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Phytochemical analysis and bioactivity of *Helicteres baruensis Jacq. (Sterculiaceae)*

Análisis fitoquímico y bioactividad de Helicteres baruensis Jacq. (Sterculiaceae)

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Palabras clave: Helicteres baruensis, bioactividad, hidrocarburos, alcaloides, terpenoides
Key words: Helicteres baruensis, bioactivity, hydrocarbons, alkaloids, terpenoids

ABSTRACT

Samples of *Helicteres baruensis Jacq*. aerial parts were extracted with methanol, and then methanol extract from the leaves was partitioned in petroleum ether and chloroform. Alkaloids, sterols and polyphenols were the secondary metabolite families detected in crude extracts and primary fractions of leaves extract. Fruit and flower extracts exhibited lethal or toxic activity against *A. salina*, while leaf and stem extracts were not toxic against this organism at concentrations lower than 1000 $\mu g \cdot mL^{-1}$. However, fractions of leaves extract showed being active, which suggest antagonism among bioactive constituents. In antimicrobial assay, Gram (+) and Gram (-) microorganisms were not sensible to crude extracts, but they were sensible to fraction of leaves extract, which confirm antagonism of this bioactivity. In this sense, aerial parts of *H. baruensis Jacq*. could be a source of secondary metabolites with pharmaceutical properties. Additionally, analyses by GC-MS of some fractions obtained from chromatographic separations of petroleum ether fraction from leaves allowed identifying several metabolites, among them some lineal saturated and unsaturated hydrocarbons such as hexatriacontane, (E)-7-tetradecene, (Z)-3-hexadecene, and (E)-3-octadecene; phthalate derivatives such as dibutyl phthalate, bis(2-methylbutyl) phthalate, and bis(2-ethylhexyl) phthalate; fatty acid derivatives such as methyl 9,12,15-octadecatrienoate and isopropyl octadecanoate; terpenoids such as cyclic 1,2-ethanediyl mercaptole (5α)-androstan-3-one, 4,8,12,16-tetramethylheptadecan-4-olide, 5,6,7,7a-tetrahydro-2(4H)-benzofuranone, 2-methylacetophenone, γ-terpinene, and 6,10,14-trimethyl-2-pentadecanone; and other constituents such as 2,4-dimethyl-9-oxadodecan-4-ol, triphenyl phosphate, and 6-methoxi-2-ethoxi-7-methyl-7H-purine. From the chloroform fraction of leaves, a compound containing steroidal nucleus was isolated, which is possibly the phytosterol stigmasterol. This is the first report of constituents on *Helicteres baruensis Jacq*. leaves in the literature, which confirms the potentiality of this plant to biosynthesize several phytochemical families.

RESUMEN

Se extrajeron muestras de partes aéreas de *Helicteres baruensis Jacq.* con metanol, y luego el extracto de metanol de las hojas se repartió en éter de petróleo y cloroformo. Alcaloides, esteroles y polifenoles fueron las familias de metabolitos secundarios detectados en extractos crudos y fracciones primarias de extracto de hojas. Los extractos de frutas y flores exhibieron actividad letal o tóxica contra A. salina, mientras que los extractos de hojas y

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tallos no fueron tóxicos contra este organismo a concentraciones inferiores a 1000 μg · mL^{-1}. Sin embargo, las fracciones de extracto de hojas mostraron ser activas, lo que sugiere antagonismo entre los componentes bioactivos. En el ensayo antimicrobiano, los microorganismos Gram (+) y Gram (-) no fueron sensibles a los extractos crudos, pero sí a la fracción de extracto de hojas, lo que confirma el antagonismo de esta bioactividad. En este sentido, partes aéreas de *H. baruensis Jacq*. podría ser una fuente de metabolitos secundarios con propiedades farmacéuticas. Además, los análisis por GC-MS de algunas fracciones obtenidas de separaciones cromatográficas de la fracción de éter de petróleo de las hojas permitieron identificar varios metabolitos, entre ellos algunos hidrocarburos lineales saturados e insaturados como el hexatriacontano, (E) -7-tetradeceno, (Z) -3 -hexadeceno y (E) -3-octadeceno; derivados de ftalato tales como ftalato de dibutilo, ftalato de bis (2-metilbutilo) y ftalato de bis (2-etilhexilo); derivados de ácidos grasos tales como 9,12,15-octadecatrienoato de metilo y octadecanoato de isopropilo; terpenoides tales como 1,2-etanodiil mercaptole (5α) -androstan-3-ona cíclico, 4,8,12,16-tetrametilheptadecan-4-olida, 5,6,7,7a-tetrahidro-2 (4H) -benzofuranona, 2-metilacetofenona, γ-terpineno y 6,10,14-trimetil-2-pentadecanona; y otros constituyentes tales como 2,4-dimetil-9-oxadodecan-4-ol, trifenilfosfano y 6-metoxi-2-etoxi-7-metil-7H-purina. De la fracción de cloroformo de las hojas, se aisló un compuesto que contenía núcleo esteroideo, que posiblemente sea el estigmasterol de fitosterol. Este es el primer informe de constituyentes sobre *Helicteres baruensis Jacq*. deja en la literatura, que confirma la potencialidad de esta planta para biosintetizar varias familias fitoquímicas.

INTRODUCTION

The genus Helicteres belongs to *Sterculiaceae* family, which has shown being a source of secondary metabolites with biological properties (Al Muqarrabun & Ahmat, 2015). This genus comprises around 60 species (Rondón & Cumana-Campos, 2007; Golberg, 2009), but much of the phytochemical and bioactivity analysis appear to be focused on *H. isora Linn* (Kumar & Kumar, 2014; Akshatha et al., 2015; Manke et al., 2015; Pandey et al., 2015; Chandirasegaran et al., 2016; Dayal et al., 2017; Kanthale & Biradar, 2017). The shrubs *H. guazumifolia Kunth* and *H. baruensis Jacq.* are the species more abundant in some regions of America, and are widely distributed in Venezuela (Rondón & Cumana, 2006; Lárez, 2007; Rondón & Cumana-Campos, 2007; Díaz & Febres, 2009; Díaz & Carrasco, 2014; Álvarez & Leite, 2016). However, they have been little studied chemically.

The aim of this study was to evaluate the secondary metabolite profile and bioactivity of extracts of *Helicteres baruensis Jacq.* (*Sterculiaceae*) aerial parts collected in Sucre state, Venezuela, through identification of some constituents responsible for that bioactivity, contributing to phytochemical knowledge of this specie.

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METODOLOGY

Sampling

Sample of *H. baruensis Jacq.* was collected in Guaracayal area, between Cumaná city and Marigüitar sector (latitude and longitude coordinates: 10.45397 and -64.1825638), Sucre state, Venezuela. Taxonomic identification was realized in the herbarium "Isidro Ramón Bermúdez Romero" of Biology Department of Universidad de Oriente, Sucre Campus, Venezuela.

Extracts and first fractions

Each botanical part (leaves, fruits, stems, and flowers) was separated from others, pulverized and extracted with methanol (puriss. p.a., Fluka Chemie, Seelze, Germany). In each case, solvent was evaporated in a rotary evaporator Heidolph (~11 mbar, 40 °C), for obtaining crude extracts (ME). Afterwards, these extracts were solubilized in methanol/distillated water (9:1 in volume) and fractionated with petroleum ether (Class 1A, Fisher Chemical, Fair Lawn, New Jersey, USA). Then, aqueous residues were extracted with pure chloroform (Fluka Chemie, Seelze, Germany), and the new aqueous residues were discarded. Anhydrous sodium sulfate (Fisher Chemical, Fair Lawn, New Jersey, USA) was added in order to dry (~5 g/100 mL of solvent) organic fractions in each case. Subsequently, each solvent was concentrated under the same conditions to obtain crude primary fractions (petroleum ether fraction-PEF and chloroform fraction-CF).

Phytochemical screening

The phytochemicals screening of plants was performed as per well-established protocols (Domínguez, 1973; Marcano & Hasegawa, 2002). Reagents used were: Dragendorff (bismuth nitrate in nitric acid and aqueous potassium iodine) for alkaloids, Liebermann-Burchard (acetic anhydride and chloroform with concentrated sulfuric acid) for sterols and triterpenoids, Baljet (picric acid in ethanol and aqueous sodium hydroxide) for sesquiterpenlactones; aqueous ferric chloride for polyphenols, gelatin for tannins, and Shinoda (magnesium and concentrated hydrochloric acid) for flavonoids.

Brine shrimp (*Artemia salina*) lethality assay

This bioassay was used to detect possible pharmacological properties of extracts. Method described by Meyer et al. (1982) was followed with some modifications. Solution of 10,000 μg·mL-1 of each extract and fraction was prepared using a mix of dimethyl sulfide and sterile sea water (1:1 v/v). Then, dilutions of 1,000.00; 100.00; 1.00; 0.10; and 0.01 μg·mL-1 were prepared. The same solvent system and dilutions were used as negative controls. Ten nauplii of A. salina brine shrimp were put into vials containing those solutions. Bioassay was carried out by triplicate. Mortality quantification after 24 h and 48 h was used

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to calculate median lethal concentration (LC_{50}) according to Stephan (1977).

Antibacterial assay

Antimicrobial activity of extracts was evaluated according Bauer et al. (1966) method. Sterile discs of filter paper (Whatman Nº 3) of 5 mm of diameter were impregnated with 10 µl of extracts (40 mg·mL-1) and put on Petri plates with Müller-Hinton agar, which were previously inoculated with microbial suspensions (108 cells·mL-1, McFarland 0.5). Plates with bacteria were pre-incubated at 5°C for 12 h and then incubated at 37°C for 24 h. Antimicrobial effect was evidenced by diameter of inhibition zone (mm) around the discs.

Chromatographic separation

Separation of constituents was carried out on column chromatography (CC) with silica gel 35-70 mesh (0.2-0.5 mm) as stationary phase in a mass relation of 30:1 (silica: extract or fraction). Mobile phase was performed based on the increasing polarity of pure solvent, such as petroleum ether (PE), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), acetone ($MeCO_2$), ethyl acetate (AcOEt), and methanol (MeOH), besides mixture of them. Thin layer chromatography (TLC) was performed on glass plates (20×20 cm2) covered with silica gel 60 mesh (0.5 mm). The eluates were joined according to separation observed under ultraviolet light (100-280 nm) and with ammonium molibdate solution (5%) in aqueous H_2SO_4 (10%).

Analysis and Characterization of fractions

Some fractions obtained after a continuous chromatographic separation were analyzed by gas chromatography-mass spectrometry (GC-MS) from Ecology Laboratory of Universidad Simón Bolívar (USB). It was used a chromatograph Hewlett Packard 5890 II, with EI 70 eV, column of methylsilicone (25 m×0.18 DI×0.18 mm thickness), T (injector) = 280 °C, Ti (oven) = 70 °C, rate of 6 °C/min, and Tf (oven) = 300 °C; which was coupled with a mass spectrometer Hewlett Packard 5971 A. Identification was made by comparison with WILEY and NIST databases.

UV-Vis, IR and 1H NMR analysis were performed on pure chloroform sub-fraction by the use of UV/VIS Lambda 11 spectrophotometer, FTIR 16 PC Pelkin Elmer spectrophotometer and 500MHz Bruker spectrometer, respectively.

RESULTS AND DISCUSSION

Yield and phytochemical screening

Crude methanol extract of leaves (ME-L) was obtained in greater proportion (14.87 %), than the other aerial part extracts, which extraction yield was among 3.35-8.68 % (table 1). For this reason, ME-L was partitioned with petroleum ether and chloroform, obtaining a non-polar fraction (PEF-L, 14.95%) and a middle-polar fraction (CF-L, 4.90 %). These results suggest that this Helicteres specie has more

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methanol soluble constituents in its leaves than in other botanical organs, which could be very polar (water soluble), followed by non-polar metabolites (lipids).

Table 1. *Yield of methanol extraction of aerial parts of H. baruensis Jacq.*

Crude extract and fraction	mass (g)	Extraction yield (%)
ME-L	37.17	14.87[a]
ME-S	4.54	6.98[a]
ME-Fr	2.31	3.35[a]
ME-Fl	0.76	8.68[a]
PEF-L	4.79	14.95[b]
CF-L	1.57	4.90[b]

ME: methanol extract, PEF: petroleum ether fraction, CF: chloroform fraction, L: leaves, S: stems, Fr: fruits, Fl: flowers. [a]: calculated based on the dry weight of respective botanical part. [b]: calculated based on the methanol extract of leaves.

As shown in table 2, phytochemical screening revealed the possible presence of alcaloids, sterols and polyphenol in some methanol crude extracts (ME) and fractions, and the absence of other phytochemical families. Alkaloids were detected in leaves (ME-L), stem (ME-S) and flower (ME-Fl), while sterols were detected in leaves (ME-L), stem (ME-S), fruit (ME-Fr), and petroleum ether and chloroform fractions from the leaves (PEF-L and CF-L respectively). Furthemore, alkaloids were detected in chloroform fraction (CF-L); whereas polyphenols were detected just in all methanol extracts (table 2). Evaluation of secondary metabolites profile of other *Helicteres* species, such as *H. isora* (Manke et al., 2015; Kanthale & Biradar, 2017) and *H. guazumifolia* (D'Armas, Vásquez & Ordaz, 2018), has shown the presence of polyphenol and alkaloid constituents, which could be a characteristic compound of this genus

Table 2. *Phytochemical families in methanol extracts and fractions of H. baruensis Jacq.*

Family of metabolite	Crude extract and fraction					
	ME-L	ME-S	ME-Fr	ME-Fl	PEF-L	CF-L
Alkaloids	+	+	–	+	–	+
Sterols	+	+	+	–	+	+
Terpenoids	–	–	–	–	–	–
Flavonoids	–	–	–	–	–	–
Polyphenols	+	+	+	+	–	–
Tannins	–	–	–	–	–	–
Cardiac glycosides	–	–	–	–	–	–
Saponins	–	–	–	–	–	–

ME: methanol extract, PEF: petroleum ether fraction, CF: chloroform fraction, L: leaves, S: stems, Fr: fruits, Fl: flowers. +: detected, –: not detected.

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Biological activity of extracts and main fractions

Table 3 shows media lethal concentration (LC50) exhibited by extracts of *H. baruensis Jacq*. against *Artemia salina*. Flowers extract (ME-Fl) showed the most lethal effect at 24 h (LC50 = 100.00 µg·mL-1), followed by fruit extract (ME-Fr, LC50 = 591,19 µg·mL-1). Extracts of leaves (ME-L) and stems (ME-S) were not lethal against A. salina (LC50 > 1000.00 µg·mL-1), however fractions obtained from ME-L exhibited lethal effect (table 3), suggesting a possible antagonism among constituents in ME-L. Results observed in this bioassay indicate that cytotoxic agents occur in *H. baruensis Jacq*, similar to those reported for *H. angustifolia* (Li et al., 2016) and *H. isora* (Dayal et al., 2017). According to Olivas-Quintero et al. (2017) this kind of activity is associated to phenolic compounds (table 1), however alkaloids and sterols are shown by their biological activity (Marcano & Hassegawa, 2002).

Table 3. *Lethal activity of crude extracts and fractions of H. baruensis Jacq. against Artemia salina at 24 h*

Crude extract	LC_{50} (µg·mL^{-1})
ME-L	> 1,000.0
ME-S	> 1,000.0
ME-Fr	591.19
ME-Fl	100.00
PEF-L	575.16
CF-L	213.44

ME: methanol extract, PEF: petroleum ether fraction, CF: chloroform fraction, L: leaves, S: stems, Fr: fruits, Fl: flowers. LC_{50}: media lethal concentration.

With respect of antimicrobial assay, methanol extracts of *H. baruensis Jacq*. did not exhibit any effect against microorganism employed (table 4). Nevertheless, chloroform fraction of ME-L inhibited very significantly the growth of *Bacillus cereus* (15 mm) and *Salmonella enteritidis* (18 mm), while ether petroleum ether fraction of ME-L showed a significant inhibition zone of 11 mm against *Salmonella enteritidis*, which confirms the antagonism among constituents in ME-L and demonstrates the strong antibacterial activity shown by the fractions of the leaves.

Separation and analysis of petroleum ether fraction

Petroleum ether fraction from *H. baruensis Jacq*. leaves extract (PEF-L, 4.79 g) was chromatographed in a glass column (CC) with silica gel 35-70 mesh, using petroleum ether (PE) and mixes of PE with $CHCl_3$ and MeOH as eluents. From this CC, 14 fractions (A1-A14, 4.31 g, 92.18%) were obtained. According to TLC analysis and chromatographic yield, fractions named A6, A7 and A9 were selected to continuous chromatographic separation.

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Table 4. *Antimicrobial activity of crude extracts of H. baruensis Jacq.*

Microorganism	Diameters of inhibition halos* (mm)					
	ME-L	ME-S	ME-Fr	ME-Fl	PEF-L	CF-L
Bacillus subtilis (CVCM 625)	–	–	–	–	–	–
Bacillus cereus (ATCC 9634)	–	–	–	–	–	15 [b]
Staphylococcus aureus (CVCM 48)	–	–	–	–	–	–
Escherichia coli (ATCC 10536)	–	–	–	–	–	–
Enterococcus faecalis (ATCC 9634)	–	–	–	–	–	–
Klebsiella pneumoniae (CVCM 438)	–	–	–	–	–	–
Pseudomonas aeruginosa (ATCC 25416)	–	–	–	–	–	–
Salmonella enteritidis (ATCC 13076)	–	–	–	–	11 [a]	18 [b]

PEF: petroleum ether extract, ME: methanol extract, CF: chloroform fraction, L: leaves, S: stems, Fr: fruits, Fl: flowers. *: included diameter of discs (5 mm), –: inactive, [a]: bacteriostatic effect, [b]: bactericidal effect.

A6 (378.0 mg, orange solid) was separated by CC in 11 subfractions (A6.1-A6.11, 376.7 mg, 99.65%), from which A6.2 (10.0 mg) was purified by preparative TLC with a mix of PE and $CHCl_3$ 4:1 v/v. The obtained subfraction, A6.2.1 (2.3 mg, 23.00 %, R_f = 0.201, uncolored oil), was analyzed by GC-MS and its chromatogram (Fig.1) showed a main constituent identified as **hexatriacontane** ($C_{36}H_{74}$ [calculated 506.9728 g mol^{-1}]; t_R = 29.28 min, 100 %; *m/z* = 506 $[M]^{+\cdot}$, 351 $[M\text{-}C_{11}H_{23}]^{+\cdot}$, 323 $[M\text{-}C_{13}H_{27}]^{+\cdot}$, 295 $[M\text{-}C_{15}H_{31}]^{+\cdot}$, 267 $[M\text{-}C_{17}H_{35}]^{+\cdot}$, 238 $[M\text{-}C_{19}H_{40}]^{+\cdot}$, 224 $[M\text{-}C_{20}H_{42}]^{+\cdot}$, 183 $[M\text{-}C_{23}H_{47}]^{+\cdot}$, 169 $[M\text{-}C_{24}H_{49}]^{+\cdot}$, 141 $[M\text{-}C_{26}H_{53}]^{+\cdot}$, 127 $[M\text{-}C_{27}H_{55}]^{+\cdot}$, 111 $[M\text{-}C_{28}H_{59}]^{+\cdot}$, 85 $[M\text{-}C_{30}H_{61}]^{+\cdot}$, 71 $[M\text{-}C_{31}H_{63}]^{+\cdot}$, 57 $[M\text{-}C_{32}H_{65}]^{+\cdot}$ base peak). This hydrocarbon has been also identified in other plant extracts by GC-MS (Anburaj et al., 2016; Bharathi & Vijaya, 2016; Darweesh & Ahmed, 2017; Rahim et al., 2018).

Subfraction A6.4 (232.1 mg) was chromatographed by CC obtaining 9 subfractions (A6.4.1-A6.4.9, 231.4 mg, 99.69%), from which fraction named A6.4.3 (33.3 mg) was separated by CC in 2 new subfractions (A6.4.3.1-A6.4.3.2, 33.1 mg, 93.99 %). Subfraction A6.4.3.2 (25.1 mg, brown oil) was analyzed by GC-MS identifying four main constituents (Fig. 2): **2,4-dimethyl-9-oxadodecan-4-ol** ($C_{13}H_{22}O_2$ [calculated 216.3602 g mol^{-1}]; t_R = 10.26 min, 15.73 %; *m/z* = 216 $[M]^{+\cdot}$, 197 $[M\text{-}H_3O]^{+\cdot}$, 143 $[M\text{-}C_4H_9O]^{+\cdot}$, 125 $[M\text{-}C_4H_{11}O_2]^{+\cdot}$, 100 $[M\text{-}C_7H_{16}O]^{+\cdot}$, 99 $[M\text{-}C_6H_{13}O_2]^{+\cdot}$ base peak, 71 $[M\text{-}C_9H_{21}O]^{+\cdot}$, 55 $[M\text{-}C_9H_{21}O_2]^{+\cdot}$); **androstan-3-one, cyclic 1,2-ethanediyl mercaptole, (5*α*)** ($C_{21}H_{34}S_2$ [calculated 350.6247 g mol^{-1}]; t_R = 19.54 min, 13.97 %; *m/z* = 350 $[M]^{+\cdot}$, 219 $[M\text{-}C_5H_7S_2]^{+\cdot}$, 176 $[M\text{-}C_8H_{14}S_2]^{+\cdot}$, 149 $[M\text{-}C_{10}H_{17}S_2]^{+\cdot}$, 121 $[M\text{-}C_{12}H_{21}S_2]^{+\cdot}$, 101 $[M\text{-}C_{13}H_{29}S_2]^{+\cdot}$ base peak, 83 $[M\text{-}C_{15}H_{23}S_2]^{+\cdot}$, 55 $[M\text{-}C_{17}H_{27}S_2]^{+\cdot}$); **methyl 9,12,15-octadecatrienoate** (linolenic acid methyl ester, $C_{19}H_{32}O_2$ [calculated 292.4562 g mol^{-1}]; t_R = 21.21 min, 12.84 %; *m/z* = 293 $[M+1]^{+\cdot}$, 292 $[M]^{+\cdot}$, 261 $[M\text{-}CH_3O]^{+\cdot}$, 236 $[M\text{-}C_4H_8]^{+\cdot}$,

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207 [M-C_5H_9O]$^{+\cdot}$, 191 [M-$C_5H_9O_2$]$^{+\cdot}$, 149 [M-$C_8H_{15}O_2$]$^{+\cdot}$, 135 [M-$C_9H_{17}O_2$]$^{+\cdot}$, 121 [M-$C_{10}H_{19}O_2$]$^{+\cdot}$, 95 [M-$C_{12}H_{21}O_2$]$^{+\cdot}$, 79 [M-$C_{13}H_{25}O_2$]$^{+\cdot}$ base peak, 67 [M-$C_{14}H_{25}O_2$]$^{+\cdot}$, 55 [M-$C_{15}H_{25}O_2$]$^{+\cdot}$), and **triphenyl phosphate** (TPP, $C_{18}H_{15}O_4P$ [calculated 326.2831 g mol^{-1}]; t_R = 21.38 min, 20.25 %; *m/z* = 326 [M]$^{+\cdot}$ base peak, 249 [M-C_6H_5]$^{+\cdot}$, 233 [M-C_6H_5O]$^{+\cdot}$, 170 [M-$C_6H_5O_3P$]$^{+\cdot}$, 141 [M-$C_{12}H_9O_2$]$^{+\cdot}$, 94 [M-$C_{12}H_9O_3P$]$^{+\cdot}$, 77 [M-$C_{12}H_{10}O_4P$]$^{+\cdot}$, 65 [M-$C_{13}H_{10}O_4P$]$^{+\cdot}$, 51 [M-$C_{14}H_{12}O_4P$]$^{+\cdot}$).

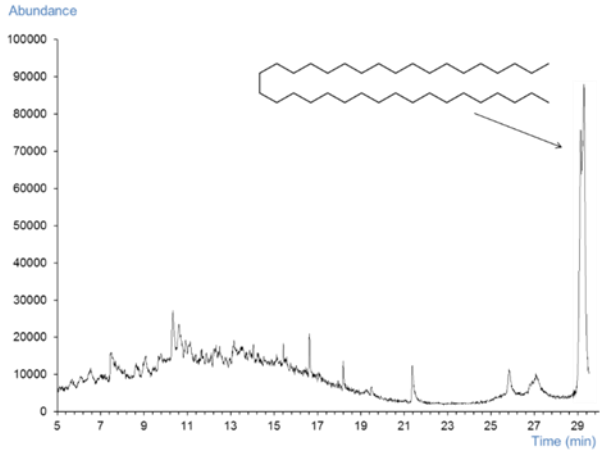

Figure 1. Chromatogram of subfraction A6.2.1, pointing identified constituent

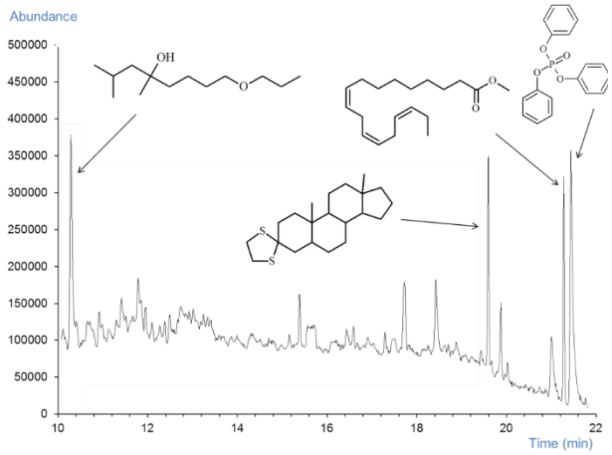

Figure 2. Chromatogram of subfraction A6.4.3.2, pointing identified constituents

None information of 2,4-dimethyl-9-oxadodecan-4-ol was found in the literature, which indicates this could be the first report of this constituent in plant extracts. The androstane derivative (androstan-3-one, cyclic 1,2-ethanediyl mercaptole, 5α) has been reported for methanolic extract of *Cinnamomum zeylanicum* Blume (Lauraceae, cinnamon bark), which was identified using GC-MS analysis (Hameed et al., 2016). Methyl linolenate was one of the main phytochemicals identified in extracts of two *Syzygium* species (Myrtaceae), which showed inhibitory activity against a virulence factor production by *Pseudomonas aeruginosa* (Musthafa et al., 2017). Besides, it was detected in high proportion together with other fatty acid methyl esters and phthalates derivatives in extracts of several *Euphorbia* species, which showed *in vitro* cytotoxicity against different cancer cell lines (Bano et al., 2017). In relation to triphenyl phosphate, it has showed potential endocrine disrupting effects via estrogen receptor α and β (Kojima et al., 2013), and has been detected as phytocompound in *Hamiltonia suaveolens* Roxb. stem extract (Kulkarni & Sathe, 2013).

Fraction A7 (343.1 mg) was separated by preparative TLC in 4 subfractions (A7.1-

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A7.4, 141.8 mg, 41.32 %), using a mix of PE and $CHCl_3$ (2:3 in volume). Subfraction named A7.2 (12.5 mg) was purified by CC. The analysis by GC-MS of the possible pure obtained subfraction (A7.2.1, crystalline solid, 11.8 mg, 94.40 %) showed that it was a mix of *n*-alkenes (Fig. 3) such as: **(*E*)-7-tetradecene** ($C_{14}H_{28}$ [calculated 196.3721 g mol^{-1}]; t_R = 15.53 min, 15.03 %; *m/z* = 196 $[M]^{+\cdot}$, 154 $[M-C_3H_6]^{+\cdot}$, 139 $[M-C_4H_9]^{+\cdot}$, 126 $[M-C_5H_{10}]^{+\cdot}$, 111 $[M-C_6H_{13}]^{+\cdot}$, 97 $[M-C_7H_{15}]^{+\cdot}$, 83 $[M-C_8H_{17}]^{+\cdot}$, 69 $[M-C_9H_{19}]^{+\cdot}$ base peak, 57 $[M-C_{10}H_{19}]^{+\cdot}$); **(*Z*)-3-hexadecene** ($C_{16}H_{32}$ [calculated 224.4252 g mol^{-1}]; t_R = 17.49 min, 29.78 %; *m/z* = 224 $[M]^{+\cdot}$, 196 $[M-C_2H_4]^{+\cdot}$, 182 $[M-C_3H_6]^{+\cdot}$, 168 $[M-C_4H_8]^{+\cdot}$, 154 $[M-C_5H_{10}]^{+\cdot}$, 139 $[M-C_6H_{13}]^{+\cdot}$, 125 $[M-C_7H_{15}]^{+\cdot}$, 111 $[M-C_8H_{17}]^{+\cdot}$, 97 $[M-C_9H_{19}]^{+\cdot}$, 83 $[M-C_{10}H_{21}]^{+\cdot}$ base peak, 69 $[M-C_{11}H_{23}]^{+\cdot}$, 54 $[M-C_{12}H_{26}]^{+\cdot}$), and **(*E*)-3-octadecene** ($C_{18}H_{36}$ [calculated 252.4784 g mol^{-1}]; t_R = 19.88 min, 46.32 %; *m/z* = 253 $[M+1]^{+\cdot}$, 238 $[M-CH_3O]^{+\cdot}$, 235 $[M-C_3H_7]^{+\cdot}$, 223 $[M-C_2H_5]^{+\cdot}$, 208 $[M-C_3H_8]^{+\cdot}$, 193 $[M-C_4H_{11}]^{+\cdot}$, 179 $[M-C_5H_{13}]^{+\cdot}$, 165 $[M-C_6H_{15}]^{+\cdot}$, 153 $[M-C_7H_{15}]^{+\cdot}$, 139 $[M-C_8H_{17}]^{+\cdot}$, 137 $[M-C_8H_{19}]^{+\cdot}$, 123 $[M-C_9H_{21}]^{+\cdot}$, 109 $[M-C_{10}H_{23}]^{+\cdot}$, 95 $[M-C_{11}H_{25}]^{+\cdot}$, 83 $[M-C_{12}H_{25}]^{+\cdot}$, 71 $[M-C_{12}H_{23}]^{+\cdot}$, 57 $[M-C_{14}H_{278}]^{+\cdot}$ base peak).

Alkenes similar to these have been reported as phytochemical constituents of several plants (Faizi et al., 2014; Dubal et al., 2015; Hussein et al., 2015; Shaheen et al., 2016; Elaiyaraja & Chandramohan, 2016; Ahmadvand et al., 2017), which could derivate or produce fatty acid.

Fraction A9 (195.3 mg) was chromatographed in a column with mixtures of PE-CH_2Cl_2 and CH_2Cl_2-Me_2CO, obtaining 6 subfractions (A9.1-A9.6, 177.3 mg, 90.92 %). According to TLC analysis and chromatographic yield, subfractions A9.1, A9.2 and A9.5 were selected to new fractionation. A9.1 (32.2 mg) was separated by preparative TLC with PE-CH_2Cl_2 (3:2 v/v) in 4 subfractions (A9.1.1-A9.1.4, 17.0 mg, 52.79 %), from which A9.1.2 (12.5 mg, light yellow oil) was analyzed by GC-MS. Chromatogram of this subfraction (Fig. 4) showed two main peaks identified as **6-methoxi-2-ethoxi-7-methyl-7H-purine** ($C_9H_{12}N_4O_2$ [calculated 208.2172 g mol^{-1}]; t_R = 10.33 min, 21.14 %; *m/z* = 208 $[M]^{+\cdot}$, 193 $[M-CH_3]^{+\cdot}$, 179 $[M-C_2H_5]^{+\cdot}$, 169 $[M-C_3H_7]^{+\cdot}$, 152 $[M-C_2H_4N_2]^{+\cdot}$, 149 $[M-C_4H_{11}]^{+\cdot}$, 137 $[M-C_3H_7N_2]^{+\cdot}$, 124 $[M-C_4H_8N_2]^{+\cdot}$, 95 $[M-C_5H_9N_2O]^{+\cdot}$, 85 $[M-C_6H_7N_2O]^{+\cdot}$, 71 $[M-C_6H_7N_3O]^{+\cdot}$, 58 $[M-C_7H_8N_3O]^{+\cdot}$ base peak, 51 $[M-C_6H_{11}N_3O_2]^{+\cdot}$), and **dibutyl phthalate** (DBP, $C_{16}H_{22}O_4$ [calculated 278.3435 g mol^{-1}]; t_R = 16.64 min, 30.25 %; *m/z* = 278 $[M]^{+\cdot}$, 223 $[M-C_4H_7]^{+\cdot}$, 206 $[M-C_5H_{12}]^{+\cdot}$, 149 $[M-C_8H_{17}O]^{+\cdot}$ base peak, 104 $[M-C_9H_{18}O_3]^{+\cdot}$, 76 $[M-C_{10}H_{18}O_4]^{+\cdot}$, 56 $[M-C_{12}H_{14}O_4]^{+\cdot}$).

Some 7H-purine derivatives has been detected in other plants extracts (Otuokere et al., 2016; Salazar et al., 2017; Yamuna et al., 2017). Identification of this phytocompound indicates that *H. baruensis* could be a source of alkaloids, which was also suggested by phytochemical screening analyses (table 2). Besides, it could be one of the constituents responsible for bioactivity showed by PEF-L (tables 3 and 4). On the other hand, dibutylphthalate (DBP) and other phthalate derivatives have been detected by GC-MS as

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phytoconstituents of several plant extracts and essential oils (Chen et al., 2011; Ordaz et al., 2011; Rodríguez et al., 2015; Verma & Kumar, 2015; Patil et al., 2016; Lakshmi & Nair, 2017; Matthew et al., 2017), however they could be contaminants on phytochemical studies (Bhakuni & Rawat, 2005).

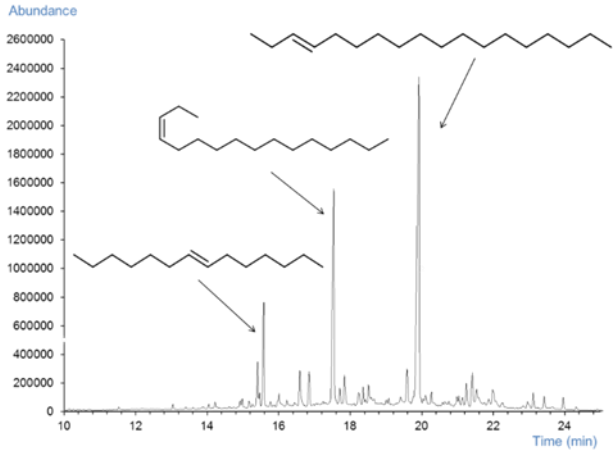

Figure 3. Chromatogram of subfraction A7.2.1, pointing identified constituents

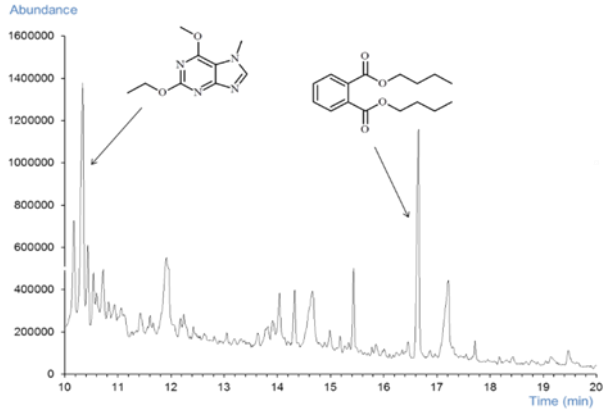

Figure 4. Chromatogram of subfraction A9.1.2, pointing identified constituents

Subfraction A9.2 (44.2 mg) was separated by CC in 4 subfractions (A9.2.1-A9.2.4, 43.6 mg, 98.64 %), using mixes of PE-CH_2Cl_2, and CH_2Cl_2-AcOEt. Subfractions A9.2.1 (5.3 mg, light yellow oil) and A9.2.2 (13.4 mg, yellow oil) were analyzed by GC-MS. Chromatogram obtained for the first fraction is shown in Fig. 5, in which were identified two main constituents as **bis(2-methylbutyl) phthalate** ($C_{18}H_{26}O_4$ [calculated 306.3966 g mol^{-1}]; t_R = 16.54 min, 31.54 %; m/z = 250 $[M\text{-}C_4H_8]^{+\cdot}$, 223 $[M\text{-}C_6H_{11}]^{+\cdot}$, 205 $[M\text{-}C_7H_{17}]^{+\cdot}$, 194 $[M\text{-}C_8H_{16}]^{+\cdot}$, 179 $[M\text{-}C_7H_{11}O_2]^{+\cdot}$, 167 $[M\text{-}C_8H_{11}O_2]^{+\cdot}$, 152 $[M\text{-}C_9H_{14}O_2]^{+\cdot}$, 149 $[M\text{-}C_{10}H_{21}O]^{+\cdot}$ base peak, 137 $[M\text{-}C_{10}H_{17}O_2]^{+\cdot}$, 124 $[M\text{-}C_{11}H_{18}O_2]^{+\cdot}$, 109 $[M\text{-}C_{12}H_{21}O_2]^{+\cdot}$, 95 $[M\text{-}C_{13}H_{23}O_2]^{+\cdot}$, 85 $[M\text{-}C_{14}H_{21}O_2]^{+\cdot}$, 71 $[M\text{-}C_{13}H_{15}O_4]^{+\cdot}$, 58 $[M\text{-}C_{16}H_{24}O_2]^{+\cdot}$, 50 $[M\text{-}C_{14}H_{24}O_4]^{+\cdot}$), and **4,8,12,16-tetramethylheptadecan-4-olide** ($C_{21}H_{40}O_2$ [calculated 324.5411 g mol^{-1}]; t_R = 21.22 min, 34.67 %; m/z = 324 $[M]^{+\cdot}$, 254 $[M\text{-}C_5H_{10}]^{+\cdot}$, 239 $[M\text{-}C_6H_{13}]^{+\cdot}$, 221 $[M\text{-}C_5H_{11}O_2]^{+\cdot}$, 196 $[M\text{-}C_7H_{12}O_2]^{+\cdot}$, 166 $[M\text{-}C_9H_{18}O_2]^{+\cdot}$, 151 $[M\text{-}C_{10}H_{21}O_2]^{+\cdot}$, 126 $[M\text{-}C_{14}H_{30}]^{+\cdot}$, 114 $[M\text{-}C_{15}H_{30}]^{+\cdot}$, 99 $[M\text{-}C_{16}H_{33}]^{+\cdot}$ base peak, 83 $[M\text{-}C_{15}H_{29}O_2]^{+\cdot}$, 69 $[M\text{-}C_{16}H_{31}O_2]^{+\cdot}$, 57 $[M\text{-}C_{17}H_{31}O_2]^{+\cdot}$). It was suggested that 4,8,12,16-tetramethylheptadecan-4-olide is a natural oxidation product from an isoprenoid compound as the α-tocopherol (vitamin E) (Rontani et al., 2007), and it has been detected in several phytochemical studies (Rodríguez et al. 2015; Duan et al., 2016; Patil et al., 2016; Madkour et al. 2017; Vats & Gupta, 2017).

Meanwhile, chromatogram of the second subfraction (A9.2.2) shows 3 main peak (Fig. 6), which were identified as **5,6,7,7a-tetrahydro-2(4H)-benzofuranone**

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(dihydroactinidiolide, $C_{11}H_{16}O_2$ [calculated 180.2435 g mol^{-1}]; t_R = 10.57 min, 47.27 %; m/z = 180 $[M]^{+\cdot}$, 165 $[M\text{-}CH_3]^{+}$, 152 $[M\text{-}CO]^{+\cdot}$, 138 $[M\text{-}C_3H_6]^{+\cdot}$, 137 $[M\text{-}C_2H_3O]^{+}$, 124 $[M\text{-}C_3H_4O]^{+\cdot}$, 111 $[M\text{-}C_5H_9]^{+}$ base peak, 109 $[M\text{-}C_4H_7O]^{+}$, 95 $[M\text{-}C_6H_{13}]^{+}$, 83 $[M\text{-}C_6H_9O]^{+}$, 81 $[M\text{-}C_5H_7O_2]^{+}$, 67 $[M\text{-}C_6H_9O_2]^{+}$, 55 $[M\text{-}C_7H_9O]^{+}$, 53 $[M\text{-}C_7H_{11}O_2]^{+}$); **isopropyl octadecanoate** ($C_{21}H_{42}O_2$ [calculated 326.5570 g mol^{-1}]; t_R = 21.23 min, 27.57 %; m/z = 298 $[M\text{-}C_2H_4]^{+\cdot}$, 256 $[M\text{-}C_5H_{10}]^{+\cdot}$, 239 $[M\text{-}C_4H_7O_2]^{+}$, 227 $[M\text{-}C_7H_{15}]^{+}$, 213 $[M\text{-}C_8H_{17}]^{+}$, 199 $[M\text{-}C_9H_{19}]^{+}$, 185 $[M\text{-}C_{10}H_{21}]^{+}$, 171 $[M\text{-}C_{11}H_{23}]^{+}$, 157 $[M\text{-}C_{12}H_{25}]^{+}$, 143 $[M\text{-}C_{13}H_{27}]^{+}$, 129 $[M\text{-}C_{14}H_{29}]^{+}$, 102 $[M\text{-}C_{16}H_{32}]^{+\cdot}$ base peak, 73 $[M\text{-}C_{18}H_{37}]^{+}$, 60 $[M\text{-}C_{19}H_{38}]^{+\cdot}$, 57 $[M\text{-}C_{18}H_{37}O]^{+}$), and **bis(2-ethylhexyl) phthalate** ($C_{24}H_{38}O_4$ [calculated 390.5561 g mol^{-1}]; t_R = 23.10 min, 15.57 %; m/z = 390 $[M]^{+\cdot}$, 279 $[M\text{-}C_8H_{15}]^{+}$, 261 $[M\text{-}C_9H_{21}]^{+}$, 180 $[M\text{-}C_{13}H_{23}O_2]^{+}$, 169 $[M\text{-}C_{14}H_{21}O_2]^{+}$, 167 $[M\text{-}C_{14}H_{23}O_2]^{+}$, 162 $[M\text{-}C_{14}H_{23}O_2]^{+}$, 149 $[M\text{-}C_{16}H_{33}O]^{+}$ base peak, 132 $[M\text{-}C_{16}H_{34}O_2]^{+}$, 113 $[M\text{-}C_{16}H_{21}O_4]^{+}$, 104 $[M\text{-}C_{17}H_{34}O_3]^{+}$, 83 $[M\text{-}C_{18}H_{27}O_4]^{+}$, 71 $[M\text{-}C_{20}H_{31}O_3]^{+}$, 57 $[M\text{-}C_{20}H_{29}O_4]^{+}$).

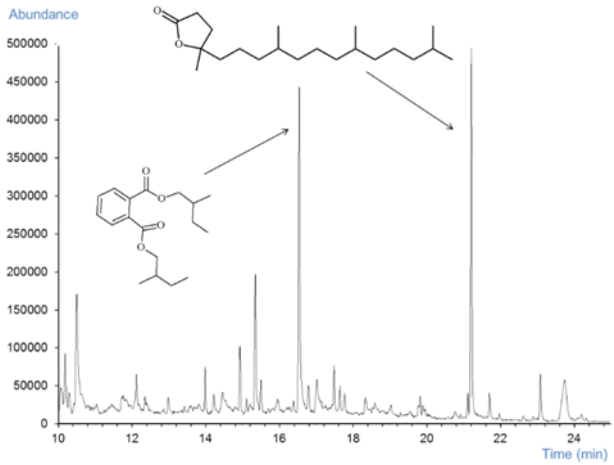

Figure 5. Chromatogram of subfraction A9.2.1, pointing identified constituents

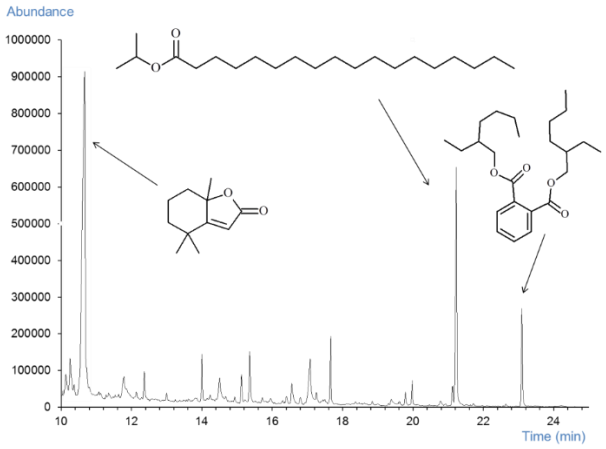

Figure 6. Chromatogram of fraction A9.2.2, pointing identified constituents

Dihydroactinidiolide is a volatile compound that could function as pheromone in animals, and has been detected in other plants (Mamadalieva et al., 2015; Ma'arif et al., 2016; Chaichowong et al., 2017). Additionally, isopropyl octadecanoate has been identified in glandular secretion of *Craesus* sawfly larvae (Boevé & Heilporn, 2008) and in pheromone composition of *Heliconius* butterflies (Schulz et al., 2008). In this sense, biosynthesis of these compounds could be for ecological interactions such as pollinizing.

Subfraction A9.5 (11.8 mg, light yellow oil) was analyzed by GC-MS, obtaining the chromatogram shown in Fig. 7, in which 3 constituents were identified as ***m*-methylacetophenone** (1-(3-methylphenyl)ethanone, $C_9H_{10}O$ [calculated 134.1751 g mol^{-1}]; t_R = 5.50 min, 25.10 %; m/z = 134 $[M]^{+\cdot}$, 119 $[M\text{-}CH_3]^{+}$ base

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peak, 95 [M-C_3H_3]$^{+\cdot}$, 91 [M-C_2H_3O]$^{+\cdot}$, 65 [M-C_4H_5O]$^{+\cdot}$, 51 [M-C_5H_7O]$^{+\cdot}$); **γ-terpinene** (1-isopropyl-4-methyl-1,4-cyclohexadiene, $C_{10}H_{16}$ [calculated 136.2340 g mol^{-1}]; t_R = 11.38 min, 21.15 %; *m/z* = 136 [M]$^{+\cdot}$ base peak, 121 [M-CH_3]$^{+\cdot}$, 109 [M-C_2H_3]$^{+\cdot}$, 93 [M-C_3H_7]$^{+\cdot}$, 83 [M-C_4H_5]$^{+\cdot}$, , 69 [M-C_5H_7]$^{+\cdot}$, 55 [M-C_6H_9]$^{+\cdot}$), and **6,10,14-trimethyl-2-pentadecanone** (hexahydrofarnesyl acetone or phytone, $C_{18}H_{36}O$ [calculated 268.4784 g mol^{-1}]; t_R = 15.41 min, 29.32 %; *m/z* = 268 [M]$^{+\cdot}$, 253 [M-CH_3]$^{+\cdot}$, 250 [M-H_2O]$^{+\cdot}$, 235 [M-CH_5O]$^{+\cdot}$, 223 [M-C_3H_9]$^{+\cdot}$, 210 [M-C_3H_6O]$^{+\cdot}$, 194 [M-C_5H_{14}]$^{+\cdot}$, 179 [M-$C_5H_{13}O$]$^{+\cdot}$, 165 [M-$C_7H_{15}O$]$^{+\cdot}$, 149 [M-$C_7H_{19}O$]$^{+\cdot}$, 137 [M-$C_8H_{19}O$]$^{+\cdot}$, 123 [M-$C_9H_{21}O$]$^{+\cdot}$, 109 [M-$C_{10}H_{23}O$]$^{+\cdot}$, 95 [M-$C_{11}H_{25}O$]$^{+\cdot}$, 85 [M-$C_{13}H_{27}$]$^{+\cdot}$, 71 [M-$C_{14}H_{29}$]$^{+\cdot}$, 58 [M-$C_{15}H_{30}$]$^{+\cdot}$ base peak, 53 [M-$C_{14}H_{31}O$]$^{+\cdot}$).

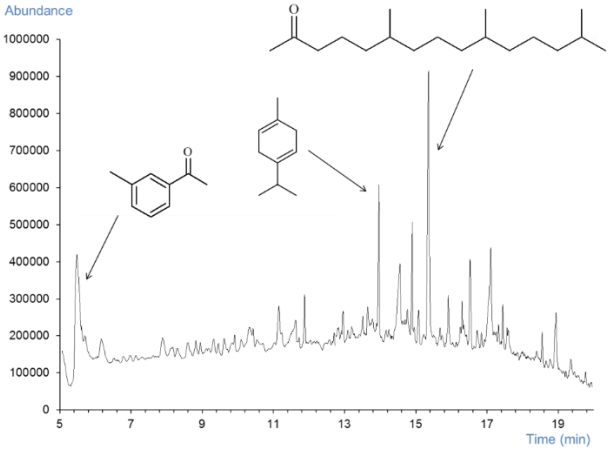

Figure 7. Chromatogram of subfraction A9.5, pointing identified constituents.

Compound named *m*-methylacetophenone has been reported among constituents of several essential oils with biological activity (Durant et al., 2014; Upadhyay, 2015; Wang et al., 2015; Sipahi et al., 2017). γ-terpinene has shown to be an active metabolite against several microorganisms (Baldissera et al., 2016; Rivera-Yáñez et al., 2017; Miladi et al., 2017), which suggested that it could be, in part, responsible for observed antibiosis effect of PEF-L (tables 3 and 4). 6,10,14-trimethyl-2-pentadecanone also could occur naturally from oxidation of vitamin E (Rontani et al., 2007) and has been detected along with 4,8,12,16-tetramethylheptadecan-4-olide in several plant extracts (Rodríguez et al. 2015; Duan et al., 2016; Madkour et al. 2017; Vats & Gupta, 2017).

Some constituents that were detected in the different chromatograms, even at lower or higher retention times, were not identified by data bases used in this study, which suggests that this species of *Helicteres* could be a source of complex and novel phytochemical components. For this reason, it will be necessary to make more researches about this genus in order to isolate and characterize those unknown metabolites.

Separation and analysis of chloroform fraction

Chloroform fraction from *H. baruensis* Jacq. leaves extract (CF-L, 1.57 g) was chromatographed in a glass column with silica gel 35-70 mesh, using petroleum ether (PE) and mixes with $CHCl_3$, acetone and MeOH as eluents. Ten (10) fractions (B1-B10, 1.51 g, 95.92%) were obtained from this CC. According to TLC analysis and chromatographic yield, fraction named B5 (72.8 mg) was selected to continuous chromatographic separation.

This fraction was separated by CC on silica

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gel 35-70 mesh in 7 subfractions (B5.1-B5.7, 70.8 mg, 92.25 %) using the same mix of solvents. Subfraction B5.4 (25.5 mg, white solid) was newly purified by CC and named B5.4.1 (7.2 mg, 28.24 %, mp. 158-166 °C). According to TLC analysis, this fraction was a possible pure compound, which showed a green coloration in the Liebermann-Burchard reaction (positive for unsaturated sterols).

The spectral analysis data of this phytosterol is shown in table 5. The UV-Vis spectrum (MeOH) of this compound exhibited a λ_{max} at 287 nm. This value is a little higher than those reported for known phytosterols like stigmasterol, campesterol and sitosterol (Siripong et al. 1992; Jain et al., 2009; Jain & Bari, 2010; Govindarajan & Sarada, 2011, Gololo et al., 2016; Pratheema et al., 2017; Antiga et al., 2018). A broad band around of 3180 cm^{-1} was observed in its IR spectrum ($CHCl_3$), which indicated the presence of –OH group. Other significant absorption bands were observed at 2770-2660 cm^{-1} (aliphatic asymmetric C-H stretching), 1520-1470 cm^{-1} (C=C), 1360-1340 cm^{-1} (CH_2), 1020 cm^{-1} (cycloalkane), and 930 cm^{-1} (deforming =C-H), which are representative for sterol skeleton. The ^1H NMR spectrum (CH_3OD) showed characteristic signals at δ 5.40, 4.62 and δ 4.40 ppm assigned to olefinic protons. Chemical shift at δ 3.19 was assigned to HC-O. Between δ 2.50-1.50 ppm appeared several signals which correspond to CH and CH_2, while presence of CH_3 groups exhibited signals at δ 1.30-0.75 ppm (table 5).

Table 5. *Spectroscopic data of subfraction B5.4.1.*

Technique	Signals
UV-Vis (CH_3OH, nm)	287
IR ($CHCl_3$, cm^{-1})	3180, 2780, 2670, 1500, 1350, 1320, 1130, 1040, 930
^{1}HNMR (CH_3OD, ppm)[a]	5.40 (H-6), 4.62 (H-23), 4.41 (H-22), 3.20 (H-3), 2.28 (H-20), 1.31 (H-19), 1.06 (H-21), 0.97 (H-29), 0.89 (H-27[b]), 0.86 (H-26[b]), 0.75 (H-18)

[a]: Assignations were made based on stigmasterol structure (figure 8), [b]: interchangeable.

The spectroscopic analysis suggested stigmasterol ($C_{29}H_{48}O$, M=412.69 $g{\cdot}mol^{-1}$, figure 8) as the possible phytocompound isolated in subfraction B5.4.1. It has been reported that stigmasterol could inhibit the proliferation and colony formation of gastric cancer SNU-1 cells, triggering both apoptosis and G2/M cell cycle arrest in SNU-1 cancer cells (Li et al., 2018). Furthermore, stigmasterol isolated from the stem bark of *Neocarya macrophylla* has shown broad-spectrum antimicrobial activity (Yusuf et al. 2018).

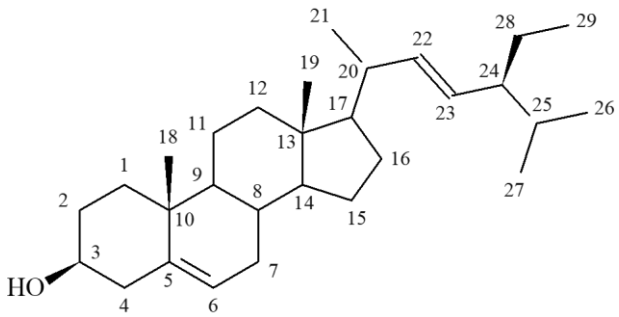

Figure 8. Chemical structure of stigmasterol

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CONCLUSION

Although the leaves of *Helicteres baruensis Jacq.* provided the highest extraction yield in methanol, this extract did not exhibit significantly bioactivity. However, its partition showed the elimination of a kind of antagonism. Among aerial parts, flowers showed being a better source of cytotoxic agents, perhaps due to alkaloid or polyphenol occurrence. Results of GC-MS demonstrated that the petroleum ether fraction from leaves is composed by several known phytochemicals, which are common in non-polar extracts of leaves or essential oils of other plants. This first report of *H. baruensis* composition indicates that this Helicteres specie could be a source of several kinds of constituents with pharmacological potential.

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