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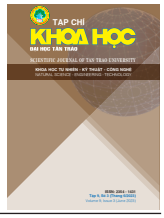

THE STUDY ON CHEMICAL CONSTITUENTS FROM THE N-HEXANE EXTRACT OF SARCANDRA GLABRA IN THAI NGUYEN PROVINCE

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Abstract

Using column chromatography, thin layer chromatography, and extraction methods with n-hexane and ethyl acetate solvents, three pure compounds were isolated from the Sacandra glabra. The chemical structures of these three compounds were determined using nuclear magnetic resonance (NMR) spectroscopy. Among them, compound 1 was identified as Stigmast-5,22-dien-3-β-ol, compound 2 as 2′,6′-dihydroxy-3′,4′-dimethoxychalcone, and compound 3 as 5-hydroxy-6,7-dimethoxyflavanone

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC CAO CHIẾT N-HEXANE CỦA CÂY SÓI RỪNG (SARCANDRA GLABRA) Ở THÁI NGUYÊN

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Tóm tắt

Bằng các phương pháp sắc ký cột, sắc ký lớp mỏng, từ cặn chiết *n*-hexan, etyl axetat của cây Sói rừng (*Sacandra glabra*) đã phân lập được 03 hợp chất sạch. Sử dụng các phương pháp phổ cộng hưởng từ hạt nhân (NMR) đã xác định được cấu trúc hóa học của 3 hợp chất: trong đó 01 hợp chất Stigmast-5,22-dien-3-β-ol (**1**) và 2′,6′-dihydroxy-3′,4′-dimetoxychalcon (**2**) và 5-hydroxy-6,7-dimetoxyflavanon (**3**).

Từ khóa

Alocasia macrorrhiza;
N-hexane; Thái Nguyên

1. Introduction

The genus *Chloranthus* is a member of the Chloranthaceae family, with around 18 species found worldwide, primarily in Asiatic and Southeast Asia nations. According to Pham Hoang Ho, the genus *Chloranthus* contains three species in Vietnam, but Vo Van Chi, count four and one of which, *Sarcandra glabra*, is also classified as *Chloranthus* and has three species. *Chloranthus brachystatus* is another name for this plant. Several varieties of plants in the genus *Chloranthus* are used in traditional medicine by many Asian and Southeast Asia peoples [1, 2, 3].

Traditional Chinese medicine uses the roots of two species, *Chloranthus serratus* and *Chloranthus japonicus*, as antifungal agents. They also used this species used in Chinese medicine to treat back pain, knee pain, boils, white fever, and colds. Often used to treat tuberculosis, back pain, and knee pain [4].

Chloranthus spicatus species are used in China to treat knives, broken bones, skeletons, and schizophrenia, the leaves are used to treat tuberculosis, and the dried plants are used for therapy [5]. Externally applied to the elderly who have been injured by falls. People in Yunnan utilize the whole plant to cure colds, rheumatism, joint pain, fall injuries, bleeding gums, epilepsy, and uterine prolapse [6].

Chloranthus serratus, *Chloranthus japonicus*, *Chloranthus japonica*, and *Chloranthus glaber* are the four most researched plant species. *Chloranthus glaber* species or other name *Sarcandra glabra* is the most interesting Chinese scientist with the various benefits of this species in the treatment of inflammatory disorders, healing boils, and effective anti-tumor fruit. Besides the advantages described above, *Sarcandra glabra* species are recognized in traditional Chinese medicine as a tea or food supplement to cure ailments, helpful in boosting memory to recover equilibrium [7].

In our nation, a few species belonging to the genus *Chloranthus* are also utilized in folk medicine. People frequently utilize *Chloranthus elatior*, to cure colds, rheumatism, arthritis, and amygdala illness. Colds, backaches, boils, and white blood cells are all treated with *Chloranthus japonicus* species. *Chloranthus spicatus*, to make tea and drink the blossoms to ease coughs. The entire aboveground section of the *Chloranthus spicatus* is used to heal knife wounds, fractured bones, rheumatism, joint pain, and epilepsy. The leaves are used to relieve labour cough. The plant's root is used to cure boils. The *Chloranthus brachystachys* also has another name *Sarcandra glabra*, which is only found in folk medicine, as roots soaked in alcohol are used to treat chest pain and pounded leaves are used to treat snakebites. Also, the leaves are utilized to cure tuberculosis. The herb is used to cure rheumatism, back pain and boils [7, 8, 9].

Around 4 plant species of the genus *Chloranthus* have been studied phytochemically, and 33 compounds, including sesquiterpenoids, have been isolated and identified [10].

Several eudesmen sesquiterpenoids have been isolated from the species *Chloranthus serratus* [11]. Besides the aforementioned compounds, eudesmen skeletal sesquiterpenes were identified from *Chloranthus japonicus* species [12]. Chloranthalactone was isolated from *C. japonica* species, as was chloranthdimeric acid from *Chloranthus japonica* and *Chloranthus glaber* [13].

The substances isolated and identified from plants of the genus *Chloranthus* mostly belong to the sesquiterpene lactone (also known as Shizukanolide) is quite diversified [14].

Shizukanolide was isolated from *Chloranthus japonicus* and *Chloranthus glaber* species, as well as 8,9-didehydro from *Chloranthus japonicus* and *Sarcandra glabra* [15, 16].

Several research on *Sarcandra glabra* species in China show that sesquiterpens and dime sesquiterpen compounds have high activity against leukemic cell lines (HL-60), lung cancer cell lines (A-549), and cytotoxicity to human liver cancer cell lines (B-7402). Several sesquiterpene glycosides are anti-hepatitis. Chinese researchers found that *Sarcandra glabra* extract had antioxidant activity *in vivo* and *in vitro*, as well as the potential to control the immune system to ease stress [17, 18].

Chloranthus glaber, sometimes known as *Sarcandra glabra*, is a plant species native to Vietnam. Crushed leaves are used to cure snakebites, a decoction to treat tuberculosis, and roots steeped in alcohol to relieve chest pain [19].

Research published results on the chemical components of *Sarcandra glabra* plants collected in Vinh Phuc recently, suggesting that the structure of chloranosid-A and sarcaglaboside G compounds was isolated and characterized. The above results have not been investigated on the chemical compostition of this plant [20].

2. Objects and research methods

2.1. Research object

Research object: *Sarcandra glabra* (Wolf), Dai Tu, Thai Nguyen, Vietnam.. Specimens are kept at the Institute of Ecology and Biological Resources - Vietnam Academy of Science and Technology.

2.2. Chemicals and equipment

Nong Lam University, Thai Nguyen University, and the Institute of Natural Product Chemistry provided chemicals and equipment.

Industrial solvents from Vietnam, Taiwan, Indonesia, and Korea are used in the extraction of raw materials, separation, and open-column run.

Pure solutions of Merck, France, and Korea are utilized for TLC and HPLC analysis, whereas analytical solvents are used for thin layer chromatography and quick column chromatography.

Analytical thin layer chromatography (TLC) was done on a pre-coated plate DC- Alufolien 60 F_{254} (Merck 105715), 0.2 mm thick; NP and RP_{18} F_{254s} (Merck).

Colour the substances present on the plate using a 10% concentrated sulfuric acid (H_2SO_4) solution sprayed on the plate and then heated on an electric burner until the color appears.

Column Chromatography (CC) was carried out using a normal-phase, reversed-phase silica gel sorbent with particle sizes of 40-63 μm, 63-200 μm, and 75 μm.

High performance liquid chromatography HPLC was performed at the Chemical Analysis Department,

Institute of Natural Product Chemistry Vietnam Academy of Science and Technology, using Agilent 1200 and Agilent 1260 systems with UV-VIS detectors.

Mass spectrometry (MS): EI-MS spectra were measured on a Bruker Dailtonics APEX II 30eV spectrometer, and HR-MS spectra were recorded in solvents using an Agilent Technologies Accurate-Mass 6530 Q-TOF LC/MS at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

Nuclear Magnetic Resonance (NMR): One-way (^{1}H-NMR, ^{13}C-NMR, DEPT) and two-dimensional (HMBC, HSQC …) from Bruker 500 MHz at the Center for Applied Spectral Methodologies, Institute of Chemistry, Vietnam Academy of Science and Technology, using appropriate solvents containing internal standard TMS.

Melting point: Melting point was measured on a Yanagimoto MP-S3 Instrument at the Analytical Chemistry department, Institute of Natural Product Chemistry.

2.3. The extract method

Fresh plant specimens were obtained and dried in a cool place before being dried at temperatures ranging from 50 to 60^{o}C until crisp and dry. To collect the complete residue, the dry sample was crushed, extracted with methanol, and distilled under decreased pressure. This whole extract was extracted using hexane, ethyl acetate, and methanol respectively.

Table 1. The weight of *Sarcandra glabra* **extracts.**

Sample collection in June 2020	Dry sample weight (g)	Extraction residue mass (g)		
		***n*-Hexan (g)**	**EtOAc (g)**	**MeOH (g)**
Leaf stem	1700	20.0 (**SGH**)	90.0 (**SGE**)	200.2 (**SGW**)

3. Results and discussion

3.1. Isolation of n-hexane extract (SgH)

Take 10.0 g of the *n*-hexane extract, separate it on a silica gel column, and then elute the column using an ethyl acetate-hexane (0-100%) solvent system. Color the solution using 5% vanillin-H_2SO_4 reagent.

*** Stigmast-5,22-dien-3-β-ol (1)**

Elute the column with the solvent system *n*-hexane-ethyl acetate (30:1), produce an amorphous solid mass, repeat separation on a silica gel column and recrystallize in *n*-hexane to yield needle-shaped crystals, colorless, mass 21 mg, R_f = 0.64, melting temperature 155-157 ^{o}C. ^{13}C-NMR (125 MHz, $CDCl_3$); δ (ppm): 36.5 (C-1); 29.21 (C-2); 71.81 (C-3); 42.32 (C-4); 140.78 (C-5); 121.70 (C-6); 37.28 (C-7); 31.93 (C-8); 51.24 (C-9); 36.52 (C-10); 24.36 (C-11); 42.32 (C-12); 31.68 (C-13); 56.79 (C-14); 26.15 (C-15); 31.57 (C-16); 56.10 (C-17); 12.05 (C-18); 19.38 (C-19); 40.45 (C-20); 21.05 (C-21); 138.29 (C-22); 129.32 (C-23); 50.17 (C-24); 33.98 (C-25); 21.09 (C-26), 19.80 (C-27); 29.21 (C-28); 12.22 (C-29).

*** 2′,6′-dihydroxy-3′,4′ dimetoxychalcon (2)**

Alter the solvent system to elute the column in a 20:1 ratio of *n*-hexane-ethyl acetate to acquire a red solid mass, then re-purify on a silica gel column to yield a red solid. Recrystallize in *n*-hexane to obtain 58 mg of red needle crystal, melting at 121-123^{o}C. ^{1}H-NMR (500MHz, $CDCl_3$, TMS, δ ppm): 7.61 (2H, dd, J = 2 Hz, H-2, H-6); 7.59 (2H, m, H-3, H-5); 7.39 (1H, m, H-4); 6.06 (1H, s, H-5′); 7.90 (1H, d, J = 15.5 Hz, H-α); 7.80 (1H, d, J = 16.0 Hz, H-β); 3.93 (3H, s, 3′-O**C**$\mathbf{H_3}$); 3.90 (3H, s, 4′-O**C**$\mathbf{H_3}$); 14.35 (2′-**OH**). ^{13}C-NMR (125MHz, $CDCl_3$, TMS, δ ppm): 135.52 (C-1); 128.88 (C-2, C-6); 127.49 (C-3, C-5); 130.12 (C-4); 106.51 (C-1′); 155.38 (C-2′); 128.55 (C-3′); 159.08 (C-4′); 89.92 (C-5′); 158.85 (C-6′); 128.38 (C-α); 142.56 (C-β); 193.30 (C-β′); 60.86 (3′-O**C**$\mathbf{H_3}$); 56.01 (4′-O**C**$\mathbf{H_3}$).

*** 5-hydroxy-6,7-dimetoxyflavanon (3)**:

A pale yellow amorphous crystalline mass was formed in the *n*-hexane/etylacetate column elution solvent system (5:1) and purified twice on the silica gel column to get a needle-shaped crystal. Recrystallized in the *n*-hexane/etyl axetat solvent system, 47 mg were obtained, melting at 159.2-161.6 ^{o}C. HR/ESI-MS (*m/z*): $[M+H]^+$ 301,10299.

^{1}H-NMR (500MHz, $CDCl_3$, TMS, δ ppm): 5.476/5.450 (2H, d, J = 13 Hz, H-2); 2.831/2.825; 2.865/2.859; 3.005/2.979; 3.038/3.013 (2H, dd, dd, dd, 3Hz, 3Hz, 13Hz, H-3a, H-3b); 6.20 (1H, s, H-8); 7.47 (2H, H-2′ và H-6′); 7.44 (2H, H-3′ và H-5′); 7.46

(1H, m, H-4′); 3.874 (3H, s, 6-OC**H**$_3$); 3.868 (3H, s, 7-OC**H**$_3$).

^{13}C-NMR (125MHz, $CDCl_3$, TMS, δ ppm): 79.5 (C-2); 45.7 (C-3); 189.1 (C-4); 155.6 (C-5); 128.8 (C-6); 158.2 (C-7); 92.4 (C-8); 155.5 (C-9); 106.0 (C-10); 138.8 (C-1′); 125.9 (C-2′ và C-6′); 128.8 (C-3′ và C-5′); 128.6 (C-4′); 61.4 (6-O**C**H$_3$); 56.2 (7-O**C**H$_3$).

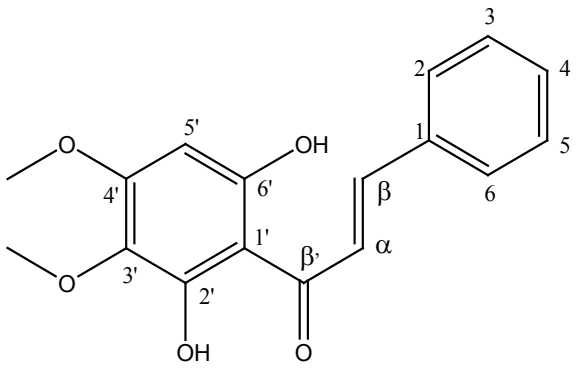
3.2. Identify the structures of the separated chemicals

3.2.1. 2',6'-dihydroxy-3',4' dimetoxychalcon

This compound, obtained in the low polar fraction of the *n*-hexane extract, is a 58 mg red-orange needle-shaped crystalline substance.

The ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra demonstrate that there are 17 carbons in the **2** molecule. There are two methyl groups belonging to methoxy radicals (MeO) at $\delta_C/_H$ 60.86/3.93 and $\delta_C/_H$ 56.01/3.90; seven quaternary carbons at C 106.51; 128.55; 135.52; 155.38; 158.85; 159.08 and 193.30; and eight carbon methane (CH) at C 89.92; 127.49; 128.38; 128.88; 130. The protons of the metin groups at H range from 6.06 to 7.90 in the ^{1}H-NMR spectrum, and the protons of the OH group are on the olefin carbon conjugated to the carbonyl group (CO).

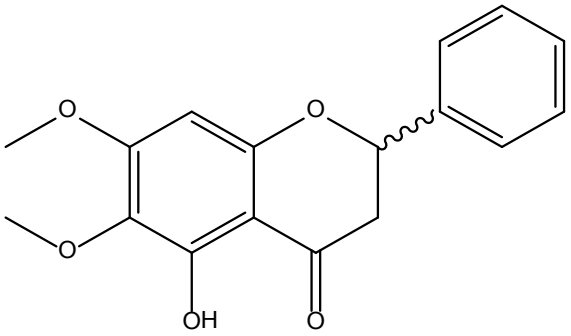
The spectral data of compound **2** are compatible with the spectral data of 2',6'-dihydroxy-3',4'-dimetoxychalcon in the literature and the study of distant interactions in the HMBC spectrum permits confirmation [21]. A chalcon is **2**: 2',6'-dihydroxy-3',4'-dimetoxychalcon



3.2.2. 5-hydroxy-6,7-dimetoxyflavanon (3)

Compound **3** was also isolated as yellow crystals from the low polar portion of the *n*-hexane extract and the *Sarcandra glabra* ethyl acetate extract (47 mg), melting at 159.2-161.6°C. It includes 17 C, of which 7 C is quaternary at δ_C: 106.0; 128.8; 138.8; 155.5; 155.6; 158.2; and 189.1; 07 groups of metin (CH) at C: 79.5; 92.4; 125.9 (2C); 128.6; and 128.8 (2C); one methylene group (CH_2) with δ_C 45.7 and two methoxy groups at δ_C 61.4; 56.2.

Through analyzing the spectral **3** as above combined with the comparison of spectral data of 5-hydroxy-6,7-dimetoxyflavanon in the literature, we can confirm that 3 is a 5-hydroxy-6,7-dimetoxyflavanon [22].



4. Conclusion

The following are the findings of phytochemical investigation on *Sacandra glabra* in Thai Nguyen: *Sacandra glabra* were extracted using column chromatography and thin layer chromatography from *n*-hexane and ethyl acetate. 03 compounds have been isolated and identified. The structures of four compounds have been determined using NMR spectroscopy methods: one compound, stigmast-5,22-dien-3-ol (**1**), and the remaining two compounds: 2',6'-dihydroxy-3',4'-dimetoxychalcon (**2**) and 5-hydroxy-6,7-dimetoxyflavonone (**3**)

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