

## Research Article

# Elucidation of Alkaloids Contained in the Fec5 Fraction of Bark of *Annickia Chlorantha*

Aimé Bertrand Madiélé Mabika<sup>1,3,4</sup>; Arnaud Wenceslas Geoffroy Tamba Sompila<sup>4,5\*</sup>; Arnold Murphy Elouma Ndinga<sup>1</sup>; Huguette Agnanié<sup>2</sup>; Valéry Thiery<sup>3</sup>; Jean Maurille Ouamba<sup>1</sup>

<sup>1</sup>Unit of Life and Plant Chemistry (UC2V), Marien Ngouabi University, Brazzaville Congo

<sup>2</sup>Laboratory of Natural Substances and Organometallic Synthesis (LASUNSO), University of Sciences and Techniques of Masuku, Franceville Gabon

<sup>3</sup>Coastline Environment and Society (LIENS), University of La Rochelle, France

<sup>4</sup>National Institute for Research in Engineering Sciences, Innovation and Technology, Scientific City, Brazzaville Congo

<sup>5</sup>Laboratory of Food and Medical Bioprocesses, National Polytechnic School, Marien Ngouabi University, Brazzaville Congo

\*Corresponding author: Arnaud Wenceslas Geoffroy Tamba Sompila

Unit of Life and Plant Chemistry (UC2V), Marien Ngouabi University, Brazzaville Congo.

Tel: +242 06.662.87.91

Email: arnaud.wens@gmail.com

Received: September 04, 2023

Accepted: October 31, 2023

Published: November 07, 2023

## Introduction

Plants have been used in traditional medicine for many millennia. Curiosity is the innate principle of the doctrine that, indicating the possibility of identifying the peculiarities and virtues of each plant by its signature, guided the first men in the choice of new preparations to be tested [1]. In the last decades there has been a growing interest in the study of medicinal plants and their traditional use in different regions of the world [2]. Today, according to the World Health Organization (WHO), nearly 80% of the population depends on traditional medicine for primary health care. Considerable economic advantages in the development of traditional medicine and in the use of medicinal plants for the treatment of various diseases have been noted world [2], hence the need to valorize traditional medicine and especially the molecules extracted from plants. In these plants, we find primary and secondary metabolites. The primary metabolites are in great number whereas the secondary metabolites

## Abstract

The aim of this study was to highlight the presence in the barks of *A. chlorantha*, new alkaloids not yet identified in the literature. FEC<sub>5</sub> fractionation method by semi-preparative HPLC revealed twelve (12) sub-fractions. Of these twelve sub-fractions, five were exploited. These five sub-fractions EC<sub>3</sub>, EC<sub>9</sub>, EC<sub>10</sub>, EC<sub>11</sub> and EC<sub>12</sub> revealed fifteen (15) alkaloids which were isolated, designated by the letters A to O, and identified mainly by mass spectrometry. The results obtained allowed us to note that the alkaloidal composition of the plant, especially of the barks, is still very poorly elucidated. The isolated alkaloids belong, for the most part, either to the group of protoberberines and tetrahydroprotoberberines or to the bisbenzyltetrahydro isoquinolines which have not yet been reported in the literature. The isolation of palmatine corroborated the results of the literature. These results showed that these sub-fractions are not totally pure. They are a mixture of compounds. The complexity of the alkaloid composition of *Annickia chlorantha* trunk bark is particularly justified by the number of alkaloids present.

**Keywords:** Bisbenzyltetrahydroisoquinoleines; Extraction; Fractionation; Isolation; Purification; Tetrahydroprotoberine

**Nomenclature:** FEC: Fraction *Annickia chlorantha*; EC: Subfraction *Annickia chlorantha*; TLC: Thin Layer Chromatography; HPLC: High Performance Liquid Chromatography; UV: UltraViolet; NMR: Nuclear Magnetic Resonance; SMHR: High Resolution Mass Spectrometry; DRC: Democratic Republic of Congo; HIV: Human Immunodeficiency Virus

are molecules having a limited distribution in the plant. The secondary metabolites play different roles, including that of defense against external aggressions. Secondary metabolism products are very numerous; more than 200.000 structures defined [3]. They include an extraordinary structural variety but are produced in small quantities [3]. These molecules mark in an original way, a species, a family or a genus of plant and sometimes allow to establish a chemical taxonomy. Phenolic compounds, terpenoids, steroids and alkaloids are examples of secondary metabolites; they have many pharmaceutical applications [3].

*Annickia chlorantha*, a plant belonging to the Annonaceae family, is one of the warm and humid climate species of tropical Africa that grows in dense forest usually at low altitude. Geographically, it is found widespread in southern Nigeria, Angola,

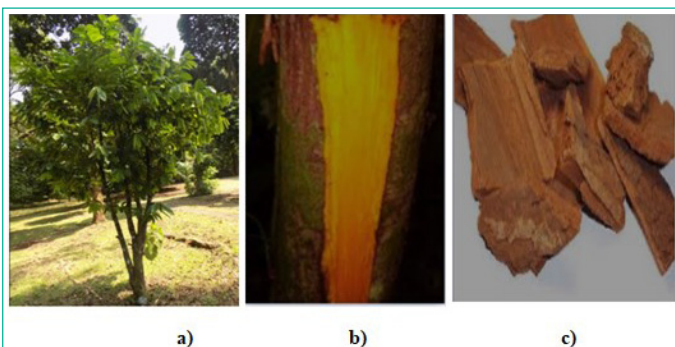
the Republic of Congo and the Democratic Republic of Congo [4]. *A. chlorantha* has been the subject of several chemical and pharmacological studies [5] and this revealed that it contains alkaloids. Some parts of the plant, especially the bark and the roots, contain alkaloids with an anti-hepatitis effect. This is a property long recognized in traditional medicine [6]. The extracts of bark of *A. chlorantha* are used in the manufacture of a drug, having a preventive and curative effect on viral hepatitis [7]. The alkaloids isolated from this plant are active against *Trypanosoma cruzi*, the American trypanosomiasis pathogen [8]. The plant's barks are also traditionally used for the treatment of rheumatism, fatigue, tuberculosis and certain ulcers [9], malaria and for deworming [10]. They are also used against diarrhea [10]. In addition, [9] revealed the anti-HIV properties of the aqueous extract of this plant. The use of the sulfur yellow bark provides an excellent dye used in the dyeing of fabrics, skins and mats in some regions, especially in Cameroon, Congo, Gabon and DRC [11,12]. The literature reports that *A. chlorantha* contains palmatin in the stem and root barks [13]. In 1975, the chemical constituents of barks were elucidated [14]. Several alkaloids were isolated [6,9]. The main alkaloids extracted from the trunk bark are quaternary protoberberines, such as palmatine, berberine, jatrorrhizine and columbamine. Two new alkaloids have been identified, namely aporphine and another alkaloid of the protoberberine type (active anti-HIV alkaloid), 7,8-dihydro-8-hydroxypalmatine have also been isolated from the bark [9].

In the same way, we have set ourselves the goal of extracting, purifying and isolating by chromatographic methods, two alkaloids of the protoberberine type and thirteen alkaloids of the bisbenzyl tetrahydrobenzyl isoquinoline types.

## Material and Methods

### Plant Material

The plant of *Annickia chlorantha* was collected in November 2012 in the department of Niari precisely in Mossendjo in southwestern Congo. The part used in this study consisted of the bark kept in the dark at room temperature (25 °C) for about five (05) days. The dry matter was then ground with an IKA-WERKE GmbH-CO-KG, D-79219 Staufen, equipped with a 0.25 mm sieve.



**Figure 1:** a) *Annickia chlorantha* plant, b) *Annickia chlorantha* trunk and c) *Annickia chlorantha* bark.

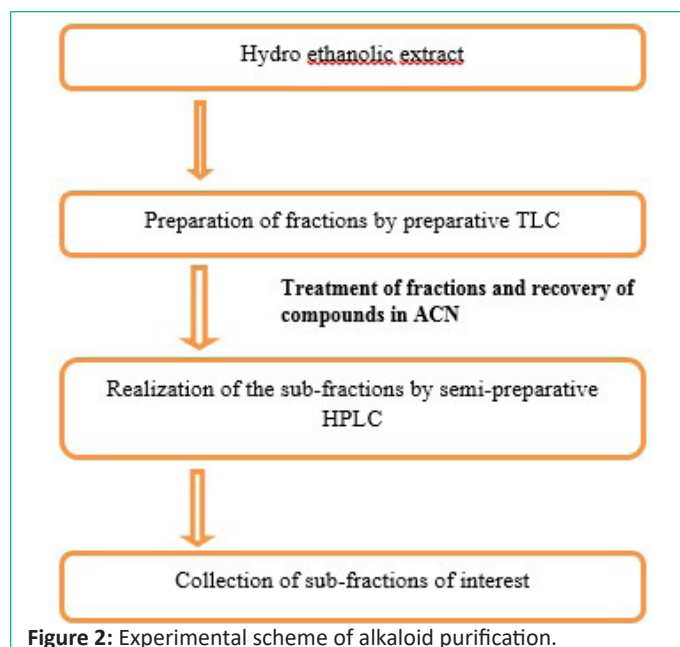
### Fractionation and Purification of Dye Extracts

#### Experimental Approach

The experimental approach (Figure 2) allowed for fractionation and purification of the dye extracts.

#### Preparative Chromatography on Thin Layer

Preparative chromatography on thin layer was used to sepa-



**Figure 2:** Experimental scheme of alkaloid purification.

rate the different compounds in the plant. The TLC plates used were Merck 60 F 254 on 1-mm-thick silica gel on 20cm x 20cm glass. The extracts were solubilized in methanol  $\geq 99.8\%$  and then deposited on the plate. The plates were eluted in a saturated tank containing a solvent mixture.

The migration systems were those used in the qualitative analysis by thin layer chromatography. Plates were developed under CAMAG UV lamp with a wavelength ranging from 254 to 366 nm. The silica containing each compound is then recovered with a spatula. The silica is desorbed in a small amount of solvent, and then filtered under vacuum to recover the compounds.

#### Analysis of Extracts by High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Waters 2996 HPLC chain equipped with a W600 pump of the same brand and a diode array UV detector (DAD-UV). The acquisition system was controlled by a Waters software. The Uptisphere C18 300°C, 250x4.6mm 5 $\mu$ m column was from Interchim (France). The mobile phase was composed of the H<sub>2</sub>O/ACN mixture in gradient elution mode consisting of (H<sub>2</sub>O: 70, 50, 0, 0, 70, 70) and (ACN: 30, 50, 100, 100, 30, 30). The working flow rate was 1 mL/min with a run time of 60 min divided (0, 10, 35, 45, 53, and 60).

#### Semi-Preparative High Performance Liquid Chromatography

The purifications were performed using an Agilent 1200 preparative HPLC system. This apparatus is equipped with two G136A pumps and an injection loop (2mL for semi-preparative column), a UV detector with diode arrays (DAD-UV) driven by a Waters software. Compound separations were performed on an Uptisphere Strategy 100 A° semi-preparative column (30 x 250mm, 10 $\mu$ m,) using an Uptisphere RP18e pre-column. The mobile phase was composed of the mixture H<sub>2</sub>O/ACOH in the proportions 99:1 v/v and ACN/ACOH in the proportions 99:1 v/v.

The gradient mode was the same as the one used in analytical mode, the only changes were observed on the flow rate (10mL/min) and the column. After collection of the fractions, the organic solvent was evaporated using a BUCHI R-201 rotary evaporator at 45°C. Then, the samples were freeze-dried to remove traces of water.

## Identification and Structure Determination of Molecules of Interest

Several analytical techniques (UV, NMR, HRMS) were used to obtain the structural information necessary to determine the structures of the isolated compounds. Indeed, the structures were established on the basis of spectroscopic evidence and by comparison of our results with previously published spectral data.

### High resolution Mass Spectrometry (HRMS)

The purified molecules were analyzed by Bruker Micro-Tof-Q 2 mass spectrometry composed of two quadrupoles and an orthogonal time-of-flight analyzer equipped with electrospray or APCI sources. The HRMS was used to determine the elemental composition (gross formula) and molecular weight of the purified product. The purified molecules were analyzed at the Centre Régional de Mesures Physiques de l'Ouest (CRMPO) in Rennes. In this study, the source used was Electrospray (ESI) and the analytical solvent was methanol.

### NMR Spectroscopy

The  $^1\text{H}$ ,  $^{13}\text{C}$ , COSY  $^1\text{H}$ - $^{13}\text{C}$  and HMBQ nuclear magnetic resonance spectra were recorded on a 400 MHz spectrometer of Jeol brand, at the Centre Commun d'Analyse (CCA) of the University of La Rochelle UMR-7266.

A few milligrams of freeze-dried sample powder were solubilized in  $d_6$  DMSO in the 5 mm diameter analytical tubes and then analyzed by 1D and 2D NMR. Chemical shifts were expressed in parts per million (ppm), relative to Tetramethylsilane (TMS), used as an internal reference. The coupling constants (J) were expressed in hertz ( $\text{H}_2$ ). Signal multiplicity was indicated as follows: s (singlet), bs (broad signal), d (doublet), dd (split doublet), ddd (split doublet), t (triplet), m (multiplet), q (quadruplet). ov (overlapped).

## Results and Discussion

### Fractionation of Extracts, Purification of Fractions and Isolation of Compounds

**Fractionation of *Annickia chlorantha* water/EtOH dye extract, purification of fractions and isolation:** The extract obtained after treatment with methanol and 10% HCl was named EEC. EEC underwent a first fractionation by preparative thin layer chromatography to give five (5) fractions  $\text{FEC}_1$ ,  $\text{FEC}_2$ ,  $\text{FEC}_3$ ,  $\text{FEC}_4$  and  $\text{FEC}_5$ . These fractions were analyzed by reverse phase chromatography coupled with an iodine bar detector (DAD). The fractions  $\text{FEC}_4$  and  $\text{FEC}_5$  presented dyeing properties. We focused our attention on the  $\text{FEC}_5$  fraction because it was obtained in significant quantity (619 mg) and would contain a large number of compounds (Figure 3). From the  $\text{FEC}_5$  fraction purified by semi-preparative HPLC on a reverse phase silica column with a polarity gradient (gradient 1), we were able to isolate twelve (12) sub-fractions all of yellow color and of which five (5) revealed the dyeing properties ( $\text{EC}_3$ ,  $\text{EC}_9$ ,  $\text{EC}_{10}$ ,  $\text{EC}_{11}$  and  $\text{EC}_{12}$ ).

HPLC analysis of the  $\text{FEC}_5$  fraction revealed the chromatographic profile and UV-visible spectra of a few compounds (Figures 4 & 5).

The UV absorption spectrum of some eluted compounds at different retention times showed maxima between 215 and 360 nm characteristic of aromatic bands.

The UV spectra of the compounds present in the  $\text{FEC}_5$  frac-

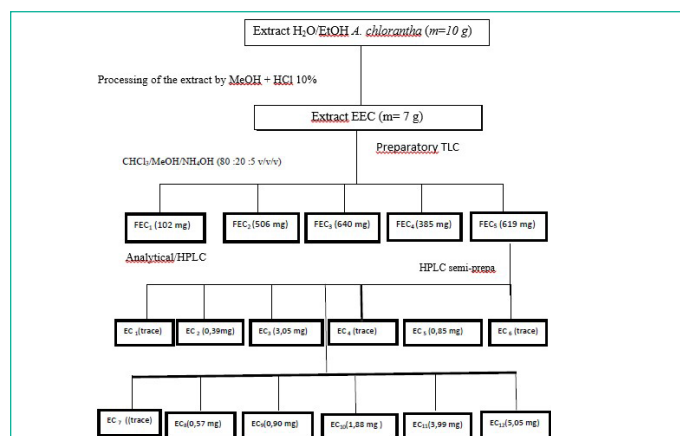
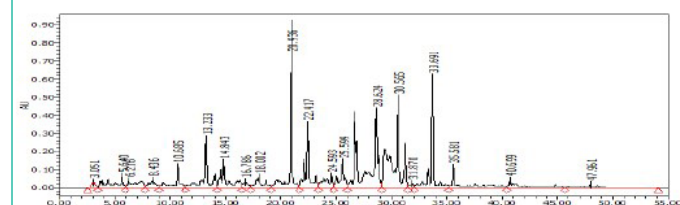


Figure 3: Fractionation, purification and isolation scheme Water/EtOH *Annickia chlorantha* extract.



**Table 1:** Different alkaloids isolated and identified from *Annickia chlorantha* barks.

Sub-fractions	Alkaloids	Raw formulas	Molecular weight (g.mol-1)	Observed peaks m/e
EC <sub>1</sub>	A	C <sub>33</sub> H <sub>37</sub> N <sub>4</sub> O <sub>6</sub>	585.737	570(M-CH <sub>3</sub> ), 554(M-OCH <sub>3</sub> ), 394(C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
EC <sub>2</sub>	B	C <sub>33</sub> H <sub>38</sub> N <sub>4</sub> O <sub>6</sub>	586.010	571(M-CH <sub>3</sub> ), 555(M-OCH <sub>3</sub> ), 409(C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> N)
EC <sub>3</sub>	C	C <sub>18</sub> H <sub>13</sub> NO <sub>3</sub>	291.089	276(M-CH <sub>3</sub> ), 260(M-OCH <sub>3</sub> )
EC <sub>4</sub>	D	C <sub>21</sub> H <sub>21</sub> NO <sub>5</sub>	367.010	337(M-2CH <sub>3</sub> ), 336(M-OCH <sub>3</sub> )
EC <sub>5</sub>	E	C <sub>35</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	538.010	522(M-CH <sub>3</sub> +H), 507(M-2CH <sub>3</sub> +H), 347(M-C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
EC <sub>6</sub>	F	C <sub>35</sub> H <sub>37</sub> N <sub>2</sub> O <sub>4</sub>	549.207	406(M-C <sub>3</sub> H <sub>7</sub> ), 491(M-CH <sub>3</sub> COCH <sub>3</sub> )
EC <sub>7</sub>	G	C <sub>36</sub> H <sub>28</sub> N <sub>2</sub> O <sub>6</sub>	584.190	569(M-CH <sub>3</sub> ), 554(M-2CH <sub>3</sub> ), 393(M-C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
EC <sub>8</sub>	H	C <sub>36</sub> H <sub>26</sub> N <sub>2</sub> O <sub>6</sub>	582.178	551(M-CH <sub>3</sub> +H), 537(M-CONH <sub>2</sub> )
EC <sub>9</sub>	I	C <sub>35</sub> H <sub>36</sub> N <sub>2</sub> O <sub>4</sub>	548.267	533(M-CH <sub>3</sub> ), 270(M-C <sub>17</sub> H <sub>12</sub> O <sub>3</sub> N)
EC <sub>10</sub>	J	C <sub>35</sub> H <sub>34</sub> N <sub>2</sub> O <sub>4</sub>	546.220	531(M-CH <sub>3</sub> ), 269(M-C <sub>17</sub> H <sub>11</sub> O <sub>3</sub> N)
EC <sub>11</sub>	K	C <sub>34</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub>	530.022	515(M-CH <sub>3</sub> ), 500(M-2CH <sub>3</sub> ), 472(M-CH <sub>3</sub> COCH <sub>3</sub> ), 259(M-C <sub>17</sub> H <sub>10</sub> O <sub>3</sub> N)
EC <sub>12</sub>	L	C <sub>36</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub>	554.220	263(M-C <sub>18</sub> H <sub>13</sub> O <sub>3</sub> N), 447(M-C <sub>7</sub> H <sub>7</sub> O)
EC <sub>13</sub>	M	C <sub>36</sub> H <sub>32</sub> N <sub>2</sub> O <sub>4</sub>	556.233	541(M-CH <sub>3</sub> ), 526(M-2CH <sub>3</sub> ), 379(M-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> N)
EC <sub>14</sub>	N	C <sub>34</sub> H <sub>32</sub> N <sub>2</sub> O <sub>4</sub>	532.235	517(M-CH <sub>3</sub> ), 428(M-C <sub>7</sub> H <sub>7</sub> O), 341(M-C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
EC <sub>15</sub>	O	C <sub>37</sub> H <sub>34</sub> N <sub>2</sub> O <sub>4</sub>	570.235	555(M-CH <sub>3</sub> ), 433(M-C <sub>8</sub> H <sub>9</sub> O <sub>2</sub> ), 393(M-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> N)

### Elucidation of the Chemical Structures of Some Molecules

**Elucidation of the chemical structures of the alkaloids of *Annickia chlorantha*:** Preliminary phytochemical tests indicated the presence of alkaloids in abundance in *Annickia chlorantha* trunk bark. From the staining extracts, fifteen (15) alkaloids were isolated designated as A to O and identified mainly by mass spectrometry. These alkaloids were extracted from the sub-fractions EC<sub>3</sub>, EC<sub>9</sub>, EC<sub>10</sub>, EC<sub>11</sub> and EC<sub>12</sub>. The results showed that these sub-fractions are not totally pure. They are a mixture of compounds.

As a result, their structure could not be determined. Apart from the compounds of crude formula C<sub>18</sub>H<sub>13</sub>NO<sub>3</sub> and C<sub>21</sub>H<sub>21</sub>NO<sub>5</sub>, the other alkaloids all belong to the same series and seem to be new in the literature of the species *Annickia chlorantha*. But at present, it has not been possible to attach them to a known structural type except for the compound of crude formula C<sub>21</sub>H<sub>21</sub>NO<sub>5</sub> from the EC<sub>3</sub> subfraction which corresponds to a known structure oxy-8-palmatine isolated from *Annickia chlorantha* barks [7,9,14,16].

### Compounds Contained in the EC<sub>3</sub> Sub-Fraction

The EC<sub>3</sub> sub-fraction is a yellow ochre powder soluble in methanol. It is a mixture of four alkaloids. It was impossible from the NMR data of this sub fraction that we elucidate in a simple way the structures of these four alkaloids. However, the NMR spectrum recorded on this impure sub-fraction shows definitely the presence of alkaloids that belong to the series of tetrahydroprotoberberines and bisbenzyl tetrahydro isoquinolines.

Alkaloids A and B: Of crude formulae C<sub>33</sub>H<sub>37</sub>N<sub>4</sub>O<sub>6</sub>Na and C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>Na, the structures could not be determined. Taking into account their molecular weight (theoretical mass) respectively 685 and 686 g/mol, it is possible that these alkaloids are attached to the series of bisbenzyl tetrahydro isoquinolines.

- Alkaloid C: With a molecular formula C<sub>18</sub>H<sub>13</sub>NO<sub>3</sub>Na and a theoretical mass of 314.079 m/z, its mass spectrum presents, in addition to the peak at m/z 314 (molecular peak), a fragment

at m/z 299 (M-15) corresponding to the loss of a CH<sub>3</sub>;

- Alkaloid D: minority compound of the EC<sub>3</sub> sub-fraction, with the molecular formula CH<sub>21</sub>H<sub>21</sub>NO<sub>5</sub>Na and theoretical mass: 390 m/z. It could be easily identified, by examination of its MSMS spectral data and by direct comparison with oxy-8-palmatine, isolated from an Annonaceae, *Annickia polycarpa* [17].

Its isolation suggested palmatine to be present among quaternary bases in *Annickia chlorantha*. Its mass spectrum revealed peaks at m/z 375 and m/z 360 corresponding to the loss of two CH<sub>3</sub> groups. Figure 6 illustrates the structure of oxy-8-palmatine, elucidated by other authors [14,16].

### Compounds Contained in the EC<sub>9</sub> Sub-Fraction

The sub-fraction EC<sub>9</sub> is a brownish yellow powder soluble in methanol. It is a mixture of two alkaloids. The latter seem to be new compounds and all are attached to the bisbenzyltetrahydroisoquinoline series.

- Alkaloid G: C<sub>36</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>Na; theoretical mass: 607.18.

MS data:

[M-.CH<sub>3</sub>+Na]<sup>+</sup>. à m/z 592

[M-CH<sub>4</sub>+Na]<sup>+</sup> à m/z 591

[M-2CH<sub>3</sub>+Na]<sup>+</sup> à m/z 577

[M- CONH<sub>2</sub>]<sub>2</sub> à m/z 563

- Alkaloid H: C<sub>36</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>Na; Theoretical mass : 605.168.

The 1H NMR spectrum of the EC<sub>10</sub> sub-fraction shows a characteristic signal 7.38 ppm (2H, m) characteristic of an aromatic ring; a singlet at 3.37ppm due to a methoxy in para or ortho; a singlet of 6 protons at 2.57 ppm due to the two N-methyl which resonate together.

These data do not allow us to attribute the signals to each compound.

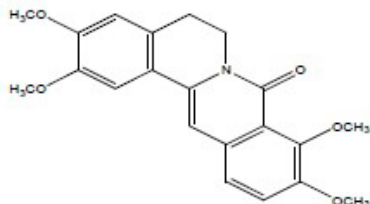
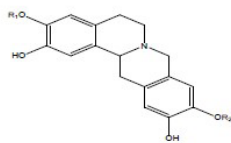
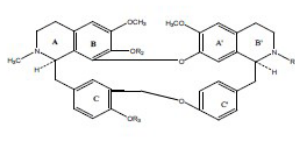


Figure 6: Structure of 8-oxy palmatine.



Tétrahydroprotoberberine



Bisbenzyltetrahydroisoquinoline

Figure 7: Basic structures of Tetrahydroprotoberberine and Bisbenzyltetrahydroisoquinoline.

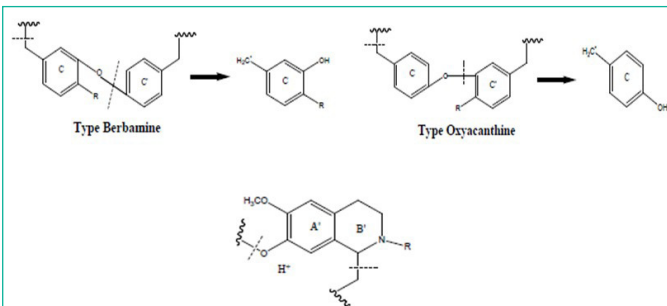


Figure 8: Characteristic fragmentation of bisbenzyl tetrahydro isoquinolines.

### Compounds Contained in the EC<sub>11</sub> Sub-Fraction

The sub-fraction EC<sub>11</sub> is a yellow powder soluble in methanol. It is a mixture of four alkaloids belonging to the same series.

- Alkaloid I : C<sub>35</sub>H<sub>36</sub>N<sub>2</sub>O<sub>4</sub>Na; Theoretical mass: 571.257

SM data:

[M-.CH<sub>3</sub>+Na]<sup>+</sup>. m/z 556,

m/z 293 (M-278), loss of C<sub>17</sub>H<sub>12</sub>O<sub>3</sub>N

- Alkaloid J: C<sub>35</sub>H<sub>34</sub>N<sub>2</sub>O<sub>4</sub>Na; theoretical mass: 569.241.

MS data:

[M-.CH<sub>3</sub>+Na]<sup>+</sup>. à m/z 554,

m/z 292 (M-277) C<sub>17</sub>H<sub>11</sub>O<sub>3</sub>N

- Alkaloid K : C<sub>34</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>Na ; Theoretical mass : 553.210

MS data:

[M-CH<sub>3</sub>+Na]<sup>+</sup>. at m/z 538

[M-2CH<sub>3</sub>+Na]<sup>+</sup> at m/z 523

[M-3CH<sub>3</sub>+Na]<sup>+</sup>. at m/z 508

m/z 495 (M-58) loss of CH<sub>3</sub>COCH<sub>3</sub>

m/z 277 (M-276) loss of C<sub>17</sub>H<sub>10</sub>O<sub>3</sub>N

m/z 262 (M-291) loss of C<sub>18</sub>H<sub>13</sub>O<sub>3</sub>N

- Alkaloid L : C<sub>36</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>Na ; Theoretical mass : 577.210

The 1H NMR spectrum shows, in addition to the two N-methyl and aromatic proton signals, two singletons of two methoxy at 3.78 and 3.91 ppm. These data do not allow us to assign the signals to each compound.

### Compounds Contained in the EC<sub>12</sub> Sub-Fraction

The sub-fraction EC<sub>12</sub> is a yellow ochre powder soluble in methanol. It is a mixture of three alkaloids belonging to the same group. The alkaloids I and J are found in this sub-fraction. The alkaloid of type I is the majority in the EC<sub>12</sub> sub-fraction.

- Alkaloid M : C<sub>36</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>Na ; Theoretical mass : 579.223

MS data:

[M-.CH<sub>3</sub>+Na] at m/z 564

[M-2CH<sub>3</sub>+Na] at m/z 549

- Alkaloid N : C<sub>34</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>Na ; Theoretical mass : 555.225

Alkaloid O : C<sub>37</sub>H<sub>34</sub>N<sub>2</sub>O<sub>4</sub>Na ; Theoretical mass: 593.225

2 N-methyl (2 singlet at 2.49 and 2.56 ppm) and two methoxy (2 singlet at 3.38 and 3.53 ppm). The characteristic signals 7.45 - 7.13 ppm (4 H, m) and 6.91 - 6.88 ppm (2 H, m) indicate the presence of aromatic rings.

These data do not allow us to attribute the signals to each compound. However they confirm that these compounds are alkaloids.

The group C and D alkaloids are protoberberines, the other alkaloids show mass spectral characteristics like those of the bisbenzyl tetrahydro isoquinoline series alkaloids (Figures 7 & 8).

Their mass spectrum revealed, in addition to the molecular peak, fragmentations characteristic of bisbenzyl tetrahydro isoquinolines with two ether bridges of the berbamine or oxyacanthine type resulting from the loss of the benzyl rings C and C' of the molecule, as well as the ions derived from them. The elimination of the tetrahydroisoquinoline unit A'B' is also noted (Figure 7).

The alkaloid composition of *Annickia chlorantha* is found to be more complex than previous work indicates.

Previous study of the barks isolated protoberberine-type alkaloids (Figure 7) that have quaternary ammonium salts [7,13]. These quaternary ammonium salts are abundant and are obviously responsible for the yellow coloring of the bark and probably also for their use in traditional medicine. Taking into account our results, it should be noted that the alkaloid composition of *Annickia chlorantha* is still very poorly elucidated. The isolated alkaloids belong, for the most part, to the group of bisbenzyl tetrahydro isoquinolines (Figure 7) which has never been reported in previous studies. The isolation of palmatine corroborates with the results of some authors [14,16]. The complexity of the alkaloid composition of *Annickia chlorantha* trunk bark is particularly justified by the number of alkaloids present. All these alkaloids belong either to the protoberberine and tetrahydroprotoberberine group or to the bisbenzyltetrahydroisoquinoline group.

The isolated and identified alkaloids of the EC<sub>3</sub> and EC<sub>4</sub> sub-fractions give a C and D type nature which are from Protoberberine types, while, the other isolated alkaloids of the sub-fractions (EC<sub>1</sub> to EC<sub>15</sub>) present mass spectral characteristics such as those of the alkaloids of the Bisbenzyl tetrahydrobenzyl isoquinoline series.

Their mass spectrum reveals, in addition to the molecular peak, classical and characteristic fragments of bisbenzyl tetra-

hydrobenzyl isoquinolines with two ether bridges of the Berbamine or Oxyacanthine type coming from the Benzylic rings C and C', as well as the ions derived from them. We also note the elimination of the Tetrahydrobenzyl isoquinoline unit A'B' (Figures 7 and 8). These alkaloids are grouped in Table 1:

### Conclusion

The alkaloidal composition of *Annickia chlorantha* turns out to be more complex than previous work indicates. These previous studies on the barks of *Annickia chlorantha* allowed the isolation of alkaloids of the Protoberberine type which are quaternary ammonium bases. In view of the results obtained, it should be noted that the alkaloid composition of *Annickia chlorantha* was still little known. The alkaloids isolated from this study, belong for the majority to the group of Bisbenzyl tetrahydrobenzyl isoquinolines which has not yet been reported in the literature. Their crude formulas were given on the basis of high resolution mass spectroscopy.

Further studies on the chlorantha species need to be conducted to structurally identify these alkaloids which do not appear to be attached to previously known structures.

### Author Statements

#### Acknowledgements

Our sincere thanks to Professor Mariamme GLABER, head of the AMES team of the Laboratoire Littoral Environnement et Société (LIENS), University of La Rochelle (France). We also thank Mr. Jean Marie MOUTSAMBOTE, Lecturer in Botany at the National School of Agronomy and Forestry (NSAF), Marien Ngouabi University, for the collection and identification of the plant.

We sincerely thank Mrs. Medine Moussounga Keener for her precious help and contribution in the editing of the English version of this article.

#### Conflict of Interest

The authors declare that we do not have any conflict of interest.

### References

- Rodrigues E. Plants of restricted use indicated by three cultures in Brazil (Caboclo-river dweller, Indian and Quilombola). *J Ethnopharmacol.* 2007; 111: 295-302.
- Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *J Ethnobiol Ethnomed.* 2006; 2: 43.
- Hartmann T. From waste products to ecochemicals: fifty years research of plant secondary metabolism [review]. *Phytochemistry.* 2007; 68: 2831-46.
- Maydell V HJ. Arbres et Arbustes du sahel. Leurs caractéristiques et leurs utilisations. *Eschbom.* 1983; 531.
- Ngantchou I, Nono J-J. Activités trypanocides comparées de quelques composés naturels isolés des plantes médicinales camerounaises. *Pharm méd Trad Afr.* 2004; 13: 133-9.
- Agomo PU, Idigo JC, Afolabi BM. Antimalarial medicinal plants and their impact on cell populations in various organs of mice. *Afr J Med Med Sci.* 1992; 21: 39-46.
- Tan PV, Nyasse B, Dimo T, Wafo P, Akahkuh BT. Synergistic and potentiating effects of ranitidine and two new anti-ulcer compounds from *Enantia chlorantha* and *Voacanga africana* in experimental animal models. *Pharmazie.* 2002; 57: 409-12.
- Nyasse B, Nkwengoua E, Sondengam B, Denier C, Willson M. Modified berberine and protoberberines from *Enantia chlorantha* as potential inhibitors of *Trypanosoma brucei*. *Pharmazie.* 2002; 57: 409-12.
- Wafo P, Nyasse B, Fontaine C, Sondengam BL. Aporphine alkaloids from *Enantia chlorantha*. *Fitoterapia.* 1999; 70: 157-60.
- Sepúlveda-Boza S, Cassels BK. Plant metabolites active against *Trypanosoma cruzi*. *Planta Med.* 1996; 62: 98-105.
- Raponda Walker A, Sillans A. Les plantes utiles du Gabon. Edition sepia. 1995; 614.
- Madiélé Mabika AB. des plantes tinctoriales d'Afrique centrale: inventaire ethnobotanique, Caractérisation analytique et biologique et développement des filières de production [thèse] de doctorat. 2015; 258.
- Samir AR, Makboul AM, Moses OF, Gabriel O, Zac G. *Boll Fac Sci.* 1989; 18: 21-9.
- Hamonnière M, Leboeuf M, Cave A, Paris RR. Alcaloïdes des Annonacées: Alcaloïdes de l'*Enantia chlorantha* Oliv, pl. méd. phytoch. 1975; 9: 296-303.
- Cardon D. Le monde des teintures naturelles. Éditions Belin. 2003; 586.
- Jössang A, Leboeuf M, Cavé A. Alkaloids of Ammonaceae XVII: alkaloids of *Enantia polycarpa* Engl. et Diels (author's transl). *Planta Med.* 1977; 32: 249-57.
- Wright CW, Phillipson JD. Biological screening of traditional preparations from some medicinal plants used antidiabète. *Phytother Res.* 1990; 4: 127-39.