Southeast-Asian J. of Sciences, Vol. 6, No. 1 (2018) pp. 96 - 102

THE RESEARCH ON THE ISOLATION OF ANTHRAGLYCOSIDE OF LEAVES OF CASSIA ANGUSTIFOLIA VALH (FABACEAE)

Le Thi Minh Thu*, **Nguyen Dinh Nga***
Tran Huu Tam† and **Vo Thi Bach Hue***

**Faculty of Pharmacy*
Ho Chi Minh University of Medicine and Pharmacy
Ho Chi Minh city , Vietnam
e-mail: vothibachhue@gmail.com

† *Center for standardization and quality control*
in medical laboratory of Ho Chi Minh city, Vietnam
email: thu.minh9x@gmail.com

Abstract

Cassia angustifolia is not widely cultivated in Vietnam, but is a valuable medicinal plant that has been studied by scientists around the world for a long time. For our country, it seems that this plant is still very new, but now it was brought to plant in Tuy Hoa, Phu Yen and initial research in the country about this plant. The effect of this tree is used to treat stools, constipation, indigestion, lobed lateral abdominal ... Many studies have demonstrated the laxative of anthraglycoside compounds derived from this plant as aglycol anthranoids (rhein, aloe emodin, chrysophanol) and anthraglycosides (rhein monoglycoside, aloe emodin mono glycoside, sennosid A, B, C, D, G, aloe-emodin dianthron glycoside). [1]

In this scientic article, the pure anthraglycoside compound Aloe emodin was isolated from 2.5 grams of total leaves of Cassia angustifolia Vahl. Their chemical structures are elucidated by analytical methods (IR, UV, MS, 1H-NMR) to determine the aloe-emodin. This is the first study on the isolation of Aloe emodin from Cassia angustifolia Valh of Vietnam in recent years.

Key words: Aloe - emodin, Cassia angustifolia, isolate anthraglycoside, chemical structure.
* Corresponding author.

1. Introduction

Cassia angustifolia is a plant from India, the study of this plant in our country is still very little. For our country, it seems to be very new, however, it was brought back to Viet Nam and initial research in the country about this plant. Studies on Cassia angustifolia focus more on the effects of the anthraglycosid laxative group as a group that promotes the excretion of the intestines to eliminate waste residues, toxic residues that are toxic to the digestive tract... The research on the isolation of anthraglycosid from Cassia angustifolia valh (Fabaceae) was carried out to contribute to standardization to isolate pure anthraglycoside compounds, to determine the structure of the anthraglycoside isolated) thanks to the method of random sampling and if a large amount of pure compound is obtained, a comparator will be established to control the quality of medicinal herbs that have been introduced into Viet Nam, contributing to the development of the ability providing pharmaceutical products for Viet Nam.

2. Materials and Methods

2.1. Materials

The research material was the leaves of Cassia angustifolia harvested in Sapa in March 2016. The leaves were washed, dried, then crushed to a powder, sieved through a sieve size of 500 ?m; Put in clean nylon bags and store in a dry place.

2.2. Methods of Analysis

Cassia angustifolia leaf powder is 5 kg which is infused with 60% ethanol. Using the rota - vapor device, concentrated solution obtained total anthraglycoside of leaves. Study the total anthraglycoside separation from the concentrated solution of Cassia angustifolia leaves.

By vacuum liquid chromatography, the concentrated solution of total anthraglycosid has been separated into fractions. These fractions were selected and tested for presence of the anthraglycoside group by thin layer chromatography (TLC), continued by classical column chromatography, purified to obtain pure anthraglycoside compounds. Determine the structure of pure anthraglycoside compounds isolated by spectroscopic techniques such as UV, IR, MS, 1H-NMR.

3. Results and Discussion

3.1. Extract the total anthraglycosid from concentrated solution of Cassia angustifolia leaf

5 kg Cassia angustifolia leaf material has been extracted by percolated method with ethanol 60%. Using the rotary- evaporatory device to retire the solvent, we have 1kg of concentrated extract of the cassia angustifolia leaf. This concentrate extract is packed in sealed PE bags and then packed in sealed bags. From 830 g of the ethanol extract according to the diagram below yielded 26 g of total anthraglycoside (performance is 3.1%).

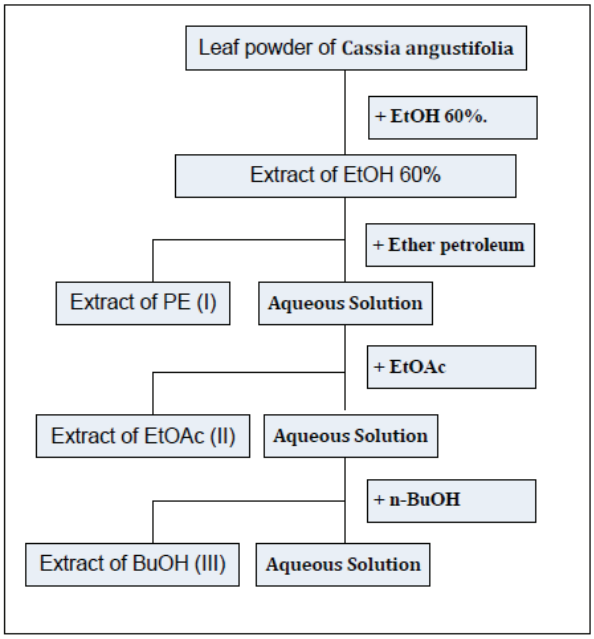


Figure 3.1. Extraction of total anthraglycoside

Control the results by thin layer chromatography and based on the nature of the anthraglycoside compound group which are dissolved in medium polar solvent . The extract of EtOAc has been used for further chemical composition study [2].

3.2. Separation of anthrglycoside segments by vacuum liquid chromatography (VLC)

EtOAc (II) extracts were separated with 2.5 g of VLC, yielding 117 parts (10 ml each) with gradually increasing polar solvents such as n-hexane; chloroform; ethyl acetate; methanol ... thin layer chromatography. Fractions 55 - 63 are chosen for more separation because they have more anthraglycoside and less impurities. (Figure 1)

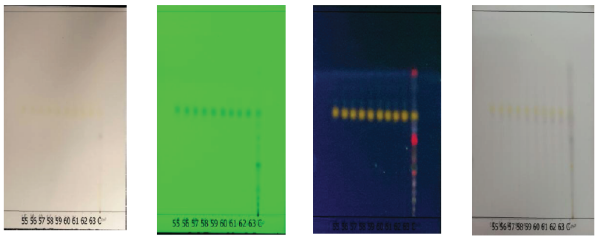


Figure 1. TLC plate showing the distinct separation of impurities after development in the mobile phase: chloroform - methanol (9:1)
C: concentrated solution of EtOAc (II)
55; 56;;63: Fractions from 55 - 63

3.3. Isolation and purification of anthraglycoside compounds

The fractions 55-58 have more precipitated yellow needle shape bottom sedimentation and sticking. particle deposition on the wall of tubes. Check them by TLC. The tubes which have equivalent spots are merged: 55 and 56 (for precipitate A), 57 and 58 (for precipitate B).

These precipitates are filtered , then wash the precipitate with chloroform, check the purity of the precipitate by TLC (A yellow stain overlaps a black mark). Continue washing the precipate B with EtOAc, check the purity of precipitate by TLC to see only a yellow spot. Fraction B are obtained anthraglycoside 21 mg. This anthraglycoside is further experimented to determine the structure.

3.4. Determine the structure anthraglycoside isolated compound

TLC: mobile phase $CHCl_3$ - MeOH (9: 1); on silica gel plate F254 (Merck), compound B for a cut are round, bright yellow color and the value of the

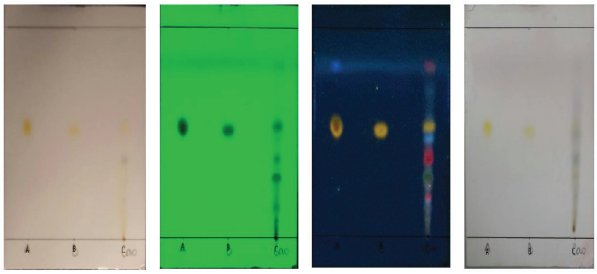


Figure 2. TLC plate showing the distinct separation of impurities after wash the precipitate with chloroform in the mobile phase: chloroform - methanol (9:1)
C: concentrated solution of EtOAc (II)
A, B: the anthraglycoside isolated

standard equivalent aloe emodin.

UV spectrum: There are 4 peaks of maximum absorption at 225.5 nm, 255.5 nm, 286 nm, 429 nm.[5]

IR spectrum: - OH (3458); -CH_2- no (2979); >C=O (1677; 1623); benzene frame (1476; 1388; 1338). [5]

MS spectrum: signal fragment with /z = 269.0479 [M-H]- suitable for bulk molecules of aloe emodin ($C_{15}H_{10}O_5$ = 270).[6]

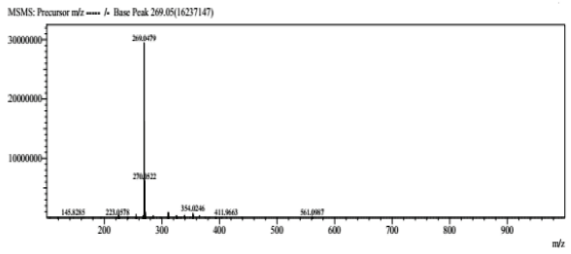


Figure 3. MS spectrum of B

^{1}H-NMR spectrum: The compound was measured with NMR spectrum (MeOD, 500 MHz) and compared the results in the reference [?].

Comment: Anthraquinone compounds isolated from cassia angustifolia leaves are aloe emodin

Table 1. The results show that NMR data of B are completely consistent with aloe emodin

C / H position	*δH, ppm, mult., (J, Hz)*
2	7.335 *d* (1.0)
4	7.810 *d* (1.0)
5	7.335 *dd* (8.0; 1.0)
6	7.757 *t* (8.0)
7	7.812 *dd* (7.0, 1.0)
11	4.722 *s*

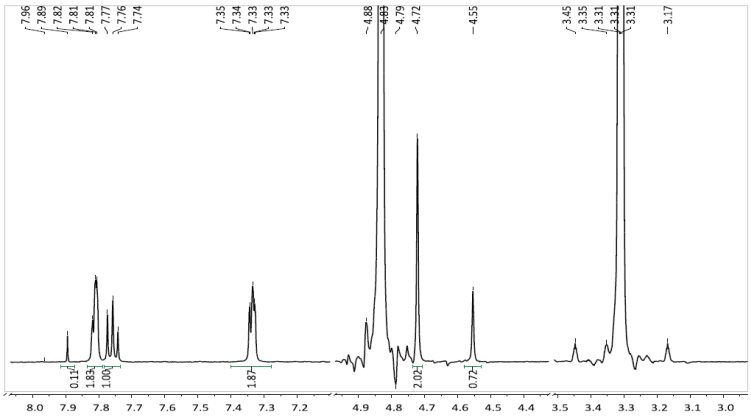
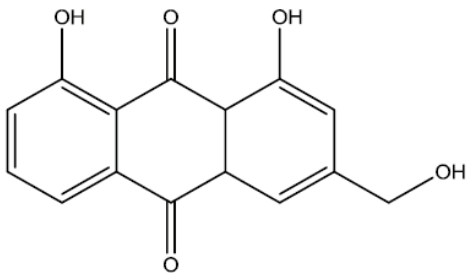


Figure 4. ^{1}H-NMR spectrum of B



Aloe emodin

4. Conclusions

From 830 g of total ethanol of cassia angustifolia leaves harvested in Sapa, isolated and obtained 26 g of total anthraglycoside. By chromatography and refining techniques, 2.5 g of total anthraglycoside isolated 21 mg of the pure anthraglycoside compound and continued to study the structure by spectral methods to determine this compound in accordance with the structure of the aloe emodin.

References

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