Chemical constituents and anti Helicobacter pylori effect of ethyl acetate fraction from *Sanchezia nobilis* Hook.F

Xuan Bui Thi*1,2, Loi Vu Duc2, Duong Le Hong2, Ngoc Tran Minh1

1National Institute of Medicinal Materials, 3B Quang Trung Street, Hoan Kiem District, Hanoi, Vietnam
2VNU School of Medicine and Pharmacy, Vietnam National University, 144 Xuan Thuy Street, Cau Giay District, Hanoi, Vietnam

**Article History:**
Received on: 17 Apr 2020
Revised on: 19 May 2020
Accepted on: 20 May 2020

**Keywords:**
*Sanchezia nobilis* Hook.F, 13-O-acetylfawcettimine, ethyl acetate fraction, anti-Helicobacter pylori, MIC

**ABSTRACT**
The leaves of *Sanchezia Nobilis* Hook. F grown in Vietnam are extracted with ethanol then distilled for ethanol retrieval under low pressure. The ethanol concentrate was shaken with n-hexane, then stirred with ethyl acetate and concentrated fractions were obtained. From the high ethyl acetate fraction, four compounds were isolated by normal phase and reversed-phase column chromatography. Their structures were determined by spectral analysis, including 1D and 2D NMR techniques (1H, 13C, DEPT, COZY, HSQC, HMBC and NOESY), High Resolution - Electro chewmical Ionization - Mass Spectroscopy (HR-ESI-MS), and identified by comparing with the recorded data. Among the isolated compounds, compound 2 (13-O-acetylfawcettimine) was first time isolated from nature; and mixture 1 (Fawcettidin), 3 (Apigenin) and 4 (Kaempferol) were first time isolated from the *Sanchezia* genus. The high ethyl acetate fraction of *Sanchezia Nobilis* Hook. F leaves were then evaluated for the anti-Helicobacter pylori (anti-HP) effect by determining the minimum inhibitory concentration method (MIC), which showed a good inhibitory, dose—dependent effect on Helicobacter pylori. The ethyl acetate fraction at 1/8 dilution showed a complete inhibitory effect on Helicobacter pylori for as long as 24 hours. At 1/128 dilution, the fraction only showed good results on inhibition after 24 hours.

**INTRODUCTION**
*Sanchezia* is a small genus with more than 50 species, mainly distributed in the subtropical and tropical area such as the USA, Africa, the Mediterranean, Oceania, India, and some Southeast Asian countries. Most species are located in tropical rainforests in Central and South America (Ecuador) (Leonard and Smith, 1964). In previous publications, the genus was studied for effects such as antibacterial, antifungal and insecticidal by the agar diffusion method with 15 strains of Gram (+) and Gram (-). The effect on six strains of fungi and *Tribolium castaneum* was not as good as the comparison antibiotics (Rafshanjani et al., 2014). Evaluation of antioxidant effect by the ORAC method showed a result similar to quercetin. The anticancer effect on a methanolic fraction from *Sanchezia speciosa* leaves extract on MCF-7, SK-MEL-5, HUVEC cell lines had the best results on CMF-7 cell lines (Paydar et al., 2013). The anticancer effect by the MTT method on Hela cells from *S. Nobilis* roots had positive results (Shaheen...
et al., 2017). Researching on anti-inflammatory effect by inhibition of albumin denaturation assay method and antioxidant effect by DPPH method had good results (Loi et al., 2017). The published report on chemical constituents of these species has been showing that they contain several groups of compounds such as flavonoids, saponins, glycosides, alkaloids, steroids, carbohydrates, tannins and phenols. A number of specific compounds were isolated, such as five matsutake alcohol compounds, in which four compounds were first time isolated from the Acanthaceae family and 1 compound was first time isolated from the nature: 3-O-β-arabinopyranosyl-(1-6)-β-glucopyranosyl-(1-6)-β-glucopyranosyl-1-octen-3-ol; 6 other compounds from the methanol extract of S. Nobilis leaves and roots, which 1 compound was first time isolated from the Acathancea genus and 1 compound was first time isolated from the Sanchezia genus (Mohamed et al., 2013; Ellah et al., 2014; J. C. Omondi and S. Omondi, 2015); 3 flavonoids and the compound 3-Methyl-1H-benz[f]indole-4, 9-dione were also isolated from Sanchezia speciosa leaves grown in Vietnam (Loi et al., 2017); 16 fatty acids were also isolated from n-hexane extract of S. Nobilis Hook. F leaves (Nhung, 2018).

In Vietnam, there are only one species has been discovered belongs to the Sanchezia genus, which is Sanchezia Nobilis Hook. F. It grows in some provinces such as Tuyen Quang, Nam Dinh, Vinh Phuc, Phu Tho, Thai Nguyen, Quang Nam, and Da Nang (Ho, 2003). In this research, we collected the total ethanol extract of S. Nobilis leaves and then separated the n-hexane, water and ethyl acetate fractions. These fractions would be continued to be isolated and determined the structure of components. We also evaluated the biological effects on peptic ulcer. In this publication, we assessed the inhibitory effect of HP’s extract by determining the minimum inhibitory concentration method (MIC).

EXPERIMENTAL PART

Materials and Methods

Plant material

The leaves of S. Nobilis Hook. F. were collected in Nam Dinh province during January 2018 and authenticated by the School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (No: 190DV18 SMP-VNU). A voucher specimen has been deposited to that place.

General experimental procedures

The ESI-MS were recorded on a Varian Agilent 1100 LCMSD mass spectrometer. The NMR [1H (500 MHz), 13C (125 MHz), and DEPT-90 and 135 MHz] spectra were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). Chemical shifts were reported in ppm downfield from TMS with J in Hz. Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB WagetechnikRapido, Germany). Optical rotation was measured on WXG-4 disc polarimeter. Analytical TLC was performed on Kieselgel 60 F254 (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F254 (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H2SO4, followed by heating with a heat gun. Column chromatography was performed on silica gel (70–230 and 230–400 mesh, Merck). Organic solvents were of analytical grade. Optical densities were read on an ELISA plate reader (Bio-rad).

Extraction and isolation

The leaves of S. Nobilis were washed, dried, chopped. 3.0 kg of chopped leaves were submerged in 12 L ethanol 80% (solvent) at room temperature in three days to collect the first extract. We added more solvent to submerge the herbs in 2-3 cm under the liquid surface (10 L per time) for two times to collect the second and the third extract.

All three extracts were mixed, paper–filtered, distilled for ethanol retrieval under low pressure, about 251.2 g concentrated ethanol extract was collected.

150 g of the concentrated ethanol extract were dispersed in distilled water, partition extracted with n—hexane and ethyl acetate (each solvent three times, 900 mL per time in 30 minutes). The n—hexane and ethyl acetate fractions were distilled for solvent retrieval under low pressure to collect these fractions: H for n—hexane (28.6 g), E for ethyl acetate (56.8 g). The remaining aqueous extract was concentrated to obtain fraction N (45.6 g).

Fraction E (20g) was dispersed in 2% tartaric acid solution (300 mL) and filtered out solid precipitate to collect the filtrate. The filtrate was alkalinized to pH 9 with NaHCO3, then extracted with dichloromethane (CH2Cl2) (4 times x 600 mL). The obtained extract was concentrated at low pressure to produce E1 extract (5g). Fraction E1 was isolated.
Table 1: The H. pylori inhibition effect levels of ethylacetate fraction

| Dilution | After 2 hours of exposure | After 6 hours of exposure | After 24 hours of exposure |
|----------|---------------------------|---------------------------|---------------------------|
| 1/4      | -                         | -                         | -                         |
| 1/8      | -                         | -                         | -                         |
| 1/16     | 102                       | -                         | -                         |
| 1/32     | 105                       | 103                       | -                         |
| 1/64     | 106                       | 104                       | -                         |

Note: (-) means that the bacteria are completely inhibited.

Figure 1: Structure of compounds 1-4

Alcaloid 1: Fawcettidin

Alcaloid 2: 13-O-acetylfawcettimin

Flavonoid 3: Apigenin

Flavonoid 4: Kaempferol

on silica gel with increasing polarization elution solvent (CH$_2$Cl$_2$: MeOH, 30:1 → 10:1) to obtain three small fractions: E1.1, E1.2, E1.3. Fraction E1.1 was isolated on reversed-phase column chromatography (YMC C-18) using acetone:methanol:28% ammonia solution (4:6:1, v/v), obtained a clean compound 1 (15mg). Fraction E1.2 was isolated on YMC C-18 using acetone:methanol:28% ammonia solution (3:3:1, v/v), obtained a clean compound 2 (12mg).

Fraction E (20g) was dispersed on a silica gel with increasing polarization elution solvent n-hexane:ethyl acetate (5:1 → 1:1, v/v, 600 mL each fraction) and followed by ethyl acetate:methanol (5:1 → 1:1, v/v, 500 mL each fraction) to obtain 5 fractions E2 ~ E5. Fraction E2 (4.1 g) was isolated on silica gel, with chloroform:methanol (30:1; 20:1; 10:1; 5:1; v/v) to obtain 4 small fractions E2.1, E2.2, E2.3, E2.4. Fraction E2.1 (0.5 g) was isolated on silica gel, with acetone:methanol (1:3; v/v) to obtain a yellow solid compound 3 (18 mg). Fraction E3 (5.4 g) was isolated on silica gel (Φ45 mm × 350 mm), with chloroform:methanol (2:1, v/v) to obtain
3 small fractions E3.1-3.3. Fraction E3.1 (1.1 g) was isolated on YMC C-18 using methanol:water (2:1, v/v) to obtain a clean compound 4 (19 mg).

**Antibacterial effect Helicobacter pylori**

Drugs and chemicals for the investigation of *H. pylori* inhibition effect include ethyl acetate extract of *S. Nobilis* leaves, *H. pylori* culture media, Pylori agar, sheep's blood, physiological saline.

37°C incubator with 10% CO₂ to culture the *H. pylori*.

**Culturing method**

Biopsies were Giemsa-stained to determine the presence of *H. pylori*. Positive samples were crushed, ground and inoculated into 5% sheep's blood agar media with *H. pylori* selective supplement. Agar plates were incubated at 37°C under microaerophilic conditions, created by GasPak™ (BD) bags. The results were read after seven days with the appearance of small, grey, transparent, 1–2 mm in diameter colonies. Reactions like urease, catalase, oxidase were used to identify the bacteria. Good growing colonies were chosen to activate in liquid media for tests.

**Antibacterial effect evaluation method**

The diluting method in liquid medium was proceeded to determine the MIC of the fractions.

Step 1: The fraction was diluted into 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, dilution, compared to the initial concentration.

Step 2: *H. pylori* were diluted in physiological saline into suspension of 10⁸ bacteria/mL, then into 10⁷, 10⁶, 10⁵, 10⁴, 10³, 10² bacteria/mL.

Step 3: The 10⁸ bacteria/mL suspension was mixed with the dilutions in step 1 in a 1:1 ratio, incubated at 37°C in 2h, then taken out, inoculated into Pylori agar using a quantitative set.

The concentrations of bacteria in step 2 were also inoculated into Pylori agar to create agar plates of standard bacterial concentrations for comparison with agar plates in step 3. The results were read after 2, 6 and 24h (Cockerill, 2010; Yee and Koo, 2000).

**RESULTS AND DISCUSSION**

**Chemical constituents of Sanchezia Nobilis**

The ethanol extract powder was isolated into n-hexane, ethyl acetate and water fractions. A part of ethyl acetate fraction was isolated by different chromatography techniques on both stationary phase and reversed-phase silica gel to isolate alkaloids 1, 2 and flavonoids 3, 4.

**Alcaloid 1: Fawcettidin**

Light yellow oil, melting point 170-172°C.

**1 H-NMR** (**CDCl₃**, 500 MHz) δ 2.97 (1H, m), 3.13 (1H, m), 1.33 (1H, m), 1.80 (1H, m), 1.55 (1H, m), 1.86 (1H, m), 2.72 (1H, d, J=1.5), 2.32-2.23 (1H, m), 1.20 (1H, m), 1.27 (1H, m), 3.13-2.97 (2H, m), 1.75 (1H, m), 1.86 (1H, m), 5.70 (1H, d, J= 5.0), 2.17-2.08 (1H, m), 1.05 (3H, d, J = 7.0).

**13 C-NMR** (**CDCl₃**, 125 MHz), δ 60.3 (C-1), 29.1 (C-2), 31.0 (C-3), 56.2 (C-4), 218.0 (C-5), 44.1 (C-6), 37.0 (C-7), 34.1 (C-8), 51.9 (C-9), 24.0 (C-10), 39.1 (C-11), 46.1 (C-12), 145.0 (C-13), 127.1 (C-14), 27.7 (C-15), 21.1 (C-16).

Compound 1 was obtained as light yellow oil. **1 H-NMR** measured in **CDCl₃** showed a typical methyl group at δ_H 1.05 (3H, d, J = 7.0 Hz). We observed characteristics of two signals of methane proton at δ_H 5.70 (1H, d, J = 5.0 Hz, H-14) and δ_H 2.72 (1H, d, J = 7.5Hz). Moreover, the characteristics of super signals are at δ_H 3.13-2.97 (m, 4H), 2.32-2.23 (m, 2H), 2.17-2.08 (m, 3H), 1.80-1.33 (m, 3H), 1.27-1.20 (m, 2H), 1.86-1.75 (m, 2H) and 1.86-1.55 (m, 2H). Analyzing the **13C-NMR** showed signals for 1 CH₃, 8 CH₂, 4 CH groups and three non-hydrogenated carbon atoms. Furthermore, **13C-NMR** contained signals corresponding to a carbon ketone δ_C 218.0 (C-5); a non-hydrogenated carbon atom δ_C 145.0 (C-13); and a methine carbon-nitrogen δ_C 127.1 (C-14); two carbon nitrogens δ_C 60.3 (C-1) and δ_C 56.2 (C-4). When compared to the previously reported researches (Dao et al., 2019; Li et al., 2015) the structure of compound 1 was inferred to be Fawcettidin. This compound was first isolated in 1963 (Burnell et al., 1963), but the structure was defined in 1970 (Ishii et al., 1970).

**Alcaloid 2: 13-O-acetylfawcettimin**

Brown yellow solid.

PT-IR (ν_max cm⁻¹): 3400 (OH), 2256 (C-H), 1651 (C=O), 1047 (C=O-C)

HR-ESI-MS: m/z 306.2064 [M+H]+, molecular formula was C₁₈H₂₇NO₃ (M = 305).

**1 H-NMR** (**CDCl₃**, 500 MHz) δ 0.92 (3H, d, J=7.0), 3.29 (1H, dt, J=13.0, 14.0), 3.24 (1H, dd, J=13.0, 14.0), 2.12 (1H, m), 1.98 (1H, m), 2.30 (2H, m), 1.54 (1H, m), 2.04 1H, (m), 2.35 (1H, d, J=12.5, 16.5), 2.18 (1H, m), 2.10 (1H, m), 1.77 (1H, m), 1.46 (1H, m), 2.94 (1H, m), 2.92 (1H, m), 2.21 (1H, m), 2.20 (1H, m), 1.98 (1H, m), 2.33 (1H, m), 1.78 (1H, m), 2.03 (1H, m).

**13 C-NMR** (**CDCl₃**, 125 MHz), δ 49.6 (C-1), 20.0 (C-2), 33.4 (C-3), 58.5 (C-4), 217.5 (C-5), 39.9 (C-6), 42.2 (C-7), 39.0 (C-8), 53.1 (C-9), 26.9 (C-10), 25.8 (C-11),
Also, compared with the reference (Katakawa et al., 2011), we could confirm that compound 2 was 13-O-acetylfawcettimin, and it was first isolation from natural.

**Flavonoid 3: Apigenin**

Light yellow powder, melting point 347, 5°C.

ESI-MS: m/z 270.9 [M+H]+, molecular formula was C15H10O5 (M=270).

1 H-NMR (CDCl3, 500 MHz), δ 6.77 (1H, s), 6.92 (1H, d, J=2.0), 6.48 (1H, d, J=2.0), 7.92 (2H, d, J=7.5), 6.93 (2H, d, J=8.5).

13 C-NMR (CDCl3, 125 MHz), δ 161.1 (C-2), 103.7 (C-3), 181.7 (C-4), 163.7 (C-5), 102.8 (C-6), 164.1 (C-7), 98.8 (C-8), 161.1 (C-9), 103.7 (C-10), 121.1 (C-1'), 128.4 (C-2'), 115.9 (C-3'), 161.4 (C-4'), 115.9 (C-5'), 128.4 (C-6').

ESI-MS of compound 3 at m/z 270.9 [M+H]+, corresponding to molecular weight M=270, was suitable for C15H10O5.

In the 1H-NMR, the aromatic ring proton signal of 2 doublets connected at δH 6.92 and 6.48 (J = 2.0 Hz) showed the correlation of HSQC with the carbon resonance at δC 102.8 (d), and 98.8 (d), assigned to H-6 and H-8 of ring A. Two doublet directly connected at δH 7.92 and 6.92 (2H, J = 8.5 Hz) showed long-distance links with 13C-NMR signal at δC 161.4 (C-4'). Therefore, assigned respectively H-2'/6' and H-3'/5' of ring B. Besides, a singlet at δH 6.77 was assigned to H-3. Long-term correlation confirmed the H-3 assignment with C-2 (δC 161.1) and C-1' (δC 121.1). 13C-NMR at δC 164.1 showed the correlation of HMBC with H-6 and H-8, assigned to C-7. In 13C-NMR and DEPT, there were signals of 8 carbon grades and 7 CH groups. Based on the data analyzed above and compared with H-NMR, 13C-NMR in reference (Ersöz et al., 2002), the structure of compound 3 was determined to be Apigenin.

**Flavonoid 4: Kaempferol**

Yellow amorphous powder, melting point 170-172°C.

ESI-MS: m/z 286.8 [M+H]+, m/z 284.8 [M-H]-, molecular formula was C15H10O4 (M=286).

1 H-NMR (CDCl3, 500 MHz), δ 6.19 (1H, d, J = 2.0), 6.43 (1H, d, J = 2.0), 8.04 (2H, dd, J = 8.5, 2.0), 6.92 (2H, d, J = 9.0, 2.0).

13 C-NMR (CDCl3, 125 MHz), δ 146.8 (C-2), 135.6 (C-3), 175.9 (C-4), 160.8 (C-5), 98.2 (C-6), 163.8 (C-7), 93.4 (C-8), 156.1 (C-9), 103.0 (C-10), 121.6 (C-1'), 129.4 (C-2'), 115.4 (C-3'), 159.1 (C-4'), 115.4 (C-5'), 129.4 (C-6').

Compound 4 was obtained as a yellow amorphous powder. ESI-MS at m/z 284.8 [M-H]- and m/z 286.8 [M+H]+, corresponding to molecular weight M=285, was suitable for C15H10O4.

1H-NMR of 4 showed the proton pairs located in the meta position on the aromatic ring at δH 6.19 (1H, d, J = 2.0 Hz) and 6.43 (1H, d, J = 2.0 Hz), corresponding with H-6 and H-8. 1H-NMR also showed the two doublet signals at δH 8.04 (2H, d, J = 2.8, 11.5 Hz, H-2' and H-6') and 6.92 (2H, d, J = 2.8, 9.7 Hz, H-3' and H-5'), corresponding with four aromatic protons in the B ring, featured for 1', 4' flavones. There were 15 C signals observed in 13C-NMR, showed by DEPT and HMQC as 14 carbon sp2 atoms and one carbonyl signal at δC 175.9. The unsaturation occupied 8/11 double bond, and the remaining 3 of unsaturation were suitable for the flavonol structure. Comparing NMR data of 4 with Kaempferol (Aisyah et al., 2017), compound 4 was determined to be Kaempferol (Figure 1).

**Evaluation of anti-Helicobacter pylori effect**

Agar plates with test samples were ethyl acetate fraction mixed with H. pylori and compared with
standard agar plates of *H. pylori* (10⁵ bacteria/mL). The *H. pylori* inhibition effect levels are shown in Table 1.

The results of the research in Table 1 showed that ethyl acetate fraction at 1/8 dilution compared to the original solution, the fraction effectively inhibited *H. pylori* after 2 hours, after 6 hours and after 24 hours of exposure. When at 1/16 concentration after 2 hours of exposure to the fraction, the intensity of bacteria decreased to 10², and after 6 hours, after 24 hours of exposure, *H. pylori* is completely inhibited. At 1/32 concentration and 1/128 concentration after 2 hours, 6 hours of exposure, the bacterial concentration also decreased, and after 24 hours, *H. pylori* is completely inhibited. Thus, the MIC of the extract fraction is 1/16.

*H. pylori* and gastric juice are considered to be the two main factors causing stomach ulcers in humans. *H. pylori* can cause inflammation and necrosis of gastric parietal cells. This bacterium also secretes the urease enzyme that breaks down urea in the stomach into ammonia, which damages the mucus layer of the stomach and produces other toxins that make the epithelial cells oedema, necrosis facilitates acid and pepsin attack causing ulcers (Malfertheiner et al., 2009; Sobic-Mikutinovic, 2015). Therefore, eliminating *H. pylori* was an important target in the treatment of gastritis with its infection. Some researches have shown that extracts are resistant to bacteria may be due to antibiotic extracts, which may also contain extracts that make the environment unsuitable for bacterial growth, or synergistically with other extracts to improve the antimicrobial effect. With the results of our research above, ethyl acetate extract from *S. Nobilis* leaves had ability to anti-HP (Konstantinopoulou et al., 2003; Wu et al., 2008) It was possible to explain the above results because the chemical constituents of the extract fraction had the effect of inhibiting bacteria including *H. pylori*, such as Apigenin and kaempferol (Wang et al., 2019; Escandón et al., 2016).

**CONCLUSIONS**

Four compounds were isolated from the ethyl acetate fraction of *Sanchezia Nobilis* Hook. F leaves extract. They were 1: Fawcettidine, 2: 13-O-acetylfawcettimine, 3: Apigenin and 4: Kaemferol. Compound 1, 3, 4 were first isolated from the *Sanchezia* genus, and compound 2 was first isolated from natural. The ethyl acetate fraction has also been shown to affect *Helicobacter pylori*.

**Conflict of Interest**

These authors have declared that there is no conflict of interest.

**Funding support**

None.

**REFERENCES**

Aisyah, L. S., Yun, Y. F., Herlina, T., Julaeha, E., Zainuddin, A., Nurafida, I., Hidayat, A. T., Supratman, U., Shiono, Y. 2017. Flavonoid Compounds from the Leaves of *Kalanchoe proliferandra* Their Cytotoxic Activity against P-388 Murine Leukemia Cells. *Natural Product Sciences*, 23(2):139–145.

Burnell, R. H., Chin, C. G., Mootoo, B. S., Taylor, D. R. 1963. Lycopodium alkaloids: Part VIII. New alkaloids from Jamaican Lycopodium species. *Canadian Journal of Chemistry*, 41(12):3091–3094.

Cockerill, F. R. 2010. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing M100-S20. *National Committee for Clinical Laboratory Standards*, 39(1):1–25.

Dao, T., Vui, D., Thu, K., Bui, T., Tung 2019. Two new abietane diterpenes huperphlegmarins A and B from Huperzia phlegmaria. *Original Article*, 11(4):396–399.

Ellah, A. E. A., Mohamed, K. M., Backheet, E. Y., Mohamed, M. H. 2014. Cinnamyl Alcohol, Benzyl Alcohol, and Flavonoid Glycosides from *Sanchezia nobilis*. *Chemistry of Natural Compounds*, 50(5):823–826.

Ersöz, T., Harput, Ü. Ş., Saracoğlu, I., Çalış, I., Ogihara, Y. 2002. Phenolic compounds from Scutellaria pontica. *Turkish Journal of Chemistry*, 26(4):581–588.

Escándón, R. A., del Campo, M., López-Solis, R., Obreque-Slier, E., Toledo, H. 2016. Antibacterial effect of kaempferol and (−)-epicatechin on Helicobacter pylori. *European Food Research and Technology*, 242(9):1495–1502.

Ho, P. H. 2003. Vietnamese Greenery II - Nguyen Hoang Ho. An illustrated Flora of Vietnam. Young Publisher.

Ishii, H., Yasui, B., Nishino, R., Harayama, T., Inubushi, Y. 1970. Structure of Serratinidine and Fawcettidine. A New Type of Lycopodium Alkaloid. *Chemical & Pharmaceutical Bulletin*, 18(9):1880–1888.

J. C. Omond and S. Omond 2015. Phytochemical analysis of 50 selected plants found in the University Botanic Garden, Maseno, Kenya for their chemotaxonomic values. *Journal of Medicinal Herbs and Ethnomedicine*, 1:130–135.

Katakawa, K., Mito, H., Kogure, N., Kitajima, M.,
Wongseripipatana, S., Arisawa, M., Takayama, H. 2011. Ten new fawcettimine-related alkaloids from three species of Lycopodium. *Tetrahedron*, 67(35):6561–6567.

Konstantinopoulou, M., Karioti, A., Skaltsas, S., Skaltsa, H. 2003. Sesquiterpene Lactones from *Anthemisaltissima* and Their Anti-Helicobacter pylori Activity. *Journal of Natural Products*, 66(5):699–702.

Leonard, E. C., Smith, L. B. 1964. *Sanchezia* and related American Acanthaceae. *Rhodora*, 66(768):313–343.

Li, P., Huang, W., Zhuo, J., Guo, Z., Cao, W., Xu, L., Ma, L., Chen, Z.-E., Kennelly, E. J., Wu, S.-B., Long, C. 2015. Seven new Lycopodium alkaloids from the aerial parts of *Phlegmariurus squarrosus*. *Tetrahedron*, 71(33):5308–5314.

Loi, V. D., Tung, B. T., Hai, N. T., Vung, N. T. 2017. In vitro antioxidant and anti-inflammatory activities of isolated compound of ethanol extract from *Sanchezia speciosa* Leonard’s leaves. *Journal of basic and clinical physiology and pharmacology*, 28(1):79–84.

Malferttheiner, P., Chan, F. K., McColl, K. E. 2009. Peptic ulcer disease. *The Lancet*, 374:1449–1461.

Mohamed, K. M., Ellah, A. E. A., Backheet, E. Y., Mohamed, M. H. 2013. Matsutake alcohol glycosides from *Sanchezia nobilis*. *Chemistry of Natural Compounds*, 48(6):930–933.

Nhung, L. T. H. 2018. The chemical constituents and biological activity of n-hexane extract from *Sanchezia speciosa* leaves. *Science and Technology*, 45:110–113.

Paydar, M., Looi, C. Y., Wong, Y. L., Moharam, B. A., Wong, W. F. 2013. In vitro Anti-oxidant and Anti-cancer Activity of Methanolic Extract from *Sanchezia speciosa* Leaves. *Pakistan Journal of Biological Sciences*, 16(20):1212–1215.

Rafshanjani, M. A. S., Parvin, S., Kader, M. A., Saha, M. R., Akhtar, M. A. 2014. In vitro antibacterial, antifungal and insecticidal activities of ethanolic extract and its fractionates of *Sanchezia speciosa* Hook F. *International Research Journal of Pharmacy*, 5(9):717–720.

Shaheen, N., Uzair, M., Ahmad, B. C., Alamgeer 2017. &it in vitro &it cytotoxicity of &it sanchezia speciosa &it extracts on human epithelial cervical cancer (hela) cell line. *Acta poloniae pharmaceutica*, 74(5):1389–1394.

Sokic-Milutinovic, A. 2015. Role of Helicobacter pylori infection in gastric carcinogenesis: Current knowledge and future directions. *World Journal of Gastroenterology*, 21(41):11654–11672.