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Phytochemical investigation and antimicrobial activity from rhizomes of *alocasia odora* K. Koch

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Abstract. *Alocasia odora* is a common plant species in Viet Nam. A phytochemical investigation of the ethyl acetate extract (EtOAc) of *A. odora* rhizomes collected in Tuyen Quang province led to the isolation and determination of five compounds, including three alkaloids, alocasin A (1), hyrtiosin B (2), hyrtiosulawesine (3), one mono-phenol, p-hydroxycinamic acid (4), and one fatty acid, myristic acid (5). The chemical structures of those compounds were determined by 1D and 2D-NMR spectroscopic, MS data and compared with those reported in the literature. Antimicrobial activity against microorganisms was evaluated using the broth microdilution method, showing that alkaloids 1 - 3 exhibited antimicrobial activity against reference strains with MIC values ranging from 8 µg/mL to 128 µg/mL, in which compound 1 was the most active with MIC values calculated between 8 µg/mL and 32 µg/mL. Compound 4-5 did not display antimicrobial activity at the studied concentration, MIC > 128 µg/mL. This is the first time the antimicrobial effect of *A. odora* has been reported.

Keywords: Alocasia odora, alkaloid, antimircobial activity

Classification numbers: 1.1.1. 1.2.1.

1. INTRODUCTION

Alocasia, belonging to the Araceae family, is a genus of more than 100 species of perennial, herbaceous, diminutive to extremely large, usually robust herbs with clear-to-milky

latex. They are distributed throughout typical subtropical Asia and were used as a common traditional medicine [1]. Previous phytochemical investigations have revealed that *Alocasia* is rich in alkaloids, carbohydrates, phenols, phytosterols, saponins, and tannins with various pharmacological activities such as antimicrobial antioxidant, antitumor and cytotoxic, hepatoprotective, anti-inflammatory, glycemic and lipidemic activities [2 - 4]. *Alocasia odora*, a widespread *Alocasia*, is a common medicinal herb known in Vietnamese as "Ráy dại, Bạc hà, Dã vu". Traditional experience of using the plant was used to treat many diseases such as liver, gout, detoxication, cutaneous diseases, malaria, and rheumatism [5]. Despite this multitude of bioactivities, a few phytochemical investigations of *A. odora* are found in the literature [6 - 7]. Therefore, this study was undertaken to identify more of the phytoconstituents and antimicrobial activity of *A. odora*. In this paper, we report the isolation and structural determination of three alkaloids (**1-3**), one mono-phenol (**4**), and one fatty acid (**5**) from the EtOAc extract of the rhizomes of *A. odora.* Three alkaloids, alocasin A (**1**), hyrtiosin B (**2**), and hyrtiosulawesine (**3**) from *A. odora* were reported for the first time.

2. MATERIALS AND METHODS

2.1. Materials

The fresh rhizomes of *A. odora* were collected in Na Hang, Tuyen Quang province in January 2021 and were identified by Dr. Nguyen Van Du, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (AO-2021) was deposited at the Laboratory of Applied Biochemistry, Institute of Chemistry, Vietnam Academy of Science and Technology.

2.2. Methods

General experimental procedures

NMR spectra were recorded on a Bruker Avance 500 and Bruker Avance III 600 spectrometers. ESI-MS spectra were obtained with a ThermoScientific LTQ Orbitrap XL spectrometer (USA). Silica gel (40 - 63 µm) and Sephadex LH-20 were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck).

Antimicrobial activity assays

Bacterial strains and culture medium

The pathogenic Gram-positive bacteria *Bacillussubtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 13709) and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442) were obtained from American Type Culture Collection. The bacterial culture medium (MHB - Mueller Hinton Broth, TSB - Tryptic Soy Broth) was purchased from Merck.

Broth microdilution assays

The antimicrobial activity of compounds was evaluated as previously described [8]. Briefly, samples were dissolved in DMSO and ionized water to concentrations of 128.0, 32.0, 8.0, 2.0 and 0.5 µg/mL. Microbacteria were kept at –80 ^{o}C. Before assaying, they were activated with culture medium and adjusted to a concentration of 5×10^5 CFU/mL. 10 µL of sample were combined with 190 µL of microbacterial solution in a 96-well plate which was further incubated within 18 - 24 h

at 37 ^{o}C. The experiment was performed in triplicate. Positive controls were wells with a bacterial suspension in growth medium, and culture medium without bacteria served as a negative control. Ampicillin and cefotaxime (Sigma-Aldrich) were used as positive controls. The results were recorded at 590 nm using a microtiter plate reader and calculated by Raw data software. The MIC value was considered to be the lowest concentration of the compound at which bacterial growth was completely inhibited under the assay conditions.

Extraction and isolation

The fresh rhizomes of *A. odora* (50 kg) were rinsed with water, cut into pieces, dried at 50 - 55 ^{o}C and then grinded to powder. The dried powder (4.0 kg) was ultrasonically soaked with *n*-hexane, EtOAc, and MeOH, respectively. This soluble extract was then concentrated under reduced pressure to give crude *n*-hexane, EtOAc and MeOH extracts. The EtOAc residue (350 g) was subjected to silica gel CC, eluted with gradient solvent systems of *n*-hexane/acetone (9:1-0:10, v/v) and acetone/MeOH (9:1-0:10, v/v) to yield 10 fractions (F1-F10). The F1 fraction (170 mg) was separated by silica gel CC employing *n*-hexane/EtOAc (20/1, v/v) to yield compound **5** (6 mg). Compound **4** (5 mg) was obtained from the F2 fraction (220 mg) after purifying by column chromatography on sephadex LH-20 using MeOH as the sovent eluent. The F5 fraction (3 g) was chromatographed on a silica gel column, eluted with a stepwise gradient solvent system of CH_2Cl_2/MeOH (10:0-0:10, v/v) to give 5 fractions (F5.1-F5.5). The F5.2 fraction (220 mg) was purified on a sephadex LH-20 column [MeOH/CH_2Cl_2 (9:1, v/v)] to obtain 5 fractions (F5.2.1-F5.2.5). Fraction F5.2.2 (22.0 mg) was separated on a preparative-TLC [CH_2Cl_2/MeOH (9:1, v/v)] to yield compound **3** (10.0 mg). Fraction F5.2.3 (30.0 mg) was separated on a preparative-TLC [CH_2Cl_2/MeOH (9:1, v/v)] to yield compound **1** (9.0 mg). The F5.3 fraction (200 mg) was purified on a sephadex LH-20 column [MeOH/CH_2Cl_2 (9:1, v/v)], to give 4 fractions (F5.3.1-F5.3.4). Using the same procedure, compound **2** (8 mg) was obtained from fraction F5.3.2 (32 mg) after purifying on a sephadex LH-20 column, eluted with MeOH.

Alocasin A (**1**)

Yellow solid, ESI-MS: *m/z* 343 $[M+H]^+$, $C_{20}H_{14}N_4O_2$ (M = 342.4). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H (ppm): 6.81 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'/H-6"), 7.31 (1H, d, *J* = 8.5 Hz, H-7'/H-7"), 7.74 (1H, d, *J* = 2.0 Hz, H-4'/H-4"), 7.92 (1H, s, H-2'/H-2"), and 8.96 (1H, s, H-3/H-6). ^{13}C-NMR (CD_3OD, 125 MHz) δ_C (ppm): 106.2 (C-4'/C-4"), 113.2 (C-7'/C-7"), 113.3 (C-6'/C-6"), 113.9 (C-3'/C-3"), 126.7 (C-2'/C-2"), 127.4 (C-9'/C-9"), 133.6 (C-8'/C-8"), 141.5 (C-3/C-6), 148.4 (C-2/C-5), and 152.8 (C-5'/C-5").

Hyrtiosin B (**2**)

Yellow solid, ESI-MS: *m/z* 321 $[M+H]^+$, $C_{18}H_{12}N_2O_4$ (M = 320.3). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H (ppm): 6.85 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'/H-6"), 7.34 (1H, d, *J* = 8.5 Hz, H-7'/H-7"), 7.78 (1H, d, *J* = 2.5 Hz, H-4'/H-4"), and 8.04 (1H, s, H-2'/H-2"). ^{13}C-NMR (CD_3OD, 125 MHz) δ_C (ppm): 107.5 (C-4'/C-4"), 113.7 (C-7'/C-7"), 114.2 (C-3'/C-3"), 114.5 (C-6'/C-6"), 128.5 (C-9'/C-9"), 132.9 (C-8'/C-8"), 138.5 (C-2'/C-2"), 155.0 (C-5'/C-5"), and 191.2 (C-10'/C-10").

Hyrtiosulawesine (**3**)

Yellow solid, ESI-MS: *m/z* 344 $[M+H]^+$, $C_{20}H_{13}N_3O_3$ (M = 343.3). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H (ppm): 6.84 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 7.16 (1H, dd, *J* = 2.5, 8.5 Hz, H-7), 7.35 (1H, d, *J* = 8.5 Hz, H-7'), 7.57 (1H, d, *J* = 8.5 Hz, H-8), 7.60 (1H, d, *J* = 2.0 Hz, H-5), 8.04 (1H, d, *J* = 2.5 Hz, H-4'), 8.18 (1H, d, *J* = 5.0 Hz, H-4), 8.45 (1H, d, *J* = 5.0 Hz, H-3), and 8.91 (1H, s, H-2'). ^{13}C-NMR (CD_3OD, 125 MHz) δ_C (ppm): 106.8 (C-5), 108.0 (C-4'), 113.3 (C-7'), 113.9 (C-6'), 113.9 (C-8), 116.1 (C-3'), 118.5 (C-4), 119.9 (C-7), 122.6 (C-4b), 129.8 (C-3'a), 132.4 (C-7'a), 132.5 (C-4a),

137.4 (C-3), 137.4 (C-8b), 137.5 (C-8a), 139.1 (C-2'), 140.4 (C-1), 152.6 (C-6), 154.4 (C-5'), and 189.9 (C-8').

***p*-Hydroxycinamic acid** (**4**): Amorphous powder, $C_9H_8O_3$ (M = 164.0). ^{1}H-NMR (CD_3OD, 600 MHz) δ_H (ppm): 7.87 (d, *J* = 9.0 Hz, H-2 & 6), 6.80 (d, *J* = 9.0 Hz, H-3 & 5), 7.76 (d, *J* = 8.4 Hz, H-7), and 7.20 (d, *J* = 8.4 Hz, H-8).

Myristic acid (**5**): White powder, ESI-MS: *m/z* 227 $[M-H]^-$, $C_{14}H_{28}O_2$ (M = 228.4). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 2.35 (t, *J* = 7.5 Hz, H-2), 1.65 (m, H-3), 1.28 (br s, H-4→H13), and 0.91 (t, *J* = 6.0 Hz, H-14)

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a yellow solid. ESI-MS showed the proton adduct ion $[M+H]^+$ at *m/z* 343 ($C_{20}H_{14}N_4O_2$). The ^{1}H-NMR spectrum of **1** indicated the presence of an ABX system at δ_H 6.81 (1H, dd, *J* = 2.0, 8.5 Hz), 7.31 (1H, d, *J* = 8.5 Hz), 7.74 (1H, d, *J* = 2.0 Hz), two singlet protons at δ_H 7.92 (1H, s), 8.96 (1H, s). Analyses of the ^{13}C-NMR spectrum with the aid of HSQC experiment of **1** indicated the signals of 10 carbons, including five sp^2 methine groups and five sp^2 quaternary carbons. This observation suggested a dimeric structure for compound **1**. The chemical shifts of C-2/C-5 (δ_C 148.4), C-8′/C-8" (δ_C 133.6), C-3/C-6 (δ_C 141.5) and C-5′/C-5" (δ_C 152.8) suggested their linkage to oxygen and nitrogen. The HMBC correlations from H-4' (δ_H 7.74) to C-6' (δ_C 113.3)/C-8' (δ_C 133.6)/C-5' (δ_C 152.8); from H-6' (δ_H 6.81) to C-4' (δ_C 106.2); from H-7' (δ_H 7.31) to C-5' (δ_C 152.8); and from H-2' (δ_H 7.92) to C-3' (δ_C 113.9)/C-8' (δ_C 133.6)/C-9' (δ_C 127.4) suggested a 3,5-disubstituted-indole moiety for compound **1**. The HMBC correlations from H-3 to C-2/C-5; from H-6 to C-2/C-5; and from H-2' to C-2/C-3′/C-8′/C-9' assigned the connections of indole and pyrazine rings. Thus, complete analyses of the 2D-NMR spectra and comparison with the literature [9] identified the structure of **1** to be an alkaloid, namely alocasin A.

Compound **2** was isolated as a yellow solid. ESI-MS showed the proton adduct ion peak at *m/z* 321 $[M+H]^+$ ($C_{18}H_{12}N_2O_4$). Analysis of the ^{13}C NMR and HSQC spectra revealed the presence of only nine carbon signals, including four sp^2 methine carbons, one carbonyl carbon and four sp^2 quaternary carbons. Moreover, the ^{1}H NMR showed proton signals of an ABX system at δ_H 6.85 (1H, dd, *J* = 2.5, 8.5 Hz), 7.34 (1H, d, *J* = 8.5 Hz), and 7.78 (1H, d, *J* = 2.5 Hz), and a singlet aromatic proton at δ_H 8.04 (1H, s). This observation suggested that its structure was a symmetrical dimer. Analysis of the 2D-NMR spectra, especially HMBC spectrum revealed the presence of an indole ring with substituents at C-3' and C-5' as follows: the HMBC correlations from H-2' (δ_H 8.04) to C-3' (δ_C 114.2)/C-8' (δ_C 132.9)/C-9' (δ_C 128.5); from H-4' (δ_H 7.78) to C-6' (δ_C 114.5)/C-8' (δ_C 132.9); from H-6' (δ_H 6.85) to C-4' (δ_C 107.5)/C-8' (δ_C 132.9); and from H-7' (δ_H 7.34) to C-5' (δ_C 155.0)/C-9' (δ_C 128.5). Detailed analysis of the NMR spectra and comparison with the reported NMR data [10] indicated that the structure of compound **2** was hyrtiosin B.

Compound **3** was isolated as a yellow solid. ESI-MS showed the proton adduct ion peak at *m/z* 344 $[M+H]^+$ ($C_{20}H_{13}N_3O_3$). The ^{1}H-NMR spectrum of **1** indicated the presence of two ABX systems at δ_H 6.84 (1H, dd, *J* = 2.5, 8.5 Hz, H-6′), 7.35 (1H, d, *J* = 8.5 Hz, H-7′), 8.04 (1H, d, *J* = 2.5 Hz, H-4′); 7.16 (1H, dd, *J* = 2.5, 8.5 Hz, H-7), 7.57 (1H, d, *J* = 8.5 Hz, H-8), 7.60 (1H, d, *J* = 2.0 Hz, H-5), a proton at δ_H 8.91 (1H, s, H-2′). Signals of two doublets of aromatic protons at δ_H 8.18 (1H, d, *J* = 5.0 Hz, H-4), 8.45 (1H, d, *J* = 5.0 Hz, H-3) were also noted. Analysis of the ^{13}C-NMR and HSQC spectra revealed the signals of 20 carbons, including one carbonyl carbon at δ_C 189.9 (C-6′), nine sp^2 methine groups, and ten quaternary carbons for compound **3**. The chemical shifts of C-6 (δ_C 152.6) and C-5′ (δ_C 154.4) suggested their linkage to oxygen, and those of C-8a, C-8b, C-1, C-3, C-2′ and C-7′a suggesting their attachment to nitrogen. The HMBC correlations

from H-5 to C-7/C-4b/C-6/C-8a; from H-7 to C-8a; from H-3 to C-4a/C-1/C-4; from H-4 to C-3/C-4b/C-8b indicated the presence of a 6-hydroxy-*β*-carboline moiety. The presence of an indole ring with substituents at C-3′ and C-5′ was determined by HMBC correlations from H-2′ to C-3′/C-7′a/C-3′a; from H-4′ to C-7′a/H-6′; and from H-7′ to C-5/C-3′a. Thus, based on the analysis of the 1D- and 2D-NMR spectra and comparison with the literature [11], the structure of **3** was identified to be an alkaloid, namely hyrtiosulawesine.

Compound **4** was obtained as an amorphous powder. Its ^{1}H-NMR spectrum showed signals of a di-substituted benzene ring revealed by signals of an A_2B_2 system [δ_H 7.87 (d, J = 9.0 Hz, H-2/6), 6.80 (d, J = 9.0 Hz, H-3/5)]. The proton signals at δ_H 7.76 (d, J = 8.4 Hz, H-7) and 7.20 (d, J = 8.4 Hz, H-8) indicated the presence of a *cis*-double bond in the chemical structure of **4**. These data led to the conclusion that **4** was *p*-hydroxycinamic acid [12].

Compound **5** was obtained as a white powder. Its molecular formula $C_{14}H_{28}O_2$ was determined by ESI-MS with a pseudomolecular negative ion peak at *m/z* 227 $[M\text{-}H]^-$. The 1H-NMR spectrum showed proton signals of the hydrocarbon chain at δ_H 2.35 (t, J = 7.5 Hz, H-2), 1.65 (m, H-3), 1.28 (br s, H-4 → H-13) and the signal of a terminal methyl group at δ_H 0.91 (t, J = 6.0 Hz, H-14). Compound **5** was identified as myristic acid by comparing the spectral data with those published in the literature [13].

Five compounds (**1**–**5**) were evaluated for antimicrobial activity against pathogenic microorganisms using the broth microdilution method (Table 1). The results showed that alkaloids **1**–**3** exhibited antimicrobial activity against bacteria strains.

Table 1. Antimicrobial activity of the isolated compounds from *Alocasia odora*.

Compounds	**MIC values (µg/mL)**			
	Gram (+) bacteria		**Gram (-) bacteria**	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
1	8	32	32	32
2	32	128	32	128
3	32	128	128	>128
4	>128	>128	>128	>128
5	>128	>128	>128	>128
Ampicillin	0.125	32		
Cefotaxime			0.5	8

Compound **1** exhibited a strong antimicrobial effect on strains *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with MIC values ranging from 8 µg/mL to 32 µg/mL. Compound **2** displayed antimicrobial activity against four strains with MIC values ranging from 32 to 128 µg/mL. Compound **3** significantly inhibited three bacteria strains *S. aureus*, *B. subtilis* and *E. coli* with MIC values of 32, 128 and 128 µg/mL, respectively, while it was inactive against *P. aeruginosa*. Compounds **4** and **5** did not inhibit microbial effect (MIC > 128 µg/mL). For ampicillin and cefotaxime, positive controls showed MICs from 0.125 µg/mL to 32 µg/mL. This is the first time *A. odora* antimicrobial capacity has been studied. The results showed the potential use of this plant in the treatment of infections and further models of research are needed to provide a scientific basis for the direction of the use of this plant. In another study, the extract from *A. odora* stems was shown to possess proliferation of skin fibroblasts and antioxidant activities [6].

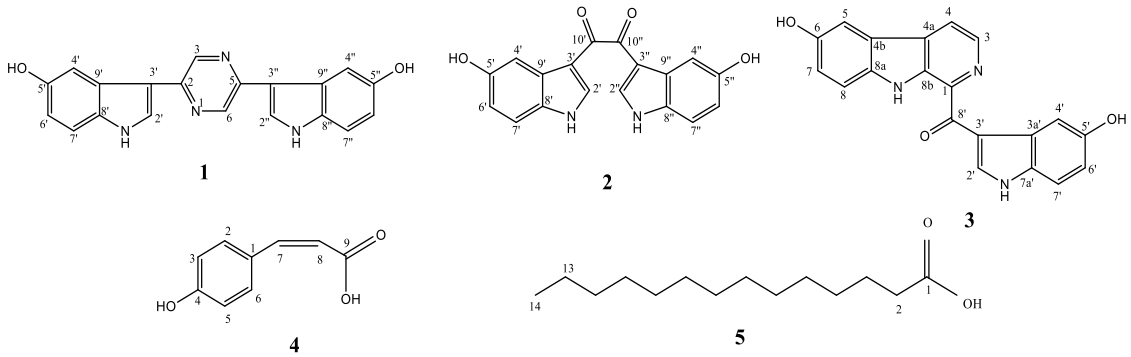

Figure 1. Chemical structures of compounds **1**-**5**.

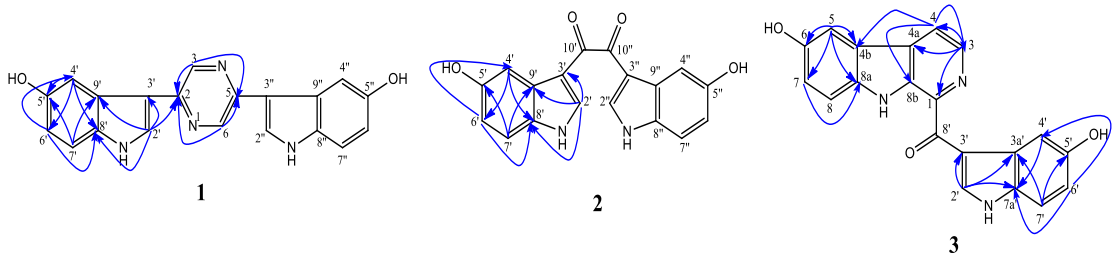

Figure 2. Key HMBC correlations of compounds **1**-**3**.

So far, seven *Alocasia* species have been studied for their antimicrobial and antifungal activities. The results showed that *A. indica* and *A. macrorrhizos* exhibited significant antimicrobial effects. Meanwhile, *A. fornicata* and *A. decipiens* showed moderate-to-good antimicrobial activities. In contrast, *A. sanderiana*, *A. denudata*, and *A. brisbanensis* extracts did not exhibit antimicrobial activities. Thus, it was believed that different extracts of the *Alocasia* species might contain different bioactive molecules that were responsible for their antimicrobial and antifungal activity [14].

4. CONCLUSIONS

From the EtOAc extract of *A. odora* rhizomes, five compounds were isolated. They were elucidated to be alocasin A (**1**), hyrtiosin B (**2**), hyrtiosulawesine (**3**), *p*-hydroxycinamic acid (**4**), and myristic acid (**5**). The antimicrobial activity of the compounds has been evaluated. Alkaloids **1**-**3** exhibited antimicrobial activity against reference strains with MIC values ranging from 8 µg/mL to 128 µg/mL. This is the first time *Alocasia odora* has been studied for antimicrobial activity.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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