Cytotoxic and Antibacterial Assessment of Stem-Barks of *Feretia apodanthera* and *Erythrophleum ivorense*; Two West African Medicinal and Socio-Economic Trees

Dieudonné Lemuh Njimoh1,a*, Germain Sotoing Taiwe2,b, Jerome Nyhalah Dinga1,c, Marcel Moyeh Nyuylam1,d, Juliette Momesaw Meyam1,e, Seraphine Ebenye Mokake3,f

1Department of Biochemistry and Molecular Biology, University of Buea, Buea, Cameroon. 2Department of Zoology and Animal Physiology, University of Buea, Buea, Cameroon. 3Department of Botany and Plant Physiology, University of Douala, Douala, Cameroon.

a*njimoh.dieudonne@ubuea.cm, btaiwe_sotoing@yahoo.fr, cdjnyhalah@yahoo.com, dmarcel.moyeh@ubuea.cm, ejuliettapowers@yahoo.com, febenyemokake@yahoo.co.uk

Keywords: *Feretia apodanthera*, *Erythrophleum ivorense*, stem-bark extracts, enriched alkaloid fraction, aqueous extract, antibacterial activity, acute toxicity, cytotoxicity, female albino rats.

Abstract. To assess the antibacterial and cytotoxic properties of stem-barks of *Feretia apodanthera* and *Erythrophleum ivorense* extracts from powdered stem-barks of *Feretia apodanthera* and *Erythrophleum ivorense* were prepared following standard techniques of marceration, filtration and evaporation. Antibacterial activity was assayed against five pathogenic bacteria strains by the well-diffusion and broth microdilution methods. Cytotoxicity was measured by acute toxicity test on female albino rats and confirmed by cell viability assay using 3T3 cell lines. Phytochemical analysis was performed following standard techniques. The aqueous/alkaloid extracts of *Feretia* *apodanthera* and the ethanol extract of *Erythrophleum ivorense* were active against the five pathogenic bacteria strains tested (diameter zone of inhibition (DZI) ranging from 5.1 to 17.8mm). The *Feretia apodanthera* extracts were the most active against *Staphylococcus aureus* (DZI 17.1-17.8mm). The MIC and MBC of the extracts of both plants ranged from 0.094mg/ml to 48mg/ml and 0.047mg/ml to 48mg/ml respectively. Extracts of *Feretia* *apodanthera* at 5000mg/Kg had no effect on the behavioural properties of rats and no death was observed. Incubation with 3T3 cell lines did not produce any cell toxicity up to 20µM and 5µM respectively for the aqueous extract and the alkaloid fraction. Incubation with higher concentrations produced cell death with IC50 of 39.41 ± 0.95µM and 38.45 ± 1.64µM respectively. Phytochemical analysis revealed the presence of various constituents. The results show for the first time that stem-bark extracts of *F. apodanthera* and *E. ivorense* possess antibacterial activities against common human pathogenic bacteria and the low/lack of toxicity as demonstrated with the *F. apodanthera* extracts justify and confirm their safe ethnomedical uses.

Introduction

Antimicrobial drug resistance remains a scourge across multiple sectors including and especially human health. The lack of vaccines against some pathogenic microorganisms, the overuse and misuse of antibiotics and other antimiobials in humans, plants and animals and together with the spread of residues of the antimicrobials on land and in water makes the problem more challenging and constantly requiring novel approaches. The *Rubiaceae* and *Fabaceae* families of plants are two of the plant-based systems widely known for their therapeutic values especially against infectious diseases. Quinine, the very first line drug against the current number one killer disease, malaria, in Africa and S.E. Asia came from the *Rubiaceae* family and though this family is especially very noticeable for its antiplasmodial activities, most of the plants in this family remain unexplored despite the wealth of information from tradi-practitioners [1]. With the increasing resistance of parasites and microorganisms to past and present drug regimens the need to explore...
other avenues for novel therapeutic entities remain an absolute necessity if lives have to be saved. While about 90% of infections found in health care services is due to bacterial infections [2] (most of them enteric infections), the antimicrobial activities of sub-saharan Rubiaceae have only been documented in a few plants including Canthium multiflorum, Keetia hispida, Mitragyna inermis, Morinda lucida, Nauclea latifolia [1] and just recently Nauclea paubeguini [3]. The antimicrobial activity of the sub-saharan Fabaceae has been widely demonstrated [4,5]. Though some of the diseases caused by various bacteria species have vaccines others do not and only rely on chemotherapy which is greatly hampered by the development of drug-resistant phenotypes. The only way forward is to search for novel therapeutic agents that can render initially resistant phenotypes susceptible and the plant-based systems have been very redoubtable in this direction.

_Ferretia apodanthera_ (F. apodanthera), a member of the Rubiaceae family of plants is widely known in literature and renown for the treatment of various preoccupying pathologies in Sub-Saharan African traditional pharmacopoeia. It is usually harvested for local use as food, medicine and cosmetic [6]. In certain areas the fleshy pulp of the ripe fruit is eaten raw as snack to quench hunger and thirst while the dried leaves are eaten as a vegetable [7]. The twigs of *Ferretia apodanthera* are used for beehive construction in rural areas of Burkina Faso to foster traditional beekeeping [8]. It is also the most persistently consumed browse species by cattle in Burkina Faso [9]. Medicinally, it is popularly used in various West African Countries carrying various vernacular names to treat various infections and health conditions (Table 1).

**Table 1. Medicinal uses of *Ferretia apodanthera* in some West African countries**

| Local names        | Country     | Traditional medicinal uses                                                                 |
|--------------------|-------------|-------------------------------------------------------------------------------------------|
| Hako kaddam, Sangu | Cameroon    | Concoctions used to treat epilepsy, infantile convulsions, anxiety, psychoses, pain and inflammation [10,11]. |
| Nassisisolok, Sitindakuan, vevei | Togo         | Stem with Leaves decoction used to treat constipation in babies [12].                        |
| Gigigree, kurukuru, jula sungalani, tusigi, celebre kapinge, ntyurungo, tiefile, gilu kile | Mali          | Root bark powder used to treat infective wounds or mixed with water and used as a wash [13]. Stem with leaves decoction used to treat conjunctivitis, stomach pains and fever chills; roots decoction used to treat dysentery, uretral pains, kidney disease; crushed dried fruits used against onchocerciasis [14]. |
| kurukuru           | Nigeria     | Decoction of the root is used to treat stomach disorders [15]. Root decoction used to treat dysentery, female gonorrhoea [16]. |
| Tounbounya         | Benin       | decoction of stem and leaves used to treat eczema [12].                                      |
| Tobida, tobi, commbi | Senegal    | Roots decoction used to treat uretral and kidney pains and lymph glands disease [17] and eye filariasis [18]. |
| Commbi             | Mauritania  | Roots decoction used against eye filariasis [18].                                            |

A decoction of the roots is used to treat gonorrhea, syphilis and leprosy; an infusion is drunk to treat stomach-ache; pounded roots powder is used to treat wounds, the crushed fruit paste is used as an antidote to snake bites [7]. The methanol extract of leaves of *Ferretia apodanthera* has been shown to exhibit a pronounced antimalarial activity [19]. Its anticonvulsant activity has also been largely documented [20,21] but its effect on pathogenic bacteria has however not been determined.

The Fabaceae, *Erythrophleum ivorense* (*E. ivorense*), is a tropical evergreen timber and medicinal tree, scattered throughout evergreen as well as moist semi-deciduous forests in West and Central Africa whose stem- and root-barks are popularly used in west African traditional pharmacopoeia to treat inflammation and various ailments including pain, convulsion, small-pox,
swellings, parasitic worms, emesis and as laxative [22, 23]. It has a high economic and socio-cultural value. Just like *F. apodanthera* and because of its high economic and socio-cultural values it is traded under various trade and local names in various countries [24]. The tree is usually harvested from the wild for medicinal use and for its very durable and internationally traded timber. The bark is usually traded locally under various appellations (including ‘sassy-bark’, ‘mancona bark’, ‘casca bark’ or ‘écorce de tali’) for various medicinal uses. The bark and sometimes the seeds are widely used as hunting, fish and ordeal poison [25]. The bark is used for tanning in Sierra Leone and Ivory Coast. Extract of the root-bark and the stem-bark have been shown to possess respectively anti-inflammatory effect in chicks [23] and anticonvulsant and sedative activities in mice [26]. Recent scientific evidence using *in vivo* experimental pain models has also confirmed the anti-nociceptive and anti-inflammatory activities of its stem-bark [27]. The antimicrobial activities of its leaf and stem-bark extracts have been assessed on reference bacterial strains but not on common pathogenic human bacteria strains [28]. These scientific findings lend credence to the folkloristic use of these two plants *Feretia apodanthera* and *Erythrophleum ivorense* in the management of various disorders. However scientific connotation regarding their safe usage to treat various ailments of microbial origin has not been given. This study therefore aims at determining the antibacterial and cytotoxic properties of stem-bark extracts of *Feretia apodanthera* and *Erythrophleum ivorense*.

**Materials and Methods**

**Plant material:** *Feretia apodanthera* and *Erythrophleum ivorense* stem-barks used in this study were harvested in Ngaoundere in the Adamawa Region of Cameroon (harvesting coordinates 7° 7′ 09″ N and 13° 55′ 34″ E). The plant collection was carried out on a private land after obtaining permission from the owner (Mr. Mamoudou waziri, resident of Ngaoundere-manwi). The species was authenticated by the National Herbarium of Yaoundé (Cameroon). The stem-barks were dried and crushed into powder and then subjected to crude extract preparation.

**Preparation of extracts from powdered plant material:** The stem-bark extracts (aqueous extract of *F. apodanthera*, ethanol, hexane, methylene chloride extracts of *E. ivorense*) were prepared from the powdered clean dry stem-bark of *F. apodanthera* and *E. ivorense* following standard techniques of maceration, filtration and evaporation with the appropriate solvents and as previously described [29,30]. The enriched alkaloid fraction of the stem-bark of *F. apodanthera* was obtained as described [30] by fractionation starting with a mixture of acetone/water at room temperature followed by evaporation *in vacuo*, resuspension of the resulting dark residue in water, successive extraction with ethyl acetate and n-butanol, concentration and resuspension of the butanol fraction and extraction with CHCl₃.

**Preparation of samples for bacterial susceptibility testing:** Plant extracts and fractions used for susceptibility testing were processed as previously described [3] at an assay concentration of 50mg/ml in Dimethyl Sulfoxide (DMSO). Gentamycin at 0.2mg/ml concentration served as the positive control. Clinical isolates of five pathogenic bacteria strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Providencia stuartii*, and *Proteus vulgaris*) previously obtained using selective media following standard protocols [31] from the regional Hospital Annex in Buea, Cameroon, were used. Identification of isolates made use of their ability to grow on selective media, their morphology, gram reaction and the different specific biochemical reactions [32].

**Determination of DZI, MIC and MBC:** The antibacterial susceptibility of the extracts measured by the diameter of zones of inhibition (DZI) and the minimal inhibitory and minimal bactericidal concentrations (MIC/MBC) were assessed by the hole-plate diffusion and broth microdilution methods respectively following standard procedures and as previously described [33] and more recently [3].
Preparation of extracts and animals for acute toxicity studies: Only the aqueous extract and the alkaloid fraction of the stem-bark of *F. apodanthera* were used for cytotoxicity test. The freeze-dried extract/fraction from the stem-bark were resuspended in distilled water at various concentrations as required in the experiments and given orally to female albino rats (weighing between 155-178g) in a volume of 10 ml/kg. The rats, housed in a controlled environment with free access to water and food, were maintained on a 12 h light-dark cycle. Ethical approval for the study was obtained from the Cameroon National Ethics Committee for animal handling and experimental procedure. The Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) was strictly followed. Animals were deprived of food twelve hours before behavioral testing conducted between 8am in the morning and 4pm in the afternoon.

Acute toxicity test: Acute toxicity was carried out according to Lorke’s method [34] using female albino rats. A set of 21 rats were divided into 7 groups of three each. Various doses (1000, 2500 and 5000 mg/kg) of the extract/fraction of *F. apodanthera* were orally administered to the rats in order to determine the range of doses producing any toxic effect. In other words, each group of three rats was administered a single dose of the aqueous extract (1000, 2500 and 5000 mg/kg; orally) and another group a single dose of the alkaloid fraction (1000, 2500 and 5000 mg/kg; orally). Two percent DMSO was administered (10 ml/kg) orally to the last group of three rats to serve as control. The behavioural pattern of the rats was observed after 1h, then intermittently every 4 h for 24hrs and thereafter over a period of 24 h. Observation lasted for two weeks after treatment for any behavioural changes or signs of toxicity and deaths.

Cell viability assay: The cytotoxic nature of the extract/fraction was further evaluated using the 3T3 cell lines (Invitrogen, France) following the manufacturer’s instructions and according to standard methods [35]. The cells, maintained at 37°C in 5% CO₂ in F-12K nutrient medium, were supplemented with 10% (v/v) heat-inactivated foetal bovine serum and 10,000 units/ml of the antibiotics streptomycin and penicillin. The 3T3 cells were seeded into 96 well micro titre plates at density of about 8 × 10^6 cells/well. After two days of culture, the cells were incubated for 24 h at 37°C with various concentrations of the aqueous extract (1, 20, 40, 80, 100µM) and the alkaloid fraction (1, 20, 40, 80, 100µM). Control wells contained cell culture medium with cells alone but no extract/fraction. Doxorubucin (3µM) was used as positive control. All assays were run in triplicates. The extent of cell viability was assessed by incubating with MTT and its conversion into purple colored MTT formazan by the living cells. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Biotek ELx-800, Mandel Scientific Inc.). The cytotoxicity was recorded as concentration causing 50% growth inhibition of 3T3 cells.

Phytochemical studies: Phytochemical studies was carried out using silica gel precoated thin layer chromatographic (TLC) plates and various phytochemical tests in order to assess the chemical composition of the aqueous extract and alkaloid fraction of *F. apodanthera* and the Ethanol, Hexane and Methylene Chloride extracts of *Erythrophleum ivorense* following standard procedures and as described [36,37].

Statistical analysis: DZI of extracts were measured as mean ± standard deviation. Cell viability assay data were expressed as mean ± standard error of the means (S.E.M.) per group. Statistical differences between control and treated groups of animals were tested by one-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc test. Differences were considered significant at P < 0.05. Graphpad Prism 5.01 for Window (Graphpad Prism Software, San Diego, CA, USA) was used.
### Results

**Stem-bark extracts of F. apodanthera and E. ivorense possess antibacterial activities:** The aqueous extract and alkaloid fraction of *F. apodanthera* and the ethanol, hexane and methylene chloride extracts of *E. ivorense* were screened against one gram positive (*Staphylococcus aureus*) and four gram negative pathogenic bacteria strains (*Escherichia coli, Proteus vulgaris, Providencia stuartii* and *Pseudomonas aeruginosa*). The DZI ranged from 0 to 17.8mm. The aqueous extract, alkaloid fraction and the Ethanol extract were active on all the five bacteria strains tested (Table 2). The hexane and methylene chloride extracts were not active on any of the strains tested. The alkaloid fraction of *F. apodanthera* had the highest diameter of zone of inhibition (17.8mm) against *Staphylococcus aureus*. *Providencia stuartii* was resistant to gentamycin reason why zero zone of inhibition was obtained. This strain is widely documented as the most resistant of all the Providencia species [38] and the isolate probably developed resistance against gentamycin.

| Extracts                                | P. stuartii | P. aeruginosa | P. vulgaris | S. aureus | E.coli |
|------------------------------------------|-------------|---------------|-------------|-----------|--------|
| Aqueous fraction of *F. apodanthera*     | 5.1         | 9.8           | 9.8         | 17.1      | 7.4    |
| Alkaloid fraction of *F. apodanthera*    | 10.3        | 8.8           | 10.8        | 17.8      | 14.3   |
| Ethanol extract of *E. ivorense*         | 8           | 11.3          | 10          | 13.1      | 8.1    |
| Hexane extract of *E. ivorense*          | 0           | 0             | 0           | 0         | 0      |
| Methylene chloride extract of *E. ivorense* | 0           | 0             | 0           | 0         | 0      |
| Positive control (gentamycin)            | -           | 14.6          | 21          | 18        | 18.6   |
| Negative control (50% DMSO)              | -           | -             | -           | -         | -      |

Values are mean standard deviation of triplicate assays. (-) represents zero DZI.

**MICs and MBCs of stem-bark extracts:** The MIC and MBC were determined by the broth microdilution assay for the various extracts in Table 2 above. The MIC values ranged from 0.094mg/ml (hexane extract against *P. vulgaris*) to 48mg/ml (ethanol extract on *P. stuartii*) while the MBC values ranged from 0.047mg/ml to 48mg/ml (Table 3). The hexane and methylene chloride extracts of *E. ivorense* did not show any activity by the hole-plate diffusion method but did yield some appreciable activities by the broth microdilution method. The difference could be accounted for by the problem of diffusion and chemical nature of extracts. The hexane extract of *E. ivorense* was the most active of the extracts by this method while the ethanol extract was the least active. Both the aqueous extract and the alkaloid fraction of *F. apodanthera* that showed good activity by the hole-plate diffusion method also yielded significant activities by the broth microdilution method. The alkaloid fraction had the same MICs (6mg/ml) for the five strains tested. The MIC is lower than the MBC for most of the test substances suggesting that the test substance in question is bacteriostatic at lower concentrations and bactericidal at higher concentrations.
Table 3. Minimal inhibitory/bactericidal concentrations (MIC/MBC) of extracts of *F. apodanthera* and *E. ivorense*

| Extract   | *P. stuartii* | *P. aeruginosa* | *P. vulgaris* | *S. aureus* | *E. coli* |
|-----------|---------------|----------------|--------------|-------------|-----------|
|           | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Aq. ext   | 5   | 3   | 3   | 3   | 4   | 2   | 3   | 6   | 4   | 2   |
| Alk. fr   | 4   | 3   | 4   | 3   | 4   | 4   | 4   | 7   | 4   | 4   |
| Eth. ext  | 1   | 2   | 3   | 2   | 3   | 1   | 3   | 2   | 3   | 1   |
| Hex. ext  | 8   | 8   | 3   | 5   | 10  | 7   | 9   | 11  | 3   | 3   |
| MeCl. ext | 3   | 5   | 4   | 3   | 4   | 2   | 5   | 6   | 4   | 3   |

1 = 48mg/ml, 2 = 24mg/ml, 3 = 12mg/ml, 4 = 6mg/ml, 5 = 3mg/ml, 6 = 1.5mg/ml, 7 = 0.75mg/ml, 8 = 0.375mg/ml, 9 = 0.188mg/ml, 10 = 0.094mg/ml, 11 = 0.047mg/ml and (-) stands for no result. Aq. ext and Alk. fr = respectively aqueous extract and alkaloid fraction of *F. apodanthera*. Eth. ext, Hex. ext and MeCl. ext stand for ethanol, hexane and methylene chloride extracts of *E. ivorense* respectively.

Chemical constituents of extracts of *F. apodanthera* and *E. ivorense*: Phytochemical screening using various phytochemistry tests and TLC revealed the presence of alkaloids, flavonoids, saponins, phenols and tannins, steroids and triterpenoids in various extracts (Table 4). The enriched alkaloid fraction gave a positive reaction with Dragendorff’s reagent demonstrating the presence of alkaloids; all other phytochemical tests were negative.

| Extract   | Flavonoids | Steroids | Saponins | Phenols/Tannins | Alkaloids | Triterpenoids |
|-----------|------------|----------|----------|-----------------|-----------|--------------|
| Aq. ext   | +++        | -        | ++       | ++              | +++       | -            |
| Alk. fr   | -          | -        | -        | -               | +++       | -            |
| Eth. ext  | +++        | -        | +        | ++              | +         | -            |
| Hex. ext  | +          | +        | +        | +               | +         | +            |
| Met. ext  | ++         | ++       | +        | +               | +         | ++           |

Aq. ext, Alk. fr, Eth. ext, Hex. ext and Met. ext = respectively aqueous extract of *F. apodanthera*, alkaloid fraction of *F. apodanthera*, Ethanol fraction of *E. ivorense*, hexane extract of *E. ivorense* and methylene chloride extract of *E. ivorense*. +++: abundant; ++: average; +: traces; -: absent.

Acute toxicity test with stem-bark extract/fraction of *F. apodanthera*: The aqueous extract and alkaloid fraction prepared from the stem-bark of *Feretia apodanthera* and orally administered to albino rats at various concentrations (1000, 2500 and 5000 mg/kg) did not have any behavioural effect, weight loss or any mortality within the period of observation which lasted for two weeks after substance administration. The aqueous extract and alkaloid fraction of *Feretia apodanthera* can therefore be considered non toxic at the doses tested.

Cell viability assay: The abilities of the aqueous extract and the alkaloid fraction of the stem-bark of *F. apodanthera* to induce cytotoxicity were further investigated using 3T3 cells and a standard MTT bioassay. Incubation of these cell lines with various concentrations of the aqueous extracts and the alkaloid fraction (1, 20, 40, 80, 100µM) up to a concentration of 20 µM and 5 µM respectively for 24h produced no cell toxicity (Table 5). The toxicity values observed were not significantly different (p>0.05) from the baseline. However, incubation of 3T3 cells with higher concentrations of the test substances (>40µM) produced higher cell death with IC<sub>50</sub> of 39.41 ± 0.95µM, 38.45 ± 1.64µM respectively, for the aqueous extract and the alkaloid fraction.
Table 5. Percentage survival of 3T3 cells as a function of various concentrations of aqueous extract and alkaloid fraction after 24 hours incubation and calculation of IC50.
Doxorubicin served as the positive control

| Treatments       | Concentration (µM) | % 3T3 cells Survival |
|------------------|--------------------|----------------------|
| Control          | -                  | 100.00               |
| Aqueous extract  | 1                  | 100.00               |
| Aqueous extract  | 20                 | 99.75                |
| Aqueous extract  | 40                 | 49.25*               |
| Aqueous extract  | 80                 | 24.96**              |
| Aqueous extract  | 100                | 12.58***             |
| Alkaloid fraction| 1                  | 100.00               |
| Alkaloid fraction| 20                 | 75.26                |
| Alkaloid fraction| 40                 | 47.98*               |
| Alkaloid fraction| 80                 | 38.45**              |
| Alkaloid fraction| 100                | 18.71***             |
| Doxorubicin      | 3                  | 01.84***             |

Data are expressed as % inhibition compared with negative control in which cell survival was considered to be 100% (means ± SD, n=24). Calculated IC50 of aqueous extract, alkaloid fraction and doxorubicin expressed as mean ± S.E.M. of the number of cells gave respectively 39.41 ± 0.95 µM, 38.45 ± 1.64 µM and 2.51 ± 0.20 µM. *, **, *** stand respectively for P<0.05, P<0.01 and P<0.001.

Discussion

Antimicrobial resistance poses a serious threat to human development, health and security. This coupled with the increasing rate of antimicrobial resistance among health-care associated and community-acquired infections over the past decade has recently prompted world leaders in their United Nations submit to commit to devise action plans on antimicrobial drug resistance (news-medical.net/news/2016; or http://www.who.int) based on the Global Action Plan on Antimicrobial resistance developed by WHO in 2015 in coordination with the FAO and the OIE [39]. This rise in bacterial and fungal infections which constitute a major health problem today is due to the fact that there are no vaccines for some infections and the emergence and widespread occurrence of multidrug resistant microbial phenotypes to past and present drug regimens. Plant-based systems have been widely exploited in traditional medicine to treat microbial infections for thousands of years. They are equally the principal sources of most conventional antimicrobials. Natural plant extracts and pure compounds isolated from plants, as well as synthetic compounds obtained by a further bioassay guided fractionation and isolation have been a good source of lead compounds for use as antimicrobials and for drug development. The present study was aimed at assessing the antibacterial and cytotoxic activities of extracts of Feretia apodanthera (a Rubiaceae) and Erythrophleum ivorense (a Fabiaceae) in order to attach a scientific connotation to its usage in African folk medicine as well as for further exploitation to identify lead compounds for drug development.

Stem-bark extracts of these two plants were screened by the hole-diffusion method against four gram negative (Escherichia coli (E.coli), Pseudomonas aeruginosa (P.aeruginosa), Proteus vulgaris (P.vulgaris) and Providencia stuartii (P.stuartii)) and one gram positive (Staphylococcus aureus (S.aureus)) human pathogenic bacteria strains giving a diameter zone of inhibition (DZI) ranging from 0 to 17.8mm. While the aqueous extract and alkaloid fraction of F. apodanthera and the ethanol extract of E. ivorense were all active on all the strains tested with activities ranging from moderate (5<DZI<10) to good (DZI>10) [31], the methylene chloride and hexane extracts of the stem-bark of E. ivorense showed no activity against any of the strains tested by this method. The
alkaloid fraction and the aqueous extract of *F. apodanthera* had the highest activities against *Staphylococcus aureus* (DZI of 17.8mm and 17.1mm, respectively) and the least (moderate activities) against *Pseudomonas aeruginosa* (DZI 8.8mm) and *Providencia stuartii* (5.1mm), respectively. While the alkaloid fraction had good activity against the rest of the strains tested, the aqueous extract had moderate activity against the rest of the strains tested. The ethanol extract of the stem-bark of *E. ivorense* showed good activity against *S. aureus* (DZI 13.1mm) and *P. aeruginosa* (DZI 11.3mm), moderate activity against *E.coli* (DZI 8.1mm) and *P. stuartii* (DZI 8mm) and moderate to good activity against *P. vulgaris* (DZI 10mm). No study to the best of our knowledge has demonstrated the antimicrobial properties of stem-bark extracts of *F. apodanthera*. Its activity against the bacterial strains of the urogenital tract confirms its usage in West African countries to treat various bacteria related infections including urinary and renal infections, gonorrhea, syphilis and stomach ache [7, 10, 17] amongst others as described above (Table 1). The antimicrobial activity and cytotoxicity of the methanolic leaf and stem bark extracts of *E. ivorense* against some reference bacterial strains has been documented [28, 40] but the antibacterial activity of its hexane, ethanol and methylene stem-bark extracts have not been documented especially against clinical pathogenic bacteria strains. Just recently the antimicrobial activity of the leaf and not the stem-bark extract against pathogenic microorganisms has been documented [41].

The five fractions were equally tested by the broth microdilution assay in order to assess their minimal inhibitory/bactericidal concentrations (MIC) against the five clinical bacteria strains. The MICs ranged from 0.094mg/ml to 48mg/ml. Both the aqueous extract and alkaloid fractions of *F. apodanthera* had similar MIC vis-à-vis *P. vulgaris* and *E. coli*, consistent with their DZI which was within the same range except for *E. coli* where the alkaloid fraction was twofold more active than the aqueous extract. Both the methylene chloride and hexane extracts of *E. ivorense* that were not active on either of the strains tested by the hole diffusion method showed activity by the broth microdilution method with MIC and MBC ranging from 0.047mg/ml to 24mg/ml. The difference could be accounted for by a number of factors including the problem of diffusion and chemical nature of the extracts. The hexane extract had the highest MIC vis-à-vis most of the strains tested with *P. vulgaris* (MIC 0.094mg/ml) being the most susceptible followed by *P. stuartii* (MIC 0.375mg/ml). The MIC was lower than the MBC for most of the test substances suggesting that the test substance in question is bacteriostatic at lower concentrations and bactericidal at higher concentrations. While the antimicrobial activity of the stem-bark of *F. apodanthera* has not been documented, antiplasmodial activity of the methanol extract of its leaves has previously been demonstrated [19] as well as its anticonvulsive activity [42].

We further analyze for the safety of the stem-bark extracts of *Ferretia apodanthera* by administering different doses to female rats and monitoring the general behaviour of the rats and any signs of toxicity and death. We showed herein that the aqueous extract and alkaloid fraction of stem-bark of *F.apodanthera* administered at various doses never showed any behavioural abnormality or death of the rat up to a dose of 5000mg/ml and up to 14days of observation. This is consistent with similar result from a previous study [21]. *Ferretia apodanthera* stem-bark may thus be considered safe partly justifying its long term and widespread usage in West African traditional folk medicine. In order to confirm this lack of toxicity, the cell viability assay was performed using 3T3 cells and a standard MTT bioassay [35]. No cell toxicity was produced after incubating the 3T3 cell lines with concentrations up to about 20µM and 5µM for the aqueous extract and the alkaloid fraction respectively. Incubation with higher concentrations of test substance (>40µM) however, produced cell death with IC₅₀ of 39.41 ± 0.95µM and 38.45 ± 1.64µM respectively for the aqueous extract and alkaloid fraction.

Lastly, the phytochemistry of the various extracts (the aqueous extract and enriched alkaloid fraction of *F. apodanthera*, the ethanol, hexane and methylene chloride extracts of *E. ivorense*) was assessed to confirm the nature of the enriched alkaloid fraction and to obtain the chemical composition of the other four extracts. As expected, various phytochemical tests never revealed the presence of any other constituent in the alkaloid fraction other than alkaloids. The aqueous fraction
however, possessed abundant amounts of flavonoids and alkaloids and some saponins, glycosides and phenols/tannins but neither steroids nor triterpenoids. This is consistent with previous findings by Taiwe et al., in 2015. The hexane and methylene chloride extracts contained only traces of these compounds including steroids and triterpenoids. This is slightly contrary to previous study [26] that found the hexane fraction of the stem-bark of *E. ivorense* to be devoid of the main compound families. However, we obtained zero activity for the hexane extract with the hole plate diffusion method and some activity with the broth microdilution assay and this should likely be due to the presence of these compound families. The ethanol extract contained an abundance of flavonoids, no steroids and triterpenoids and traces of alkaloids, saponins and phenols and tannins. Previous studies duelled mostly on the methanol [40], ethyl acetate, dichloromethane and hexane extracts [26] of the stem-bark of *E. ivorense* and not on the ethanol and methylene chloride fractions and revealed the presence or absence of the main compound families. The presence of these compounds could account for the antimicrobial activity of the active plant parts.

**Conclusion**

The stem-barks of *Ferretia apodanthera* and *E. ivorense* possess antimicrobial activities especially against human pathogenic bacteria and are worthy of further exploration to identify novel compounds for antimicrobial drug development. This is the first time antibacterial activity (especially against human pathogenic bacteria) has been associated to these plant parts. The phytochemistry of the two plants reveals the presence of various compounds probably responsible for their antibacterial activity. Acute toxicity and cell viability assays showed that the stem-bark extracts of *Ferretia apodanthera* are safe or of low toxicity justifying their safe usage in West African traditional folk medicine.

**Conflict of interests**

The authors declare that there is no conflict of interests regarding the publication of this work.

**Acknowledgement**

This work was supported by the RMA from the Cameroon Ministry of Higher Education (MINESUP) to DLN and GST.

**References**

[1] S.D. Karou et al., Sub-Saharan Rubiaceae: A review of their traditional uses, phytochemistry and biological activities, Pak. J. Biol. Sci. 14(3) (2011) 149-169.

[2] WHO, Global Strategy for Containment of Antimicrobial Resistance (Online). Available: http://www.who.int/csr/resources/.

[3] D.L. Njimoh et al., Antimicrobial activities of a plethora of medicinal plant extracts and hydrolates against human pathogens and their potential to reverse antibiotic resistance, Int. J. Microbiol. 2015 (2015).

[4] M. Rosado-Vallado et al., Antimicrobial activity of Fabaceae species used in Yucatan traditional medicine, Fitoterapia. 71(5) (2000) 570-573.

[5] D.B. Santana et al., Activity of Fabaceae species extracts against fungi and *Leishmania*: vatacarpan as a novel potent anti-*Candida* agent, Revista Brasileira de Farmacognosia. 25(4) (2015) 401-406.

[6] H.M. Burkil, The useful plants of West Tropical Africa, Royal Botanic Gardens, Kew. 1985-2004.
[7] C.K. Ruffo, A. Birnie, B. Tengnas, Edible wild plants of Tanzania, Regional Land Management Unit, Nairobi, 2002.

[8] S. Paul et al., Plants used in traditional beekeeping in Burkina Faso, Open Journal of Ecology. 3(5) 2013) 354-358.

[9] H.H. Hansen, L. Sanou, B.M.I. Nacoulma, Tree leaves in the diet of free-ranging ruminants in three areas of Burkina Faso, Livest. Res. Rural Dev. 20(3) (2008).

[10] J.E. Adjanohoun et al., Traditional medicine and pharmacopoeia, in: Contribution to Ethnobotanical and Floristic Studies in Cameroon, Porto-Novo: Organization of African Unity Scientific, Technical and Research Commission, Centre National de Production de Manuels Scolaires, 1996.

[11] M. Arbonnier, Arbres, arbustes et lianes des zones sèches d’Afrique de l’Ouest, Paris: Centre de coopération internationale en recherche agronomique pour le développement/Muséum national d’histoire naturel/union mondiale pour la nature, 2000.

[12] E.J. Adjanohoun et al., Contributions aux études ethnobotaniques et floristiques au Togo, Médecine Traditionnelle et Pharmacopée, Agence de Coopération Culturelle et Technique, Paris, France, 1986.

[13] K. Inngjerdingen et al., An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa, J. Ethnopharmacol. 92 (2004) 233-244.

[14] D. Malgras, Arbres et arbustes guérisseurs des savanes maliennes, Editions Karthala, 22-24, boulevard Arago, 75013 Paris, 1992.

[15] H.S.N. Hussain, Y.Y. Karatela, Traditional medicinal plants used by Hausa tribe of Kano State of Nigeria, Int. J. Crude Drug Res. 27(4) (1989) 211-216.

[16] J.G. Adam, N. Echard, M. Lescot, Plantes médicinales Hausa de l'Ader (République du Niger), Journal d'Agriculture Tropicale et de Botanique Appliquée. 19(8) (1972) 259-399.

[17] J. Kerharo, J.G. Adam, Plantes médicinales et toxiques des Peuls et des Toucouleurs du Sénégal, Journal d'Agriculture Tropicale et de Botanique Appliquée. 11(10) (1964) 384-444.

[18] A.S. Bâ, L'art vétérinaire en milieu traditionnel africain, Agence de Coopération culturelle et technique (ACCT), 1994.

[19] C. Ancolio, Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali, Phytotherapy Research. 16(7) (2002) 646-649.

[20] G.S.Taiwe et al., Anticonvulsant effects of iridoid glycosides fraction purified from Feretia apodanthera Del. (Rubiaceae) in experimental mice models of generalized tonic-clonic seizures, BMC Complement Altern. Med. 16 (2016) 285.

[21] G.S. Taiwe et al., Extracts of Feretia apodanthera Del. demonstrated anticonvulsant activities against seizures induced by chemicals and maximal electroshock, Epilepsy Research., 127(2016) 30-39.

[22] F. Arriaga et al., Structural Tali timber (Erythrophleum ivorense A. Chev., Erythrophleum suaveolens Brenan.): Assessment of strength and stiffness properties using visual and ultrasonic methods, Holz als Roh- und Werkstoff. 64(5) (2006) 357-362.

[23] F.A. Armah et al., Erythroivorensin: A novel anti-inflammatory diterpene from the root-bark of Erythrophleum ivorense (A Chev.), Fitoterapia. 105 (2015) 37–42.

[24] H.G. Richter, M.J. Dallwitz, Commercial timbers: descriptions, illustrations, identification, and information retrieval [Online]. Available: http://delta-intkey.com.
[25] PROTA, Plant Resources for Tropical Africa [Online]. Available: www.prota4u.info/Prota.org.

[26] O.K. Wakeel et al., Anticonvulsant and sedative activities of extracts of *Erythrophleum ivorense* stem bark in mice, Asian J. Biomed. Pharm. Sci. 04(28) (2014) 44-47.

[27] O.K. Wakeel et al., Evaluation of anti-nociceptive and anti-inflammatory activities of *Erythrophleum ivorense* stem bark in experimental animals, European J. Biomed. Pharm. Sci. 3(3) (2016) 84-89.

[28] L. Adu-Amoah et al., Antimicrobial and cytotoxicity studies of the methanolic extracts of *Erythrophleum ivorense* leaf and stem bark, Planta Med. 79 (2013).

[29] ICS-UNIDO (United Nations Industrial Development Organization and the International Centre for Science and High Technology), Extraction Technologies for Medicinal and aromatic Plants, 2008.

[30] N.N. Azwanida, A Review on the extraction methods use in medicinal plants, principle, strength and limitation, Med. Aromat. Plants. 4 (2015) 196.

[31] NCCLS, Antimicrobial Susceptibility Testing/SC3, National Committee for Clinical Laboratory Standards, Villanova, Pa, USA, 3rd edition, 1990.

[32] T. Hussain et al., Biochemical characterization and identification of bacterial strains isolated from drinking water sources of Kohat, Pakistan, Afr. J. Microbiol. Res. 7(16) (2013) 1579-1590.

[33] J.H. Jorgensen, M.J. Ferraro, Antimicrobial susceptibility testing: a review of general principles and contemporary practices, Clin. Infect. Dis. 49(11) (2009) 1749-1755.

[34] D. Lorke, A new approach to practical acute toxicity testing, Arch. Toxicol. 54(4) (1983) 275-287.

[35] T.L. Riss et al., Cell Viability Assays, Assay Guidance Manual, 2016.

[36] T. Mohammed, C. Teshale, Preliminary phytochemical screening and evaluation of antibacterial activity of *Dichrocepala integrifolia* (L.f) O. Kuntze, J. Intercult. Ethnopharmacol. 1(1) (2012) 30-34.

[37] A. Debella, Preliminary screening techniques of secondary metabolites, in: Manual for Phytochemical Screening of Medicinal Plants, Ethiopian Department of Drug Research, Ethiopian Nutrition Research Institute, Addis Ababa, Ethiopia, 2002, pp. 38-57.

[38] M. Tumarello et al., ESBL-producing multidrug-resistant *Providencia stuartii* infections in a university hospital, J Antimicrob Chemother. 53(2) (2004) 277-282.

[39] WHO, Global Action plan on Antimicrobial Resistance, 2015. [Online]. Available: http://www.who.int/antimicrobial-resistance/global-action-plan/en/.

[40] L. Adu-Amoah et al., Toxicity assessment of *Erythrophleum ivorense* and *Parquetina nigrescens*, Toxicology Reports. 1 (2014) 411-420.

[41] S.O. Cédric et al., Phytochemical screening, evaluation of antioxidant and antimicrobial properties of *Erythrophleum ivorense* A. Chev (Leguminosae) and *Megaphrynium macrostachyum* Benth (Marantaceae), medicinal plants from Gabon, Int. J. Biosci. 8(6) (2016) 43-53.

[42] G.S. Taiwe et al., Effects of a lyophilized aqueous extract of *Feretia apodanthera* Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice, Epilepsy & Behavior. 43 (2015) 100-108.