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Lipophilic Components from the Ecuadorian Plant *Schistocarpha eupatorioides*

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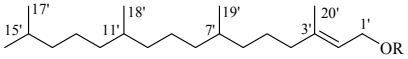
Phytochemical investigation of secondary metabolites of the Ecuadorian plant *Schistocarpha eupatorioides* (Fenzl) Kuntze (Asteraceae) afforded three phytyl fatty acid esters along with a mixture of unidentified polyprenols, the very well known sterols β-sitosterol and stigmasterol, and their corresponding fatty acid esters and glucosyl derivatives. The structures of the compounds were elucidated on the basis of various spectroscopic means. In addition, a volatile fraction was separated the composition of which, comprising sesquiterpene hydrocarbons as the main fraction, was determined by GC-MS.

Keywords: *Schistocarpha eupatorioides*, Asteraceae, triquinane sesquiterpenes, phytyl fatty acid esters, acylglucosylated sterols.

Amongst the wide family of Asteraceae, the Neotropical genus *Schistocarpha* is a member of the tribe Heliantheae and it is noteworthy in the tribe, together with the genus *Neurolaena,* because of having a pappus of many capillary bristles. The genus is widely distributed from Central Mexico southward through Central America into the northern Andes from Venezuela and Colombia to Bolivia [1]. The species *S. eupatorioides* (Fenzl) Kuntze [sin. *S. oppositifolia* (Kuntze) Rydb.] is common throughout the range of the genus, extending farther north in Mexico and farther south in South America than any other species of the genus [1]. The plant is commonly found also in Ecuador, where secondary forests are the preferred habitats. A sap infusion of the plant is used in Andean folk medicine to cure internal ulcers and diarrhoea, whereas topical applications are believed to heal wounds.

There are very few phytochemical studies on the genus *Schistocarpha*: in a couple of papers, Bohlmann and coll. reported the isolation of polyacetylenic compounds from *S. bicolor* Less, *S. longiligula* Rydb., and *S. oppositifolia* (Kuntze) Rydb. [2,3]. In continuation of our ongoing studies on Ecuadorian plants, we describe in this paper the results of the first phytochemical investigation of lipophilic fractions obtained from the CH_2Cl_2 extract of *S. eupatorioides*, reporting the isolation and structure elucidation of three phytyl fatty acid esters (**1-3**), along with a mixture of unidentified polyprenols, the very well known sterols β-sitosterol and stigmasterol, and their corresponding fatty acid esters and glucosyl derivatives. The structures of these compounds were elucidated on the basis of MS, ^{1}H and ^{13}C NMR spectra. Moreover, a volatile fraction was separated the composition of which, comprising sesquiterpene hydrocarbons as the main fraction, was determined by GC-MS.



1 R = $CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6CO$
2 R = $CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6CO$
3 R= $CH_3(CH_2)_{14}CO$

Figure 1. Phytyl esters isolated from *Schistocarpha eupatorioides*.

The ^{1}H NMR spectrum of compound **1** was typical of an esterified acyclic terpenoid alcohol. In fact, it showed a series of H_3 doublets (J 6.5 Hz) in the range of δ 0.8−0.9, attributable to four secondary methyl groups, a broad singlet (3H) at δ 1.72, assignable to an olefinic methyl, a doublet (2H, J = 7.1 Hz) at δ 4.62, coupled (COSY) to a vinylic proton at around δ 5.40, that was attributed to the methylene group of an esterified primary allylic alcoholic group. The feature of the ester moiety was clearly

indicated by a series of signals typical of a long-chain unsaturated fatty acid, such as methylene group resonances at δ 1.24-1.40, a methyl triplet at δ 0.88, and a methylene triplet at δ 2.32 which is diagnostic for a methylene adjacent to an ester carbonyl group [4]. Particularly diagnostic was a triplet at δ 2.84 (J = 5.5 Hz), integrating for 4H and assigned to two methylene groups in bis-allylic position, and a multiplet at δ 5.25−5.48, superimposed on the CH at ca. δ 5.4 and integrating for 6H, which was indicative of three conjugated double bonds in an aliphatic straight-chain. Compound **1** was definitely identified as (*E*)-phytyl linolenate by methanolysis and subsequent separation of (*E*)-phytol [5] (^{1}H and ^{13}C NMR spectra, NOESY spectrum) and methyl (9*Z*, 12*Z*, 15*Z*)-linolenate (identified by GC-MS). Phytyl (9*Z*, 12*Z*)-linoleate (**2**) and palmitate (**3**) were separately identified in a similar way, by ^{1}H NMR spectroscopy and gas chromatography coupled with mass spectrometry (GC-MS) analysis of the products resulting from saponification. Thus the ^{1}H NMR spectrum of **2** was almost identical to the spectrum of ester **1**, except for the numbers of bis allylic protons at δ 2.80 and olefinic hydrogens at δ 5.25−5.48, which were consistent with only two double bonds in a long-chain fatty acid ester moiety. These signals were, of course, lacking in the ^{1}H NMR spectrum of palmitate **3** that, in the olefinic region, showed only the triplet for phytol 2'-H at δ 5.35 (J =7.1 Hz).

Previously, phytyl esters were found in bacteria, dinoflagellates, chlorophytes, mosses, grasses, and some higher plant species [10-22]. The relative abundance and the type of fatty acids linked to phytol vary from organism to organism. In algae, the predominant esterifying acids were the polyunsaturated 16:3 n−3 (15%), 18:5 n−3 (6%), 20:5 n−3 (36%), and 22:6 n−3 (17%) species [7], while in freshwater chlorophytes the 18:3 n−3 and 18:2 n−6 acids were the most abundant ones [9]. In plants, long-chain phytyl esters occur in leaf waxes that have been proposed to play an essential role in the epicuticular transport barrier which hinders the diffusion of water and solutes across the plant cuticle [22]. Interestingly, phytyl esters were found to accumulate in leaves in response to stress factors as frost [13] and drought [16], and during plant senescence [21]. This increase is accompanied by a marked change in phytyl ester composition. In *Arabidopsis* spp., for example, while C-16 and C-18 phytol esters are predominant in seedlings, during senescence phytyl esters are prevalently formed by short chain, saturated fatty acids and hexatrienoic acid (16:3 n−3) [21]. Thus, it has been suggested that plants contains an enzymatic machinery, distinct from *de novo* synthesis, for redirecting free phytol released from chlorophyll degradation into chloroplast lipid metabolism [21]. The types of phytyl ester found in *S. eupatorioides* are therefore indicative of healthy leaf conditions.

Phytyl esters have usually been examined as mixtures by GC-MS, before and after saponification, with the aid of commercial fatty acid methyl ester or authentic synthetic

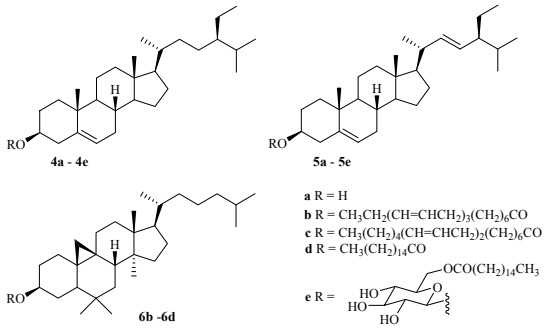


Figure 2: Sterols and triterpenes isolated from *Schistocarpha eupatorioides*.

Table 1. Chemical composition of the volatile hydrocarbon fraction from CH_2Cl_2 extract of *Schistocarpha eupatorioides*.

Compound	RT	RI_{calc}	RI_{lit}	%[a]	IM[b]
unidentified	16.58	1307		0.1	
Silphiperfol-5-ene (**7**)	17.20	1326	1328	0.59	1,2
Silphinene (**8**)	17.81	1345	1347	7.66	1,2
unidentified	17.99	1351		0.21	
unidentified	18.20	1358		0.06	
unidentified	18.78	1376		1.28	
Modeph-2-ene (**9**)	19.05	1384	1383	54.7	1,2
α-Isocomene (**10**)	19.23	1390	1388	19.6	1,2
unidentified	19.43	1396		0.06	
β-Isocomene (**11**)	19.81	1408	1408	9.45	1,2
α-Cedrene (**12**)	19.98	1414	1411	0.29	1,2
unidentified	20.18	1420		0.91	
β-Cedrene (**13**)	20.24	1422	1420	1.70	1,2
Kaurene (**14**)	27.42	2098		3.20	3

[a]Percent calculated by FID peak-area normalization, all relative response factors being taken as one. [b]Identification method: 1- relative retention indices; 2-Adams mass spectral data; 3-Wiley mass spectral data.

samples prepared from a mixture of *cis*/*trans* phytol [21]. Their individual separation has rarely been attempted and demanded the use of silver-ion chromatography [9,12,15]. In this paper we succeeded in the separation of esters **1-3** by simple reversed-phase column chromatography, which allowed determining, for the first time, the complete NMR data of each naturally occurring phytyl ester. They were consistent with incomplete NMR data reported in the literature [12, 20].

Synthetic esters of phytol with fatty acids have been patented as anti-inflammatory and antiulcer agents [23]. This gives credit to the use of an infusion of *S. eupatorioides* leaves by Andean people to heal ulcers [24].

β-Sitosterol **4a** and stigmasterol **5a**, common higher plants sterols, were identified by comparison with authentic samples. They were isolated as an almost equimolecular mixture, as shown by the characteristic olefinic pattern signals for the olefinic protons 22-H/23-H (two dd's at δ 4.95−5.25) for stigmasterol, and 5-H (m at δ 5.38) for both sterols. The same sterols, in inseparable mixtures with tentatively identified cycloartenol derivatives **6b**-**6d**, were found as esters **4b**−**4d** and **5b**−**5d** of α-linolenic, linoleic, and palmitic acids, identified by GC-MS after methanolysis. In addition, palmitic acid was also found esterified to the 6'-OH group of 3-*O*-β-glucosyl ß-sitosterol and stigmasterol **4e** and **5e** [25]. The acylglucosyl sterol

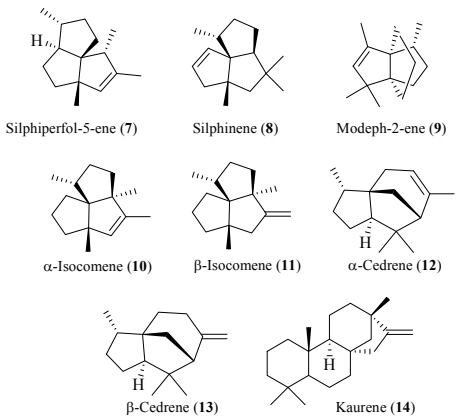


Figure 3: Terpenoid hydrocarbons identified in the volatile fraction from CH_2Cl_2 extract of *Schistocarpha eupatorioides*.

mixture was identified by acid hydrolysis, followed by NMR analysis of the free sterols and GC-MS of methyl palmitate and persilylated D-glucose. The signals of the H_2-6 protons of the glucosyl moiety at δ 4.28 (br d, J = 11.8 Hz) and 4.44 (dd, J = 11.8 and 4.5 Hz) were downfield shifted by ca. 0.6 ppm compared to glucose, suggesting esterification of the hydroxymethylene group.

The volatile hydrocarbon fraction from the CH_2Cl_2 extract of *S. eupatorioides* was characterized by GC and GC-MS analysis. Fourteen compounds were detected in the chromatogram (Table 1) and eight, accounting for a total of 97.2% of the total substances in the fraction, were identified by comparing the experimental gas chromatographic relative retention indices RI [26] and MS fragmentation patterns with corresponding reference data [27]. The most abundant component (ca. 55%) was modheph-2-ene (**9**), the first carbocyclic [3.3.3]propellane to be identified from natural sources [28]. Also the presence of rare triquinane sesquiterpenes silphiperfol-5-ene (**7**) [28b] (0.59%), silphinene (**8**) [29b] (7.66%), α.isocomene (**10**) [29b] (19.6%), and β-isocomene (**11**) [29b] (9.45%) was worthy of note. Kaurene (**14**) (3.2%) was the only diterpene detected in the fraction.

A biogenetic pathway from the triquinane sesquiterpenes from the caryophyllenyl ion was originally proposed by Bohlmann, based on the co-occurrence of the triquinane sesquiterpenes isocomene, modhephene, silphiperfolenes and silphinenes along with caryophyllene in *Silphium perfoliatum* L. (Asteraceae) [29a]. More recently, Weyerstahl found sesquiterpenes of the same types and of at least three other triquinane skeletons in the essential oil of *Echinops giganteus* var. *lelyi* C. D. Adams (Asteraceae) [29b]. On the basis of these data he proposed a biosynthetic route that included all of these constituents, showing a possible interrelationship between them [29b]. The volatile fraction of *S. eupatorioides* proved to be one of the richest source of triquinane sesquiterpenes known so far and gives additional support to the biosynthetic relationship proposed by Weyerstahl.

Though *S. eupatorioides* has no antifungal-related ethnopharmacological uses [30], the quantitative and qualitative composition of the volatile fraction of prompted us to test its antimicrobial activity. In fact, the appreciable antimicrobial activity of the essential oils of *Silphium trifoliatum* L., *Silphium integrifolium* Michx., and *Leontopodium alpinum* Cass. (Asteraceae) was attributed to the presence of triquinane sesquiterpenes [31]. *In vitro* antimicrobial activity of the volatile fraction from *S. eupatorioides* was evaluated against 3 bacterial species (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538) and 2 species of fungi (*Aspergillus niger* ATCC 16404 and *Candida albicans*) by microdilution method. The MIC and the MBC (MFC for fungi) were measured [32,33]. The hydrocarbon fraction resulted active, although weakly (MIC = 2.6 mg/mL), only against *Bacillus subtilis* and it was capable of inhibiting the growth or reproduction of a bacterial colony for a period up to 2 days, as observed by the MBC test.

Experimental

General experimental procedures: IR spectra were recorded on an FT-IR Perkin Elmer Paragon 1000 PC spectrometer as neat film on NaCl discs. ^{1}H and ^{13}C NMR spectra were determined in the indicated solvent on a Bruker DXP 300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C), respectively. ^{1}H and ^{13}C chemical shifts (δ, ppm) are relative to the solvent signals used as references [$CDCl_3$: δ_C (central line of t) 77.16; residual $CHCl_3$ in $CDCl_3$: δ_H 7.26; the abbreviation s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad are used throughout; coupling constants (*J*) are reported in Hz. The number of H-atoms attached to each C-atom was determined by DEPT experiments, using a standard pulse sequence. Positive ion mode ESIMS analysis was performed on a Thermo-Finnigan LCQ HRESIMS analyses were acquired on a Bruker-Daltonics Apex II FT-ICR mass spectrometer. TLC was performed on 0.25 mm silica gel 60 (GF_{254}, Merck) or RP-18 (F_{254}s, Merck), aluminum-supported plates. Compounds were visualized under UV light (254 and 366 nm) or stained by spraying with a 0.5% solution of vanillin in H_2SO_4–EtOH (4:1), followed by charring. Preparative flash column chromatography (FCC) was performed on Kieselgel 60 (40–63 μm, Merck) or reversed phase LiChroprep RP18 (25–40 μm, Merck). The GC-MS analyses were performed with an ITS40 Finnigan coupled to a Perkin Elmer Autosystem gas chromatograph equipped with an HP–5 (Crosslinked 5% PH ME Siloxane) fused silica capillary column (30 m, i.d. = 0.25 mm, f.t. = 0.25 μm), using He as carrier gas (1.00 cc min^{-1}). The injector temperature was 250°C and the detector (FID) was operated at 280°C. Transfer line temperature was 280°C, ion source was at 230°C, EIMS, 70 eV. A standard solution of n-alkanes (C_7-C_{26}) was used to obtain the relative retention indices, calculated according to Van Den Dool [25]. Sil-Prep® (HMDS:TMCS:Py, 3:1:9), used for preparing the TMS

derivatives of sugars, was purchased from Grace (Deerfield, IL, USA).

Plant material: The leaves of *S. eupatorioides* used in this work were collected in the protected forest "JATUN-SACHA", Napo Province (Ecuador). The plant was identified by Mr. Milton Tirado, botanist at the National Herbarium of Quito. A voucher sample has been deposited with the number 0046 at the Herbarium of the Faculty of Chemical Sciences, Laboratory of Phytopharmacy, Universidad Central at Quito.

Extraction and isolation: Air dried leaves of *S. eupatorioides* (0.81 Kg) were finely cut and extracted successively by maceration (3 × 2.5 L, 24 h) with CH_2Cl_2, followed by EtOH–H_2O 70:30 v/v (3 × 2.5 L, 8 h). The extracts were concentrated at reduced pressure to afford 13.1 and 42.6 g of dried residues, respectively. A portion of the CH_2Cl_2 extract (12 g) was partitioned between pentane (200 mL) and MeCN (200 mL). The MeCN layer was extracted with pentane (4 × 200 mL) and combined pentane fractions re-extracted with MeCN (1 × 300 mL). Separate evaporation of the two MeCN layers afforded two residues N1 and N2, 0.64 and 2.8 g, respectively. Concentration of the pentane layer afforded a precipitate P (5.4 g) and a supernatant that was distilled at atmospheric pressure under a CO_2 stream, to remove volatiles from chlorophylls. The condensed volatile fraction V (2.3 g), a slightly smelling pale yellow oil, was analyzed by GC-MS (Table 1). The column oven was programmed from 60°C (1 min) to 260°C at 5°C min^{-1}, then kept at 260°C for 10 min. Precipitate P was separated over a Kieselgel 60 column (220 g). Elution with an increasing gradient of EtOAc in hexane afforded 11 subfractions (from P1 to P11). P1 (247 mg) was identical to V (GC-MS). Further column chromatography (CC) of P2 (2.16 g) over silica gel (110 g) with a gradient of Et_2O in pentane, followed by a pentane–EtOAc gradient, from 25:1 until 100% EtOAc, afforded a volatile fraction P2/1 identical to V (408 mg), followed by 12 subfractions P2/2−P2/13. Multiple CC of P2/2 (0.86 g) over silica gel (eluents: hexane–EtOAc 99:1; hexane–Et_2O 99:1) and RP18 (eluent: a gradient of Me_2CO in MeOH, from 100% MeOH to 100% Me_2CO) delivered, in the order: a) a mixture of unidentified polyprenols (15 mg) of undetermined MWs, in which methyl-substituted *trans* double bonds predominated over *cis* ones by about 4:1; b) a mixture of phytyl esters (46 mg); c) a mixture (0.204 g) of acyl sterols and triterpenes (NMR) that could not be separated between each other by multiple chromatographic separations either over silica gel and RP18. Transesterification of this mixture with 6% MeONa in MeOH at reflux for 5h, followed by CC over silica gel (eluent: hexane–Et_2O 95:5 v/v), afforded a mixture of fatty acids methyl esters, followed by a mixture of stigmasterol, ß-sitosterol, and cycloartenol. Methyl esters were identified as methyl palmitate, linoleate, and linolenate by GC-MS and comparison with authentic samples. The column oven temperature was programmed from 60°C (2 min) to 250°C at a rate of 3°C min^{-1}, then kept at 250°C for 10 min. The mixture of phytyl esters was fractioned over a RP18 column (5 g). Elution with a gradient of Me_2CO in MeOH, from 100% MeOH to 100% Me_2CO, gave, in the order, **1** (2.5 mg), **2** (13 mg), and **3** (21 mg). Repeated CC separations of fractions P10 (84 mg) and P11 (67 mg) on silica gel and RP18 afforded a mixture (15 mg) of 3-*O*-(6'-*O*-palmitoyl)-β-D-glucopyranosyl stigmasterol and ß-sitosterol, **4e** and **5e**.

Phytyl (9*Z*, 12*Z*, 15*Z*)-linolenate (1)

Colorless oil.

R_f: 0.43 (RP18, Me_2CO–MeOH, 2:1).

^{1}H NMR ($CDCl_3$): 0.8−0.9 (12 H, 4×d, *J* = 6.6 Hz, 4 phytyl Me groups), 0.88 (3H, t, *J* = 6.0 Hz, H_3-18), 1.0–1.6 (m, 27H overall, H_2-(4–7), H_2-5', H_2-6', H_2-8', H_2-9', H_2-10', H_2-12', H_2-13', H_2-14', H-7', H-11', H-15'), 1.5–1.7 (2H, m, H_2-3), 1.72 (3H, br s, H_3-3'), 1.95–2.15 (6H, m, H_2-8, H_2-17, and H_2-4'), 2.32 (2H, t, *J* = 7.0 Hz, H_2-2), 2.84 (4H, distorted t, *J* = 6.0 Hz, H_2-11, H_2-14), 4.62 (2H, d, *J* = 7.1 Hz, H_2-1'), 5.25−5.48 (7H, m, H-9, H-10, H-12, H-13, H-15, H-16, H-2').

^{13}C NMR ($CDCl_3$): 173.0 (C-1), 142.1 (C-3'), 131.9 (CH), 130.2 (CH), 128.2 (CH), 127.7 (CH), 127.1 (CH), 118.3 (C-2'), 61.1 (C-1'), 39.7 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 37.2 (2×CH_2), 36.5 (CH_2), 34.1 (CH_2), 32.7 (CH), 32.6 (CH), 29.6 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.8 (CH), 27.2 (CH_2), 25.6 (CH_2), 25.5 (CH_2), 25.0 (2×CH_2), 24.7 (CH_2), 24.3 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 20.6 (CH_2), 19.6 (2×CH_3), 16.4 (CH_3), 14.3 (CH_3).

Positive ion ESI-FT-ICR-MS: *m/z* $[M + H]^+$ calcd for $C_{38}H_{69}O_2$: 557.5298; found: 557.5311.

Phytyl (9*Z*, 12*Z*)-linoleate (2)

Colorless oil.

R_f: 0.40 (RP18, Me_2CO–MeOH, 2:1).

^{1}H NMR ($CDCl_3$): 0.8−0.9 (12 H, 4×d, *J* = 6.5 Hz, 4 phytyl Me groups), 0.88 (3H, t, *J* = 6.0 Hz, H_3-18), 1.0–1.5 (m, 33H overall, H_2-(4–7), H_2-(15–17), H_2-5', H_2-6', H_2-8', H_2-9', H_2-10', H_2-12', H_2-13', H_2-14', H-7', H-11', H-15'), 1.55–1.7 (2H, m, H_2-3), 1.7 (3H, br s, H_3-3'), 1.9–2.2 (6H, m, H_2-8, H_2-14, and H_2-4'), 2.30 (2H, t, *J* = 7.0 Hz, H_2-2), 2.78 (2H, distorted t, *J* = 6.0 Hz, H_2-11), 4.60 (2H, d, *J* = 7.1 Hz, H_2-1'), 5.25−5.48 (5H, m, H-9, H-10, H-12, H-13, H-2'). The data are consistent with incomplete ^{1}H NMR data reported in the literature [12].

^{13}C NMR ($CDCl_3$): 173.2 (C-1), 142.2 (C-3'), 130.1 (CH), 130.0 (CH), 128.1 (CH), 127.8 (CH), 118.3 (C-2'), 61.2 (C-1'), 39.7 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 37.2 (2×CH_2), 36.5 (CH_2), 34.1 (CH_2), 32.7 (CH), 32.6 (CH), 31.5 (CH), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.8 (CH), 27.2 (CH_2), 25.6 (CH_2), 25.5 (CH_2), 25.0 (2×CH_2), 24.7 (CH_2), 24.3 (CH_2), 22.6 (CH_3), 22.6 (CH_2), 22.5 (CH_3), 19.6 (2×CH_3), 16.4 (CH_3), 14.1 (CH_3).

Positive ion ESI-FT-ICR-MS: *m/z* $[M + H]^+$ calcd for $C_{38}H_{71}O_2$: 559.5454; found: 559.5468.

Phytyl palmitate (**3**)

Colorless oil.

R_f: 0.36 (RP18, Me_2CO–MeOH, 2:1).

^1H NMR ($CDCl_3$): 0.8−0.9 (12 H, 4×d, J = 6.6 Hz, 4 phytyl Me groups), 0.88 (3H, t, J = 6.0 Hz, H_3-18), 1.0-1.5 (m, 43H overall, H_2-(4–15), H_2-5', H_2-6', H_2-8', H_2-9', H_2-10', H_2-12', H_2-13', H_2-14', H-7', H-11', H-15'), 1.55–1.7 (2H, m, H_2-3), 1.72 (3H, br s, H_3-3'), 1.9–2.2 (2H, m, H_2-4'), 2.30 (2H, t, J = 7.0 Hz, H_2-2), 4.60 (2H, d, J = 7.1 Hz, H_2-1'), 5.35 (1H, brt, J = 7.1 Hz, H-2').

^{13}C NMR ($CDCl_3$): 174.1 (C-1), 142.7 (C-3'), 118.4 (C-2'), 61.2 (C-1'), 39.6 (CH_2), 39.3 (CH_2), 37.3 (CH_2), 37.2 (2×CH_2), 36.6 (CH_2), 34.6 (CH_2), 32.7 (CH), 32.6 (CH), 32.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.8 (CH), 25.0 (CH_2), 24.7 (2×CH_2), 24.2 (CH_2), 22.7 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.6 (2×CH_3), 16.2 (CH_3), 14.2 (CH_3). The data are consistent with incomplete ^1H and ^{13}C NMR data reported in the literature [12, 20].

Positive ion ESI-FT-ICR-MS: m/z $[M + H]^+$ calcd for $C_{36}H_{71}O_2$: 535.5454; found: 535.5469.

Separate methanolysis (5% MeONa in boiling MeOH) of each phytyl ester **1**−**3** gave (*E*)-phytol [5] and, respectively, methyl (9*Z*, 12*Z*, 15*Z*)-linolenate, (9*Z*, 12*Z*)-linoleate, and palmitate (GC-MS), identical with authentic samples.

Acid hydrolysis of 3-O-(6'-O-palmitoyl)-β-D-glucopyranosyl stigmasterol and ß-sitosterol, 4e and 5e: A solution of the glucosylated sterols **4e** and **5e** (5 mg) in 2N aqueous CF_3COOH (1 mL) was heated for 2 h at 80°C. The aqueous layer was extracted with $CHCl_3$ and the organic layer was treated with ethereal CH_2N_2. After chromatographic separation, stigmasterol and ß-sitosterol were identified by NMR, and methyl palmitate by GC-MS analysis, in comparison with authentic samples. CF_3COOH was removed from the aqueous layer by repeated evaporation with MeCN. The residue coeluted with glucose on comparison with standard sugars (TLC on silica gel; eluent: *n*-BuOH/toluene/pyridine/H_2O, 5/1/3/3). Moreover, after silylation with Sil-Prep®, it coeluted in GC with the TMS derivatives of glucose: α-anomer at 18.8 min, β-anomer at 19.43 min (HP-5 capillary column; oven temperature programmed from 60 to 280°C at a rate of 10°C/min). A positive optical rotation of an aqueous solution indicated the D-configuration of glucose.

Antimicrobial test: *In vitro* antimicrobial activity of the volatile hydrocarbon fraction from *S. eupatorioides* was evaluated against 3 bacterial species (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538) and 2 species of fungi (*Aspergillus niger* ATCC 16404, *Candida albicans*) by microdilution method. The MIC and the MBC (MFC for fungi) were measured [32, 33]. The MIC was the lowest oil solution concentration inhibiting observable microbial growth; the MBC (or MFC) were the lowest concentration resulting in >99.9% reduction of the initial inoculum. For MIC determination bacterial cultures, grown for 24 h, were suspended in Luria-Bertani (LB) broth, whereas one-week old fungal cultures were suspended in Sabouraud broth. Cell suspensions were adjusted to a turbidity of a 0.5 McFarland standard. The volatile hydrocarbon fraction was suspended in Sabouraud culture broth or Luria-Bertani broth for fungi or bacteria, respectively, with 10% Tween 80 and distributed in 96 microwell plates; sample concentrations from 2.6 to 33 mg/mL were tested. Reference compounds, Penicillin-G for bacteria and Amphotericin B for fungi, were tested at concentrations from 0.001 to 0.05 mg/mL. Each test was replicated three times. The plates were incubated for 24 h at 25°C for *B. subtilis*, *S. aureus* and *A. niger* and at 37°C for *E. coli*, and *C. albicans*. Observations were made for further 3 days in order to evaluate the stability of the activity. MBC was determined transplanting the cells from the wells where no growth was observed to cultural medium free of the tested sample.

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