ISOLATION OF STIGMASTEROL AND *β*-SITOSTEROL FROM *OCIMUM TENUIFLORUM* L. (LAMIACEACE)

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Abstract: Some of the extracts were got from *Ocimum tenuiflorum* L. (Lamiaceace) by soaking and liquid-phase extraction such as n-hexane, chloroform, ethyl acetate and methanol extracts. The aim of this study is to isolation the compound in n-hexane extract from the aerial parts of the plant and then identify and characterize its structure. The isolation and purification afforded white needle-like crystals which were characterized on the basis of spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and HSQC) and in comparison with literature data. The compound was concluded as a mixture of stigmasterol and *β*-sitosterol.

Keywords: *Ocimum tenuiflorum* L., stigmasterol, *β*-sitosterol, extracts.

1. INTRODUCTION

Bioactive natural compounds isolated from the plants still get the concern of many researchers. *Ocimum tenuiflorum* L. (Lamiaceace) is an aromatic plant that plays an important source for essential oils and used for food, perfumery, cosmetic and pharmaceutical industries [2],[7]. All parts of the plant, from leaves, seeds, roots and even the total plant, are used in traditional medicine for treating infections, skin diseases, cold, cough, malarial fever [3], [8]. *Ocimum tenuiflorum* L. extracts were shown to have antibacterial activities against *E. coli*, *S. aureus* and *P. aeruginosa*, anti-stress, antioxidant, antifungal,…[2], [4], [8]. This is the first research of extraction and isolation from the aerial parts of *Ocimum tenuiflorum* L. from Quang Nam province.

2. EXPERIMENTAL

2.1. Plant materials

Wild variety of *Ocimum tenuiflorum* L. was collected in Quang Nam province. The species identification was authenticated by Mr. Do Xuan Cam, botanical taxonomist, Hue University of Agriculture and Foresty. A voucher spicemen is preserved in the library of natural compounds, Department of Chemistry, Hue University of Education. The aerial parts of the plant were manually separated which was then air dried, dried in an oven at 40-50 ^{0}C for 24h and powdered in a fine size.

2.2. Extraction and Isolation

The powdered aerial parts of *Ocimun tenuiflorum* L. (800 g) were extracted with 800 mL of methanol (MeOH) at room temperature for 24 hours and repeated this for several times until the colour of the extract was almost colourless. The whole extract was then filtered through filter

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paper and the filtrate was then evaporated under reduced pressure at 40-50^{o}C using a rotary vacuum evaporator to provide 29.1 g of a gummy concentrate of the crude extract and 17.0 g of a green prepcipitate. The methanol gum was dissolved in a solution of 50% methanol in water . It was partitioned with n-hexane, followed by chloroform ($CHCl_3$) and ethyl acetate (EA). All the extracts were filtered through a filter paper and then concentrated by using a rotary vacuum evaporator to provide n-hexane (1.6 g), then chloroform (4.9 g) and finally with ethyl acetate (5.3 g) extractives. A portion of the green precipitate was partitionally dissolved in n-hexane, chloroform and ethyl acetate to give the three solution respectively. This n-hexane solution after filtration were evaporated *in vacuo* using a rotary evaporator to give 3.4 g of n-hexane extract.

2.3. Chromatographic separation

The n-hexane extract (3.4 g) was fractionated on a silica gel column, eluting with hexane-acetone (solvent system: gradient from 10:0 to 10:5, v/v), to yield 05 major fractions (RHE01-RHE05). Fractions RHE02 (green oil, 0.56 g) were rechromatographed over a silica gel column, eluting with hexane-acetone (gradient from 10:1 to 10:4, v/v), to afford 76 mg of a compound (labelled ETH01) (2.2 % w/w).

All the fractions eluted from the column is spotted on TLC plates using precoated aluminium with silica gel 60 F_{254}. Then the TLC plates were run by specific solvent system and viewed individually under UV light and vanilin-H_2SO_4 in ethanol reagent.

2.4. Spectroscopic characterization

Various spectroscopic techniques were employed for determining the structure of the isolated compounds, including IR, ^{1}H-, ^{13}C-NMR and HSQC. The IR spectrum was recorded on FT-IR Perkin Elmer at Department of Chemistry, Hue University of Education. ^{1}H-NMR, ^{13}C-NMR and HSQC spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR spectrometer at Institute of Chemistry, Vietnam Acedemy of Science and Technology.

3. RESULTS AND DISCUSSION

The IR spectrum showed absorption peaks at 3442.9 cm^{-1}(O-H stretching); a group peaks from 2956.9 cm^{-1} to 2864.3 cm^{-1} (aliphatic C-H stretching); 1635.6 cm^{-1} (C=C absorption peak), 964.4 cm^{-1} (CHR=CHR, *trans* configuration) and other absorption peaks includes 1462.0 cm^{-1} (CH_2); 1028.1 cm^{-1}(cycloalkane) and 802.4 cm^{-1}(CHR=CR_2). These absorption frequencies resemble the absorption frequencies observed for stigmasterol.

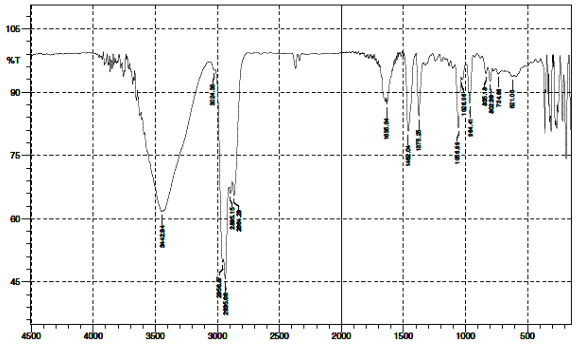

Figure 1. IR spectrum of **ETH01**

Due to the combined results from ^{1}H-NMR, ^{13}C-NMR and HSQC spectra, **ETH01** is a mixture of stigmasterol (**1a**) and *β*-sitosterol (**1b**). The ^{1}H-NMR spectrum of **ETH01** shows the signal of 2 protons at δ_H 5.35 (4H, m, =C**H**, H-6 (**1a**), H-6(**1b**)), 2 protons at δ_H 5.16 (3H, d, =C**H**, H-22) and at δ_H 5.02 (3H, d, =C**H**, H-23). Besides, the multiplet signal of 2 protons at δ_H 3.53 (4H, m, -C**H**-OH, H-3(**1a**), H-3(**1b**)). These are the featured peaks of stigmasterol and *β*-sitosterol. Based on the integrals and disparity in single hydrogen peaks of H-6 (**1a**), H-6 (**1b**), H-22, H-23 in ^{1}H-NMR spectrum, **ETH01** yielded an approximately 3:1 mixture of (**1a**) and (**1b**), respectively.

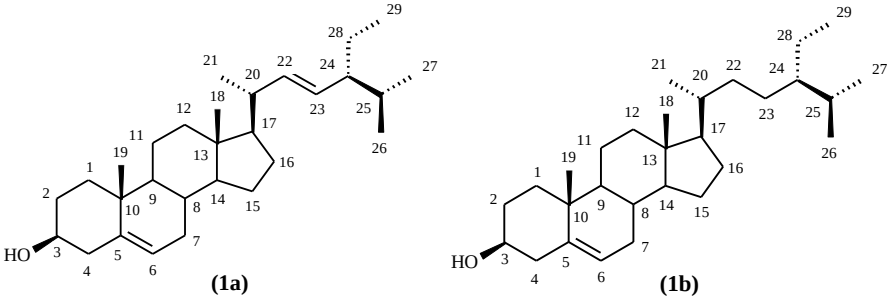

Figure 2. Compounds **ETH01** isolated from *Ocimum tenuiflorum* L. including stigmasterol (**1a**) and *β*-sitosterol (**1b**)

^{1}H-NMR spectrum of stigmasterol shows two multiplets at δ 3.53 and δ 5.35 typical for H-3 and H-6 of a steroidal nucleus. Two olefinic protons appeared at δ 5.16 (1H, dd) and 5.02 (1H, dd) which were identical with the chemical shift of H-22 and H-23 respectively of stigmasterol [1]. The spectrum also displayed two singlets at δ 0.70 and δ 1.01 assignable for H-18 and H-19 respectively. In addition, two doublets at δ 0.84 (3H, d) and 0.83 (3H, d) could belong to the two methyl groups at H-26, H-27 and another doublet at δ 1.02 (3H, d) for H-21. On the other hand, one triplet at δ0.80 (3H, t) could be ascribed to the primary methyl group attached H-29 The ^{13}C-NMR spectrum showed 29 carbons including an oxymethine carbon at δ 71.82, and two olefinic carbons appeared at δ 138.31 and 129.31 which were assigned to the chemical shift of C-22 and C-23 respectively of stigmasterol. In comparison of the HSQC for stigmasterol we confirmed that this compound was having six methyl (CH_3) groups, nine methylene (CH_2), eleven methine (CH) and three quaternary carbons (Cq) groups.

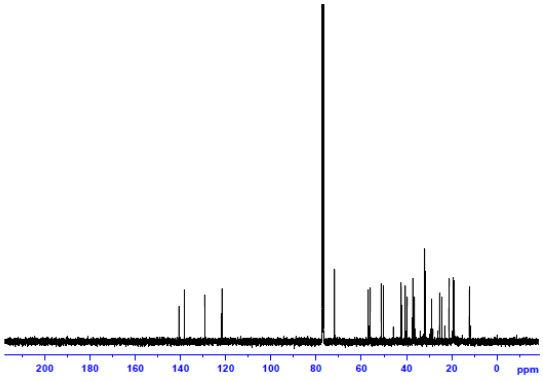

Figure 3. ^{13}C-NMR spectrum of **ETH01**

The ^{1}H-NMR data of *β*-sitosterol were very similar to those of stigmasterol except the presence of the two signals of methylene group for H-22 and H-23 instead of two olefinic protons signals.

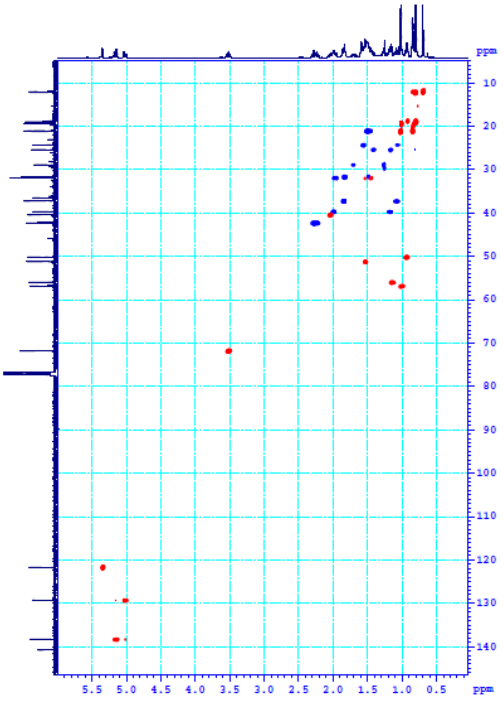

Figure 4. HSQC spectrum of **ETH01**

The above results characterized the feature of the structure of stigmasterol and *β*-sitosterol. The chemical shift values from NMR of stigmasterol and *β*-sitosterol were showed in table 1. These assignments were in good agreements with the reported data [1], [9].

β-Sitosterol and stigmasterol are usually in a mixture form. It is very difficult to obtain stigmasterol or *β*-sitosterol in pure state if they exist in a mixture [5], [6], [9]. The only difference between the two compounds is the presence of the double bond of C22=C23 in stigmasterol and the single bond of C22-C23 in *β*-sitosterol.

Table 1. ^{1}H and ^{13}C-NMR chemical shift values of stigmasterol (**1a**) and *β*-sitosterol (**1b**) recorded in $CDCl_3$

Position of carbon	**(1a)**		**(1b)**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	37.28		37.28	
2	31.69		31.69	
3	71.82	3.53 m	71.82	3.53 m
4	42.33		42.29	
5	140.78		140.78	
6	121.76	5.35 m	121.71	5.35 m

7	31.92		31.92	
8	31.92		31.92	
9	50.19		50.19	
10	36.53		36.53	
11	21.09		21.07	
12	39.71		39.71	
13	42.24		42.33	
14	56.89		56.89	
15	24.37		24.37	
16	28.91		28.91	
17	55.99		55.99	
18	12.06	0.70 s	12.06	0.68 s
19	19.4	1.01 s	19.40	1.01 s
20	40.47		36.53	
21	21.09	1.02 d	18.99	0.93 d
22	**138.31**	**5.16 dd**	**31.83**	**1.28 m**
23	**129.31**	**5.02 dd**	**25.40**	**1.15 m**
24	51.25	1.53	42.29	0.92 d
25	31.88		28.91	
26	21.22	0.84 d	18.99	0.84 d
27	18.99	0.83 d	19.40	0.85 d
28	25.40	1,15	21.13	
29	12.24	0.80 t	12.15	0.86 t

4. CONCLUSIONS

ETH01 was isolated from the extract of aerial parts of *Ocimum tenuiflorum* L. which is a mixture of stigmasterol and β -sitosterol. The structure of **ETH01** was identified on the basis of spectroscopic methods and by comparing with the reported literature. The complete spectral assignments of the two isolated compounds were made based on IR, ^{1}H and ^{13}C-NMR, HSQC spectroscopic data. This is the first report of isolation of stigmasterol and β-sitosterol from *Ocimum tenuiflorum* L. collected in Quang Nam province.

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