Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine

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Abstract

Background: Infectious diseases caused by multiresistant microbial strains are on the increase. Fighting these diseases with natural products may be more efficacious. The aim of this study was to investigate the in vitro antimicrobial activity of methanolic, ethylacetate (EtOAc) and hexanic fractions of five Cameroon medicinal plants (Piptadeniastum africana, Cissus aralioides, Hileria latifolia, Phyllanthus muellerianus and Gladiolus gregasius) against 10 pathogenic microorganisms of the urogenital and gastrointestinal tracts.

Methods: The fractions were screened for their chemical composition and in vivo acute toxicity was carried out on the most active extracts in order to assess their inhibitory selectivity. The agar well-diffusion and the micro dilution methods were used for the determination of the inhibition diameters (ID) and Minimum inhibitory concentrations (MIC) respectively on 8 bacterial species including two Gram positive species (Staphylococcus aureus, Enterococcus faecalis), and six Gram negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Shigella flexneri, Salmonella typhi) and two fungal isolates (Candida albicans, Candida krusei). The chemical composition was done according to Harbone (1976), the acute toxicity evaluation according to WHO protocol and the hepatic as well as serum parameters measured to assess liver and kidney functions.

Results: The chemical components of each plant’s extract varied according to the solvent used, and they were found to contain alkaloids, flavonoids, polyphenols, triterpenes, sterols, tannins, coumarins, glycosides, cardiac glycosides and reducing sugars. The methanolic and ethylacetate extracts of Phyllanthus muellerianus and Piptadeniastum africana presented the highest antimicrobial activities against all tested microorganisms with ID varying from 8 to 26 mm and MIC from 2.5 to 0.31 mg/ml. The in vivo acute toxicity study carried out on the methanolic extracts of Phyllanthus muellerianus and Piptadeniastum africana indicated that these two plants were not toxic. At the dose of 4 g/kg body weight, kidney and liver function tests indicated that these two medicinal plants induced no adverse effect on these organs.

Conclusion: These results showed that, all these plant’s extracts can be used as antimicrobial phytomedicines which can be therapeutically used against infections caused by multiresistant agents. Phyllanthus muellerianus, Piptadeniastum africana, antimicrobial, acute toxicity, kidney and liver function tests, Cameroon Traditional Medicine

Keywords: Phyllanthus muellerianus, Piptadeniastum africana, antimicrobial, acute toxicity, kidney and liver function tests, Cameroon Traditional Medicine
Background
Infectious diseases are considered a major threat to human health, because of the unavailability of vaccines or limited chemotherapy. They account for approximately one half of all deaths in tropical countries [1]. Infectious diseases which ranked 5th in 1981, became the 3rd leading cause of death in 1992, with an increase of 58% [2]. Most of the current antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects, and their widespread overuse has led to increasing clinical resistance of previously sensitive microorganisms and to the occurrence of uncommon infections [3]. Historically plants have provided a good source of anti-infective agents and the search for plants with antimicrobial activities has increasingly gained importance during recent years. With the advent of ever-increasing resistant bacteria and fungi strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics. Herbal medicines have widely been used and now form an integral part of the primary health care in many countries. They may constitute a reservoir of new antimicrobial substances to be discovered. Use of herbal medicines in Africa represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases as stipulated by Diallo et al. [4]. In Cameroon, many plant species are used as traditional medicine to treat infectious diseases, and several interesting openings have originated for further inquiry following in vitro antimicrobial activity evaluation [5]. A previous epidemiological study on the characterization of urogenital tract microbial agents amongst women in the South West Region of Cameroon, revealed the presence of numerous multidrug resistant strains to currently used antibiotics in the treatment of urogenital tract infections [6]. Fighting local infections with natural products will be more advantageous and affordable to most patients. Our study was based on five selected medicinal plants described by Adjanohoun et al. [7] as traditional medication for various infectious ailments. The aim of this study was to evaluate the in vitro antimicrobial properties of methanolic, ethylacetate (EtOAc) and hexanic extracts of these five medicinal plants against eight Gram positive and Gram negative multidrug resistant bacteria species and two Candida isolates as well as to evaluate their in vivo acute toxicity on albino rats. The selection of these plants was based on their use in traditional medicine to cure current infectious diseases including urogenital tract infections [6].

Methods
Plants extraction and phytochemistry
Fresh plant parts were collected in August 2007. The medicinal plants used included the leaves of Piptadeniastum africana (Hook. f.) Bren. (Mimosaceae), the leafy liana of Cissus aralioides (WELW EX BAKER) (Vitaceae), the leaves of Hileria latifolia (Lam.) H.Walt (Phytolacceae), the stem barks of Phyllanthus muellerianus (O.Ktze) Exel. (Euphorbiaceae) and the bulbs of Gladiolus gregasius BAKER (Iridaceae). Piptadeniastum africana leaves were collected from Melong (Littoral Region of Cameroon) whereas others plants materials were collected from Dschang (West Region of Cameroon). These plants were identified at the Cameroon National Herbarium in Yaoundé. Fresh materials were dried at room temperature for about 3 weeks and crushed. Fractionation was done by macerating the powder obtained above into 6 L of hexane for two days. After filtration, the filtrate was evaporated to dryness under reduced pressure using a rotary vacuum evaporator. The resulting residues were successively macerated into the same volume (6 L) of ethylacetate (EtOAc) and methanol (MeOH). The extraction yield were evaluated (Table 1) and the dried crude extracts were stored at +4°C. The Phytochemical screening of the crude extracts was carried out using standard methods described elsewhere [8,9]. Each plant extract was screened for the presence of different classes of compounds including alkaloids, flavonoids, polyphenols, sterols, triterpenes, coumarins, anthraquinones, tannins, anthocyanins, saponins, glycosides.

Antimicrobial tests
Hexane, EtOAc and methanol extracts of the 5 medicinal plants were tested against a total of 8 bacterial species including two Gram positive species (Staphylococcus aureus, Enterococcus faecalis), and 6 Gram negative (Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Shigella flexneri, Salmonella typhi) and 2 fungal isolates (Candida albicans, Candida krusei) from urogenital and GIT specimens obtained at the Buea Regional Hospital in Cameroon [6]. These isolates were characterized morphologically and biochemically using API galleries [6].

The susceptibility tests were performed using the agar well diffusion method as previously described [10-12]. Stock cultures were maintained at 4°C on Nutrient Agar (Oxoid, England) slopes. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (Oxoid, England) for bacteria species and Sabouraud dextrose broth (Oxoid, England) for Candida species. They were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with distilled water to achieve an optical density corresponding to 2.0 × 10^6 colony forming units per ml (CFU/ml) for bacteria species and 2.0 × 10^5 CFU/ml for Candida species. The medium was punched with six millimeters diameter wells and filled with 120 μl of the test sample. The concentration of the extracts employed was 50 mg/ml, prepared in 10% v/v
aqueous Dimethyl Sulfoxide (DMSO). Simultaneously, Gentamycin (Sigma, USA) and Nystatin (Sigma, USA) were used as positive controls at a concentration of 0.2 mg/ml for bacteria and yeast cells respectively with 10% v/v aqueous/DMSO as negative control. The plates were incubated at 37°C for 24 hours, and inhibition zones formed around the wells were measured (mm) using a caliper. All tests were done in duplicate and the results were recorded as the mean diameter of the zones of growth inhibition surrounding the discs.

The Minimum Inhibitory Concentration (MIC) was determined by broth microdilution technique using 96-well plates [10,3]. Culture media were supplemented with 5% glucose and 1% phenol red end point indicator. After filling each well with 100 μL of broth, dry test samples previously diluted in DMSO were prepared to make a final concentration of 720 mg/ml. These solutions (100 μl) were added to the first well of each microtiter line. Successive dilutions were done by transferring the mixture/dilution (100 μl) from the first to the eleventh well. An aliquot (100 μl) was discarded from the eleventh well. The twelfth well served as control since no sample (extract, or reference antibiotics) was added in it. The microbial suspension (100 μl at 2.0 × 10^6 CFU/ml for bacteria species and 2.0 × 10^5 cells/ml for Candida species), obtained from an overnight growth was added to each well. The final concentration of the extracts used to evaluate the antimicrobial activity ranged from 160 to 0.15 mg/ml and reference drugs from 0.960 to 0.003 mg/ml. Tests were incubated aerobically at 37°C for 24 hours before being read. The end point was made by visual observation of growth. This was revealed by a colour change indicator from red to yellow. The MIC was considered as the lowest sample’s concentration that prevented visible growth or changed in color from red to yellow due to the formation of acidic metabolites corresponding to microbial growth.

Toxicity study

Experimental animals

Adult Wistar albino rats (120 - 140 g); were bred at the animal breeding house of Phytorica Lab in Douala. They were all clinically healthy and maintained in standard environmental conditions of temperature (28.0 ± 2°C). They were fed a standard diet and tap water ad libitum. The bioassay was conducted in accordance with the internationally accepted principles described by WHO [13] for laboratory use and care. Rats were deprived of food but not of water 12 h prior to administration of the test substance. Ethical clearance for animal experimentation was obtained from the Faculty of Health Sciences Institutional Review Board (Ref. No.: 2010/003/UB/FHS/IRB) at the University of Buea, Cameroon.

Acute toxicity

Acute toxicity was evaluated on extracts showing important antimicrobial properties; they were selected based on their MIC and the spectrum of antimicrobial activities. For each tested extract the procedure was as follow: The animals were separated in six groups of five males and five females including one control (group 1) and five treated (groups 2-6). The treated groups (2-6) received 4; 8; 12; 16; 20 g/kg of body weight (bw) each respectively of oral single dose of each extract. The control group received tap water and or 20% aqueous DMSO at an equivalent volume. Observations were made and recorded systematically after

| Plant Material            | Solvent | Extraction yield (%) | Alk | Tri | St | Ant | Fla | Pol | Sap | Tan | Cou | Gly | CG | RS |
|---------------------------|---------|----------------------|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|----|----|
| *Piptadeniastrum africana* (Leaves) | HEX     | 0.60                 | +++ | +++ | +  | -   | -   | -   | -   | +   | +   | -   | -  |
|                           | ETA     | 0.15                 | ++  | +++ | +  | -   | -   | -   | +   | +   | +   | +   | +  |
|                           | MEOH    | 1.75                 | -   | +   | +  | ++  | +   | ++  | +   | ++  | ++  | +   | +  |
| *Cissus aralioides* (Leaves) | HEX     | 0.26                 | ++  | ++  | +  | -   | -   | -   | +   | +   | +   | +   | +  |
|                           | ETA     | 0.66                 | ++  | ++  | -  | -   | -   | -   | +   | +   | +   | +   | +  |
|                           | MEOH    | 0.65                 | +   | +   | +  | -   | -   | -   | +   | ++  | ++  | +   | +  |
| *Hileria latifolia* (Leaves) | HEX     | 0.56                 | -   | ++  | +  | -   | -   | -   | -   | -   | -   | -   | -  |
|                           | ETA     | 1.55                 | -   | ++  | +  | -   | -   | -   | -   | +   | -   | -   | -  |
|                           | MEOH    | 4.05                 | -   | +   | +  | -   | -   | -   | +   | ++  | +++ | ++  | +  |
| *Phyllanthus muellerianus* (stem bark) | HEX     | 0.34                 | -   | ++  | +  | -   | -   | -   | -   | +   | +   | -   | +  |
|                           | ETA     | 0.53                 | -   | -   | -  | -   | -   | -   | -   | -   | -   | -   | -  |
|                           | MEOH    | 1.81                 | +   | +   | +  | ++  | +   | +   | +   | +   | +   | +   | -  |
| *Gladiolus gregasius Baker* (Bulbs) | HEX     | 0.71                 | ++  | ++  | +  | -   | -   | -   | -   | +   | +   | -   | +  |
|                           | ETA     | 0.73                 | ++  | ++  | +  | -   | -   | -   | -   | +   | +   | +   | +  |
|                           | MEOH    | 8.95                 | +   | +   | +  | -   | -   | +   | ++  | ++  | +++ | ++  | +  |

Key: HEX: hexane; ETA: Ethylacetate; MEOH: Methanol; Alk: Alkaloids; Tri: Triterpens; St: Sterols; Ant: Anthraquinons; Fla: Flavonoids; Pol: Polyphenols; Sap: Saponins; Tan: Tannins; Cou: Coumarins; Gly: Glycosides; CG: Cardiac glycosides; RS: Reducing sugars.

+++: abundantly found; ++: averagely found; +: trace.
1 and 2 hours of extract administration. The visual observations included changes in respiratory pattern, motility and skin sensitivity, diarrhea, behavioral pattern, weight gain, food and water consumption. The number of survivors was noted after 48 hours and these were then maintained for a further 5 days, after which they were sacrificed by decapitation through an incision on the jugular vein to collect blood; sera were obtained after blood clotting and centrifugation at 3000 rpm for 10 min and used for biochemical analyses. The liver was also excised, rinsed in ice-normal saline solution and 20% (W/V) liver homogenates were prepared in a 0.1M Tris-HCl (pH 7.4) buffer solution. After centrifugation at 3000 rpm for 20 min the supernatant was separated and used for biochemical analysis. The medium lethal dose (LD₅₀) was determined using the Behrens and Karber method [14]. Evaluation of the toxicity degree was based on previously described methods [15-17].

**Biochemical estimation**

Sera were assayed for liver function tests (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) [18]), and kidney function tests (creatinin and urea [19]). Liver homogenates were assayed for aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) [18], malondialdehyde (MDA) [20] and glutathion (GSH) [21].

**Statistical analysis**

One way analysis of variance (ANOVA) was applied for determining the statistical significance in various markers level between the control and the tested group. The level of significance was set at 0.05 and 0.01 [22].

**Results**

**Phytochemical screening**

Results presented in Table 1 below, show that all plant’s extracts presented differences in phytochemical composition. Alkaloids, triterpenes, sterols, flavonoids, tannins, coumarins, anthraquinions and reducing sugars were the main classes of compounds encountered in these plants. However, anthraquiniones were not present in all the fractions of *P. africana*, *C. aralioides* and *H. latifolia*. The hexanic, ethylacetate and methanolic extracts presented a differential composition of the classes of chemical compounds tested positive in these plants. Alkaloids, triterpenes, sterols were abundantly found in hexane and ethylacetate extracts, whereas flavonoids, anthraquiniones were averagely present in ethylacetate and methanolic fractions. The fractions of *Piptadentiastrum africana* were all found to contain triterpenes, sterols, tannins, coumarins, and glycosides. The fractions (hexanic, ethylacetate and methanolic extracts) of *Cissus aralioides* were tested positive to alkaloids, triterpenes, sterols, tannins, coumarins, glycosides and cardiac glycosides, but the ethylacetate and methanolic extracts of this plant further contained reducing sugars. *Hileria latifolia* fractions were all tested positive to triterpends, sterols and coumarins, but in addition saponins, glycosides and reducing sugars were found only in the methanolic fraction. The hexanic, ethylacetate and methanolic extracts of *Phyllanthus muellerianus* (leaves) all contained triterpends, sterols, anthraquinions, coumarins, glycosides, cardiac glycosides and reducing sugars. The chemical composition of the 3 fractions of the bulbs of *Gladiolus gregasius* Baker showed that they all contained alcaloids, triterpends, sterols, anthraquiniones, glycosides and cardiac glycoside, but reducing sugars and saponins were further present in the methanolic fraction.

**Antimicrobial activity**

All plant extracts exhibited antibacterial and anticandidal activities. The ID and MIC obtained against Gram negative, Gram positive bacteria species, and yeast strains (*C. albicans*, *C. krusei*) are shown in Tables 2 and 3 respectively. Generally for all tested medicinal plants, the hexanic extract was poorly active than the ethylacetate and methanolic extracts except for *Gladiolus gregasius*. The ethylacetate and methanolic extracts of *Piptadentiastrum africana* induced important inhibitory activities on tested Gram negative and Gram positive microorganisms with inhibition zone diameters (ID) varying from 8 to 26 mm and MIC from 2.5 to 0.31 mg/ml. The highest IDs (26 mm) were obtained with *S. flexneri* and *S. typhi* which are Gram negative strains, whereas the most sensitive Gram positive were *S. aureus* and *Candida albicans*, (ID = 25 and MIC = 0.31 mg/ml) against the methanolic extract. The ethylacetate fraction was less active when compared to the methanolic (with ID = 14 mm, MIC = 0.31 mg/ml on *S. aureus* and *Candida albicans*). In general the most important inhibition obtained was the methanolic fraction followed by the ethylacetate, and the lowest being the hexanic fraction which on many Gram negative microbial agents induced little or no inhibition (table 3).

*Cissus aralioides* had the highest inhibitory activity which was obtained with its EtOAc extract on *P. aeruginosa* (ID = 27 mm; MIC = 0.31 mg/ml) and on *S. aureus* (ID = 23 mm; MIC = 0.31 mg/ml). In general, all Gram negative bacteria species except *Proteus mirabilis* were sensitive to the 3 fractions of this plant, the most active fraction being the ethylacetate fraction. Amongst the Gram negative bacteria strains tested using the 3 fractions of *Hileria latifolia*, *P. aeruginosa* and *S. typhi* were considerably sensitive to both the ethylacetate (ID = 25 mm; MIC = 0.62 mg/ml) and the methanolic (ID = 26 mm; MIC = 0.62 mg/ml) fractions. The highest inhibitory activity on Gram positive strains was obtained with ethylacetate (23 ≤IDS≤25 mm; 0.31≤MIC≤0.62 mg/ml) and
methanolic (23≤ID≤25 mm; 0.31≤MIC≤0.62 mg/ml) fractions; its hexanic fraction induced only mild activity (10≤ID≤12 mm; 5≤MIC≤20 mg/ml). Ethylacetate and methanolic extracts of *Phyllanthus muellerianus* were found to be the most active fractions as compared to the hexanic. The methanolic and ethylacetate extracts of *Phyllanthus muellerianus* showed the largest antimicrobial spectrum as they were active against all the microorganisms investigated with the most important ID (16 to 33 mm) and MIC (0.07 to 1.25 mg/ml). *P. aeruginosa*,

| Table 2 Inhibition zone diameters (ID in mm) and minimal inhibitory concentration (MIC in mg/ml) of hexanic, ethylacetate and methanolic extracts of five medicinal plants against Gram – bacteria species |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Plant material                  | Extracts                        | *Proteus mirabilis*             | *Escherichia coli*              | *Shigella flexneri*             | *Pseudomonas aeruginosa*        | *Klebsiella pneumoniae*         | *Salmonella typhi*              |
|                                 |                                 | ID                              | MIC                             | ID                              | MIC                             | ID                              | MIC                             |
| *Piptadeniastum africana*       | Hexane                          | 8                               | >20                             | 0                               | >20                             | 10                              | 20                              |
|                                 | EtOAc                           | 8                               | 10                              | 20                              | 17                              | 12                              | 1.25                            |
|                                 | MeOH                            | 14                              | 2.5                             | 22                              | 1.25                            | 21                              | 1.21                            |
| *Cissus aralioides*             | Hexane                          | 10                              | >20                             | 11                              | >20                             | 10                              | >20                             |
|                                 | EtOAc                           | 10                              | 5                               | 21                              | 2.5                             | 19                              | 5                               |
|                                 | MeOH                            | 9                               | 10                              | 9                               | 10                              | 10                              | 1.25                            |
| *Hileria latifolia*             | Hexane                          | 10                              | 20                              | 10                              | 20                              | 12                              | 20                              |
|                                 | EtOAc                           | 15                              | 12.5                            | 15                              | 1.25                            | 26                              | 0.62                            |
|                                 | MeOH                            | 13                              | 1.25                            | 19                              | 1.25                            | 26                              | 0.31                            |
| *Phyllanthus muellerianus*      | Hexane                          | 10                              | 10                              | 10                              | 20                              | 14                              | 10                              |
|                                 | EtOAc                           | 16                              | 1.25                            | 24                              | 0.62                            | 22                              | 1.25                            |
|                                 | MeOH                            | 24                              | 1.25                            | 26                              | 0.31                            | 27                              | 0.31                            |
| *Gladiolus gregasius*           | Hexane                          | 12                              | >20                             | 11                              | >20                             | 15                              | 20                              |
|                                 | EtOAc                           | 10                              | 10                              | 12                              | 10                              | 14                              | 20                              |
|                                 | MeOH                            | 0                               | >20                             | 9                               | 20                              | 0                               | >20                             |
| Reference Drugs*                |                                 | 14                              | 0.12                            | 16                              | 0.12                            | 17                              | 0.03                            |

*Gentamycin was used as reference drug for bacteria species. ID: Inhibition zone diameters in mm; MIC: and minimal inhibition concentration. Note: Extracts and reference drugs were tested at the concentration of 50 mg/ml and 0.2 mg/ml respectively for the determination of IDs*

| Table 3 Inhibition zone diameters (ID in mm) and minimal inhibitory concentration (MIC in mg/ml) of hexanic, ethylacetate and methanolic extracts of five medicinal plants against Gram positive bacteria species and yeasts strains |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| PLANT MATERIAL                  | Extracts                        | *Staphylococcus aureus*         | *Enterococcus faecalis*         | *Candida albicans*             | *Candida krusei*               |
|                                 |                                 | ID                              | MIC                             | ID                              | MIC                             | ID                              | MIC                             |
| *Piptadeniastum africana*       | Hexane                          | 0                               | >20                             | 0                               | >20                             | 9                               | 20                              |
|                                 | EtOAc                           | 16                              | 0.62                            | 17                              | 1.25                            | 14                              | 1.25                            |
|                                 | MeOH                            | 22                              | 0.31                            | 23                              | 0.62                            | 25                              | 0.31                            |
| *Cissus aralioides*             | Hexane                          | 10                              | >20                             | 10                              | >20                             | 8                               | 20                              |
|                                 | EtOAc                           | 23                              | 0.31                            | 21                              | 1.2                             | 21                              | 1.25                            |
|                                 | MeOH                            | 9                               | 10                              | 10                              | 5                               | 12                              | 0.62                            |
| *Hileria latifolia*             | Hexane                          | 12                              | 20                              | 12                              | 20                              | 11                              | 10                              |
|                                 | EtOAc                           | 21                              | 0.62                            | 23                              | 0.62                            | 19                              | 1.25                            |
|                                 | MeOH                            | 23                              | 0.62                            | 25                              | 0.62                            | 24                              | 1.25                            |
| *Phyllanthus muellerianus*      | Hexane                          | 12                              | 10                              | 11                              | 10                              | 14                              | 10                              |
|                                 | EtOAc                           | 23                              | 0.31                            | 25                              | 0.31                            | 23                              | 0.62                            |
|                                 | MeOH                            | 26                              | 0.31                            | 28                              | 0.07                            | 28                              | 0.31                            |
| *Gladiolus gregasius*           | Hexane                          | 13                              | 20                              | 9                               | 20                              | 12                              | 20                              |
|                                 | EtOAc                           | 12                              | 20                              | 12                              | 5                               | 12                              | 5                               |
|                                 | MeOH                            | 12                              | 20                              | 12                              | 5                               | 12                              | 5                               |
| Reference Drugs*                |                                 | 25                              | 0.06                            | 17                              | 0.06                            | 22                              | 0.01                            |

*Gentamycin and Nystatin were used as reference drugs for bacteria species and yeasts strains respectively. ID: Inhibition zone diameters in mm; MIC: and minimal inhibition concentration. Note: Extracts and reference drugs were tested at the concentration of 50 mg/ml and 0.2 mg/ml respectively for the determination of IDs*
E. faecalis and C. krusei (MIC = 0.07 mg/ml) were the most sensitive species to the MeOH extract whereas the hexanic fraction was less active.

Hexane, EtOAc and MeOH extracts of G. gregasius showed only mild activity compared to other plant extracts and its methanolic extract was not active on P. mirabilis, S. flexneri, P. aeruginosa and K. pneumonia. The MIC values confirmed results obtained by the inhibition zone (Tables 2 and 3) ranging from 0.07 to >20 mg/ml.

In general, EtOAc and MeOH fractions of Piptadeniastrum africana and Phyllantus muellerianus happened to be the most active extracts with regard to their spectra of activity and the important inhibitory properties they induced.

Acute toxicity
Acute toxicity was carried out on two medicinal plants: Piptadeniastrum africana and Phyllantus muellerianus. They were selected based on their antimicrobial spectrum and efficacy. Because the ethylacetate and the methanolic extracts of these two plants were similar in terms of chemical composition and efficacy, the methanolic extracts of these two medicinal plants were used for toxicological evaluations; moreover these extracts were water soluble. During the observation period, no death of rats was neither recorded in the control nor in the treated groups. Visual observations of rats were made and recorded systematically 1 and 2 h after extract administration, including changes in respiratory, motility and skin sensitivity, diarrhea, behavioral pattern, weight gain, food and water consumption. These parameters changed mildly in groups 5 (16 g/kg bw) and 6 (20 g/kg bw) of male and female rats compared to the control. The pathological examination of the liver showed no visual abnormality in all groups at the end of the experiment.

Table 4 presents results of serum parameters for Piptadeniastrum africana. This extract administered at the dose of 4 g/kg bw showed no significant difference (P > 0.05) as compared to the treated group with regards to all the parameters tested. At the dose of 8 g/kg bw, an increased significant level (P < 0.05) of transaminases (ASAT and ALAT) was obtained, whereas serum creatinine and urea levels were not different from the control (P < 0.05).

Administered at 12, 16 and 20 g/kg bw, all liver (ASAT and ALAT) and kidney (creatinine and urea) function tests increased significantly (P < 0.05 and P < 0.01).

Table 5 presents results of the effect of Piptadeniastrum africana on different liver biochemical parameters of male and female rats. As in Table 4, significant changes (P < 0.05 and P < 0.01) induced by this extract appeared only at high doses (8; 12; 16; 20 g/kg bw). An increased malondialdehyde was noted only at the dose of 8 g/kg bw, whereas, significant reduction in GSH level was obtained at 12 g/kg bw. Reduced levels of transaminases (ASAT and ALAT) in the hepatocytes were also observed at the dose of 8 g/kg bw.

Table 6 presents the effect of the methanolic extract of Phyllanthus muellerianus on different sera biochemical parameters of male and female rats. Sera biochemical parameters increased significantly only at doses starting from 8 g/kg bw. Sera transaminases increased markedly (P < 0.05) in male and female rats when the methanolic extract was administered at the dose of 8 g/kg bw. Administered at the dose of 4; 8; 12 g/kg bw, this extract had no significant effect on the levels of urea and creatinine but induced significant increase of these two parameters on the tested animals (P < 0.05 and P < 0.01) only at high doses.

Table 4 Effect of the methanolic extract of Piptadeniastrum africana on different sera biochemical parameters of male and female rats

| Dose(g/kg) | Sex     | ALAT (UI) | ASAT (UI) | Creatinine (mg/l) | Urea (mg/l) |
|-----------|---------|-----------|-----------|-------------------|-------------|
| 0         | Male    | 74.60 ± 11.64 | 38.83 ± 0.18 | 9.00 ± 1.58 | 168.20 ± 1.58 |
|           | Female  | 74.50 ± 10.00 | 34.00 ± 4.21 | 10.22 ± 2.04 | 165.81 ± 0.42 |
| 4         | Male    | 81.65 ± 2.92  | 38.66 ± 2.37 | 12.50 ± 4.21 | 162.20 ± 1.58 |
|           | Female  | 79.40 ± 2.15  | 37.28 ± 1.91 | 11.37 ± 4.32 | 158.11 ± 1.42 |
| 8         | Male    | 89.65 ± 5.48* | 44.20 ± 2.52* | 11.25 ± 1.11 | 169.30 ± 1.22 |
|           | Female  | 88.10 ± 4.21* | 42.32 ± 2.53* | 12.70 ± 1.22 | 179.08 ± 1.91 |
| 12        | Male    | 131.10 ± 1.73** | 82.50 ± 1.52** | 25.00 ± 2.53* | 191.21 ± 2.00* |
|           | Female  | 80.01 ± 2.10* | 43.00 ± 1.21* | 24.95 ± 2.14* | 188.18 ± 1.45* |
| 16        | Male    | 154.25 ± 6.21** | 93.41 ± 11.14** | 36.36 ± 1.85** | 214.21 ± 1.58** |
|           | Female  | 124.01 ± 4.57** | 92.58 ± 8.24** | 40.15 ± 4.33** | 195.82 ± 0.92** |
| 20        | Male    | 152.33 ± 6.23** | 114.33 ± 6.12** | 46.25 ± 2.25** | 208.21 ± 1.18** |
|           | Female  | 146.01 ± 3.27** | 113.98 ± 3.21** | 45.29 ± 5.41** | 205.58 ± 1.25** |

ANOVA *Significant difference between test groups and control at P < 0.05  
** Significant difference between test groups and control at P < 0.01
doses (16 and 20 g/kg bw). Table 7 presents results of the effect of the methanolic extract of *Phyllanthus muellerianus* on different liver biochemical parameters of male and female rats. Significant decrease in hepatocyte transaminases (P < 0.05) as well as of GSH (P < 0.01) was only observed starting at 8 g/kg bw and 12 g/kg bw respectively. MDA serum level also significantly increased when administered at the dose of 8 g/kg bw.

**Discussion**

Successful prediction of chemical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent. In this study, extraction was done using organic solvent such as hexane, methanol and EtOAc. This is because these solvents are easily evaporated and permit an easier estimation of extract concentration, which is difficult to obtain with water as solvent. Results of the analysis of five medicinal plants extracts using these solvents showed that their chemical content differs depending on the nature of solvent used. Identical classes of chemical compounds are found in the hexanic, methanolic and EtOAc extracts of *P. africana*; however, flavonoids, polyphenols, doses (16 and 20 g/kg bw). Table 7 presents results of the effect of the methanolic extract of *Phyllanthus muellerianus* on different liver biochemical parameters of male and female rats. Significant decrease in hepatocyte transaminases (P < 0.05) as well as of GSH (P < 0.01) was only observed starting at 8 g/kg bw and 12 g/kg bw respectively. MDA serum level also significantly increased when administered at the dose of 8 g/kg bw.

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cardiac glycosides and reducing sugars were mostly found in the methanolic and EtOAc fractions. Alkaloids were absent in the methanolic fraction and this could be justified by its poor polarity. Both Gram negative and Gram positive were sensitive to ethylacetate and methanolic fractions of the five medicinal plants rich in flavonoids, polyphenols and anthraquinons. This is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls; more lipophilic flavonoids may also disrupt microbial membranes, [23,24]. The presence of coumarins in the various fractions of \textit{P. muellerianus}, \textit{H. latifolia}, \textit{C. araliaoides} and \textit{P. africana}, may explain their important inhibitory activity against \textit{Candida albicans} and \textit{Candida krusei}. This anticandidal activity of coumarins has been demonstrated \textit{in vitro} and \textit{in vivo} against vaginal candidiads by Thornes [25]. Success of extracts from \textit{P. africana}, \textit{C. araliaoides} and \textit{G. gregasius} against both Gram positive and Gram negative agents is likely dependent on their content in alkaloids able to intercalate between DNA strands [26]. The presence of sterols and triterpens has been confirmed in another study carried out in Cameroon by Mbouangouere et al. [27] who isolated terpenes such as sitosterol, β-amyrin and eicosane; however they found no alkaloids in this plant. The absence of alkaloids in their study could be due to the fact that, their investigation was done on a defatted fraction of the methanol/CH$_2$Cl$_2$ extract. The hexanic fraction induced only mild activity compared to the ethylacetate and methanolic fractions to which all microbial strains tested were sensitive. This could indicate that important bioactive principles of \textit{Piptadeniastrum africana} are of polar nature. Polar secondary metabolites like flavonoids, coumarins, anthraquinons, phenols and glycosides have been found to possess important antimicrobial properties [5,28]. In accordance, Mbouangouere et al. [27] obtained inhibitory activity on Gram positive and Gram negative strains (\textit{Escherina coli}, \textit{Bacillus subtilis}, \textit{Shigella flexenari}, \textit{Staphylococcus aureus}, \textit{Pseudomonas aeruginosa}, \textit{Salmonella typhi}) with ID varying between 9-16 mm when tested at 3 mg/ml. In the present study, we obtained a similar activity with \textit{P. africana} against \textit{S. aureus}, \textit{P. aeruginosa}, \textit{C. albicans} and \textit{C. krusei} with ID varying between 14 and 26 mm. In a previous epidemiological study, these microorganisms were found to be highly resistant to ampicillin and augmentin [6]. With regards to these results we can deduce that this plant’ extract, \textit{P. africana}, is a good source of antimicrobial agents with interesting activity on multi resistant strains. 

Looking at it toxicity parameters, no death of rats was neither recorded in the control nor in treatment groups during acute toxicity indicating an LD$_{50}$ > 5 g/Kg bw. From these results, it can be concluded that \textit{P. africana} is not toxic with regard to the threshold of toxic substances (5 g/kg) as previously stipulated [15-17].

Results of sera and liver biochemical parameters indicated that the variation of transaminase (ALT and AST) activities are associated with hepato-cellular damage, although marked increased in these parameters were only observed at a higher dosage (≥8 g/kg bw). The concurrent

| Dose (g/kg) | Sex | ALAT (UI/g of liver) | ASAT (UI/g of liver) | MDA (mmol/g of liver) | Glutathione (GSH) (μmol/g of liver) |
|------------|-----|----------------------|----------------------|-----------------------|-------------------------------------|
| 0          | Male 74.60 ± 11.64 | 40.83 ± 0.18 | 10.00 ± 1.58 | 4.12 ± 1.11 |
|            | Female 74.50 ± 10.00 | 42.00 ± 4.21 | 10.25 ± 204 | 4.62 ± 1.24 |
| 4          | Male 71.66 ± 2.92 | 42.66 ± 2.37 | 12.50 ± 5.27 | 4.31 ± 2.23 |
|            | Female 79.50 ± 2.15 | 40.28 ± 1.95 | 13.27 ± 4.52 | 4.48 ± 1.21 |
| 8          | Male 79.36 ± 5.48* | 43.00 ± 1.82* | 16.25 ± 3.44* | 4.12 ± 1.11 |
|            | Female 78.10 ± 4.21* | 43.12 ± 1.51* | 14.70 ± 4.21* | 4.62 ± 1.24 |
| 12         | Male 61.00 ± 1.73* | 30.50 ± 1.82** | 24.00 ± 4.33** | 2.32 ± 1.25** |
|            | Female 60.00 ± 3.10* | 30.01 ± 2.31** | 14.95 ± 3.12* | 3.16 ± 1.84** |
| 16         | Male 44.33 ± 6.21** | 24.33 ± 13.14** | 26.15 ± 2.55** | 2.19 ± 1.23** |
|            | Female 44.01 ± 3.57** | 23.98 ± 8.24** | 23.21 ± 5.11** | 3.02 ± 1.34** |
| 20         | Male 22.33 ± 6.21** | 24.23 ± 10.14** | 45.25 ± 2.15** | 2.31 ± 1.13** |
|            | Female 24.01 ± 3.57** | 22.68 ± 6.24** | 46.29 ± 5.21** | 2.22 ± 2.14** |

ANOVA *Significant difference between test groups and control at P < 0.05.
** Significant difference between test groups and control at P < 0.01.
significant increase of ALT and AST activities in sera and decrease ALT and AST activities in hepatocytes after treatment of the male and female rats above 8 g/kg bw clearly indicate leakages of liver cell contents into the blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. The creatinine and urea levels of treated rats increased significantly as compared to the control blood stream. 

Conclusions

In general, the highest antimicrobial activities were obtained with Phyllanthus muellerianus and Pitadeniastum africana extracts. However, all the five medicinal plants, Pitadeniastum africana, Cissus aralioides, Hilaria latifolia, Phyllanthus muellerianus and Gladiolus gregasius tested are antibiotic and antifungal agents and could be used in the treatment of various urogenital and gastrointestinal ailments caused by multiresistant microbial agents. It is also clear from this study that, the antimicrobial activity of these five medicinal was mainly found in the methanolic and ethylacetate fractions. The antimicrobial activities of Cissus aralioides and Hilaria latifolia have not been studied before. This study has also revealed that Pitadeniastum africana and Phyllanthus muellerianus are not toxic at therapeutic doses with good antimicrobial properties. This study is an important step towards clinical evaluation in order to produce improved phytomedicine in the treatment of multiresistant microbial infections. Alongside...
further chemical analysis involving CG-MS, HPLC-MS or ESI-MS analysis will enhance our comprehension on the nature of the chemical compounds contain in the extracts.

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Authors’ contributions
JCNA as principal investigator designed and implemented the project and initiated the writing of the manuscript. VBP assisted in designing the project and revised the manuscript. HLF assisted in the in vitro antimicrobial testing and in revising the paper. DSN assisted in the identification of microbial strains, the statistical analysis of data and in revising the paper. ALN provided technical assistance during antimicrobial testing. PFN assisted in the identification of the microbial strains used and contributed in the write up. EAA assisted in designing the project. VBP carried out the antimicrobial and toxicological tests. BS contributed in the identification of the microbial strains used and contributed in the in vivo acute toxicological investigations in the Phytotopia Lab in Douala. All the authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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