

NEW FINDINGS OF DOCTORAL DISSERTATION

Name of Doctoral Candidate: Doan Xuan Dinh

Dissertation Title: “Research on botanical characteristics, chemical composition and some biological effects of *Sedum sarmentosum* Bunge, Crassulaceae”.

Speciality: Medicinal Materials - Traditional Pharmacy

Code of speciality: 9720206

Name of academic advisor:

1. Assoc. Prof. Nguyễn Thượng Dong, PhD.
2. Assoc. Prof. Phạm Thị Nguyệt Hằng, PhD.

Name of academic institute: National Institute of Medicinal Materials

1. Objectives:

- To elucidate the botanical characteristics and microanatomical structure of the stem of *Sedum sarmentosum* Bunge, alongside a qualitative analysis of groups of substances it contains.
- To identify the principal groups of compounds present in *Sedum sarmentosum* Bunge, followed by the isolation and structural determination of select compounds.
- Assess the acute and subchronic toxicity profiles of the whole extract of *Sedum sarmentosum* Bunge, and examine its hepatoprotective effects as well as the anti-cervical cancer potential of certain isolated compounds.

2. Methods

2.1 Botanical Research

- Conduct a comprehensive appraisal of the scientific nomenclature of the research species by analyzing its morphological characteristics and comparing these with existing taxonomic literature and classification keys.

- Utilize microscopic techniques to determine the microanatomical characteristics of the stem and the distinct features of the medicinal powder derived from the species.

2.2 Chemical Research

- Method of chemical compounds extraction and isolation:

+ Sequentially extract layers of substances based on the increasing polarity of the solvent. Coarsely ground research samples were subjected to exhaustive extraction using methanol as the solvent. The extracted solvent was subsequently distilled and recovered, then combined with water. This mixture underwent liquid-liquid extraction in succession with solvents of progressively increasing polarity: n-hexane; dichloromethane, ethyl acetate. Solvent recovery of the fractions resulted in the following residues: n-hexane residue (residue A), dichloromethane residue (residue B), ethyl acetate residue (residue C), and aqueous layer residue (residue D). These fractions were subsequently utilized for the isolation of pure active compounds.

+ The process of isolating compounds from selected fractions predominantly employed column chromatography, complemented by various chromatographic techniques, including medium-pressure liquid chromatography (MPLC), high-performance liquid chromatography (HPLC), preparative high-performance liquid chromatography, and preparative thin-layer chromatography. The fractions obtained during the isolation process were monitored using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

- Methodology for the determination of the chemical structure of compounds:

The structural elucidation of isolated compounds was conducted using physical parameters and advanced spectroscopic techniques, including ESI-MS, HR-EI-MS, 1D-NMR and 2D-NMR, COSY, HMBC, NOESY, among others. This analysis was further corroborated by comparing the results with existing literature.

2.3 Research on toxicity and biological effects

- Assess acute and subchronic toxicity in accordance with the guidelines for acute drug toxicity testing issued by the Ministry of Health, supplemented by relevant literature.

- Assessing the hepatoprotective effect in a model of acute hepatitis induced by Paracetamol (with silymarin as a positive control): Methodology for quantifying ALT and AST enzyme activities.

- The cytotoxicity evaluation against the HeLa cancer cell line for isolated pure substances was conducted at Toyama University, Japan, following the methodology described by Lombe et al. (2018).

3. Result and Conclusions

3.1 The botanical characteristics of *Sedum sarmentosum* Bunge

- Elucidated in detail, accompanied by photographic documentation, are the botanical characteristics of *Sedum sarmentosum* Bunge, (Crassulaceae).

- Microscopic characteristics of the stem were delineated, and a comprehensive description of the characteristics of the above-ground part's powder of *Sedum sarmentosum* Bunge was provided, contributing to the standardization of medicinal herbs.

3.2 The chemical composition of *Sedum sarmentosum* Bunge

From the above ground part, the researchers isolated 18 pure compounds, including 1 megastigman (SSH2 - *Sarmentol A*), 5 megastigman glycosid (SSH3 - *Myrsiniosid A*, SSH4 - *Simplicifloranosid*, SSH7 - *Sedumosid I*, SSH9 - *Sedumosid C* và SSH24 - *Sedumosid K*), 1 flavon (SSH1 - *Luteolin*), 5 flavon glycosid (SSH8 - *Isorhamnetin-3,7-O-di-β-D-glucosid*, SSH10 - *2-phenylethyl-D-rutinosid*, SSH12 - *3'-Methoxy-3,5,4'-trihydroxyflavon-7-neohesperidosid*, SSH13 - *Quercetin-3-O-β-D-glucopyranose* và SSH17 - *3'-methoxyluteolin-7-O-β-D-glucopyranosid*), 1 lignan glycosid (SSH25 - *Lariciresinol-9-O-β-D-glucopyranosid*), 2 alcohol (SSH22 - *Tyrosol* và SSH29 - *3,4-dimethoxybenzyl*

alcohol) và 3 acid phenolic (SSH19 - *Acid Ferulic*, SSH20 - *Acid p-Hydroxybenzoic* và SSH21 - *Acid trans-p-coumaric*), including a newly identified substance, SSH24, designated as **Sedumosid K**.

3.3 Toxicological and biological effects of Sedum sarmentosum Bunge

- The research findings indicate that *Sedum sarmentosum* Bunge exhibits no acute or sub-chronic toxicity at the administered oral dose.

- Researching the hepatoprotective effect in a model of acute liver injury induced by paracetamol, the study demonstrated that the total methanol extract of *Sedum sarmentosum* Bunge at doses of 0.5 and 1 g/kg body weight in mice exerted hepatoprotective effects. These effects were evidenced by a reduction in ALT activity at both dose levels and AST activity at the 0.5 g/kg body weight dose.

- The hepatoprotective effect of the total methanol extract of *Sedum sarmentosum* Bunge on a model of paracetamol-induced acute liver injury was investigated at two dosage levels, 0.5g and 1g/kg body weight in mice. The study revealed that the total methanol extract of *Sedum sarmentosum* Bunge exhibited hepatoprotective activity in the model of paracetamol-induced acute liver injury, as evidenced by its ability to reduce ALT activity at the dose of 0.5g/kg body weight and AST at the dose of 1g/kg body weight.

- To evaluate the cytotoxic effects on HeLa cancer cells, testing was conducted on twelve compounds (SSH24, SSH9, SSH3, SSH4, SSH7, SSH2, SSH8, SSH13, SSH19, SSH21, SSH20, and SSH22) at the laboratory of Professor Suresh, Toyama University, Japan. The results indicated that all twelve compounds isolated from *Sedum sarmentosum* Bunge exhibited pronounced cytotoxic effects on HeLa cells at a concentration of 200 μ M, achieving statistical significance ($p < 0.001$) with cell death rates ranging from 92.5% to 100%. At a concentration of 100 μ M, compounds 10, 14, 15, and 16 induced cell death rates of 30%, 26%, 18%, and 24%, respectively. Conversely, at a concentration of 50 μ M, none of the twelve compounds demonstrated cytotoxic effects on HeLa cells.

Therefore, all twelve compounds isolated from *Sedum sarmentosum* Bunge exhibited cytotoxicity against HeLa cervical cancer cells, albeit to varying degrees.

Scientific Supervisors

July... 2024 in Ha Noi
PhD Candidate

**Assoc. Prof. Nguyen
Thuong Dong, PhD**

**Assoc. Prof. Pham Thi
Nguyet Hang, PhD**

Doan Xuan Dinh