-fractions will be placed in different reaction tubes and subjected TLC method to determine the type of isolate based on the similarity of retention time (R_f). The isolate(s) was collected in beaker glass then evaporated in the room temperature to get the dry isolate(s). All isolate(s) were stored in refrigerator before conducting inhibition activity assay and identification of compound group.

Inhibition assay of *S. mahagoni* L. Jacq. to α -glucosidase enzyme

Bioactivity of the isolate of *S. mahagoni* L. Jacq seeds was quantitatively determined by using spectrophotometric stop rate determination method which adapted from Masitha (2011) and Ibrahim *et al.* (2014) [10,12]. As much as 10 μ L of each isolate or acarbose, at different concentrations (0.0625-1 ppm), was incubated with 250 μ L of *p*-nitrophenil α -D-glucopyranoside (pNPG) solution in 490 μ L of phosphate buffer (pH 6.8) at 37°C for 15 min. Thereafter, 250 μ L of α -glucosidase enzyme solution (1 U mL⁻¹) in 100 mmol L⁻¹ of phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37°C for 20 min. The reaction has been stoped by adding 2000 μ L of Na₂CO₃ 200 mmol L⁻¹. The absorbance of the released *p*-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitor. It could be calculated by the formula in Eqs. (1) [10].

$$\%inhibitory\ act. = \left[1 - \frac{A_{sample}}{A_{control}} \right] \times 100\% \quad (1)$$

The capacity of inhibitory activity of the sample was expressed in IC₅₀. It could be calculated by using linear regression equation, $y=a+bx$, where x is concentration of the sample and y is the % inhibitory activity. The value of IC₅₀ was calculated by Eqs. (2) [10].

$$IC_{50} = \frac{50-a}{b} \quad (2)$$

Identification of compound group

The identification of the compound groups was performed with FTIR spectrophotometer to the isolate with the better inhibition capacity. This was generally used as a result from absorption of energy by stretching and bending of any bonding of the functional group. The performance of FTIR instrument is much efficient, quick result, high sensitivity, and requires very small quantity of the sample [16]. All researchers who investigate the constituent contained in the natural products always use IR spectra to predict their group of compounds. They suggest that IR spectroscopy is the main and the powerful enough method to determine the group of compounds to support this study.

III RESULT AND DISCUSSION

The research was begun by drying the sample for a week. Its water content has to being reduced because of efficiency reason of solvent consuming in maceration step. Then, the cover of the seeds should be taken off to get the seed. Therefore, the seeds have to being cut into smaller size in which seem like a powder form to increase the surface area of the sample. Larger surface area would increase the possibility of interaction between extract and solvent. As much as 200 g of the sample was extracted through maceration because the organic compound able to be broken when meet higher temperature on this step. Maceration was the better method for *S. mahagoni* L. Jacq seeds than reflux [8]. The step was conducted for 4 d followed by periodical stirring to increase the rate of the solvent's mobility.

Every 24 h, the solvent has been replaced to ensure maximum migration of the chemicals. Before change the solvent, the sample was filtered while the residue treated re-maceration. The filtrate collected in erlenmeyer then dried with rotary evaporator at 60°C as long as 2 h. The temperature lowers than the boiling point of the solvent (78°C) to avoid sample losing. The step gave oily, amorphous, and yellowish extract with rendement as 32.4%. The dried extract thus dissolved into ethanol:water (1:2) to make a suspension. Therefore, it was separated with *n*-hexane, ethyl acetate, and methanol (1:1:1) combination to attract the suitable compounds. The extract was shaking for 5 min to ensure complete mixing and became two layers. Then, it was separated with separating funnel and evaporated with rotary evaporator for about an hour at 40°C.

Table 1 Results of phytochemical assay for the extract of *S. mahagoni* L. Jacq seeds

No	Type of Test	Observation	Result
1	Alkaloid		
	a. Dragendorff	Brown-orange precipitate	+
	b. Wagner	Brown precipitate	+
2	Saponin	Bubble appear	+
3	Tanin	Muddy-white solution	+
4	Polyphenol	Dark blue solution	+
5	Flavonoid	No change in color	-
6	Quinone	No change in color	-
7	Steroid	Brown solution	+
8	Triterpenoid	Red solution	+

Remark: (+) indicates the presence and (-) indicates the absence of the target compound

Table 2 Results of inhibitory activity test of the isolates of *S. mahagoni* L. Jacq seeds to α -glucosidase enzyme

Concentration (ppm)	Yellow Isolate						Colorless Isolate					
	Absorbance			Value of IC ₅₀			Absorbance			Value of IC ₅₀		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
1	1.81	1.72	1.78				1.53	1.63	1.91			
0.5	1.87	1.73	1.86				1.40	1.61	1.71			
0.25	1.77	1.72	1.92	23.131	30.080	4.8225	1.26	1.38	1.61	-1.515	-1.884	-1.509
0.125	1.91	1.72	1.96				1.25	1.33	1.47			
0.0625	1.73	1.67	1.95				1.24	1.37	1.45			
IC ₅₀ average= 19.345 ppm							IC ₅₀ average = -1.636 ppm					

Table 3 Data of analysis of yellow isolate with IR spectrophotometer

No	Wave Number (cm ⁻¹)	Intensity	Functional Group
1	3337.563	strong	stretching of -OH
2	1636.472	middle	stretching of C=C
3	1339.212	weak	bending of C=O cyclic carbonyl
4	1052.674	middle	bending of C-O ether
5	994.819	middle	bending of furan

After that, both extracts was evaporated in the room temperature for 24 h and gained yellow and white precipitate. Phytochemical assay has been practiced to the extract following the standard procedures for natural product screening. The specific reagent for each secondary metabolite compound is used in this qualitative test through observing the color changing of the sample. Table 1 show the observation recorded. The separation of the sample depends on the polarity order of the solvent by liquid-liquid extraction. This step gave 1 g of hexane fraction and 28 g of polar fraction. The further treatment, isolation with TLC and CC technique, was subjected to the polar fraction since it was the most active fraction as anti-hyperglycemic from the *genus* of *swietenia* seeds as confirmed by Dewanjee *et al.* (2009) and Maiti *et al.* (2009) [17,18].

Two organic solvents as mobile phase for TLC, chloroform:ethanol, following the suggestion from Dewanjee *et al.* (2009) who has successfully isolated swietenine from *S.*

macrophylla seeds for hypoglycemic therapy [17]. The best proportion is 1:1 based on the trial treatment to the sample. It was also used as mobile phase in CC which gave twenty five sub-fractions. Based on TLC-based-determination, the sub-fractions form two isolates with different color, colorless and yellow isolate. To ensure that the isolates were pure, TLC evaluation has been subjected to both isolates and gave single node for each of them. The isolates has also been determined their capacity of inhibitory activity. The result is shown in the Table 2 which pose a much different in inhibition activity. When converted into IC₅₀ value, the value for yellow isolate is 19.345 ppm while the value for acarbose is 0.044 ppm. In contrast, the colorless isolate has a very poor inhibition activity with IC₅₀ value is -1.636 ppm.

The activity of the sample has a little different with the result of Aliyan (2012) who successfully trained to petroleum eter fraction of *S. macrophylla* seed with IC₅₀ as 15.44 ppm

[11]. The experiment involves separation step through different polarity based solvent partition. Masitha (2011) also subjected for ethanol extract of *S. mahagoni* L. Jacq without separation and purification and successfully reported the best result ($IC_{50} = 7.03$ ppm) [10]. Depending on the trend, we can propose that bioactive compound contained on the mahagony seed may has a better bioactive potential as anti-diabetic agent in the crude form than the other form with either separation or purification step but it still need more deep investigation. Further research would verify this statement.

Investigation the compound group

The step is an analysis of functional group which contained in the isolate with the better activity, the yellow isolate, using IR spectrophotometry. A drop of the sample placed on the NaCl crystalline plat and then pressed with another plat. After finished, it inserted into the spectrometer to be treated by infrared rays. Table 3 shows by which the absorption of energy at the specific length of wave which done by some functional groups. Any atom or functional group will undergo stretching or bending when interacts with IR ray [22]. The first wave number is 3337.563 cm^{-1} in which the O-H bonding gives respond to the treatment. We can predict that the isolate has OH group. It belongs to alcohol while OH in the carboxylic acid has an extreme pattern of the spectra because of the hydrogen bonding more complex than the others. The second is 1636.472 cm^{-1} which included to responding area for C=C non-aromatic class with range about $1700\text{--}1600\text{ cm}^{-1}$. It can also predict that the compound has a conjugation system because its vibration frequency lower relatively [22,23]. The third is 1339.212 cm^{-1} in which the bending vibration of cyclic carbonyl could be taken place [23]. It might be as the sign of the presence of lactone ring where effect of cyclic

form and resonance able to be the reason why its vibration frequency falls to lower frequency significantly [16,23]. The fourth is 1052.674 cm^{-1} where the responding of C-O ether occupied ($1000\text{--}1200\text{ cm}^{-1}$). The last one is 994.829 cm^{-1} which included to the fingerprint area for furan ring [24]. The analysis of compound group in this research is depended on the particular information that it contains OH, chain type, and C=C bonding. If it is comparing with flavonoid structure, the data is not suitable to the flavonoid family because flavonoid is a phenolate compound with formula as $C_6\text{--}C_3\text{--}C_6$ (two aromatic rings) [22]. Since alkaloid is a compound consists of carbon, hydrogen, nitrogen, and sometimes also oxygen [22], the data do not suggest to this family because of no nitrogen appeared in the spectra.

Tanin is a polyphenol compound which contains aromatic and OH groups. The data is far from both of the group. Quinone is a colored compound and has chromophore unit likely benzaquinone, a compound consists of two carbonyl groups which conjugated to two doubled bond of carbon-carbon. The data do not show them therefore it cannot be classified to the quinone family. Finally, the highest possibility for the compound contained in the isolate is going to a derivative of terpenoid group. The data of IR spectra tend to show that the group contains lactone and furan ring in which their characteristic nearly similar with some literatures. This statement has verified by comparing the data with previous reports about IR spectra data from several limonoid compounds of *Swietenia* genus; i.e. Lin *et al.*, 2009; Cheng *et al.*, 2014; Zhang *et al.*, 2014; and Ma *et al.*, 2017 [26,27,28,29]. It proves that the compound may be classified to limonoid group, a class of compound which is included as derivative of highly oxygenated triterpenoid group [30].

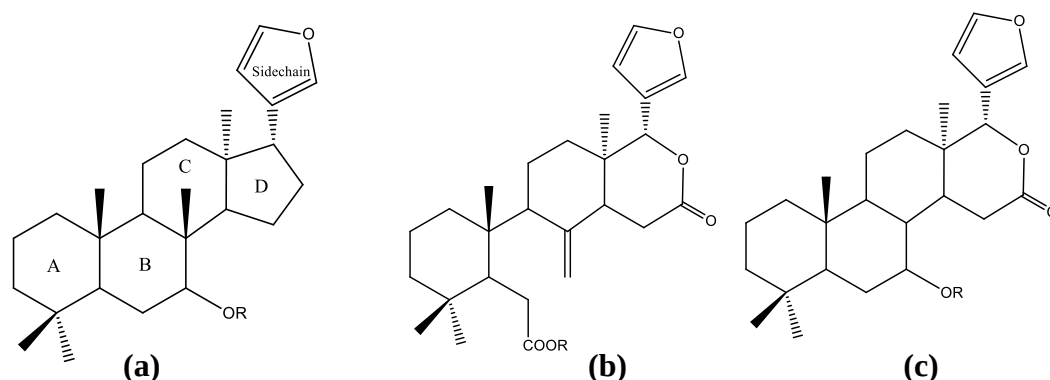


Figure 1 (a) General structure of meliaceae limonoid group; (b) structure of ring B,D-seco limonoid; and (c) structure of ring D-seco limonoid [31]

Table 4 Classification of meliaceous limonoid group founded in the *S. mahagoni* L. Jacq [32]

Class	Type	Ring A	Ring B	Ring C	Ring D	Sidechain
Ring B,D-seco limonoid	Mexicanolide	Intact	Recyclised	Intact	Lactone	Furan
Ring B,D-seco limonoid	Secomahoganin	Intact	Open	Intact	Lactone	Furan
Ring B,D-seco limonoid	Andirobin	Intact	Open	Intact	Lactone	Furan
Ring B,D-seco limonoid	Phragmalin	Modified	Recyclised	Intact	Lactone	Furan
Ring D-seco limonoid	Gedunin	Intact	Open	Intact	Lactone	Furan

The result of this identification has a great agreement with several previous reports before in which they said that the major constituents contained in the seeds of *S. mahagoni* L. Jacq, a member of *Meliaceae* family, is meliaceous limonoid group. The usual definition of a limonoid is a triterpene derivative in which the side chain has become a furan ring by the loss of four carbon atoms, hence an alternative name, tetranortriterpenoids, for the limonoids [33]. Structurally, the limonoids are derived from tetracyclic triterpenes which have oxidatively changes interspersed with molecular rearrangements.

The compounds of limonoid group which have founded from *S. mahagoni* L. Jacq belong to vary types based on variation of the skeletons (Table 4). There are mexicanolide type, secomahoganin type, andirobin type, phragmalin type, and gedunin type in which furan ring as their unchanged type of sidechain [28,31,32]. Thus, the presence of the limonoid group contained in the *S. mahagoni* L. Jacq could be recognized by the bending motion of lactone and furan group in fingerprint area of the IR spectra [31,32,34].

CONCLUSION

The ethanolic extract of *S. mahagoni* L. Jacq seed consists of several secondary metabolites which is proven by phytochemical assignment. The sample has given two isolates where yellow isolate has IC₅₀ as 19.345 ppm but the colorless isolate was inactive. The compound contained in the yellow isolate belongs to tetranortriterpenoid group which verified by FTIR analysis. Further investigations were needed to determine the comparison of inhibitory activity between crude extract and pure isolate of *S. mahagoni* L. Jacq seeds and identification the specific constituents related to the bioactivity.

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