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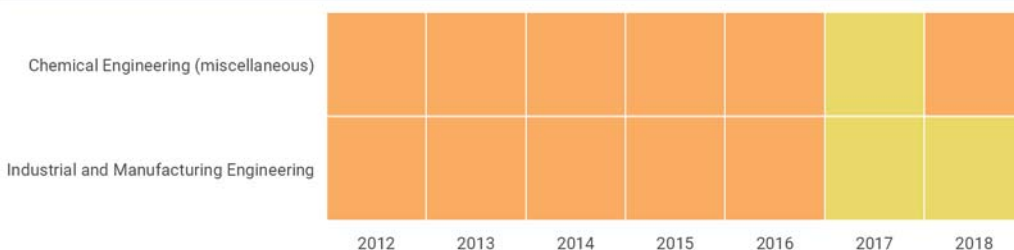
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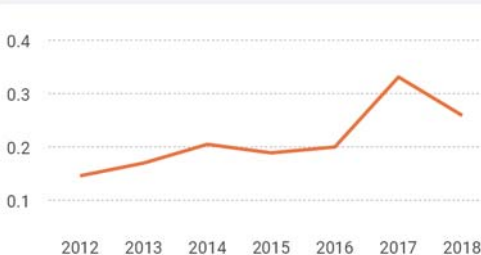
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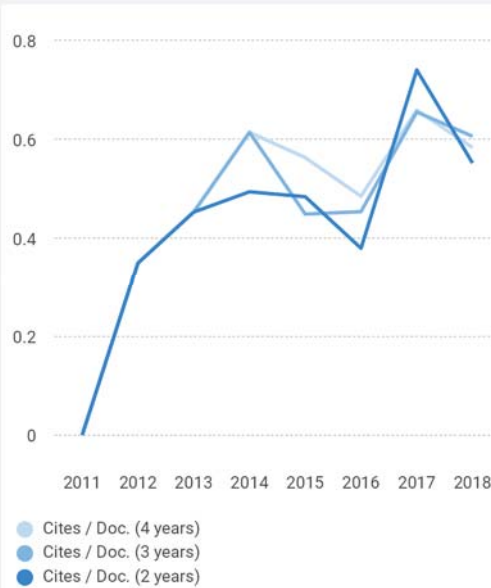
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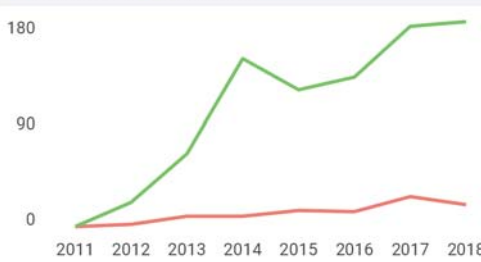
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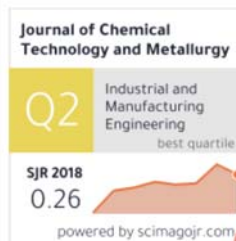
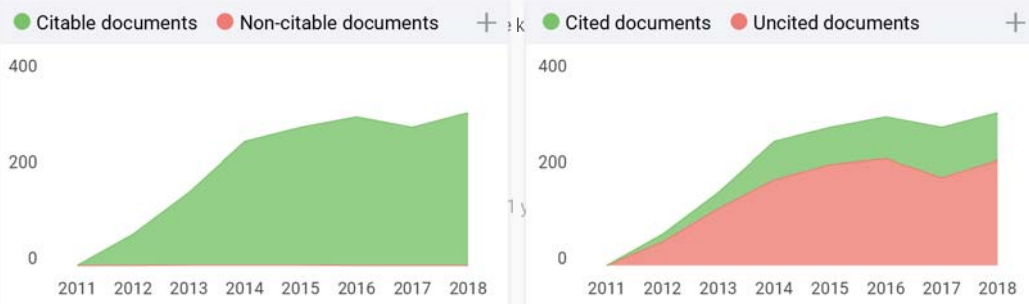
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CONFUSARIN AND NUDOL, TWO PHENANTHRENE GROUP COMPOUNDS, FROM *Dioscorea esculenta* L. AND THEIR ANTIOXIDANT ACTIVITIES

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ABSTRACT

Two phenanthrene group compounds namely: confusarin and nudol are isolated from *Dioscorea esculenta* L. This species belongs to Dioscoreaceae family. The two compounds extraction is conducted by the maceration method using methanol solvent and partition with *n*-hexane and ethyl acetate. The ethyl acetate extract is purified using various chromatographic techniques yielding pure compounds. The latter structure is determined by spectroscopic methods including UV/Vis, IR, 1D and 2D NMR. Confusarin and nudol are tested for their antioxidant activity against DPPH radical scavenging. They show IC_{50} of $19,63 \pm 0,09$ and $37,91 \pm 0,08$ ppm, correspondingly.

Keywords: phenanthrene, confusarin, nudol, *Dioscorea esculenta* L., antioxidant.

INTRODUCTION

Dioscorea esculenta L. with the local name “gembili” is one of species belonging to *Dioscorea* genus. In Indonesia, this plant is one of species used by the community as a substitute for rice [1]. *Dioscorea*, a genus in Dioscoreaceae family, is comprised of about 600 species. It has been reported that various kinds of phenolic compounds are contained in *Dioscorea* genus, such as: stilbenoid, dihydrostilbene, phenanthrene, dihydrophenanthrene, diarylheptanoid and acetophenone [2 - 6]. Secondary metabolite of compounds from *Dioscorea* genus show [7-11] an interesting biological activity, such as: antiinflammatory, antibacterial, antimicrobial, anticancer, antiallergenic, antifungal and antioxidant ones.

Based on previous studies, the methanol extract of *D. esculenta* shows an antioxidant activity [12]. But a phytochemical study of the secondary metabolite compounds as well as an antioxidant activity of pure compounds from this species has not been so far reported. The present communication presents two phenolic compounds, namely confusarin (1) and nudol (2) isolated from *D. esculenta* and verifies their

antioxidant activity using DPPH reagent.

EXPERIMENTAL

Gravitation column chromatography (GCC) was carried out using Merck Si gel 60 (700-200 mesh), radial chromatography was conducted out using Merck Si gel 60 PF₂₅₄, while pre-coated Si gel plates (Merck Kieselgel 60 GF₂₅₄, 0.25 mm) were used for TLC analysis. UV and IR spectra were recorded with a Shimadzu UV-1800 and FT IR Spectrum One Perkin-Elmer instruments, respectively. ¹H and ¹³C-NMR spectra were obtained with JEOL ECA 400, operating at 400 (¹H) and 100 (¹³C) MHz, using residual and deuterated solvent peaks as reference standards. The determination of the antioxidant activity was based on the inhibition of free radical against DPPH using a spectroscopy method.

Samples of *Dioscorea esculenta* tuber were collected from Pengampon District, Kabuh, Jombang, East Java, Indonesia. The plant was identified by the staff of the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia, and a voucher specimen were deposited in the herbarium.

The powdered tuber of *Dioscorea esculenta* L. (10,0 kg) was maserated with methanol for 2 x 24 h and then concentrated under reduced pressure to give a gummy brownish extract. The methanol extract was partitioned into n-hexane and ethyl acetate soluble fractions. A portion (5.2 g) of the ethyl acetate fraction was fractionated using GLC (n-hexane : ethyl acetate, increasing polarity) to give two group fractions A & B by combining fractions with similar TLC profiles. Fraction B (159 mg) was subjected to further partition using radial chromatography (n-hexane:chloroform = 8:2, 7:3, 1:1, 3:7, chloroform and chloroform:ethyl acetate = 9:1). It gave three sub-fractions B1, B2, and B3. The purification of B2 fraction by radial chromatography (n-hexane : chloroform = 7:3, 1:1, 3:7 and chloroform) gave compound **1** (3,3 mg). Fraction B3 yielded compound **2** (19.6 mg) using the same methods (n-hexane : ethyl acetate = 9:1 and 8:2 as eluent), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity assay was used to determine compound's inhibition capacity. Its reaction principle based on the mechanism of free radicals inhibition by hydrogen transfer provided the determination of the sample antioxidant activity expressed in IC₅₀ (Inhibition Concentration 50 %). The

procedure used was as follows: a total of 500 µL of the test solutions of a varying concentration (10 ppm - 500 ppm), 500 µL of 0.2 M acetate buffer of pH 5.5, and 1000 µL of methanol were mixed in a test tube; 500 µL of 5x10⁻⁴M DPPH were added to this mixture; the latter was homogenized using a vortex in a dark room (resistant to UV light) and incubated for 30 min. Then the mixture UV absorbance was measured by a spectrophotometer at λ_{max} of 517 nm. Ascorbic acid was used as a positive control [13]. To prevent the sample from light disturbance, the test tube wrapped with aluminum foil. The inhibition capacity was evaluated in correspondence with the following equation:

$$\% \text{ inhibition} = \frac{A. \text{blanko} - A. \text{sampel}}{A. \text{blanko}} \times 100\%$$

RESULTS AND DISCUSSION

Confusarin (**1**) – an yellow powder, mp 220.8°C (dec.); UV spectrum (MeOH) λ_{max} nm (log ε) : 264 (4.41), 286 (3.82), 308 (3.64), 350 (2.94) and 367 nm (2.97); spectrum ¹H-NMR (CDCl₃, 400 MHz) and ¹³C-NMR (CDCl₃, 100 MHz) - see Table 1.

Table 1. ¹H-NMR and ¹³C-NMR data (CDCl₃) of compounds **1** and **2**.

No	Compound 1		Compound 2	
	δ _H	δ _C	δ _H	δ _C
1	7.19(s)	108.1	7.18(s)	108.2
2	-	142.3	-	147.5
3	-	136.1	-	140.9
4	-	145.4	-	150.7
4a	-	114.4	-	133.5
4b	-	119.8	-	118.9
5	9.19 (d, <i>J</i> =9.2)	123.9	9.36(d, <i>J</i> =9.1)	128.5
6	7.29(d, <i>J</i> =9.2)	116.1	7.19(dd, <i>J</i> =9.1, 2.8)	116.5
7	-	140.9	-	153.2
8	-	135.1	7.16(d, <i>J</i> =2.8)	111.9
8a	-	121.6	-	129.6
9	7.85(d, <i>J</i> =9.1)	119.3	7.49(d, <i>J</i> =8.8)	126.4
10	7.60(d, <i>J</i> =9.1)	127.3	7.53(d, <i>J</i> =8.8)	127.4
10a	-	122.7	-	124.3
3-OCH ₃	4.10(s)	61.3	4.11(s)	61.4
4-OCH ₃	3.96(s)	61.9	3.98(s)	59.8
8-OCH ₃	3.97(s)	59.7	-	-
2-OH	6.00(s)	-	6.03(s)	-
7-OH	5.80(s)	-	5.34	-

Nudol (**2**) - an amorphous pale yellow solid; UV spectrum (MeOH) (MeOH) λ_{\max} nm (log ϵ): 211 (3.88), 258 (4.39), 283 (3.73), 347 (2.69) dan 363 nm (2.75); spektrum IR ν_{\max} (cm^{-1}): 3273 (OH), 2935 and 2850 (CH aromatic), 1618-1357 (C=C aromatic) and 1287 - 1159 (C-O-C ether). Spectrum $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) - see Table 1.

The $^1\text{H-NMR}$ spectrum of Confusarin (**1**) reveals that the three aromatic protons, that are a pair of doublet signals ($J = 9.2$ Hz) at δ_{H} 9.19 and 7.29 ppm, a singlet signal at δ_{H} 7.19 ppm and a pair of doublet signals ($J = 9.1$ Hz) at δ_{H} 7.85 and 7.60 ppm could be assigned to penta-substituted phenanthrene. The spectrum indicates the presence of five oxyaryl protons through three singlet signals from methoxy groups at δ_{H} 4.10 ppm, 3.97 ppm and 3.96 ppm and two hydroxyl groups at δ_{H} 6.00 ppm and 5.80 ppm. This means that there are three methoxy and two hydroxyl groups attached to the aromatic ring.

The spectrum $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) shows 17 perfectly separated carbon signals. They refer to nine quaternary carbon atoms (δ_{C} 145.4 ppm, 142.3 ppm, 140.9 ppm, 136.1 ppm, 135.1 ppm, 119.8 ppm, 114.4 ppm, 122.7 ppm and 121.6 ppm), five methine carbons (δ_{C} 127.3 ppm, 123.9 ppm, 119.3 ppm, 116.1 ppm and 108.1 ppm) and three methyl carbons from a methoxy group (δ_{C} 61.9 ppm, 61.3 ppm and 59.7 ppm). Five from the nine quaternary carbon atoms are of an oxyaryl carbons type (δ_{C} 145.4 ppm, 142.3 ppm, 140.9 ppm, 136.1 ppm and 135.1 ppm). The data presented shows that compound 1 belongs to the phenanthrene derivatives group.

Further information of the methoxy and hydroxyl group position in compound 1 phenanthrene is obtained from HMQC and HMBC analyses. The doublet signal ($J = 9.2$ Hz) at δ_{H} 9.19 ppm (H-5) is correlated with two quaternary carbon signals (δ_{C} 121.6 ppm and 114.4 ppm) and one oxyaryl carbon signal (δ_{C} 140.9 ppm). This data confirms the carbon signals (δ_{C} 140.9 ppm, 121.6 ppm and 114.4 ppm) at position C-4a, C-7 and C-8a. The correlation of the signal singlet at δ_{H} 7.19 with two quaternary carbon signals (δ_{C} 114.4 ppm and 122.7 ppm) and two oxyaryl carbon signals (δ_{C} 136.1 ppm and 142.3 ppm) attributes δ_{C} of 122,7 ppm to position C-10a, while δ_{C} of 114,4 ppm - to position C-4a. Meanwhile the two

oxyaryl carbons (δ_{C} 136.1 ppm and 142.3 ppm) occupy positions C-2 and C-3. The absence of a correlation between the signals at δ_{H} 7.19 ppm and δ_{C} 119.8 ppm (C-4b) indicates δ_{H} 7,19 ppm attribution to H-1 position. The UV, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ data of compound 1 verify that it is 2,7-dihydroxy-3,4,8-trimethoxyphenantrene (Fig. 1). A compound of this structure named confusarin has been obtained from *Eria confuse* [14] but for the first time it is found in *Dioscorea esculenta*.

The IR spectrum of Nudol (**2**) shows a strong absorption band in the range of 1618 cm^{-1} -1357 cm^{-1} referring to aromatic rings, that of 1287 cm^{-1} -1159 cm^{-1} indicative of C-O-C ether and that at 3273 cm^{-1} attributed to hydroxyl groups. The absence of carbonyl groups is evident. The $^1\text{H-NMR}$ spectrum (CDCl_3 , 400 MHz) whose data is listed in Table 1 shows the presence of three signals of an aromatic type: ABX system signals at δ_{H} 9.36 ppm (d, $J = 9.1$ Hz), 7.19 ppm (dd, $J = 9.1$ and 2.8 Hz) and 7.16 ppm (d, $J = 2.8$ Hz), *ortho* doublet signals at δ_{H} 7.53 ppm and 7.49 ppm ($J = 8.8$ Hz) and a singlet signal of *penta* substituted benzene at δ_{H} 7,18 ppm. Two methoxy aromatic signals referred to two singlet protons at δ_{H} 4,11 ppm and 3,98 ppm are also outlined. In addition, there are two hydroxyl aromatic signals at δ_{H} 5,34 ppm and 6,03 ppm. Based on the molecular formula of compound $\text{C}_{16}\text{H}_{14}\text{O}_4$ and presence of four oxyaryl carbons (δ_{C} 153.2 ppm, 150.7 ppm, 147.5 ppm and 140.8 ppm), it can be suggested that the isolated compound is two hydroxy and two methoxy substituted phenanthrene at C-2, C-3, C-4 and C-7 positions, respectively.

The $^{13}\text{C-NMR}$ spectrum (CDCl_3 , 100 MHz) reported in Table 1 shows 16 perfectly separated carbon signals. The signals refer to eight quaternary carbons (δ_{C} 153.2 ppm, 150.7 ppm, 147.5 ppm, 140.9 ppm, 133.5 ppm, 129.6 ppm, 124.3 ppm and 118.9 ppm), four quaternary

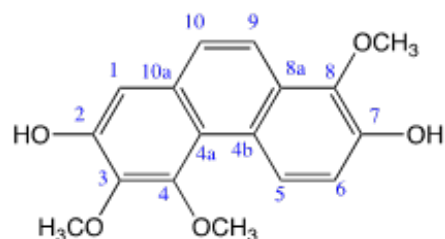


Fig. 1. Chemical structure of confusarin (**1**).

oxyaryl carbons (δ_c 153,2 ppm; 150,7 ppm; 147,5 ppm and 140,8 ppm), six methin carbons (δ_c 128.5 ppm, 127.4 ppm, 126.4 ppm, 116.5 ppm, 111.9 ppm and 108.2 ppm), and two methyl carbons from a methoxy group (δ_c 61,4 ppm and 59,8 ppm). The data of ^{13}C -NMR analysis indicates that compound **2** belongs to the phenanthrene derivatives group.

The position of the methoxy and the hydroxyl group of compound **2** phenanthrene is determined on the ground of HMQC and HMBC analyses. The correlation between the singlet signal referring to an aromatic proton (H-1) at δ_H 7,18 ppm with two oxyaryl carbon signals (δ_c 147.5 ppm and 140.9 ppm) and one methin signal (δ_c 127.4 ppm) indicates that the position of the methin carbon δ_c 127.4 ppm is at C-10. The two oxyaryl carbons (δ_c 147.5 ppm and 140.9 ppm) occupy positions C-2 and C-3. The correlation between the hydroxyl singlet signal at δ_H 6.03 ppm, the two oxyaryl carbon signals (δ_c 147.5 ppm and 140.9 ppm) and the methin carbon signal (δ_c 108.2 ppm) confirms that OH position is at C-2, while that of the methin carbon (δ_c 108.2 ppm) is at C-1.

The position of $-\text{OCH}_3$ at C-3 is indicated by the correlation between the singlet methoxy group signal at δ_H 4.11 ppm and that of the oxyaryl carbon at δ_c 140.9 ppm. This in turn means that the position of the oxyaryl carbons (signals at δ_c 147.5 ppm and 140.9 ppm) is at C-2 and C-3. The correlation between the methoxy group signal (δ_H 3,98 ppm) and the oxyaryl carbon signal (δ_c 150,7 ppm) as well as the absence of a correlation between the ABX proton system signals at δ_H 9.36 ppm (d, $J=9.1$ Hz), 7.19 (dd, $J=9.1$ and 2.8 Hz) and 7.16 ppm (d, $J=2.8$ Hz) indicate that the position of the methoxy group (δ_c 150.7 ppm) is at C-4, while that of the hydroxyl group is at C-7. The UV, IR, ^1H -NMR, and ^{13}C -NMR data referring to compound **2** postulate that it is 2,7-dihydroxy-3,4-dimethoxyphenantrene (Fig. 2). A compound of this structure named nudol has been obtained from *Eulophia nuda* [15] but for the first time it is found in *Dioscorea esculenta*.

The antioxidant activity of compounds **1** and **2** against DPPH radical reagent is estimated to IC_{50} 19,63 \pm 0,09 and IC_{50} 37,91 \pm 0,08 ppm, correspondingly. The values pointed out provide to assume that the methoxy group at C-8 in compound **2** is responsible for the higher

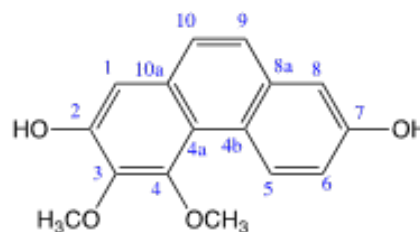


Fig. 2. Chemical structure of nudol (**2**).

antioxidant activity displayed. Ascorbic acid of IC_{50} of 10,95 \pm 0.08 ppm is used as a reference in the antioxidant activity test.

CONCLUSIONS

Confusarin and nudol had been isolated from tuber of *dioscorea esculenta* L. The two isolated compounds showed an antioxidant activity of IC_{50} 19,63 \pm 0,09 and 37,91 \pm 0,08 ppm, respectively.

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