Alkaloids piperine in dichloromethane fraction of red galangal rizhome (Alpinia purpurata)

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Alkaloids piperine in dichloromethane fraction of red galangal rizhome (*Alpinia purpurata*)

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Abstract. Red galangal rizhome (*Alpinia purpurata*) was extracted with dichloromethane using maceration methods. The crude extract was fractionated using vacuum column chromatography with a mixture of ethyl acetate:n-hexane (2:3) to give two fractions (F1 and F2). Fraction 2 was fed into the silica gel of column chromatography tailed by recrystallization to produce crystalline (17 mg). The purity was tested by using melting point apparatus and thin-layer chromatography method for any solvent. Their structures were established by spectroscopic methods (UV-Vis, FTIR, ^{1}H-NMR and ^{13}C-NMR). Result of analysis gave melting point of crystals around 130°C. The UV-Vis spectrometer obtained maximum wavelength 344 nm. FTIR analysis showed C=O carbonyl, C-O ether, C-N, and C=C aromatic. Analysis by ^{1}H-NMR spectrometer showed that bioactive compounds have a 19 protons. Analysis by ^{13}C-NMR spectrometer showed that bioactive compounds have a 17 carbon atoms. The results of identification showed that bioactive compounds in dichloromethane fraction of red galangal rhizome was a compound piperine alkaloids types with a chemical formula of $C_{17}H_{19}O_3N$.

Keywords: *Alpinia purpurata*, red galangal rizhome, Piperine, alkaloids

1. Introduction

Red galangal rhizome (*Alpinia purpurata* K. Schum) is one of herbs that broadly been used as raw materials for bio-pharmacy products or material sources of the drugs [1]. *Alpinia purpurata* K. Schum (family Zingebaeraceae) is typical plant for tropical and subtropic regions of Asia.[2].

Phytochemical analysis for Rhizome *Alpinia purpurata* showed positive results for alkaloids (14.9%), phenols (9.5%), flavonoids (0.85%) and tannins (13.8%) [3]. Investigation of the flavonoids compound contained by kaempferol-3-owned glucoronide, kaempferol-3-uliocronide and rutin of ethanol extract has been done using HPLC [4]. Ethanolic extracts of *Alpinia purpurata* showed highest inhibitory activity against *Enteobacter aerogens* [5]. Other chemical contents contained within hexane and dichloromethane are fatty alcohol, kumatakerin, sitosteryl-3-O-6-palmitoyl-β-D-glucoside and β-galactoside sitosteryl [6]. Based on literature above, despite the limitations of phytochemical studies of red galangal rhizome (*Alpinia purpurata*) species that grows in Indonesia, in this research we started further exploration about isolation and bioactive compounds identification from red galangal dichloromethane fraction.

2. Materials and Methods

The equipment used in this research were vials bottle, capillary pipettes, thin layer chromatography, column chromatography, vacuum rotary evaporator (IKA basic RF 10), blender, mixer, spatula, analytical balance (Ohaus), Fisher Jones melting point apparatus, UV-Vis Spectroscopy (Spectroquant

® Pharo 300), FTIR (Government employees), and NMR (Agilent 500 MHz with console system DD2 operated on the frequency of 500 MHz (^{1}H) and 125 MHz (^{13}C). The materials used in this research were methanol, acetone, ethyl acetate, dichloromethane, n-hexane, chloroform, ether, silica gel G60 F_{254} from *e. Merck*. Red galangal's rhizomes (*Alpinia purpurata*) collected from Semarang, Central Java, Indonesia. Rhizomes specimen was stored in Wet Laboratory of Tropical Disease Diponegoro University.

2.1. Isolation and Constituents Identification

Dried rhizomes of *Alpinia. purpurata* (2000 g) were extracted with maceration methods using dichloromethane solvent followed by reduced pressure evaporation to give 75,91 g crude product. The crude product was fractionated with column vacuum chromatography using a dichloromethane to give two fractions (F1 and F2). We used F2 for further treatment because the amount of crude fraction obtained was higher than crude fraction of F1. We partitioned F2 using gravity column chromatography with mixture of ethyl acetate: n-hexane (2:3) as eluent, followed by recrystallization to produce crystals approximately 17 mg.

2.2. Characterization of Bioactive Compounds

Crystals obtained from isolation of F2 (17 mg) characterized using UV-Vis spectrometer, FTIR, ^{1}H-NMR, and ^{13}C-NMR.

3. Results and Discussion

3.1. Isolation and Identification

The extraction process was done using maceration method using methanol solvent, producing thick reddish brown extract for about 75.91 grams, obtained from 2 kg dry powder of red galangal rhizome with a yield of 4.2172% (w/w). Condensed extracts were then fractionated using vacuum column chromatography with dichloromethane as eluent, resulting two fractions: (F1=0.12 g and F2=1.83 g). F2 fraction was then separated using gravity column chromatography (GCC) method using ethyl acetate: n-hexane (2:3) as eluent to acquired 0.2136 grams of solid. Recrystallization was carried out with ether to produce 17 mg of yellow blanch crystals. Identification of crystal purity was done by measurement of melting point and acquired melting point of 130°C.

3.2. Characterization of Bioactive Compounds

Characterization result of pure compound using UV Spectrometer shown in Fig. 1.

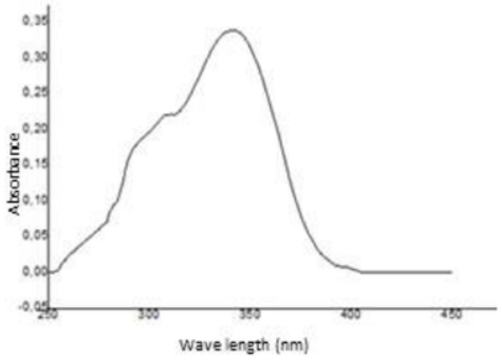

Figure 1. UV-Vis spectrum of pure compound.

UV-Vis analysis resulting maximum wavelength ($\lambda_{methanol}$=344 nm). The analysis result using Spectrophotometer UV-Vis reinforced past studies from Adosraku *et al.* [7] and Deepthi *et al.* [8] which showed that piperine compounds have maximum wavelength at 343 nm using methanol solvent. From this result, we assume that the pure compound is piperine but further analysis need to be done.

Further analysis using IR spectroscopy shown in Fig. 2.

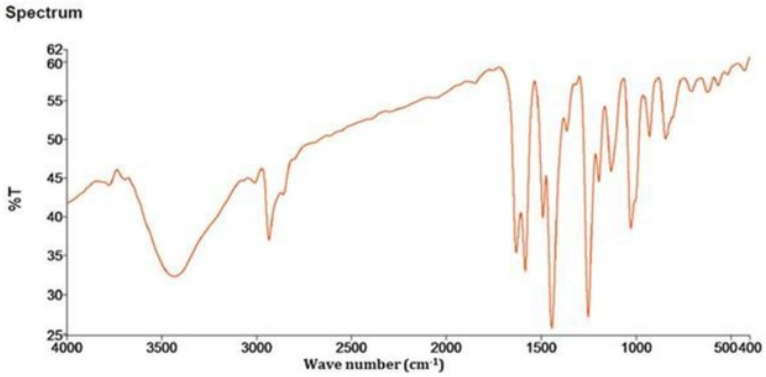

Figure 2. Infrared spectrum of pure compound.

The absorption patterns of FTIR of pure compound showed in table 1.

Table 1. Absorption patterns of pure compound using FTIR spectrometry.

Wavenumber (cm^{-1})	Vibration types
3435.29	O-H stretch
2937.27	Asymmetrical aliphatic C-H stretch
2863.05	Symmetrical aliphatic C-H stretch
1631.66	Amide C=O stretch
1584.59	Aromatic C=C stretch
1443.91	Aliphatic C-H bending
1365.28	C-N bending
1027.30	Ether C-O stretch
929.10; 845.26; 704.65	para, meta, orto substitution on aromatic ring

FTIR result showed that pure compound contains functional groups: C-N, amide C=O, aromatic C=C, ethers C-O, and aliphatic C-H. These data reinforced structure of piperine compound. Our assumption was confirmed from previous study from Deepthi *et al.* [8] which identify the piperine alkaloid from *Piper longium* that shows the functional group absorption of C=O at 1635 cm^{-1} wavenumbers and aromatic C=C at 1580 cm^{-1}. As well as research from Sirat and Liamen [9] which has been identified piperine from *Alpinia purpurata* which shows absorption of amide C=O at 1640 cm^{-1} as well as C=C absorption at 1595 cm^{-1} and 1500 cm^{-1}. Based on data obtained, it was believed that pure crystalline contains alkaloid compound known as piperine whose structure is presented in Fig. 3.

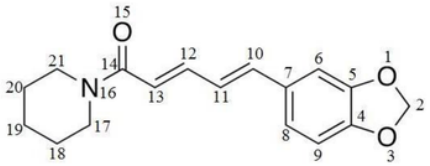

Figure 3. Piperine structure.

NMR characterization of pure compound (piperine) was done in order to confirmed the data obtained beforehand. NMR characterization of pure compound shown in Fig. 4.

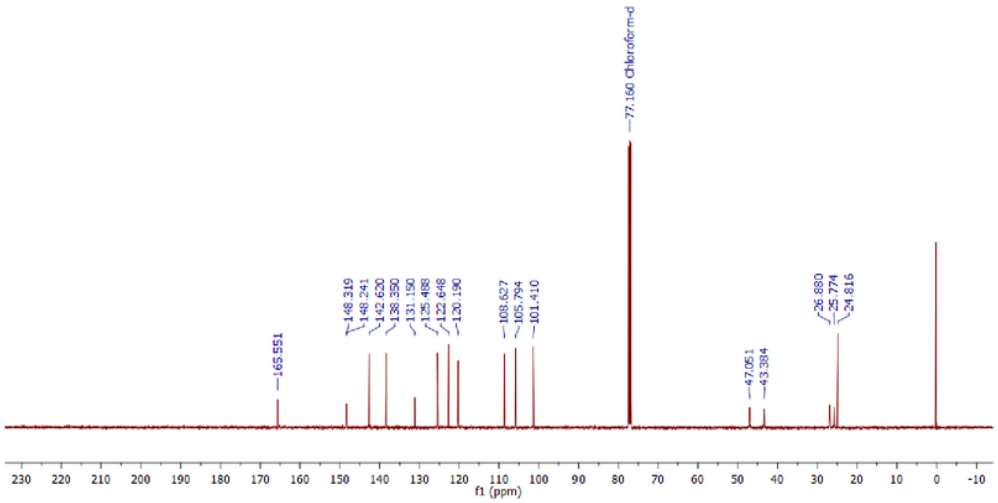

Figure 4. ^{13}C-NMR spectrum.

The spectrum of ^{13}C-NMR from compound isolation informed existence of 4 carbon atoms as C-quartener appearing at δ 165.551 ppm (C-14); δ 148.241 ppm (C-5); δ 148.319 ppm (C-4), and δ 131.150 ppm (C-7). There are seven =CH- carbon atoms appearing at δ 142.620 ppm (C-12), δ 138.350 ppm (C-10), δ 125.488 ppm (C-8), δ 122.648 ppm (C-11), δ 120.190 ppm (C-13), δ 108.627 ppm (C-9), and δ 105.794 ppm (C-6). There are also six -CH_2- carbon atoms at δ 101.410 ppm (C-2), δ 47.051 ppm (C-17), δ 43.384 ppm (C-21), δ 26.880 ppm (C-18), δ 25.774 ppm (C-20), and δ 24.816 ppm (C-19).

Characterization by H NMR produce spectra shown in Fig. 5.

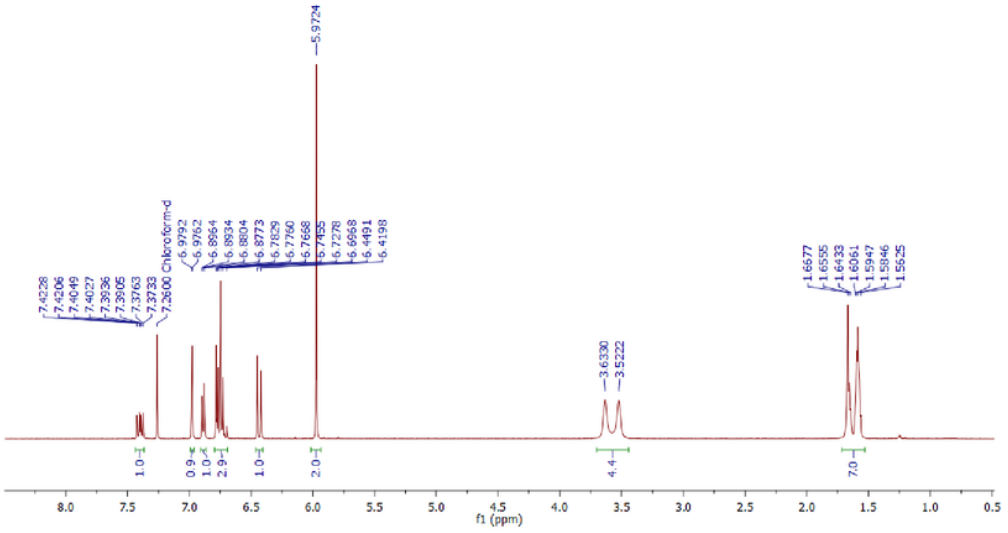

Figure 5. [1]H-NMR spectrum.

Fig. 5 shows the [1]H-NMR spectrum, which give several peaks, at δ 1,5625-1,6677 ppm (6H, m, overlapping peaks from H-19, H-20 and H-18); δ 3,5222-3,6330 ppm (4H, d, H-21 and H-17); δ 5.9724 ppm (2H, s, H-2); δ 6,4198- 6,4491 ppm (1H, d, H-13); δ 6.6968 -6,7829 ppm (3H, d, overlapping peaks from H-11, H-10 and H-9); δ 6.8773-6.8964 ppm (1H, dd, H-8); δ 6,9762- 6,9792 ppm (1H, d, H-6); δ 7,3733- 7,4228 ppm (1H, ddd, H-12). The chemical shifts of [1]H-NMR and [13]C-NMR are in accordance with the position of the proton and carbon atoms from piperine.

4. Conclusion

Bioactive compound isolated from dichloromethane fraction of red galangal is a type of alkaloids known as piperine which has 130°C melting point, 344 nm maximum wavelength and chemical formula $C_{17}H_{19}O_3N$.

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