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Isolation of Piperin From the Fruit of Piper *Retrofractum*

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Abstract

Methyl Ester Sulfonate had been prepared From Ketapang Seed Oil and was used as Surfactant. The optimum This paper will described the isolation of major compound from MeOH extract from the fruit of *Piper retrofractum*. Using several chromatography techniques including liquid vacuum chromatography and thin layer chromatography, and further purification using re-cristalization technique, Piperine, an alkaloids compound, was isolated from this extract. The structure of this compound was determined using spectroscopic methods including FTIR, 1D-NMR and 2-D NMR.

Keywords: P.retrofractum, alkaloids, piperine, structure elucidation

Abstrak (Indonesian)

Pada makalah ini akan diuraikan mengenai pemisahan dan pemurnian senyawa utama dari ekstrak MeOH buah cabe jawa (*Piper retrofractum*). Dengan menggunakan beberapa tehnik kromatografi termasuk kromatografi cair vakum dan kromatografi lapis tipis, kemudian di murnikan lebih lanjut dengan teknik rekristalisasi, maka Piperin, suatu senyawa turunan alkaloid berhasil dipisahkan dari ekstrak ini. Penentuan struktur senyawa piperin dilakukan dengan metode spektroskopi termasuk FTIR, NMR satu dimensi (NMR ^{1}H dan ^{13}C), dan NMR dua dimensi (HMBC dan HMQC).

Keywords: P.retrofractum, alkaloid, piperin, elusidasi struktur

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INTRODUCTION

Piper retrofractum (Piperaceae) or known as Javanese long pepper is a relatively less known spices. Nevertheless, in traditional medicine scope, this plant is often used as an anti-flatulent, expectorant, or antitussive agent [1]. A literature survey disclosed that a number biological studies have been carried out on this plant extract such as antioxidant, anti-fungal, cytotoxic and also shown the α-glucosidase inhibitory activity [2-8], and revealed that piperidine alkaloids, amides, and phenylpropanoid are the major secondary metabolites isolated. In continuation of our works aimed at finding responsible metabolites for medicinal properties of Indonesian traditional medicine plant, we have examined fruit samples of *P. retrofractum* and have isolated piperine. This paper will describe the isolation and structure elucidation of piperine.

MATERIALS AND METHODS

General

IR spectra were measured with a Shimadzu 8400 FTIR spectrometers (KBr).^{1}H and ^{13}C NMR spectra were recorded with a AGILENT 500 MHz operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual (δH 7.26) and deuterated solvent (δC 77.1) peaks of chloroform-*d* as reference standards. VLC (vacuum liquid chromatography) was carried out using Merck silica gel 60 GF254; for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF254, 0.25 mm thickness) were used. Solvents used for extraction and preparative

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chromatography were of technical grade and distilled before use.

Plant Material

Fruit samples of *P. retrofractum* were collected from Lembang District, Wes Java, Indonesia in the month of February 2016. The plant was identified by Staf at Herbarium Bandungense, Biology Department, Universitas Pendidikan Indonesia, and the voucher specimen has deposited at the herbarium.

Extraction and Isolation

The dried and powdered fruit of *P. retrofractum* (2.0 Kg) was macerated in MeOH. 30 g of the total MeOH extract (180 g) was fractionated by VLC (silica gel, n-hexane-EtOAc = 9:1 → 0:10) into four major fraction A – D. Fraction D (7.5 g) was refractionated using the same method (silica gel, n-hexane-EtOAc = 6:4 → 5:5) into another five fraction D1- D5. Crystallization to fraction D2-D4 (mixtured solvent: n-hexane-EtOAc) afforded compound **1** (0.4 g).

RESULT AND DISCUSSION

Maceration of the dried powdered fruit of *P. retrofractum* in MeOH yielded a brown extract. Fractination of the extract by VLC on silica gel gave four major fractions A-D. From TLC analysis, the major constituents of this extract were concentrated in fraction D which was further fractionated using same method, followed with crystallization using mixture solvent (n-hexane-EtOAc) to give compound **1**. Compound **1** was identified, based on the analysis of 1D NMR (^{1}H and ^{13}C) and 2D NMR (HMQC and HMBC) data, as piperine.

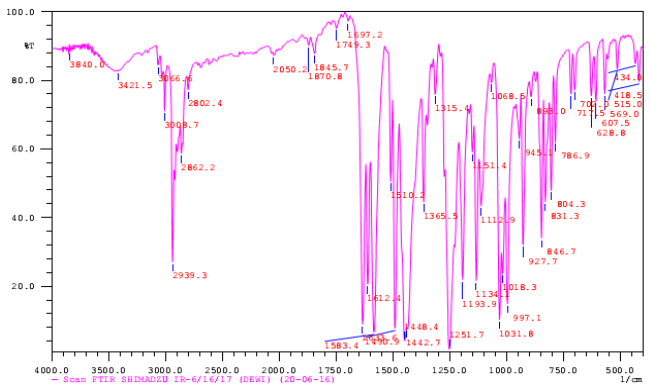

Figure 1. IR spectra of purified compound of *Piper retrof ractum*

The ^{13}C-NMR spectrum was shown in Figure 2. From the figure, The ^{13}C-NMR spectrum of **1** disclosed the presence of 17 carbon resonances. There are six CH_2 at δC 101.3, 46.9, 43.3, 26.8, 25.7, and 24.7 ppm as evident clearly from the HMQC spectra (Fig. 2b). The HMQC spectra also indicated the presence of seven CH at δC 105.7, 108.5, 120.1, 122.5, 125.4, 138.2 and 142.5 ppm. Peak at δC 165.4 supported the presence of carbonyl, and peak at δC 131.0 ppm being assignable to a quarternary carbon as well as peak at δC 148.1, and 148,2 ppm.

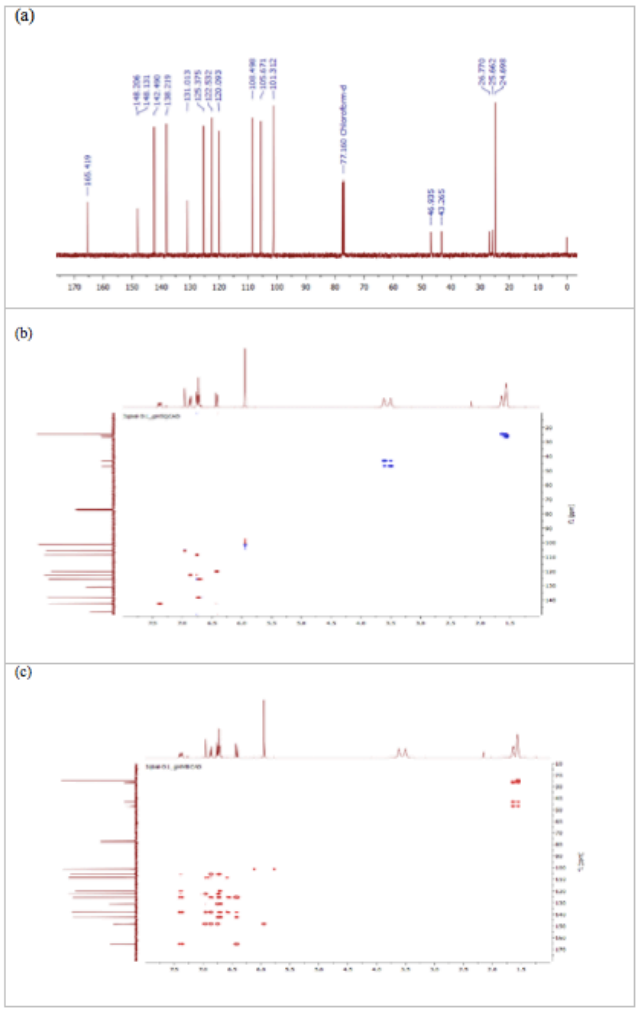

Fig 2 (a-c): (a) ^{13}C NMR, (b) HMQC and (c) HMBC spectra of purified compound of *Piper retrofractum*

The ^{1}H NMR spectra also exhibited proton signals at δH 6.41 (d, *J*=14.8Hz, 1H), 7.32 (dd, *J*=17 dan 14.8 Hz, 1H), 6.70 (dd, *J*=17 dan 17Hz, 1H), 6.73 (d, *J*=17Hz, 1H), 6.85 (m, 1H), 6.74 (d, *J*=8Hz, 1H), 6.94 (s, 1H) and a methylene-di-oxy proton at δH 5.49 (s, 2H). Those proton signals together with carbonyl signal at 13C NMR are characteristic for piperoil moiety.

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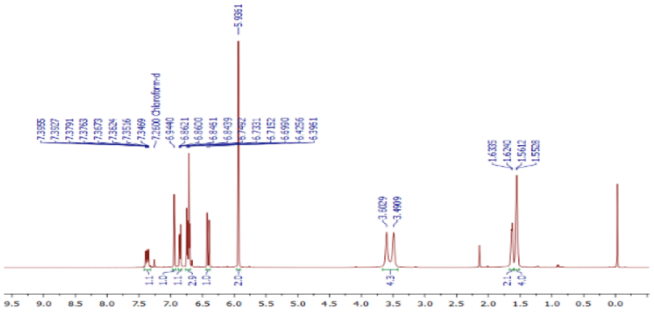

Fig 3. ^{1}H NMR spectra of purified compound of *Piper retrofractum*

Furthermore, the presence of five CH_2 signal at δC 24.7 – 46.9 ppm, supported with the ^{1}H NMR spectrum which exhibited signal at δH 1.56 (d, *J*=4.2Hz; 4H), 1.63 (d, *J*=4.7 Hz; 2H), 3.50 (brs,2H), 3.60 (brs, 2H) ppm, are characteristic for piperidine moiety. The Figure 3 showed the ^{1}H NMR spectra of purified compound of *Piper retrofractum*.

Table 1. NMR data (chloroform-d6) of piperine

No. C	δH (mult, *J* in Hz, integration) (ppm)	δC (ppm)	δC (ppm) of standard piperin*
1'	3.50 (brs, 2H)	46.9	46.8
2'	1.56 (d, *J*=4.2; 2H)	26.8	26.7
3'	1.63 (d, *J* =4.75; 2H)	24.7	24.6
4'	1.56 (d, *J* =4.2; 2H)	25.7	25.6
5'	3.50 (brs, 2H)	43.3	43.1
1	-	165.4	165.3
2	6.41 (d, *J*=14.8; 1H)	120.1	120.0
3	7.32 (dd, *J*=17 dan 14.8; 1H)	142.5	142.4
4	6.70 (dd, *J*=17 dan 17; 1H)	125.4	125.3
5	6.73 (d, *J*=17. 1H)	138.2	138.1
6	-	131.0	130.9
7	6.85 (m,1H)	122.5	122.4
8	6.74 (d, *J*=8. 1H)	108.5	108.4
9	-	148.2	148.1
10	-	148.1	148.0
11	6.94 (s. 1H)	105.7	105.6
-O-CH_2-O-	5.49 (s. 2H)	101.3	101.2

Figure 4 showed the 1D and 2D NMR spectral analysis of **1,** while Figure 5 revealed the purified compound to be a known piperidine alkaloids as piperine.

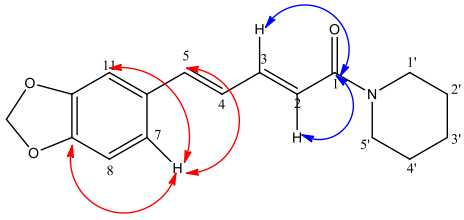

Fig. 4 HMBC correlation of purified compound of *Piper retrofractum*

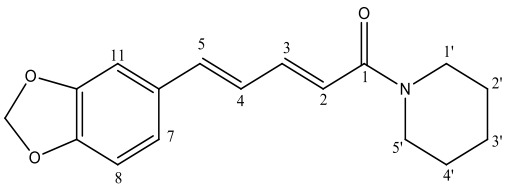

Fig. 5 Compound **1** (Piperine)

CONCLUSION

From the MeOH extract of the fruit of *Piper retrofractum*. We already succeed to isolate the major compound of this extract. The isolated compound was piperine, a well-known piperidine alkaloids.

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