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Methyl Ferulate from Methanol Extract of Indonesian Sausage Fruit (*Kigelia africana*)

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Abstract. Sausage tree (*Kigelia africana)* is a medicinal plant of Bignoniaceae family which can be used as anticancer. Study on *K. africana* as an anticancer activity is still restricted to its crude extract while the structure of its active compound is still limited. On the other hand, *K. africana* in Indonesia has not been used as a medicine. It is only used as an ornamental plant because of its unique fruit shape. Therefore, it is interesting to conduct a research on isolation of active compounds from *K. africana* having anticancer activity. Simplicia of *K. africana* fruit was macerated in methanol and fractionated using vacuum liquid chromatography obtained 6 fractions (A-F). Fraction C was further fractionated using radial chromatography yielded 5 sub-fractions. Sub-fractions C3 and C4 were purified by preparative thin layer chromatography and obtained cinnamate derivative. Based on NMR and LC-MS spectral data, cinnamate derivative was identified as methyl ferulate. This study reported the isolation of methyl ferulate from *K. africana* which can be examined to its anticancer activity in further research.

INTRODUCTION

The Bignoniaceae family is one of the plant families having an important role in traditional medicine. The genus of this family which have been widely studied are *Stereospermum*, *Oroxylum*, *Kigelia*, and *Markhamia*. One of well-known species of the *Kigelia* genus is *K. africana* which commonly known as a sausage tree because the shape of its fruit is similar to sausage. *K. africana* is a tropical African plant that is widely planted and spread in South, Central, and Western Africa [1,2]. It has synonym as *K. pinnata* (Jacq.) DC. [3]. According to phytochemical evaluation in previous studies, methanol extract of *K. africana* in Australia contained phenolic, flavonoid, tannin, and alkaloid [4]. Furthermore, the methanol extract of the fruits and leaves of *K. africana* from the African plant contained triterpenoids and lignin [5]. Oyebanji *et al*. [6]. also reported that methanol extract of *K. africana* fruit in Africa contained saponins, cardiacglycosides, flavonoids, and tannins. While the methanol extract of *K. africana* fruit grown at PT Alam Indah Bunga Nusantara Cianjur, Indonesia contains phenolic, flavonoid, saponin, tannin, triterpenoid, and alkaloid [7].

K. africana has bioactivity as an antioxidant, antibacterial, antifungal, and hepatoprotective activity that can inhibit the oxidation process to protect the body cells from oxidation damage [4,8,9,10]. In addition, *K. africana* also showed the potency as an anticancer, which has been investigated by Yani *et al*. [7]. Yani *et al*. [7] reported that the ethyl acetate fraction (EtOAc) from the methanol extract of *K. africana* fruit had an IC_{50} value of 2.52 µg/mL and the C fraction from the purification results of the EtOAc fraction had an IC_{50} value of $3.2x10^{-7}$ µg/mL which was classified as very active in inhibits the growth of MCF-7 breast cancer cells. Moreover, previous research had also been reported that dichloromethane extract of sausage fruit showed cytotoxic activities against G361 skin cells [11], Caki-2 renal cells, and WHMel2 skin cells [12], MCF-7, and MDA-MB-468 breast cells [13]. In spite of its bioactivity, sausage tree in Indonesia has not been used as a medicine, it only used as an ornamental plant.

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Moreover, study on sausage fruit as an anticancer source is still limited to its crude extracts and the structure of its active compound has not been reported, yet. To our knowledge, difference in geographical location will affect the presence of secondary metabolites. Therefore, it drives our interest to conduct the phytochemical investigation on Indonesian sausage fruit. The present study assessed to isolate the compounds contained in *K. africana*.

MATERIALS AND METHODS

General Experimental procedures

Apparatus was used in this research were distillation apparatus, rotary evaporator, ultraviolet (UV) lamp 254 and 366 nm, and analytical balance. Various chromatography techniques were used included vacuum liquid chromatography (VLC) using silica 60 G (Merck) for column packed and silica 60 (0.2–0.5 mm) (Merck) for sample adsorbed, radial chromatography (RC) using silica 60 PF254 containing gypsum (Merck), and preparative Thin Layer Chromatography (TLC) using silica 60 GF254 (Merck). For TLC analysis, pre-coated silica gel plates (Merck silica 60 GF254 and 0.25 mm thickness) were used. For structure elucidation, UV spectra were measured with UV-Vis Pharmaspec 1700 Shimadzu, FTIR with Parkin Elmer Spectrum One, LC-MS with Waters Acquity. ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in CD_3OD using Agilent 500 instrument.

Extraction and Isolation

The dried and powdered *K. Africana* fruit were macerated in MeOH at room temperature and the MeOH extract was evaporated under reduced pressure to give a semisolid residue (14 g). Tannins were separated by adding acetone to the MeOH extract, decanted, and the filtrate was evaporated (10 g). The filtrate was further fractionated on a silica gel VLC column, eluted with *n*-hexane:EtOAc of increasing polarity (9:1 to 3:7; v/v) to give 6 major fractions (A–F). Re-fractionation of the C fraction (113.7 mg) with a RC eluted with *n*-hexane:diethyl ether (9:1 to 1:1; v/v) gave five sub-fractions (C1-C5), then purification of C3 and C4 fractions by preparative TLC with *n*-hexane:CH_2Cl_2:MeOH (12:7:1; v/v) yielded CD-1 compound (17 mg).

RESULT AND DISCUSSION

CD-1 compound was isolated as yellowish solid and showed the absorption peaks at λ_{max} of 238.50, 291.50, and 322.50 nm in MeOH which indicated a cinnamate derivative. LC chromatogram of CD-1 compound exhibited several peaks from retention time of 8.00-18.00 minutes with the highest abundance peak showed at a retention time of 8.58 minutes which was thought to be a dominant compound. The MS spectra of its retention time were determined possibility as methyl-2-methoxy-5-hydroxycinnamate having $C_{11}H_{12}O_4$ molecular formula with m/z 209.0814 (Figure 1).

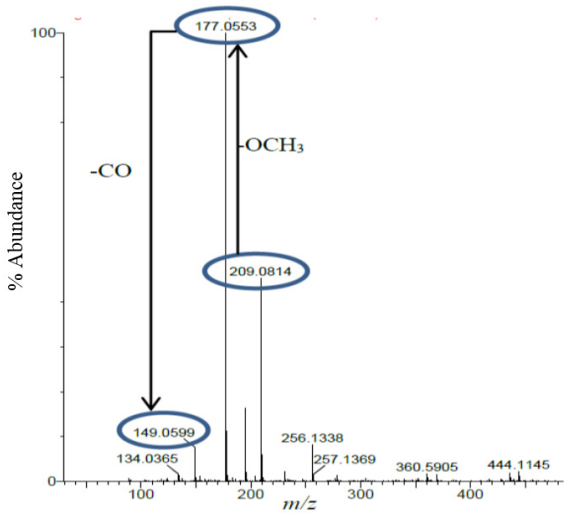

FIGURE 1. The mass spectrum of CD-1 peak with an 8.58 minute retention time

^{1}H NMR spectrum of CD-1 compound showed that there are suspected aromatic groups, at the chemical shifts (δ_H) of 7.06 (1H, *dm*, 2.1 Hz), 7.02 (1H, *dd*, 8.0 Hz; 2.1 Hz), and 6.93 ppm (1H, *do*, 8.2 Hz) which correspond to ABX system in aromatic group (Figure 2). Furthermore, at δ_H 6-9 ppm, in addition to aromatic signals there were also two other signals at δ_H 7.57 and 6.31 ppm with integration 1 proton on each signal. Both of these signals have a coupling constant of 15.9 Hz which corresponds to signal from trans-alkenes [14].

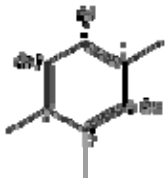

FIGURE 2. ABX system of aromatic ring of CD-1

According to HSQC spectrum, the H-2 proton is correlated with 120.92 ppm (C-2) carbon, H-3 proton correlated with 110.99 ppm (C-3) carbon, and H-6 proton correlated with 113.49 ppm (C-6). The HMBC spectrum displayed ^{1}H–^{13}C long-range correlations between one H of trans-alkenes (δ_H 7.57 ppm) with C aromatics at δ_C 120.92 (C-2) and 113.49 ppm (C-6), indicating that trans-alkene is thought to be bound to benzene at C-1 . In the ^{13}C spectrum there are 4 quaternary carbon (these carbon is not correlated with the proton on the HSQC spectrum), at the chemical shifts (δ_C) 127.42, 147.44, 150.47, and 168.21 ppm. The quaternary carbon at δ_C 168.21 ppm is a typical signal for carbonyl group. Then in ^{1}H NMR there are two singlet signals with integration equal to 3H at δ_H 3.76 and 3.88, indicating the presence of methoxy. One of the methoxy (δ_H 3.76 ppm) correlates with carbonyl indicating presence of ester. From its HMBC correlations, this ester attached to trans-alkene. The other methoxy group (δH 3.88 ppm) correlates with carbon at δ_C 150.47 ppm where that carbon also correlates with C-2 benzene, indicating the methoxy is bound to C-4 benzene. There is still 1 substituent in benzene that is not yet known. The predicted unknown substituent is OH, because the UV-Vis results the compound have a hydroxyl group.

Based on NMR interpretation and reinforced by the results of UV-Vis, FTIR, and LC-MS showed that the CD-1 compound is methyl ferulate (Figure 3). Table 1 summarized the NMR spectral data of CD-1 compound compared with previous researchers Woo and Lee [15] and Masuda *et al.* [16] who interpreted methyl ferulate from *Allium victorialis leaves* and synthesis result, respectively. According to Table 1, there is a high similarity between the NMR spectral data from isolation with the literature. Based on these data it can be concluded that the main compound in the fraction of CD-11 is a methyl ferulate, a cinnamate derivative. Masuda *et al*. [16] reported that methyl ferulate inhibited the accumulation of the hydroperoxide of ethyl linoleate for 5 hours concentration-

dependently. The antioxidant mechanism for methyl ferulate was considered based on the chemical structure due to it has one phenolic hydroxyl group.

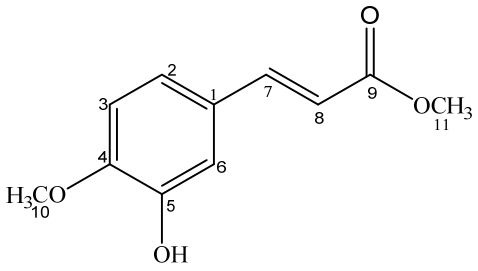

FIGURE 3. Methyl Ferulate

TABLE 1. Comparison of NMR spectrum with other researcher

C	CD-1 compound		Woo dan Lee [15]		Masuda *et al.* [16]
	δ_H (intg, *mult*, J)	δ_C	δ_H (intg, *mult*, J)	δ_C	δ_H (intg, *mult*, J)
1		127.42		126.50	
2	7.02 (1H, *dd*, 8.0 Hz; 2.1 Hz)	120.92	7.05 (1H, *dd*, 8.0 Hz; 2.0 Hz)	122.80	7.08 (1H, *dd*, 8.0 Hz; 2.0 Hz)
3	6.93 (1H, *d*, 8.2 Hz)	110.99	6.80 (1H, *d*, 8.0 Hz)	115.30	6.93 (1H, *d*, 8.0 Hz)
4		150.47		149.40	5.85 (1H, *brs*)
5		147.44		148.20	
6	7.06 (1H, *d*, 2.1 Hz)	113.49	7.17 (1H, *d*, 2.0 Hz)	110.50	7.03 (1H, *d*, 2.0 Hz)
7	7.57 (1H, *d*, 15.9 Hz)	145.31	7.60 (1H, *d*, 16 Hz)	145.60	7.62 (1H, *d*, 15.0 Hz)
8	6.31 (1H, *d*, 15.9 Hz)	114.24	6.35 (1H, *d*, 15.5 Hz)	114.00	6.28 (1H, *d*, 15.0 Hz)
9		168.21		168.50	
10	3.88 (3H, *s*)	54.91	3.90 (3H, *s*)	55.20	3.92 (3H, *s*)
11	3.76 (3H, *s*)	50.62	3.75 (3H, *s*)	50.80	3.80 (3H, *s*)

CONCLUSION

In this research, one compound CD-1 was successfully isolated from methanol extract of sausage fruit. The structure of CD-1 was identified by using UV-Vis, FTIR, NMR, and LC-MS as a cinnamate derivative which was thought to be methyl ferulate.

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