Inhibits Diabetes from *Plectranthus amboinicus* (Lour.)

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Article Info
ISSN (online): 3049-0421
Volume: 02
Issue: 02
March-April2025
Received: 22-02-2025
Accepted: 20-03-2025
Page No: 43-47

Abstract
Objective: To investigate the chemical composition of *Plectranthus amboinicus* (Lour.) collected from Dak Lak Province using combined chromatography, which has isolated two compounds.
Subject and Research Methods: Investigation of the chemical composition of *Plectranthus amboinicus* (Lour.) collected from Dak Lak Province.
Research Methods: Combined use of modern spectroscopic methods (Mass Spectrometry (EI-MS), Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR)).
Results and Discussion: The chemical composition of *Plectranthus amboinicus* (Lour.) collected from Dak Lak Province was investigated. Using combined chromatography, two compounds were isolated. Modern spectroscopic methods (Mass Spectrometry (EI-MS), Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR)) were used to determine the structures of the two compounds, which were identified as β-sitosterol and Stigmasterol. These two compounds are commonly found in various plant species and have also been isolated for the first time in studies on *Plectranthus amboinicus*. Compound 1 exhibited the ability to inhibit α-amylase and α-glucosidase enzymes with IC_{50} values of 1375.50±92.45 and 966.31±80.01 µg/ml, respectively; compound 2 showed inhibitory activity on α-amylase and α-glucosidase enzymes with IC_{50} values of 1205.93±100.24 and 1025,24±67,52 µg/ml, respectively.

DOI: https://doi.org/10.54660/IJPGRR.2025.2.2.43-47

Keywords: *Plectranthus amboinicus*, α-amylase, α-glucosidase, *β-sitosterol, stigmasterol*

Introduction

Plectranthus amboinicus (Lour.) Spreng, commonly known as the Cuban oregano or Indian borage, belongs to the Lamiaceae family, which consists of 202 genera and about 3500 species [1], mainly distributed in tropical regions. In Vietnam, there are over 41 genera and approximately 146 species, and this plant is widely cultivated [2-3]. In Vietnam, it is also known by other names such as "rau tần dày lá" or "rau thơm lùn". For a long time, the leaves of this plant have been used as a seasoning in food preparation, offering a spicy taste, aromatic smell, and non-toxicity. In folk medicine, the leaves are used to treat conditions such as cough, fever, diarrhea, as a disinfectant, or applied to insect bites. Apart from these uses, *Plectranthus amboinicus* is one of the plants that contains essential oils. In Vietnam, there have been several reports on the chemical composition of its essential oils [4-6].

Studies on *Plectranthus amboinicus* in Cu Chi by authors Lu Thi Mong My and Nguyen Thi Bich Thuyen, as well as research in Can Tho on its chemical composition and antimicrobial activity, have been conducted. Other international researchers have also shown interest in studies on *Plectranthus amboinicus*. These studies have partly explained why medicinal formulations made from this plant have been used for a long time [7-12]. However, the number of health-related products derived from *Plectranthus amboinicus* is still limited, and there has been little attention or research into developing them for widespread use.

Research History

As of now, there have been no studies conducted on the isolation and structural identification of compounds from *Plectranthus amboinicus* in Vietnam, especially in Dak Lak, Vienames province. Therefore, with the goal of identifying the differences and adding to the data on *Plectranthus amboinicus*, we conducted this study to determine the chemical composition from the extract of this plant, contributing to the effective utilization and exploitation of this species in the region.

Research Methods

- **Study Location:** *Plectranthus amboinicus* was collected in Dak Lak province.
- **Study Subject:** The plant was identified by Dr. Truong Ba Phong, Department of Biology, Faculty of Natural Sciences & Technology, Tay Nguyen University.

Research Methods

- **Sampling Method:** The plant samples were collected at an appropriate time during the year. Fresh samples were cleaned, left in a well-ventilated area, or dried at 40°C. The samples were then processed using a selective extraction method with suitable solvents, resulting in a mixture of compounds for the research, as outlined in the experimental section.
- **Methods for analyzing, separating mixtures, and isolating compounds:** To analyze, separate, and isolate compounds, the following chromatography methods were used:
  - **Column Chromatography:** Using silica gel with particle size of 230-400 mesh.
  - **Thin Layer Chromatography:** Analysis was performed on pre-coated silica gel Merck 60 F_{254} glass plates with a thickness of 0.2 mm.
  - **Visualization:** Using iodine vapor and UV light at 254 nm and 365 nm wavelengths.
  - **Chemicals:** The solvents for extracting plant samples were of analytical-grade purity, with high-purity solvents used for thin layer chromatography and column chromatography. The solvents used included methanol, methyl chloride, ethyl acetate, and distilled water.

Isolation of Compounds: The aerial parts of *Plectranthus amboinicus* (Lour.) weighing 3.0 kg, collected from Dak Lak, in September 2022, were chopped and air-dried at room temperature. The dried plant material was then extracted with methanol for 15 days. The solvent was evaporated to obtain a methanol extract residue (568 g). The methanol extract was partitioned with water and then successively extracted with n-hexane, ethyl acetate, and butanol. The corresponding extracts were collected after solvent evaporation, yielding 31 g, 213 g, and 157 g of respective fractions.

α-Amylase and α-Glucosidase Inhibition Activity

α-Amylase Inhibition: The α-amylase inhibition activity of β-sitosterol (1) and stigmasterol (2) was measured according to the method of Dao Thi Xuan Trang and colleagues with modifications [17]. The procedure involved incubating a mixture of 50 µl phosphate buffer solution (pH 7), 50 µl of the pure compound, and 50 µl α-amylase enzyme solution (3U) at 37°C for 5 minutes. Then, 50 µl starch solution was added, and the mixture was incubated for another 15 minutes. Afterward, 200 µl concentrated HCl was added to stop the reaction, and 300 µl iodine reagent was added to detect the remaining starch after the reaction. The absorbance of the starch-iodine complex was measured at 660 nm. Acarbose was used as a positive control.

α-Glucosidase Inhibition: The α-glucosidase inhibition activity of β-sitosterol (1) and stigmasterol (2) was also evaluated using the method of Đái Thị Xuân Trang and colleagues [17]. The procedure involved incubating a mixture of 100 µl phosphate buffer solution (100 mM, pH 6.8), 20 µl α-glucosidase enzyme (1U), and 40 µl of the pure compound at 37°C for 15 minutes. Then, 40 µl of p-nitro-phenyl-α-D-glucopyranoside (5 mM) was added, and the mixture was incubated for an additional 20 minutes. Finally, 100 µl Na_2CO_3 (0.1 M) was added to stop the reaction. The absorbance of the released p-nitrophenol was measured at 405 nm.

Ethics in Research

The study was conducted with the approval of local healthcare facilities, authorities at various levels, and the participants involved in the research. The study ensured the confidentiality of information and respected the voluntary participation of the subjects. In addition to data collection, participants received free testing and treatment consultation. The research followed the approval process of the Ethics Committee of Tay Nguyen University.

Results

Isolation and structural identification of compounds

The ethyl acetate extract was separated using column chromatography with silica gel, employing a gradient solvent system of chloroform/ethyl acetate, increasing the concentration of ethyl acetate at the following ratios: 0% to 100%. This process resulted in the isolation of 8 fractions.

Fragmentation and characterization of compounds

1. Fraction 1 yielded compound 1 (80 mg), while Fraction 4 was further separated on a smaller column using a solvent system of n-hexane/acetone in ratios of 11:1, 5:1, and 44:1, resulting in the isolation of compound 2 (55 mg).
2. **Compound 1**: Crystalline needles, melting point: 136-138°C.
   - **EI-MS**: m/z 414 [M]+ (20), 413 (41), 398 (28), 397 (100), 395 (32), 383 (11), 361 (11), 257 (3), 255 (6.3), 151 (5.6), 139 (11).
   - **^{1}H-NMR (500 MHz, $CDCl_3$)** (δ ppm): 5.31 (1H, m, H-6); 3.51 (1H, m, H-3); 1.01 (3H, s, 19-CH3); 0.92 (3H, d, J = 6.2 Hz, 21-CH3); 0.84 (3H, d, J = 7.0 Hz, 29-CH3); 0.83 (3H, d, J = 6.5 Hz, H-26); 0.81 (3H, d, J = 6.5 Hz, 27-CH3); 0.68 (3H, s, 18-CH3).
   - **^{13}C-NMR (125 MHz, $CDCl_3$)** (δ ppm): 140.8 (C-5); 121.7 (C-6); 71.8 (C-3); 56.8 (C-14); 56.1 (C-17); 50.2 (C-9); 45.9 (C-24); 42.3 (C-4, C-13); 39.8 (C-12); 37.3 (C-1); 36.5 (C-10); 36.2 (C-20); 33.9 (C-22); 31.9 (C-7); 31.7 (C-8); 29.7 (C-2); 29.2 (C-25); 28.3 (C-16); 26.1 (C-23); 24.3 (C-15); 23.1 (C-28); 21.1 (C-11); 19.8 (C-19); 19.4 (C-27); 19.1 (C-26); 18.8 (C-21); 11.9 (C-18, C-29). *(Table 3.1)*.

Compound 2: Crystalline needles, melting point: 154°C -

156°C.

- **EI-MS**: m/z 412 [M]+ (7), 300 (7), 255 (11), 231 (4), 213 (8), 173 (7), 145 (20), 133 (20), 83 (49.3), 55 (100), 43 (90).
- **^{1}H-NMR (500 MHz, $CDCl_3$)** (δ ppm); **^{13}C-NMR (125 MHz, $CDCl^3$)** (δ ppm). *(Table 3.2)*.

Structural Elucidation of Compound (1)

In the EI-MS spectrum, the molecular ion peak at m/z 414 $[M]^+$ corresponds to the molecular formula of compound (1), which is $C_{29}H_{50}O$. This molecular formula was further supported by the detailed analysis of the MS and NMR spectra, which provided information about the structural features of the compound.

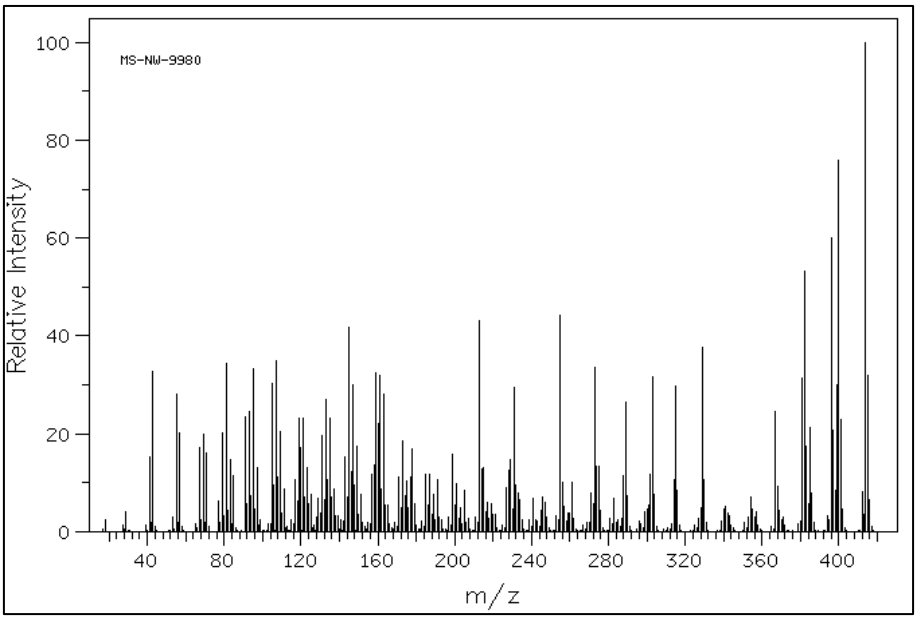

Fig 1: Collision-intensity mass spectrum (EI-MS) of compound (1)

Analysis of the 1H-NMR and 13C-NMR spectra combined with the DEPT spectrum of compound (1) reveals the following proton signals: at δH 3.51 ppm (H-3), which is the proton attached to C-3 (δC 71.4 ppm); the signal at δH 5.31 ppm corresponds to the proton attached to C-6 (δC 120.3 ppm) at the C=C double bond. Additionally, signals for 6 methyl groups are observed at the following positions: δ_H 0.67, 0.86, 0.90, 1.00, 1.13, and 1.18. Based on the spectral data obtained and compared with the literature [12], we can confirm that compound (1) is β-sitosterol. This compound is very common in plants. Previous studies on in vitro and in vivo models have demonstrated that β-sitosterol has anti-inflammatory properties, promotes angiogenesis, and exhibits hypoglycemic effects [13-16].

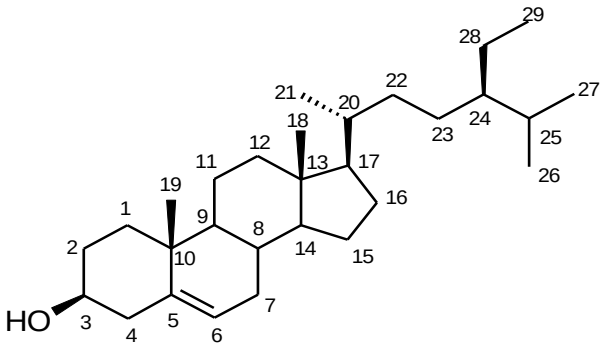

Fig 2: Chemical Structure of Compound (1) (β-sitosterol)

Interpretation of the Structural Identification of Compound (2)

Upon visual inspection, compound (2) is observed to be a colorless needle-shaped crystal with a melting point ranging from 154°C to 156°C. The ^{1}H-NMR and 13C-NMR spectra of compound (2) reveal the presence of hydroxyl groups attached to C-3, corresponding to the signals δ_{Ha}-3 3.28 ppm and δ_C-3 71.77 ppm.

Additionally, signals for 3 protons from the methylene group of 2 conjugated double bonds are observed, with characteristic signals at δ_H-6 5.33 ppm, δ_H-22 5.13 ppm, and δH-23 5.02 ppm.

On the other hand, the EI-MS spectrum of compound (2) also shows the molecular ion peak m/z 412 $[M]^+$, which is consistent with the molecular formula $C_{29}H_{48}O$. Comparing the spectral data of compound (2) with reference materials and combining it with the analysis of the obtained spectra, we find that it perfectly matches the compound stigmasterol.[18] Stigmasterol exhibits various biological activities such as anti-inflammatory, anti-cancer, and anti-diabetic properties. These compounds may have pharmaceutical applications or serve as precursors for synthesizing new biologically active compounds [19-20].

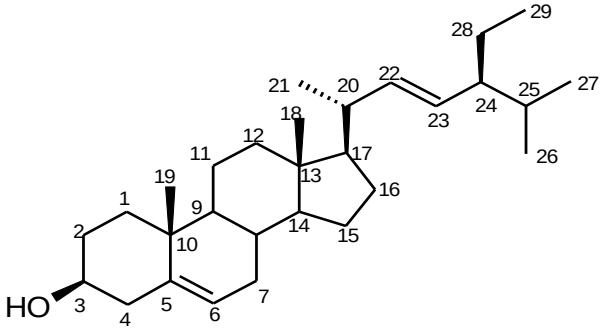

Fig 3: Chemical Structure of Compound 2 (Stigmasterol)

Results of α-amylase and α-glucosidase Inhibition Activity Evaluation

The results of the inhibition activity tests for α-amylase and α-glucosidase enzymes, which are related to diabetes treatment, of the two compounds β-sitosterol and stigmasterol, isolated from the plant *Plectranthus amboinicus*, are shown in Table 1. The results indicate that compound 1 exhibited the ability to inhibit α-amylase and α-glucosidase enzymes, with IC_{50} values of 1375.50±92.45 µg/ml and 966.31±80.01 µg/ml, respectively. Compound 2 exhibited inhibition of α-amylase and α-glucosidase enzymes, with IC_{50} values of 1205.93±100.24 µg/ml and 1025.24±67.52 µg/ml, respectively. The IC_{50} values show that both compounds 1 and 2 demonstrate weaker activity compared to the standard acarbose (with IC_{50} values of 15.88±1.32 µg/ml and 7.19±1.05 µg/ml, respectively).

Table 1: IC_{50} (µg/ml) Values for α-amylase and α-glucosidase Enzyme Inhibition Activity of Compounds 1 and 2

Sample	IC_{50} Values (µg/ml)	
	Enzyme α-amylase	Enzyme α-glucosidase
1	1375.50±92.45	966.31±80.01
2	1205.93±100.24	1025,24±67,52
Acarbose	15,88±1,32	7,19±1,05

Conclusion

From the methanol extract of the Plectranthus amboinicus plant, two compounds were isolated using a combined chromatography method. The compounds were identified through modern spectroscopy techniques: Mass Spectrometry, Nuclear Magnetic Resonance Spectroscopy, and their structures were determined as β-sitosterol and stigmasterol. These two compounds are commonly found in various plant species and were isolated for the first time from Plectranthus amboinicus in Dak Lak. They exhibit anti-diabetic effects. Further research on the chemical composition and biological activity evaluation of this plant is essential and is currently being conducted by us.

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