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Quantitative Analysis Of Scopolamine In Brugmansia Suaveolens By HPLC-MS Method

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***Abstract*—Scopolamine is a tropane alkaloid which typically found in plant belonging Solanaceae family. From the aerial part of *Brugmansia suaveolens*, scopolamine was isolated by chromatographic methods. Its chemical structure was confirmed by HR-ESI-MS, NMR spectra, and as well as comparison with reported literature. Content of scopolamine in several part of *B. suaveolens* such as roots, leaves, and flowers was analyzed by HPLC-MS. Results showed that the flowers contained highest scopolamine amount (0.2738%, w/w), over teen times in the leaves.**

***Keywords*—Brugmansia suaveolens; scopolamine; quantitation; HPLC-MS.**

I. INTRODUCTION

Brugmansia suaveolens is a flowering plant of the Solanaceae family. It was known as angel's trumpet due to the shape and beautiful appearance of the flower. Chemical investigation of *B. suaveolens* have focused in essential oils, isolation of flavonol glycoside and alkaloids [1, 2]. Among alkaloids, scopolamine was reported as one of major tropane alkaloid natural occurring in this plant. This compound was suggested to protect *B. suaveolens* against insects [3]. In addition, scopolamine was also known to involve in broad range of pharmacological activity such as anticholinergic activity which effects on nervous system. However, several side effects of scopolamine have been archived. It might cause blurred vision, dilated pupils, feeling restless, or difficult breathing [3, 4]. Therefore, a suitable dose of scopolamine is important in medication and the estimation of content of scopolamine also avoid unexpected side effects.

This work deals with the isolation, chemical structure determination of scopolamine in a Solanaceae plant, *Brugmansia suaveolens* growing at the North of Vietnam. The content of scopolamine in several part of this plant such as roots, leaves, and flowers was also analyzed by HPLC-MS method.

II. MATERIALS AND METHODS

A. General experiment procedures

Optical rotation was measured on a Jasco DIP-370 automatic polarimeter (Jasco, Kyoto, Japan). NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker BioSpin, Bremen, Germany) using TMS as an internal standard. HPLC analysis was performed on an Agilent infinity 1290 LC system and high resolution electrospray ionization mass spectra were acquired on an Agilent 6530 Accurate Mass QTOF LC/MS system (Agilent technology, Santa Clara, CA, USA). Column chromatography was performed using silica gel (Merck, Whitehouse Station, NJ, USA) and reverse phase C18 resins (YMC Ltd., Kyoto, Japan). Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-C18 F_{254S} plates (0.25 mm, Merck). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

B. Plant materials

The whole plant of *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl were collected at Lao Cai Province, Vietnam in March 2015. Its scientific name was identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources, Hanoi, Vietnam. After collection, samples were divided into roots, leaves, flowers and aerial part. Each part was dried, powdered and kept in the plant storage room until used.

C. Purification of scopolamine

The dried powdered aerial part of *B. suaveolens* (5.0 kg) was ultrasonically extracted in methanol (3 times, each 10 L in 60 minutes at 50°C). After removal of methanol *in vacuo*, a dark solid methanol extract (300 g) was obtained. This extract was then suspended in 2 L of distilled water and successively partitioned with dichloromethane and ethyl acetate (each 3 L) to give corresponding dichloromethane extract (30 g), ethyl acetate (12 g) and water layer. The ethyl acetate extract was roughly separated on a silica gel column chromatography, eluting with gradient solvent system of dichloromethane/methanol (0-100% volume of methanol) to give six fractions (E1-E6). The E2 fraction was repeatedly chromatographed on a silica gel column, eluting with isocratic solvent system of ethyl acetate/methanol/water (20/1/0.05, v/v/v) to obtain four fractions E2A-E2D. The E2A fraction was loaded on a reverse phase C18 resins column chromatography and eluted with methanol/water (2/1, v/v) to give scopolamine (62 mg). The purity of isolated

scopolamine was determined by HPLC-DAD and found to be 98.3%.

- Scopolamine: Molecular formula: $C_{17}H_{21}NO_4$; White amorphous powder; $[\alpha]_D^{25}$ = -22.7 (c 0.05, MeOH); HR-ESI-MS *m/z*: 304.1540 $[M+H]^+$ (calcd for $C_{17}H_{22}NO_4$, 304.1549); ^{1}H-NMR δ_H (ppm): 2.99 (1H, br s, H-1), 3.51 (1H, br d, J = 2.5 Hz, H-2), 3.03 (1H, br d, J = 2.5 Hz, H-3), 3.10 (1H, br s, H-4), 2.05 (1H, br d, J = 15.5, H-5a), 2.12 (1H, br d, J = 15.5 Hz, H-5b), 4.96 (1H, br s, H-6), 1.42 (1H, br d, J = 15.5 Hz, H-7a), 1.60 (1H, br d, J = 15.5 Hz, H-7b), 2.42 (3H, s, N-CH_3), 3.81 (2H, H-2′ and H-3′a), 4.18 (1H, dd, J = 11.5, 11.5 Hz, H-3′b), 7.29-7.37 (5H, m, H-Ph); ^{13}C-NMR δ_H (ppm): 58.3 (C-1), 56.9 (C-2), 56.6 (C-3), 58.4 (C-4), 30.3 (C-5), 67.5 (C-6), 30.4 (C-7), 40.8 (N-CH3), 172.6 (C-1′), 55.7 (C-2′), 64.3, (C-3′), 137.3 (Ph-1), 129.1 (Ph-2,6), 129.7 (Ph-3,5), 128.6 (Ph-4).

D. Preperation of extract and standard samples

A stock scopolamine solution, standard sample, was prepared by weighting and subsequent dissolving in methanol to give 1.0 mg/mL stock solution. Seven level of calibration samples were prepared from stock solution by dilution with methanol to give series solutions of 300, 200, 150, 100, 50, 25, 10 µg/mL.

Finely powdered roots, leaves, and flowers of *B. suaveolens* (5 g for leaves and flowers; 10 g for roots) were extracted four times with methanol by sonication (each 10 mL, 20 minutes in room temperature). After centrifugation (13,000 rpm) for 5 minutes, methanol solution was combined and filled up to final volume in a 50 mL volumetric flask. Resulted solution was used for quantitative analysis.

E. UHPLC-DAD-QTOF-MS analysis

UHPLC analysis was carried out on an Agilent Infinity 1290 series HPLC system, equipped with quaternary pump, autosampler, column oven and diode array detector (Agilent, Waldbronn, Germany). A Zorbax Eclipse Plus-C18 column (2.1 × 50 mm, 1.8 µm particle size) guarded with its narrow bore guard column (2.1 × 12.5 mm, 5.0 µm) was used. The mobile phase consisted of 0.1% formic acid in water (v/v, bottle A,) and 0.1% formic acid in acetonitrile (v/v, bottle B). HPLC sequence was acquired by a gradient elution (0 min: 95% A, 15 min: 5% A, 19.9 min: 5% A, 20 min: 95% A; post time: 2 min 95% A) with a flow rate of 0.4 mL/min. Column oven was set at 40 °C. Samples were loaded with injection volume of 0.1 µL and the HPLC chromatogram was monitored by detection wavelength at 210 nm and 254 nm, respectively.

Mass spectra were acquired on an Agilent 6530 Accurate Mass QTOF connected to the UHPLC system as described above. MS parameters were set including ESI positive mode, nebulizer gas 25.0 psi, dry gas 10.0 L/min at 325°C, capillary voltage 4.0 kV, in the mass scan range of 100–1000 *m/z* with a scan rate of 1 Hz [5, 6].

III. Results and Discussion

Dried aerial part of *B. suaveolens* was extracted with methanol and successively separated with dichloromethane, ethyl acetate to give corresponding dichloromethane, ethyl acetate, and water residues. The ethyl acetate extract was subjected to various chromatographic technique to obtain compound **1**.

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined to be $C_{17}H_{21}NO_4$ on the basis of quasi molecular ion peak m/z 304.1540 (calcd for $C_{17}H_{22}NO_4$, 304.1549) in the HR-ESI-MS, indicating eight indices of hydrogen deficiency. The ^{1}H-NMR spectrum of **1** showed five aromatic protons at δ_H 7.29-7.37 (5H, m), oxygenated methin protons at δ_H 3.51 (1H, br d, J = 2.5 Hz, H-2), 3.03 (1H, br d, J = 2.5 Hz, H-3), 4.96 (1H, br s, H-6), 3.81 (H-3′a), and 4.18 (1H, dd, J = 11.5, 11.5 Hz, H-3′b). A nitrogen bearing methyl group was observed at δ_H 2.42 (3H, s) and δ_C 40.8. Analysis of ^{13}C-NMR and DEPT spectra revealed signals of 17 carbons including a carbonyl carbon δ_C 172.6 (C-1′); six aromatic carbons of a phenyl group at δ_C 137.3 (Ph-1), 129.1 (Ph-2,6), 129.7 (Ph-3,5), 128.6 (Ph-4); two epoxy carbon at δ_C 56.9 (C-2), 56.6 (C-3); three nitrogen bearing carbons at δ_C 58.3 (C-1), 58.4 (C-4), 40.8; two oxygenated carbons at δ_C 67.5 (C-6), 64.3, (C-3′); two

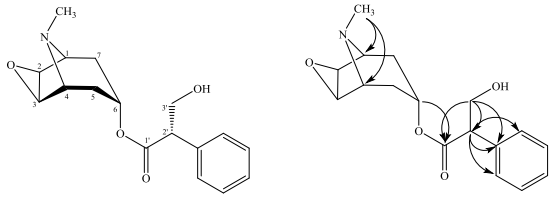

Fig. 1. *Chemical structure of scopolamine (**1**) and important HMBC correlations*

TABLE I. Recovery of Scopolamine in Spiked B. Suaveolens Extracts

Extract	Low spike		Medium spike		High spike	
	Added (µg)	Recovery (%)	Added (µg)	Recovery (%)	Added (µg)	Recovery (%)
Roots	20.0	107.3	100.0	103.8	200.0	99.2
Leaves	20.0	102.5	100.0	98.6	200.0	94.1
Flowers	20.0	99.3	100.0	98.5	200.0	95.7

TABLE II. CONTENT OF SCOPOLAMINE IN THE ROOTS, LEAVES, AND FLOWERS OF B. SUAVEOLENS

Compound	Roots	Leaves	Flowers
Scopolamine	91.3±13.7	216.9±6.4	2738.4±71.8

Results are expressed as mean±SD (µg scopolamine per gram of dried material).

aliphatic methylenes at δ_C 30.3 (C-5), 30.4 (C-7); and a methin at δ_C 55.7 (C-2′). In the HMBC spectra of **1**, correlations between N-methyl proton δ_H 2.42 and C-1 (δ_C 58.3)/C-4 (δ_C 58.4) suggested for N-methyl tropane-type alkaloid structure (Fig. 1). The presence of a β-hydroxy-α-phenyl propanoic acid moiety was observed by HMBC correlations of H-3′ (δ_H 4.18/3.81) to C-1′ (δ_C 172.6)/ C-2′ (δ_C 55.7)/ Ph-1 (δ_C 137.3). Connection between β-hydroxy-α-phenyl propanoic acid moiety and tropane skeleton was established at C-6, confirming by the downfield shifted of H-6 and HMBC correlation of H-6 (δ_H 4.96)/C-1′ (δ_C 172.6). Consequently, compound **1** was determined to be scopolamine. The NMR data of **1** was good consistence with those of scopolamine in the literature [7]. Next, purity of isolated scopolamine (98.3%) was examined by HPLC-DAD method. It was then used as a standard for quantitative of scopolamine content in several part of *B. suaveolens* including roots, leaves, and flowers.

Seven calibration level solutions of scopolamine standard were prepared and injected to UHPLC-DAD-QTOF system. Exact ion chromatography of scopolamine (*m/z* 304.1543, Δ_{amu} 20 ppm) was extracted and used to plot calibration curve, describing relationship of peak areal versus concentration. A linear regressive equation was established. Where there, y and x values were peak areal and concentration (µg/mL), respectively. The regression parameters including slope, intercept, and correlation coefficient (R^2) were calculated by linear regression analysis using Microsoft Excel. Experiments were carried out in triplicate. Limit of detection (LOD) value was calculated by 3.3 times of ratio between intercept standard deviation and slope averaged value meanwhile limit of quantification (LOQ) was determined by 10 times of this ratio. According above approach, LOD and LOQ values were determined to be 0.37 and 1.11 µg/mL. The averaged regressive equation ($y = 119.87x + 8.67$, $R^2 = 0.9997$) was obtained and used for method validation and quantitative analysis.

Precision was determined individually in each extract (roots, leaves, and flowers) by analyzing the scopolamine content intra- and inter days (three consecutive days). Results indicated that relative standard deviation (RSD) of scopolamine were not much different and in range of 1.2 to 4.6%, showing acceptable stability of scopolamine in analysis conditions. Next, accuracy was examined at three concentration levels of scopolamine (high, medium, and low concentrations) spiked into the leaves extract samples. As shown in the Table I, recovery percentages were found from 94.1 to 107.3%.

Quantitative analysis of scopolamine was then applied for methanol extract of the roots, leaves, and flower of *B. suaveolens*. Each part was exactly weighted (5.0 gram for leaves and flowers, 10.0 gram for roots), extracted in methanol, filled up in volumetric flask and subjected to UHPLC analysis in the same conditions. The scopolamine peak was monitored in HPLC chromatogram by both DAD detector and QTOF-MS analysis. Exact ion chromatograms of scopolamine in the sample were extracted and used for calculation of its content in the samples. As shown in the Table II, the content of scopolamine in the dried roots, leaves, and flowers were 0.0091, 0.0217, and 0.2738% (w/w), respectively. The results showed that content of scopolamine was highest in the flowers and smallest in the roots of *B. suaveolens*. The content of scopolamine in flowers was over teen times in the leaves. The flowers of *B. suaveolens* highly produce scopolamine which could be explain in part the use of *B. suaveolens* in folk medicinal remedies. It also warned that the rich of scopolamine or other tropane ankaloids in the flower of *B. suaveolens* easily cause over dose, resulting unexpected effects during usage.

In conclusion, tropane alkaloid, scopolamine could be found in the roots, leaves, and flowers of *B. suaveolens*. Of these, content of scopolamine was highest in the flowers. Quantitative analysis of scopolamine in several part of *B. suaveolens* provided in part a guild for the usage of them to avoid side effects of scopolamine.

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