

STUDY ON BARRINGTONIA ASIATICA KURZ

Asmaedy Samah *

ABSTRAK

Telah dilakukan suatu penelitian tentang *Barringtonia asiatica* Kurz. Dua senyawa saponin telah dapat dipisahkan dari benih *Barringtonia asiatica* Kurz. Titik lebur mereka adalah 243–245°C untuk senyawa R₁ dan 266–268°C untuk senyawa R₂. Spektrum IR menunjukkan bahwa senyawa R₂ memiliki functional groups yang terdiri atas OH, CH, C = C, C – O – C dan CH₃ sedangkan senyawa R₁ memiliki functional groups OH, C–H, C = C dan C–O–C dalam strukturnya.

INTRODUCTION

Various surveys carried out by several author^{1,6,9} reported that a great number of plants found in West Sumatera contained substances, known to have therapeutic effects, such as alkaloids, steroids, flavonoids, triterpenoid, phenolics and saponins. Out of 88 medicinal plants⁹, traditionally used as anthelmintics in West Sumatera, 20 of them were found to contain alkaloids, saponins, steroids, flavonoids, phenolics and triterpenoids compounds. From about 30 species of medicinal plants⁶ traditionally used as antidiarrheae, 10 of them had been phytochemically screened and found to contain alkaloids, steroids, triterpenoids, phenolics, flavonoids and tannin.

This great number of plants grown in West Sumatera might serve as a potential source for medicinal compounds and merit further research. The following is a report about a study of a specific plant which was reported about in the literature⁴ as having several economic values. The objectives of this study was to characterize the major compounds it contained and to undertake preliminary tests concerning its biological activity. The plant was identified as *Barringtonia asiatica*

Kurz, family Lecythidaceae⁷ or Myrta-ceae⁴.

Barringtonia asiatica Kurz is also known as *Barringtonia speciosa*, Forst⁴. It has several names in the various regions it is found. It is called "butun" in Java and "butung" in South Sulawesi. It is also called "putat gajah", "putat laut", "putat ayer" or "pertun" in other regions of Indonesia.

According to Burkill⁴, the plant has several economic values. The fruits are used in many places to intoxicate fish, for which purpose they are pulped and thrown into the rivers. The extract of the bark has been used for spraying caterpillar of *Parasa herbifera*, but it was found to be only a little toxic — much less so than the root of Derris. The saponin compound is the biologically active compound used for intoxicating fish. It is the purpose of this research to characterize further the saponin of *Barringtonia asiatica* Kurz.

THE EXPERIMENT

1. Plant collection and Identification.

Fruits of *B. asiatica* Kurz were collected from Pulau Persumpahan and Pulau Baronjong, two small islands situated

* Andalas University, Padang.

about 6 miles across Padang coast. West Sumatera. The seeds were taken out of the fruits and allowed to dry while keeping them away from direct sunlight. The dried seeds were then grounded and pulverized and kept in exicators until the extraction treatment. The identification of the plant was carried out at the Department of Biology, Andalas University, Padang.

2. Extraction Procedure

The solvents used in the extraction procedure are all of analytical grades. The dried-pulverized seeds were extracted by macerating three times for 2–3 days each with aethanol 98% as solvent. All extracts were collected and combined, and then concentrated under reduced pressure by using a rotary evaporator. The crude extracts obtained were then fractionated with other solvents of different polarities. The fractionation were done by extracting the residue with solvents of increasing polarity. The scheme of extraction and fractionation procedures is presented in Fig. 1. Each fractionated extracts were then concentrated under reduced pressure in a rotary evaporator, and identified for any group of compound it contained and their toxicity on fish.

3. Biological Activity Test

The toxicity test of the substances contained in each of the crude extracts was conducted using the widely known "ikan seribu" or *Lebistes reticulatus* Peter⁸. The following technique was applied.

An appropriate amount of crude extract was diluted in a jar with 2 liters of

water and mixed to become a homogeneous mixture. In this mixture, 10 fishes were introduced and observed for the number of deaths within 24 hours. As a control, another 10 fishes were also kept in a jar containing a mixture of 2 ml aethanol and 2 liters of water.

4. Identification Methods.

The identification of any compounds present in each extract was performed by using the following methods. The Alkaloids were identified by the Culvenor and Fitzgerald method⁵, the saponins by foam formation test, the steroid-triterpenoids by Liebermann–Burchard's test and concentrated sulphuric acid reagent, and the pehnolic compounds by ferric chloride reagent³. The results are presented in Table 1.

5. The Isolation of Sapogenins.

For the isolation of sapogenins, the methanol fraction was further hydrolyzed with hydrochloric acid 2N and then extracted four times with chloroform. The chloroform extracts were collected together and then washed with a solution of 5% sodium hydroxide and distilled water. The chloroform extract was then concentrated under reduced pressure and taken into TLC treatment. When a suitable solvent was obtained, the chloroform extract was separated by column chromatography. By using petroleum aether or aethyl acetat, alone or in a mixture of various solvent compositions, five crystalline substances can be separated. However, only two of them were obtained in appropriate amounts and identified further. The characterization of these substances were done by melting points and UV and IR spectroscopy methods.

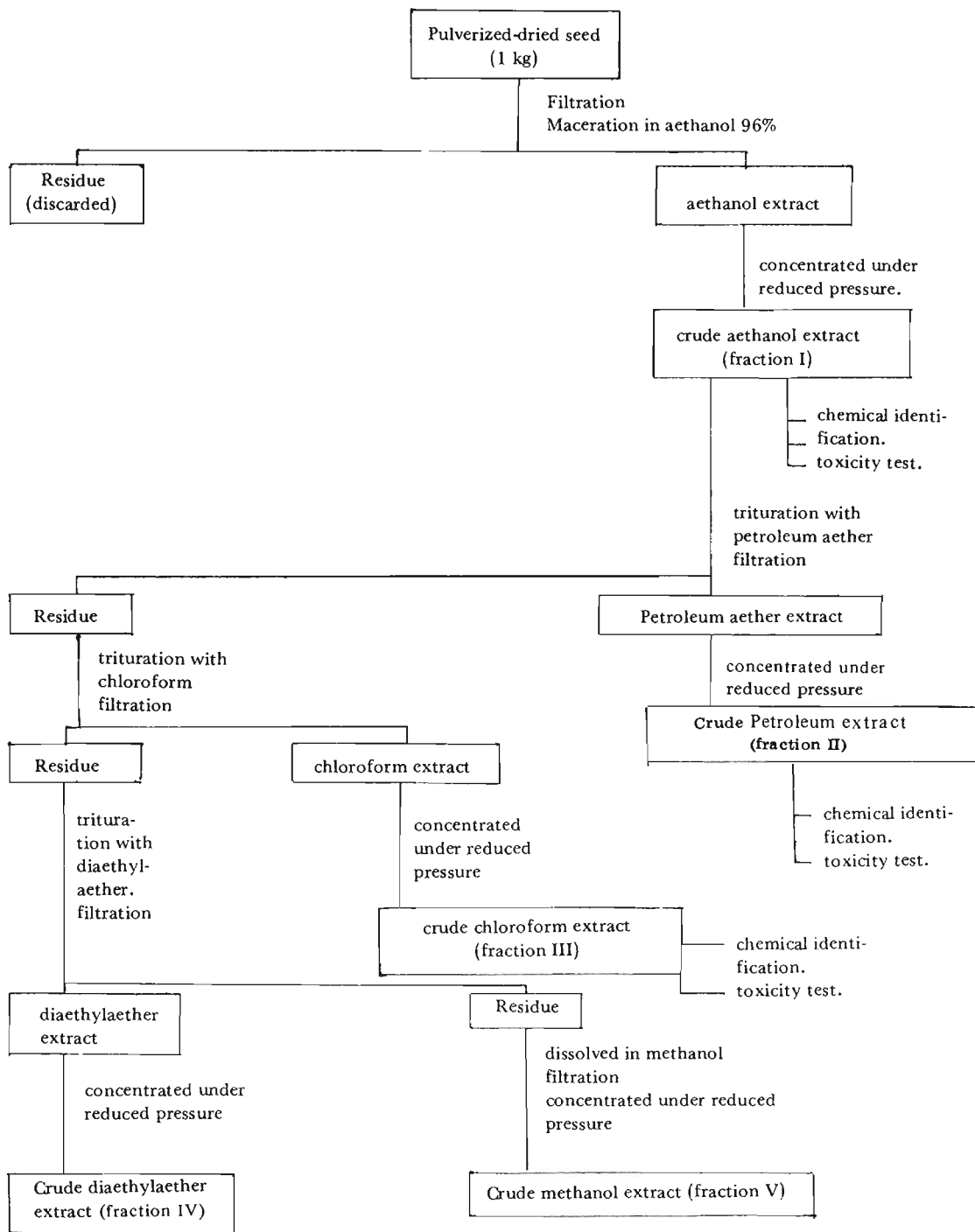


Fig. 1. Extraction scheme of *B. asiatica* Kurz.

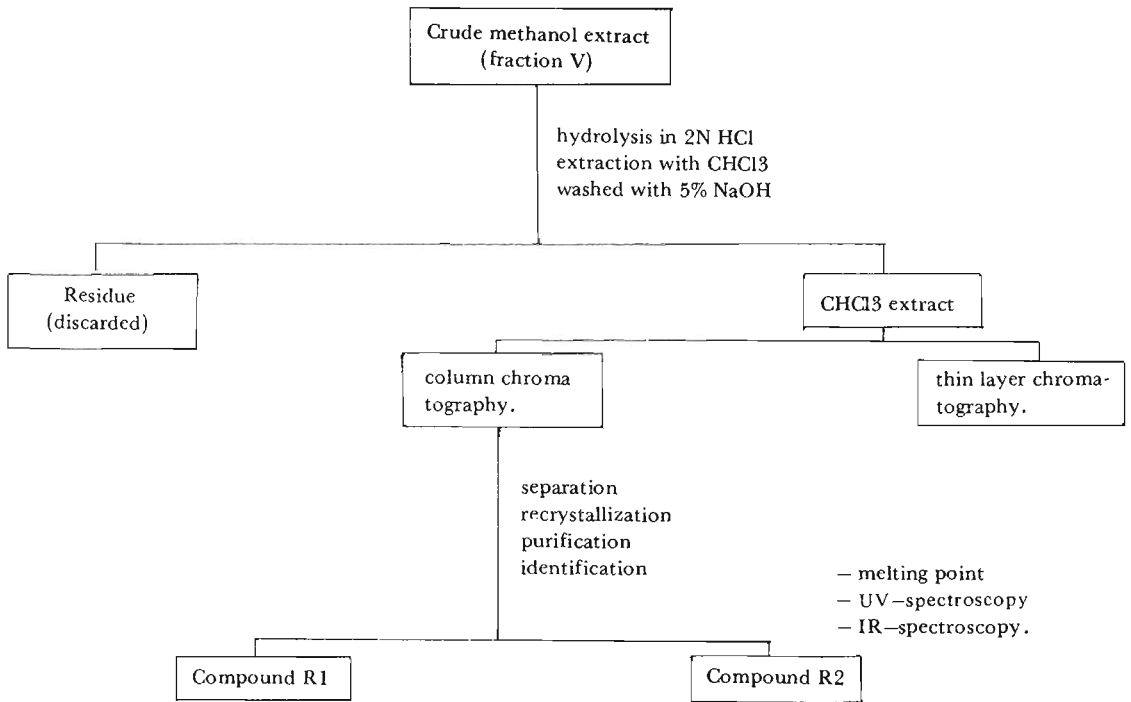


Fig. 2. The scheme of sapogenin isolation from *B. asiatica* Kurz.

RESULTS AND DISCUSSION

Table 1 shows the results of the chemical identification performed on each crude extract obtained after fractionation with various solvents of increasing polarities. The seeds of *B. asiatica* Kurz seem not to contain alkaloidal compounds, because the addition of Mayer's reagent to the solution of each crude extract in 2N sulphuric acid aqueous solution did not give a precipitate. Steroids and triterpenoids compounds were present in every fraction since it gave purplish-red colour with Liebermann-Burchard's reagent and orange colour with the 2N sulphuric acid solution. The saponin and the phenolics compounds were present in the seeds of *B. asiatica* Kurz which can be fractionated and found only in the methanol fraction. Since this study was to characterize the saponin compound in this plant, only the methanol fraction was taken for further treatment.

Table 2 shows the results of toxicity test of every fraction of the crude extracts. It is consistent with the result of chemical identification. No saponin compound were present in the petroleum aether, chloroform, and aether fractions which resulted in no toxic effect on the fish. The concentration of saponin in the methanol fraction was validated by the lower dose of the methanol fraction causing toxic effect on fish as compared to the initial aethanol fraction.

The methanol fraction was taken for the isolation of the saponin since it contained a relatively greater amount of saponin. The saponin was hydrolyzed with 2N hydrochloric acid solution. The amount of the crude extract of the methanol fraction used for isolation was about 20 gr (see fig. 2). The sapogenin released after acid treatment was extracted with chloroform and then concentrated under reduced temperature. By doing so, about 7 gr of sapogenin had been separated as a crude sapogenin. Treating

Table 1. Chemical identification of crude extracts of the seed of *B. asiatica* Kurz fractionations with solvents.

Expected compound/ reagents	crude extract of fraction no.				
	I	II	III	V	IV
1. Alkaloids - Mayer's reagent	(-)	(-)	(-)	(-)	(-)
2. Steroids-terpenoids - Liebermann-Burchard - Conc. sulphuric acid	pr or	pr or	pr or	pr or	pr or
3. Saponin - Foam test	(+)	(-)	(-)	(-)	(+)
4. Phenolics - Ferric chloride solution	dg	(-)	(-)	(-)	dg

* I. Aethanol fraction,

III. Chloroform fraction,

V. Methanol fraction.

II. Petroleum aether fraction

IV. Aether fraction,

pr = purplish-red, or = orange, (-) no reaction, (+) positive reaction, dg = deep green.

the crude sapogenin of pre-coated silicagel F254 (E. Merck) with a mixture of petroleum aether and aethylacetate (1+1) as solvent, three different substances had been separated with their R_f values 0.65, 0.40 and 0.25 respectively.

aethyl acetate. This compound, labelled as R1, has a characteristic melting point of 243–245^o C and a wavelength of maximum absorption in methanol at 203 nm ($\log a = 0,22$). The R2 sapogenin has several specific peaks absorption on IR

Table 2. Toxicity test of crude extracts of the seeds of *B. asiatica* Kurz obtained from fractination with various solvents on *Lebistes reticulatus* Peter (fish).

Crude extract of fraction No. *)	Conc (mg/2 l)	No. of fish used	% of fish died	duration (hours)
I	45	10	100	1
	23	10	100	4
	18	10	100	9
	13	10	100	13
	6	10	100	24
	4	10	0	24
II	15	10	0	24
III	3	10	0	24
IV	—	—	—	—
V	23	10	100	24
	11	10	100	5
	4	10	100	20
	2	10	—	24

The crude sapogenin was then taken into column chromatography treatment by using silicic acid as a stationary phase and a mixture of various compositions of petroleum aether and aethylacetate as solvents. The use of mixture of petroleum aether + aethyl acetate (3+1) as solvent had resulted in a pure sapogenin compound after recrystallization in

spectroscopic analysis. The presence of —OH, —CH, C = O, C = C and C—O—C functional groups in the R2 sapogenin were indicated by peaks of absorption at the wave number of 3500, 3025, 1750, 1480, and 1400, and 1100 and 1060 cm^{-1} respectively. The UV— and IR— absorption spectra were presented in Fig. 3 and Fig. 4. respectively.

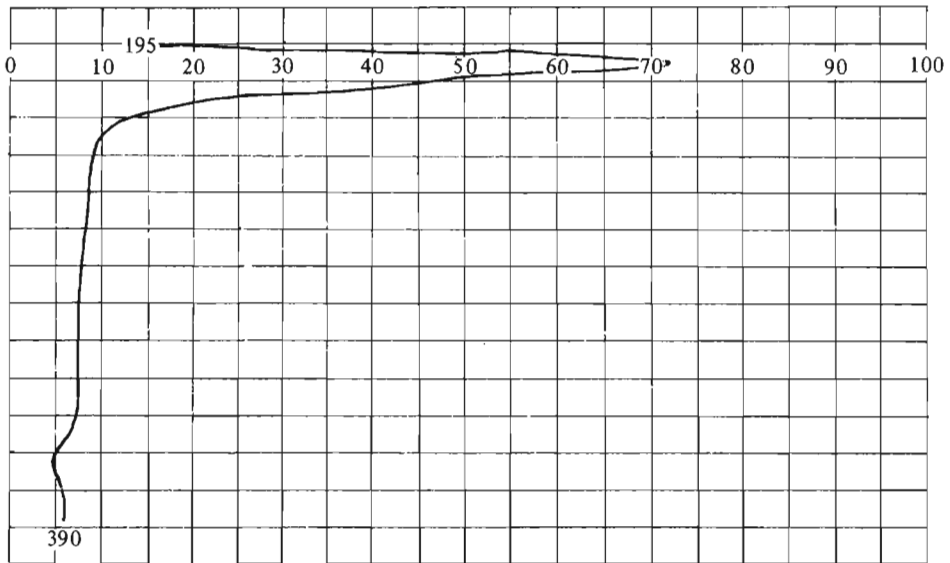


Fig. 3 The UV—Absorption Spectrum of Compound R1 of *B. asiatica* Kurz.

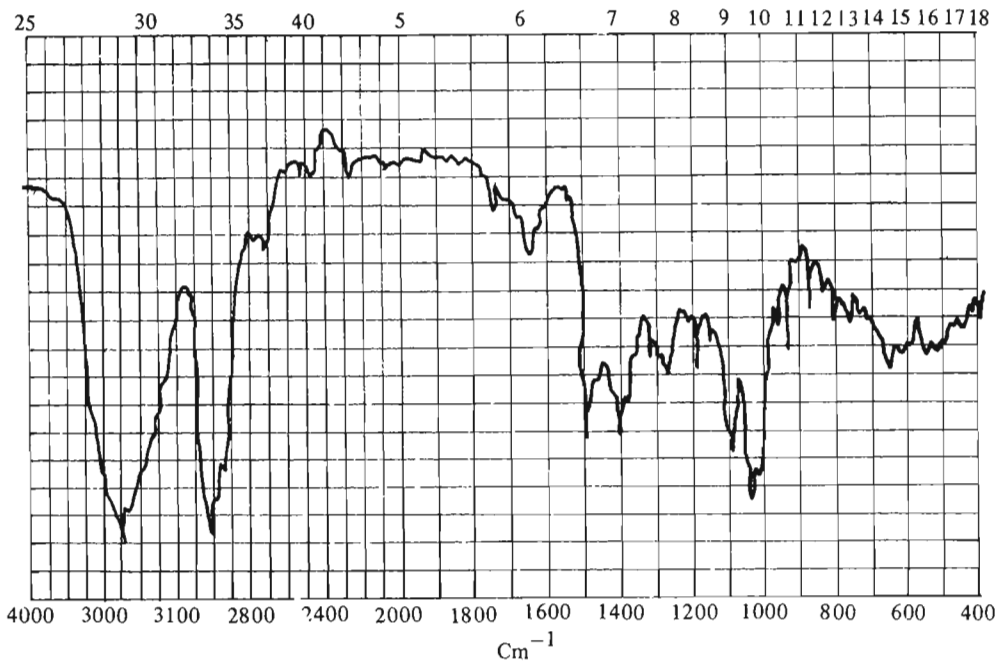


Fig. 4. The IR—Absorption Spectrum of Compound R1 of *B. asiatica* Kurz

Other sapogenin had also been separated with a mixture of petroleum aether and aethyl acetate (1+1) as solvent system on column chromatography treatment. This compound was labeled as R2 and after several recrystallization treatments in aethyl acetate and petroleum aether, identified as colorless crystalline substances having the following properties : melting point 266 – 268° C, ma-

ximum absorption in methanol at 202 nm (log a = 1.06), functional group present as molecular structures – OH, –CH, C=C, C–O–C and –CHS3 each with peaks of absorption in IR–spectrum at 3500, 3025, 1650–1480–1400, 1040 and at 650 cm⁻¹ respectively. The UV and IR– absorption spectra were presented in Fig. 5 and Fig. 6 respectively.

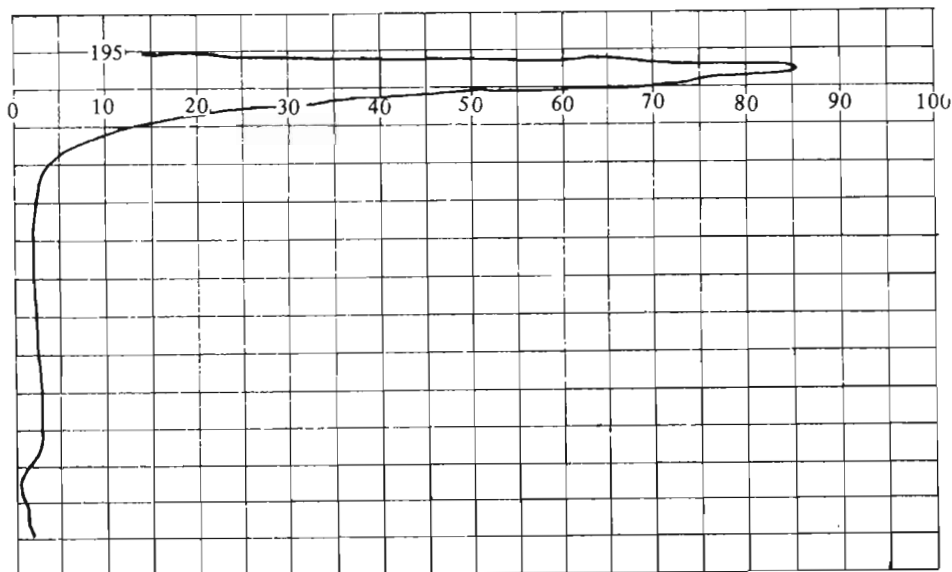


Fig. 5. UV–Absorption Spectrum of Compound R2 of *B. asiatica* Kurz

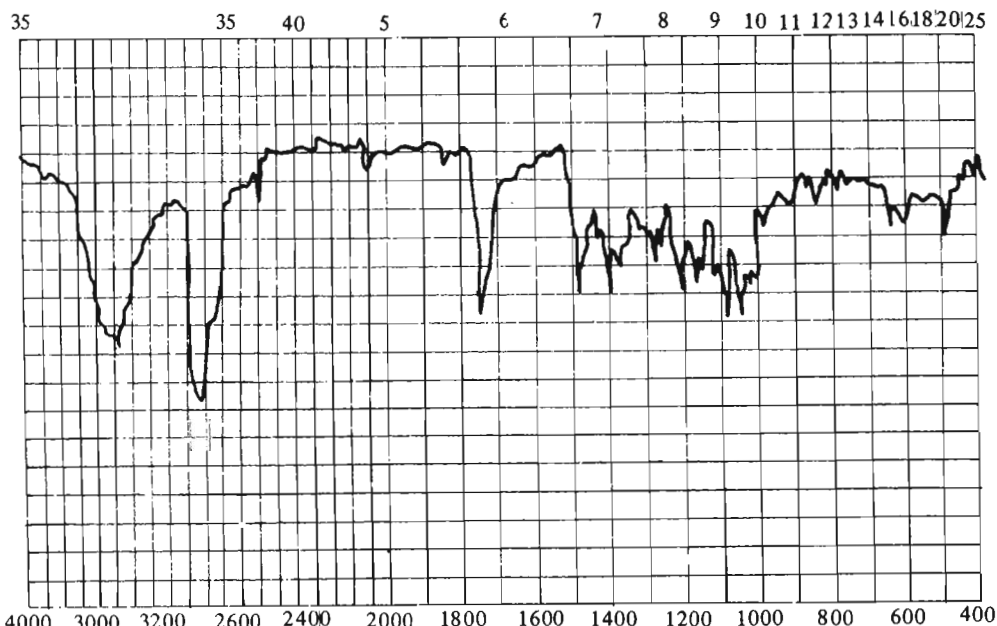


Fig. 6. The IR–Absorption Spectrum of Compound R2 of *B. asiatica* Kurz

Although only three major substances can be identified on the first TLC treatment of the crude sapogenin, five-crystalline substances had been separated on the following column chromatography treatment. Since only of these crystalline substances were obtainable in appropriate amount ($R_1 = 36$ mg and $R_2 = 125$ mg), the other three compounds, in the form of yellowish crystalline substances, could not be characterized further.

CONCLUSION

Two sapogenin compounds had been separated from the seeds of *Barringtonia asiatica* Kurz with their melting points of $243-245^{\circ}$ C for compound R_1 and $266-268^{\circ}$ C for compound R_2 . The IR spectra of these compounds indicated that the R_2 compound has the functional groups of OH, CH, C = C, C—O—C and CH₃ and R_1 compound has the functional groups of OH, C—H, C=C and C—O—C in its structure. However, the conclusion proposed here need to be verified with other spectroscopic methods such as the NMR and the mass spectroscopies, before the complete structure of each sapogenin isolated can be elucidated.

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