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# LC-MS based analysis reveal antimicrobial compounds from Gabonese pharmacopoeia: chemical characterisation and cytotoxicity evaluation

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Plants used in traditional medicine represent an important source of new compounds. *Halea ledermannii* (*H. ledermannii*) (Krause) Verdc. (Rubiaceae), *Gossypium barbadense* (*G. barbadense*) (Malvaceae), *Pycnanthus angolensis* (*P. angolensis*) (Myristicaceae), *Drypetes gossweileri* (*D. gossweileri*) S. Moore (Euphorbiaceae) and *Scyphocephalum ochocoa* (*S. ochocoa*) Warb (Myristicaceae) are five plants widely used in traditional Gabonese medicine as antimicrobials. However, little is known about the active compounds associated with their biological activities. Based on botanical studies and the claims of traditional healers regarding the antimicrobial effects of these plants, a study to evaluate the antimicrobial activity and phyto-chemical profile of aqueous extracts of three plants (bark of *H. ledermannii*, *G. barbadense* root bark and *P. angolensis* bark) and methanolic extracts of two plants (*S. ochocoa* stem bark and *D. gossweileri* root bark). Under the guidance of LC-MS detection, identified twenty seven (27) potentially active compounds. Eight (8) of these belong to the quinovic acid-type triterpenoid sap-onins identified in the aqueous extract of *H. ledermannii*, eleven (11) are dibenzofurans, chroman and stigmaterol detected in the methanolic extract of *S. ochocoa* and eight (8) compounds in the methanolic extract of *D. gossweileri* are friedelin, drypemolundein B and gossweilone, to name but a few. In parallel, the five extracts were tested on reference bacterial strains: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 278533, *Salmonella typhi* ATCC 13311, *Klebsiella pneumoniae* ATCC 700603, *Shigella flexneri* ATCC 24570. Three of these extracts (aqueous extract of *H. ledermannii* and two methanolic extracts of *S. ochocoa* stem bark and *D. gossweileri* root bark) showed moderate activity against *Staphylococcus aureus* ATCC 25923, with inhibition zones of  $12.3 \pm 0.5$  mm,  $10.1 \pm 0.5$  mm and  $7.6 \pm 0$  mm respectively. In addition, we assessed the toxicity of the three extracts that showed antimicrobial activity using an invertebrate model, *Galleria mellonella* (GM). We found that the LD varied according to the concentration of the plant material. The aqueous extract of *H. ledermannii* and the methanolic

extracts of *S. ochocoa* and *D. gossweileri* were not toxic to *G. mellonella*. The LD<sub>50s</sub> (mg/mL) obtained were 93.2 mg/mL [717.2 g/kg body weight (bw)] and 100 mg/mL (762.3 g/kg bw), 95.4 mg/mL [721.1 g/kg body weight (bw)].

#### KEYWORDS

LC-MS based analysis, antimicrobial compounds, cytotoxic activity, traditional Gabonese medicine, LC-MS

## Introduction

Natural products derived from plants, alongside synthetic products based on combinatorial chemistry and genomics, remain an essential source for the discovery of new compounds (Newman and Cragg, 2007). In sub-Saharan Africa, particularly Gabon, the practices of traditional healers have contributed to the collection of ethnobotanical data and suspicions of bioactivity of several plants in humans, thus offering advantages and assets for the implementation of genuine discovery of compounds targeted against tropical infectious diseases (Karaman et al., 2003; Kokoska et al., 2002). The emergence of multi-resistant bacterial pathogens and the increase in antimicrobial resistance drive the need for extensive and sustained investigations towards discovering new antimicrobials. The collection of plants used by traditional healers, testing for antimicrobial properties of the plant extracts, chemical characterization of molecules and evaluation of cytotoxicity are the main steps of discovering new compounds derived from natural products. These steps are often challenging, especially when generating comprehensive and stringent preclinical toxicity data that will lead to first use in humans (Ignasiak and Maxwell, 2017). The invertebrate model *Galleria mellonella* (GM) (Ogungbemi and van Gestel, 2018; Megaw et al., 2015) is technically easy to transfer from one laboratory to another. It is being investigated as a potential biological alternative to the rat and mouse models (Allegra et al., 2018; Moya-Anderico et al., 2021).

We carried out a study of extracts from five plants; namely *Hallea ledermannii* (Krause) Verdc. Bark (Rubiaceae), *Scyphocephalum ochocoa* Warb stem bark (Myristicaceae), *Gossypium barbadense* root bark (Malvaceae), *Pycnanthus angolensis* bark (Myristicaceae) and *Drypetes gossweileri* S. Moore root bark (Euphorbiaceae) (List, 2010). They have been found in the swampy riverine forests and rainforests of Africa and are used by healers for a variety of ailments, including skin and microbial infections, dysentery, pain and as a febrifuge (Burkill, 1997), abscesses, anaemia, general fatigue, infections and respiratory tract infections including tuberculosis (Tchouya et al., 2015; Ngouela et al., 2003).

Here, we report the results of our investigations to identify the compounds that induced antimicrobial effects the plant extracts using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) guided method consisting on the characterization of specialised metabolites contained in the plant extracts. To fine-tune the identification of the compounds, ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC/HRMS-MS) analyses were carried out on the three active extracts for quinovic acid-type triterpenoid saponins, dibenzofurans, chroman and stigmaterol constituting the main family of compounds in these extracts. The toxicity profiles of the identified compounds using the GM as a model

were analysed. To our knowledge, this study is the first study to present a comprehensive characterisation of these three extracts.

## Materials and methods

### Plant material

Specimens of *H. ledermannii* (Rubiaceae) *G. barbadense* (Malvaceae), *P. angolensis* (Myristicaceae), *D. gossweileri* (Putranjivaceae) and *S. ochocoa* (Myristicaceae) referenced as HL\_01, GB\_01, PA\_01, DG\_01 and SO\_02, respectively were made kindly available to us the National Institut de Pharmacopée et de Médecine Traditionnelle (IPHAMETRA) Gabon Libreville. Botanical identification was confirmed by Prof. Paul Henri Bourobou Bourou and Mr. Raoul Niangadouma of the Institut de Pharmacopée et de Médecine Traditionnelle (IPHAMETRA). The dried plant material obtained was then pulverized using a Retsch1 MM 400 ball mill. (Monitoring and Control Pty Ltd., Haans, Germany). at a frequency of 30.0 Hz for 120 min frequency of 30.0 Hz for 120 s to obtain fine brown powders. To ensure particle size consistency, these powders were sieved using a 500 mm mesh (Endcotts Filters Ltd., London, United Kingdom).

### Extraction of secondary metabolites

The resulting powders of *H. ledermannii* (245 g) *G. barbadense* (205 g), *P. angolensis* (147 g), *D. gossweileri* (345 g) and *S. ochocoa* (305 g) respectively were extracted by maceration with various solvents. The first extraction was performed in hexane, followed by ethyl acetate, dichloromethane, methanol and water. Extraction with each solvent was carried out at room temperature in the laboratory for 24 h. The extracts obtained were filtered using filter paper and then evaporated using a rotary evaporator. Only aqueous extracts were freeze-dried.

### Data dependent LC-HRMS<sup>2</sup> analyses

HPLC-QTOF-HRES analyses were achieved by coupling the LC system to a hybrid quadrupole time-of-flight mass spectrometer Agilent 6,530 (Agilent Technologies, Massy, France) equipped with an ESI source, operating in positive ion mode. Source parameters were set as follows: capillary temperature at 320°C, source voltage at 3500 V, and sheath gas flow rate at 10 L.min<sup>-1</sup>. The divert valve was set to waste for the first 3 min. MS scans were operated in full-scan mode from *m/z* 100 to 1700 (0.1 s scan time) with a mass resolution of 11,000 at *m/z* 922. A MS<sub>1</sub> scan was followed by MS<sub>2</sub> scans of the three most intense ions above an absolute threshold of 5,000 counts. The selected parent ions were fragmented with one collision energy fixed at

45 eV and an isolation window of 1.3 amu. The calibration solution contained two internal reference masses (purine, C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>, *m/z* 121.050873, and HP-921 [hexakis-(<sup>1</sup>H,<sup>1</sup>H,<sup>3</sup>H-tetrafluoropentoxy) phosphazene], C<sub>18</sub>H<sub>18</sub>O<sub>6</sub>N<sub>3</sub>P<sub>3</sub>F<sub>24</sub>, *m/z* 922.0098). A permanent MS/MS exclusion list criteria was set to prevent oversampling of the internal calibrant. LC-UV and MS data acquisition and processing were performed using MassHunter Workstation software (Agilent Technologies, Massy, France).

In order to clarify the concordance of the raw formulae obtained with the MS<sup>2</sup> spectra and the compounds present in the databases with a view to dereplication, we used the identification certainty rules proposed by Dormann et al. (2012). These rules indicate a degree of confidence for the identification of compounds, divided into 5 levels (1–5). They are based on the agreement of several parameters:

- retention time (the authors recommend co-injection);
- precise mass measurement (HR-MS; identical crude formulae, matching isotopic masses);
- matching MS/MS and UV spectra. Level 1 corresponds to reliable identification of a compound.

For this level, the MS and MS<sup>2</sup> spectra and the retention time are compared with a physically available reference substance, which is not our case. Level 2 is proposed for satisfactory comparison with data in a database (level 2a), or for satisfactory interpretation of MS and MS/MS data (level 2b). For this level, the retention times between an analyte and a reference are not necessarily concordant, indicating a similarity (sometimes relative in structural terms) but not allowing the identity of an experimental match to be affirmed with certainty. The other levels (3, 4 and 5) are not described in detail. The use of this classification is indicative.

## Antimicrobial assays

### Bacteria strains

In this study, the following reference strains of bacteria: *Pseudomonas aeruginosa* ATCC 278533, *Salmonella thyphi* ATCC 13311, *Klebsiella pneumoniae* ATCC 700603, *Shigella flexneri* ATCC 24570 were used as gram negative and as gram positive *Staphylococcus aureus* ATCC 25923 and clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) were used. The reference bacteria were purchased from American Type Culture Collection (ATCC). These germs were brought to room temperature and then suspended in Brain Heart Infusion nutrient broth (Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, United Kingdom) in order to revive them. 10 µL of each reference bacterium are added to 1 mL of BHI then incubated at 37°C overnight.

### Preparation of bacteria

After incubation, bacterial suspensions containing gram negative bacteria (*Pseudomonas aeruginosa* ATCC 278533, *S. thyphi* ATCC 13311, *K. pneumoniae* ATCC 700603), were cultured on MacConkey agar (Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, United Kingdom), the gram-negative bacterium *S. flexneri* ATCC 24570 was inoculated on Hektoen enteric agar (Becton, Dickinson and company Spark, MD 21152 United States). The gram-positive bacteria *staphylococcus aureus* was inoculated on Columbia blood agar (Oxoid

Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, United Kingdom); in order to obtain pure colonies.

## Antimicrobial activity evaluation of plant extracts by Kirby Bauer method

### Preparation of inoculum

Kirby Bauer's method (1, 2) was used to assess the antimicrobial activities of plant extracts. A pure colony of identical morphology of each bacterium is suspended in 4 mL of physiological water (NaCl). The turbidity of this bacterial suspension is measured at 0.5 McFarland, this corresponds to 1.5 × 10<sup>8</sup> CFU (Karaman et al., 2003).

### Challenge of plant extracts with bacteria

Bacterial solution was collected with a clean cotton swab and dipped into Mueller Hinton agar (Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, United Kingdom), in which the antimicrobial tests were performed. The agar was dried for 5 min. We made 6 mm disks from filter paper. These disks were individually soaked with 5 µL of each plant extract at an initial concentration of 1 mg/mL. The soaked discs were then placed on Petri dishes containing Mueller Hinton medium. Petri dishes were then incubated at 37°C overnight. The zone of inhibition was measured in millimeters using a ruler.

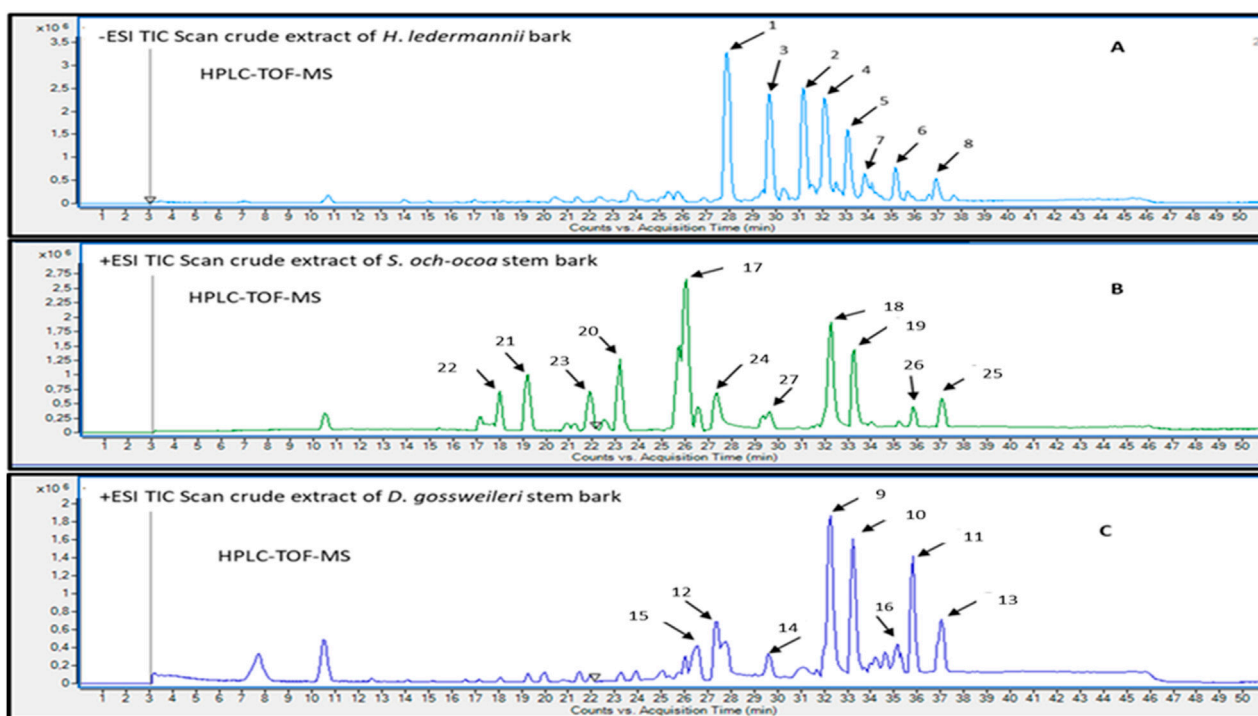
## Assessment of acute toxicity using the *Galleria mellonella* model

Cytotoxicity was evaluated on the *G. mellonella* (GM) larvae. GM larvae were obtained from the Medical University of Vienna, Clinical Department of Infections and Tropical Medicine Control Center 3P Währinger Gürtel 18–20, 1090 Vienna. They were stored at 15°C before use. Dead larvae and larvae with dark spots or presenting signs of melanization were removed. A selection of larvae with masses between 0.2 and 0.5 g were chosen for testing. For each concentration, three groups of six (6) larvae were tested. Using a PBS solution, three extract concentrations were obtained: 20 mg/mL; 60 mg/mL and 100 mg/mL. Injection of 20 µL of each substance was performed using a 0.3 mL Terumo® Myjector® U-100 syringe. Injections were made below the last prominent left leg. A group of 6 larvae injected with sterile PBS was considered as a control. All larvae were incubated at 37°C in the dark. Larval mortality was monitored every 24 h for 120 h. An individual was considered dead when it failed to reorient itself or respond to various stimuli. The 50% lethal dose (LD50) was determined by taking the average weight of a group of 15 larvae from the formula:

## Results

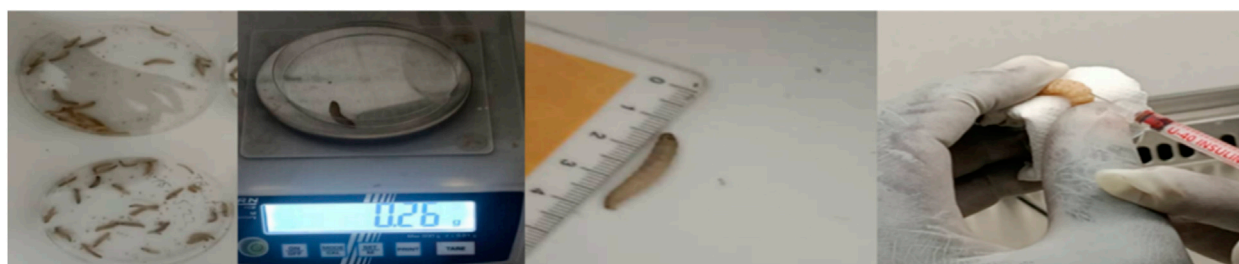
### LC-MS based analysis of the three active extracts

LC-MS analysis in this study was only carried out on the three plants that showed potential activity. In order to rapidly identify the



**FIGURE 1**  
 A total ion chromatogram (TIC) in positive and negative ion mode of the aqueous extract and methanolic extract of the three active extracts, obtained on an Agilent 6530 Q/ToF (Scan rang). [(A) ESI TIC Scan crude extract of *H. ledermannii*; (B) ESI TIC Scan crude extract of *S. ochocoo* and (C) ESI TIC Scan crude extract of *D. gossweileri*].

$$LD50 \left( \frac{g}{kg} \text{ masse larvaire} \right) = \frac{\text{Volume (mL)} \times LD50 \left( \frac{mg}{mL} \right) \times 0,001}{\text{Mean weight of larves (kg)}} * 100$$



Group of 50 larva (weighs 0,2-0,5g) (A); Measuring the length of a larva (B); Injection of GM larva with plant extracts through the last left proleg (c)

compounds potentially responsible for this activity, aqueous extracts of *H. ledermannii* bark, and *D. gossweileri* root bark and methanolic extracts of *S. ochocoo* stem bark were rigorously analyzed by LC-HRMS/MS in positive and negative ionization mode, using data-dependent acquisition.

A Total Ion Chromatogram (TIC) of each crude extract showing peaks corresponding to a chemical compound in the extract is

presented in (Figure 1). A manual inspection of the resulted MS/MS spectra led to the putative identification of chemical constituents of the crude extracts (Figures 2–4). The names of the putatively identified compounds are recorded in (Tables 1–3). Known compounds were annotated using search in the most recent version of the dictionary of natural products database (Natural Products database, 2023; Scifinder, 2023; PubChem, 2023).

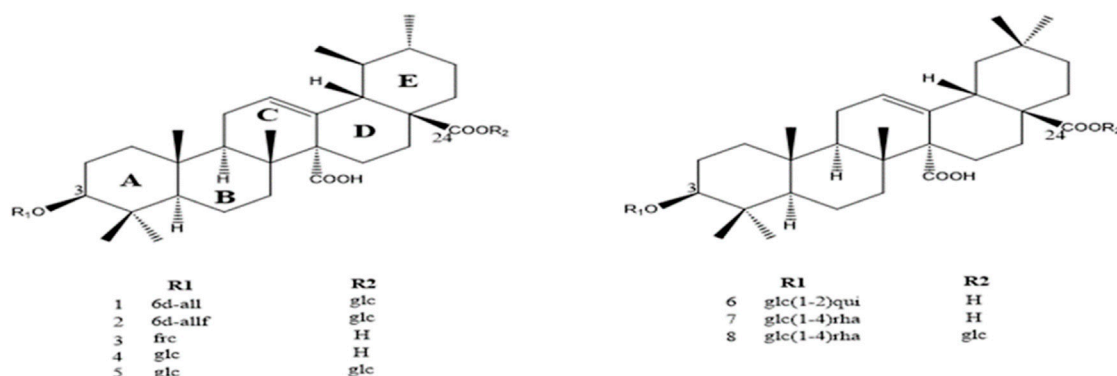


FIGURE 2 Structure of the compounds isolated from the bark of *Hallee ledermannii* (Krause) Verdc. (Rubiaceae) (6 d-all, 6-deoxy-β-D allopyranosyl; 6 d-allf, 6-deoxy-β-D allofuranosyl; Frc, fucopyranoside, glc, glucopyranoside, H, hydrogen; glc (1–2) rha, glucopyranosyl-(1→4) rhamnopyranoside; glc (1–2) rha, glucopyranosyl quinovic acid).

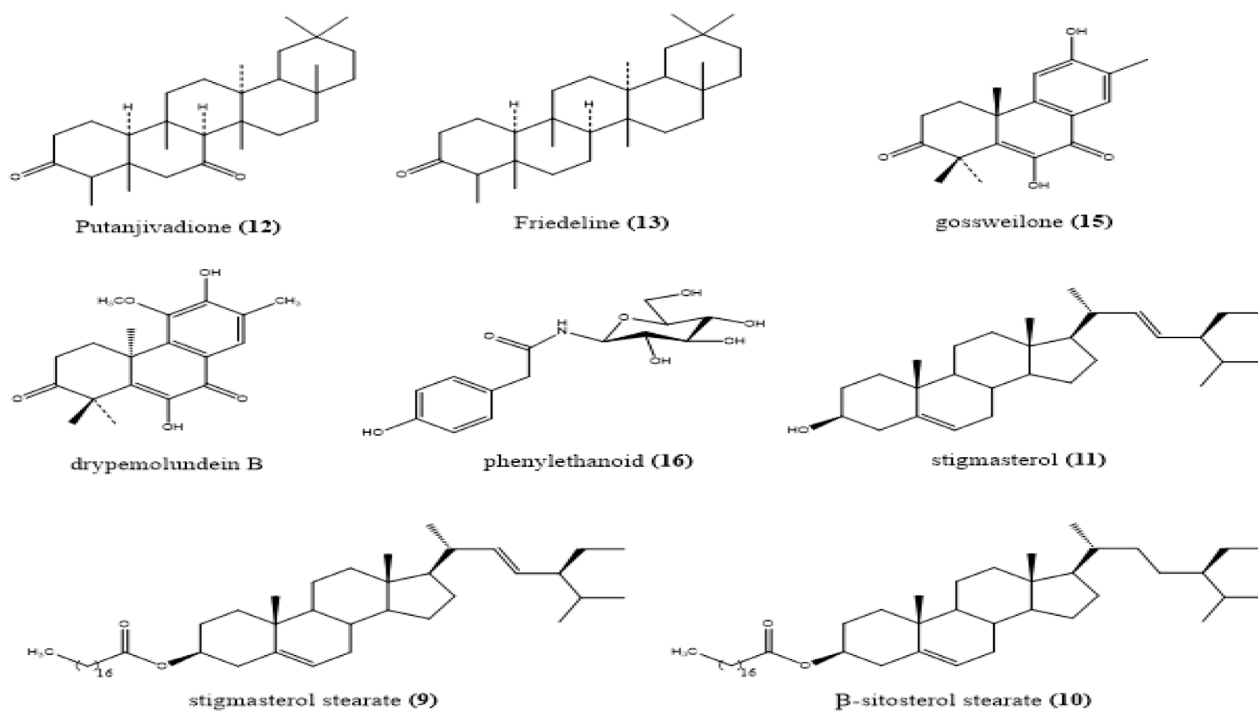
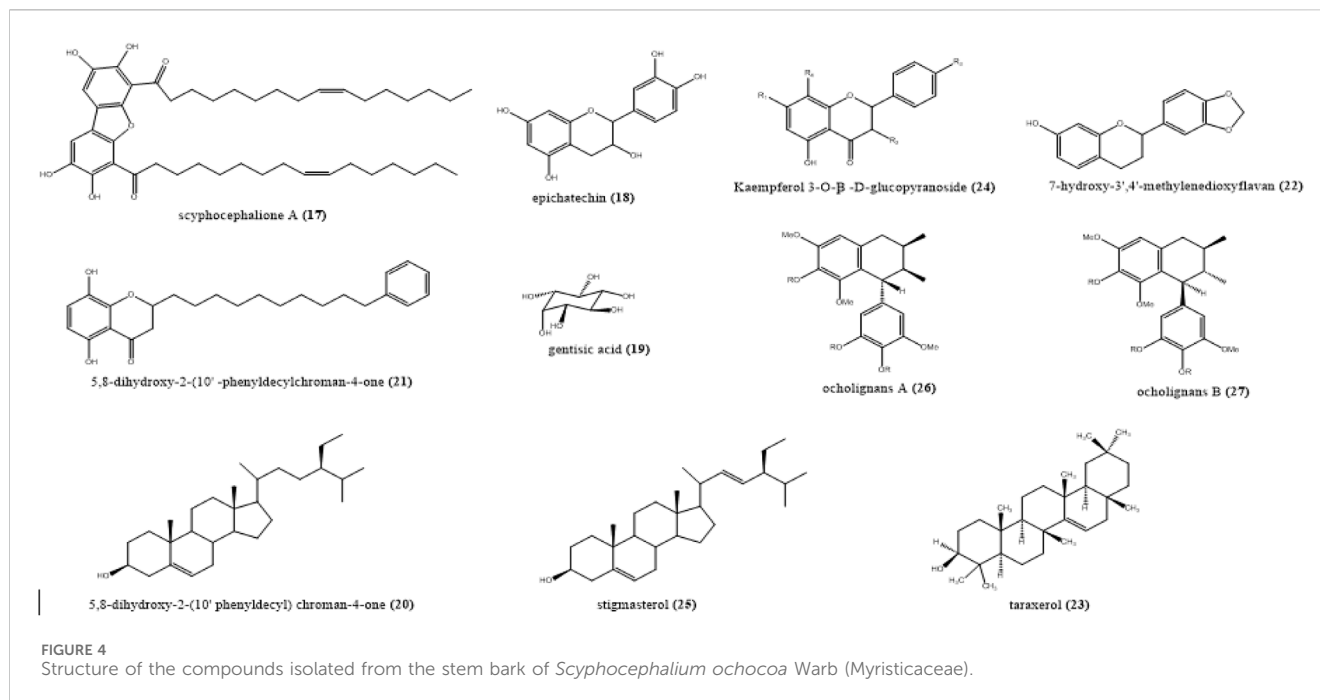


FIGURE 3 Structure of the compounds isolated from the root bark of *Drypetes gossweileri*.

The total ion chromatogram (TIC) of the aqueous extract of *H. ledermannii* bark (A) showed eight distinct peaks (ranging from a retention time (tR) of 28.7–37.5 min) (Figure 1). A comparison between the MS data obtained and those published for compounds previously isolated from the bark of *H. ledermannii* was carried out for dereplication purposes (Figure 2). The peak 1, 2, 6 and 7 showed an ion at  $m/z$  793.4382 [M-H]<sup>-</sup>,  $m/z$  955.4903 [M-H]<sup>-</sup>,  $m/z$  793.4377 [M-H]<sup>-</sup> and  $m/z$  793.4380 [M-H]<sup>-</sup> corresponding to the molecular formula C<sub>42</sub>H<sub>66</sub>O<sub>14</sub>, C<sub>48</sub>H<sub>76</sub>O<sub>19</sub>, C<sub>42</sub>H<sub>65</sub>O<sub>14</sub>, and C<sub>41</sub>H<sub>66</sub>O<sub>11</sub> respectively. While peaks 3, 4, 5 and 8 showed ions at  $m/z$  779.4219 [M-H]<sup>-</sup>,  $m/z$  672.4240 [M-H]<sup>-</sup>,  $m/z$  795.1209 [M-H]<sup>-</sup>,

$m/z$  648.8219 [M-H]<sup>-</sup>, corresponding to the molecular formula C<sub>41</sub>H<sub>64</sub>O<sub>14</sub>, C<sub>39</sub>H<sub>60</sub>O<sub>9</sub>, C<sub>42</sub>H<sub>66</sub>O<sub>14</sub> and C<sub>36</sub>H<sub>56</sub>O<sub>10</sub> respectively.

The base peak chromatogram of the HPLC/HRMS analysis presented in Figure 1 on the extract obtained from the root bark of *D. gossweileri* (C) detected (8) main chromatographic peaks in the [M + H]<sup>+</sup> ionic mode. Manual inspection of the MS/MS spectra obtained led to the putative identification of the chemical constituents of the extract. The 8 compounds are identified (Figure 4): stigmasterol stearate (9), β-sitosterol stearate (10) and β-sitosterol (11) at  $m/z$  680.302 [M + H]<sup>+</sup>,  $m/z$  654.319 [M + H]<sup>+</sup> and  $m/z$  414.313 [M + H]<sup>+</sup>, respectively. As for the triterpenoids,



**TABLE 1** Identification of compound from the aqueous extract of *H. ledermannii* bark by LC-ESI-MS in the negative ion mode.

Peak	RT (min)	$m/z$ [M-H] <sup>-</sup>	Molecular formulas [M]	Ions tentative identification	Confidence level
1	25.76	793.4377	C <sub>42</sub> H <sub>65</sub> O <sub>14</sub>	cincholic acid-3-O-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranoside	3
2	31.79	955.4903	C <sub>48</sub> H <sub>76</sub> O <sub>19</sub>	quinovic acid-3-O-6-deoxy-β-D-allofuranosyl-28-O-β-D-glucopyranoside	3
3	29.85	779.4219	C <sub>41</sub> H <sub>64</sub> O <sub>14</sub>	cincholic acid-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside	3
4	32.45	672.4240	C <sub>39</sub> H <sub>60</sub> O <sub>9</sub>	quinovic acid-3-O-6-deoxy-β-D-allopyranosyl-28-O-β-D-glucopyranoside	3
5	33.27	795.1209	C <sub>42</sub> H <sub>66</sub> O <sub>14</sub>	cincholic acid-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-28-β-D-glucopyranoside	3
6	35.62	793.4377	C <sub>42</sub> H <sub>65</sub> O <sub>14</sub>	quinovic acid-3-O-β-D-fucopyranoside	3
7	34.20	793.4380	C <sub>41</sub> H <sub>66</sub> O <sub>11</sub>	quinovic acid-28-O-β-D-glucopyranoside	3
8	37.81	648.8219	C <sub>36</sub> H <sub>56</sub> O <sub>10</sub>	quinovic acid-3-O-β-D-quinovopyranoside	3

putanjivadiene an ion  $m/z$  442.345 (Calcd for C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>) (12), friedelin (13) with an ion of  $m/z$  426.302 (Calcd for C<sub>30</sub>H<sub>50</sub>O) and drypemelundin B (14) with an ion  $m/z$ : 456.7152. (Calcd for C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>). Diterpenes were identified, namely Gossweilone (15) with an ion  $m/z$  299.145 (Calcd for C<sub>18</sub>H<sub>20</sub>O<sub>4</sub>).

Using the same procedure, with the methanolic extract of *S. ochocoa* stem bark (C), several compounds were putatively identified, namely dibenzofurans such as scyphocephalus (17)  $m/z$  703.4563 [M + H]<sup>+</sup> corresponding to the molecular formula C<sub>44</sub>H<sub>64</sub>O<sub>7</sub>; 5,8-dihydroxy-2-(10'-phenyldecyl) chroman-4-one (20)  $m/z$  396.2292 [M + H]<sup>+</sup> C<sub>25</sub>H<sub>32</sub>O<sub>4</sub>; a sterol such as stigmasterol (25)  $m/z$  412.690 [M + H]<sup>+</sup> C<sub>29</sub>H<sub>48</sub>O;

7-hydroxy-3',4'-methylenedioxyflavan (22)  $m/z$  270.2919 [M + H]<sup>+</sup> C<sub>16</sub>H<sub>14</sub>O<sub>4</sub> and 2, 7'-cycloignans as ocholignans A (26) corresponding to the  $m/z$  375.1630, molecular formula C<sub>21</sub>H<sub>26</sub>O<sub>6</sub> [M + H]<sup>+</sup> (Table 3).

## Biological activity of the five (5) crude extracts

The 5 crude extracts obtained from the plants studied were tested biologically. Growth inhibition was observed on methicillin-resistant *Staphylococcus aureus* (MRSA). For 3 of them, namely the aqueous extracts of *H. ledermannii* and the methanolic extract of

TABLE 2 Tentative identification of compound from the methanolic extract of *D. gossweileri* stem bark by LC–ESI-MS in the positive ion mode.

Peak	RT (min)	m/z [M-H] <sup>+</sup>	Molecular formulas [M]	Ions tentative identification	Confidence level
9	32.756	680.302	C <sub>44</sub> H <sub>64</sub> O <sub>7</sub>	stigmasterol stearate	3
10	33.798	654.319	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	β-sitosterol stearate	3
11	35.806	414.313	C <sub>7</sub> H <sub>6</sub> O <sub>6</sub>	stigmasterol	3
12	27.467	442.345	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	putanjivadione	3
13	37.254	426.302	C <sub>30</sub> H <sub>50</sub> O	friedelin	3
14	29.823	456.7152	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	drypamolundein B	3
15	26.798	299.145	C <sub>18</sub> H <sub>20</sub> O <sub>4</sub>	gossweilone	3
16	35.665	448.4321	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	phenylethanoid	3

TABLE 3 Tentative identification of compound from the methanolic extract of *S. ochocoa* stem bark by LC–ESI-MS in the positive ion mode.

Peak	RT (min)	m/z [M-H] <sup>+</sup>	Molecular formulas [M]	Ions tentative identification	Confidence level
17	4.356	703.4563	C <sub>44</sub> H <sub>64</sub> O <sub>7</sub>	scyphocephalione A	3
18	3.798	290.2712	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	epicatechin	3
19	4.506	154.1380	C <sub>7</sub> H <sub>6</sub> O <sub>6</sub>	gentisic acid	3
20	4.867	396.2292	C <sub>25</sub> H <sub>32</sub> O <sub>4</sub>	5,8-dihydroxy-2-(10'-phenyldecyl)chroman-4-one	3
21	5.854	414.7119	C <sub>29</sub> H <sub>50</sub> O	β-sitosterol	3
22	5.223	270.2919	C <sub>16</sub> H <sub>14</sub> O <sub>4</sub>	7-hydroxy-3',4'-methylenedioxyflavan	3
23	5.198	426.7240	C <sub>30</sub> H <sub>50</sub> O	taraxerol	3
24	5.965	448.4321	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	kaempferol 3-O-β -D-glucopyranoside	3
25	6.456	412.690	C <sub>29</sub> H <sub>48</sub> O	Stigmasterol	3
26	1. 854	373.1630	C <sub>29</sub> H <sub>48</sub> O	Ocholignans A	3
27	2.342	373.1630	C <sub>29</sub> H <sub>48</sub> O	Ocholignans B	3

TABLE 4 Susceptibility of gram-positive and gram-negative bacteria against aqueous extract of *H. ledermannii*, *G. barbadense*, *P. angolensis* and the methanolic extract of *S. ochococoa* and *D. gossweileri*.

Plants	Extracts	Inhibition zone diameters (mm)				
		Gram-negative				Gram-positive
		Ps. aeruginosa ATCC 278533	Sal. Thyphi ATCC 13311	Kl. Pneumoniae ATCC 700603	<i>Shigella flexneri</i>	Staph. Aureus ATCC 25923
<i>H. ledermannii</i> (bark)	Aqueous	—	—	—	—	12.3 ± 0.5 mm
<i>D. gossweileri</i> (root bark)	Methanolic	—	—	—	—	7.6 ± 0 mm
<i>S. ochocoa</i> (Stem bark)	Methanolic	—	—	—	—	10.1 ± 0.5 mm
<i>P. angolensis</i> (bark)	Aqueous	—	—	—	—	—
<i>G. barbadense</i> (root bark)	Aqueous	—	—	—	—	—
Positive control	Ceftriazone	22	39	18	29	22
Negative control	DMSO	—	—	—	—	—

TABLE 5 LD<sub>50</sub> values for the aqueous extract of *H. ledermannii*, *D. gossweileri* and *S. ochocoo* methanolic tested on *Galleria mellonella* model.

Plants	Parts used	Extracts	LD <sub>50</sub>	
			(mg/mL)	(g/Kg of bw)
<i>H. ledermannii</i>	Bark	Aqueous	93.2 ± 1	717.2 ± 1
<i>D. gossweileri</i>	Root bark	Methanolic	95.4 ± 0.5	721.1 ± 05
<i>S. ochocoo</i>	Stem bark	Methanolic	100 ± 0	762.3 ± 0

*D. gossweileri* and *S. ochocoo* with inhibition zones of 12.3 ± 0.5 mm, 7.6 ± 0 mm and 10.1 ± 0.5 mm respectively (Table 4). The other two extracts were found to be non-susceptible.

Following this analysis, we assessed the toxicity of the three extracts using the invertebrate model, *G. mellonella* (GM). We found that the LD varied with the concentration of the plant material (Table 5). The aqueous extract of *H. ledermannii*, and the methanolic extract of *D. gossweileri*, *S. ochocoo* were non toxic to GM. The LD<sub>50</sub> (mg/mL) obtained were 93.2 mg/mL [717.2 g/kg body weight (bw)], 95.4 mg/mL (721.1 g/kg bw) and 100 mg/mL (762.3 g/kg bw) respectively.

## Discussion

Due to the prevalence of multi-resistant bacteria and their low sensitivity to antibiotics, all the extracts were tested for their antibacterial activity. They were tested at a concentration of 250 µg/mL against four reference strains, namely *Pseudomonas aeruginosa* ATCC 278533, *S. thyphi* ATCC 13311, *K. pneumoniae* ATCC 700603, *S. flexneri* ATCC 24570 were used as gram negative and as gram positive *Staphylococcus aureus* ATCC 25923. The positive control was ceftriaxone and the negative control was DMSO. *Staphylococcus aureus* ATCC 25923 was the only gram-positive bacterium tested. This bacterium was only sensitive to three of the five extracts. The aqueous extracts of *H. ledermannii*, with a zone of inhibition of 12 mm, was the extract that showed the highest antibacterial activity. This result is similar to that found by Adesegun et al. (2012). Who evaluated the activity of leaf extract of *H. ledermannii*. The methanolic extract of *S. ochocoo*, with a zone of inhibition of 10 mm. This is almost half the zone of inhibition of the antibiotic ceftriaxone used as a positive control. This result is similar to that obtained by Tchouya et al. (2015) and Foundikou et al. (2018). And the methanolic extract of *D. gossweileri*, was moderately active with a zone of inhibition of 7 mm. This result is similar to a DCM extract of *D. gossweileri* bark showed antibacterial activity with MIC values of 0.25–1.00 mg/mL against *Staphylococcus aureus* (Wansi et al., 2016). The results showed that none of the extracts showed activity against gram-negative bacteria (Table 4).

The toxicity test was carried out on the three plants with antimicrobial activity: the aqueous extract of *H. ledermannii*, the methanolic extract of *S. ochocoo* and the methanolic extract of *D. gossweileri*. This test was carried out over 4 days and in triplicate. Although the differences were not statistically significant, the mortality rates obtained with the 100 mg/mL concentrations were visually higher than those obtained with the 20 and 60 mg/mL concentrations (SI). The results showed that the LD<sub>50</sub> of the

aqueous extract of *H. ledermannii* 93.241 mg/mL (717.2 g/kg) was slightly lower than that of the methanolic extract of *D. gossweileri* 95.41 mg/mL (721.1 g/kg) and *S. ochocoo* 100 mg/mL (762.3 g/kg). The compounds contained in the aqueous extract of *H. ledermannii* are therefore more toxic. However, compared to other medicinal plant extracts, the toxicity of the aqueous extract of *H. ledermannii* and the methanolic extract of *S. ochocoo* and *D. gossweileri* is very low. In deed, for example, the hydroethanol extract of *Citrus officinalis*, whose toxicity was as-sessed on the *G. mellonella* model, was 4.87 mg/mL (90 g/kg larval mass) (Mbarga et al., 2021). Extracts of total alkaloids from the plant *Artemisia annua* showed a toxicity of 200 g/kg; the isolated anti-plasmodial molecules were judged to be non-toxic to humans (Sunmonu and Afolayan, 2013). Comparing the results of the studies cited with our own, the toxicity of the extracts studied is much lower than that of *Artemisia annua*, leading to the conclusion that the various molecules that could be extracted from it would be harmless to humans.

Many structurally diverse secondary metabolites have already been isolated from *H. ledermannii*. In our study, Using the molecular formula obtained by MS/MS only one result corresponding to the quinovic acid triterpenoid saponin compounds previously described from the stem bark of *Hallea ledermannii* (Table 1) (Koffi et al., 2023; Kang et al., 2003). On the basis of these results, we can assume that quinovic acid-type triterpenoid saponins could be one of the compounds responsible for the antibacterial activity of this extract.

Manual inspection of the MS/MS spectra of methanolic extract of *D. gossweileri* stem bark led to the putative identification of the chemical constituents of the extract; A total of 8 compounds were identified. These metabolites belong to families of natural compounds, namely steroids, diterpenes and triterpenoids. Among the steroids, stigmasterol stearate, β-sitosterol stearate and β-sitosterol, were putatively identified. Compounds, 10 and 11 were previously isolated by Matochko (2010) and Ata et al., in 2011. We have the putanjivadiene, friedelin and drypemelundin B. All these compounds had been described by Ngouela et al. (2003); Wandji et al. (2003) or by Wandji et al. (2000) in *D. gossweileri* stem bark extracts (Ngouela et al., 2003; Wandji et al., 2000). Diterpenes were identified, namely Gossweilone. This compound had already been isolated previously by Ngouela et al. (2003) in methanolic extract of *D. gossweileri* stem bark.

All compounds have been previously described in *S. ochocoo* (Foundikou et al., 2018). In another study, ocholignan A was found to possess significant *in vitro* antibacterial activity against methicillin-resistant Gram-positive *Staphylococcus aureus* ATCC 33591 and *Staphylococcus aureus* 78–13607A, with a MIC of 16 µg/mL, respectively (Hu et al., 2005). Based on these results, we can speculate that cyclolignans, like ocholignans, could be one of



the compounds responsible for the antimicrobial activity of the extract.

## Conclusion

To the best of our knowledge, our work reports the chemical and biochemical study of five plants used in traditional medicine in Gabon. First of all, it was shown that three of the five plants, namely: *H. ledermannii*, *D. gossweileri* (Putranjivaceae) and *S. ochocoa* can biosynthesize antimicrobial compounds. In addition, using the LCMS/MS data base approach, we were able to easily identify (27) compounds likely to be responsible for the biological activity observed, namely quinovic acid-type triterpenoid saponins detected in the stem bark of *Hallea ledermannii*; steroids such as stigmasterol stearate,  $\beta$ -sitosterol stearate,  $\beta$ -sitosterol, putanjudione, friedelin and drypemelundin B in extracts of *D. gossweileri* root bark extracts and in the ethanolic. Extract of *S. ochocoa* Scyphocephalus, chroman 5,8-dihydroxy-2-(10 phenyldecyl)chroman-4-one methylenediether 7-hydroxy-3',4'-methylenedioxyflavan 6 and 2, 7-cyclolignans as ocholignans A. Our study highlighted that the three extracts can produce various compounds with antimicrobial activity against *Staphylococcus aureus*, providing important documentation of the antibacterial activity of these compounds. In addition, the aqueous extract of the bark of *H. ledermannii* and the methanolic extract of the stem bark of *S. ochocoa* and *D. gossweileri* root bark extracts were found to be non-cytotoxic on the *G. mellonella* model.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

EON'n: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft. PE: Investigation, Methodology, Writing—review and editing. JE: Methodology, Software, Writing—review and editing. AB: Investigation, Writing—review and editing. RK: Conceptualization, Investigation, Methodology, Project administration, Writing—review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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