**In vitro** antimicrobial activity of the methanol extract and compounds from the wood of *Ficus elastica* Roxb. ex Hornem. aerial roots

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**A B S T R A C T**

*Ficus elastica* Roxb. ex Hornem., an edible plant belonging to the family of Moraceae, is traditionally used against skin infections and allergies besides having diuretic properties. This study aimed at investigating the antimicrobial activity of the wood of *F. elastica* aerial roots against a set of bacteria (*Staphylococcus aureus*; *Escherichia coli*, *Pseudomonas aeruginosa* and *Providencia stuartii*; *Candida albicans*). A mixture of linear aliphatic alkanes with n-hexacosane as major compound, β-sitosterol, biochanin A, sitosteryl 3-O-β-D-glucopyranoside (1), elastamide (2), elastiquinone (3) and ficusoside B (4) were purified and characterized. Antimicrobial activities, expressed as minimum inhibitory concentration (MIC), indicated that the methanol extract showed MIC of 39.1 μg/mL; the lowest values were obtained for 3 and 4, with MIC as low as 4.9 μg/mL, smaller than the values of reference antibiotics (25 μg/mL). Furthermore, as most of the studied samples exhibited Minimum Micbicidal Concentration/Minimum Inhibitory Concentration (MMC/MIC) ratios lower than 4, a microbicidal effect was clearly exhibited. The overall results provided evidence that the wood of *F. elastica* aerial roots, as well as some of its isolated components might be potential sources of new antimicrobial drugs.

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### 1. Introduction

Infectious diseases are one of the major causes of death worldwide with almost one third of all deaths in low-income countries (World Health Organisation, 2012). They remain a serious health problem worldwide, especially with the growing phenomenon of antibiotic resistance. It has been reported that about 80% of the world population is dependent (fully or partially) on plant-based drugs (Veeresham, 2012). This situation is more complicated in Africa as the population is relatively poorer and relies on medicinal plants to cure several ailments. Despite the discovery of many drugs from natural origin, the search for new antimicrobial agents is still necessary. Such researches could increase the number of drugs which are less toxic and more effective. For instance, in the years from 1981 to 2014, of the 112 antibacterial agents (small molecules) approved, ~73% are natural products (unmodified structures) or natural product derivatives (Newman and Cragg, 2016).

The Moraceae plant family includes *Ficus* as one of the main plants with biological activities already described such as antiplasmodial (Muregi et al., 2003), antioxidant (Phan et al., 2012), anticancer (Mbosso et al., 2012, 2015, 2016a, 2016b), antimicrobial (Mbosso et al., 2012, 2015, 2016a), antiluver (Galati et al., 2001), antidiarrhoeal (Mandal and Kumar, 2002), anti-pyretic (Rao et al., 2002), and gastroprotective (Rao et al., 2008). Note that the latex of some species of *Ficus* is exploited in traditional folk medicine for its antihelmintic parasiticidal property of this plant has been ascribed to the presence of ficin (Pistelli et al., 2000) and it was also demonstrated that the latex of *Ficus elastica* Roxb. ex Hornem. (Moraceae) showed a significant antischistosomal activity (Seif el-Din et al., 2014). The parasiticidal property of this plant has been ascribed to the presence of ficin (Pistelli et al., 2000) and it was also demonstrated that the latex of *Ficus elastica* Roxb. ex Hornem. (Moraceae) showed a significant antischistosomal activity (Seif el-Din et al., 2014).

In our continuous search for bioactive compounds in Cameroon plants, we focused herein on *F. elastica* (the rubber tree) which is a widely-spread evergreen tree up to 30 m tall. Its leaf extract is used for treating skin infections and allergies, as well as a diuretic agent (Phan et al., 2012). The phytochemical investigation
of this taxon revealed the presence of 6,10,14-trimethyl-2-pentadecanone (25.9%) and geranyl acetone (9.9%) in the leaf oil as main constituents (Ogunwande et al., 2011); emodin, sucrose, morin and rutin with antimicrobial activities from the MeOH extract of the leaves (Hassan et al., 2003), feroxidin, quercitrin, kaempferin, and rutin with antimicrobial activities from the MeOH extract of leaves (Phan et al., 2012), n-alkanes, friedelin, friedelinol, linear aliphatic primary alcohols, linear fatty, phytosterols, betulinic acid, ursolic acid, sitosterol 3-0-β-D-glucopyranoside, fucosamide, fucoside B and fucoside with anticancer and antimicrobial activities from the bark of aerial roots (Mbosso et al., 2016a). In our previous studies on the Cameroonian F. elastica, Ficusoside B endowed with anticancer activity was isolated from the bark of aerial roots (Mbosso et al., 2016a). 

In the present study, we examine the antimicrobial activity of isolated molecules as well as the MeOH extract of wood of F. elastica aerial roots. To the best of our knowledge, the antimicrobial evaluation of the wood of F. elastica aerial roots is reported here for the first time against pathogenic bacteria and yeast strains.

2. Materials and methods

Cyclohexane (99 + %), ethyl acetate (99 + %) and MeOH (99 + %) were purchased from Chemlab and DMSO (99.7%) was from Acros.

2.1. Plant material

The wood of F. elastica aerial roots was collected from Yaoundé (Cameroon) in December 2007. The plant's identification was established by a member of the National Herbarium of Cameroon (NHC), where a voucher specimen (No. 65646 HNC) was deposited. After Air-drying, the plant material (wood of F. elastica aerial roots) was crushed into a fine powder by using an electric grinder.

2.2. Extraction and isolation

Macerate of the dried aliquot (5.50 kg) was obtained using methanol (20 L) twice for 48 h at room temperature (27 ± 2 °C) (Mohamad et al., 2011). After filtration (Whatman Number One) and evaporation at low pressure in a rotary evaporator (bath at 40 °C), 15 g of extract was obtained (Mbosso et al., 2016a). The crude MeOH extract FEBr (14.5 g) was subjected to silica gel column chromatography (CC) (cyclohexane/EtOAc/MeOH gradient of increasing polarity) to afford four fractions on the basis of TLC composition. Further purification through successive column chromatography yielded several pure molecules belonging to many classes of compounds. Products previously isolated from wood of F. elastica aerial roots included a mixture of linear aliphatic alkanes with n-hexacosane as major compound, ϖ-sitosterol, biochanin A, sitosteryl 3-O-β-D-glucopyranoside (1), elasticamide (2), elastiquinone (3) and fucoside B (4) (Mbosso et al., 2016a).

2.3. Antimicrobial assay using microbroth dilution method

Six microorganisms, i.e. one Gram-positive (Staphylococcus aureus); four Gram-negative bacteria specimens (Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa) and one yeast (Candida albicans) were tested. All these microbial strains were obtained from urine samples and vaginal swabs of in-patients suffering from urogenital tract infections at the Tiko Cottage Hospital (Cameroon). Microbial strains were identified through their growing properties using specific culture media, as well as through microscopic and biochemical characteristics described as follows. Gram positive (S. aureus RN4220) and Gram negative (E. coli JM109) reference strains were submitted to the same microscopic and biochemical characteristic procedure) as controls. We were convinced of the species determination of isolates only if those of the reference samples were confirmatory. Concerning Candida species: The sample was cultured on Sabouraud chloramphenicol agar, and the isolate was tested for the evidence of production of germ tube in human serum, a test which differentiates Candida albicans from other candida species. Fresh plates of test bacteria species were made from the isolated cultures obtained on agar slants. Discrete colonies of fresh cultures of the different isolates were then picked and suspended in 5 ml of Nutrient broth (NB, Oxoid), in well-labeled sterile bottles, and incubated for 24 h at 37 °C prior to antimicrobial susceptibility testing. Fluconazole (Sigma, USA), and gentamycin (Sigma, USA) were used as reference antibiotics against yeasts and bacteria species, respectively.

Table 1

| Name | Structure | Reference |
|------|-----------|-----------|
| Sitosteryl 3-O-β-D-glucopyranoside | | |
| Elasticamide | | Mbosso et al. (2016a) |
| Elastiquinone | | |
| Ficusoside B | | |
The Minimum Inhibitory Concentration (MIC), considered as the lowest concentration of the sample that inhibits the visible growth of microorganisms, was determined by the microbroth dilution method (Carbonnelle et al., 1987; Berghe and Vlietinck, 1991) in Mueller Hinton (for antibacterial activity) or Sabouraud broth (for antifungal activity) supplemented with 10% glucose and 0.5% phenol red. For susceptibility testing, in a first step supplemented Mueller Hinton or Sabouraud broth (50 μL) was distributed from the first to the twelfth row on a 96 well microplate. The dry extract was initially dissolved in DMSO (20%) (100 μL) and subsequently in Mueller Hinton broth, to reach a final concentration of 10.0 mg/mL for FEBr and for 1, 2, 3 and 4 (the extract nomenclature is detailed on footnotes of Table 2). Solutions (50 μL) were added to the first well of each microtiter line. Successive dilutions were then carried out by transferring the mixture/solution (50 μL) from the first to the eleventh well. An aliquot (50 μL) was discarded from this eleventh well. The twelfth well was left as control since no sample (extract, compounds, or reference antibiotics) was added. A microbial suspension (of isolates as well as of reference strains), i.e. 50 μL sample (extract, compounds, or reference antibiotics) was added. A final concentration was reached after the following incubation: 18 to 24 h for bacteria and 24 to 48 h for fungi. The MIC was determined by the microbroth dilution method as the lowest concentration of the sample that inhibits the visible growth of a microbe and MMC is considered as the lowest concentration of the sample capable of causing the deaths of at least 99.99% of a tested inoculum.

### Table 2

| Concentration (μg/mL) | E. coli | P. aeruginosa | S. aureus | C. albicans | G. f. P. stuartii | P. vulgaris |
|-----------------------|---------|---------------|-----------|-------------|----------------|------------|
| 1                     | 78.1    | 78.1          | 78.1      | 78.1        | 78.1          | 78.1       |
| 2                     | 39.1    | 39.1          | 39.1      | 39.1        | 39.1          | 39.1       |
| 3                     | 19.5    | 19.5          | 19.5      | 19.5        | 19.5          | 19.5       |
| 4                     | 9.8     | 9.8           | 9.8       | 9.8         | 9.8           | 9.8        |
| 5                     | 39.1    | 39.1          | 39.1      | 39.1        | 39.1          | 39.1       |
| 6                     | 25      | 25            | 25        | 25          | 25            | 25         |
| 7                     | /       | /             | /         | /           | /             | /          |
| 8                     | /       | /             | /         | /           | /             | /          |
| 9                     | /       | /             | /         | /           | /             | /          |
| 10                    | /       | /             | /         | /           | /             | /          |
| 11                    | /       | /             | /         | /           | /             | /          |
| 12                    | /       | /             | /         | /           | /             | /          |

| Concentration (μg/mL) | P. mirabilis | E. faecalis | S. marcescens |
|-----------------------|--------------|-------------|--------------|
| 1                     | 78.1         | 78.1        | 78.1         |
| 2                     | 39.1         | 39.1        | 39.1         |
| 3                     | 19.5         | 19.5        | 19.5         |
| 4                     | 9.8          | 9.8         | 9.8          |
| 5                     | 39.1         | 39.1        | 39.1         |
| 6                     | 25           | 25          | 25           |
| 7                     | /            | /           | /            |
| 8                     | /            | /           | /            |
| 9                     | /            | /           | /            |
| 10                    | /            | /           | /            |
| 11                    | /            | /           | /            |
| 12                    | /            | /           | /            |

### Results and discussion

Compounds tested in this study included steroidal glucosides known as sitosterol 3-O-β-D-glucopyranoside (1), elasticamide (2), elastiquinone (3) and ficusoside B (4).

They were isolated and characterized from wood of *F. elastica* aerial roots and subsequently, a notable anti-proliferative effect on 6 human cancer cell lines (Mbosso et al., 2016a). The isolated compounds, as well as the crude extract from wood of *F. elastica* aerial roots were tested for their antimicrobial activities on a panel of microbial strains and the results are reported in Table 2.

In the literature, various criteria are applied to determine the susceptibility of extracts and isolated compounds as microbial inhibitors. In our case, the antimicrobial activity of a crude plant extract has been defined as significant with MIC below 100 μg/mL, moderate with MIC between 100 μg/mL and 625 μg/mL, and low with MIC values more than 625 μg/mL. Tests were incubated aerobically at 37 °C for 24 and 48 h. Gentamycin was already investigated elsewhere (Sklenickova et al., 2010; Liu et al., 2011) and consequently these molecules were not tested in the present study. Compounds 1-4 as well as the crude extract from the wood of *F. elastica* aerial roots were tested for their antimicrobial activities on a panel of microbial strains and the results are reported in Table 2.
compounds 1–4 were further tested for their antimicrobial properties. The defined threshold values for each molecule are defined as follows: MIC below 10 μg/mL (significant activity), 10 ≤ MIC ≤ 100 μg/mL (moderate activity) and MIC > 100 μg/mL (low activity) (Kuete, 2010). Results summarized in Table 2 indicated that MICs ranged from 4.9 to 78.1 μg/mL.

More specifically, elasticamide 2 inhibited the growth of 100% of the six tested strains with MICs spanning 19.5–78.1 μg/mL and can be considered as a moderate antibacterial agent (Kuete, 2010). In a previous report, we isolated fucosamide from the bark of F. elastica aerial roots. This ceramide revealed a significant activity with MIC of 3 μg/mL against Staphylococcus saprophyticus and low bactericidal properties against Klebsiella pneumoniae (MIC 380 μg/mL), Escherichia coli and Enterococcus faecalis (MIC 190 μg/mL). Note that MIC values obtained for reference gentamycin were of 980 μg/mL against Staphylococcus saprophyticus and Enterococcus faecalis; 7810 μg/mL against Klebsiella pneumoniae and Escherichia coli (Mbosso et al., 2012). Similar results were previously mentioned for structurally closed skeletons (Fischer et al., 2012; Poupamane, 2012).

Elastiquinone 3 inhibited the growth the six tested microorganisms with the MICs in the range of 4.9–78.1 μg/mL and demonstrated a significant activity with a MIC of 4.9 μg/mL against P. stuartii and P. aeruginosa, 9.8 μg/mL against P. vulgaris and S. aureus and a moderate activity (MIC of 19.5 μg/mL) against E. coli and C. albicans. Results in the same order were previously obtained for similar agents (Manojlovic et al., 2000; Comini et al., 2011).

Ficusoside B 4 also prevented the growth of the six strains with the same MICs range found for elastiquinone 3. However, compound 4 was the most active against E. coli, P. vulgaris, S. aureus and C. albicans with a MIC of 4.9 μg/mL. These values were even smaller than MIC of used reference antibiotics (25 μg/mL). Molecules 3 and 4 can be considered as significant antibacterial agents as MIC values below 10 μg/mL were obtained against 4/6 of the tested microorganisms (Kuete et al., 2010). In a previous study, we isolated spathoside from the stem bark of Spathodea campanulata P. Beauv., a cerebroside which showed a significant activity against K. pneumoniae (MIC 6.25 μg/mL), but moderate activity against Staphylococcus aureus, Streptococcus faecalis, P. aeruginosa, Shigella flexneri (MIC 12.5 μg/mL), Bacillus subtilis (MIC 25 μg/mL) and Bacillus cereus. However, no sign of growth inhibition was seen for E. coli and Shigella dysenteriae (Mbosso et al., 2008). In a previous study, we separated an elasticoside from the bark of Ficus elastica aerial roots, another cerebroside which showed a moderate activity against E. faecalis (MIC 30 μg/mL), but low activity against many microorganisms such as Staphylococcus saprophyticus (MIC 130 μg/mL), K. pneumoniae (MIC 250 μg/mL), Trichophyton rubrum, C. albicans, E. coli, Salmonella typhimurium, S. aureus and Staphylococcus epidermidis (MIC 500 μg/mL) (Mbosso et al., 2012). Other cerebrosides have also been reported to display antimicrobial activities (Cateni et al., 2003; Chen et al., 2003; Shu et al., 2004).

Sitosterol 3-O-β-D-glucopyranoside 1 inhibited the growth of the five tested bacteria with the MICs ranging from 19.5 to 78.1 μg/mL. Similar results were previously described for sugar derivative 1 which has already demonstrated antibacterial activity against S. aureus (Soo-Hwan et al., 2003; Phan et al., 2005), S. aureus, S. flexneri (MIC 12.5 μg/mL), S. faecalis, P. aeruginosa, S. dysenterae (MIC 25 μg/mL) (Mbosso et al., 2010), S. aureus (MIC 12.5 μg/mL), S. faecalis (MIC 6.3 μg/mL), P. aeruginosa (MIC 3.2 μg/mL) and S. flexneri (MIC 12.5 μg/mL) (Mbosso et al., 2008). Compound 1 also showed a moderate antifungal activity against C. albicans (MIC 78.1 μg/mL) which is of the same order as other studies found in the literature: Candida tropicalis (MIC 100 μg/mL), Cryptococcus neoformans (MIC 50 μg/mL) (Tamakou et al., 2011), and C. albicans, Cryptococcus neoformans, Aspergillus fumigatus (MIC 125 μg/mL) (Awouafack et al., 2013).

Molecules 2–3 demonstrated a more pronounced antifungal activity (MICs 19.5 μg/mL) while Ficusoside B 4 possessed a significant antifungal activity with a MIC of 4.9 μg/mL against C. albicans.

Data in Table 2 indicated that most MMC/MIC ratios for the crude extract were below 4, signifying the microbiocidal effects on the microorganisms (Carbonnelle et al., 1987; Mbaveng et al., 2008, 2011). A keen look at the MICs and MMCs indicated that compounds 1 and 2 are bactericidal against all tested microorganisms. However, compounds 3 and 4 rather exerted bacteriostatic (MMC/MIC ≥ 4) effects (Mbaveng et al., 2008, 2011) on 50% and 66.66% of the tested microorganisms, respectively. These data suggest that the secondary metabolites of the wood of F. elastica aerial roots may interact synergistically to produce the observed effects. Regarding the involvement of microorganisms in treatment failures and the re-emergence of infectious diseases (Blot et al., 2007; Falagas and Bliziotis, 2007; Kuete, 2010; Kuete et al., 2011a), the antimicrobial activity of methanol wood extract F. elastica aerial roots as well as that of compounds 1–4 could be considered promising. To the best of our knowledge, the antimicrobial effect of 2–4 against Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans is being reported for the first time. Data reported herein, therefore, provide additional information on the potential of various parts of this plant to fight microorganisms and more particularly, elastiquinone 3 and fucososide B 4 as the main active antimicrobial agents of the wood of Ficus elastica aerial roots extract.

4. Conclusions

The methanol extract showed a good inhibition with the lowest MIC value (39.1 μg/mL) on the entire studied organisms except for P. stuartii. The most active molecules were elastiquinone 3 and fucososide B 4 whose MIC against P. stuartii, P. aeruginosa and E. coli, P. vulgaris, S. aureus, C. albicans, respectively, was 4.9 μg/mL. The results of the present study are important, taking into account the implication of the studied microorganisms in therapeutic failure. The results of our study are consistent with those reported for other Ficus species such as methanol extract from the stem bark of F. ovata (Kuete et al., 2009), methanol extract from the roots of F. polita (Kuete et al., 2011b), CHCl₃/MEOH 1:1 crude extract of bark of F. elastica aerial roots (Mbosso et al., 2012) and methanol extract from fruits of F. Bubu (Mbosso et al., 2016b). These data indicate that the methanol wood extracts of F. elastica aerial roots as well as some of its constituents, and mostly elastiquinone and fucososide B deserve more attention in the future development of potential antimicrobial drugs to fight MDR bacterial and yeast infections.

Competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sajb.2017.03.026.

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