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Isolasi dan Karakterisasi Senyawa dari Residu Distilasi Minyak Kayu Manis (*Cinnamomum burmanii*)

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ABSTRACT

This study aimed to isolate and characterize compounds from the distillation residue of cinnamon oil from Loksado, South Kalimantan. Cinnamon (Cinnamomum burmanii) distillation residue was extracted with methanol as solvent. The methanol extract was fractionated by liquid vacuum chromatography to obtain fractions A, B, C, and D. The crystals contained in fraction C were washed with cold n-hexane to obtain 5.4 mg of yellow isolate (FC1). FC1 isolates were characterized by UV-Vis, IR, ^{1}H-NMR, and ^{13}C-NMR spectrophotometers. UV spectra showed a maximum wavelength at 307, 316, and 321 nm indicating the presence of a conjugated aromatic system. The infrared spectra showed -C=O, -OH, C-O, C-H, C-N, and C=N groups. The ^{1}H-NMR spectra showed the presence of aromatic protons at 6.38 ppm (1H, d, J=9.5 Hz), 7.67 ppm (1H, d, J=9.5 Hz), 7.29 ppm (1H, d, J=8 Hz), 7.44 ppm (1H, d, J=8 Hz), and 7.49 ppm (1H, t) and there was a methyl proton (acetyl group) at H 2.13 ppm (3H,s). The ^{13}C-NMR spectra showed the presence of a C=O ketone group at 207.26 ppm and there were 9 C-sp^2 at 116.9; 119.0;124.6; 128.1;132.0;143.7; 154.3; 161.0 ppm, which δ_C 161.0 ppm was C-oxyaryl. Based on UV, IR, ^{1}H and ^{13}C-NMR spectra data, FC1 isolate was suggested as an isoquinoline alkaloid substituted by OH and acetyl groups.

Keywords: *distillation residu, Cinnamomum burmanii, alkaloid, isoquinoline.*

ABSTRAK

Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi senyawa dari residu distilasi minyak kayu manis asal Loksado, Kalimantan Selatan. Residu distilasi kayu manis (Cinnamomum burmanii) diekstraksi dengan pelarut metanol. Ekstrak metanol difraksinasi secara kromatografi vakum cair sehingga diperoleh fraksi A, B, C, dan D. Kristal yang terdapat pada fraksi C dicuci dengan n-heksana dingin sehingga diperoleh isolat berwarna kuning (disebut FC1) sebanyak 5,4 mg. Isolat FC1 dikarakterisasi dengan spektrofotometer UV-Vis, IR, ^{1}H-NMR dan ^{13}C-NMR. Spektra UV menunjukkan terdapat panjang gelombang maksimum pada 307, 316, dan 321 nm menunjukkan adanya sistem terkonjugasi atau aromatis. Spektra infra merah menunjukkan terdapat gugus -C=O, -OH, C-O, C-H, C-N dan C=N. Spektra ^{1}H-NMR memperlihatkan adanya proton aromatis pada pergeseran kimia δ_H 6,38 ppm (1H, d, J=9,5 Hz), 7,67 ppm (1H, d, J=9,5 Hz), 7,29 ppm (1H, d, J=8 Hz), 7,44 ppm (1H, d, J=8 Hz), dan 7,49 ppm (1H, t) serta terdapat proton metil (gugus asetil) pada δ_H 2,13 ppm (3H,s). Spektra ^{13}C-NMR menunjukkan adanya gugus C=O keton pada pergeseran δ_C 207,26 ppm dan terdapat 9 C-sp^2 pada δ_C 116,9; 119,0;124,6; 128,1;132,0;143,7; 154,3; 161,0 ppm, dimana δ_C 161,0 ppm merupakan C-oksiaril. Berdasarkan data spektra UV, IR,^{1}H dan ^{13}C-NMR maka isolat FC1 disarankan sebagai senyawa alkaloid isokuinolin yang tersubstitusi oleh gugus OH dan asetil.

Kata Kunci: residu distilasi, kayu manis, alkaloid, isokuinolin.

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1. INTRODUCTION

Cinnamon (*Cinnamomum burmanii*) belongs to the Lauraceae family and is one of the leading commodities of South Kalimantan, particularly the South Hulu Sungai area. The productivity of cinnamon from the Loksado sub-district was 1,184.43 tons, and the Haruyan sub-district was 0.9 tons. Another cinnamon producer area was the Kotabaru district, with 24 tons (BPS South Kalimantan, 2014).

Cinnamon enhances the food and beverages flavor and is a raw material in cosmetics. Additionally, cinnamon is used in traditional medicine for various purposes, including treating toothache, cough, diarrhea, malaria, eliminating bad breath, preventing bleeding, and improving intestinal health (Al-Dhubiab, 2012; Rao & Gan, 2014). Several studies have shown that cinnamon has various activities such as antioxidant (Matthew & Abraham, 2006; Choi & Hwang, 2005; Prasad et al., 2009), antibacterial (Shan et al., 2007; Prasetyaningrum et al., 2012), anti-inflammatory (Khatib et al., 2005), antidiabetic (Kim et al., 2006), and anticancer (Lu et al., 2010).

These various bioactivities and the distinctive aroma of cinnamon are caused by the various compounds, such as essential oils, consisting of various compounds from the monoterpenoid, sesquiterpenoid, or phenylpropanoid groups. In addition to essential oils, cinnamon contains other compounds such as flavonoids, saponins, tannins, alkaloids, steroids, and lignans (Safratilofa, 2016; Yuan et al., 2017).

Cinnamon essential oil from Loksado contains 9 compounds, namely cinnamaldehyde (75.59%), cinnamyl acetate (17.40%), bornyl acetate (1.74%), copaene (1.63%), 1,8-cineol (1.35%), -terpineol (0.77%), limonene (0.59%), pinene (0.48%), and bicyclo 3.1.1 heptane (0.44%) (Astuti et al., 2018). Prasetyaningrum et al. (2012) stated a total phenol content of 28.563 mg/mL in cinnamon extract (C. burmanii), while in the residual distillation, there was a total phenol content of 15.975 mg/mL. Several phenolic compounds are identified in Cinnamomum, namely 5-hydroxyethyl salicylate, syringaldehyde, p-hydroxybenzoic acid, isovanylic acid, protocatechuic acid, protocatechuic aldehyde, vanillin, vanillic acid, trans-cinnamaldehyde, cinnamyl alcohol, cinnamic acid, sinapaldehyde, litseachromolaevanes A, 4 -hydroxy-1,10-seco-muurol-5-ene-1,10-dione, pinoresinol (PRO), syiringaresinol, coumarin, 4-hydroxymelein, kaempferol, (-)-(2R,3R)-5,7- dimethoxy-3′,4′-methylenedioxy-flavan-3-ol (MFO), and decumabic acid (Li et al., 2018). Several glycosides flavonoid, including quercetin 3-O-(3″,4″-di-trans-p-kumaroyl)-α-L-rhamnopyranoside and kaempferol 3-O-(3″,6″-di-trans-p-coumaroyl)-ß-D-glucopyranoside has been isolated from *C. cassia* (Liu et al., 2018). A lignan compound, namely cinnaburmanin A, has been isolated from the roots of *C. burmanii* (Yuan et al., 2017). The benzylisoquinoline alkaloid, cinnamolauryn, has been isolated from the Cinnamomum plant (Gellert & Summons, 1969). The alkaloids cinnaretamine, crykonisine, corydaldina, glaziovina, and zenkerina have been isolated from *C. philippinense* (Li et al., 2012).

Cinnamon essential oil (cinnamon oil) can be obtained by distillation. The distillation process produces dregs or residues that most likely still contain non-volatile chemical compounds such as flavonoids, saponins, tannins, or alkaloids. These compounds have various bioactivities. Flavonoids have antioxidant, anti-inflammatory, antimutagenic, and anticancer activities (Panche et al., 2016). Alkaloids act as an antibacterial, anti-inflammatory, antifungal, analgesic, and antiviral (Bribi, 2018). Saponins have anticancer and anticholesterol activity (Thakur et al., 2011). Tannins are used as lung anticancer therapy (Rajasekar et al.,

2021). Based on the description above, it can be seen that flavonoid compounds, alkaloids, saponins, and tannins have various bioactivities, and it is suspected that these compounds are still present in the cinnamon distillation residue. Therefore, it is necessary to isolate and characterize the chemical compounds from the cinnamon distillation residue from Loksado.

2. MATERIALS AND METHODS

2.1. Materials

The materials used were the distillation residue of cinnamon bark from Loksado Hulu Sungai Selatan District, silica gel 60 (Merck.), silica gel 60G (Merck.), TLC plate Silica gel GF254 (Merck.), methanol p.a (Merck.), chloroform p.a (Merck), and redistilled technical quality solvents such as methanol, n-hexane, and ethyl acetate. The tools used were glassware (measuring glass, Erlenmeyer, beaker (Pyrex), Liquid Vacuum Chromatography (KVC) column, Buchner funnel d=2 cm, porcelain dish, filter paper, analytical balance, rotary evaporator, UV-Vis spectrophotometer (Hitachi u-2100), FTIR Spectrophotometer (Thermo Nicolet IS 10), NMR spectrometer (Jeol Type JNM-ECA 500)).

2.2. Extraction

One kilogram of chopped cinnamon bark distillation residue was air-dried and then ground into a coarse powder. Cinnamon residue was macerated with methanol for 24 hours and filtered. Maceration was repeated three times. The filtrate was concentrated with a rotary evaporator and continued by heating on a waterbath until 39.79 g of methanol extract was obtained.

2.3. Search for eluent

A small amount of methanol extract was dissolved in methanol and then spotted on a Silica Gel GF254 TLC (Thin Layer Chromatography) plate and then eluted in various eluent compositions. The eluents were n-hexane:chloroform (1:9; 1:4; 3:7, 1:1) and n-hexane:ethyl acetate (2:3; 1:1).

2.4. Isolation and purity analysis.

Fractionation of cinnamon methanol extract was carried out by Vacuum Liquid Chromatography (KVC) with 60G silica gel as stationary phase (t=5 cm and column d=7 cm). The mobile phase used a gradient elution system, namely n-hexane with increased polarity with chloroform, then continued with ethylacetate single eluent. A total of 10 g of methanol extract was fractionated by KVC, obtained 4 combined fractions, namely fractions A (vials 1), B (vials 2-3), C (vials 5-10), and D (vials 11-12). There were yellow crystals in the evaporator flask at fraction C when concentrated with a rotary evaporator. Crystals were collected and washed with cold n-hexane. The washed crystal was called FC1. The rotary evaporator flask containing fraction C was rinsed with chloroform and then concentrated with a rotary evaporator, called fraction FC2. Furthermore, the FC2 fraction (60 mg) was fractionated by gravity column chromatography with n-hexane:chloroform (1:1) as eluent to produce 2 fractions, namely FC2a and FC2b fractions. FC1 and FC2b fractions were spotted on the same TLC plate with n-hexane:chloroform (1:1) eluent and showed the same Rf value. The FC1 fraction purity test was carried out by TLC in various eluents, namely n-hexane:chloroform (1:1); n-hexane:ethylacetate (1:1) and chloroform:ethylacetate (1:1), and 2-dimensional TLC with eluent 1, namely n-hexane:ethylacetate (1:1).and eluent 2, namely n-hexane:chloroform (1:1).

2.5. Structure characterization

The structural characterization of FC1 isolates was carried out using spectrophotometer UV, infrared, ^{1}H-NMR, and ^{13}C-NMR.

3. RESULTS AND DISCUSSION

3.1. Isolation of FC1 Compound

The yield of methanol extract of cinnamon distillation residue was 3.98%. The eluent selection was carried out before fractionating the methanol extract. TLC chromatograms of methanol extracts in various eluent compositions are presented in Fig. 1.

Fig. 1f reveals that n-hexane:chloroform (1:1) TLC eluent produces better separation than n-hexane:ethyl acetate (1:1) (Fig. 1e) so that n-hexane:chloroform eluent was used as eluent for fractionation of methanol extract using KVC. Fig. 1a, 1b, 1c, and 1d show that the methanol extract was not separated properly.

Fractionation of methanol extract by KVC method employed a gradient elution system. The elution process started with single eluent of n-hexane, followed by n-hexane:chloroform (9:1), (8:2), (7:3), and ended with ethyl acetate.

Fig. 2 shows the chromatogram of the methanol extract fractionated by KVC. Based on the TLC chromatogram pattern, the resulting fractions were grouped into 4 combined fractions, namely Fraction A, Fraction B, Fraction C, and D. There were crystals on the walls of the flask at the concentration of fraction C with a rotary evaporator, then separated and washed with cold n-hexane, called FC1. A total of 5.4 mg of FC1 isolate in the form of a yellow crystalline solid was obtained.

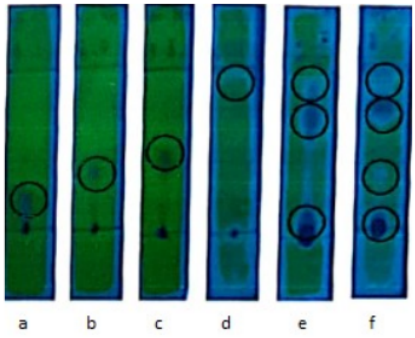

Figure 1. Chromatogram of methanol extract of cinnamon distillation residue, viewed under UV light 254 nm, stationary phase TLC silica gel GF254, mobile phase TLC (a) n-hexane:chloroform (1:9), (b) n-hexane :chloroform (1:4), (c) n-hexane:chloroform (3:7), (d) n-hexane:ethylacetate (2:3), (e) n-hexane:ethylacetate (1:1), (f) n-hexane:chloroform (1:1)

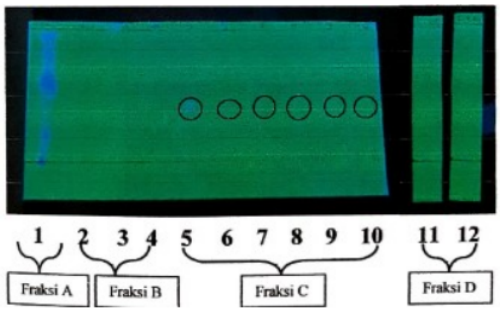

Figure 2. Chromatogram of the fractionated methanol extract of cinnamon distillation residue, viewed under UV light 254 nm, the stationary phase was Silica Gel TLC GF254, the mobile phase was n-hexane:chloroform (1:1)

The remaining fraction C in the evaporator flask was dissolved with chloroform, dried, and 60 mg FC2 was obtained. Furthermore, FC2 was fractionated by gravity column chromatography (KKG) using the fixed eluent n-hexane:chloroform (1:1) to produce 2 fractions, namely FC2(a) and FC2(b) (Fig. 3).

In Fig. 3, it can be seen that FC2(b) has one stain, so to determine whether FC2(b) and FC1 are the same compounds, the two isolates were spotted on the same TLC plate. Fig. 4 shows that FC1 and FC2(b) have the same Rf, so it can be said that FC1 and FC2(b) are the same compounds.

The purity test was carried out on FC1 isolates in different eluents and 2-dimensional TLC. All TLC chromatograms (Fig. 5 and 6) showed one spot, so it can be stated that the FC1 isolate was pure enough to proceed with structural characterization

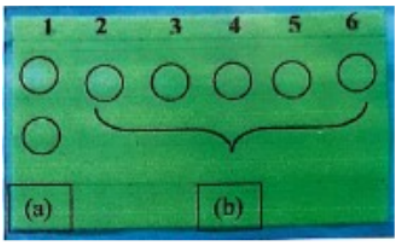

Figure 3. Chromatogram of the fractionated FC2 fraction, viewed under UV light 254 nm, the stationary phase was Silica Gel TLC GF254, the mobile phase was n-hexane:chloroform (1:1)

Figure 4. FC1 and FC2(b) TLC chromatograms, viewed under UV light at 254 nm, the stationary phase was Silica Gel TLC GF254, the mobile phase was n-hexane:chloroform TLC (1:1)

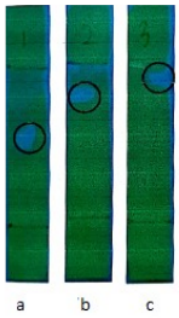

Figure 5. FC1 TLC chromatogram, viewed under UV light 254 nm, stationary phase was Silica Gel, mobile phase was GF254. (a) n-hexane:chloroform (1:1), (b) n-hexane:ethylacetate (1:1) , (c) chloroform:ethylacetate (1:1)

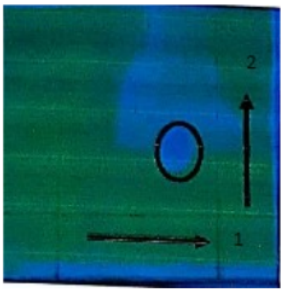

Figure 6. 2 Dimensional FC1 TLC chromatogram, viewed under UV light at 254 nm, stationary phase was Silica Gel GF254, mobile phase was 1 n-hexane:ethylacetate (1:1), 2 n-hexane:chloroform (1:1)

3.2. Compound Characterization of FC1

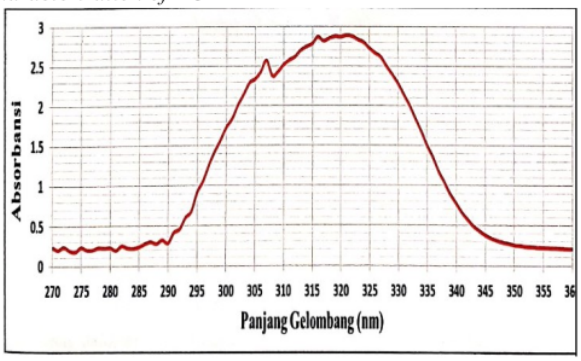

Figure 7. UV Spectra of FC1

The UV spectra of the FC1 isolate (Fig. 7) showed maximum wavelengths at 321, 316, and 307 nm. The presence of absorption at wavelengths in the UV region indicates the presence of a conjugated system chromophore or an aromatic ring, according to the simple isoquinoline framework as reported by Saidi et al. (2011). Isoquinoline alkaloid compounds, namely papralline, isolated from the *Cryptocarya rugulosa* plant (Lauraceae), showed maximum wavelengths at 325, 317, 312 nm (Saidi et al., 2011). Sulaiman et al. (2011) reported that the UV spectra of the *Litsea lancifolia* (Lauraceae) plant had a maximum wavelength at 307 nm and were a compound of N-alyllaurolitsine, an alkaloid with a benzylisoquinoline framework.

The infrared spectra show the presence of some characteristic functional group vibrations. The absorption at 3415.45 cm^{-1} is caused by the vibration of the hydroxy functional group (-OH), which is strengthened by the presence of C-O vibrations at 1174.37 cm^{-1}. Absorption at wave number 1697.76 cm^{-1} indicates the presence of a carbonyl group (C=O). A characteristic vibration of C=C indicates the aromatic ring at 1618.95; 1560.62; 1486.74 cm^{-1}, which is also supported by the =C-H stretching vibration at a wavenumber of 3057.10 cm^{-1} and =C-H bending at 686.87 to 994.33 cm^{-1}. The aliphatic –C-H vibration appears at wave number 2954.41 cm^{-1}. Absorption at wave number 1397.20 cm^{-1} indicates the presence of C-N vibrations. The imine vibration (C=N) was found at a wavenumber of 1599.94 cm^{-1}, in accordance with the imine vibration (1599 cm^{-1}) typical of isoquinoline alkaloids as in the study of Sulaiman et al. (2011). The presence of aromatic C=C, =C-H functional groups, C-N, and C=N vibrations support the isoquinoline alkaloid framework in FC1.

The ^{1}H-NMR spectra showed several signals at a 2.13 – 7.67 ppm chemical shift. Aromatic protons appeared at a chemical shift of 6.38 – 7.67 ppm. The chemical shift H of 6.38 ppm (1H, d, J=9.5) and 7.67 ppm (1H, d, J=9.5) indicates the aromatic proton at the ortho position. Chemical shift 7.29 ppm (1H, d, J=8); 7.49 ppm (1H, t), and 7.44 ppm (1H, d, J=8) indicates the presence of 3 protons side by side. The methyl protons present in the acetyl group appear at a chemical shift of 2.13 (3H, s) (Pavia et al., 2015).

The ^{13}C-NMR spectra showed the presence of 9 aromatic carbon atoms (Csp^2) at the chemical shift δ_C 116.9; 117.1; 119.0; 124.6; 128.1; 132.0; 143.7; 154.3; 161.0 ppm. This amount of aromatic carbon (Csp^2) corresponds to the carbons in the isoquinoline skeleton alkaloids. The chemical shift δ_C at 161.0 ppm is oxyaryl carbon (Rodrigueza et al., 2008). The chemical shift at 31.16 ppm was an aliphatic -CH_3 carbon, while the ketone carbonyl carbon appeared at a chemical shift of 207.2 ppm (Pavia et al., 2015).

Based on the UV, infrared, ^{1}H-NMR, and ^{13}C-NMR spectra data, the structure of FC1 isolate is estimated to be an alkaloid compound with an isoquinoline skeleton substituted by an OH group and an acetyl group, namely 1-(5-hydroxyisoquinoline-1-yl)ethanol, as shown in Fig. 8.

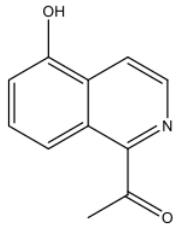

Figure 8. Structure of FC1 isolate

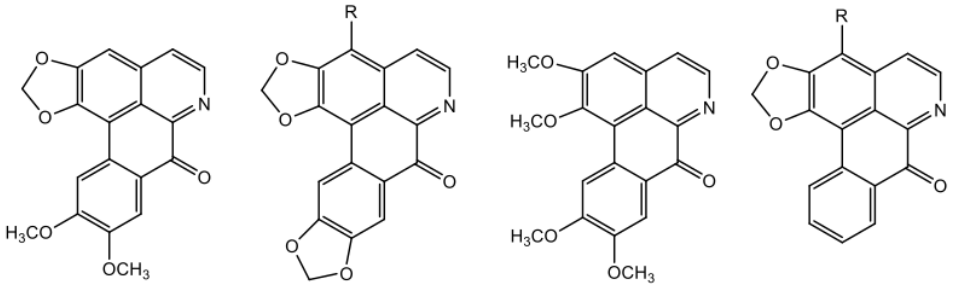

Figure 9. Structure of some isoquinoline alkaloids in the family Lauraceae (Custodio & da Veiga Jr, 2014).

Alkaloid compounds with an isoquinoline framework are commonly found in the Lauraceae family. The structure of several alkaloid compounds that have been isolated

from plants of the Lauraceae family, namely dicentrinone, cassamedin, cassameridine, catafillin, atherospermidine, and lyriodenine are shown in Fig. 8. FC1 isolate has a simpler structure than the isoquinoline alkaloid compounds that have been reported from the Cinnamomum plant. Several Cinnamomum plant species containing isoquinoline alkaloids are *C. insularimontanum* and *C. camphora* (Custodio & da Veiga Jr, 2014).

4. CONCLUSION

FC 1 isolate was in the form of yellow crystals. FC1 isolate was an alkaloid with an isoquinoline skeleton, which was substituted by– OH and acetyl groups.

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