

Isolation and Structure Elucidation of Secondary Metabolites from *Echinophora platyloba* DC from Iran

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Abstract

Background: *Echinophora platyloba* DC (Apiaceae) is one of the two endemic species of *Echinophora* genus in Iran. It has many folkloric uses and several biological activities but there is no information about its phytoconstituents.

Objective: In this work some of the secondary metabolites were isolated, purified and identified from n-hexane, ethyl acetate and methanol extracts from the aerial parts and root of this plant for the first time.

Methods: Resulting extracts were subjected to column chromatography using EtOAc/n-hexane as eluent. Further purifications were carried out using preparative TLC and recrystallization techniques. Characterization of compounds were established using spectroscopic data (¹H-NMR, ¹³C-NMR, MS and FT-IR), and by comparing with the literature.

Results: Stigmasterol, Sitosterol, Stigmasterol-β-D-glycoside and Saccharose were purified from these extracts and their chemical structures were characterized.

Conclusion: Several phytosterols were isolated from various extracts of aerial parts and great amount of saccharose was purified from the sweet root of the plant.

Keywords: *Echinophora platyloba*, Saccharose, Sitosterole, Stigmasterol, Stigmasterol glycoside

Introduction

Historically, plants have provided a source of inspiration for novel drugs. Many plant derived medicines have made large contributions to human health and are used worldwide for the treatment of diseases [1]. There are about 20,000 species of medicinal plants using in traditional medicines and these are potential reservoirs for new drugs. Traditional medicine in some countries like Iran has a major therapeutic role. Traditional healers have been using different plants to treat people for thousands of years [2].

Echinophora is a genus of Apiaceae family which just 10 species throughout the world which has been defined as: *E. tenuifolia*, *E. anatolica* Boiss, *E. vadiaus* Boiss, *E. tournefortiijoub*, *E. trichophylla* Sm, *E. spinosa*, *E. orientalis*, *E. sibthorpiana*, *E. cinerea* and *E. platyloba*. The last four of these species are native to Iran [3]. There are many folkloric herbs that people use them without modern scientific evidences. One of them is *Echinophora platyloba* DC, which is one of the endemic species of this genus in Iran [4]. The Persian name of this plant is “Khosharizeh”.

An important application of *E. platyloba* in Iran (Chaharmahal va Bakhtiari province) is using of it for food seasoning and also it is used to prevent the fungal growth on some food like tomato paste and pickled cucumber. Traditionally it is used as flavouring agent for soup, meat, and dairy products in Azerbaijan province, Iran [5]. *E. platyloba* has also medicinally important properties such as antifungal, antibacterial and anti-primary dysmenorrhea properties [6]. The effectiveness

of the ethanol extract of *E. platyloba* on *Candida albicans* has been demonstrated [2]. *E. platyloba* extracts could reduce the severity of premenstrual syndrome [7].

A previous study has shown the presence of components such as flavonoids, alkaloids and saponins in *E. platyloba* [8] but there is a lack of information on the phytochemicals of this plant. Rahimi et al. obtained the essential oil of the aerial parts of *E. platyloba* by hydrodistillation and analyzed the chemical composition by GC and GC-MS [10]. The volatile compounds of *E. platyloba* have been determined by headspace solid phase micro extraction in combination with capillary GC-MS [11]. Hashemi et al. analyzed the volatile components of aerial parts of *Echinophora cinerea* Boiss by the hydrodistillation-solvent micro extraction (HD – SME) method coupled to GC-MS [9].

Sterols are very important structural components of plasma membranes and precursors of steroidal hormones in both animals and plants. The biosynthesis of these compounds has been extensively investigated for elucidation of the origin of structural diversity in plant sterols [12, 13]. Sterols are integral components of the membrane lipid bilayer [14]. Wide structural variety of phytosterols such as Sitosterol, stigmasterol and campesterol were known and their structures are not completely identical to those of animals and fungi.

To the best of our knowledge, no detailed phytochemical studies on *E. platyloba* have been reported. The present research is an attempt to isolation and identification of some phytoconstituents of the aerial parts and root

of this species.

Materials and methods

All reagents were purchased from Merck (Germany) and used without further purification. Infrared spectra were recorded in KBr and were determined on a Perkin Elmer FT-IR spectrometer. ¹H-NMR spectra were recorded on Bruker Avance AC-400MHz using CDCl₃, D₂O and MeOD as the deuterated solvents. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. All melting points are uncorrected and measured in open glass-capillaries using Stuart melting point apparatus. Silica gel (230 – 400 mesh, Merck, Germany) was employed for column chromatography. GC/MS analyses were carried out on a “Trace GC-Trace MS” instrument (England) with electron ionization source, quadropole analyzer, capillary DB-5MS column and Helium as carrier gas.

Plant material

The fresh aerial parts and roots of the plant *Echinophora platyloba* DC were collected from East Azerbaijan province, Bonab, Iran, in July 2011 and identified by plant systematic expert Dr. Fatemeh Mahmoodi Kordi and a voucher specimen (10-1390) has been deposited in the herbarium of the department of Biology, Azarbaijan Shahid Madani University, Tabriz, Iran.

Extraction and Isolation

The dried aerial parts (1.5 kg) and roots (1 kg) of *E. platyloba* were ground to very small size powder and extracted with n-hexane

(3 × 10 L) individually for 3 weeks at room temperature and filtered off. The residue was extracted with ethyl acetate (3 × 10 L) for 3 weeks too. The residue was also extracted with methanol (3 × 10 L). Concentration of solutions of n-hexane, ethyl acetate and methanol under reduced pressure led to the related solvent extracts 44 g, 38 g and 40 g, respectively. The n-hexane portion was subjected to column chromatography on silica gel with a gradient elution of n-hexane/EtOAc (1:0 → 0:1) as eluent to afford nine fractions: F1 (1 g), F2 (1.3 g), F3 (1.6 g), F4 (0.8 g), F5 (0.2 g), F6 (0.1 g), F7 (4 g), F8 (1.0 g) and F9 (0.4 g). F7 was subjected to column chromatography (smaller column) with n-hexane/ethyl acetate (7:3→0:1) and finally purified by preparative TLC with n-hexane/ethyl acetate (2:8) to afford impure compounds 1 and 2. These impure crudes were separately further purified two times by preparative chromatography with chloroform/acetone (19:1) to afford pure compound 1 (11 mg, R_f = 0.45) and 2 (5 mg, R_f = 0.40).

The methanol extract was loaded on a chromatography column on silica gel with a gradient of n- EtOAc/methanol (1:0→3:7) as eluent to afford four fractions: F1 (0.1 g), F2 (0.15 g), F3 (12.0 g), F4 (1.1 g). F3 was purified by recrystallization and resulted to pure compound 3 (900 mg).

The ethyl acetate fraction was subjected to silica gel column chromatography with a gradient of n-hexane/EtOAc (1:0→0:1) and EtOAc/methanol (1:0→6:4) as eluent to afford eleven fractions: F1 (0.2 g), F2 (0.3 g), F3 (1.1 g), F4 (0.5 g), F5 (0.3 g), F6 (4.5 g), F7 (1.2



g), F8 (0.5 g), F9 (1.1 g), F10 (0.8 g) and F11 (0.2 g). F6 was subjected to column chromatography with EtOAc/methanol (1:0→5:5) and finally purified by preparative thin layer chromatography with n-hexane/EtOAc (1:9) to afford impure compound 4. This impure crude was purified by preparative TLC with chloroform/methanol (4:1) to afford pure compound 4 (120 mg, R_f = 0.70).

Results

The phytochemical investigation of n-hexane, ethyl acetate and methanol extracts obtained from the aerial part and root of

E. platyloba afforded four natural compounds (Figure 1). Sitosterole and stigmasterol were isolated from the n-hexane extract. Stigmasterol-β-D-glycoside was isolated from the ethyl acetate extract. Large amounts of saccharose was isolated and identified from the methanol extract of the root of *E. platyloba*.

Discussions

The structures of compounds 1–4 were elucidated by detailed spectroscopic analysis and comparison of their spectroscopic data with those reported in the literature:

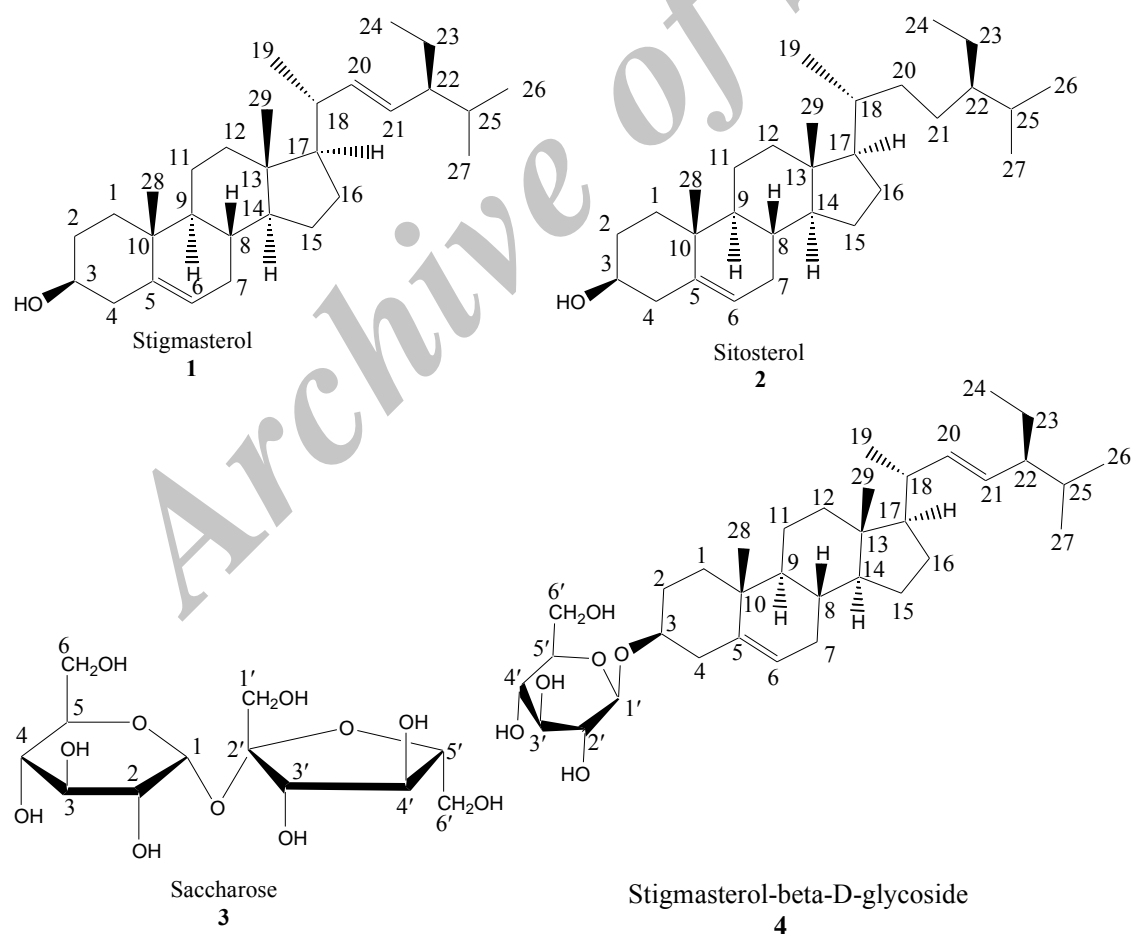


Figure 1- Compounds 1–4 isolated from the *Echinophora platyloba* DC.

Compound 1

Stigmasterol was isolated as a white powder (m.p = 160-163°C). The molecular formula of this compound was determined to be C₂₉H₄₈O by the positive ion at *m/z* 412.51 [M]⁺ in the GC-MS and by the comparison of IR, ¹H-NMR (400 MHz, CDCl₃, δ, ppm, *J*/Hz) and ¹³C-NMR (100 MHz, CDCl₃, δ, ppm) spectra with those reported in the literature. The IR spectrum displayed absorptions at 3200–3450, 1582, 1574 and 1211 cm⁻¹, consistent with the presence of hydroxyl group, C-C double bonds and C-O single bond respectively. The occurrence of two double bonds containing three olefinic hydrogens in the molecule could be easily deduced from the ¹H-NMR [δ 5.06 (1H, dd, *J* = 8.4 and 8.8 Hz, =C(20)H), 5.18 (1H, dd, *J* = 8.4 and 8.8 Hz, =C(21)H), 5.38 (1H, m, =C(6)H)] and ¹³C-NMR [δ 140.77C (5), 138.35C (20), 129.28C (21), 121.73C (6)]. The signals of other protons in the ¹H-NMR spectrum were also assigned as: 3.52-3.58 (1H, quintet, *J* = 6.0 and 4.8 Hz, CH-OH), 0.71 (6H, d, *J* = 7.2 Hz, C (25) H (CH₃)₂) and 1.17-2.31 (37H, m, aliphatic-H). The signals of all the carbons in the ¹³C-NMR spectrum were observed at: δ= 24.39(C29), 25.43(C18), 26.07(C27), 28.27(C19), 29.15(C26), 31.68(C23), 31.91(C11), 33.96(C15), 36.17C(C28), 36.53(C16), 37.27(C2), 39.70(C8), 39.79(C7), 40.53(C10), 42.33(C1), 43.32(C12), 45.84(C22), 50.14(C13), 50.16(C4), 51.26(C25), 55.96(C9), 56.07(C24), 56.78(C17), 56.88(C14), 71.82(C3), 121.73C(6), 129.28C(21), 138.35C(20), 140.77C(5).

Compound 2

Sitosterol was purified as a white powder (m.p = 137-140°C). The molecular formula of this compound was determined to be C₂₉H₅₀O by the positive ion at *m/z* 414.50 [M]⁺ in the GC-MS and molecular structure was elucidated by comparison of IR, ¹H-NMR (400 MHz, CDCl₃, δ, ppm, *J*/Hz) and ¹³C-NMR (100 MHz, CDCl₃, δ, ppm) spectra with those reported in the literature. The ¹H and ¹³C-NMR spectrum of this compound is very similar to compound 1. There are two olefinic bounds in the compound 1 (C5=C6 and C20=C21) but there is just one double bound inside compound 2 (C5=C6). This was deduced from the ¹H-NMR [5.38 (1H, m, =C (6) H)] and ¹³C-NMR [δ 140.77 (C5), 121.75 (C6)]. The ¹H NMR spectra of compound 2 also showed one olefinic proton at δ 5.36 instead of three in 1 at δ 5.06, 5.18 and 5.38. The signals of other protons in the ¹H-NMR spectrum were also assigned as: 3.53 [(1H, tdd, *J* = 4.5, 4.2, 3.8 Hz (C3)), 5.36 [(1H, t, *J* = 6.4 Hz (C5)), 0.93 [(3H, d, *J* = 6.5 Hz (C19)), 0.84 [(3H, t, *J* = 7.2 Hz (C24)), 0.83 [(3H, d, *J* = 6.4 Hz (C26)), 0.81 [(3H, d, *J* = 6.4 Hz (C27)), 0.68 [(3H, s, (C28))] and 1.01 [(3H, s, (C29)]. The signals related to all of the carbon atoms in the ¹³C-NMR spectrum were observed at: δ= 37.5 (C1), 31.9 (C2), 72.0 (C3), 42.5 (C4), 140.77 (C5), 121.75 (C6), 32.1 (C7), 32.1 (C8), 50.3 (C9), 36.7 (C10), 21.3 (C11), 39.9 (C12), 42.6 (C13), 56.9 (C14), 26.3 (C15), 28.5 (C16), 56.3 (C17), 36.3 (C18), 19.2 (C19), 34.2 (C20), 26.3 (C21), 46.1 (C22), 23.3 (C23), 12.2 (C24), 29.4 (C25), 20.1 (C26), 19.6 (C27), 19.0 (C28), 12.0 (C29).



Compound 3

Saccharose was isolated as colorless crystals (m.p = 180.5-183.5°C). The molecular structure of this compound was determined to be C₁₂H₂₂O₁₁ by the positive ion at *m/z* 342.30 [M]⁺ in the MS. IR (KBr): 3387, 3386, 2971, 2913, 1209 cm⁻¹ (O-H broad). ¹H-NMR spectrum (400 MHz, D₂O, δ, ppm, *J*/Hz): 5.32 (1H, d, anomeric H (C1)), 4.12 (1H, d, C(3')H-OH), 3.95 (1H, t, C(5)H-OH), 3.73-3.76 (7H, m), 3.61 (2H, s, C(1')H₂OH), 3.48 (1H, d, *J* = 10.01 Hz), 3.45 (1H, d, *J* = 3.81 Hz). ¹³C-NMR spectrum (100 MHz, D₂O, δ, ppm): 57.44 (C6), 59.56 (C1'), 60.78 (C6'), 61.83 (C4), 68.67 (C2), 70.53 (C5), 71.86 (C3), 72.02 (C4'), 73.44 (C3'), 75.84 (C5'), 91.64 (C1), 103.15 (C2').

Compound 4

Stigmasterol-β-D-glycoside was isolated as white powder (m.p = 270-274°C). The molecular formula of this compound was determined as C₃₅H₅₈O₆ by the positive ion at *m/z* 574.42 [M]⁺ in the MS. IR (KBr): 3421 cm⁻¹ (OH), 2959 and 2868 cm⁻¹ (aliphatic CH), 1520 cm⁻¹, 1104 and 1163 cm⁻¹ (C-O bonds). ¹H-NMR spectrum (400 MHz, MeOD, δ, ppm, *J*/Hz): 5.09 (1H, dd, *J* = 8.38 and 8.75 Hz, =C(22)H), 5.12 (1H, dd, *J* = 8.38 and 8.77 Hz, =C(23)H), 5.33 (1H, m, =C(6)H), 3.17 (2H, d, -CH₂OH), 4.33 (1H, d, -CH anomeric, *J* = 7.8 Hz), 3.32-3.36 (3H, m), 2.20-2.35 (2H, m), 1.95-2.10 (3H, m), 1.40-1.91 (10H, m), 0.95-

1.31 (18H, m), 0.78-0.90 (6H, m), 0.78 (6H, d). ¹³C NMR spectrum (100 MHz, MeOD, δ, ppm): 24.41 (C29), 25.47 (C18), 26.17 (C27), 28.37 (C19), 29.45 (C26), 31.81 (C21), 31.99 (C11), 34.16 (C15), 36.33 (C28), 36.73 (C16), 37.46 (C2), 39.90 (C8), 40.23 (C7), 41.36 (C10), 43.30 (C1), 45.88 (C20), 50.74 (C13), 51.16 (C4), 51.66 (C25), 56.36 (C9), 56.37 (C24), 56.78 (C17), 57.35 (C14), 72.67 (C3), 121.99 (C6), 129.48 (C23), 138.45 (C22), 140.83 (C5). And ¹³C NMR spectrum of glycoside portion: δ = 61.28 (C6'), 67.37 (C4'), 69.73 (C2'), 71.42 (C5'), 90.04 (C3'), 104.15 (C1').

Conclusion

In summary, three natural compounds were isolated from the n-hexane and ethyl acetate extracts of the aerial parts of *E. platyloba*. Two of these compounds were identified as sitosterole and stigmasterol from the n-hexane extract. Stigmasterol-β-D-glycoside was isolated from the ethyl acetate extract. Large amounts of saccharose was isolated and identified from the methanol extract of the root of this plant.

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