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*Original Research*

# Antimicrobial and Antibiotic-Resistance Reversal Activity of Some Medicinal Plants from Cameroon against Selected Resistant and Non-Resistant Uropathogenic Bacteria

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**Abstract**

**Background and Aim**: Antibiotics' resistance is the leading cause of complications in the treatment of urinary tract infections. This study aimed to screen the antimicrobial potential of 8 plants from Cameroon against multi-resistant uropathogenic (MRU) bacteria and to investigate their antibioresistance reversal properties. **Method**: Bioactive compounds were extracted from leaves of *Leucanthemum vulgare*, *Cymbopogon citratus*, *Moringa oleifera* and *Vernonia amygdalina*; barks of *Cinchona officinalis* and *Enantia chlorantha* barks and seeds of *Garcinia lucida* and leaves and seeds of *Azadirachta indica* using water and ethanol as solvents. The extracts were tested against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* 10231 using the well diffusion and the broth microdilution methods. The antibiotic-resistance reversal activity was assessed against selected MRU bacteria. The phytochemical composition and the elemental composition of the most active extracts were assessed respectively using HPLC-MS/MS and X-ray fluorescence (XRF) spectrometry. **Results**: Among the most active plants, in decreasing order of antimicrobial activity we found ethanolic (EE) and aqueous extracts (AE) of *E. chloranta* bark (ECB), EE of *L. vulgare* leaves and *G. lucida* seeds. The best synergies between common antibiotics and extracts were found with EE-ECB which well-modulated kanamycin nitrofurantoin and ampicillin. All the compounds identified in EE-ECB were alkaloids and the major constituents were palmatine (51.63%), columbamine+7,8-dihydro-8-hydroxypalmatine (19.21%), jatrorrhizine (11.02%) and pseudocolumbamine (6.33%). Among the minerals found in EE-ECB (S, Si, Cl, K, Ca, Mn, Fe, Zn and Br), Br, Fe and Cl were the most abundant with mean fluorescence intensities of 4.6529, 3.4854 and 2.5942 cps/uA respectively. **Conclusions**: The ethanol extract of the bark of *E. chlorantha* has remarkable, broad-spectrum antimicrobial and contains several palmatine derivatives.

**Keywords:** medicinal plants; antimicrobial; synergy test; antibiotics; multiresistant; uropathogenic bacteria

## 1. Introduction

Antibiotic resistance is defined as the ability of a bacteria to resist the inhibitory or destructive activity of an antimicrobial to which it was not resistant [1–3]. This adaptation phenomenon is mainly due to the enzymatic degradation of antibiotics by bacteria, the modification of the antibiotic target, the change in membrane permeability, and alternative metabolic pathways [4].

Antibiotic resistance is a public health problem with an impact on human and animal health, agriculture, the economy and the environment [1–4]. Recent estimates have shown that antibiotic resistance is responsible for 700,000 annual deaths worldwide, 230,000 of which have resulted from multidrug-resistant bacteria [2]. The World Health Organization estimates that if nothing is done to address this problem, drug-resistant diseases may cause 10 million deaths each year by 2050 and damage to the economy as catastrophic as the 2008–2009 global financial crisis [2]. Furthermore, economically (linked directly or not to agriculture and animal breeding), antimicrobial resistance could force up to 24 million people into extreme poverty by 2030 [2]. The search for new antimicrobials is therefore essential to address this worldwide public health issue [1–4]. This situation affects all areas requiring the use of antibiotics including the management of diseases such as urinary tract infections (UTIs).

UTIs are very common infections in the human population (especially in women) and can be defined as any infection, commonly of bacterial origin, which occurs in any part of the urinary system [3]. Nowadays, UTIs are serious public health issues and are responsible for nearly 150 million disease cases every year worldwide [3]. Most UTIs (80–90%) are caused by *Escherichia coli* while other germs like *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Streptococcus*, and *Enterococcus faecalis* are rarely involved [5].

Resistance to antibiotics has made these infections more difficult to treat. Medicinal plants are among the most promising solutions to address this problem and each year, studies carried out in the 4 corners of the globe are intended to exploit their antimicrobial potential. In this context, the Cameroonian flora, known for its abundance of plants with multiple therapeutic virtues, can significantly contribute to this fight against antibiotic resistance and the development of new antimicrobials.

In this study, the herbal medicines investigated were leaves of *Cymbopogon citratus* (DC.) Stapf (C. *citratus*), *Moringa oleifera* Lam (M. *oleifera*), *Leucanthemum vulgare* (*L. vulgare*) and *Vernonia amygdalina* Delile (*V. amygdalina*); barks of *Cinchona officinalis* (*C. officinalis*) and *Enantia chlorantha* Oliv (*E. chlorantha*); barks and seeds of *Garcinia lucida* Vesque (*G. lucida*) and Leaves and seeds of *Azadirachta indica* (Neem) (*A. indica*). These medicinal plants are among the most famous in Cameroon. For example, *C. officinalis* is a shrub of the Rubiaceae family whose bark is well known for its very bitter taste and its antimalarial properties. This plant is rich in alkaloids such as quinine, dihydroquinine, cinchonidine, epiquinin, quinidine, dihydroquinidine, cinchonine and epiquinidine [6,7]. *G. lucida* Vesque is also a well-known herbal medicine whose seeds, fruits and barks were reported to possess cardioprotective and nephroprotective effects [8] and are recognized to be useful in the treatment of gastric and gynecological infections, diarrheas, cure for snake bites as well as an antidote against poison [9]. Otherwise, *E. chlorantha* also called Epoue (Baka), Peye (Badjoue), and Nfol (Bulu), is used in the management of various infections including dysentery, malaria, typhoid fever, jaundice, wounds, high blood pressure, urinary infection, leprosy spots and convulsions [10]. Furthermore, *A. indica* known as Neem, is a monoecious tree of the Meliaceae family whose oil produced from its seeds is widely used for its medicinal properties in the northern part of Cameroon. It is known that compounds in Neem extracts have anti-inflammatory, anti-hyperglycaemic, anti-carcinogenic, antimicrobial, immune-modulator, anti-mutagenic, antioxidant, anti-ulcer, and antiviral effects [11,12]. Recent studies by Baildya *et al*. [12] even found 19 compounds from this plant which may be used as anti-COVID-19. Finally, *M. oleifera*, *V. amygdalina* and *C. citratus* are all edible and medicinal plants. Every part of the *M. oleifera*, from the leaves to the roots, has been reported to possess potential health benefits [13]. Besides its nutritional properties, *M. oleifera* is traditionally used to treat skin infection, asthma, diabetes, diarrhea, arthritis, inflammation, cough, fever, and headache. It has also been reported to have antioxidant, anti-inflammatory, antitumor, antimicrobial, hepatoprotective and anti-arthritic properties [14–16]. *V. amygdalina* (known in Cameroon under the popular name of Ndolè) have been reported to have anticancer and antitumor activity [17,18]; antihepatotoxic activity [19]; hypoglycemic activity [20]; antibacterial activity [21]; anti-inflammatory [22] as well as antioxidant property [23]. Moreover, *C. citratus* (lemongrass) is widely used as a tea and is rich in minerals, vitamins, macronutrients (including carbohydrate, protein, and small amounts of fat) and its leaves are a good source of various bioactive compounds such as alkaloids, terpenoids, flavonoids, phenols, saponins and tannins that confer *C. citratus* leaves pharmacological properties such as anti-cancer, antihypertensive, anti-mutagenicity, anti-diabetic, antioxidant, anxiolytic, anti-nociceptive and anti-fungal [24]. Like *C. citratus*, all plants investigated in this study have various phytocompounds such as terpenoids and xanthones products, alkaloids (such as protoberberines and phenanthrene alkaloids), aporphines, zeatin, quercetin, $\beta$-sitosterol, caffeoylquinic acid and kaempferol, saponins, sesquiterpenes, flavonoids, steroid glycosides, and lactones [9,13,20,25,26]. These multiple compounds make these plants an exploitable source for the development of new antimicrobials.

Therefore, the aim of this study was to evaluate the antimicrobial potential of aqueous and hydro ethanolic extracts of thirteen samples (bark, leaf, seed) of the eight above-mentioned plants from Cameroon and to assess their synergy with common antibiotics against various multiresistant uropathogenic bacteria.

## 2. Materials and Methods

### *2.1 Vegetal Material*

The vegetal materials used in this study were the same used in our previous study [27]. They were barks and seeds of *G. lucida* Vesque, leaves of *C. citratus* (DC.) Stapf, *L. vulgare, M. oleifera* Lam and *V. amygdalina* Delile; barks of *C. officinalis* and *E. chlorantha* Oliv; and Leaves and seeds of *A. indica* (Neem).

### *2.2 Microbial Strains*

Three reference cultures (purchased from American Type Culture Collection) were used to screen the antimicrobial activity of the different vegetal materials. They were *S. aureus* ATCC 6538 as Gram positive model, *E. coli* ATCC 25922 as Gram negative model and *C. albicans* ATCC 10231 as fungi model. To assess the synergy between common antibiotics and the extracts, 11 strains of uropathogenic bacteria provided by the Department of Microbiology and Virology of the Peoples' Friendship University of Russi were used. These strains were *A.* xylosoxidans 4892, *C. freundii* 426, *E. avium* 1669, *E. coli* 1449, *K. oxytoca* 3003, *K. rizophilia.* 1542, *M. catarrhalis* 4222, *M. morganii* 1543, *P. aeruginosa* 3057, *S. aureus* 1449 and *S. agalactiae* 3984. From frozen stock, the different strains were subcultured twice in BHI broth for bacteria and SAB broth for *C. albicans* ATCC 10231.

### *2.3 Chemicals and Media*

Brain Heart Infusion Broth (BHIB), Muller Hinton Agar (MHA), and Sabouraud Dextrose Broth (SDB) were purchased from HiMedia™ Laboratories Pvt. Ltd., India while Dimethyl sulfoxide (DMSO) was purchased from BDH Laboratories, VWR International Ltd., USA.

### *2.4 Preparation of Plant Extracts*

Plant extracts were prepared following the protocol described in previous studies by Mbarga *et al.* [27]. Briefly, fifty grams of plant material was weighed and introduced into separate conical flasks containing 450 mL of the solvents which were distilled water and ethanol/water (80:20, v/v), The mixture were shaken at 200 rpm for 24 h and 25 °C (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany), filtered (Whatman n°1), and concentrated at 40 °C using a rotary evaporator (IKA RV8, Germany) The semi-solid extracts obtained were kept at 4 °C for analyses.

### *2.5 Screening of Antibacterial Activity of Plant Extracts*

#### 2.5.1 Inoculum Preparation

Strains were cultured for 24 h in their appropriate medium and temperature. Cells were collected by centrifugation (7000 ×g, 4 °C, 10 min), washed twice with sterile saline, resuspended in 5 mL of sterile saline and adjusted to 0.5 McFarland.

#### 2.5.2 Preparation of Antimicrobial Solution

The different dried extracts were dissolved in DMSO 5% (v/v) in order to obtain a stock solution of 521 mg/mL. The solution was sterilized by microfiltration (0.22 $\mu$m).

#### 2.5.3 Realization of the Test

The antimicrobial activity of the extracts was screened using the well diffusion method previously described by Mbarga *et al.* [28]. Briefly, 100 $\mu$L of the inoculum was spread at the surface of sterile MHA (for bacteria) or SDA (for *C. albicans*). Wells of 6 mm diameter were digged in the Petri dishes and filled with 20 $\mu$L (at 100 mg/mL) of each extract. The Petri dishes were incubated at 37 °C for 24 h and the inhibition diameters were measured. All trials were performed in triplicate and sterile DMSO 5% (v/v) was used as negative control.

#### 2.5.4 Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

MIC is the lowest concentration of antibacterial agent that completely inhibits the bacterial growth. The MIC of the different extracts was assessed using the broth microdilution method [29,30]. Briefly, the wells of a U-bottom 96-well microplates were filled with 100 $\mu$L of sterile BHI. 100 $\mu$L of extract (100 mg/mL) was added to the first row (columns 1–10). Then, 100 $\mu$L of 5% DMSO was added into wells of columns 11 and 12. Serial dilutions were performed by transferring 100 $\mu$L from the wells of the first row to the wells of the second row and so forth, resulting in the concentrations presented in Fig. 1 (Ref. [28]). 10 $\mu$L of the inoculum was added in all wells excepted column 11 where 10 $\mu$L of sterile saline free of culture was added. and this served as a positive control. For column 12, 10 $\mu$L of inoculum was added, which served as a negative control. Finally, the plates were covered and incubated at 37 °C for 24 h. After incubation, MIC was considered the lowest concentration of the tested material that inhibited the visible growth of the bacteria. MBCs were determined by subculturing the wells without visible growth (with concentrations $\geq$MIC) on MHA plates. Inoculated agar plates were incubated at 37 °C for 24 h. MBC was considered the lowest concentration that did not yield any bacterial growth on agar.

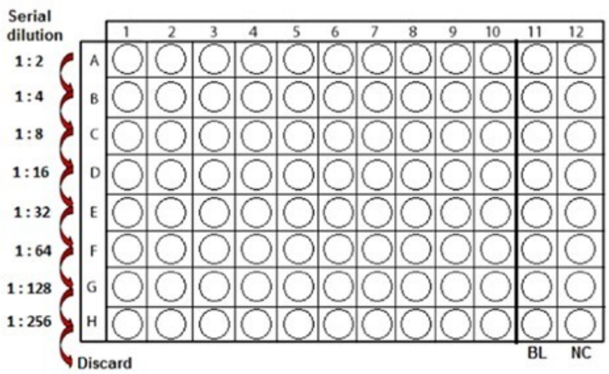



**Fig. 1. Serial dilution process in microbroth dilution method [28].**

#### 2.5.5 Tolerance Level

The tolerance level of the different pathogens to the plant extracts used in this study was performed according to the method described by Mondal *et al.* [31]. The following formula was used:

$$Tolerance = \frac{MBC}{MIC} \quad (1)$$

**Table 1. Interpretation criteria for antibiotic sensitivity [4,32].**

| Antibiotics | Inhibition diameters (mm) | | |
|---|---|---|---|
| | R | I | S |
| Ciprofloxacin, 30 $\mu$g/disk (CIP) | d $\leq$15 | 16–20 | d $\geq$21 |
| Cefazolin, 30 µg/disk (CAZ) | d $\leq$14 | 15–17 | d $\geq$18 |
| Amoxicillin, 30 $\mu$g/disk (AMC) | d $\leq$13 | 14–17 | d $\geq$18 |
| Ceftriaxone, 30 $\mu$g/disk (CTR) | d $\leq$13 | 14–20 | d $\geq$21 |
| Trimethoprim, 30 $\mu$g/disk (TR) | d $\leq$13 | 14–15 | d $\geq$16 |
| Tetracyclin, 30 $\mu$g/disc (TE) | d $\leq$14 | 15–18 | d $\geq$19 |
| Nitrofurantoin, 200 $\mu$g/disk (NIT) | d $\leq$13 | 14–17 | d $\geq$18 |
| Ampicillin, 25 $\mu$g/disk (AMP) | d $\leq$13 | 14–16 | d $\geq$17 |
| Imipenem, 10 $\mu$g/disc (IMP) | d $\leq$13 | 14–15 | d $\geq$16 |
| Cefazolin/clavulanic acid, 30/10 per disk (CAC) | d $\leq$14 | 15–17 | d $\geq$18 |
| Fosfomycin, 200 $\mu$g/disc (FO) | d $\leq$12 | 13–15 | d $\geq$17 |

D, inhibition diameter.

Tolerance value $\geq$16, indicates that the antibacterial efficacy is considered as bacteriostatic whereas tolerance value $\leq$4 indicates a bactericidal activity.

### *2.6 Modulation of Common Antibiotics with Extracts*

#### 2.6.1 Susceptibility of the Strains Used to Antibiotics

The susceptibility of the uropathogenic bacteria to antibiotics was assessed with the modified Kirby–Bauer's disk method exactly as described in previous study by Mbarga *et al*. [29] and the inhibition diameters were interpreted referring to the Clinical & Laboratory Standards Institute [32]. The antibiotics used are presented in Table 1 (Ref. [4,32]). Resistant R, Intermediate I, and Susceptible S interpretations were obtained automatically using algorithms written in Excel software [Microsoft Office 2016 MSO version 16.0.13628.20128 (32 bits), USA] with the parameters described in Table 1 [4].

#### 2.6.2 Modulation in Solid Media by Disc Diffusion Method and Assessment of Increase in Fold Area

The antibiotics which gave inhibition diameters of less than 20 mm after susceptibility test against the uropathogenic bacteria were modulated with 5 mg/mL of each extract. The test was performed as described by Rolta *et al*. [33] with slight modifications. Briefly, in Petri dish, inoculated the test bacteria, a sterile disc paper and an antibiotic disc were placed aseptically. Then, 20 $\mu$L of the considered extract was slowly deposited on each of the two discs. After 24 h of incubation at 37 °C, the inhibition diameters were recorded and interpreted by calculating the increase in fold area (IFA) [34] using the following formula:

$$IFA = \frac{Y^2 - X^2}{X^2} \quad (2)$$

Where, "A" is the increase in fold area, "Y" the inhibition diameter of extract + antibiotic and "X" is the inhibition diameter of antibiotic alone.

#### 2.6.3 Modulation in Liquid Media with Checkboard Method and Determination of the Fractional Inhibitory Concentration (FIC)

The checkerboard method, commonly used for the determination of synergy between the antibiotics and natural antibacterial compounds, was used for the antibiotic modulation assay [28]. Modulations of ampicillin, benzylpenicillin, cefazolin, ciprofloxacin, nitrofurantoin, and kanamycin were performed with extracts whose MIC were successfully determined (Not those with MIC <2 or MIC >256). The fractional inhibitory concentration (FIC) index was calculated, as described in previous studies [28]. Briefly, the individual MICs of the antibiotics (MIC-ATB) and the extract (MIC-extr) against the two targeted strains (*S. aureus* ATCC 6538 and *E. coli* ATCC 25922) were first determined using the microdilution method as described above. Then, the new MIC values (MIC′-ATB and MIC′-extr) were determined after combining the two substances. Combinations of antibiotics + extracts were prepared by mixing the two antimicrobial solutions in 50:50 (v: v) proportions with initial concentrations equivalent to 4MIC against the microorganism tested. To assess the interaction between the antibiotic and the extract, the FIC was determined using the using the formula:

$$FIC = FICA + FICB,$$

$$\text{with: } FICA = \frac{MIC'ATB}{MICATB} \text{ and } FICB = \frac{MIC\text{ extr}}{MIC\text{ extr}}.$$

The FIC index was interpreted as follows FIC $\leq$0.5, synergy; 0.5 $\leq$ FIC $\leq$ 1, addition of effects; 1 $\leq$ FIC $\leq$ 4, indifference and for FIC >4, Antagonism.

### *2.7 HPLC-MS/MS Analysis of the Most Active Plants*

2.7.1 Sample Preparation

1.0 mg of the dried ethanolic extract was placed in an Eppendorf, 1.0 mL of a mixture of methanol:water (70:30) was added, and extraction was carried out in an ultrasonic bath for 30 minutes. The complete dissolution of a sample was noted, and it was transferred to a chromatographic vial for analysis.

2.7.2 Analysis Conditions

Extract was analyzed by 6030 series HPLC-MS/MS (Agilent, USA). HPLC (Agilent 1290), consisting of a binary pump, an autosampler, and a thermostatted column compartment, was performed with a Shim-pack FC-ODS C18 column (150 $\times$ 2.0 mm $\times$ 3.0 $\mu$m). The flow rate was 0.25 mL/min. The sample cooler and the column temperature were set at 5 °C and 30 °C, respectively. Injection volume was 10 $\mu$L. Gradient elution was performed with 0.1% (v/v) formic acid (A) and acetonitrile (B). The gradient of mobile phase B was used: 5% (5 min)-30% (30 min)-70% (40 min)-90% (45 min)-5% (47 min)-5% (50 min). Mass spectrometric detection was achieved with an ESI source operating in positive mode using nitrogen as the nebulizer gas. Mass Hunter software was used to operate the mass spectrometer (Agilent, USA). The parameters of the mass spectrometer were set as follows: nebulizer gas flow, 3 L/min; drying gas, 10 L/min; drying gas temperature, 320 °C; fragmentor voltage, 135 V; capillary voltage, 4000 V; collision induced dissociation pressure, 230 kPa. Identification of extract components was performed by MS, MS/MS data and comparing with the previously reported results in the literature. Quantification was accomplished by area normalization method (the ionization coefficients of the compounds were taken equal to 1).

### *2.8 Mineral Composition of the Most Active Plants*

An EDX-7000 Shimadzu energy dispersive X-ray fluorescence (XRF) spectrometer was used to identify the minerals present in the *E. chlorantha* extract . The equipment settings were as followed: range of measured elements — 11Na - 92U; X-ray generator — a tube with a Rh-anode, air cooled; voltage 4–50 kV, current 1–1000 $\mu$A; irradiated area — a circle of 10 mm in diameter; silicon drift detector (SDD), counting method — a digital counting filter; the content of elements according to the value of intensity; automatic change of filters emitting the wavelengths of the corresponding elements; chamber size 300 mm $\times$ 275 mm $\times$ 100 mm. The X-ray fluorescence spectrum for each measurement was recorded at the same device settings: mylar film, collimator width - 10 mm, exposure time - 100 sec, atmosphere - air; the number of repeated measurements for one sample n = 3. To process the obtained results, we used the OriginPro 2017 software (OriginLab, Northampton, MA, USA). The results obtained using the XRF method were presented in values of irradiation intensity expressed in cps/$\mu$A.

### *2.9 Statistical Analysis*

All the experiments were carried out at least in triplicate. The statistical significance was set at $p \leq 0.05$. *T*-test was carried out using the statistical software XLSTAT 2020 (Addinsof Inc., New York, USA) and the graphs were plotted by SigmaPlot 12.5 (Systat Software, San Jose, CA, USA).

## 3. Results and Discussion

### *3.1 Inhibition Zone of Extract against Tested Bacteria*

Fig. 2 presents the inhibition diameters of the different extracts on the tested pathogens. All extracts were not active against the pathogens. It is the case of the extracts (both ethanolic and aqueous extracts) from *C. officinalis* and *G. lucida* leaves. The rest of extracts were actives with inhibition diameters ranging from 5 to 36 mm.

Taking into consideration the extraction solvents, the highest inhibition diameters were mainly recorded with ethanol as solvent. Ethanol therefore appears as the solvent which extracted more antimicrobial compounds compared to water although the extraction yields were globally more important with water as solvent [27]. This observation could be ascribed to the insoluble nature of metabolites extracted with ethanol as solvent opposite to water. Indeed, most of bioactive compounds endowed with antimicrobial activity such as flavonoids, polyphenols, tannins and alkaloids are generally insoluble in water [35–37]. In a study conducted by Mouafo *et al.* [35], it was highlighted that ethanol extracted more antimicrobial compounds from plant materials opposite to water. A similar conclusion was also stated by Evbuomwan *et al*. [38].

With regards to the part of the plant material, extracts from bark were more actives independently of the pathogens and the extraction solvents. The highest activities on both bacterial and yeast strains were noticed with bark from *E. chloranta*. This could be attributed to the presence of high amount of antimicrobial alkaloids such as protoberberines (berberine, canadine, palmatine, jatrorrhizine, columbamine and pseudocolumbamine), phenanthrene alkaloids (atherosperminine and argentinine) and aporphines (7-hydroxydehydronuciferine and 7-hydroxydehydronornuciferine) in that plant as reported in the literature [26]. Several studies also highlighted the interesting antibacterial and antifungal properties of dried and fresh barks from *E. chlorantha* [10,39,40]. The extracts from *E. chloranta* therefore appears as a source of antimicrobials with a broad-spectrum activity. Similarly, ethanolic extracts of *L. vulgare* and *V. amygdalina* showed a broad-spectrum antimicrobial activity against all the tested pathogens. This could be ascribed to the richness of these extracts in phytochemicals such as saponins, sesquiterpenes, flavonoids, steroid glycosides, and lactones [20] for which recent studies reported its antibacterial activity [21].

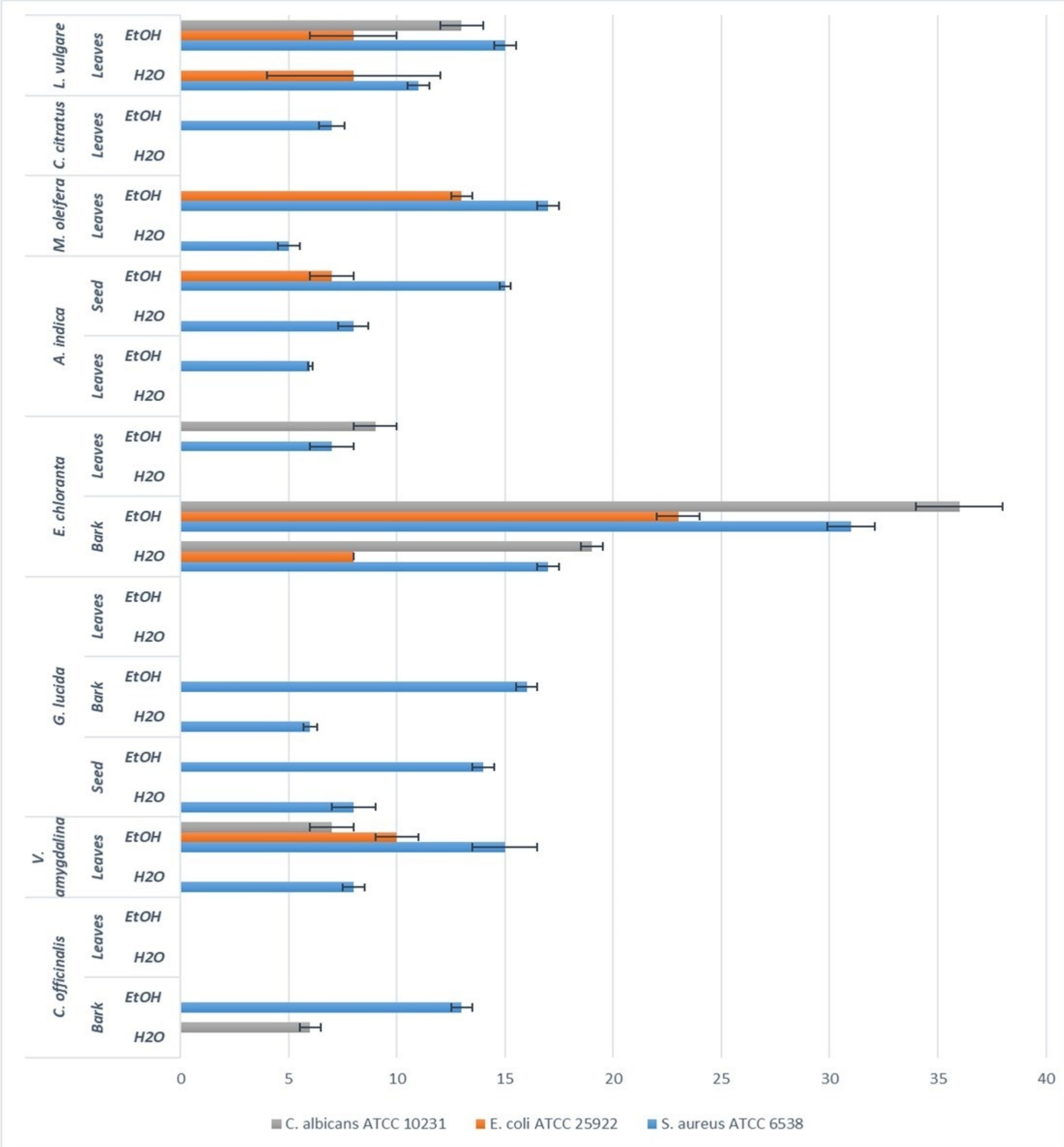



**Fig. 2. Inhibition diameter (mm) resulting from the screening of antimicrobial activity by well diffusion method with 100 mg/mL of each extract.**

Amongst leaves extracts, those derived from *M. oleifera* were more actives against the bacterial strains *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 while extract from *L. vulgare* leaves was more active against *C. albicans* ATCC 10231. This result could be explained by the great variability of the phytochemical composition of the two vegetal materials and thus, the possible different action mechanisms against microorganisms.

When the tested pathogens are considered, it appears from Fig. 2 that *S. aureus* ATCC 6538 was more sensitive than *E. coli* ATCC 25922. This observation can be explained by the nature and composition of both cell wall and membranes which differ between Gram positive and Gram-negative bacteria. In fact, Gram-negative bacteria possess a lipopolysaccharides layer in their external membrane. This layer act as a barrier against the permeability antimicrobials [41].

A surprising observation was noticed in this study as the yeast *C. albicans* ATCC 10231 was more sensitive to the ethanolic and aqueous extracts of both bark and leaves extracts from *E. chlorantha* compared to the bacterial strains *S. aureus* ATCC 6538 and *E. coli* ATCC 25922. This result might arise from the antimicrobial action mechanisms of the bioactive compounds found in that plant. In fact, eukaryotic cells are known for their ability to resist to several antimicrobials as opposite to prokaryotic cells, they possess less phospholipids in their membranes. Phospholipids due to their anionic nature are mostly involved in the preliminary interaction with antimicrobial which will ease their penetration into cells [42,43].

**Table 2. Minimal inhibitory concentration (MIC, mg/mL), Minimum bactericidal concentration (MBC, mg/mL) and Ratio MBC/MIC of the different plant extracts against the three references pathogens.**

| Plants | Part used | Solvents | *S. aureus* ATCC 6538 | | | *E. coli* ATCC 25922 | | | *C. albicans* ATCC 10231 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC |
| *Cynchona officinalis* | Bark | $H_2O$ | 256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 64 | 256 | 4 | 256 | >256 | - | 256 | >256 | - |
| | Leaves | $H_2O$ | >256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| *Vernonia amygdalina* | Leaves | $H_2O$ | 128 | 256 | 2 | 256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 32 | 128 | 4 | 64 | 256 | 4 | 256 | >256 | - |
| *Garcinia lucida* | Seed | $H_2O$ | 64 | 256 | 4 | 128 | 256 | 2 | 256 | >256 | - |
| | | EtOH 80% | 8 | 64 | 8 | 8 | 128 | 16 | 256 | 256 | 1 |
| | Bark | $H_2O$ | 128 | 256 | 2 | 256 | 256 | 1 | >256 | >256 | - |
| | | EtOH 80% | 32 | 128 | 4 | 128 | 256 | 2 | 256 | >256 | - |
| | Leaves | $H_2O$ | >256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | >256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| *Enantia chlorantha* | Bark | $H_2O$ | 8 | 32 | 4 | 8 | 64 | 8 | 4 | 16 | 4 |
| | | EtOH 80% | <2 | 4 | - | <2 | 8 | - | <2 | 4 | - |
| | Leaves | $H_2O$ | 128 | 256 | 2 | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 32 | 256 | 8 | >256 | >256 | - | >256 | >256 | - |
| *Azadirachta indica* | Leaves | $H_2O$ | >256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 128 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | Seed | $H_2O$ | 64 | 128 | 2 | 256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 8 | 32 | 4 | 64 | >256 | - | 256 | >256 | - |
| *Moringa oleifera* | Leaves | $H_2O$ | 256 | >256 | - | 128 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 16 | 64 | 4 | 32 | 256 | 8 | >256 | >256 | - |
| *Cymbopogon citratus* | Leaves | $H_2O$ | 256 | >256 | - | >256 | >256 | - | >256 | >256 | - |
| | | EtOH 80% | 128 | >256 | - | 256 | >256 | - | >256 | >256 | - |
| *Leucanthemum vulgare* | Leaves | $H_2O$ | 32 | 128 | 4 | 64 | 256 | 4 | 32 | 256 | 8 |
| | | EtOH 80% | 8 | 8 | 1 | 16 | 32 | 2 | 8 | 16 | 2 |

### *3.2 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)*

All the extracts inhibited the pathogens with MIC values which varied significantly from one plant material to another. As previously observed with qualitative tests, the extract of *E. chloranta* was the most active independently of the solvents and the tested pathogens. As shown in Table 2, the ethanolic extract of *E. chloranta* with MIC value lower than 2 mg/mL against the three pathogens was the most active extract. This important activity could result from the presence of alkaloids such as palmatine, coloumbamine and jatrorrhizine in its composition [44]. Indeed, these compounds have the ability to penetrate cells and intercalate DNA of microorganism leading to their death [45]. MIC values higher than 256 mg/mL were observed with some aqueous and ethanolic extracts against the different pathogens. This observation suggests that further analysis at concentrations higher than 256 should be performed in order to quantify the antimicrobial activity of these extracts. Moreover, extracts which have showed no activity in well diffusion qualitative test were active in liquid medium against the pathogens. Thus, suggesting that these extracts might contain antimicrobial compounds which cannot diffuse in the Muller Hinton agar.

With water as solvent, the most important activity was recorded with extract from *E. chloranta* for which MIC of 8 mg/mL was noticed against *S. aureus* ATCC 6538 and *E. coli* ATCC 25922, and 4 mg/mL against *C. albicans* ATCC 10231. These activities were higher compared to those reported by Adesokan *et al*. [46]. They obtained with aqueous extract of *E. chloranta*, MIC values of 25 and 100 mg/mL against *S. aureus* and *E. coli*, respectively. This can be ascribed to several factors which influence the plant composition such as climate and soil composition, as well as to the tested strains.

The majority of extracts showed MIC values higher or equal to 256 mg/mL against the yeast strain *C. albicans* ATCC 10231 independently of the extraction solvent. Only ethanolic (MIC = 8 mg/mL) and aqueous (MIC = 32 mg/mL) extracts of *L. vulgare* leaves, and the ethanolic (MIC <2 mg/mL) and aqueous (MIC = 4 mg/mL) extracts of *E. chloranta* bark. This observation suggests that *C. albicans* ATCC 10231 was the most resistant strain to the different extracts independently of the solvent. This resistance could be ascribed to their membrane composition which is different to those of bacteria. In fact, the higher amount of anionic phospholipids in the membrane of bacteria ease their interaction with antimicrobial compounds and thus increase their sensitivity [42,43].

An observation of Table 2 revealed that *S. aureus* ATCC 6538 was the most sensitive strain as lower MIC values of most extracts were generally recorded against that strain. Thus, it clearly appears that the bacterial cell walls and membranes are involved in the antimicrobial activity mechanism of these extracts. This conclusion is different to that stated by Etame *et al*. [10] who found no significant difference in the MIC values of plant extracts against Gram positive and Gram-negative bacteria.

According to the classification established by Kuete [47] and Kuete and Efferth [48], the different extracts could be considered as deserving a weak antimicrobial activity independently of the extraction solvent and the tested strain as they scored MIC value higher than 0.625 mg/mL.

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MIC) of the different extracts against three pathogenic strains were assessed and results are presented in Table 2. The MBC values ranged from 4 to more than 256 mg/mL. The strongest MBC value (4 mg/mL) against *C. albicans* ATCC 10231 and *S. aureus* ATCC 6538 was obtained with the ethanolic extract of *E. chloranta* bark. Against *E. coli* ATCC 25922, the strongest MBC value of 8 mg/mL was recorded with the same ethanolic extract of *E. chloranta* bark. Globally, lowest MBC values were recorded against *S. aureus* ATCC 6538, thus confirming its higher sensitivity to the different plant extracts.

The MBC values against *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 obtained in this study with the aqueous extracts of *E. chloranta* were lower than that reported by Adesokan *et al*. [46]. The authors found with aqueous extract of *E. chloranta*, MBC values of 90 and 130 mg/mL against *S. aureus* and *E. coli*, respectively. In the same way, the MBC values of the ethanolic extract of *V. amygdalina* leaves against *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 were lower than the 100 and 200 mg/mL obtained respectively against *E. coli* and *S. aureus* by Evbuomwan *et al*. [38] using the ethanolic extract from the same plant. This difference could be explained by the variability of phytochemical profile of the plant according to the geographical origin. Besides, the fact that microbial strain developed different resistance mechanism to antimicrobials as highlighted by several authors in the literature [49,50] could also explained the variability in the MBC values.

It is established in the literature that an antimicrobial compound is considered as bactericidal/fungicidal against a microbial strain when the ratio MBC/MIC or MIC/MIC is $\leq$4 [51,52]. Based on this classification, the ethanolic extract of *C. officinalis* bark and *M. oleifera* leaves, the aqueous extract of *G. lucida* seeds, *E. chloranta* leaves and bark, the aqueous and ethanolic extracts of *V. amygdalina* leaves, *G. lucida* bark, *A. indica* seeds, and *L. vulgare* leaves can be considered as bactericidal against *S. aureus* ATCC 6538. The ethanolic extract of *V. amygdalina* leaves, the aqueous extract of *G. lucida* seeds, the ethanolic and aqueous extracts of *G. lucida* bark and *L. vulgare* leaves can be considered as bactericidal against *E. coli* ATCC 25922. The ethanolic extract of *G. lucida* seeds and *L. vulgare* leaves, and the aqueous extracts of *E. chloranta* bark can be considered as fungicidal against *C. albicans* ATCC 10231.

### *3.3 Synergestic Effect between Common Antibiotics and Plant Extracts Using Checkboard Method*

The use of combination therapy has been suggested as a new approach to improve the efficacy of antimicrobial agents by screening crude extracts from medicinal plants with good indications for use in combination with antibiotics [53]. The checkboard method was applied to assess the synergy between some conventional antibiotics and plant extracts which showed a valid MIC. After determining the MIC of the plant materials (Table 2), we determined the MICs and MBCs of the antibiotics (ampicillin, benzylpenicillin, cefazolin, ciprofloxacin, nitrofurantoin, and kanamycin) against *S. aureus* ATCC 6538 and *E. coli* ATCC 25922. As shown in Table 3, the MICs of the different antibiotics varied from 4–64 $\mu$g/mL while the MBCs varied from 4–256 $\mu$g/mL.

**Table 3. MIC and MBC of antibiotics used for modulation assay in liquid media.**

| Pathogens | MIC (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | CIP | NIT | BP | AMP | Ka | CZ |
| *E. coli* ATCC 25922 | 4 | 32 | 16 | 32 | 64 | 16 |
| *S. aureus* ATCC 6538 | 4 | 16 | ND | 4 | 32 | ND |
| | MBC (µg/mL) | | | | | |
| *E. coli* ATCC 25922 | 16 | 256 | 64 | 64 | 256 | 64 |
| *S. aureus* ATCC 6538 | 8 | 128 | ND | 4 | 32 | ND |

AMP, ampicillin; BP, benzylpenicillin; CZ, cefazolin; CIP, ciprofloxacin; NIT, nitrofurantoin; and Ka, kanamycin; MIC, minimum inhibitory concentrations; MBC, minimum bactericidal concentration; ND, not determined.

Antibiotics with high and quantified MICs values against the both pathogens (ampicillin, nitrofurantoin, and kanamycin) were selected for modulation assays. Similarly, regarding the plant extracts, we decided to work with extracts with low and determined MICs. Thus, it was performed a modulation of ampicillin, nitrofurantoin, and kanamycin with aqueous extract of *E. chlorantha* bark and ethanolic extract of *G. lucida* seed, *A. indica* seed and *L. vulgare* leaves. As shown in Table 4, the fractional inhibitory concentration (FIC) ranged from 0.125 to 0.750. No antagonism (FIC >4) or indifference ($1 \leq$ FIC $\leq 4$) was noted between the extracts and the antibiotics. However, it was found an additional effect ($0.5 \leq$ FIC $\leq 1$) in

**Table 4. Fractional inhibitory concentrations (FIC) of the combinations of extracts and antibiotics against *S. aureus* ATCC 6538 and *E. coli* ATCC 25922.**

| Plants | ATB | *S. aureus* ATCC 6538 | | | | | *E. coli* ATCC 25922 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC extr (mg/mL) | MIC ATB (µg/mL) | MIC' extr (mg/mL) | MIC' ATB (µg/mL) | FIC | MIC extr (mg/mL) | MIC ATB (µg/mL) | MIC' extr (mg/mL) | MIC' ATB (µg/mL) | FIC |
| | AMP | | 4 | 0.5 | 1 | 0.313 | | 32 | 0.5 | 2 | 0.125 |
| *E. Chlorantha* bark $H_2O$ | Ka | 8 | 32 | 0.5 | 2 | 0.125 | 8 | 64 | 1 | 8 | 0.250 |
| | NIT | | 16 | 1 | 2 | 0.250 | | 32 | 0.5 | 4 | 0.188 |
| | AMP | | 4 | 2 | 1 | 0.500 | | 32 | 2 | 4 | 0.375 |
| *G. lucida* seed EtOH | Ka | 8 | 32 | 4 | 4 | 0.625 | 8 | 64 | 4 | 16 | 0.750 |
| | NIT | | 16 | 2 | 4 | 0.500 | | 32 | 2 | 8 | 0.500 |
| | AMP | | 4 | 1 | 1 | 0.375 | | 32 | 4 | 8 | 0.313 |
| *A. Indica* seed EtOH | Ka | 8 | 32 | 4 | 4 | 0.625 | 64 | 64 | 8 | 16 | 0.375 |
| | NIT | | 16 | 1 | 2 | 0.250 | | 32 | 4 | 2 | 0.125 |
| | AMP | | 4 | 2 | 1 | 0.500 | | 32 | 4 | 4 | 0.375 |
| *L. vulgare* leaves EtOH | Ka | 8 | 32 | 2 | 2 | 0.313 | 16 | 64 | 8 | 16 | 0.750 |
| | NIT | | 16 | 1 | 2 | 0.250 | | 32 | 2 | 4 | 0.250 |

ATB, antibiotics; FIC, fractional inhibitory concentration; MIC, minimum inhibitory concentrations; MBC, minimum bactericidal concentration; AMP, ampicillin; BP, benzylpenicillin; CZ, cefazolin; CIP, ciprofloxacin; NIT, nitrofurantoin; and Ka, kanamycin. FIC $\leq$0.5, synergy; $0.5 \leq$ FIC $\leq 1$, additive effects; $1 \leq$ FIC $\leq 4$, indifference and FIC >4, Antagonism.

some plant + antibiotic combinations such as Kanamycin + (*G. lucida* seed or *A. Indica* seed) which had a FIC index of 0.625 against *S. aureus*. Regarding *E. coli*, an additional effect (FIC index = 0.750) in the combinations Kanamycin + *G. lucida* seed and Kanamycin + *L. vulgare* leaves, was also found. Except for the 4 cases of combinations above mentioned, all the other plant + antibiotic combinations exhibited synergistic effect (FIC $\leq$0.5) against the two test microorganisms. The lower is the FIC index, better is the synergy [53]. The best synergies were therefore found with *E. chloranta* bark which well-modulated Kanamycin (FIC = 0.125 against *S. aureus* and 0.250 against *E. coli*), nitrofurantoin (FIC = 0.250 against *S. aureus* and 0.188 against *E. coli*) and ampicillin (FIC = 0.125 against *E. coli*). A good synergy between *A. indica* seed and nitrofurantoin (FIC = 0.125) was also noticed. The results gathered from this study prove that common antibiotics such as ampicillin, nitrofurantoin and kanamycin could be successfully combined with plant extracts and demonstrate better antimicrobial activity materialized here by good FIC and reduction of MIC (more than 50% in all combinations). It has been reported that some plant-derived compounds can enhance the *in vitro* activity of some antibiotics by directly attacking the same site as the antibiotic or multiple sites at once [54]. For the 3 antibiotics used for modulation in liquid medium, it is well known that kanamycin inhibits protein synthesis by tightly binding to the conserved A site of 16S rRNA in the 30S ribosomal subunit [55]; Ampicillin acts as an irreversible inhibitor of transpeptidase, an enzyme essential the cell wall synthesis [56], and nitrofurantoin is a broad-spectrum antibacterial agent, active against the majority pathogens [57]. Thus, the protoberberins and phenanthrene alkaloids which have been reported as being the major constituents of *E. chloranta* bark [26] and which inhibit protein synthesis [58] may have had a cumulative effect when combined with kanamycin or an inhibitory effect on two targets (Protein synthesis mechanism and cell wall synthesis) at the same time when combined with ampicillin, which therefore explains the good modulation between *E. chlorantha* bark and kanamycin or ampicillin. Similarly, the broad-spectrum antibacterial properties of Azadirachtin which is one of the major constituents of *A. indica* [59,60] may also explain the 16-fold reduction of the MIC of nitrofurantoin (from 32 to 2 $\mu$g/mL against *E. coli*) when associated with *A. indica* seed. Finally, although the combinatory assays gave positive results against both Gram + and Gram - models, further studies are needed to assess the bonds formed between the extracts and the antibiotics tested and their implication in the mechanism of action. Similarly, further preclinical and clinical trials are required to evaluate the cytotoxicity and safety issues of these combinations before they can be recommended as antimicrobials drug in the fight against antibiotic resistance issue.

### *3.4 Susceptibility to Antibiotics of the Test Uropathogenic Bacteria and Modulation of Common Antibiotic with Extracts in Solid Media*

The solid-medium modulation test using commercial antibiotic discs is a less complex means for synergy testing. In this study, after having observed in liquid medium (checkboard method) that most of the extracts had a synergistic effect with antibiotics on non-resistant bacteria, we undertook to perform modulation tests in solid medium to assess the extent to which extracts from the tested plants could potentially enhance the performance of conventional antibiotics against a wide range of resistant bacteria. So, we started by determining the effectiveness of antibiotic discs alone. The sensitivity of the eleven uropathogenic bacteria used in this study to eleven (11) antibiotics was determined (Table 5) and the multidrug resistance (MDR) index of each bacterium was calculated. No bacteria were resistant to imipenem and amoxiclav. Regarding the other antibiotics, we found resistance in 10/11 bacteria to ampicillin, 8/11 to trimethoprim and tetracyclin, 6/11 to cefazolin + clavulanic acid, 5/11 to ceftazidime, 4/11 to nitrofurantoin and 1/11 to ceftriaxone and ciprofloxacin. The highest MDR index (0.54) was found in *E. coli* 1449 which was resistant to ampicillin, ceftazidime, cefazoline + clavulanic acid, ceftriaxone, tetracycline and trimethoprim. *St. agalactiae* 3984 and *K. rizophilia* 1542 scored the same MDR index of 0.45. *K. rizophilia* 3984 was resistant to Ampicillin, Ceftazidime, Cefazoline + clavulanic, Nitrofurantoin and tetracycline while *St. agalactiae* 3984 was resistant to trimethoprim, ampicillin, ceftazidime, cefazoline + clavulanic and tetracycline The lowest MDR index (0.27) was recorded with *C. freundii* 426 and *S. aureus* 1449. This result is consistent with those obtained by other authors on the resistance of clinical strains to antibiotics [61–63].

Various means have been implemented in recent years to provide effective solutions to the antibioresistance issue, and the studies carried out target bacteria that are resistant or not. Here we focused on evaluating the modulatory effect of ethanolic extracts of plant materials on antibiotics which presented an inhibition diameter lower than 20 mm (Table 5) against uropathogenic bacteria. Tables 6,7,8,9,10 present, respectively, in the form of increase in fold area (IFA), the modulating effect of plant extracts with ampicillin (AMP) (Table 6), ceftazidime (CAZ) (Table 7), tetracycline (TE) (Table 8), nitrofurantoin (NIT) (Table 9) and trimethoprim (TR) (Table 10). As with synergy tests using the checkboard method on non-resistant bacteria (Table 5), we found that *E. chlorantha* bark (ECB) (indenpendently of the solvent used) had the best modulating properties on most of the antibiotics tested. Its IFA was significantly different ($p < 0.05$) to that obtained with the other extracts. The ECB-AMP combination induced an increase in inhibition diameters of more than 35% in all bacteria tested and made *P. aeruginosa* 3057, *K. rizophilia* 1542 and *M. catarrhalis* 4222 more susceptible to AMP with respective AFIs of 8.00, 3.84 and 3.00. The ECB-CAZ combination has also demonstrated an interesting increase of the susceptibility to CAZ and we found IFAs of 3.00; 3.00, 4.90 and 6.11 respectively against *K. rizophilia* 1542, *St. agalactiae* 3984, *E. coli* 1449, and *S. aureus* 1449 (Fig. 3A). Similarly, *E. avium* 1669, *K. oxytoca* 3003 (Fig. 3B), *M. morganii* 1543 and *St. agalactiae* 3984 which were resistant to tetracycline became susceptible to that antibiotic after combination with ECB.

The ECB-TR and ECB-NIT combinations also showed a strong increase in activity especially against *Ac. Xylosoxidans* 4892 (both) *K. rizophilia* 1542 (only ECB-NIT) *P. aeruginosa* 3057 (both), and *K. oxytoca* 3003 (only

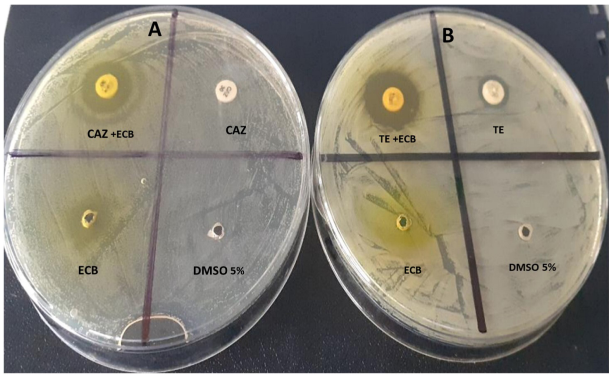



**Fig. 3. Modulation of ceftazidime (CAZ) and tetracycline (TE) with *E. Chlorantha* bark (ECB) respectively against *S. aureus* 1449 (A) and *K. oxytoca* 3003 (B).**

ECB-TR). Otherwise, *L. vulgare* leaves (LVL) and *G. lucida* seed (GLS) have also demonstrated some promising results when combined with certain antibiotics. For example, the combinations LVL-AMP, LVL-CAZ, LVL-CAZ, LVL-TE, LVL-TE, LVL-TE, LVL-TE, LVL-TR, LVL-TR and LVL-TR were respectively very active *against P. aeruginosa* 3057, *E. coli* 1449, *P. aeruginosa* 3057, *E. avium* 1669, *K. oxytoca* 3003, *M. morganii* 1543, *St. agalactiae 3984*, *E. avium* 1669, *P. aeruginosa* 3057 and *St. agalactiae* 3984. Interestingly, *C. citratus* well-modulated trimethoprim while with other antibiotics the activity was lower compared to ECB, LVL and GLS.

Generally, the activity of antibiotics to which bacteria were resistant was interestingly increased with *E. chlorantha* bark, *L. vulgare* leaves and *G. lucida* seed while the action of ATB-extract combinations was moderate with *G. lucida* bark and weak with the other extracts. Besides mechanisms previously mentioned for checkboard method, another explanation of the modulation effect observed in this study in solid media could be the fact that some plant-derived compounds enhance the activity of antimicrobial compounds by inhibiting MDR efflux systems in bacteria [54]. Bacterial efflux pumps are responsible for a significant level of resistance to antibiotics in pathogenic bacteria [5]. Indeed, efflux pumps allow bacteria to flush antibiotics out of bacterial cells and therefore reduce their sensitivity to conventional antibiotics [5]. It is likely that ethanolic extracts from our plants may contain potential efflux pump inhibitors which are likely to be broad considering that the synergistic effect of the extract was observed on both Gram-positive and Gram-negative organisms. Several studies reported the isolation of some broad-spectrum efflux pump inhibitors from plant materials [5,54,62,64]. A good example of this is the work of Smith *et al*. [64] who reported one efflux inhibitor (ferruginol) from the cones of *Chamaecyparis lawso-niana*, which inhibited the activity of the quinolone resistance pump (NorA), the tetracycline resistance pump (TetK) and the erythromycin resistance pump (MsrA ) in *Staphylococcus aureus*.

**Table 5. Inhibition diameters (mm) of antibiotics against uropathogenic bacteria.**

| Pathogens | NIT | TE | CTR | AMC | FO | CAZ | IPM | CAC | CIP | AMP | TR | MDR Index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ac. Xylosoxidans* 4892 | 6 ± 0 (R) | 11 ± 0 (R) | 23 ± 2 (S) | 36 ± 4 (S) | 6 ± 0 (R) | 16 ± 0 (I) | 32 ± 3 (S) | 16 ± 1 (I) | 20 ± 2 (I) | 20 ± 1 (S) | 6 ± 0 (R) | 0.36 |
| *C. freundii* 426 | 21 ± 1 (S) | 30 ± 3 (S) | 27 ± 2 (S) | 35 ± 1 (S) | 40 ± 2 (S) | 12 ± 0 (R) | 37 ± 1 (S) | 10 ± 0 (R) | 30 ± 2 (S) | 6 ± 0 (R) | 22 ± 2 (S) | 0.27 |
| *E. avium* 1669 | 21 ± 1 (S) | 6 ± 0 (R) | 30 ± 4 (S) | 25 ± 3 (S) | 31 ± 3 (S) | 23 ± 1 (S) | 27 ± 4 (S) | 24 ± 2 (S) | 15 ± 0 (R) | 6 ± 0 (R) | 6 ± 0 (R) | 0.36 |
| *E. coli* 1449 | 24 ± 3 (S) | 11 ± 0 (R) | 8 ± 0 (R) | 27 ± 1 (S) | 30 ± 1 (S) | 7 ± 0 (R) | 22 ± 2 (S) | 12 ± 0 (R) | 26 ± 1 (S) | 6 ± 0 (R) | 6 ± 0 (R) | 0.54 |
| *K. oxytoca* 3003 | 20 ± 1 (S) | 8 ± 0 (R) | 22 ± 0 (S) | 24 ± 1 (S) | 25 ± 3 (S) | 15 ± 1 (I) | 27 ± 3 (S) | 6 ± 0 (R) | 30 ± 2 (S) | 6 ± 0 (R) | 6 ± 0 (R) | 0.36 |
| *K. rizophilia* 1542 | 10 ± 0 (R) | 13 ± 0 (R) | 22 ± 0 (S) | 22 ± 1 (S) | 28 ± 2 (S) | 10 ± 0 (R) | 23 ± 1 (S) | 6 ± 0 (R) | 30 ± 1 (S) | 13 ± 1 (R) | 21 ± 2 (S) | 0.45 |
| *M. catarrhalis* 4222 | 12 ± 1 (R) | 22 ± 2 (S) | 24 ± 1 (S) | 36 ± 3 (S) | 27 ± 2 (S) | 16 ± 0 (I) | 27 ± 1 (S) | 21 ± 0 (S) | 32 ± 4 (S) | 10 ± 0 (R) | 15 ± 1 (I) | 0.18 |
| *M. morganii* 1543 | 15 ± 0 (I) | 6 ± 0 (R) | 33 ± 2 (S) | 17 ± 1 (I) | 13 ± 0 (R) | 23 ± 1 (S) | 22 ± 0 (S) | 23 ± 1 (S) | 22 ± 2 (S) | 10 ± 0 (R) | 6 ± 0 (R) | 0.36 |
| *P. aeruginosa* 3057 | 6 ± 0 (R) | 13 ± 1 (R) | 21 ± 1 (S) | 16 ± 0 (I) | 27 ± 1 (S) | 15 ± 0 (I) | 34 ± 4 (S) | 22 ± 2 (S) | 30 ± 1 (S) | 6 ± 0 (R) | 6 ± 0 (R) | 0.36 |
| *S. aureus* 1449 | 16 ± 1 (I) | 25 ± 3 (S) | 18 ± 2 (I) | 27 ± 3 (S) | 27 ± 2 (S) | 6 ± 0 (R) | 25 ± 1 (S) | 6 ± 0 (R) | 26 ± 3 (S) | 6 ± 0 (R) | 6 ± 0 (R) | 0.27 |
| *St. agalactiae* 3984 | 18 ± 2 (S) | 6 ± 0 (R) | 27 ± 1 (S) | 27 ± 2 (S) | 27 ± 2 (S) | 10 ± 0 (R) | 30 ± 2 (S) | 12 ± 1 (R) | 18 ± 1 (I) | 6 ± 0 (R) | 6 ± 0 (R) | 0.45 |

AMC, Amoxycillin; AMP, Ampicillin; CZ, Cefazolin; CAC, Cefazolin/clavulanic acid; CAZ, Ceftazidime; CTR, Ceftriaxone; CIP, Ciprofloxacin; FO, Fosfomycin; IMP, Imipenem; NIT, Nitrofurantoin; TE, Tetracyclin; TR, Trimethoprim; MDR, multidrug resistance.

**Table 6. Increase in the fold area in the modulation of ampicillin with ethanolic extract of the different plant materials.**

| Pathogens | *C. officinalis* | | *V. amygdalina* | *G. lucida* | | | *E. chlorantha* | | *A. indica* | | *M. oleifera* | *C. citratus* | *L. vulgare* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bark | Leaves | Leaves | Seed | Bark | Leaves | Bark | Leaves | Leaves | Seed | Leaves | Leaves | Leaves |
| *C. freundii* 426 | 0.31 | 0.10 | 0.00 | 0.53 | 0.36 | 0.10 | 1.47 | 0.14 | 0.34 | 0.42 | 0.15 | 0.05 | 0.42 |
| *E. avium* 1669 | 0.31 | 0.10 | 0.10 | 0.53 | 0.36 | 0.00 | 1.47 | 0.14 | 0.31 | 0.42 | 0.15 | 0.05 | 0.65 |
| *E. coli* 1449 | 0.27 | 0.09 | 0.27 | 0.46 | 0.31 | 0.09 | 1.25 | 0.12 | 0.31 | 0.36 | 0.13 | 0.04 | 0.57 |
| *K. oxytoca* 3003 | 0.32 | 0.10 | 0.00 | 0.56 | 0.27 | 0.00 | 1.56 | 0.14 | 0.11 | 0.44 | 0.16 | 0.05 | 0.70 |
| *K. rizophilia* 1542 | 0.72 | 0.21 | 0.96 | 1.25 | 0.56 | 0.21 | 3.84 | 0.30 | 0.42 | 0.96 | 0.32 | 0.10 | 1.59 |
| *M. catarrhalis* 4222 | 0.58 | 0.17 | 0.36 | 1.01 | 0.46 | 0.17 | 3.00 | 0.25 | 0.46 | 0.78 | 0.27 | 0.09 | 1.28 |
| *M. morganii* 1543 | 0.46 | 0.14 | 0.00 | 0.78 | 0.36 | 0.07 | 2.24 | 0.20 | 0.36 | 0.60 | 0.21 | 0.07 | 0.98 |
| *P. aeruginosa* 3057 | 1.30 | 0.36 | 1.25 | 2.36 | 1.15 | 0.17 | 8.00 | 0.52 | 1.01 | 1.78 | 0.56 | 0.17 | 3.07 |
| *S. aureus* 1449 | 0.27 | 0.13 | 0.00 | 0.72 | 0.38 | 0.06 | 2.06 | 0.18 | 0.21 | 0.56 | 0.20 | 0.06 | 0.91 |
| *St. agalactiae* 3984 | 0.23 | 0.11 | 0.49 | 0.63 | 0.34 | 0.06 | 1.78 | 0.16 | 0.10 | 0.49 | 0.17 | 0.06 | 0.79 |

**Table 7. Increase in the fold area in the modulation of Ceftazidime with ethanolic extract of plant materials.**

| Pathogens | *C. officinalis* | | *V. amygdalina* | *G. lucida* | | | *E. Chlorantha* | | *A. indica* | | *M. oleifera* | *C. citratus* | *L. vulgare* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bark | Leaves | Leaves | Seed | Bark | Leaves | Bark | Leaves | Leaves | Seed | Leaves | Leaves | Leaves |
| *Ac. Xylosoxidans* 4892 | 0.27 | 0.13 | 0.13 | 0.73 | 0.32 | 0.03 | 1.64 | 0.13 | 0.06 | 0.56 | 0.56 | 0.06 | 1.07 |
| *C. freundii* 426 | 0.46 | 0.00 | 0.17 | 1.09 | 0.44 | 0.10 | 2.36 | 0.09 | 0.09 | 1.25 | 1.01 | 0.00 | 1.53 |
| *E. coli* 1449 | 1.94 | 0.31 | 0.65 | 1.94 | 0.65 | 0.31 | 4.90 | 0.31 | 0.15 | 1.47 | 0.31 | 0.15 | 3.01 |
| *K. oxytoca* 3003 | 0.60 | 0.00 | 0.14 | 0.78 | 0.35 | 0.00 | 1.78 | 0.00 | 0.00 | 0.78 | 0.44 | 0.00 | 1.15 |
| *K. rizophilia* 1542 | 0.96 | 0.21 | 0.69 | 1.25 | 0.44 | 0.00 | 3.00 | 0.21 | 0.10 | 1.56 | 0.96 | 0.10 | 1.89 |
| *M. catarrhalis* 4222 | 0.72 | 0.27 | 0.41 | 0.49 | 0.32 | 0.00 | 1.64 | 0.00 | 0.06 | 0.72 | 0.41 | 0.13 | 1.07 |
| *P. aeruginosa* 3057 | 0.28 | 0.14 | 0.44 | 0.71 | 0.35 | 0.14 | 1.78 | 0.14 | 0.00 | 0.87 | 0.60 | 0.07 | 1.15 |
| *S. aureus* 1449 | 3.00 | 0.78 | 0.56 | 1.31 | 0.56 | 0.36 | 6.11 | 1.25 | 0.36 | 3.00 | 0.78 | 0.78 | 4.69 |
| *St. agalactiae* 3984 | 0.96 | 0.21 | 0.32 | 1.25 | 0.32 | 0.44 | 3.00 | 0.44 | 0.10 | 1.25 | 0.96 | 0.10 | 1.89 |

**Table 8. Increase in the fold area in the modulation of tetracycline with ethanolic extract of plant materials.**

| Pathogens | *C. officinalis* | | *V. amygdalina* | *G. lucida* | | | *E. chlorantha* | | *A. indica* | | *M. oleifera* | *C. citratus* | *L. vulgare* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bark | Leaves | Leaves | Seed | Bark | Leaves | Bark | Leaves | Leaves | Seed | Leaves | Leaves | Leaves |
| *Ac. Xylosoxidans* 4892 | 0.80 | 0.00 | 1.36 | 2.47 | 1.04 | 0.19 | 2.64 | 0.62 | 0.40 | 1.68 | 0.00 | 0.40 | 1.39 |
| *E. avium* 1669 | 1.70 | 0.17 | 1.72 | 4.44 | 2.18 | 0.00 | 5.25 | 1.25 | 1.01 | 3.69 | 0.17 | 0.25 | 4.44 |
| *E. coli* 1449 | 0.77 | 0.19 | 1.36 | 1.12 | 0.81 | 0.00 | 2.64 | 0.62 | 0.40 | 1.68 | 0.00 | 0.19 | 1.12 |
| *K. oxytoca* 3003 | 1.53 | 2.30 | 2.30 | 3.20 | 1.67 | 0.00 | 6.11 | 1.51 | 0.36 | 3.69 | 0.00 | 0.78 | 3.69 |
| *K. rizophilia* 1542 | 0.52 | 0.14 | 0.50 | 1.14 | 0.61 | 0.08 | 2.13 | 0.51 | 0.42 | 1.37 | 0.16 | 0.33 | 0.92 |
| *M. morganii* 1543 | 1.43 | 0.00 | 1.20 | 2.36 | 1.45 | 0.00 | 7.03 | 2.06 | 0.78 | 3.69 | 0.00 | 0.00 | 3.00 |
| *P. aeruginosa* 3057 | 0.51 | 0.16 | 1.11 | 2.00 | 0.59 | 0.00 | 2.13 | 0.51 | 0.51 | 1.37 | 0.51 | 0.16 | 1.61 |
| *St. agalactiae* 3984 | 1.38 | 0.00 | 1.25 | 2.36 | 1.45 | 0.17 | 4.44 | 0.78 | 0.00 | 3.69 | 0.00 | 0.36 | 3.00 |

**Table 9. Increase in the fold area in the modulation of nitrofurantoin with ethanolic extract of plant materials.**

| Pathogens | *C. officinalis* | | *V. amygdalina* | *G. lucida* | | | *E. chlorantha* | | *A. indica* | | *M. oleifera* | *C. citratus* | *L. vulgare* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bark | Leaves | Leaves | Seed | Bark | Leaves | Bark | Leaves | Leaves | Seed | Leaves | Leaves | Leaves |
| *Ac. Xylosoxidans* 4892 | 1.13 | 0.15 | 0.95 | 1.64 | 0.83 | 0.00 | 7.03 | 0.78 | 0.61 | 4.48 | 0.21 | 0.00 | 0.58 |
| *K. oxytoca* 3003 | 0.63 | 0.00 | 1.21 | 0.93 | 1.51 | 0.10 | 1.40 | 0.32 | 1.37 | 0.96 | 0.63 | 0.06 | 0.77 |
| *K. rizophilia* 1542 | 1.10 | 0.10 | 1.09 | 2.97 | 0.76 | 0.16 | 3.41 | 1.25 | 0.68 | 1.27 | 0.28 | 0.30 | 1.15 |
| *M. catarrhalis* 4222 | 1.52 | 0.72 | 0.98 | 1.03 | 1.21 | 0.00 | 2.67 | 0.00 | 0.74 | 0.78 | 0.38 | 0.05 | 1.01 |
| *M. morganii* 1543 | 0.68 | 0.13 | 0.69 | 0.83 | 0.81 | 0.00 | 2.00 | 0.14 | 0.74 | 1.43 | 0.48 | 0.06 | 0.88 |
| *P. aeruginosa* 3057 | 1.06 | 0.81 | 0.97 | 1.55 | 1.02 | 0.00 | 3.31 | 0.78 | 0.99 | 3.64 | 0.31 | 0.05 | 1.83 |
| *S. aureus* 1449 | 0.93 | 0.29 | 1.00 | 1.47 | 0.79 | 0.00 | 1.85 | 0.00 | 0.86 | 1.08 | 0.86 | 0.16 | 0.85 |
| *St. agalactiae* 3984 | 1.06 | 0.67 | 1.07 | 1.08 | 1.10 | 0.15 | 1.60 | 0.36 | 0.41 | 0.50 | 0.31 | 0.11 | 1.02 |

**Table 10. Increase in the fold area in the modulation of trimethoprim with ethanolic extract of plant materials.**

| Pathogens | *C. officinalis* | | *V. amygdalina* | *G. lucida* | | | *E. chlorantha* | | *A. indica* | | *M. oleifera* | *C. citratus* | *L. vulgare* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bark | Leaves | Leaves | Seed | Bark | Leaves | Bark | Leaves | Leaves | Seed | Leaves | Leaves | Leaves |
| *Ac. Xylosoxidans* 4892 | 1.63 | 0.78 | 1.42 | 1.07 | 1.47 | 0.56 | 7.49 | 1.28 | 1.01 | 2.47 | 0.46 | 2.06 | 0.78 |
| *E. avium* 1669 | 1.13 | 1.72 | 0.90 | 2.91 | 0.70 | 1.78 | 1.86 | 0.82 | 0.36 | 0.70 | 1.25 | 2.04 | 3.69 |
| *E. coli* 1449 | 1.96 | 0.00 | 1.61 | 2.03 | 1.32 | 2.67 | 3.87 | 1.75 | 0.56 | 1.32 | 1.87 | 1.69 | 2.36 |
| *K. oxytoca* 3003 | 2.06 | 0.40 | 0.77 | 1.67 | 1.12 | 0.36 | 3.13 | 0.50 | 0.78 | 1.12 | 1.71 | 1.78 | 1.78 |
| *M. morganii* 1543 | 1.18 | 1.78 | 0.87 | 1.30 | 0.92 | 0.00 | 2.46 | 0.64 | 0.36 | 1.92 | 1.56 | 1.65 | 1.25 |
| *P. aeruginosa* 3057 | 1.78 | 0.45 | 1.25 | 2.45 | 1.28 | 0.78 | 3.77 | 1.28 | 1.25 | 2.28 | 1.05 | 1.67 | 3.00 |
| *S. aureus* 1449 | 2.06 | 0.43 | 0.75 | 1.84 | 0.86 | 1.51 | 2.31 | 0.50 | 2.36 | 0.86 | 1.79 | 1.78 | 2.06 |
| *St. agalactiae* 3984 | 1.56 | 0.27 | 0.94 | 2.97 | 0.76 | 0.78 | 2.06 | 0.86 | 1.78 | 0.76 | 1.15 | 1.78 | 3.34 |

**Table 11. Phytochemical composition of the optimized ethanolic bark extract of *Enantia Chlorantha*.**

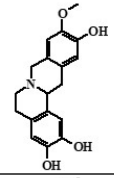
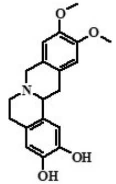
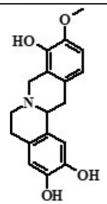
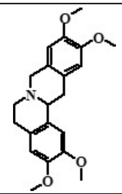
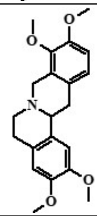
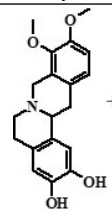
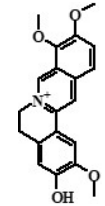
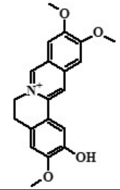
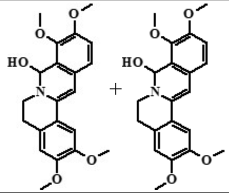
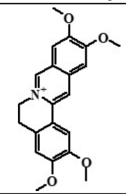
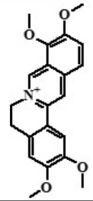
| № | Name | RT, min | Structure | m/z, MS spectra | m/z, MS/MS spectra (CE 30 eV) | % |
|---|---|---|---|---|---|---|
| 1 | **Compound 1** | 15,24 |  | 314 | 269, 253, 237, 211, 209, 192, 175, 160, 145, 143, 137, 121, 107 | 1.86 |
| 2 | **Compound 2** | 17,68 |  | 328 | 283, 269, 253, 237, 189, 174, 151, 121, 107 | 0.64 |
| 3 | **Compound 3** | 19,66 |  | 314 | 269, 253, 237, 211, 209, 192, 175, 145, 143, 137, 121, 107 | 0.69 |
| 4 | Pseudorotundine | 20,33 |  | 356 | 192, 190, 177 | 1.68 |
| 5 | Tetrahydropalmatine (Rotundine) | 23,54 |  | 356 | 192, 190, 177 | 3.12 |
| 6 | **Compound 6** + Unknown compound | 24,19 |  + ??? | 328 (78%), 368 (22%) | 283, 268, 252, 237, 189, 174, 145, 121, 107 + 353, 352, 338, 336, 324, 310, 307 | 1.83 |

**Table 11. Continued.**

| № | Name | RT, min | Structure | m/z, MS spectra | m/z, MS/MS spectra (CE 30 eV) | % |
|---|---|---|---|---|---|---|
| 7 | Jatrorrhizine | 24,73 |  | 338 | 323, 322, 308, 294, 279 | 11.02 |
| 8 | Pseudocolumbamine | 25,88 |  | 338 | 323, 322, 308, 294, 279, 265 | 6.33 |
| 9 | Columbamine + 7,8-dihydro-8-hydroxypalmatine | 26,29 |  | 338 (95%), 370 (5%) | 323, 322, 308, 306, 294, 279, 277, 265 + 355, 354, 340, 326, 312, 311 | 19.21 |
| 10 | Pseudopalmatine | 28,19 |  | 352 | 336, 320, 308, 294, 292, 279 | 1.99 |
| 11 | Palmatine | 28,98 |  | 352 | 336, 322, 320, 308, 294, 292, 278 | 51.63 |

Finally, the management of bacterial infections should be done by aggressive empiric therapy with at least two antimicrobial agents [54]. Empiric combination antimicrobial therapy is usually applied to expand antibacterial spectrum and reduce the selection of resistant mutants during treatment [54]. In addition, combinations of agents that exhibit synergy or partial synergy could potentially improve the outcome for patients with difficult to treat infections [54]. However, this approach while viable, has limitations because it might not be effective for a long period of time due the possible alteration in the susceptibility of bacteria. Therefore, the development of new classes of antimicrobial compounds is of significant importance.

## 4. Phytochemical Profile of the Ethanolic Extract from *E. chloranta* Bark

The HPLC–MS/MS chromatogram of the ethanolic extract from *Enantia chlorantha* bark showed a total of 11 peaks (Fig. 4). The compounds corresponding to these peaks were identified based on their retention time, peak area (%), height (%) and mass spectral fragmentation (m/z, MS and m/z, MS/MS) (Table 11). After comparing the data obtained with that previously reported in the literature [65,66], it was found thar all the identified compounds belonged to the alkaloids family and the major constituents were palmatine (51.63%), columbamine + 7,8-dihydro-8-hydroxypalmatine (19.21%), jatrorrhizine (11.02%) and pseudocolumbamine (6.33%). Four (4) compounds (compounds 1, 2, 3 and 6) were not clearly identified and seemed to have been never reported in the literature.The structures of these compounds were only predicted since without isolation and NMR research, reliable description of new component structure is impossible. However, the composition of this plant explains its strong antimicrobial activity because the compounds it contains have already been individually reported for their antimicrobial activities in other studies [40,44,46].

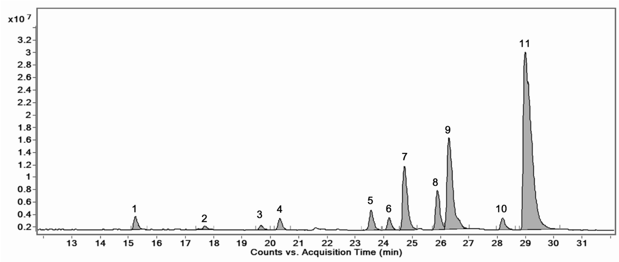



**Fig. 4. HPLC-MS/MS chromatogram of the optimized extract of *Enantia chloranta* bark.**

In addition, X-ray fluorescence spectrum made it possible to qualitatively assess the microelement present in O-ECB. As shown in Table 12, the minerals found were sulfur (S), Silicon (Si), Chlorine (Cl), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn) and Bromine (Br). The highest mean fluorescence intensities were recorded for Br (4.6529 cps/uA), Fe (3.4854 cps/uA) and Cl (2.5942 cps/uA), which could mean that these minerals are the most abundant in O-ECB.

**Table 12. Mineral composition of optimized ethanolic bark extract of *Enantia chlorantha*.**

| Chemical element | Mean fluorescence intensity, cps/uA | Standard deviation |
|---|---|---|
| Si | 0.0581 | 0.0024 |
| S | 0.2052 | 0.0028 |
| Cl | 2.5942 | 0.0097 |
| K | 0.6511 | 0.0010 |
| Ca | 0.6185 | 0.0253 |
| Mn | 0.1482 | 0.0033 |
| Fe | 3.4854 | 0.0127 |
| Zn | 0.1624 | 0.0014 |
| Br | 4.6529 | 0.0114 |

## 5. Conclusions

We found that only *L. vulgare* leaves, *G. lucida* seed, and *E. chlorantha* bark possessed exploitable and promising antimicrobial properties. *E. chlorantha* bark was the most active and had strong activity against both Gram-positive and Gram-negative bacteria as well as fungi. The results of this study clearly demonstrated that *E. chloranta* bark, *L. vulgare* leaves and *G. lucida* seed act synergistically with most common antibiotics and hence increases drug efficacy. Finally, under the limitations of this study, it can be concluded that *E. chlorantha* bark, which contains a high proportion of alkaloids, especially palmatine and its derivatives, should be considered for further studies in the search for new antimicrobials.

## Abbreviations

MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; EE, Ethanolic extract; AE, Aqueous extract.

## Author Contributions

MMJA designed the research study. MMJA, AKLD, PIV, DMS & SIP performed the research. MMJA analyzed the data. SLA, MMJA, KP, MR, HTM, IAMM, YNV and SIP wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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