TẠP CHÍ KHOA HỌC ĐẠI HỌC TÂN TRÀO

ISSN: 2354 - 1431

http://tckh.daihoctantrao.edu.vn/

STUDY FOR THE CHEMICAL COMPOSITION OF *CANAVALIA CATHARTICA* COLLECTED IN CAN GIO DISTRICT- HO CHI MINH CITY

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https://doi.org/10.51453/2354-1431/2024/1251

Article info

Received:19/5/2024

Revised: 23/7/2024

Accepted: 25/8/2024

Keywords:

Canavalia cathartica, β-sitosterol, daucosterol, ursolic acid.

Abstract:

Canavalia cathartica, commonly known as maunaloa is a species of flowering plant in the legume family, Fabaceae. Leaves and branches of *Canavalia cathartica* were collected in Can Gio, Ho Chi Minh city. From dichlomethane extract of the aerial parts of *Canavalia cathartica* Du Petit-Thouars. led to the isolation and structural elucidation of three compounds including ursolic acid **1**, β-sitosterol **2**, daucosterol **3**. Their structures were elucidated by the analysis of the IR, MS and NMR 1D and 2D spectra.

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NGHIÊN CỨU THÀNH PHẦN HOÁ HỌC LOÀI ĐẬU CỘ BIỂN (*CANAVALIA CATHARTICA)* THU HÁI Ở CẦN GIỜ - THÀNH PHỐ HỒ CHÍ MINH

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https://doi.org/10.51453/2354-1431/2024/1251

Thông tin bài viết

Ngày nhận bài: 19/5/2024

Ngày sửa bài: 23/7/2024

Ngày duyệt đăng: 25/8/2024

Từ khóa:

Canavalia cathartica , β-sitosterol, daucosterol, ursolic acid.

Tóm tắt

Lá và cành loài Đậu Cộ biển (*Canavalia cathartica* Thouars.), thu hái tại Cần Giờ, Thành phố Hồ Chí Minh, Việt Nam, đã được tiến hành nghiên cứu hóa học thực vật. Từ nghiên cứu này, hai sterol β-sitosterol **và** daucosterol, một triterpen ursolic acid đã được phân lập thành công. Cấu trúc của các hợp chất này được xác định bằng phổ cộng hưởng từ hạt nhân NMR, phổ hồng ngoại IR và phổ khối ion hóa phun điện tử ESI-MS. Nghiên cứu đã cung cấp những phát hiện mới về hóa học thực vật của loài *Canavalia cathartica*, đồng thời mở rộng hiểu biết về các thành phần hóa học của loài này, góp phần hiểu rõ, định hướng nghiên cứu sâu hơn về hoạt tính sinh học và ứng dụng của chúng.

1. INTRODUCTION

Canavalia cathartica, commonly known as maunaloa is a species of flowering plant in the legume family, Fabaceae. It has a paleotropical distribution, occurring throughout tropical regions in Asia, Africa, Australia and many Pacific Islands. *Canavalia cathartica* is a biennial or perennial herb with thick, twining, climbing stems. The seeds and pods are used as famine foods in coastal India. It's considered to be an underutilized wild plant with the potential to serve as a protein and carbohydrate rich food crop. When compared to edible legumes, it has rich protein content. It grows rapidly, tolerates challenging habitat types such as sandy, saline soils, etc. *Canavalia cathartica* contains anti-nutrients and requires some processing before using it as a food. Farmers use *Canavalia cathartica* as green manure and mulch and host it in their fields for fixation of nitrogen [3,4]. In 2018, the research team of Saraswathi K and his colleagues announced that the species of Seaweed has an effective effect against free radicals.

In Vietnam, *Canavalia cathartica* is a wild species commonly found in coastal areas from Quang Ninh to Ca Mau. Research on chemical composition has not been studied much so far. From dichlomethane extract of the aerial parts of *Canavalia cathartica* Du Petit-Thouars. led to the isolation and structural elucidation of three compounds including ursolic acid **1**, β-sitosterol **2**, daucosterol **3.**

2. EXPERIMENTAL

2.1. General experimental procedures

NMR experiments were performed on Bruker 500 MHz spectrometers using TMS as an internal standard. ESI-MS data were measured on an Agilent 1100-LC/MSDTrapSL mass spectrometer. IR spectrum (KBr) was recorded on an IMPACT-410 machine from Nicolet. TLC was conducted using Silica gel 60 F254 (0.25mm, Merck). Column chromatography involved the use of Silica gel 60 (230-400 mesh, Merck) for the first column, and silica gel 60, 40-63 μm (Merck) and Sephadex LH20 for the subsequent columns. Preparative HPLC was carried out on a Waters 600 apparatus with an ODS column (C18, 250 × 20 mm; Inertsil Pak). TLC spots were visualized under UV light and by spraying with 5% H_2SO_4 in alcohol followed by heating.

2.2. Plant material

Leaves and branches of *Canavalia cathartica* were collected in Can Gio district in 2013 by Botanist Ngo Van Trai, Institute of Medicinal Materials, and identified the scientific name as *Canavalia cathartica* Du Petit-Thouars., belonging to the Fabaceae family. Specimens are kept at the Organic Synthesis Department, Institute of Chemistry - Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The powdered leaves and branches of *Canavalia cathartica* sample (1.2 kg) was subjected to extraction to isolate pure compounds. Triplicate extractions were performed using 70% ethanol (EtOH) over three days. The combined ethanolic extracts were concentrated under reduced pressure, yielding 176 g of crude extract. This extract was partitioned between *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol after the addition of water. Each fraction was concentrated under reduced pressure at 45°C to obtain respective residues.

The CH_2Cl_2 fraction (29.0 g) was further processed using silica gel column chromatography (CC) with a solvent gradient (*n*-hexane:EtOAc, from 95:5 to 1:9), yielding 10 fractions (F1-F10). Fraction 2 appeared as a crystalline solid, washed several times with cold MeOH to obtain 30 mg of compound **2**. Fraction 5 (710 mg) was separated by sephadex LH-20 column chromatography with an elution solvent system of *n*-hexane:CH_2Cl_2:MeOH=1:1:2. To evaporate part of the solvent, a crystalline solid appears. Washing the solid several times with cold methanol and cold dichlomethane yielded 10 mg of compound **1**. Fraction 9 appeared as a crystalline solid, washing several times with cold MeOH yielded 20 mg of compound **3**.

The chemical structure of the compounds was determined based on physical parameters, mass spectrometry data, combined nuclear magnetic resonance spectroscopy and comparison with published documents.

Ursolic acid (1). White powder, IR (KBr, υ cm^{-1}): 3414 cm^{-1} (-OH acid), 2928 cm^{-1} (-CH alkan), 1692 cm^{-1} (C=O acid). ESI-MS *m/z*: 479.23 (90%) $[M+Na]^+$, 455.27 (100%) $[M-H]^-$, công thức phân tử $C_{30}H_{48}O_3$. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm), *J* (Hz): Table 1

β-*sito sterol* (2). White needle-shaped crystals. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 5.38-5.36 (1H, m); 3.56-3.52 (1H, m); 2.33-22.5 (2H, m); 2.05-1.97 (2H, m); 1.89-1.82 (3H, m); 1.70-1.65 (2H, m);

1.54-1.11 (24H, m); 1.07 (3H, s); 1.00 (3H, d, J = 6.7); 0,87 (3H, t, J = 7.1); 0.86 (6H, br s); 0.70 (3H, s).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ (ppm): 140.79; 121.72; 71.82; 56.80; 56.10; 50.17; 45.88; 42.35; 42.33; 39.81; 37.28; 36.53; 36.16; 33.98; 31.93; 31.69; 29.20; 28.26; 26.14; 24.32; 23.10; 21.11; 19.82; 19.41; 19.06; 18.80; 12.00; 11.87.

Daucosterol (3). White powder. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm), J (Hz): 5.32 (1H, br s); 4.39 (1H, t, J = 5.7); 4.83 (3H, m); 4.22 (1H, d, J = 7.8); 3.64 (1H, dd, J = 5.5; 10.1); 3.38-3.48 (2H, m); 3.10-3.14 (2H, m); 3.05-3.08 (2H, m); 2.87-2.91 (1H, m); 2.34-2.38 (1H, m); 2.10-2.17 (1H, m); 1.90-1.97 (3H, m); 1.62-1.49 (1H, m); 1.43-1.49 (4H, m); 1.38-1.41 (5H, m); 1.23 (3H, s, H-19); 1.00 (3H, d, J = 6.7); 0.96 (6H, br s); 0.90 (3H, d, J = 6.5); 0.81 (3H, d, J = 6.8); 0.80 (3H, d, J = 6.9); 0.65 (3H, s).

^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 140.4; 121.1; 100.7; 76.9; 76.7; 76.6; 73.4; 70.0; 61.0; 56.1; 55.4; 49.5; 45.1; 41.8; 38.2; 36.7; 36.1; 35.4; 33.3; 31.3; 31.3; 28,6; 27.7; 25.4; 23.8; 22.5; 20.5; 19.6; 19.0; 18.9; 18.5; 11.7; 11.6.

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. ESI-MS mass spectrum shows pseudomolecular ion peaks at m/z 479.23 $[M+Na]^+$ (positive ion) and 455.27 $[M-H]^-$ (negative ion), combined with NMR spectrum data to allow determine the molecular formula as $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum of compound **1** shows the signal of an olefin proton at δ_H 5.12 (1H, br s), a hydroxymethine group at δ_H 3.01-2.99 (1H, m), seven methyl groups covering consisting of five tertiary methyl groups at δ_H 0.67; 0.75; 0.86; 0.89; 1.04 (3H, s) and two secondary methyl groups at δ_H 0.81 (3H, d, J = 6.5 Hz); 0.90 (3H, d, J = 6.5 Hz) and a proton of the hydroxyl group at δ_H 4.30 (1H, d, J = 4.5 Hz, 3-OH). The ^{13}C-NMR and DEPT spectra show the presence of 30 carbons in the molecule including one carbon of the acid group at δ_C 178.27, one hydroxymethine carbon at δ_C 76.85, two olefin carbons >C=CH- at δ_C 138.19 and 124.58, seven methyl carbons, nine methylene carbons, five methine carbons and five quaternary carbons. From the above spectral analysis data, it shows that compound **1** is a ursane frame triterpenoid. Comparing the ^{1}H-, ^{13}C-NMR spectrum data of **1** with ursolic acid in reference [8,9] found complete agreement. Therefore, the structure of **1** is confirmed to be ursolic acid, a common compound in the plant kingdom that exhibits a diverse spectrum of activities such as antibacterial, antifungal, anti-inflammatory, hepatoprotective, and anti-inflammatory properties. cancer and tumors... [10].

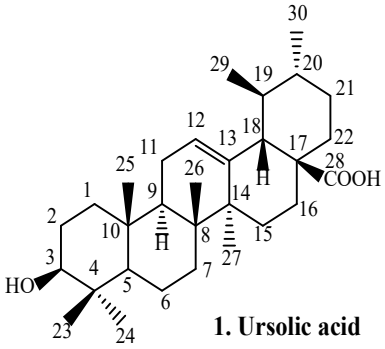
Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compound 1

Vị trí	Compound 1 (DMSO-d_6)		Ursolic acid [8] (DMSO-d_6)	
	δ_H (ppm, J/Hz)	δ_C/ppm	δ_H (ppm, J/Hz)	δ_C/ppm
1		38.24		38.2
2		26.98		26.98
3	3.01-2.99 (1H, m)	76.85	3.01-2.99 (1H, m)	76.85
4		38.37		38.3
5		54.79		54.7
6		18.00		18.0
7		30.19		30.1
8		39.10		39.10
9		47.02		47.02
10		36.53		36.53
11		23.81		23.81
12	5.12 (1H, br s)	124.58	5.12 (1H, br s)	124.58
13		138.19		138.19
14		41.64		41.64
15		32.71		32.71
16		22.85		22.85
17		46.83		46.83
18	2.10 (1H, d, J = 11.5 Hz)	52.38	2.10 (1H, d, J = 11.5 Hz)	52.38
19		38.44		38.44
20		38.50		38.50

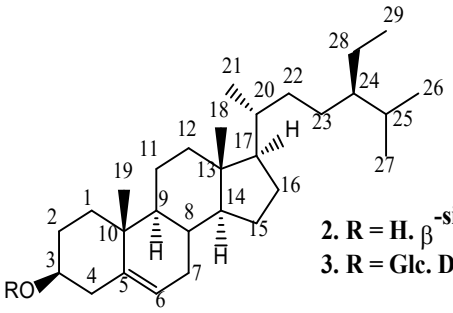
Vị trí	Compound 1 (DMSO-d_6)		Ursolic acid [8] (DMSO-d_6)	
	δ_H (ppm, *J*/Hz)	δ_C/ppm	δ_H (ppm, *J*/Hz)	δ_C/ppm
21		27.54		27.54
22		36.32		36.32
23	0.89 (3H, s)	28.26	0.89 (3H, s)	28.26
24	0.67 (3H, s)	16.91	0.67 (3H, s)	16.91
25	0.86 (3H, s)	16.07	0.86 (3H, s)	16.07
26	0.75 (3H, s)	15.22	0.75 (3H, s)	15.22
27	1.04 (3H, s)	23.27	1.04 (3H, s)	23.27
28		178.27		178.27
29	0.81 (3H, d, *J* = 6.5 Hz)	17.01	0.81 (3H, d, *J* = 6.5 Hz)	17.01
30	0.90 (3H, d, *J* = 9.5 Hz)	21.07	0.90 (3H, d, *J* = 9.5 Hz)	21.07
3-O<u>H</u>	4.30 (1H, d, *J* = 4.5 Hz)		4.30-4.29 (1H, m)	
19-O<u>H</u>				
28-O<u>H</u>	11.88 (1H, s)			

Compound **2** was isolated as white needle-shaped crystals. The ^{1}H-NMR spectrum shows signals of two singlet methyl groups at δH 1.07 (3H, s) and 0.70 (3H, s), one triplet methyl group at δ_H 0.87 (3H, t, *J* = 7 ,1 Hz) along with three other methyl groups at δ_H 1.00 (3H, d, *J* = 6.7 Hz) and 0.86 (6H, br s). In addition, the ^{1}H-NMR spectrum also shows the signal of an olefin proton at δ_H 5.38-5.36 (1H, m) and an oxygen-bearing methine signal at δ_H 3.56-3.52 (1H, m). The signals of the remaining protons overlap in the range δ_H 2.33-1.11. The ^{13}C-NMR spectrum showed signals of two olefin carbons (δ_C 140.79; 121.72) and one oxymethine group at δ_C 71.82. Signals of six methyl groups appeared at δ_C 19.82; 19.41; 19.06; 18.80; 12.00; 11.87. The remaining signals of six methine groups and nine methylene groups are in the range δ_C 56.80-21.11. Comparing the NMR spectrum data with reference [11], it was determined that this is β-sitosterol with the molecular formula $C_{29}H_{50}O$. This is a sterol that exists quite commonly in plants. This compound has high biological activity, has anti-cancer, anti-inflammatory, anti-tussive, heart-protective, anti-cancer, anti-arthritic effects, etc.

Compound **3** was isolated as a white powder. The ^{1}H-NMR spectrum shows signals of six methyl groups at δ_H 0.65 (3H, s); 0.80 (3H, d, *J* = 6.9 Hz); 0.81 (3H, d, *J* = 6.8 Hz); 0.90 (3H, d, *J* = 6.5 Hz); 0.82 (3H, d, *J* = 7.0 Hz) and 0.84 (3H, d, *J* = 6.5 Hz), a signal of an olefin proton appears at δ_H 5.37 (1H, dd, *J* = 3.0; 3.0 Hz), signal of an oxymethine at δ_H 3.57 (1H, m), a proton β-anomer at δ_H 4.41 (1H, d, *J* = 7.5 Hz) , the signal of an oxymethylene at δ_H 3.84 (1H, dd, *J* = 12.0; 3.0 Hz) and 3.76 (1H, dd, *J* = 12.0; 4.5 Hz) this predicts predict the presence of a monosaccharide. The ^{13}C-NMR spectrum shows the presence of 35 carbons, including 29 carbons of aglycon and 6 carbons of a sugar. The ^{13}C-NMR spectrum confirmed the signal of olefin bond at δ_C 140.2 (C-5) and 122.1 (C-6); signal of six methyl groups at δ_C 11.8 (C-18); 19.6 (C-19); 19.1 (C-21); 18.9 (C-26); 18.6 (C-27) and 11.7 (C-29) along with a signal of an oxymethine group of the aglycon at δ_C 70.1 (C-3). In addition, the spectrum has signals of a carbon anomer at δ_C 101.0 (C-1'), an oxymethylene at δ_C 61.8 (C-6') along with four oxymethine groups in the sugar region at δ_C 75.6 (C -2'); 76.3 (C-3'); 73.4 (C-4') and 79.1 (C-5'), combining ^{1}H-NMR spectral data with the interaction constant $J_{H\text{-}1'/H\text{-}2'}$ = 7.5 Hz confirms the presence of β-glucopyranose sugar. Comparing the NMR spectral data with the compound daucosterol, the results were completely consistent.



1. Ursolic acid



2. R = H. β-sitosterol

3. R = Glc. Daucosterol

4. CONCLUSION

This study marks the first phytochemical investigation of the leaves and branches of *Canavalia cathartica* collected from Can Gio district, Ho Chi Minh city, Vietnam. Through this research. From the dichlomethane extract of *Canavalia cathartica*, we have isolated and determined the chemical structure of three compounds ursolic acid, β-sito sterol, and daucosterol. The structures of these compounds were elucidated using modern spectroscopic techniques, including IR, ESI-MS and NMR. A literature search shows that all three of these substances were isolated for the first time from this species.

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