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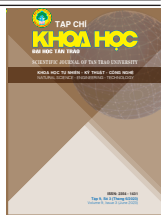

CHEMICAL COMPOSITION OF THE N-HEXANE EXTRACT OF ALOCASIA MACRORRHIZA IN TUYEN QUANG PROVINCE

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Abstract

The species *Alocasia macrorrhiza* is a representative representative of the Ayaceae family. In recent publications on the chemical composition of this plant, it contains cerebroside components, this class of substances has quite good activities of anti-inflammatory, anti-ulcer and antibacterial, one of the common inflammatory agents. variable. Folk medicine has used a number of species to treat diseases, especially Alocasia macrorrhiza species, used to treat diseases such as flu, colds, high fever, sunstroke, tuberculosis, rheumatism, joint pain, cysts, boils, scabies, fire burns, poisonous insects and venomous snake bites. Using column chromatography, thin layer chromatography, and extraction methods with the *n*-hexane fraction, two compounds have been isolated from *Alocasia macrorrhiza* L.: 6-β-hydroxyipolamid (**1**) and Verbenalin (**2**).

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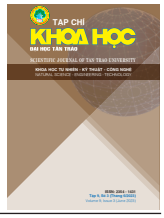

THÀNH PHẦN HÓA HỌC CAO CHIẾT N-HEXANE CỦA CỦ RÁY (ALOCASIA MACRORRHIZA) Ở TUYÊN QUANG

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Alocasia ***macrorrhiza; n-hexane; Tuyên*** *Quang*

Tóm tắt

Loài *Alocasia macrorrhiza* là đại diện tiêu biểu cho các thực vật họ Ráy. Trong các công bố mới đây về thành phần hóa học của cây này có chứa các thành phần cerebrosid, lớp chất này có các hoạt tính khá tốt về kháng viêm, chống lở loét và kháng khuẩn, một trong các tác nhân gây viêm khá phổ biến. Y học dân gian đã sử dụng một số loài để trị bệnh, đặc biệt sử dụng nhiều nhất là loài *Alocasia macrorrhiza* , được dùng để chữa các bệnh như: cúm, cảm mạo, sốt cao, trúng nắng, lao phổi, phong thấp, đau nhức khớp, sa nang, mụn nhọt, ghẻ lở, bỏng lửa, trùng độc và rắn độc cắn. Bằng các phương pháp sắc ký cột, sắc ký lớp mỏng, từ phân đoạn *n*-hexane đã phân lập được 2 hợp chất từ cây Ráy *(Alocasia macrorrhiza* L.*)* là: 6-β-hydroxyipolamid (**1**) vàVerbenalin (**2**).

1. Introduction

The Araceae family comprises widely distributed 107 genus, 2824 species throughout the world. According to Pham Hoang Ho, there were 108 species in Indochina in 1994 [1]. Between 1995 and 2009, 38 new species were discovered in Indochina, according to Nguyen Van Du's statistics, of which Vietnam had 28 species, 8 species in Laos, 2 species in Cambodia [2].

In Vietnam, the The Araceae family has approximately 30 genus and over 100 species. There are about 11 species of Alocasia genus in Vietnam. Some compounds were discovered including fatty acids, tecpenoids, ancaloids, cerebrosid, and glycozid... [3, 4]

12 compounds were isolated from the *Alocasia macrorrhiza* L. plants of Vietnam by author Nguyen Quyet Tien and his colleagues. Among these are a KNO3 inorganic compound (content > 2% compared to the initial dry sample), two fatty acids: palmitic acid and sucinic acid, four cerebrozids (A-D) in which the chemical structure of two cerebrozids (A (A, C). There are two cerebrozid B and D hasn't been determined, two sterols, and two sterol-glucozites [5, 6].

Alocasia macrorrhiza is a plant that has anti-inflammatory activity. Central Pharmaceutical Enterprise 24, Ho Chi Minh City, has successfully prepared a 2% *Alocasia* cream product from its ethyl acetate extract to treat leprosy patients effectively and believes that the active ingredient of the processing of this product is a flavonoid. Recent publications on the chemical composition of this plant contain cerebrosid ingredients, which have quite a good anti-inflammatory, anti-ulcer, and antibacterial activity, one of the common inflammatory agents [7, 8, 9].

Folk medicine has used a variety of species to treat diseases, particularly *Alocasia macrorrhiza* L., which has been used to treat diseases such as flu, colds, high fever, sunburn, pulmonary, and leprosy low, joint pain, capsules, pimples, scabies, burns, poison, and bites from poisonous snakes [9, 10, 11]. According to recent research, the *Siaogo macrorrhiza* contains a variety of compounds including starch, vitamins, amylase, lectin, chlorophyll-protein complex, niacin, tiamin, sterol, cianit, calcium-oxalate, KNO_3 and some trypsin inhibitors and lymphocyte stimulation [2]. Besides the above compounds, there are lipid compounds, fatty acids, triglochinins and glycolipids: trigalactosldiglycerides, tetragalactosyldiglycerides. Besides the above beneficial biological activities, some *Alocasia* species are neurotoxic, inhibiting some hormones such as trypsin or stimulating lymphocyte leukocytes when used as real source products [12].

2. Subjects and methods of research

2.1. The research subjects

Raw materials for research are the rhizome of *A. macrorrhiza* of the plant collected in Yen Son district, Tuyen Quang province in June 2021, with the scientific name of *Alocasia macrorrhiza* L. Biological Resources - Vietnam Academy of Science and Technology.

2.2. Extraction method

Following collection, the plant samples are chopped, dried in the shade at 45-50 ^oC, and crushed. In an ultrasonic device, dry sample powder is soaked in ethanol three times (60 minutes) at 40-50 ^oC. The ethanol extract is stored under reduced pressure before being diluted with water and redistributed via *n*-hexan solvents, ethyl acetate or chloroform, and methanol. To get the corresponding extracts of *n*-hexan extract, ethyl acetate extract, or chlorofom extract and methanol, the extracts are dried and stored under reduced pressure solvent.

2.3. Isolation compound method

Mainly research methods and equipment include:

• Thin layer chromatography (SKLM/TLC): Thin layer chromatography is performed on the thin, pre-coated version of DC-ALUFOLIEN 60 F254 (Merck 1,05715), RP18 F254S (Merck 1,05715), and RP18 F254S (Merck 1,05715). (Merck). Detecting ultraviolet lights at two wavelengths of 254 nm and 368 nm or using a reagent is a 10% vanillin/H_2SO_4 solution that is sprayed evenly on the thin, dried and heated on the magnetic stove until the colour.

• Thin layer chromatography: thin layer chromatography is done on the thin, thin version of Silicagel 60 g F254 (Merck 1 1,05875), discovered a dumpling with ultraviolet lights of two wavelengths of 254 nm and 368 nm, or cutting the thin edge to spray the reagent is 10% vanillin/H_2SO_4 solution, heat to detect a substance, combine the thin version to determine the substance area, then scrape the silica gel layer, adsorption and refining in the solvent.

• Column chromatography (CC): Adsorbent with regular and reversed-phase silica gel is used in column chromatography. The particle size of silicagel is 0.040-0.063 mm. Reversed-phase silica gel (Octadecylsilyl-Ods/C18) or YMC (CHEM) of Institute of Natural Product Chemistry.

2.4. Compound chemical structure determination methods

• Nuclear spectrum: NMR spectrum measured on Bruker AM300, Bruker AM600, and Avance 500 machines from the Korean Basic Science Research Institute (KBSI) and the Institute of Chemistry, Institute of Science and Technology Vietnam. TMS is the internal standard (tetrametylsilan).

• Melting point (MP): Melting point is determined using the Kofler Micro-Hotstage at the Institute of Natural Product Chemistry, Institute of Science and Technology Vietnam

3. Results and discussions

3.1. Collect extracts

Fresh rhizomes plants are sliced, dried, and crushed before being soaked and exhausted with ethanol at room temperature until the color is uncolored. The pressure decreases as the ethanol extract is stored at 50 ^oC. After that, the ethanol extract is extracted with *n*-hexan, ethyl acetate, and methanol solvents. The above extracts (*n*-hexan, ethyl acetate and methanol) are made with Na_2SO_4 anhydrous, filtered and chased solvent with under the decreasing pressure and temperature < 50 ^oC. Sediment is dried and weighed to determine the weight. Thus, three epidemics were extracted from the sample: *n*-hexan, ethyl axetat, and methanol, with the corresponding symbols: **AMH**, **AME**, and **AMW**.

Table 1. Shows the volume of the tree's segment extraction (*Alocasia macrorrhiza*)

Part	Sample weight (g)	Extracts		
		n-hexan (g)	EtOAc (g)	MeOH (g)
		AMH	AME	AMW
Root	1700	8.6	10.5	116.5

3.2. Compounds isolated from n-Hexane extract

The following steps are taken to isolate pure substances from plant extracts:

After washing several times with cold *n*-hexan (at low temperatures < 50°C), 1.24 g of gray-white solids were obtained from 7.5 g of *n*-hexan sediment. Dissolve this solid block in *n*-hexan, add activated carbon, and boil for 1 hour before filtering and chasing the solvent and allowing it to translate at room temperature. To obtain 70.6 mg of HC11, the needle-shaped precipitate is filtered and washed with cold hexane.

n-Hexan extract (**AMH**) is expelled from solvents and taken to the SiO_2 column to continue the remaining substances. After crystallizing in hexan for the compound **1**, the column segment with the solvent system of ethyl acetate/*n*-hexan (1:25) is expelled from the solvent to get a white solid block (98.6 mg). Continue to wash with an ethyl acetate/*n*-hexan (1:15) solvent system, then dissolve in methanol to crystallize 90 mg of compound **2**.

3.2. Determine the structure of isolated compounds

3.2.1. 6-β-hydroxyipolamid (1)

The compound **1** was received in the form of colorless powder with melting point 192-193°C. A single olefin proton signal as of singlet occurs on the ^{1}H-NMR spectrum at δ 7.51 (1H, *s*, H-3), demonstrating a pair of three-position connections. This signal's rather strong migration towards the low field indicates that it is positioned near powerful electronic suction groups. Besides the characteristic signals of a glucose molecule at δ 4.61 (1H, d, *J* = 8.0 Hz, H-1'), 3.22 (1H, dd, *J* = 8.0, 9.0 Hz, H-2'), 3.40 (1H, t, *J* = 9.0 Hz, H-3'), 3.52 (1H, H-4'), 3.35 (1H, H-5'), 3.92 (1H, dd, *J* = 2.0, 12.0 Hz, H_a-6'), 3.68 (1H, dd, *J* = 6.0, 2.0 Hz, H_b-6'). There is also a signal of a methylene group at δ 1.91 (1H, dd, *J* = 8.0, 12.5 Hz, H-7α) và 2.01 (1H, dd, *J* = 6.0, 12.5 Hz, H-7β), two oximetin groups at δ 5.86 (1H, s, H-1) và 4.07 (1H, d, *J* = 6.0, 8.0 Hz, H-6), a metin group at δ 2.59 (1H, *s*, H-9) and two methyl group at δ 1.05 (3H, *s*, H-10) and 3.75 (3H, *br s*, OMe). The existence of the methoxy group was verified by the singlet signal at δ 3.75. Using the above signals and the fundamental frame structure (Iridoid) of compounds found in *Verbena* species, it is possible to determine that the **1** chemical is likewise an Iridoid glycoside.

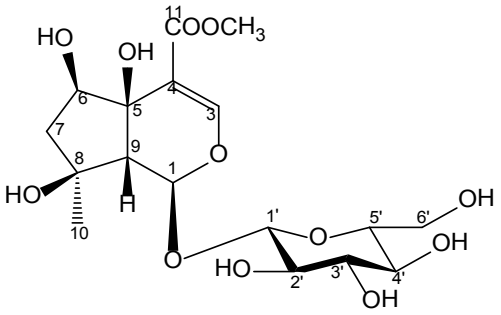

Image 1. Structure of compound 1

The signal of 17 carbon atoms occurs in the ^{13}C-NMR spectrum of the **1** complex, including 6 carbon from the sugar molecule and the remaining 11 carbon from the Aglycon component of the Iridoid structure. The double bond signal at 154.07 (C), 114.37 (CH), and the carbonyl group (ester) signal at 168.37 (C=O), 51.84 (OMe) are extremely common. Compounds having an iridoid structure, a double bond at C-3/C-4, and a methyl carbonyl group linked to C-4 are shown. The signal at δ_C 94.03(C-1)/δ_H 5.86 (1H, s) is suitable for the C-1 location, which contains the H-1 proton at. Hence, the two quaternary carbons coupled to the oxygen atom at δ 70.40 (C-5), 74.65 (C-8) (found by DEPT spectroscopy) must belong to the fifth ring of the iridoid structure. The signal on the 1H-NMR spectrum of this proton appears as a singlet with the iridoid structure, the only methyl group connected to C-8, suggesting that a hydroxyl group must be coupled to C-8. The HMBC interaction between the H-3 proton δ (7.51) and the oxygen-carrying quaternary carbon at δ 70.40 identified another hydroxyl group that was linked to C-5. The last hyroxy group of the aglycon must be attached to C-6 because the signal of the methyl group associated with the quaternary carbon δ (1.05, 3H, s, H-10) interacts with the methylene carbon at δ 48.11 (C-7) but not with carbon at δ 75.50 on the HMBC spectrum.

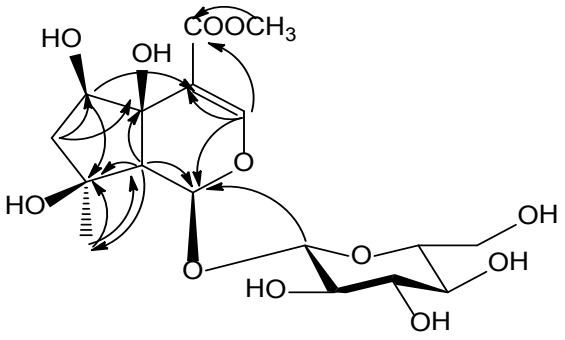

Image 2. 1's major HMBC interactions

The sugar molecule's junction is totally determined by the distant interaction signal in the HMBC spectrum between the H-1' proton (δ 4.61) and the C-1 carbon (δ 94.03), as well as the interaction of H-1 vs C-1'. Carbon chemical shift values of δ 99.76 (C-1'), 74.40 (C-2'), 77.42 (C-3'), 71.74 (C-4'), 78.40 (C-5'), and 62.87 (C-6') are perfectly adequate for the glucose molecule, as are the corresponding H values. The interaction constant value $J_{H\text{-}1'/H\text{-}2'}$ = 8.0 Hz indicates that both H-1' and H-2' protons occupy *axial* locations. Comparing the NMR spectrum values of **1** molecule (chemical shift, interaction constant value, peak shape) with the corresponding published spectral values of 6*β*-hydroxyipolamiid (table 4.1) to discover exact match in all spots. This result, which confirmed the molecule, was identical to ESI MS mass spectrometry results in the presence of peak *m/z* 445 [M+Na], corresponding to molecular mass M = 422 and molecular formula $C_{17}H_{26}O_{12}$. A 6*β*-hydroxyipolamiid is **1**. A compound was isolated from the species *Stachytarpheta mutabilis* in 1983, however this is the first time this compound has been identified from the genus *Verbena*, according to our research [11].

3.2.2. Verbenalin (2)

The compound **2** was obtained as a whitish powder. The ^{1}H-NMR spectrum shows a doublet signal of a single olefin proton at δ 7.48 (*J* = 1.5 Hz, H3), indicating a triple potential double bond (>C=CH-), a doublet signal of one proton of the dioximetin group at 5.25 (*J* = 6.0 Hz, H1), three protons of metin (CH) at 3.53 (1H, br dd, *J* = 8, 0, 1.5 Hz), 2.50 (1H, m), 2.24 (1H, m) and the signal of a methylene group (CH_2, H7a,b) at δ 2.00 (1H, dd, *J* = 4.5, 18.5 Hz), 2.57 (1H, dd, *J* = 8.5, 18.5 Hz) all appear in the higher field region. Moreover, there are two methyl signals at δ 3.74 (CH_3O) and 1.24 (H10) that both integrate for three protons. Although the signal at 1.24 shows as a doublet (*J* = 6.5 Hz) on the highest field side, suggesting that this methyl group is connected to a metin carbon (CH), the signal at 3.74 appears as an appropriate singlet for a methoxy group. The presence of a glucose molecule is also indicated by the doublet signal of the proton attached to the carbon anomer at 4.69 (*J* = 8.0 Hz), the signal of the other four oximetin groups at 3.22 (1H, dd, *J* = 8.0, 9.0 Hz), 3.38 (1H, d, *J* = 9.0 Hz), 3.31 (1H, H-4'), 3.32 (1H), and two signals of the oximethylene group at 3.92 (1H, dd, *J* = 2.0, 12.0 Hz, H_a-6'), 3.66 (1H, dd, *J* = 5.5, 12, 0 Hz, H_b-6'). The high value of the interaction constant between H-1' and H-2' of the sugar molecule (*J* = 8.0 Hz) suggests that both of these protons occupy *axial* positions in the cyclohexane ring, are present. the β-glycosidic ether bond between the sugar molecule and the aglycon. The spectrum findings shown above are also compatible with the existence of an iridoid backbone in Verbena species with an extra carbonyl group (C11) and a glucose molecule. [12].

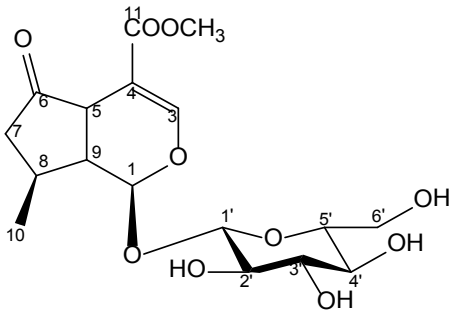

Image 3. Structure of compound 2

4. Conclusion

Two compound were identified from *Alocasia macrorrhiza* L. fractions with tested antibacterial activity: two compounds have been isolated from *Alocasia macrorrhiza* L.: 6-β-hydroxyipolamid (**1**) and Verbenalin (**2**).

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