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Phytochemical Identification by TLC and GC-MS of Crude Hydroethanolic Extracts of Four Medicinal Plants from Côte D'ivoire

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Abstract

This work was carried out to determine the chemical composition of crude hydroethanolic extracts of organs of 4 medicinal plants from Côte d'Ivoire. The phytochemical screening of these extracts was carried out by TLC. It highlighted the copresence of phenolic acids, alkaloids, coumarins, flavonoids and tannins. The identification of the molecules in the hydroethanolic extracts of the different plants was carried out by GC-MS after derivatization with MSTFA. The relevant results revealed the existence of several compounds including phenolic acids (gallic acid, protocatechic acid), coumarin (scopolin) and cyclic polyol (inositol). The copresence of these organic compounds would be responsible for the therapeutic virtues of these plants.

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Introduction

The therapeutic properties of plants can be explained by the improvement of the performance of structural determination methods for the discovery of new active ingredients (Newman *et al.*, 2007). A number of analytical methods, among which are proposed for the separation and determination of phytochemicals mainly by high performance liquid chromatography (HPLC) coupled with UV detection (Justesen *et al.*, 1998).

However, compared to mass spectrometry (MS), UV-Visible spectrometry does not provide sufficient structural identification (Chen *et al.*, 2001). Thus, coupling techniques are acclaimed for quickly characterizing the chemical constituents of natural

extracts without chemical separation. They represent significant time savings, especially when the quantities of samples available are low (Kadja, 2014). Therefore, gas chromatography coupled with mass spectrometry (GC-MS) can provide more accurate results. The analysis of non-volatile and thermolabile phytochemicals by GC-MS supposes their conversion into volatile and thermotolerant by chemical silylation (Zuo *et al.*, 2002). Indeed, derivatization is an ideal procedure for GC-MS analysis. Compared to their original compounds, the trimethylsilylated derivatives are more volatile, less polar and more thermotolerant.

This study, which is part of the search for biologically active molecules of plant origin, relates to *Nauclea latifolia* (Rubiaceae), *Cochlospermum planchonii*

(Cochlospermaceae), *Piliostigma thonningii* (Cesalpiniaceae) and *Argemone mexicana* (Papaveraceae). These 4 medicinal plants were selected on the basis of ethnobotanical, chemotaxonomic and biological considerations.

Materials and Methods

The plant material consists of the roots of *Nauclea latifolia* (1) and *Cochlospermum planchonii* (2), the bark of the trunk of *Piliostigma thonningii* (3) and the whole plant of *Argemone mexicana* (4). These plants were selected from an ethnobotanical survey of 5 traditional healers in the north of Côte d'Ivoire. The harvest was made at Korhogo (9° 27' 28" North, 5° 37' 46" West). The plants were identified at the herbarium of the National Center of Floristics (NCF) (5° 20' 11" North, 4° 01' 36" West) located at Félix Houphouët-Boigny University.

The plant material was cleaned and dried for 2 days, first out of direct sunlight, then for 7 days in air-conditioned room (18°C) and finally in an oven (45°C) for 3 days. It was then pulverized with an electric grinder (RETSCH, type SM 100) to obtain powders which were used for the preparation of the various extracts to be tested.

Hydroethanolic extraction

Vegetable powder (10 g) was macerated in 100 mL of EtOH (80%) for 24 hours with constant stirring. The operation was repeated 3 times with the same marc. After filtration on Büchner, the macerates obtained were collected and concentrated at 40°C with a rotary evaporator (BÜCHI Waterbath B-480). The extracts obtained were stored for 24 h in the refrigerator at 4°C for the precipitation of lipophilic compounds. After decantation, the crude hydroethanolic extracts were obtained (Marston *et al.*, 2006).

Selective extraction

Each hydroethanolic extract (60 mL) was successively exhausted by fractionation with (3 × 20 mL) petroleum ether, diethyl ether and ethyl acetate.

The organic fractions were first concentrated with a rotary evaporator (BÜCHI Waterbath B-480) and dried in an oven (40°C) for 24 h. The various selective extracts as well as the aqueous phases were stored in the refrigerator at 4°C (Markham, 1982).

Phytochemical screening by TLC

Screening by TLC of the selective extracts was carried out according to Ladyguina *et al.*, (1983), Georgievskii *et al.*, (1990), Dawson *et al.*, (1991), Mamyrbékova-Békro *et al.*, (2008), as cited in Kabran *et al.*, (2011).

GC-MS analysis

Different samples' silylation

To 3 mg of each extract were added 0.5 mL of distilled CH₂Cl₂ and 0.2 mL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). After an overnight incubation at room temperature with stirring, the CH₂Cl₂-MSTFA mixture was evaporated and then dried. The dry residue was recovered in CHCl₃ (1 mL) and injected into GC-MS (Yilmaz *et al.*, 2009; Kadja, 2014).

GC-MS analysis

The analysis was performed with a gas chromatograph coupled to a SHIMADZU brand mass spectrometer, model QP2010SE with a Zebron ZB-5ms column 20 m long, with an internal diameter of 0.18 mm and a thickness 0.18 µm stationary phase film. Helium was used as the carrier gas, with a linear velocity of 0.9 mL/s. The oven temperature (70-270°C) was programmed at 4°C/min and maintained at 270°C for 20 min. Those of the injector and the detector were respectively fixed at 280°C and 290°C. The injection was carried out in sharing mode. The mass spectrometer parameters for the electronic impact mode are the ionization source temperature 230°C, the electron energy 70 eV, the scan speed 50 scans/s and the acquisition speed 10,000 uma/s. The different volatile phytochemicals were identified by comparison of their retention times (RT) and mass spectra with the spectral data of the reference compounds of the device (NIST 08.LIB libraries). Each determination was made in duplicate (Kadja, 2014; Ouattara, 2017).

Results and Discussions

Preliminary chemical composition

Tables (1 –3) present the TLC results of the various selective extracts.

The different phytochemicals observed in the diethyl ether extracts of the 4 plants are phenolic acids,

alkaloids, coumarins, flavonoids and tannins. Flavonoids are present in all extracts with an abundance in the E₁^I extract with regard to the number of molecular fingerprints corresponding to the phytochemicals' families (Table 1). Coumarins are seen in all extracts except E₂^I. Phenolic acids and tannins are exclusively present in the extracts E₁^I and E₂^I. As for the alkaloids, they were observed only in the extracts E₁^I and E₄^I (Rf = 0.06).

Table 2 reveals the presence of the tannins in the ethyl acetate extracts except the extract E₄^{II}, which contains only flavonoids. Coumarins, on the other hand, were observed only in the extract E₁^{II}.

Table 3 indicates that the compounds revealed in the water extracts are phenolic acids, alkaloids, coumarins, flavonoids and tannins.

All the results of identification of secondary metabolites by TLC are reported in Table 4.

With regard to this table, it appears that the roots of *Nauclea latifolia* contain phenolic acids, alkaloids, coumarins, flavonoids and tannins. Ngo *et al.*, (2009) reported that the roots of the Cameroonian species have the same phytochemical composition. Puri (1964) and Hotelier *et al.*, (1975) isolated tannins, flavonoids and

alkaloids of the indolequinolizidine type from all organs of *N. latifolia*. The presence of these bioactive secondary metabolites seems to justify the use of *N. latifolia* in the treatment of malaria, dysentery and diarrhea (Akabue *et al.*, 1982; Benoit-Vical *et al.*, 1998; Okwu *et al.*, 2009), hypertension (Akabue *et al.*, 1982; Nworgu *et al.*, 2008), sores, coughs and gonorrhoea (Madubunyi, 1995).

C. planchonii contains phenolic acids, coumarins, flavonoids and tannins. Nafiu *et al.*, (2011) also showed the presence of anthraquinones, phenolic compounds, flavonoids, phlobatannins and tannins in the water extract of the root of *C. planchonii*. What may appear to justify the use of *C. planchonii* in the treatment of stomach disorders, typhoid fever and urinary tract infections (Yakubu *et al.*, 2010; Nafiu *et al.*, 2011; as cited in Isah *et al.*, 2013).

The bark of *Piliostigma thonningii*'s trunk contains only coumarins, flavonoids and tannins. Preliminary phytochemical studies by Akindahunsi *et al.*, (2005) on *P. thonningii* reveal high levels of flavonoids and tannins. Maydell (1983) has shown that the bark of *P. thonningii* contains up to 18% tannins. These different bioactive molecules justified the use of *P. thonningii* in the treatment of many pathologies of viral origin such as herpes, influenza, bronchopulmonary diseases and HIV virus (Bombardelli *et al.*, 1992).

Table.1 Phytochemicals identified in diethyl ether extracts

Extract	Rf, color: compound identified
E ₁ ^I	0.93, br ^a - y ^N - bl ^{Al} - y ^K : <i>fla</i> (br ^a - y ^N - bl ^{Al})/ <i>coum</i> (y ^K); 0.85, g ^{Am} - bl ^K : <i>fla</i> (g ^{Am})/ <i>coum</i> (bl ^K); 0.80, bl ^a - y ^N - bl ^{Al} : <i>fla</i> ; 0.77, bl ^{Am} : <i>fla</i> ; 0.70, br ^a - g ^{Al} - gr ^F : <i>fla</i> (br ^a - g ^{Al})/ <i>tan</i> (gr ^F); 0.64, bl ^N - bl ^{Am} - bl ^K : <i>fla</i> (bl ^N - bl ^{Am})/ <i>coum</i> (bl ^{Am}); 0.62, y ^a : <i>fla</i> ; 0.55, y ^K : <i>coum</i> ; 0.53, g ^F : <i>pha</i> ; 0.49, y ^a - bl ^{Al} - bl ^F : <i>fla</i> (y ^a - bl ^{Al})/ <i>pha</i> (bl ^F); 0.40, bl ^N - bl ^{Al} : <i>fla</i> ; 0.30, bl ^{Am} : <i>fla</i> ; 0.26, g ^N - bl ^{Am} - bl ^{Al} - bl ^K : <i>fla</i> (g ^N - bl ^{Am} - bl ^{Al})/ <i>coum</i> (bl ^K); 0.20, bl ^a - bl ^N : <i>fla</i> ; 0.15, bl ^K : <i>coum</i> ; 0.12, y ^a - g ^N - bl ^{Am} - bl ^{Al} : <i>fla</i> ; 0.06, o ^D : <i>alc</i>
E ₂ ^I	0.92, bl ^a : <i>fla</i> ; 0.90, bl ^a : <i>fla</i> ; 0.71, gr ^F : <i>tan</i> ; 0.62, bl ^N : <i>fla</i> ; 0.52, bl ^F : <i>pha</i>
E ₃ ^I	0.90, g ^a - y ^N : <i>fla</i> ; 0.85, bl ^{Am} : <i>fla</i> ; 0.81, y ^{Am} : <i>fla</i> ; 0.74, g ^K : <i>coum</i> ; 0.69, bl ^a : <i>fla</i> ; 0.62, bl ^a - bl ^N : <i>fla</i> ; 0.44, bl ^a : <i>fla</i>
E ₄ ^I	0.91, y ^N : <i>fla</i> ; 0.78, o ^a : <i>fla</i> ; 0.71, o ^a : <i>fla</i> ; 0.61, y ^N : <i>fla</i> ; 0.22, y ^N : <i>fla</i> ; 0.14, y ^N : <i>fla</i> ; 0.07, y ^K : <i>coum</i> ; 0.06, o ^D : <i>alc</i>

br/brown; y/yellow; bl/blue; g/green; gr/grey; o/orange; a/without UV 366 nm developer; N/ Neu at UV 366 nm; Al/AlCl₃ at UV 366 nm; K/KOH at UV 366 nm; Am/Ammonia at UV 366 nm; F/ FeCl₃ in the visible; D/Druggendorff in the visible; *fla*/flavonoid; *coum*/coumarin; *alc*/alkaloid; *pha*/phenolic acid; *tan*/tannin.

E₁^I: *Nauclea latifolia* diethyl ether extract; E₂^I: *Cochlospermum planchonii* diethyl ether extract; E₃^I: *Piliostigma thonningii* diethyl ether extract; E₄^I: *Argemone mexicanadiethyl* ether extract.

Table.2 Phytocompounds identified in ethyl acetate extracts

Extract	Rf, color: compound identified
E ^{II} ₁	0.96, bl ^N : fla; 0.93, y ^a - bl ^{Am} - g ^K : fla (y ^a - bl ^{Am})/coum (g ^K); 0.83, g ^K : coum 0.78, bl ^a - g ^{Am} - bl ^{Al} - bl ^F : fla (bl ^a - g ^{Am} - bl ^{Al})/tan (gr ^F); 0.75, g ^N : fla; 0.69, bl ^a : fla 0.63, bl ^{Am} - g ^K : fla (bl ^{Am})/coum (g ^K); 0.58, bl ^a - g ^N : fla; 0.54, g ^{Al} : fla; 0.49, y ^a - g ^N - g ^K : fla (y ^a - g ^N)/coum (g ^N); 0.42, y ^N - bl ^{Al} : fla; 0.38, bl ^a : fla; 0.33, y ^{Am} - g ^K : fla (y ^{Am})/coum (g ^K); 0.31, y ^a - y ^N : fla; 0.24, y ^a : fla; 0.16, y ^a : fla
E ^{II} ₂	0.98, gr ^F : tan; 0.92, g ^N : fla; 0.87, p ^a : fla; 0.69, p ^a : fla; 0.50, r ^N : fla; 0.11, gr ^F : tan
E ^{II} ₃	0.98, gr ^F : tan; 0.74, g ^{Am} : fla; 0.67, y ^a : fla; 0.60, y ^a : fla; 0.55, br ^N : fla; 0.41, y ^{Am} : fla; 0.31, bl ^{Al} : fla; 0.13, gr ^F : tan
E ^{II} ₄	0.73, o ^N : fla; 0.71, o ^a : fla; 0.53, y ^N - y ^{Al} : fla; 0.42, y ^a : fla; 0.29, g ^{Al} : fla

br/brown; y/yellow; bl/blue; g/green; gr/grey; o/orange; p/purple; r/red; a/without UV 366 nm developer; N/ Neu at UV 366 nm; Al/AlCl₃ at UV 366 nm; K/KOH at UV 366 nm; Am/Ammonia at UV 366 nm; F/ FeCl₃ in the visible; D/Druggendor'ff in the visible; fla/flavonoid; coum/coumarin; alc/alkaloid; pha/phenolic acid; tan/tannin.

E^{II}₁: *Nauclea latifolia* ethyl acetate extract; E^{II}₂: *Cochlospermum planchonii* ethyl acetate extract; E^{II}₃: *Piliostigma thonningii* ethyl acetate extract; E^{II}₄: *Argemone Mexicana* ethyl acetate extract.

Table.3 Phytocompounds revealed in aqueous extracts

Extract	Rf, color: compound identified
E ^{III} ₁	0.92, g ^N - g ^{Am} : fla; 0.89, bl ^F : pha; 0.84, g ^N - g ^{Am} : fla; 0.77, g ^N : fla; 0.74, y ^{Al} : fla 0.67, bl ^{Al} : fla; 0.64, y ^a - g ^N - bl ^{Am} - g ^K : fla (y ^a - g ^N - bl ^{Am})/coum (g ^K); 0.56, g ^N - bl ^K : fla (g ^N)/coum (bl ^K); 0.53, bl ^{Al} : fla; 0.50, bl ^a - g ^{Am} : fla; 0.46, y ^{Al} - bl ^K : fla (y ^{Al})/coum (bl ^K); 0.41, y ^a : fla; 0.36, bl ^{Al} - g ^K : fla (bl ^{Al})/coum (g ^K); 0.33, bl ^N : fla; 0.31, g ^{Am} : fla; 0.27, y ^{Al} : fla; 0.17, y ^a : fla; 0.06, o ^D : alc
E ^{III} ₂	0.77, o ^a : fla; 0.64, y ^a : fla; 0.61, gr ^F : tan; 0.58, g ^K : coum; 0.56, y ^a : fla; 0.47, bl ^{Am} - bl ^{Al} : fla; 0.33, bl ^F : pha; 0.30, bl ^K : coum; 0.26, y ^a - bl ^{Al} : fla; 0.18, bl ^F : pha; 0.16, bl ^{Am} - y ^{Al} : fla
E ^{III} ₃	0.60, g ^K : coum; 0.54, bl ^{Am} : fla; 0.38, g ^{Am} : fla; 0.11, g ^K : coum
E ^{III} ₄	0.83, o ^N : fla; 0.76, o ^a : fla; 0.64, bl ^a - bl ^{Am} - v ^K : fla (bl ^a - bl ^{Am})/coum (g ^K) 0.59, y ^N : fla; 0.57, y ^a - bl ^{Al} : fla; 0.51, y ^a : fla; 0.47, o ^N - y ^{Al} : fla; 0.43, y ^N : fla 0.37, bl ^K : coum; 0.27, y ^a - o ^N - bl ^{Al} : fla; 0.24, o ^D : alc; 0.20, g ^K : coum

y/yellow; bl/blue; g/green; gr/grey; o/orange; a/without UV 366 nm developer; N/ Neu at UV 366 nm; Al/AlCl₃ at UV 366 nm; K/KOH at UV 366 nm; Am/Ammonia at UV 366 nm; F/ FeCl₃ in the visible; D/Druggendor'ff in the visible; fla/flavonoid; coum/coumarin; alc/alkaloid; pha/phenolic acid; tan/tannin.

E^{III}₁: *Nauclea latifolia* aqueous extract; E^{III}₂: *Cochlospermum planchonii* aqueous extract; E^{III}₃: *Piliostigma thonningii* aqueous extract; E^{III}₄: *Argemone Mexicana* aqueous extract.

Table.4 Summary of phytocompounds identified by TLC

Compound identified	<i>N. latifolia</i>			<i>C. planchonii</i>			<i>P. thonningii</i>			<i>A. mexicana</i>		
	E ^I ₁	E ^{II} ₁	E ^{III} ₁	E ^I ₂	E ^{II} ₂	E ^{III} ₂	E ^I ₃	E ^{II} ₃	E ^{III} ₃	E ^I ₄	E ^{II} ₄	E ^{III} ₄
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Phenolic acids	+	-	+	+	-	+	-	-	-	-	-	-
Coumarins	+	+	+	-	-	+	+	-	+	+	-	+
Tannins	+	+	-	+	+	+	-	+	-	-	-	-
Alkaloids	+	-	+	-	-	-	-	-	-	+	-	+

Table.5 Phytocompounds identified by GC-MS in extract E₁

Peak	R.T (min.)	Percent age (%)	Molar masses (M)	Ion identified (m/z)	Compound identified
1	9.633	0.65	526	333 [M- TMS - 8 CH ₃] ⁺ ; 307 [M-3 TMS] ⁺ ; 277 [M-3 TMS - 2 CH ₃] ⁺ ; 217 [M- 3 TMS - 6 CH ₃] ⁺ ; 117 [COO-TMS] ⁺ ; 73 [TMS] ⁺	Ribonic acid
2	9.753	1.93	554	437 [M- COO-TMS] ⁺ ; 347 [M- OTMS - TMS - 3 CH ₃] ⁺ ; 217 [M- 4 TMS - 3 CH ₃] ⁺ ; 73 [TMS] ⁺	3,4,5,6-tetrahydroxy-2-oxohexanoic acid
3	9.793	5.81	540	540 [M] ⁺ ; 437 [C ₁₇ H ₄₁ O ₅ Si ₄] ⁺ ; 231 [M-3 TMS - 6 CH ₃] ⁺	D-Fructose
4	9.830	1.50	540	103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	
5	9.883	12.36	370	370 [M] ⁺ ; 355 [M-CH ₃] ⁺ ; 223 [M-C ₄ H ₉ O ₂ Si - 2 CH ₃] ⁺ ; 193 [M- OTMS - TMS - CH ₃] ⁺ ; 73 [TMS] ⁺	Protocatechic acid
6	10.023	17.17	360	345 [M-CH ₃] ⁺ ; 255 [M- C ₃ H ₉ SSi] ⁺ ; 167 [M- 2 OTMS - CH ₃] ⁺ ; 73 [TMS] ⁺	4,6-dihydroxy-pyrimidine-2-thiol
7	10.190	2.88	540	525 [M-CH ₃] ⁺ ; 291 [M- 3 TMS - 2 CH ₃] ⁺ ; 217 [M-3 TMS - OTMS - CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	α -D-galactopyranose
8	10.273	0.99	538	523 [M-CH ₃] ⁺ ; 419 [M- OTMS - 2 CH ₃] ⁺ ; 331 [M- TMS - TMSO - 3 CH ₃] ⁺ ; 246 [M- 4 TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Inosose
9	10.560	3.73	540	217 [M-3 TMS - OTMS - CH ₃] ⁺ ; 157 [M- 3 TMS - TMSO - 5 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	β -D-glucopyranose
10	10.690	4.52	612	434 [M-2 OTMS] ⁺ ; 305 [M- 4 TMS - CH ₃] ⁺ ; 217 [M- 5 TMS - 2 CH ₃] ⁺ ; 73 [TMS] ⁺	Inositol
11	11.123	0.62	846	450 [M- TMSO - TMS - 2 CH ₃] ⁺ ; 361 [M-2 OTMS - TMS - 2 CH ₃] ⁺ ; 345 [M-3 OTMS - 2 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	Scopoline
12	12.763	5.24	600	481 [M- OTMS - 2 CH ₃] ⁺ ; 467 [M- TMS - 4 CH ₃] ⁺ ; 409 [M- 2 TMS - 3 CH ₃] ⁺ ; 392 [M- 2 OTMS - 2 CH ₃] ⁺ ; 319 [M-2 OTMS - TMS - 2 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Thymol- β -D-glucopyranoside
13	13.057	1.24	600		
14	13.293	30.47	846		
15	13.337	10.88	846	451 [M- 5 TMS - 2 CH ₃] ⁺ ; 361 [M-4 TMS - 2 OTMS - CH ₃] ⁺ ; 331 [M-4 TMS - 2 OTMS - 3 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	D-Turanose

Table.6 Phytocompounds identified by GC-MS in extract E₂

Peak	R.T (min.)	Percent age (%)	Molar mass (M)	Ion identified (m/z)	Compound identified
6	5.017	0.13	188	173 [M- CH ₃] ⁺ ; 131 [M- C ₃ H ₅ O] ⁺ ; 115 [M-TMS] ⁺ ; 73 [TMS] ⁺	4-hydroxy-4-methylpentan-2-one
9	6.107	0.26	234	117 [COO-TMS] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	Butan-1,3-diol
11	7.063	0.26	254	254 [M] ⁺ ; 166 [M- TMS- CH ₃] ⁺ ; 73 [TMS] ⁺	Pyrocatechol
12	7.527	0.69	212	212 [M] ⁺ ; 182 [M-2 CH ₃] ⁺ ; 167 [M-3 CH ₃] ⁺ ; 152 [M-4 CH ₃] ⁺ ; 108 [M-OTMS - CH ₃] ⁺	2-methylthiophenol
13	7.623	0.26	254	85 [C ₆ H ₁₃] ⁺ ; 71 [C ₅ H ₁₁] ⁺ ; 57 [C ₄ H ₉] ⁺ ; 43 [C ₃ H ₇] ⁺	2,6,10-trimethylpentadecane
17	8.423	0.36	144	129 [M- CH ₃] ⁺ ; 75 [M- C ₅ H ₉] ⁺ ; 59 [M- C ₅ H ₉ O] ⁺	2-methylbut-3-en-2-ol
18	8.713	0.13	154	139 [M- CH ₃] ⁺ ; 111 [M- C ₃ H ₇] ⁺ ; 97 [M- C ₄ H ₉] ⁺ ; 55 [C ₃ H ₃ O] ⁺	Dec-1-en-3-one
26	10.180	0.56	540	231 [M- 3TMS- 6CH ₃] ⁺ ; 217 [M- 3TMS- OTMS- CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	α- D-galactopyranose
28	10.500	0.26	458	458 [M] ⁺ ; 443 [M-CH ₃] ⁺ ; 179 [M- 3 TMS - 4 CH ₃] ⁺ ; 73 [TMS] ⁺	Gallic acid
29	10.553	0.47	540	231 [M- 3TMS- 6CH ₃] ⁺ ; 217 [M- 3TMS-OTMS- CH ₃] ⁺ ; 73 [TMS] ⁺	D- glucose
32	11.083	0.92	612	612 [M] ⁺ ; 305 [M- 4TMS - CH ₃] ⁺ ; 217 [M- 5TMS - 2CH ₃] ⁺ ; 129 [M- 6 TMS - 3 CH ₃] ⁺ ; 73 [TMS] ⁺	Inositol
35	11.970	0.43	190	190 [M] ⁺ ; 145 [M- COOH] ⁺ ; 77 [C ₆ H ₅] ⁺	2-cyclopentyl-2-phenylethanoic acid
37	12.847	0.43	368	368 [M] ⁺ ; 191 [C ₁₃ H ₁₉ O] ⁺ ; 57 [C ₄ H ₉] ⁺	Antioxydant 425
39	13.280	1.84			
40	13.320	0.49	600	600 [M] ⁺ ; 585 [M-CH ₃] ⁺ ; 452 [M-TMS- 5CH ₃] ⁺ ; 364 [M- 2TMS- 6CH ₃] ⁺ ; 319 [M- 2 OTMS- TMS-2CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Thymol-β-D-glucopyranoside
41	13.603	0.14			
43	13.853	0.18			

Table.7 Phytocompounds identified by GC-MS in extract E₃

Peak	R.T (min.)	Percent age (%)	Molar mass (M)	Ion identified (m/z)	Compound identified
1	6.243	1.84	322	205 [C ₈ H ₂₁ O ₂ Si] ⁺ ; 117 [C ₅ H ₁₃ OSi] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Butan-1,2,3-triol
2	6.737	19.08	308	205 [M - CH ₂ -O-TMS] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Glycerol
13	9.823	1.66	540	437 [C ₁₇ H ₄₁ O ₅ Si ₄] ⁺ ; 231 [M- 3TMS- 6CH ₃] ⁺ ; 217 [M- 3 TMS- OTMS -CH ₃] ⁺ ; 73 [TMS] ⁺	D-Fructose
14	9.887	5.94	612	305 [M- 4TMS -CH ₃] ⁺ ; 217 [M- 5 TMS- 2CH ₃] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Inositol

Table.8 Phytocompounds identified by GC-MS in extract E₄

Peak	R.T (min.)	Percent age (%)	Molar mass (M)	Ion identified (m/z)	Compound identified
2	5.227	1.87	148	131 [M- OH] ⁺ ; 73 [TMS] ⁺ ; 45 [C ₂ H ₅ O] ⁺	Propan-1,2-diol
3	6.155	0.84	322	307 [M- CH ₃] ⁺ ; 205 [M- C ₅ H ₁₃ OSi] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Butan-1,2,3-triol
4	6.699	11.14	308	293 [M- CH ₃] ⁺ ; 263 [M- 3 CH ₃] ⁺ ; 205 [M -CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Glycerol
6	7.980	0.42	322	307 [M- CH ₃] ⁺ ; 205 [M- C ₅ H ₁₃ OSi] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Butan-1,2,3-triol
7	8.088	0.51	410	307 [M - CH ₂ -O-TMS] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Butan-1,2,3,4-tétraol
11	9.286	2.97	512	291 [M- 2 TMS - 5 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	Arabitol
12	9.387	0.44	460	267 [M- 2 OTMS - CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 73 [TMS] ⁺	Uridine
13	9.510	5.66	438	217 [M- 2 TMS - 5 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	D- Ribofuranose
15	9.713	1.59	568	347 [M- 2 TMS - 5 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	D-glycero-L-manno-heptano-γ-lactone
16	9.755	2.81	554	405 [M- OTMS - 4 CH ₃] ⁺ ; 217 [M- 4 TMS - 3 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	3,4,5,6-tetrahydroxy-2-oxohexanoic acid

17	9.786	0.84	526	511 [M- CH ₃] ⁺ ; 437 [M- OTMS] ⁺ ;	3-Desoxy-D-
18	9.833	4.58		217 [M- 3 TMS - 6 CH ₃] ⁺ 103 [CH ₂ -	glycero-hexitol
19	9.933	1.07	482	O-TMS] ⁺ ; 89 [OTMS] ⁺ ; 73 [TMS] ⁺	
20	10.123	0.35		437 [M- 3 CH ₃] ⁺ ; 363 [M- OTMS - 2	Methylgluco
23	10.315	5.62	614	CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ;	furanside
21	10.155	1.06	540	89 [OTMS] ⁺ ; 73 [TMS] ⁺	
22	10.197	0.59	438	496 [M- TMS - 3 CH ₃] ⁺ ; 393 [M- 2	Glucitol
24	10.350	1.31	614	TMS - 5 CH ₃] ⁺ ; 306 [M- 3 TMS -	
25	10.522	2.09	540	OTMS] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ;	
26	10.597	0.59	540	73 [TMS] ⁺	
28	10.916	2.77	328	306 [M- 3 TMS - CH ₃] ⁺ ; 103 [CH ₂ -	α-D-
30	11.387	0.35	342	O-TMS] ⁺ ; 89 [OTMS] ⁺ ;	galactopyranose
35	11.751	0.70	356	73 [TMS] ⁺	
36	12.270	0.74	281	305 [M- TMS - 4 CH ₃] ⁺ ; 231 [M-	α-Arabinopyranose
42	13.795	0.44	600	OTMS - TMS - 3 CH ₃] ⁺ ; 217 [M- 2	
43	13.893	0.40		TMS - 5 CH ₃] ⁺ ; 73 [TMS] ⁺	
44	15.393	1.32	430	393 [M- 2 TMS - 5 CH ₃] ⁺ ; 307 [M- 4	Dulcitol
45	15.847	14.19	384	TMS - CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ;	
49	17.488	1.85	486	89 [OTMS] ⁺ ; 73 [TMS] ⁺	
				467 [M- TMS] ⁺ ; 217 [M- 3 TMS -	D-glucose
				OTMS - CH ₃] ⁺ ; 73 [TMS] ⁺	
				407 [M- TMS - 4 CH ₃] ⁺ ; 332 [M- 2	β-D-
				OTMS - 2 CH ₃] ⁺ ; 103 [CH ₂ -O-	galactofuranose
				TMS] ⁺ ; 73 [TMS] ⁺	
				328 [M] ⁺ ; 313 [M- CH ₃] ⁺ ;	Palmitic acid
				73 [TMS] ⁺	
				327 [M- CH ₃] ⁺ ; 208 [M- OTMS - 3	Octadecan-1-ol
				CH ₃] ⁺ ; 57 [C ₄ H ₉] ⁺ ; 43 [C ₃ H ₇] ⁺	
				356 [M] ⁺ ; 341 [M- CH ₃] ⁺ ;	Stearic acid
				313 [M- C ₃ H ₇] ⁺ ; 73 [TMS] ⁺	
				281 [M] ⁺ ; 238 [M- C ₃ H ₇] ⁺ ;	Oleamide
				72 [C ₃ H ₆ NO] ⁺	
				481 [M- TMSO - 2 CH ₃] ⁺ ; 437 [M-	Thymol-β-D-
				TMS - 6 CH ₃] ⁺ ; 103 [CH ₂ -O-TMS] ⁺ ;	glucopyranoside
				89 [OTMS] ⁺ ; 73 [TMS] ⁺	
				327 [M- TMS - 2 CH ₃] ⁺ ;	Octadecan-1,2-diol
				73 [TMS] ⁺ ; 43[C ₃ H ₇] ⁺	
				369 [M- CH ₃] ⁺ ; 341 [M- C ₃ H ₇] ⁺ ; 73	Henicosan-1-ol
				[TMS] ⁺ ; 57 [C ₄ H ₉] ⁺ ; 43 [C ₃ H ₇] ⁺	
				486 [M] ⁺ ; 471 [M- CH ₃] ⁺ ;	β-sitosterol
				73 [TMS] ⁺	

A. mexicana, contains only alkaloids, coumarins and flavonoids. Yuh-Chwen *et al.*, (2003) have shown the presence of isoquinoline and benzylisoquinoline alkaloids in the plant. Other types of alkaloids, namely berberine, tetrahydroberberine, protopine and benzophenanthridine have also been isolated from the

plant (Kenneth *et al.*, 2001). The existence of these secondary phytochemicals in *A. mexicana* would attest to its use in the treatment of leprosy, malaria, jaundice, rheumatism, pain, inflammation, skin diseases, fever, warts, dysentery, tumors and helminthiasis (Nadkarni *et*

al., 1976; Chopra *et al.*, 1979; as cited in Warriar *et al.*, 1996).

GC-MS analysis of hydroethanolic extracts

The GC-MS chromatogram of *Nauclea latifolia* hydroethanolic extract (E₁) presents 15 peaks (Table 5) representing phytochemicals among which carbohydrates (62.74%), thiols (17.17%), phenolic compounds have been identified (12.98%), alcohols (4.52%) and carboxylic acids (2.58%).

We were able to identify from the GC-MS chromatogram of *Cochlospermum planchonii* hydroethanolic extract (E₂) 14 compounds (Table 6) represented by 17 peaks. The phytochemicals identified are carbohydrates (4.11%), phenolic compounds (1.64%), alcohols (1.54%), carboxylic acids (0.43%), ketones (0.26%) and hydrocarbons (0.26%).

The GC-MS chromatogram of *Piliostigma thonningii* hydroethanolic extract (E₃) shows 15 peaks, 4 of which were identified. Those are alcohols (26.86%) and carbohydrates (1.66%) (Table 7).

Thirty (30) phytochemicals (Table 8) identified in *Argemone Mexicana* hydroethanolic extract (E₄) were distributed as follows: 53.50% alcohols, 13.49% carbohydrates, 6.28% carboxylic acids, 1.85% sterols, 0.74% amides and amines (0.44%).

GC-MS characterization by silylation of the various hydroethanolic extracts revealed the copresence of several phytochemicals, which can be grouped into 10 families: carboxylic acids, alcohols, amides, amines, ketones, phenolic compounds, carbohydrates, hydrocarbons, sterols and thiols.

Carbohydrates and alcohols were found in all extracts. Indeed, sugars and their derivatives are the class of molecules most reactive to derivatization (Villas-Bôas *et al.*, 2011). Analysis of the sugars carried out by certain authors made it possible to obtain high derivatization results, with coefficients of variation <5% reflecting good repeatability of the reaction (glucose, fructose, ribose, xylitol, 2-deoxyglucose) (Koek *et al.*, 2006). Thiols are only present in E₁. As for sterols, amides and amines, they are only present in E₄. Carboxylic acids are absent from E₃ while ketones and hydrocarbons are contained in E₂.

Otherwise, the analysis by GC-MS made it possible to identify the phenolic acids, were highlighted by

phytochemical screening by TLC. These include protocatechic acid (12.36%) in *Nauclea latifolia* and gallic acid (0.26%) in *Cochlospermum planchonii*. In addition, scopolin (0.62%) has been identified in *Nauclea latifolia*. It is a coumarin with vascular protective, anti-inflammatory, antiparasitic, analgesic, anticoagulant, antioxidant and anti-oedematous properties (Ito *et al.*, 2005; Kalkhambar *et al.*, 2007; Win *et al.*, 2008; as cited in Hitara *et al.*, 2009). Also, the presence of the antioxidant 425 (m/z = 368) is noted in *Cochlospermum planchonii*. Furthermore, the inositol which has been identified in the two plants does not belong to the families of secondary metabolites. However, it has pharmacological properties. It is involved in many biological processes such as insulin (Lerner, 2002), the strengthening of the cell membrane (Kukuljan *et al.*, 1997), the breakdown of fat and the reduction of cholesterol (Rapiejko, 1986).

In conclusion, the main objective of this work was to carry out a qualitative study using the TLC and then to identify by GC-MS the phytochemicals derived from organ extracts from four medicinal plants from Côte d'Ivoire. To do this, 4 plant matrices, namely *Nauclea latifolia* (Rubiaceae), *Cochlospermum planchonii* (Cochlospermaceae), *Piliostigma thonningii* (Cesalpiniaceae) and *Argemone mexicana* (Papaveraceae) were the subject of an ethnobotanical survey.

Phytochemical screening of extracts obtained by means of TLC has highlighted the coexistence of phenolic acids, alkaloids, coumarins, flavonoids and tannins. Flavonoids and coumarins are present in all the plants studied, but dominant in *Nauclea latifolia*.

GC-MS analysis of the crude hydroethanolic extracts revealed the copresence of 10 families of phytochemicals. Protocatechic acid (12.36%), scopolin (0.62%) and gallic acid (0.26%) were identified in *Nauclea latifolia* and *Cochlospermum planchonii*, respectively.

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