JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

Jurnal Ilmiah Berkala: Sains dan Terapan Kimia
Vol. 17 No. 1, Januari 2023

Isolation of Pinostrobin Compound in Temu Kunci (*Kaempferia Pandurata Roxb*) Rhizome

Isolasi Senyawa *Pinostrobin* pada Rimpang Temu Kunci (*Kaempferia Pandurata Roxb*)

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ABSTRACT

Isolation and identification of Pinostrobin compounds in Temu Kunci Rhizomes *(Kaempferia pandurata* Roxb) were conducted in this study. The isolation and identification methods used were extraction, crystallization & recrystallization, Thin Layer Chromatography, and (4) Infrared (IR) Testing. The extraction results were followed by crystallization and recrystallization processes. The recrystallization produced a yellow solid isolate. A Thin Layer Chromatography test was carried out to determine the purity of the isolate using the eluent Chloroform : n-hexane of 6:4, Chloroform : Ethylacetate of 7:3. A comparison of the Retention Factor (Rf), and the Rf value of the phinostrobin standard indicates that the positive test sample contained the Pinostrobin compound. The Infrared spectrum indicates that the sample belongs to the flavonol group. It is confirmed from the functional groups of the isolates that are compatible or identical to the functional groups in the pinostrobin compound.

Keywords: Temu Kunci Rhizome, Pinostrobin, Thin Layer Chromatography, Crystallization, IR Spectrophotometer.

ABSTRAK

Telah dilakukan isolasi dan identifikasi senyawa Pinostrobin dalam Rimpang Temu Kunci (Kaempferia pandurata Roxb). Secara umum metode isolasi dan identifikasi yang dilakukan yaitu: (1) Ekstraksi, (2) Kristalisasi & Rekristalisasi, (3) Kromatografi Lapis Tipis dan (4) Pengujian Infra Merah (IR). Hasil ekstrasi yang diperoleh dilakukan proses kristalisasi dan rekristalisasi. Hasil rekristalisasi menghasilkan isolat padat berwarna kuning. Untuk mengetahui kemurnian isolat dilakukanlah uji Kromatografi Lapis Tipis menggunakan eluen Kloroform:n-heksana = 6:4; Kloroform:Etilasetat = 7:3. Berdasarkan perbandingan nilai Faktor Retensi (Rf) dengan nilai Rf dari standard phinostrobin, mengindikasikan sampel uji positif mengandung senyawa Pinostrobin. Hasil spektrum Infra Merah dapat diduga bahwa sampel merupakan golongan flavonol, hal ini dilihat dari gugus-gugus fungsi dari isolat yang didapatkan sesuai atau identik dengan gugus-gugus fungsi pada senyawa pinostrobin.

Kata kunci: *Rimpang Temu Kunci, Pinostrobin, Kromatografi Lapis Tipis, Kristalisas, Spektrofotometer IR.*

Received: September 13, 2022; **Accepted**: January 4, 2023: **Available online**: February 15, 2023

1. INTRODUCTION

The rhizome of Temu Kunci (*Kaempferia pandurata* Roxb) is widely used as a traditional medicine in Indonesia for dry cough, canker sores, large intestine disorders, bloated stomach, voiding dysfunction, cervicitis, dysentery, and tumours/cancer (Bail *et al.,* 2015). Based on research conducted in Bangkok, the temu kunci (*kaempferia pandurata* Roxb) rhizome extracted with diethyl ether resulted in a relatively large concentration of the flavonoid compound pinostrobin, namely 20 grams/800gram powder or 2.5% and 1% alpinetin (Handayani *et al.,* 2018). Pinostrobin acts as an antioxidant and relaxes smooth muscle. Due to the high concentration of pinostrobin, its isolation as a pure substance can be performed quickly. The polarity of pinostrobin is reduced due to intra-molecular hydrogen bonds between the carbonyl group at C-4 and the hydroxy group at C-5. Therefore, the extraction can be carried out with less polar solvents such as chloroform and n-hexane (Nugraha *et al.,* 2012).

Pinostrobin is a secondary metabolite compound of the flavonoid group. Pinostrobin is a non-polar compound. Therefore, non-polar compounds such as n-hexane, chloroform, and ether are needed for extraction.

Based on its structure, pinostrobin can be identified by UV-Vis spectroscopy. Pinostrobin consists of two absorption bands, band I (325 nm) and band II (287 nm) and bathochromic shift when added with an $AlCl_3$ shear reaction of 20 - 26 nm to indicate the presence of an OH substituent at the C-5 position. IR spectroscopy is used to identify the functional groups. RMI Proton and Carbon spectroscopy is used to see the type and number of H and C atoms of Pinostrobin, and Mass spectroscopy to see the Relative Molecular Mass (Mr) and fragmentation of 5-hydroxy-7-methoxy flavanone or pinostrobin (Silverstein *et al.,* 1981).

2. MATERIALS AND METHODS

2.1. Materials

The materials used were 1.5 kg of Temu Kunci Rhizome, n-hexane, Chloroform, Aquades, and Ethyl Acetate. Tools used were analytical balance, TLC plate, separating funnel, rotary evaporator, filter paper, chromatographic chamber, glass jar, small capillary tube, hot plate, and oven.

2.2 Sample preparation

Temu Kunci rhizomes were purchased from the local market. It was cleaned and thinly sliced. The thin slices were dried in an oven at 60°C for three days. Dried slices were mashed using a blender. Then maceration was carried out for three days using n-hexane as solvent. The next step was evaporating the solvent with a rotary evaporator.

2.3 Crystallization and Recrystallization

The recrystallization process was carried out by adding n-hexane solvent to the thick extract and heating it. The recrystallization process was carried out five times.

2.4 IR Spectroscopy Test

The isolation results were then identified using Perkin Elmer's FT-IR Frontier to analyze the functional groups. FT-IR needed a solid sample; therefore, sample preparation was needed. The sample was mixed with KBr powder (5 – 10% of KBr powder samples) and then made into KBr pellets (KBr pills) using the "mini hand press".

3. RESULTS AND DISCUSSION

About 1.5 kg temu kunci rhizomes purchased from the local market were cleaned from soil and other impurities with clean water. After cleaning, the rhizome was thinly sliced to facilitate faster drying. Thin slices of temu kunci rhizome were dried in an oven at 60°C for three days. The dried sliced temu kunci rhizome was mashed with a blender. From 1.5 kg of dry temu kunci rhizome, 135 grams of brown temu kunci powder was obtained.

The temu kunci powder was macerated using n-hexane solvent and carried out for three days. The maceration aims to remove all the chemical components contained in the simplicia. The solvent n-hexane was used because the compound isolated, pinostrobin, is non-polar. It can be seen from the structure of the pinostrobin. Its polarity is reduced due to intra-molecular Hydrogen bonds between the carbonyl group at C-4 and the hydroxyl group at C-5. Therefore, extraction can be carried out with less polar solvents such as n-hexane and chloroform (Harborne J.B., 1992). After maceration, a brown macerate was obtained. The obtained macerate was heated on an electric stove until boiled. The heating aims to dissolve the isolated compound. It was then filtered with filter paper in a hot state. A brown extract was obtained.

The next step was to evaporate the solvent by aerating it using a rotary evaporator. The result was a thick green extract. The viscous extract was recrystallized using n-hexane. The recrystallization process was carried out by adding n-hexane to the thick extract. The heating aimed to dissolve the viscous extract. The completely mixed solution was left for one night to form pure yellow crystals. The recrystallization was carried out five times. Recrystallization is a technique for purifying a solid substance from a mixture of impurities. It is carried out by recrystallizing the substance after being dissolved in a suitable solvent (Day and Underwood, 2001). N-hexane was chosen for recrystallization because the crystals formed, indicated to be Pinostrobin compounds, are non-polar. Therefore no-polar solvent is the suitable choice. It is also following the "Like Dissolve like" rule.

The purity of the yellow crystal was tested using Thin Layer Chromatography (TLC). First, the crystal was dissolved using chloroform. Chloroform was used because chloroform is semipolar. This compound can dissolve polar and nonpolar compounds. Qualitative and quantitative thin layer chromatography (TLC) analysis of curcumin used the stationary phase plate/ TLC plate. Prior to sampling, the stationary phase was activated by preheating at 110°C for 15 minutes. It aims to increase the absorption power of the stationary phase. The TLC plate was made of 3 cm x 3 cm, and the start and finish lines were determined. The starting line is where the sample is pointed and the start of the bearer. The starting line is spaced between one sample and another so that the spots formed do not overlap to avoid difficulties in analysis. In this study, the distance of the sampling point was 1 cm. The finish line is the final boundary line of the solvent during the carrier process. The starting line was 1 cm from the plate's base, and the finish line was 1 cm from the end of the plate.

Thin layer chromatographic analysis was performed with eluents of Chloroform, Chloroform : n-hexane of 6:4, and Chloroform : Ethylacetate of 7:3. It showed a single spot, indicating that the isolated compound is pure (Markam 1988) with Rf values of 0.84 and 0.64, respectively. This Rf result was then compared with the Rf value of the standard Pinostrobin compound with the same eluent and ratio. The comparison obtained the same value for two eluent systems, namely chloroform and chloroform : ethyl acetate

(7:3). This indicates that the isolated compound is identical to the standard compound, which is a pinostrobin compound. The isolation results were then identified using Perkin Elmer's FT-IR Frontier to see the functional groups.

Identification by IR spectroscopy obtained a wave number of 3447.34 cm^{-1} (indicating -OH with its Hydrogen bond with C=O at C-4), 3012.32 cm^{-1} (showing -C-H stretch of aromatic), 1621.78 cm^{-1}, 1650.83 cm^{-1} (represents C=O ketones hydrogen bonding with -OH at C-5), 1498.12, 1580.37 cm^{-1} (indicating C=C aromatic), 2910.92 cm^{-1} (showing -C-H methyl and ethyl), and 3631.47, 3736.89 (indicating a phenol group).

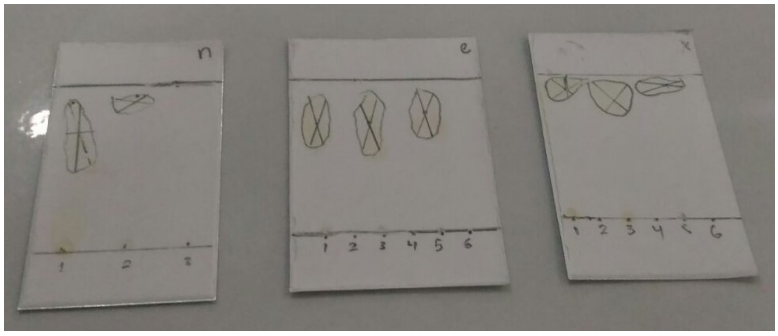

Figure 1. thin layer chromatography results

Table 1 Result of Comparison Rf Value

Eluen	Comparison (ml)	Solution	RF(cm)
Chloroform	10	Standar	0.87
		Isolasi	0.84
Chloroform : n-heksan	6:4	Standar	0.94
		Isolasi	0.68
Chloroform : Etil asetat	7:3	Standar	0.78
		Isolasi	0.72

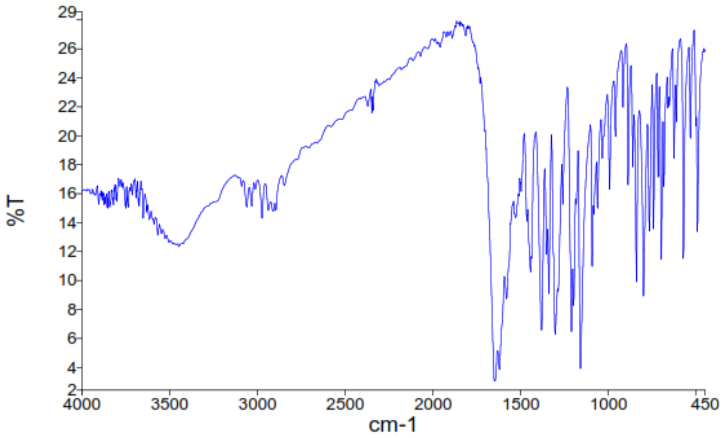

Table.2 Result of FT-IR spectrophotometer

No	Functional Group	Group of compounds	Frequency Range	Isolation results (cm^{-1})

1	O-H	alcohol and phenol	3200-3500 (hydrogen bond) 3500-3700(free)	3447.34 3631.47 ; 3736.89
2	C = O	ketone	1650 – 1780	1621.78 ; 1650.83
3	C - H	aromatic	3000 – 3100	3012.32
4	C - H	methyl and ethylene	2850 – 3000	2910.92
5	C = C	aromatic	1450 – 1600	1498.12 ; 1580.37

4. CONCLUSIONS

Based on the purity test using the comparison of Rf values in Thin Layer Chromatography (TLC), the isolates obtained were identical to Pinostrobin compounds. It is confirmed by Infrared (IR) spectroscopy. The isolates obtained display a peak graph (Peak) identical to phinostobin compounds. Therefore, the isolates obtained are pinostrobin compounds from the temu kunci plant. Based on these results, further research is needed to ensure that the structure of the compounds in the isolates is 100% pinostrobin compounds.

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