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Identification of some constituents of *Helicteres guazumifolia* Kunth (Malvaceae) leaves from Sucre state, Venezuela

Haydelba D'Armas, Victoria Vásquez, Shailili Moreno and Gabriel Ordaz

Abstract
Some constituents of *Helicteres guazumifolia* Kunth leaves extracts, which were obtained by maceration in petroleum ether and methanol, were proposed to separate and identify. Chemical separation was performed by column and thin layer chromatography, while identification was done by Gas Chromatography-Mass Spectrometric analysis (GC-MS) and 1D-2D Nuclear Magnetic Resonance experiments (^{1}H, ^{13}C, DEPT-135°, HMQC, HMBC, COSY ^{1}H-^{1}H). Several fatty constituents were isolates from non-polar fractions of this *Helicteres* specie, such as: methyl *cis*-13-docosenoate, 1-docosanol, and decyl decanoate. Other constituents were identified by GC-MS as 2-dodecanone, methyl hexadecanoate, butyl (2-methylbutyl) phthalate, ethyl hexadecanoate, *n*-eicosane, di (2-ethylhexyl) phthalate, (22*E*, 24*R*)-stigmasta-4,22-dien-3-one, δ-stigmast-4-en-3-one, benzophenone, (4-methylphenyl) phenylmethanone, 6,10,14-trimethylpentadeca-2-one, dibutylphtalate, 4,8,12,16-tetramethylheptadecan-4-olide, and cyclic 1,2-ethanediyl acetal (5α)-4,4-dimethyl-cholestan-3-one. Results suggest that *H. guazumifolia* Kunth could biosynthesize several chemical families, including fatty acid, hydrocarbon, terpenoid, steroid, and phenolic derivatives, which could suggest its possible ethnomedical uses.

Keywords: *Helicteres*, GC-MS, lipids, isoprenoids, NMR

Introduction
Species of the *Helicteres* genus (Malvaceae, previously included in family Sterculaceae) are characterized by having distinctive fruits, which are spiral capsules composed of five unilocular carpels and may be ovoid to ellipsoid or subcylindrical (Golberg, 2009; Cowie, 2011) [1, 2]. They are widely distributed around the world and their pharmacological potential has gained prominence, especially with *H. isora* and *H. angustifolia* that have a long history of use in traditional Chinese medicine; while about 149 compounds have been isolated from several of *Helicteres* species, including terpenoids, sterols, and phenolic compounds, among others (Fernandes *et al*., 2020) [3].
The specie *H. guazumifolia* Kunth is particularly abundant in several regions of America, such as Mexico (Bravo *et al*., 2016; Notario *et al*., 2020) [4, 5], Costa Rica (Goldberg, 2009) [1], Brazil (Fernandes and Oliveira, 2018; Stavis *et al*., 2020) [6, 7], Colombia (Angarita *et al*., 2014; Sanmartín-Sierra *et al*., 2016) [8, 9], and Venezuela (Lárez, 2007; Rondón and Cumana-Campos, 2007; Fariñas *et al*., 2011, Díaz and Carrasco, 2014) [10-13]. Population of these regions seems to use this specie as medicinal plant (da Costa *et al*., 2020) [14], and although its ethno-botanical uses have not been indicated, they may be related to its effects on fertility and women's health (Yazbek *et al*., 2016) [15].
Previous study of extracts obtained from aerial parts of *H. guazumifolia* Kunth showed slight antimicrobial and antifungal activities, and strong toxic activity against *Artemia salina* (D'Armas *et al*., 2020) [16]. Furthemore, it has been reported the chemical compositions of its leaves essential oil, which was constituted mainly by diisobuthylphtalate, pentadecanal, 2-chloroethyl linoleate, hexahydrofarnesyl acetone, and isophytol, among others (Ordaz *et al*., 2011) [17]. For that, the aim of this study was to isolated and characterize some phytochemical constituents from petroleum ether and methanol extracts of *H. guazumifolia* Kunth leaves collected in Sucre state, Venezuela.

Materials and Methods
Collection and identification of plant materials
Sample of *H. guazumifolia* was collected in the way between Cumaná city and San Juan de Macarapana sector (10°38'44''N, 63°02'20''W; 43 mamsl), Sucre state, Venezuela.

Taxonomic identification was realized at the herbarium "Isidro Ramón Bermúdez Romero", Biology Department, Universidad de Oriente, Sucre Campus, Venezuela.

Extracts

Samples of dried and powdered *H. guazumifolia* Kunth leaves were extracted with petroleum ether. Then, solvent was separated and evaporated under vacuum in a rotary evaporator Heidolph (~11 mbar, 40 °C), obtaining crude petroleum ether extract (PEE). The vegetal residues were re-extracted with methanol. Solvent was separated and anhydrous sodium sulfate was added to dry (~5 g/100 mL of solvent). Then, filtrated solvent was concentrated in the same conditions to obtain crude methanol extract (ME).

Separation

PEE of *H. guazumifolia* Kunth leaves (5.36 g) was chromatographed in column (CC), using silica gel 35-70 mesh as stationary phase in a relation in mass of 30:1 respect to extract. Mobile phase was performed on basis to increasing polarity with mixes of solvents in a relation in volume, starting with petroleum ether-dichloromethane (1:0, 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9, 0:1), then dichloromethane-ethyl acetate (1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 0:1), and finally ethyl acetate-methanol (1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 0:1). They were obtained 124 elutes, which were grouped in 15 fractions (E1-E15, 2.35 g, 43.77% w/w). Thin layer chromatography (TLC) on glass plates (20×20 cm^2) covered with silica gel 60 mesh (0.5 mm) was used to join elutes according to separation observed under ultraviolet light and with ammonium molibdate solution (5% w/v) in aqueous H_2SO_4 (5% v/v). Chromatography of ME of *H. guazumifolia* Kunth leaves (5.08 g) was performed similarly with mixes of petroleum ether-ethyl acetate-methanol in a relation in volume of 1:0:0, 4:1:0, 3:2:0, 1:1:0, 2:3:0, 1:4:0, 0:1:0, 0:4:1, 0:3:2, 0:1:1, 0:2:3, 0:1:4, and 0:0:1. It yielded 101 elutes, which were grouped in 10 fractions (M1-M10, 3.16 g, 62.24% w/w). Continuous separation was performed by CC and preparative TLC (1.0 mm of silica gel thickness).

Characterization

Some fractions obtained after a continuous chromatographic separation were analyzed by Infrared Spectroscopic (using a FTIR 16 PC spectrometer Perkin Elmer), Gas Chromatography-Mass Spectrometry (GC-MS, in a chromatograph Hewlett Packard 5890 II with EI 70 eV, column of methylsilicone of 25 m×0.18 DI×0.18 mm thickness, T (injector) = 280 °C, T_i (oven) = 70°C, rate of 10°C/min, and T_f (oven) = 300 °C; coupled with a mass spectrometer Hewlett Packard 5971 A; total running time was 45 min.), and 1D (^1H, ^{13}C) and 2D (HMBC, HMQC, COSY ^1H-^1H) Nuclear Magnetic Resonance (NMR) (using spectrometers Bruker 600 MHz and Varian 500 MHz). ^1H and ^{13}C spectrum were made at 500.13 MHz and 125.75 MHz, respectively, in solution of deuterated chloroform ($CDCl_3$), expressing chemical shifts in ppm respect to tetramethylsilane (TMS).

Identification by GC-MS was made by comparison of obtained values with those of WILEY and NIST databases, while assignations of chemical shift on NMR experiments were made by comparison with theoretical ^1H and ^{13}C NMR spectrums at Spectral Database for Organic Compounds (AIST, 2017) [24], and NMR predictor available on-line (Aires-de-Sousa *et al*., 2002; Steinbeck *et al*., 2003; Binev and Aires-de-Sousa, 2004; Binev *et al*., 2004; Banfi and Patiny, 2008; Castillo *et al*., 2011) [19-24].

Results

Separation and analysis of fractions from PEE

According to yield and TLC analysis of the fractions from PEE, E2 and E3 were selected to be partitioned. Fraction E2 (157.9 mg) was chromatographed in column (silica gel 35-70 mesh; petroleum ether-dicloromethane-ethyl acetate: 1:0:0, 4:1:0, 3:2:0, 1:1:0, 2:3:0, 1:4:0, 0:1:0, 0:7:3, 0:1:1, 0:3:7, 0:0:1), obtaining 5 fractions (E2.1-E2.5, 96.39% w/w). Fraction E2.2 (58.6 mg) was separated by CC (silica gel 37-70 mesh, petroleum ether-dicloromethene: 1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 0:1) in 5 fractions (E2.2.1-E2.2.5, 87.37% w/w), from which E2.2.5 (15.3 mg) was chromatographed by TLC (silica gel 60 mesh, petroleum ether-dicloromethene: 2:3), obtaining 4 new fractions (E2.2.5.1-E2.2.5.4, 73.46% w/w). Fraction E2.2.5.2 (5.3 mg) was a white solid and showed to be a pure compound according TLC analysis (R_f = 0.26), reason for which it was analyzed by NMR.

Chemical shifts are shown in table 1, which are consistent with an unsaturated fatty acid methyl ester. The ^{13}C-NMR spectrum of fraction E2.2.5.2 showed 15 signals, δ_C 174.30 ppm corresponded to a quaternary carbon of the carbonyl group (C=O), δ_C 130.04-129.79 ppm were signals assigned to nucleus of a C=C bond (methynes, according to DEPT-135°), δ_C 51.42 ppm was assigned to the primary carbon of the methoxy group (-OCH_3). Rocking methylene signals were observed between δ_C 34.17 ppm and δ_C 22.72 ppm, and the terminal methyl group appeared at δ_C 14.12 ppm of the spectrum. Signals at δ_H 5.38-5.28 ppm (*m*) on the ^1H-NMR spectrum were assigned to the unsaturated protons, while the signal at δ_H 3.65 ppm (*s*) was attributed to methoxy protons. Chemical shifts around δ_H 2.28 ppm (*t*), δ_H 1.98 ppm (*d*), δ_H 1.60 ppm (*s*), δ_H 1.25 ppm (*m*) and δ_H 0.86 ppm (*t*), correspond to α-carbonyl methylene protons, α-methylene protons respect to double bond (α-CH_2), β-carbonyl methylene protons, methylene protons of chain, and the terminal methyl protons, respectively. Comparison of these experimental spectral data with theoretical spectrums for several monounsaturated fatty acid methyl esters available at AIST (2017) [24] led us to establish methyl *cis*-13-docosenoate (methyl erucate, figure 1) as the compound isolated in fraction E2.2.5.2.

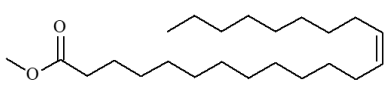

Fig 1: Chemical structure of methyl *cis*-13-docosenoate, possible compound isolated as fraction E2.2.5.2 from petroleum ether extract of *H. guazumifolia* Kunth leaves

Table 1: Chemical shifts of NMR (^1H and ^{13}C) spectrums of fraction E2.2.5.2 obtained from petroleum ether extract of *H. guazumifolia* kunth leaves

δ_C (ppm)	DEPT 135° [a]	δ_H (ppm)	COSY ^1H-^1H [a]
174.30	-(C=O)- (C1)	-	
130.04 *	-CH=CH- (C13, C14)	5.34-5.30 (*m*)	H12, H15
129.79 *			

51.42	-OCH_3 (C23)	3.65	
34.17	α-CH_2 to C=O (C2)	2.31-2.25 (*t*)	H3
31.95	β-CH_2 to CH_3 (C20)	1.28-1.23 (*m*)	
29.82 *	-$(CH_2)_n$- (C7, C16)		H13, H14
29.73 *	-$(CH_2)_n$- (C7, C8, C9)		
29.57 *	-$(CH_2)_n$- (C6, C18)		
29.37 *	-$(CH_2)_n$- (C5, C10, C17, C19)		
29.19	γ-CH_2 to C=O (C4)		H3
22.71	α-CH_2 to CH_3 (C21)		H22
27.27	α-CH_2 to C=C (C12, C15)	2.00-1.98 (*d*)	H13, H14
25.01	β-CH_2 to C=O (C3)	1.59 (*s*)	H2, H4
14.12	-CH_3 (C22)	0.88-0.83 (*t*)	H21

*Interchangeable. [a] Assignations were made on basis to theoretical spectrums of methyl *cis*-13-docosenoate structure (figure 1), SDBS No. 7657 (AIST, 2017) [24].

Fraction E2.3 was analyzed by GC-MS, identifying six majority compounds (figure 2): 2-dodecanone ($C_{12}H_{24}O$ [calculated 184.3184 g mol^{-1}]; RT = 19.55 min, Area = 8.84%, Match = 94%; *m/z* = 184 $[M]^{+\cdot}$, 85 $[M\text{-}C_7H_{15}]^{+\cdot}$, 71 $[M\text{-}C_8H_{17}]^{+\cdot}$, 58 $[M\text{-}C_9H_{18}]^{+\cdot}$ base/main peak, 43 $[M\text{-}C_{10}H_{21}]^{+\cdot}$), methyl hexadecanoate ($C_{17}H_{34}O_2$ [calculated 270.4507 g mol^{-1}]; RT = 20.71 min, Area = 15.52%, Match = 98%; *m/z* = 270 $[M]^{+\cdot}$, 239 $[M\text{-}CH_3O]^{+\cdot}$, 227 $[M\text{-}C_3H_7]^{+\cdot}$, 199 $[M\text{-}C_5H_{11}]^{+\cdot}$, 185 $[M\text{-}C_6H_{13}]^{+\cdot}$, 171 $[M\text{-}C_7H_{15}]^{+\cdot}$, 129 $[M\text{-}C_{10}H_{21}]^{+\cdot}$, 87 $[M\text{-}C_{13}H_{27}]^{+\cdot}$, 74 $[M\text{-}C_{14}H_{28}]^{+\cdot}$ base/main peak, 57 $[M\text{-}C_{13}H_{25}O_2]^{+\cdot}$, 43 $[M\text{-}C_{14}H_{27}O_2]^{+\cdot}$), butyl(2-methylbutyl) phtalate ($C_{17}H_{24}O_4$ [calculated 292.3701 g mol^{-1}]; RT = 21.43 min, Area = 23.83%, Match = 96%; *m/z* = 292 $[M]^{+\cdot}$, 223 $[M\text{-}C_5H_9]^{+\cdot}$, 205 $[M\text{-}C_5H_{11}O]^{+\cdot}$, 149 $[M\text{-}C_9H_{19}O]^{+\cdot}$ base/main peak, 57 $[M\text{-}C_{13}H_{15}O_4]^{+\cdot}$), ethyl hexadecanoate ($C_{18}H_{36}O_2$ [calculated 284.4772 g mol^{-1}]; RT = 21.70 min, Area = 13.11%, Match = 98%; *m/z* = 284 $[M]^{+\cdot}$, 241 $[M\text{-}C_3H_7]^{+\cdot}$, 213 $[M\text{-}C_5H_{11}]^{+\cdot}$, 199 $[M\text{-}C_6H_{13}]^{+\cdot}$, 185 $[M\text{-}C_7H_{15}]^{+\cdot}$, 143 $[M\text{-}C_{10}H_{21}]^{+\cdot}$, 129 $[M\text{-}C_{11}H_{23}]^{+\cdot}$, 115 $[M\text{-}C_{12}H_{25}]^{+\cdot}$, 88 $[M\text{-}C_{14}H_{28}]^{+\cdot}$ base/main peak), *n*-eicosane ($C_{20}H_{42}$ [calculated 282.5475 g mol^{-1}]; RT = 21.79 min, Area = 5.98%, Match = 95%; *m/z* = 282 $[M]^{+\cdot}$, 155 $[M\text{-}C_9H_{19}]^{+\cdot}$, 141 $[M\text{-}C_{10}H_{21}]^{+\cdot}$, 127 $[M\text{-}C_{11}H_{23}]^{+\cdot}$, 113 $[M\text{-}C_{12}H_{25}]^{+\cdot}$, 99 $[M\text{-}C_{13}H_{27}]^{+\cdot}$, 85 $[M\text{-}C_{14}H_{29}]^{+\cdot}$, 71 $[M\text{-}C_{15}H_{31}]^{+\cdot}$, 57 $[M\text{-}C_{16}H_{33}]^{+\cdot}$ base/main peak, 43 $[M\text{-}C_{17}H_{35}]^{+\cdot}$, 29 $[M\text{-}C_{18}H_{37}]^{+\cdot}$), and di(2-ethylhexyl)phthalate (DEHP, $C_{24}H_{38}O_4$ [calculated 390.5561 g mol^{-1}]; RT = 29.06 min, Area = 28.90%, Match = 91%; *m/z* = 390 $[M]^{+\cdot}$, 279 $[M\text{-}C_8H_{15}]^{+\cdot}$, 167 $[M\text{-}C_{12}H_{15}O_4]^{+\cdot}$, 149 $[M\text{-}C_{16}H_{34}O]^{+\cdot}$ base/main peak, 57 $[M\text{-}C_{20}H_{29}O_4]^{+\cdot}$, 43 $[M\text{-}C_{21}H_{31}O_4]^{+\cdot}$). Furthermore, the IR spectrum showed signals due to O-H (3308 cm^{-1}), C-H (2900-2890 cm^{-1}), C=O (1735 cm^{-1}), C-O (1172 cm^{-1}), and C-H (730 cm^{-1}) bonds.

Separation of fraction E3 (100.0 mg) was made by CC (silica gel 35-50 mesh, petroleum ether-ethyl acetate-methanol: 1:0:0, 4:1:0, 3:2:0, 1:1:0, 2:3:0, 1:4:0, 0:1:0, 0:7:3, 0:1:1, 0:3:7, 0:0:1), yielding 5 fractions (E3.1-E3.5, 50.10% w/w). Fraction E3.2 (11.6 mg, beige solid) was separated by CC (silica gel 35-50 mesh, petroleum ether-ethyl acetate: 1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 0:1) in 4 new fractions (E3.2.1-E3.2.4, 55.78% w/w). Fraction E3.2.4 (4.4 mg) was purified by preparative TLC (silica gel 60 mesh, petroleum ether-ethyl acetate: 7:3), recovering a possible pure compound (fraction E3.2.4.1, 3.9 mg, 88.64% w/w, R_f = 0.70). This fraction was analyzed by ^1H and ^{13}C NMR.

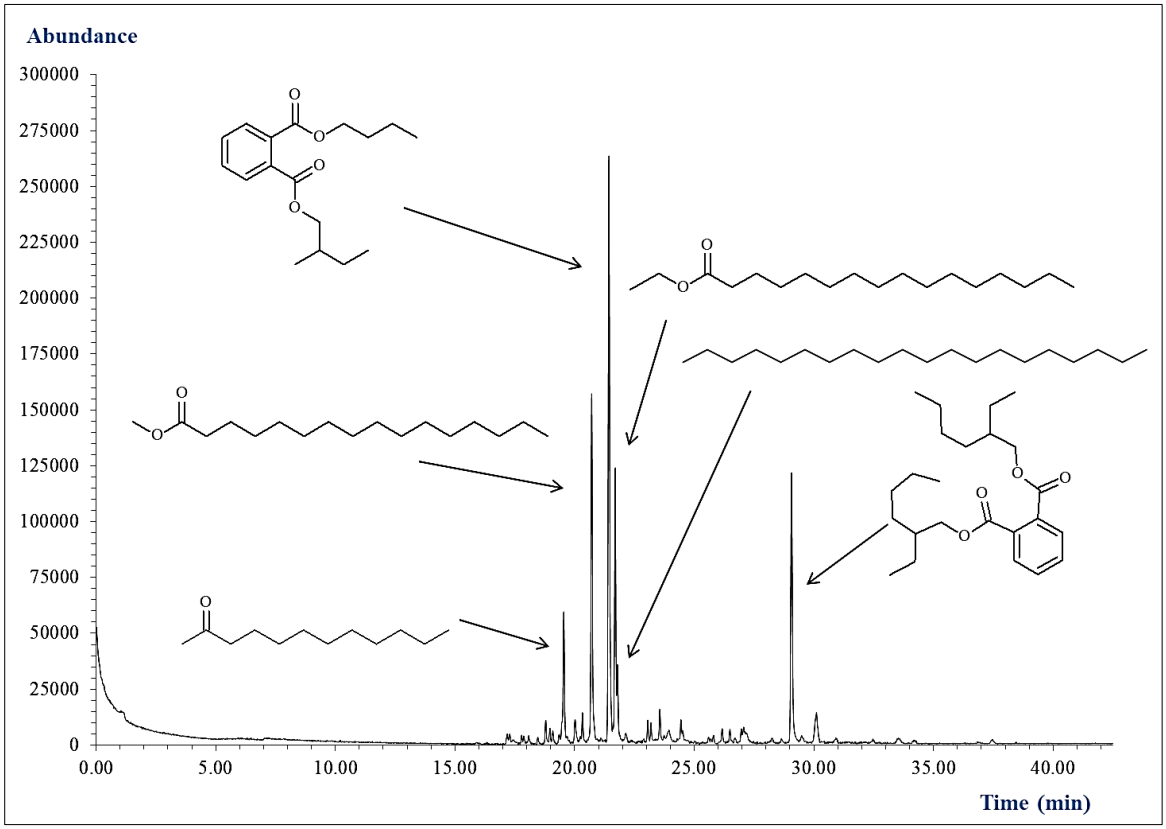

Fig 2: Chromatogram of fraction E2.3, pointing the main constituents.

Experimental spectral data of the fraction E3.2.4.1 is shown in table 2. The ^{13}C-NMR spectrum showed 9 signals that almost all (δ_C 63.16-22.71 ppm) correspond to methylene nucleuses according to DEPT-135° experiment, except δ_C 14.11 ppm, which corresponds to terminal methyl (-CH_3). Signals at δ_H 3.63 ppm (*t*) and 1.55 ppm (*m*) of oxygenated methylene protons and neighbor methylene protons, respectively appeared in ^{1}H-NMR spectrum; δ_H 1.24 ppm (*m*) of other methylene protons, and δ_H 0.87 ppm (*t*) of terminal methyl protons. These signals were consistent with a fatty alcohol, and their comparison with data of theoretical and predicted spectrums for several long chain alcohols, the compound isolated in fraction E3.2.4.1 could be 1-docosanol (figure 3).

Fig 3: Chemical structure of 1-docosanol, possible compound isolated as fraction E3.2.4.1 from petroleum ether extract of *H. guazumifolia* Kunth leaves

Table 2: Chemical shifts of NMR (^{1}H and ^{13}C) spectrums of fraction E3.2.4.1 obtained from petroleum ether extract of *H. guazumifolia* kunth leaves

δ_C (ppm)	DEPT 135° [a]	δ_H (ppm)	COSY ^{1}H-^{1}H [a]
63.16	α-CH_2 to OH (C1)	3.65-3.60 (*t*)	H2
32.91	β-CH_2 to OH (C2)	1.55-1.53 (*m*)	H1
31.97	β-CH_2 to CH_3 (C20)	1.24 (*m*)	
29.72	-$(CH_2)_n$- (C5-C18)		
29.48	δ-CH_2 to OH (C4)		
29.39	γ-CH_2 to CH_3 (C19)		
25.81	γ-CH_2 to OH (C3)		
22.71	α-CH_2 to CH_3 (C21)		H12
14.11	-CH_3 (C22)	0.89-0.84 (*t*)	H11
-	-	1.58 (H-O)	

[a] Assignations were made on basis to theoretical spectrums of 1-docosanol (figure 3), SDBS No. 7647 (AIST, 2017) [24].

Fraction E3.4 was also purified by preparative TLC (silica gel 60 mesh, petroleum ether-dicloromethane: 3:2). Fraction recovered was other possible pure compound (E3.4.1, 3.4 mg, 60.63%, R_f = 0.69) and was analyzed by NMR (table 3). The NMR experiments indicated a carbonyl nucleus (δ_C 173.94 ppm), an oxygenated methylene (δ_C 63.38 ppm; δ_H 4.03 ppm), α-CH_2 to carbonyl (δ_C 34.41 ppm; δ_H 2.27 ppm), β-CH_2 to O bridge (δ_C 28.64 ppm; δ_H 1.59 ppm), other saturated methylenes (δ_C 31.91-25.02 ppm; δ_H 1.23 ppm), and terminal methyl groups (δ_C 14.10 ppm; δ_H 0.86 ppm). These signals are consistent with a wax structure (ester of a fatty acid with a long chain alcohol). According to experimental and theoretical data, the component isolated in fraction E3.4.1 could be decyl decanoate (figure 4).

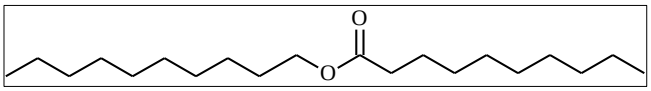

Fig 4: Chemical structure of decyl decanoate, possible compound isolated as fraction E3.4.1 from petroleum ether extract of *H. guazumifolia* Kunth leaves

Table 3: Chemical shifts of NMR (^{1}H and ^{13}C) spectrums of fraction E3.4.1 obtained from petroleum ether extract of *H. guazumifolia* Kunth leaves

δ_C (ppm)	DEPT 135° [a]	δ_H (ppm), HMQC	HMBC [a]
173.94	-(C=O)- (C1)	-	-
64.38	-CH_2O- (C11)	4.05-4.02 (*t*)	H12
34.41	α-CH_2 to C=O (C2)	2.28-2.25 (*t*)	-
28.64	α-CH_2 to -CH_2O- (C12)	1.61-1.56 (*m*)	H11, H13-H15
31.91	β-CH_2 to CH_3 (C8, C18)	1.23 (*m*)	H4-H10, H14-H20
29.64*	γ-CH_2 to CH_3 (C7)		
29.52*	γ-CH_2 to CH_3 (C17)		
29.46*	γ-CH_2 to C=O (C4)		H2, H3, H5-H7
29.34*	γ-CH_2 to –CH_2O- (C14)		H13, H15-H17
29.26*	-$(CH_2)_n$- (C5-C6)		H3-H9
29.15*	-$(CH_2)_n$- (C15-C16)		H13-H19
25.93	β-CH_2 to –CH_2O- (C13)		H11
25.02	β-CH_2 to C=O (C3)		H2
22.67	α-CH_2 to CH_3 (C9, C19)		H6-H8, H10, H16-H18, H20
14.10	-CH_3 (C10, C20)	0.87-0.85 (*t*)	-

*Interchangeable. [a] Assignations were made on basis to theoretical spectrums of decyl decanoate (figure 4), SDBS No. 7580 (AIST, 2017) [24].

Analysis of fractions from ME

According to TLC analyses, fractions M2 (7.9 mg) and M3 (18.2 mg) seemed to contain a few compounds, which showed very close R_f. Due to their low mass, both fractions were selected to be studied by GC-MS. Chromatogram of fraction M2 (figure 5) displayed two main peaks at 29.75 min (39.28%) and 32.70 min (60.72%), which corresponded to two steroidal constituents, (22*E*, 24*R*)-stigmasta-4,22-dien-3-one ($C_{29}H_{46}O$ [calculated 410.6749 g mol^{-1}]; Match = 97%; *m/z* = 410 $[M]^{+\cdot}$, 367 $[M\text{-}C_3H_7]^{+\cdot}$, 298 $[M\text{-}C_8H_{16}]^{+\cdot}$, 271 $[M\text{-}C_{10}H_{19}]^{+\cdot}$, 147 $[M\text{-}C_{18}H_{31}O]^{+\cdot}$, 123 $[M\text{-}C_{21}H_{35}]^{+\cdot}$, 95 $[M\text{-}C_{22}H_{35}O]^{+\cdot}$, 81 $[M\text{-}C_{24}H_{41}]^{+\cdot}$, 69 $[M\text{-}C_{25}H_{41}O]^{+\cdot}$, 55 $[M\text{-}C_{26}H_{43}]^{+\cdot}$ base/main peak, 43 $[M\text{-}C_{26}H_{39}O]^{+\cdot}$), and δ-stigmast-4-en-3-one (sitostenone, $C_{29}H_{48}O$ [calculated 412.6908 g mol^{-1}]; Match = 99%; *m/z* = 412 $[M]^{+\cdot}$, 370 $[M\text{-}C_3H_6]^{+\cdot}$, 281 $[M\text{-}C_8H_{19}O]^{+\cdot}$, 207 $[M\text{-}C_{14}H_{21}O]^{+\cdot}$, 124 $[M\text{-}C_{20}H_{32}O]^{+\cdot}$ base/main peak, 43 $[M\text{-}C_{26}H_{41}O]^{+\cdot}$), respectively.

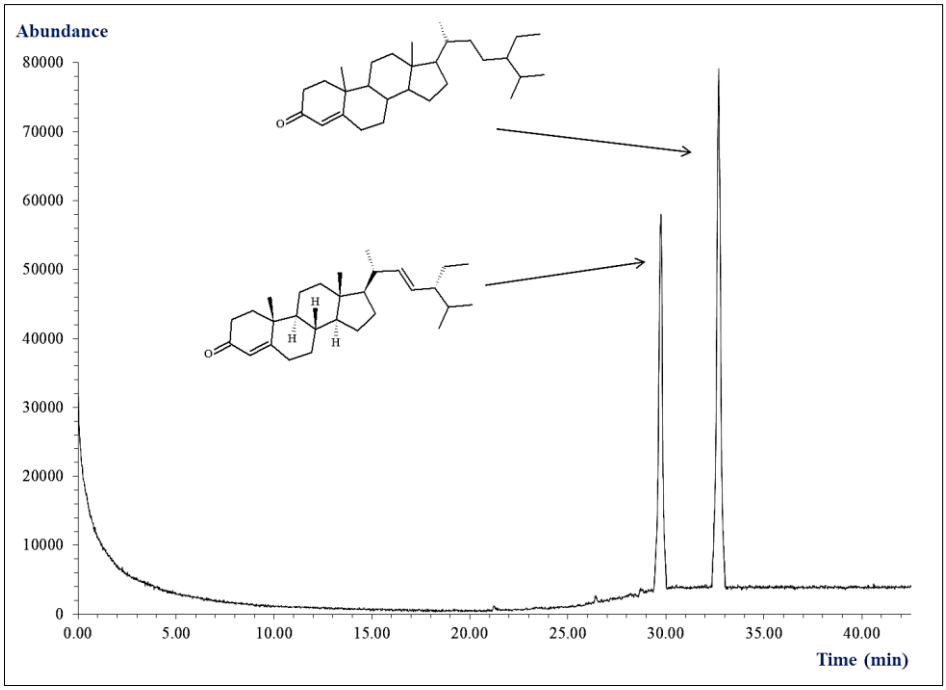

Fig 5: Chromatogram of fraction M2, pointing the main constituents

Chromatogram of fraction M3 (figure 6) displayed 6 main peaks, related to the compounds benzophenone (diphenylmethanone, $C_{13}H_{10}O$ [calculated 182.2179 g mol^{-1}]; RT = 16.13 min, Area = 15.40%, Match = 96%; *m/z* = 182 $[M]^{+\cdot}$, 105 $[M\text{-}C_6H_5]^{+\cdot}$ base/main peak, 77 $[M\text{-}C_7H_5O]^{+\cdot}$, 51 $[M\text{-}C_9H_7O]^{+\cdot}$), 4-methylbenzophenone ((4-methylphenyl)phenylmethanone, $C_{14}H_{12}O$ [calculated 196.2445 g mol^{-1}]; RT = 18.25 min, Area = 7.71%, Match = 97%; *m/z* = 196 $[M]^{+\cdot}$, 181 $[M\text{-}CH_3]^{+\cdot}$, 119 $[M\text{-}C_6H_5]^{+\cdot}$ base/main peak, 105 $[M\text{-}C_7H_7]^{+\cdot}$, 91 $[M\text{-}C_7H_5O]^{+\cdot}$, 77 $[M\text{-}C_8H_7O]^{+\cdot}$, 65 $[M\text{-}C_9H_7O]^{+\cdot}$, 51 $[M\text{-}C_{10}H_9O]^{+\cdot}$), 6,10,14-trimethylpentadeca-2-one (hexahydrofarnesyl acetone $C_{18}H_{36}O$ [calculated 268.4778 g mol^{-1}]; RT = 19.31 min, Area = 43.33%, Match = 95%; *m/z* = 268 $[M]^{+\cdot}$, 225 $[M\text{-}C_3H_7]^{+\cdot}$, 210 $[M\text{-}C_3H_6O]^{+\cdot}$, 194 $[M\text{-}C_4H_{10}O]^{+\cdot}$, 85 $[M\text{-}C_{13}H_{27}]^{+\cdot}$, 71 $[M\text{-}C_{14}H_{29}]^{+\cdot}$, 58 $[M\text{-}C_{15}H_{30}]^{+\cdot}$, 43 $[M\text{-}C_{16}H_{33}]^{+\cdot}$ base/main peak), dibutylphtalate (DBP, $C_{16}H_{22}O_4$ [calculated 278.3435 g mol^{-1}]; RT = 21.10 min, Area = 7.43%, Match = 96%; *m/z* = 278 $[M]^{+\cdot}$, 223 $[M\text{-}C_4H_7]^{+\cdot}$, 205 $[M\text{-}C_5H_{13}]^{+\cdot}$, 149 $[M\text{-}C_8H_{17}O]^{+\cdot}$ base/main peak, 57 $[M\text{-}C_{12}H_{13}O_4]^{+\cdot}$), 4,8,12,16-tetramethylheptadecan-4-olide (5-methyl-5-(4,8,12-trimethyltridecyl)dihydrofuran-2(3H)-one, $C_{21}H_{40}O_2$ [calculated 324.5411 g mol^{-1}]; RT = 26.34 min, Area = 10.58%, Match = 99%; *m/z* = 324 $[M]^{+\cdot}$, 254 $[M\text{-}C_5H_{10}]^{+\cdot}$, 184 $[M\text{-}C_{10}H_{20}]^{+\cdot}$, 114 $[M\text{-}C_{15}H_{30}]^{+\cdot}$, 99 $[M\text{-}C_{16}H_{33}]^{+\cdot}$ base/main peak, 83 $[M\text{-}C_{17}H_{37}]^{+\cdot}$, 69 $[M\text{-}C_{18}H_{39}]^{+\cdot}$, 55 $[M\text{-}C_{18}H_{37}O]^{+\cdot}$), and cyclic 1,2-ethanediyl acetal (5α)-4,4-dimethyl-cholestan-3-one ($C_{31}H_{54}O_2$ [calculated 458.7593 g mol^{-1}]; RT = 28.62 min, Area = 6.67%, Match = 90%; *m/z* = 430 $[M\text{-}C_2H_4]^{+\cdot}$, 340 $[M\text{-}C_6H_{14}O_2]^{+\cdot}$, 125 $[M\text{-}C_{22}H_{37}O_2]^{+\cdot}$, 99 $[M\text{-}C_{26}H_{47}]^{+\cdot}$ base/main peak, 55 $[M\text{-}C_{27}H_{47}O_2]^{+\cdot}$).

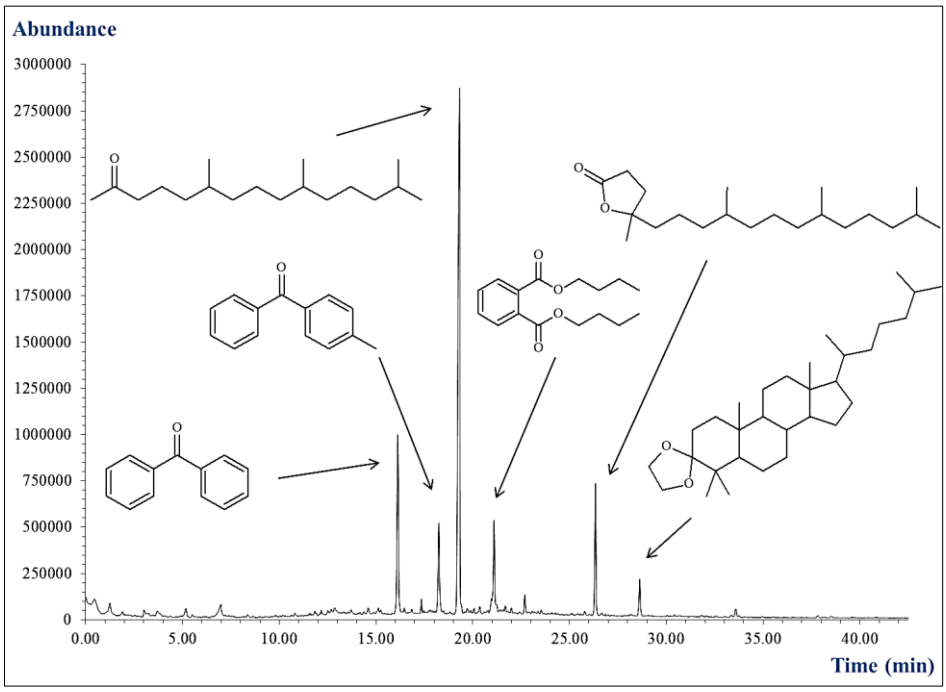

Fig 6: Chromatogram of fraction M3, pointing the main constituents

Discussion

Three lipid derivatives were isolated from *H. guazumifolia* Kunth petroleum ether extract, which were characterized by NMR as methyl *cis*-13-docosenoate, 1-docosanol and decyl decanoate, respectively. Methyl *cis*-13-docosenoate (or methyl erucate) has been reported in the petroleum ether extract of *Luffa echinata* Roxb fruit (Rachana *et al*., 2019) [25], and bioactive oils of *Horsfieldia glabra* seeds (Waman *et al*., 2021) [26], and the *Brassica nigra* seed (Olgun *et al*., 2017) [27]. Fatty acid methyl esters from vegetable oils have shown antimicrobial and antioxidant activities, possibly due to the presence of unsaturated ones (Pinto *et al*., 2017) [28].

The fatty alcohol 1-dodecanol is well known by its antiviral bioactivity against herpes simplex viruses 1 and 2 (Pope *et al*., 1996; Leung and Sacks, 2005) [29, 30]. It has been isolated from the bark of *Rhamnus caroliniana* chloroform extract (Mekala *et al*., 2017) [31], and identified in extracts of *Kielmeyera Coriacea* leaf (Figueiredo *et al*., 2014) [32] and *Gomphrena decumbens* Jacq. (Yamuna *et al*., 2017) [33]. While decyl decanoate has been identified as the main constituent of the extracts of abdominal tergite glands of virgin honeybee queens (*Apis Mellifera* L.), indicating its potential effect as pheromone (Espelie *et al*., 1990; Trhlin and Rajchard, 2011; Villar *et al*., 2019) [34-36].

Other fatty acid derivatives were identified by GC-MS, such as methyl and ethyl palmitate; phenolic compounds, such as phthalates and benzophenone derivatives; some steroidal and acyclic terpenoids, and other hydrocarbons. Phthalate derivatives had been commonly pointed as contaminants in the process of purification in natural product studies (Bhakuni and Rawat, 2005; Venditti, 2018) [37, 38]. DBP, DEHP and other phthalate derivatives are known as toxic components, especially on reproductive system (Pan *et al*., 2006) [39]. These compounds also could be absorbed from water and soil into the plant root and be accumulated for them, thus identification of phthalates in plant extracts or essential oils could be a signal of environmental pollution (Manayi *et al*., 2014) [40].

However, other studies suggest that phthalates derivatives could occur naturally, which are different of synthetic ones in terms of the abundance of ^{14}C and its bond structure, leading to its varied activities in the biological system (Zhang *et al*., 2018; Narayan 2020) [41, 42]. The compound di-2-ethylhexyl phthalate (DEHP), identified in this study, has been isolated from several organisms that appear to biosynthesize it naturally, such as *Calotropis gigantea* (plant), *Streptomyces* sp. (bacteria) and *Penicillium janthinellum* (fungi), showing interesting antibacterial and antitumor activities (Ortiz and Sansinenea, 2018) [43]. For that, it is difficult to confirm if phthalates identified here have a natural origin or are contaminants, but their presence might mean, in the first case, a potential source of bioactive compounds, and, in the second, a possible pollution in the area of recollection of sample or in the experimental processes.

Steroidal constituents (22*E*, 24*R*)-stigmasta-4,22-dien-3-one and δ-stigmast-4-en-3-one have also been identified together in bioactive extracts of *Cenchrus setigerus* (Singariya *et al*., 2014) [44] and *Boophone haemanthoides* (Ibrakaw *et al*., 2021) [45].

Conclusions

Results confirm that *Helicteres guazumifolia* is a source of several phytochemical compounds, especially lipids (fatty acids, fatty alcohols, waxes, sterols and isoprenoids), which are widely distributed in plants and could be used by themselves due to their ecological interactions. Furthermore, the compounds isolated and identified in this study are known for their biological activities. In this sense, this specie could represent an important ethnobotanical resource.

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References

1. Goldberg L. Patterns of nectar production and composition, and morphology of floral nectaries in *Helicteres guazumifolia* and *Helicteres baruensis* (Sterculiaceae): two sympatric species from the Costa Rican tropical dry forest. Rev. biol. Trop 2009;57(S1):161-177.
2. Cowie I. New taxa and notes on *Helicteres* L. (Malvaceae: Helicteroideae) from the Northern Territory, Australia. The Beagle, Records of the Museums and Art Galleries of the Northern Territory 2011;27:27-54.
3. Fernandes D, de Assis E, Souza M, Vanderlei P, Vanderlei M. *Helicteres* L. Species (Malvaceae Sensu Lato) as source of new drugs: a review. Química Nova 2020;43(6):787-803. DOI: https://doi.org/10.21577/0100-4042.20170533
4. Bravo O, Sánchez-González A, de Nova J, Pavón N. Composición y estructura arbórea y arbustiva de la vegetación de la zona costera de Bahía de Banderas, Nayarit, México [Composition and structure of trees and shrubs of the vegetation of the coastal area of Bahía de Banderas, Nayarit, Mexico]. Botanical Sciences 2016;94(3):603-623.
DOI: https://doi.org/10.17129/botsci.461
5. Notario S, Retana O, Vargas J. Mantenimiento de fauna silvestre durante la temporada de secas mediante parcelas de manejo [Sustaining wildlife during dry season through management plots]. Tropical and Subtropical Agroecosystems 2020;23(1):15(12).
6. Fernandes A, Oliveira A. Flora das cangas da Serra dos Carajás, Pará, Brasil: Malvaceae [Flora of the canga of the Serra dos Carajás, Pará, Brazil: Malvaceae]. Rodriguésia 2018;69(3):1237-1254.
DOI: http://dx.doi.org/10.1590/2175-7860201869325
7. Stavis V, Ribeiro P, Gardenal B. Estrutura de fragmento de floresta estacional em área de ecótono, Mato Grosso do Sul [Strucuture of an seasonal forest fragment at an ecotono area, Mato Grosso do Sul, Brazil]. Brazilian Journal of Development 2020;6(8):59197-59213. DOI:10.34117/bjdv6n8-366
8. Angarita H, Sanmartín S, Mercado G. Nuevos registros corológicos para Sucre (Sanguaré-Colombia) [New chorological record to Sucre (Sanguaré-Colombia)]. Revista Colombiana de Ciencia Animal-RECIA 2014;6(2):327-334.
9. Sanmartín-Sierra D, Angarita-Hernández D, Mercado-Gómez J. Estructura y composición florística del bosque seco tropical de Sanguaré-Sucre (Colombia) [Structure and Floristic Composition of Tropical Dry Forest of Sanguaré-Sucre (Colombia)]. Ciencia en Desarrollo 2016;7(2):43-56.

10. Lárez A. Claves para identificar malezas asociadas con diversos cultivos en el Estado Monagas, Venezuela [Keys to identify weeds associated with different plant crops in the Monagas State, Venezuela]. Revista Científica UDO Agrícola 2007;7(1):91-121.
11. Rondón J, Cumana-Campos L. Revisión taxonómica delgénero *Helicteres* L. (sterculiaceae) en Venezuela [Taxonomic revisión of genus *Helicteres* L. (Sterculiaceae) in Venezuela]. Acta Botánica Venezuelica 2007;30(1):163-190.
12. Fariñas J, Vásquez M, Cumana L, Barrios R, Leonett L, Rodríguez G, Mark D. Flórula de plantaciones de palma aceitera establecidas en el estado Monagas, Venezuela [Florula of oil palm plantations established in Monagas State, Venezuela]. Revista Científica UDO Agrícola 2011;11(1):71-82.
13. Díaz W, Carrasco S. Florística de comunidades vegetales de un sector de Matanzas, Puerto Ordaz, estado Bolívar, Venezuela [Floristic composition of plant communities in a sector of Matanzas, Puerto Ordaz, State of Bolívar, Venezuela]. Boletín del Centro de Investigaciones Biológicas 2014;48(3):224-241.
14. da Costa L, da Silva S, Schaffrath V. Usos das plantas do cerrado pela comunidade rural Nossa Senhora da Guia, no município Mato-Grossense de Cáceres, Brasil [The use of Cerrado´s plants by the Nossa Senhora da Guia rural community, city of Cáceres, Mato Grosso, Brazil]. Revista GeoPantanal 2020;28:111-125.
15. Yazbek P, Tezoto J, Cassa F, Rodrigues E. Plants used during maternity, menstrual cycle and other women'shealth conditions among Brazilian cultures. Journal of Ethnopharmacology 2016;179:310-331. DOI: http://dx.doi.org/10.1016/j.jep.2015.12.054
16. D'Armas H, Vásquez V, Ordaz G. Phytochemical screening and bioactivity analysis of extracts from *Helicteres guazumifolia* Kunth (Sterculiaceae). International Journal of Herbal Medicine 2018;6(1):47-50.
17. Ordaz G, D'Armas H, Yáñez D, Moreno S. Composición química de los aceites esenciales de las hojas de *Helicteres guazumifolia* (Sterculiaceae), *Piper tuberculatum* (Piperaceae), *Scoparia dulcis* (Arecaceae) y *Solanum subinerme* (Solanaceae), recolectadas en Sucre, Venezuela [Chemical composition of essential oils from leaves of *Helicteres guazumifolia* (Sterculiaceae), *Piper tuberculatum* (Piperaceae), *Scoparia dulcis* (Arecaceae) and *Solanum subinerme* (Solanaceae) from Sucre, Venezuela]. Revista de Biología Tropical 2011;59(2):585-595.
18. Aires-de-Sousa J, Hemmer M, Gasteiger J. Prediction of ^{1}H NMR chemical shifts using neural networks. Analytical Chemistry 2002;74(1):80-90. DOI: https://doi.org/10.1021/ac010737m
19. Steinbeck C, Krause S, Kuhn S. NMR Shift DB - Constructing a free chemical information system with open-source component. Journal of Chemical Information and Computer Sciences 2003;43(6):1733-1739. DOI: https://doi.org/10.1021/ci0341363
20. Binev Y, Aires-de-Sousa J. Structure-based predictions of ^{1}H NMR chemical shifts using feed-forward neural networks. Journal of Chemical Information and Computer Sciences 2004;44(3):940-945. DOI: https://doi.org/10.1021/ci034228s
21. Binev Y, Corvo M, Aires-de-Sousa J. The impact of available experimental data on the prediction of ^{1}H NMR chemical shifts by neural networks. Journal of Chemical Information and Computer Sciences 2004;44(3):946-949. DOI: https://doi.org/10.1021/ci034229k
22. Banfi D, Patiny L. www.nmrdb.org: Resurrecting and processing NMR spectra on-line. International Journal of Chemistry 2008;62(4):280-281. DOI: https://doi.org/10.2533/chimia.2008.280
23. Castillo A, Patiny L, Wist J. Fast and accurate algorithm for the simulation of NMR spectra of large spin system. Journal of Magnetic Resonance 2011;209(2):123-130. DOI: https://doi.org/10.1016/j.jmr.2010.12.008
24. AIST, National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds SDBS 2017. http://sdbs.db.aist.go.jp
25. Rachana G, Pathak A, Tailang M. Extraction isolation and analytical characterization of phytoconstituent of fruit of *Luffa echinata* Roxb. Journal of Pharmacognosy and Phytochemistry 2019;8(3):2041-2046.
26. Waman A, Bohra P, Roy T, Shivashankara K. Seed morphological and biochemical studies in certain wild nutmegs. Trees 2021. https://doi.org/10.1007/s00468-021-02091-1
27. Olgun Ç, Özkan O, Güney B, Pattabanoglu E, Güney K, Gür M. Chemical composition and antimicrobial activity in cold press oil of fennel, anise, white and black mustard seeds. Indian Journal of Pharmaceutical Education and Research 2017;51(3):S200-S204. DOI: http://dx.doi.org/10.5530/ijper.51.3s.12
28. Pinto M, Araújo S, Morais M, Sá N, Lima C, Rosa C *et al*. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. Anais da Academia Brasileira de Ciências 2017;89(3):1671-1681. DOI: http://dx.doi.org/10.1590/0001-3765201720160908
29. Pope L, Marcelletti J, Katz L, Katz D. Anti-herpes simplex virus activity of n-docosanol correlates with intracellular metabolic conversion of the drug. Journal of Lipid Research Volume 1996;37:2167-2178.
30. Leung D, Sacks S. Docosanol: a topical antiviral for herpes labialis. Expert Opinion on Pharmacotherapy 2004;5:12:2567-2571. DOI: https://doi.org/10.1517/14656566.5.12.2567
31. Mekala A, Satyal P, Setzer W. Phytochemicals from the Bark of *Rhamnus caroliniana*. Natural Product Communications 2017;12(3):403-406.
32. Yamuna P, Abirami, P, Vijayashalini P, Sharmila M. GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of Gomphrena decumbens Jacq. Journal of Medicinal Plants Studies 2017;5(3):31-37.
33. Figueiredo C, Matsuo A, Massaoka M, Girola N, Azevedo R, Rabaça A *et al*. Antitumor activity of *Kielmeyera Coriacea* leaf constituents in experimental melanoma, tested *in vitro* and *in vivo* in syngeneic mice. Advanced Pharmaceutical Bulletin 2014;4(S1):429-436. DOI: https://dx.doi.org/10.5681/apb.2014.063
34. Espelie K, Butz V, Dietz A. Decyl decanoate: a major component of the tergite glands of honeybee queens (*Apis mellifera* L.). Journal of Apicultural Research 1990;29(1):15-19. DOI: https://doi.org/10.1080/00218839.1990.11101192
35. Trhlin M, Rajchard J. Chemical communication in the honeybee (*Apis mellifera* L.): a review. Veterinarni Medicina 2011;56(6):265-273.
36. Villar G, Hefetz A, Grozinger, C. Evaluating the effect of honey bee (*Apis mellifera*) queen reproductive state on

pheromone-mediated interactions with male drone bees. Journal of Chemical Ecology 2019;45:588-597.
DOI: https://doi.org/10.1007/s10886-019-01086-0
37. Bhakuni D, Rawat D. Bioactive marine natural products. Anamaya/Springer, India 2006, P215.
38. Venditti A. What is and what should never be: artifacts, improbable phytochemicals, contaminants and natural products. Natural Product Research (Natural Product Letters) 2018;34(7):1014-1031.
DOI: https://doi.org/10.1080/14786419.2018.1543674
39. Pan G, Hanaoka T, Yoshimura M, Zhang S, Wang P, Tsukino H *et al*. Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): A cross-sectional study in China. Environmental Health Perspectives 2006;114(11):1643-1648.
DOI: https://doi.org/10.1289/ehp.9016
40. Manayi A, Kurepaz-Mahmoodabadi M, Gohari A, Ajani Y, Saeidnia S. Presence of phthalate derivatives in the essential oils of a medicinal plant *Achillea tenuifolia*. DARU Journal of Pharmaceutical Sciences 2014;22(1):1-6. DOI: https://doi.org/10.1186/s40199-014-0078-1
41. Zhang H, Hua Y, Chen J, Li X, Bai X, Wang H. Organism-derived phthalate derivatives as bioactive natural products. Journal of Environmental Science and Health, Part C 2018;36(3):125-144. https://doi.org/10.1080/10590501.2018.1490512
42. Narajan R. Bioactive natural derivatives of phthalate ester. Critical Reviews in Biotechnology 2020;40(7):913-929.
DOI: https://doi.org/10.1080/07388551.2020.1789838
43. Ortiz A, Sansinenea E. Di-2-ethylhexylphthalate May Be a Natural Product, Rather than a Pollutant. Journal of Chemistry 2018, P7.
DOI: https://doi.org/10.1155/2018/6040814
44. Singariya P, Kumar P, Kumar K. Isolation of new steroids of Kala Dhaman grass (*Cenchrus setigerus*) and evaluation of their bioactivity. Brazilian Archives of Biology and Technology 2014;57(1).
DOI: https://doi.org/10.1590/S1516-89132014000100010
45. Ibrakaw A, Boatwright J, Lesch T, Cupido C, Hussein A. Triterpenes and other minor chemical constituents of *Boophone haemanthoides* F.M. Leight (Amaryllidaceae). South African Journal of Botany 2021;136:35-39.
DOI: https://doi.org/10.1016/j.sajb.2020.06.025