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In vitro antibacterial and antibiotic-potentiation activities of five edible plant extracts and mode of action against several MDR Gram-negative phenotypes

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Abstract

Background: Bacterial infections involving the multidrug resistant (MDR) strains are among the top leading causes of death throughout the world. Healthcare system across the globe has been suffering from an extra-ordinary burden in terms of looking for the new and more potent antimicrobial compounds. The aim of the present study was to determine the antibacterial activity of some Cameroonian edible plants (*Garcinia lucida* bark, *Phoenix dactylifera* pericarps, *Theobroma cacao* pod, *Solanum macrocarpon* leaves and *Termitomyces titanicus* whole plant) and their antibiotics-potentiation effects against some MDR Gram-negative bacteria phenotypes expressing efflux pumps (*Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Providencia stuartii* strains).

Methods: The antibacterial activities of plant extract alone and in combination with usual antibiotics were carried out using the micro-dilution method. The effects of the most active plant extract (*Garcinia lucida* bark) on H^+-ATPase-mediated proton pumps and on bacterial growth kinetic were performed using experimental protocols, while qualitative reference methods were used to highligh the major groups of secondary metabolites present in the extracts.

Results: Qualitative phytochemical screening of plant extracts indicated that all analysed secondary metabolites were present in *Theobroma cacao* and *Termitomyces titanicus* while one (saponins) of them was absent in *Garcinia lucida* and *Solanum macrocarpon*. Only three of them (polyphenols, flavonoids and saponins) were detected in *Phoenix dactylifera*. Antibacterial essays showed that *G. lucida* was the most active plant as it inhibited the growth of all studied bacteria with strong activity (MIC<100 µg/mL) against *E. coli* ATCC8739, significant activity (100≤MIC≤512 µg/mL) against 80% of bacteria and moderate activity (512<MIC≤2048 µg/mL) against *E. coli* AG100A and *E. aerogenes* (EA289 and CM64). It was followed by *T. cacao* and *S. macrocarpon* extracts which exhibited an antibacterial potential against 95% and 80% of bacterial strains, respectively. These three extracts exhibited a bactericidal effect on a few bacteria. Extracts from *T. titanicus* and *P. dactylifera* were less active as they moderately (512<MIC≤2048 µg/mL) inhibited the growth of 35% and 10% of bacteria. All extracts selectively potentiated the activities of all antibiotics with improvement activity factors (IAF) ranging from 2 to 256. *G. lucida*, *T. cacao* and *S. macrocarpon* potentiated the activities of 100%, 89% and 67% of antibiotics respectively against more than 70%, suggesting that they contain bioactive compounds which could be considered as efflux pumps inhibitors. Whereas *T. titanicus* and *P. dactylifera* improved the activities of almost 40% and 20% of antibiotics, respectively. This increase of activities also characterizes synergistic effects between antibiotics and these bioactive compounds. *G. lucida* extract at all tested concentrations, strongly inhibited the growth of bacterial strain *E. coli* ATCC8739 and exhibited an inhibitory effect on this bacterial H^+-ATPase-mediated proton pumps increasing the pH of the medium.

Conclusion: The overall results indicated that food plants among which *G. lucida*, *T. cacao* and *S. macrocarpon* could have a benefit interest in combatting resistant types of bacteria.

Keywords: Food plants; infectious diseases; MDR bacteria; efflux pumps; antibiotics; secondary metabolites.

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Background

The control of infectious diseases is badly endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. Today's, microbial infections, resistance to antibiotic drugs, have been the best challenges, which threaten the health of societies. Microbial infections are responsible for millions of deaths every year worldwide. In 2013, 9.2 million deaths have been reported because of infections, i.e. about 17% of total deaths [1,2]. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death [3]. For several decades, antibiotics have been critical in the fight against infectious diseases caused by bacteria and other microbes [4]. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the twentieth century. However, diseases-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem [3]. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial agents. Another part of the problem is due to increasing use and misuse of existing antibiotics in human and veterinary medicine. When are underused, overused or misused, the process of antibiotic resistance is increased [5]. Bacteria resist to antibiotics through several mechanisms or strategies including chemical modification of antibiotic, its inactivation through physical removal from the cell reducing its intracellular concentration, modification of target size so that it is not recognized by the antibiotic [6,7]. Many bacteria developing resistance such as *Escherichia*, *Klebsiella*, *Pseudomonas, Enterobacter or Staphylococcus* species are become a serious clinical problem throughout the world [8,9]. At the present time, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most used for treatment [3]. Healthcare system across the globe has been suffering from an extra-ordinary burden in terms of looking for the new and more potent antimicrobial compounds. Natural products are important sources of medicinal compounds with various pharmacological properties. A wide variety of organisms among which food plants produce such bioactive compounds and some of these natural substances have been shown to be able to kill bacteria or be able to potentiate the activities of usual antibiotics [10-15]. Combination therapies may result in the administration of a low dose of commercial antimicrobial which might reduce drug toxicity and improve efficacy. Moreover, many food plants including *Garcinia lucida*, *Phoenix dactylifera*, *Solanum macrocarpon*, *Theobroma cacao* and *Termitomyces titanicus* (Table 1) are used in indigenous medicine to treat infectious diseases and other illness. For this reason, the present work was carried out to evaluate the antibacterial properties of the above mentioned Cameroonian dietary plants and the effects of their combination with some commonly used antibiotics as well as their mechanisms of action against several multidrug resistant Gram-negative phenotypes.

Methods

Plant collection

Plants used in this study were constituted of five Cameroonian edible plants including part of *Garcinia lucida* (bark), *Phoenix dactylifera* (pericarps), *Solanum macrocarpon* (leaves), *Theobroma cacao* (pod) and *Termitomyces titanicus* (whole plant). They were collected in Bamboutos and Menoua Divisions, West Region of Cameroon between September and October 2019 and then identified at the National Herbarium (Yaoundé, Cameroon) where voucher specimens were deposited under the reference numbers (Table 1). Some information concerning the traditional use as well as previous biological activities of these plants are summarized in this Table 1.

Microorganisms and culture media

Microorganisms were constituted of twenty multidrug resistant Gram-negative bacterial phenotypes overexpressing efflux pumps. These bacteria provided some from American Type Culture Collection (ATCC) and others from laboratory of UMR-MD1 of University of Mediterranean, Marseille (France) included reference and clinical isolates of *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Providencia stuartii* and *Pseudomonas aeruginosa* strains. They were maintained on agar slant at 4 ℃ and sub-cultured on a fresh appropriate agar plates 24 hrs prior to any antimicrobial test. Their main characteristics are summarized in Table 2.

Two culture media were used. The Mueller Hinton Agar for bacterial activation and the Mueller Hinton Broth for bacterial conservation as well as for determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs).

Chemicals

Nine antibiotics belonging to different families and commonly used in infectious diseases treatment including Oxacillin (OXA), Thiamphenicol (THI), Erythromycin (ERY), Gentamycin (GEN), Doxycycline (DOX), Ciprofloxacin (CIP), Ofloxacin (OFL), Azithromycin (AZT), Flucloxacillin (FLC) were used. *para*-Iodonitrotetrazolium chloride (INT) was used for colorimetric detection of living bacteria and dimethylsulfoxide (DMSO) for extracts and antibiotics dissolution. All these substances provided from Sigma-Aldrich (St. Quentin Fallavier, France).

Plants extraction

Freshly collected plants were washed with water, then dried safe from sun at room temperature. After crushed these dried samples, powders obtained were soaked in the methanol solvent in the proportions 1:3 m/v for 48 hrs and stirred three times per days. After filtration using Whatman N°1 filter paper, obtained solution was concentrated at 65°C temperature under reduced pressure to give the crude extract that was also dried at room temperature under sterile conditions to complete evaporation of methanol. This crude extract was then kept at 4°C for further tests. The extraction yield (EY) of each plant was calculated using the following formulation EY= (mass of crude extract / mass of powder) x100 (Table 3).

Phytochemical analysis

Plant extracts were submitted to a qualitative phytochemical screening for identification of the main classes of secondary metabolites or bioactive components responsible of the antibacterial properties of each plant. These tests were carried out using a colorimetric method as described by [16].

Antibacterial assays

Bacterial susceptibility to plant extracts

Antibacterial activities of tested samples were performed through the determination of the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) using the broth micro-dilution methods as described by [17,18]. One hundred microliter of MHB culture medium were introduced in a sterile 96-wells microplate followed by 100 µl of sample solution in first wells and serial dilutions. Wells contents were completed to 200 µl by introducing 100 µl of bacterial inoculum 10^6 UFC/mL final concentration and microplate was covered and incubated at 37°C for 18 hrs. After incubation, 40 µl of INT 0.2 % were introduced and microplate was reincubated at 37°C for 30 min. During this time, INT reacts with viable bacteria to yield a pink colour complex. MIC of each sample was defined as the lowest concentration that prevented the change of this colour and which resulted in the complete inhibition of bacterial growth. Ciprofloxacin (tested at a final concentration of 256 µg/mL) and DMSO 2.5% were used as positive and negative controls, respectively. MBCs were determined by adding 50 µl from the previous wells content that did not received INT and that correspond to MICs values, to 150 µl of MHB contained in news plates. After incubation at 37°C for 48 hrs and addition of 40 µl of INT 0.2 %, MBC of each sample was determined as described above. Each assay was performed in triplicate and two independent times (Table 4).

Plant extract was considered to have strong activity if MIC<100 µg/mL, significant activity if 100≤MIC≤512 µg/mL, moderate activity if 512<MIC≤2048 µg/mL and weak activity if MIC>2048 µg/mL. Moreover, plant extract was considered to bactericidal effect if MBC/MIC≤4 and bacteriostatic effect if MBC/MIC>4 [19].

Determination of MICs of antibiotics in combination with sample extracts

Conventional antibiotics (tested at a final concentration of 256 µg/mL) were associated with tested plant extracts and the effects of these combinations were determined calculating the improvement activity factors (IAFs). Tests were done using broth micro-dilution assays [18]. Antibiotics solutions (100 µl) were serially diluted in a 96-wells microplate and 50 µl of extract solution followed by 50 µl of bacterial inoculum ($4x10^6$ UFC/ml) were then added. The microplate was then covered and incubated at 37°C for 18 hrs after which 40 µl of INT 0.2 % were introduced and the MICs of antibiotics alone and those of antibiotic-extract combinations were determined as described above. Preliminary tests were performed against *Pseudomonas aeruginosa* PA124 strain which was the most resistant bacteria and extracts were tested at MIC/2, MIC/4, MIC/8 and MIC/16 (results are summarized in Table 1 of supplementary file). Two concentrations of extracts (MIC/2 and MIC/4) were chose to be tested against the other studied bacteria including *E. coli* (ATCC8739 and AG102), *E. aerogenes* (ATCC13048 and CM64), *K. pneumoniae* (ATCC11296 and KP55), *P. aeruginosa* (PA01 and PA124), *P. stuartii* (ATCC29916 and NEA16). The improvement activity factors (IAF) of each combination were determined by calculating the MIC of antibiotic alone / MIC of combination. Each assay was also done in triplicate and two independent times (Tables 5-9). Extract and antibiotic were considered to have synergistic, indifference or antagonistic effects if IAF≥2, IAF=1 or IAF≤0.5 respectively [20].

Mechanisms of action

Effect of G. lucida on bacterial growth kinetic

The effect of *G. lucida* extract which was the most active sample was investigated on *E. coli* ATCC8739 strain, the most sensitive and a reference studied bacterium using optical density measurements with respect to time. Extract was tested at different concentrations MIC/2, MIC and 2xMIC and ciprofloxacin was used as positive control while DMSO (2.5% v/v) and bacterial inoculum ($1.5x10^8$ UFC/ml) were used as negative controls. Optical densities (OD) were read at 600 nm wavelength using a spectrophotometer and the growth kinetic of this bacterium was followed-up for a period of 18 hrs. The experiment was carried out using a described method by [21] with some modifications. A quantity (500 µl) of bacterial suspension from preculture of 24 hrs followed by 500 µl of tested samples were added to 450 mL of MHB (1/100 v/v dilution) culture medium and the overall was incubated at 37°C under magnetic agitation. After 18 hrs of incubation, aliquots of 1 ml from the preparation were deducted at regular interval times of 2 hrs from 0 to 18 hrs and introduced in a spectrophotometric tab for optical densities reading. From the obtained results, bacterial growth curves [OD = f (times)] were plotted using Microsoft Excel software (Figure 1).

Effect of G. lucida on bacterial H^+-ATPase-mediated proton pumps

G. lucida extract was also tested to evaluate its capacity to inhibit the H^+-ATPase-mediated proton pumps of *E. coli* ATCC8739 strain. This assay was done using an experimental method described by [22]. A fresh bacterial colony was dissolved in 20 mL of MHB culture medium and incubated at 37°C under magnetic agitation for 18 hrs. Aliquots of 1 mL from this bacterial preculture were deducted and added to MHB to afford 100 mL final volume (1/100 v/v dilution), and then re-incubated at 37°C for 18 hrs under magnetic agitation. One hundred millilitre from this bacterial culture was centrifuged at 4000 rds/min for 30 min at 4°C. Recuperated gut was washed with sterile distilled water then with KCl 50 mM and was dissolved in 50 mL KCl 50 mM. obtained bacterial suspension was conserved at 4°C for 18 hrs (for glucose starvation), after which the pH was adjusted to 6.48 by adding HCl or NaOH solution. Then, 0.5 mL of tested sample was added to 4 mL of this bacterial culture ($1.5x10^8$ UFC/mL) and the mixture was incubated at 37°C for 10 min, after which, 0.5 mL of glucose 20% was added in order to initiate the acidification of the environment. DMSO (2.5% v/v) constituted the negative control. The pH values of tested samples were read at room temperature (25°C) every 10 min for 1 hr, using a pH-meter. The curves [pH = f (times)] were labelled using Microsoft Excel software (Figure 2).

Results

Phytochemical screening

Qualitative phytochemical analysis revealed the presence of selected main classes of secondary metabolites in tested plant extracts. Two extracts, *Theobroma cacao* and *Termitomyces titanicus* contained all the six analysed bioactive constituents which are alkaloids, polyphenols, flavonoids, triterpenes, steroids and saponins. Among them, saponins were absent in *Garcinia lucida* and *Solanum macrocarpon* extracts meanwhile, *Phoenix dactylifera* extract was found to has only three of them, polyphenols, flavonoids and saponins (Table 3).

Determination of antibacterial activities of plant extracts

Table 4 shows minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of tested samples. This table indicates that samples have presented different level of antibacterial activities with MICs values ranging from 64 to 2048 µg/mL. *Garcinia lucida* extract inhibited the growth of all studied bacteria. It showed strong activity (MIC<100 µg/mL) against *Escherichi coli* ATCC8739, significant activity (100≤MIC≤512 µg/mL) against 80% of bacteria and moderate activity (512<MIC≤2048 µg/mL) against 15% of bacteria. It was followed by *Theobroma cacao* extract which inhibited the growth of 95% of studied bacterial strain with significative and moderate activities against 45% and 50% of them respectively. Furthermore, *G. lucida* showed bactericidal effects (MBC/MIC≤4) against 55% of bacteria including each specie type and *T. cacao* showed this effect against 25% of bacteria mainly four *E. coli* strains and *Providencia stuartii* NEA16 strain. *Solanum macrocarpon* presented an inhibition spectrum against 80% of bacteria with strong activity on *Enterobacter aerogenes* EA298, significative and moderate activities against 25% and 50% of bacteria, respectively. This extract showed bactericidal effects against 25% of bacteria including three *E. coli* strains (ATCC8739, AG102 and MC4100) and two *E. aerogenes* strains (ATCC13048 and EA289). Extracts from *Termitomyces titanicus* and *Phoenix dactylifera* were less active as the moderately inhibited the growth of 35% and 10% of bacteria respectively without presenting any bactericidal effect. The antibacterial power of *G. lucida* is compared to that of reference antibiotic ciprofloxacin which inhibited the growth of all studied bacteria and presented a bactericidal effect against more of them. Notice that *E. coli* ATCC8739 and *Pseudomonas aeruginosa* PA124 were respectively the most susceptible and the most resistant strains.

Effects of the combination of antibiotics with plant extracts

Tables 5-9 shows minimal inhibitory concentrations of antibiotics alone and antibiotics combined to tested plant extracts. The improvement activity factors (IAF, in parenthesis in the tables) give information about the close link between antibiotics and bioactive components of extracts. This concerns the type of effect including synergism (IAF≥2), indifference (IAF=1) or antagonism (IAF≤0.5) which could exist between them. From these tables, it is noted that all extracts increased the activity of all antibiotics on a number of studied bacteria with IAF values ranging from 2 to 256. Extracts from *Garcinia lucida* at the two sub-inhibitory concentrations (MIC/2 and MIC/4) potentiated the activity of all antibiotics (100%) against all bacterial strains (100%). It showed a strong synergistic effect (IAF≥16) with Oxacillin, Thiamphenicol, Erythromycin, Gentamicin and Ciprofloxacin against most bacteria. No antagonistic effect was observed between this extract and used antibiotics (Table 5). *Theobroma cacao* also improved the activity of 89% of antibiotics against more than 70% of bacteria and its strong synergistic effects were mostly observed with Gentamicin and Ciprofloxacin (Table 6). Extract from *Solanum macrocarpon* potentiated the antibacterial activity of 67% of antibiotics against more than 70% of studied bacterial strains. It also showed strong synergistic effects with some antibiotic including Oxacillin, Erythromycin, Gentamicin and Ciprofloxacin against most studied bacteria. Whereas it showed antagonistic effects with Flucloxacillin on some bacterial strains (Table 7). Extracts from *Termitomyces titanicus* and *Phoenix dactylifera* enhanced the inhibitory power of 40% and 20% of used antibiotics respectively against more than 70% of bacteria. They selectively presented strong synergistic effects with few antibiotics against some bacteria and antagonistic effects mostly with Erythromycin (Tables 8 and 9). Indifferent effects were also obtained in many cases with all tested extracts. Notice that preliminaries essays were carried out against *Pseudomonas aeruginosa* PA124 strain at four different concentrations of extracts (MIC/2, MIC/4, MIC/8 and MIC/16) at the conclusion of which synergistic effects were mostly obtained at MIC/2 and MIC/4 of the extracts (see Table 1 of supplementary file). For this reason, these two concentrations were selected for the other bacteria (Tables 5-9).

Effect of Garcinia lucida extract on bacterial growth kinetic

The effect on bacterial growth kinetic of *Garcinia lucida* extract, which was the best tested sample, was evaluated on *E. coli* ATCC8739 strain, the more sensitive bacteria (Figure 1). This figure shows that with negative controls (curves of inoculum alone and inoculum treated with DMSO 2.5%), bacterial growth started after 2 hrs and the bacterial multiplication become more pronounced till 12 hrs of time after which the number of bacteria in the medium stabilized itself and remained constant till end of experiment. Meanwhile, when treated with tested sample at different concentrations, bacteria grow slowly after 10 hrs for MIC/2 and 14 hrs for MIC and 2xMIC of extract because of weak multiplication of bacterial cell. This multiplication decreases when the concentration of extract increases. In presence of reference antibiotic, there is no growth of this bacteria during the time of experiment (see supporting graphics G1).

Effect of Garcinia lucida extract on bacterial H^+-ATPase--mediated proton pumps

The mechanism of action of *Garcinia lucida* extract tested at MIC concentration on H^+-ATPase-mediated proton pumps of *E. coli* ATCC8739 strain was also investigated (Figure 2). It was observed that when the solution was treated with DMSO 2.5%, the negative control, the pH values decreased from 6.2 to 4.9 during the time of incubation. Whereas the reverse situation was obtained when the medium was treated with tested extract whose pH values gradually increased from 6.2 to 7.5 at the end of experiment. It is important to mention that studied bacterial species grows well in acidic condition of environment and that increase of pH is unfavourable for its survival (see supporting graphics G2).

Discussion

Antibacterial activities of plant extracts

The first part of the present work consisted at evaluating of the antibacterial potential of some dietary plants (Table 4) and the effects of their association with commonly used antibiotics (Tables 5-9). The second part aimed at determining the mechanisms of action of the most active sample. The antibacterial activities were carried out by determining the minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of tested samples. Some criteria concerning MIC values were defined to categorize the antimicrobial power of a natural substance [19]. *Garcinia lucida* which inhibited the growth of all studied bacteria showed strong activity (MIC<100 µg/mL) against *E. coli* ATCC8739 and significant activity (100≤MIC≤512 µg/mL) against 80% of bacteria. Previous studies on *G. lucida* seeds as well as *Garcinia kola* seeds also showed significant activity against the most of used bacterial strains. Moreover, the antibacterial potential of the bark and seeds of *G. lucida* methanolic extracts against

Escherichia coli, *Enterobacter aerogenes* et *Pseudomonas aeruginosa* have been reported. Stem back extracts of this tested plant has been also shown to display strong and significant antimicrobial potential against some fungi and bacteria [23,24,25]. In the present work, from the 80% of bacteria inhibited, *Solanum macrocarpon* leaves extracts exhibited strong activity on *Enterobacter aerogenes* EA 298 and showed moderate antibacterial activity (512<MIC≤2048 µg/mL) against 25% of other strains. Previous studies reported antimicrobial screening of the leaves of *S. macrocarpon* showing their highest inhibitory activity against few pathogens. Alternatively, antimicrobial activities as well as antioxidant and other pharmacological properties of *Solanum* species have been reported [26,27,28]. Next to these two samples, *Theobroma cacao* extracts also displayed an inhibitory potential against all studied bacterial strains excepted *P. aeruginosa* PA124 with significant activity against 45% of bacteria. Previous studies on different parts of *T. cacao* reported their antimicrobial potential against sensitive as well as resistant pathogens and their antioxidant properties. Pectin from *T. cacao* showed dose-dependent moderate activity against Gram-positive and Gram-negative microorganisms [29-33]. The other two tested extracts moderately exhibited selected antibacterial activities against 10% and 35% of used strains for *Phoenix dactylifera* and *Termitomyces titanicus* respectively. In contrary to results obtained in this work, leaves and fruits from *P. dactylifera* (data palm) extracts have been found to display strong and moderate antimicrobial potential against some drug sensitive microorganisms. Some previous literature works also reported the antioxidant activities of this plant [34-37]. Weak inhibitory power of this plant obtained herein is due to the fact that studied bacteria were multidrug resistant phenotypes. The antimicrobial activity of *T. titanicus* has not yet be reported in literature but plants of the same specie showed some properties as antioxidant, antitumor, immunomodulators and antimicrobial [38]. Its inhibitory activity is reported in this work for the first time. A part of all these properties on tested samples, they are traditionally used in treating infectious diseases and other illness (Table 1).

Bioactive compounds of plant

Natural products are important sources of medicinal compounds or bioactive components which are responsible for the pharmaceutical and therapeutic values of plants [39,40]. Phytochemical analysis showed the presence of some mean secondary metabolites in plants used in the present study (Table 3). Alkaloids, flavonoids, polyphenols, triterpenes, steroids and saponins were found in *T. cacao* and *T. titanicus* while saponins among them were absent in *G. lucida* and *S. macrocarpon*. Only flavonoids, polyphenols and saponins were present in *P. dactylifera*. Several studies also reported the presence of all or some of these phytochemicals in used plants. Moreover, bioactive compounds have been isolated in some of these plants and have shown antimicrobial and antioxidant properties [34,41,42]. Examples are terpenes (putranjivic acid, methyl putranjivate, his intermediate lactone, friedelin, cycloartenol), Dihydrochelerithrine, 6-acetonyldihydrochelerithrine and lucidamine isolated from *G. lucida* that showed bacterial growth inhibition. Polyphenols contents from *S. macrocarpon* and *P. dactylifera* and peptin from *T. cacao* were found to possess pharmacological activities [24,30,43]. Antimicrobial potential of a natural product depends not only to the presence of bioactive compounds but also to their quantities or concentrations. Furthermore, these compounds in plants can display synergic or antagonistic effects. This can explain the fact that in the present study, *G. lucida* has highest inhibitory activity than *S. macrocarpon* and *T. cacao*.

Antibacterial activities of plant extract-antibiotic combination

Since studied bacteria are MDR phenotypes overexpressing efflux pumps (EPs), tested plant extracts at sub-inhibitory concentrations were combined with conventional antibiotics to make these microorganisms more susceptible. Numerous natural products have also exhibited potent synergism against the drug-resistant bacteria when used in combination with various types of antibiotics [44,45,46]. It was reported that a substance which is capable to highly improve the activity of almost 70% of extruded antibiotics against 70% of bacteria is considered an efflux pumps inhibitor (EPI) [47]. According to obtained results, *G. lucida* and *T. cacao* extracts could contain bioactive compounds acting as EPIs (Tables 5 and 6) and avoiding the removal of antibiotics from the cell cytoplasm. The intracellular concentration of the antibiotic is therefore high to be able to inhibit the bacterial growth. It had been reported that 5'-méthoxyhydnocarpine (5'-MHC) a flavonolignan isolated from *Berberis fremontii* plant, exhibited an inhibiting effect of efflux pumps NorA expressed by *S. aureus* restoring the activity of certain antibiotics [48]. In this work, many synergistic (IAF≥2) cases were observed. These synergistic effects result of the simultaneous or conjugated action of the combined substances at different target sites of bacterial cell. Antimicrobial agents exhibit their action inhibiting the synthesis of genetic materials, plasma membrane, cell wall, metabolism of folic acid and proteins [49,50]. For the best of our knowledge, no previous studies on the antibiotic-potentiation of plants used in the present work did not yet reported. This manuscript could constitute the first report for their combination with antibiotics against pathogens. Antagonisms (IAF≤0.5) observed is some few cases could result to the competition between bioactive compounds of plant and antibiotic on the same target site of bacterial cell, avoiding each one to display its inhibiting role. Moreover, indifferent effects (IAF=1) obtained in some cases indicate that the inhibitory effect of extract has not changed against concerned bacteria and could not thus influence the antibacterial activity of antibiotic.

Effect of G. lucida extract on E. coli ATCC8739 growth kinetic

Bacteria grow and multiply themselves following many stages of development which include lag, exponential, stationery, and decline phases. In normal growth conditions and without treating with an antimicrobial substance, it was shown the lag-phase duration is generally 2 hrs and the exponential-phase rate is very high [51]. In this work, curves obtained with negative controls (inoculum alone and DMSO 2.5%/inoculum mixture) showed well studied bacterial growth respecting the above different growth phases. When treated with tested samples (*G. lucida* extract which was the most active), growth was highly inhibited and exponential-phase (also call steady-state growth) was almost inexistent (Figure 1). For *E. coli* growing in broth medium, this phase has been estimated to end when optical density at 600 nm (OD_{600}) is between 0.6 and 1.0 [52]. Similar results were obtained here with negative controls. Lag-phase consists of bacterial adaptation to its new environment and enzymes synthesis for substrates metabolism contained in nutrients. This provides energy to be used by bacteria for multiplication and growth at the exponential-phase. This energy decreases at the stationary-phase as metabolized substrates also decrease causing bacterial death whose number is equal to that of living bacteria. After 24 hrs of incubation, complete lack of nutrients is accompanied by a very increased number of

death and toxins production in the environment thus marking the decline-phase [51,53]. In presence of tested sample at all concentrations, lag-phase is prolonged till after 12 hrs and the number of living bacteria at the steady-state growth is very low (OD less than 0.34 for MIC/2 and less than 0.2 for MIC and 2xMIC). This indicates the highest inhibitory effect of tested *G. lucida* extract and its bactericidal character against the studied bacterium, *E. coli* ATCC8739 strain as shown in Table 4.

Effect of G. lucida extract on E. coli ATCC8739 H^+-ATPase-mediated proton pumps

Antimicrobial agents exhibit their inhibitory effects through many mechanisms of action. The effect of *G. lucida* extract in inhibiting the H^+-ATPase-mediated proton pumps of *E. coli* ATCC8739 strain was investigated (Figure 2). Bacterial survival is highly dependent of a high concentration of intracellular hydrogen ions. It is well established that bacteria conserve and transduce metabolic energy by means of an electrochemical gradient of hydrogen ions across the cytoplasmic membrane [54]. Furthermore, it is accepted that secondary transport systems coupled to protons mediate the movement of K^+ and Na^+ ions. Proton movement across the membrane is the primary event not only for energy metabolism but also for performing this homeostatic work. The maintenance of a constant internal ion composition is indispensable to all living cell [54,55]. Influx of protons via a secondary K^+/H^+ or Na^+/H^+ antiporter can be excluded, and such that antiporter can be energized by the membrane potential. In several bacteria, acidification of the cytoplasmic pH has been attributed to secondary porters that exchange K^+ or Na^+ for H^+ and cytoplasmic acidification appear to be required for the growth of *Escherichia* species in alkaline medium. The inhibition of these H^+-ATPase proton pumps leads to the decrease of extracellular H^+ protons and to the increase of pH. It is reported that the minimum pH supporting bacterial proliferation for an *Escherichia coli* strain is 4.4 [56]. In the present work, compared to negative control whose pH decreased (from 6.2 to 4.9) during the time of experiment, tested sample provoked an increase of pH (from 6.2 to 7.5). This indicates that tested extract sample induced the inhibition of H^+-ATPase-mediated proton pumps of studied bacterial strain suggesting that this mechanism could constitute one of the ways by which this extract exhibits his antibacterial activity. Other studies have demonstrated the inhibiting effects of H^+-ATPase-mediated proton pumps of the same studied bacterial strain by dietary plant extracts [57].

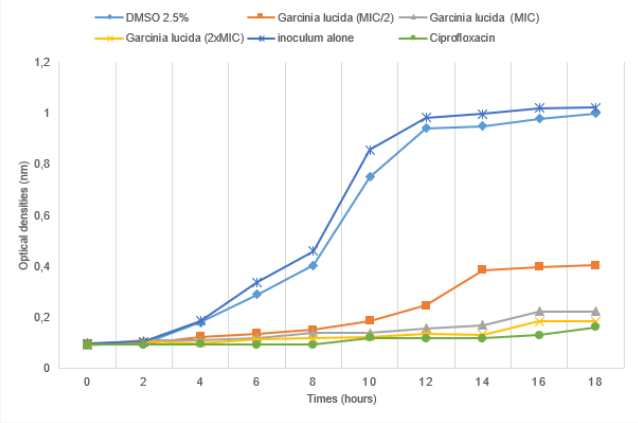

Figure 1. Effect of *Garcinia lucida* extract at different concentrations on growth kinetic of *Escherichia coli* ATCC8739

These results show that DMSO at 2.5% concentration used to dissolve tested samples, did not inhibit the growth of studied bacterial strains and did not influent the activity of these samples

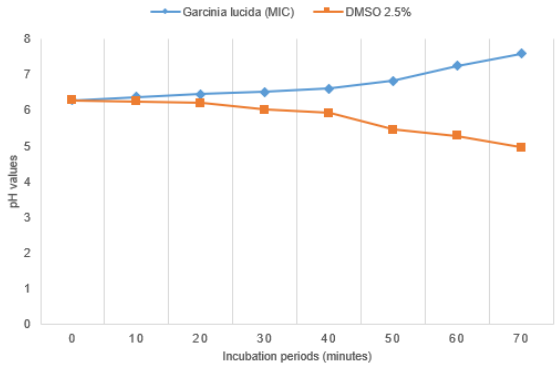

Figure 2. Effect of *Garcinia lucida* extract on *Escherichia coli* ATCC8739 H^+-ATPase-mediated proton pumps

Increase of pH values or acidic conditions of the medium favourites the growth of tested bacterial meanwhile, decrease of pH values or basic conditions allows his growth inhibition

Table 1. Plant samples, their extractive yields, traditional use, and biological activities

Plants and reference numbers	Family	Extractive yields (%)	Traditional usage	Biological activities	Identified or isolated bioactive compounds
Garcinia lucida 17973 NHC	Clusiaceae	37.40	Used as venom and poison antidote and aphrodisiac stimulant; treats gastroenteritis and gynaecologic diseases [58]	Methanolic extracts against *St, Sa, Ca, Ea, Kp, Pa, Pp* and *Ec* [24,25]	6-acetonyldihydrochelerithrine, Dihydrochelerithrine and lucidamine [43]
Phoenix dactylifera 14473 NHC	Arecaceae	36	Used as detergent and astringent for the treatment of sore throat, alcoholic intoxications, and gonorrhoea; treats paralysis, fever, nervous disorders and malaria [59,60,61]	Aqueous, methanolic and acetone extracts against *Ec, Pa, Sp, Sa, Bc* and *Sm* [34,36]	Alkaloids, tannins, steroids, flavonoids, saponins [34]
Solanum macrocarpon 21364 SFR/Cam	Solanaceae	7.18	Treats articular rheumatisms, cardiac diseases, dyspepsia, constipation and gastro-oesophageal ebb [26,62]	Ethanolic extracts against *Ec, Sa, Ca, An* [26]	Alkaloids, saponins, flavonoids and tannins [41]
Theobroma cacao 66394 NHC	Sterculiaceae	19.90	Used to relieve symptoms linked to cardiovascular, gastrointestinal, and nervous diseases. It is also used as diuretic, immunostimulant, cardiotonic [63,64]	Methanolic and acetone extracts against *Sd, Kp, Sm, Pa, Pm, Ec, Sa, Se, Ef* [31,65]	alkaloids, anthraquinones, cardiac glycosides, phenolic compounds and saponins. [42] (Santos et al., 2014).
Termitomyces titanicus /	Lyophyllaceae	17.33	Not found but the same species plants were found to have potential for treating neurodegenerative and rheumatic disorders, constipation, fever, gastrointestinal problems, ulcers, haemorrhoids, abdominal pain and stomach-ache [38]	*Termitomyces* species as potential uses as antioxidant, antitumor, immunomodulators and antimicrobial [38]	Phenolic compounds, fatty acid amide, polysaccharides, saponins, ergostane and neurogenic cerebrosides were isolated from *Termitomyces* species [38]

Ea : Enterobacter aerogenes ; Kp : Klebsiella pneumoniae ; Pm : Proteus mirabilis ; An : Aspergillus niger ; Sa : Staphylococcus aureus ; Ec : Escherichia coli ; Pa : Pseudomonas aeruginosa ; Sp : Salmonella paratyphi ; St : Salmonella typhi ; Sd : Shigella dysenteriae ; Sp :Streptococcus pyogenes ; Se : Staphylococcus epidermidis ; Ef: Enterococcus faecalis ; Sm : Serratia marcescens Bp : Bacillus pumilus ; Bc : Bacillus cereus ; Ca *: Candida albicans ; Pp : Pseudomonas pseudoalcaligenes* HNC : National Herbarium of Cameroon SRF/Cam : Society of forest reserve of Cameroon Extractive yield of each sample was obtained by calculating the crude extract weight / powder weight

Table 2. Studied bacterial strains and their characteristics

Species	Types	Characteristics	References
Escherichia coli	ATCC8739	Reference strain	[66]
	AG100A	*E. coli* K-12 expressing △acrAB: KAN^r	[67]
	AG102	△acrAB mutant AG100, owing acrF gene markedly over expressed TET^r	[68]
	AG100A$_{Tet}$	△acrAB mutant AG100, with over-expressing *acrF* gene; TET^r	[69]
	W3110	Wild type *E. coli* K-12	[70]
	MC4100	Wild type *E. coli* expressed ABC pumps KAN^r	
Enterobacter aerogenes	ATCC13048	Reference strain	[66]
	EA27	Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN^r, AMP^r, NAL^r, STR^r, TET^r	[71,72]
	EA289	KAN sensitive derivative of EA27	
	EA294	EA289 expressing acrA: KAN^r	[71]
	EA298	EA289 expressing *tolC* : KAN^r	[71,73]
Klebsiella pneumoniae	ATCC11296	Reference strain	
	Kp55	Clinical MDR isolate, TET^r, AMP^r, ATM^r, CEF^r	[66]
	Kp63	Clinical MDR isolate, TET^r, CHL^r, AMP^r, ATM^r	[74]
Providencia stuartii	NEA16	Clinical MDR isolate, AcrAB-TolC	[66]
	PS299645	Clinical MDR isolate, AcrAB-TolC associated to types OMPF and OMPC porins	
Pseudomonas aeruginosa	PA 01	Reference strain	[66]
	PA 124	Clinical MDR isolate, expressing *MexAB-OprM*	[67]

KAN^r, TET^r, AMP^r, NAL^r, STR^r, ATM^r, CEF^r, CHL^r : resistant (r) to kanamycin, tetracycline, ampicillin, nalidixic acid, streptomycin, aztreonam, cefepime, chloramphenicol, respectively; MDR : Multidrug-resistant ; AcrAB-TolC, AcrAB and TolC are efflux pumps.

Table 3. Phytochemical analysis of plant extracts

Phytochemicals	Plant extracts				
	Garcinia lucida	*Theobroma cacao*	*Solanum macrocarpon*	*Termitomyces titanicus*	*Phoenix dactylifera*
Alkaloids	+	+	+	+	-
Polyphenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Triterpenes	+	+	+	+	-
Steroids	+	+	+	+	-
Saponins	-	+	-	+	+

(-): absence of phytochemicals (+): presence of phytochemicals

Table 4. Minimal inhibitory and bactericidal concentrations of tested samples

Bacterial strains	**Extracts samples**															**Ciprofloxacin**		
	Phoenix dactylifera			***Garcinia lucida***			***Theobroma cacao***			***Solanum macrocarpon***			***Termitomyces titanicus***					
	MIC	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**
Escherichi coli																		
ATCC8739	2048	-	>1	64	64	1	256	1024	4	128	512	4	1024	-	>2	0.5	1	2
AG100A	-	nt	nd	2048	-	>1	128	-	>16	512	-	>4	-	nt	nd	4	32	8
AG102	-	nt	nd	128	1024	8	1024	1024	1	1024	1024	1	2048	-	>1	2	8	4
MC4100	-	nt	nd	128	128	1	1024	2048	2	1024	1024	1	-	-	>1	4	8	2
AG100A$_{Tet}$	-	nt	nd	256	2048	8	512	2048	4	-	nt	nd	1024	2048	2	8	32	4
W3110	-	nt	nd	256	2048	8	1024	-	>2	1024	-	>2	2048	-	>1	2	2	1
Enterobacter aerogenes																		
ATCC13048	-	nt	nd	128	128	1	512	-	>4	1024	2048	2	-	nt	nd	1	8	8
EA289	-	nt	nd	1024	2048	2	1024	-	>2	1024	2048	2	-	nt	nd	2	16	8
EA294	-	nt	nd	128	256	2	256	2048	8	128	-	>16	-	nt	nd	2	8	4
EA27	-	nt	nd	256	1024	4	1024	-	>2	1024	-	>2	2048	-	>1	4	64	16
EA298	-	nt	nd	128	-	>16	256	-	>8	64	-	>32	-	nt	nd	2	4	2
CM64	-	nt	nd	2048	-	>1	2048	-	>1	256	-	>8	-	nt	nd	4	32	8
Klebsiella pneumoniae																		
ATCC11296	-	nt	nd	128	512	4	256	-	>8	512	-	>4	-	nt	nd	8	64	8
Kp55	-	nt	nd	256	1024	1	2048	-	>1	1024	-	>2	-	nt	nd	8	16	2
Kp63	-	nt	nd	256	2048	8	2048	-	>1	-	nt	nd	2048	-	>1	16	128	8
Providencia stuartii																		
ATCC29916	-	nt	nd	128	128	1	1024	-	>2	1024	-	>2	1024	-	>2	16	64	4
NEA16	-	nt	nd	128	1024	8	1024	2048	2	2048	-	>2	-	nt	nd	16	128	8
PS2636	1024	-	>2	256	1024	4	512	-	>4	-	nt	nd	-	nt	nd	2	8	4
Pseudomonas aeruginosa																		
PA01	-	nt	nd	128	512	4	256	-	>8	1024	-	>2	-	nt	nd	8	32	4
PA124	-	nt	nd	256	2048	8	-	nt	nd	-	nt	nd	-	nt	nd	32	256	8
PSBS (%)	10			100			95			80			35			100		

MIC : minimal inhibitory concentration MBC : minimal bactericidal concentration R : MBC / MIC ratio (a sample is considered as bacteriostatic or bactericidal when this ratio is >4 or ≤4 respectively) (-) : MIC or MBC > 2048 µg/mL nt : not tested nd : not determined (as no MIC and MBC values were not observed till 2048 µg/mL) PSBS : percentage of susceptible bacteria to substances DMSO 2.5% used as negative control does not showed inhibitory effect against all bacteria

Table 5. MICs of antibiotics associated with *Garcinia lucida*

Antibiotics	MICs of plant extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	ATCC29916	NAE16	PA01	PA124	
Oxacillin	0	32	32	64	64	64	8	64	64	64	32	
	MIC/2	0.125(256)	0.125(256)	4(16)	0.25(256)	16(4)	0.25(32)	0.25(256)	0.25(256)	0.25(256)	1(32)	**100**
	MIC/4	0.25(128)	0.25(128)	16(4)	0.25(256)	16(4)	0.25(32)	4(16)	0.5(128)	16(4)	8(4)	**100**
Thiamphenicol	0	2	2	4	4	32	16	16	8	2	32	
	MIC/2	1(2)	0.125(16)	4(1)	0.25(16)	8(4)	0.125(128)	0.125(128)	0.125(64)	0.125(16)	4(8)	**90**
	MIC/4	1(2)	0.5(4)	4(1)	0.25(16)	8(4)	0.5(32)	1(16)	0.25(32)	0.25(8)	4(8)	**90**
Erythromycin	0	1	2	16	16	16	8	16	16	16	2	
	MIC/2	0.125(8)	1(2)	2(8)	0.125(128)	0.125(8)	0.5(16)	0.125(128)	0.125(128)	1(16)	2(1)	**90**
	MIC/4	0.25(4)	2(1)	4(4)	0.25(64)	0.125(8)	0.5(16)	0.5(32)	0.25(64)	4(4)	2(1)	**70**
Gentamicin	0	2	1	16	8	16	8	16	8	16	4	
	MIC/2	0.125(16)	0.125(8)	0.25(64)	0.125(64)	0.125(128)	0.25(32)	0.125(128)	0.125(64)	0.125(128)	0.25(16)	**100**
	MIC/4	0.25(8)	0.25(4)	0.25(64)	0.25(32)	0.5(32)	0.5(16)	0.25(64)	0.25(32)	0.25(64)	0.25(16)	**100**
Ciprofloxacin	0	1	2	1	4	8	8	16	16	8	32	
	MIC/2	0.125(8)	0.25(8)	0.25(4)	0.125(16)	0.5(16)	0.25(32)	0.25(64)	0.25(64)	0.25(32)	8(4)	**100**
	MIC/4	0.25(4)	0.25(8)	0.25(4)	0.25(16)	0.5(16)	0.5(16)	2(8)	0.25(64)	4(2)	16(2)	**100**
Doxycycline	0	2	8	2	2	4	8	2	16	4	16	
	MIC/2	0.125(16)	0.5(16)	2(1)	0.25(8)	0.5(8)	0.5(16)	2(1)	4(4)	0.125(32)	0.125(128)	**80**
	MIC/4	0.125(16)	2(4)	2(1)	0.25(8)	0.5(8)	2(4)	2(1)	4(4)	0.5(8)	0.25(64)	**80**
Azithromycin	0	4	16	16	16	4	4	4	16	4	64	
	MIC/2	4(1)	1(8)	0.25(64)	0.125(128)	0.5(8)	0.25(16)	1(4)	16(1)	2(2)	0.25(128)	**80**
	MIC/4	4(1)	1(8)	1(16)	0.25(64)	1(4)	4(1)	2(2)	16(1)	2(2)	2(32)	**70**
Ofloxacin	0	2	2	2	16	4	2	2	16	4	1	
	MIC/2	0.5(4)	0.125(16)	0.125(16)	0.25(64)	0.25(16)	0.5(4)	0.125(16)	8(2)	2(2)	0.125(8)	**100**
	MIC/4	0.5(4)	0.25(8)	0.5(4)	0.5(32)	0.5(8)	2(1)	0.25(8)	16(1)	4(1)	0.5(2)	**70**
Flucloxacillin	0	16	2	32	4	32	8	2	32	4	32	
	MIC/2	0.5(32)	2(1)	4(8)	0.25(16)	4(8)	0.25(32)	0.125(16)	32(1)	4(1)	0.125(256)	**70**
	MIC/4	0.5(32)	2(1)	4(8)	0.5(8)	4(8)	4(2)	0.25(8)	32(1)	4(1)	0.25(128)	**70**

The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5] IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination 0: MICs values of antibiotics tested alone PBS : percentage of bacterial susceptibility *E. coli: Escherichia coli* *E. aerogenes: Enterobacter aerogenes* *K. pneumoniae: Klebsiella pneumoniae* *P. aeruginosa: Pseudomonas aeruginosa* *P. stuartii: Providencia stuartii* The MIC of extract sample is those showed in Table 4

Table 6. MICs of antibiotics associated with *Theobroma cacao*

Antibiotics	**MICs of plant extract**	**Bacterial strains and concentrations of antibiotics**										**PBS (%)**
		E. coli		***E. aerogenes***		***K. pneumoniae***		***P. stuartii***		***P. aeruginosa***		
		ATCC8739	**AG102**	**ATCC13048**	**CM64**	**ATCC11296**	**KP55**	**ATCC29916**	**NAE16**	**PA01**	**PA124**	
Oxacillin	0	32	32	64	64	64	8	64	64	64	32	
	MIC/2	4(8)	1(32)	32(2)	8(8)	16(4)	0.5(16)	32(2)	32(2)	16(4)	64(0.5)	**90**
	MIC/4	8(4)	1(32)	32(2)	8(8)	16(4)	0.5(16)	64(1)	32(2)	32(2)	64(0.5)	**80**
Thiamphenicol	0	2	2	4	4	32	16	16	8	2	32	
	MIC/2	1(2)	0.125(16)	4(1)	0.125(32)	16(2)	16(1)	16(1)	0.25(32)	2(1)	64(0.5)	50
	MIC/4	2(1)	0.5(4)	4(1)	1(4)	16(2)	16(1)	16(1)	0.25(32)	2(1)	64(0.5)	40
Erythromycin	0	1	2	16	16	16	8	16	16	16	2	
	MIC/2	2(0.5)	0.125(16)	16(1)	2(8)	8(2)	1(8)	2(8)	0.125(128)	64(0.25)	1(2)	**70**
	MIC/4	2(0.5)	0.25(8)	16(1)	2(8)	16(1)	1(8)	2(8)	0.25(64)	64(0.25)	2(1)	50
Gentamicin	0	2	1	16	8	16	8	16	8	16	4	
	MIC/2	0.125(16)	0.125(8)	0.25(64)	0.125(64)	4(4)	0.125(64)	16(1)	0.125(64)	16(1)	0.25(16)	**80**
	MIC/4	0.5(4)	0.125(8)	0.25(64)	0.25(32)	8(2)	0.25(32)	16(1)	0.125(64)	16(1)	2(2)	**80**
Ciprofloxacin	0	1	2	1	4	8	8	16	16	8	32	
	MIC/2	0.125(8)	0.125(16)	0.25(4)	0.25(16)	0.5(16)	0.25(32)	8(2)	0.125(128)	0.5(16)	8(4)	**100**
	MIC/4	0.25(4)	0.25(8)	0.5(2)	0.25(16)	2(4)	0.25(32)	8(2)	0.25(64)	2(4)	16(2)	**100**
Doxycycline	0	2	8	2	2	4	8	2	16	4	16	
	MIC/2	0.125(16)	0.125(64)	0.5(4)	1(2)	0.25(16)	0.125(64)	1(2)	8(2)	0.25(16)	2(8)	**100**
	MIC/4	0.25(8)	0.5(16)	1(2)	2(1)	1(4)	0.25(16)	1(2)	8(2)	0.25(16)	2(8)	**90**
Azithromycin	0	4	16	16	16	4	4	4	16	4	64	
	MIC/2	0.125(32)	2(8)	2(1)	8(2)	0.25(16)	0.125(32)	0.25(16)	8(2)	0.5(8)	64(1)	**80**
	MIC/4	0.5(8)	2(8)	2(1)	16(1)	0.25(16)	1(4)	0.5(8)	16(1)	2(2)	64(1)	60
Ofloxacin	0	2	2	2	16	4	2	2	16	4	1	
	MIC/2	0.125(16)	0.125(16)	0.25(8)	0.25(64)	0.25(32)	0.5(4)	0.25(8)	16(1)	2(2)	1(1)	**80**
	MIC/4	0.25(8)	0.25(8)	0.25(8)	1(16)	0.25(32)	2(1)	0.25(8)	16(1)	2(2)	1(1)	**70**
Flucloxacillin	0	16	2	32	4	32	8	2	32	4	32	
	MIC/2	4(4)	1(2)	8(4)	0.5(8)	4(8)	0.5(16)	0.125(16)	32(1)	4(1)	4(8)	**80**
	MIC/4	4(4)	1(2)	8(4)	0.5(8)	4(8)	4(2)	0.25(8)	321)	4(1)	4(8)	**80**

The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5] IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination 0: MICs values of antibiotics tested alone PBS : percentage of bacterial susceptibility *E. coli: Escherichia coli* *E. aerogenes: Enterobacter aerogenes* *K. pneumoniae: Klebsiella pneumoniae* *P. aeruginosa: Pseudomonas aeruginosa* *P. stuartii: Providencia stuartii* The MIC of extract sample is those showed in Table 4

Table 7. MICs of antibiotics associated with *Solanum macrocarpon*

Antibiotics	MICs of plant extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	ATCC29916	NAE16	PA01	PA124	
Oxacillin	0	32	32	64	64	64	8	64	64	64	32	
	MIC/2	0.125(256)	0.125(256)	2(32)	2(32)	4(16)	0.25(32)	8(8)	32(2)	0.25(256)	16(2)	**100**
	MIC/4	0.25(128)	0.25(128)	8(8)	2(32)	4(16)	0.25(32)	8(8)	32(2)	0.25(128)	32(1)	**90**
Thiamphenicol	0	2	2	4	4	32	16	16	8	2	32	
	MIC/2	0.125(16)	0.125(16)	0.125(32)	0.5 (8)	8(8)	2(8)	8(2)	0.125(64)	0.125(16)	32(1)	**100**
	MIC/4	0.25(8)	0.5(2)	1(4)	1(4)	8(8)	4(4)	16(1)	0.25(32)	0.25(8)	32(1)	**80**
Erythromycin	0	1	2	16	16	16	8	16	16	16	2	
	MIC/2	0.25(4)	0.125(16)	0.25(64)	2(8)	0.125(8)	0.25(32)	0.25(64)	0.5(32)	0.125(128)	1(2)	**100**
	MIC/4	1(1)	0.25(8)	2(8)	2(8)	0.25(4)	2(4)	0.5(32)	0.5(32)	0.25(64)	1(2)	**90**
Gentamicin	0	2	1	16	8	16	8	16	8	16	4	
	MIC/2	0.125(16)	0.125(8)	0.25(64)	0.125(64)	0.25(64)	0.125(64)	16(1)	0.25(32)	0.125(128)	4(1)	**80**
	MIC/4	0.25(8)	0.25(4)	2(8)	0.25(32)	0.5(32)	0.25(64)	16(1)	0.25(32)	0.25(64)	4(1)	**80**
Ciprofloxacin	0	1	2	1	4	8	8	16	16	8	32	
	MIC/2	0.25(16)	0.25(8)	0.125(8)	0.125(32)	2(4)	0.25(32)	0.125(128)	0.125(128)	0.25(32)	16(2)	**100**
	MIC/4	0.25(16)	0.25(8)	0.25(4)	0.25(16)	4(2)	0.25(32)	0.25(64)	0.25(64)	0.25(32)	32(1)	**90**
Doxycycline	0	2	8	2	2	4	8	2	16	4	16	
	MIC/2	0.125(16)	16(0.5)	2(1)	0.25(8)	0.25(16)	8(1)	2(1)	2(8)	0.5(8)	0.25(64)	60
	MIC/4	0.5(4)	16(0.5)	2(1)	2(1)	0.5(8)	8(1)	2(1)	16(1)	0.5(8)	2(8)	40
Azithromycin	0	4	16	16	16	4	4	4	16	4	64	
	MIC/2	1(4)	4(4)	0.25(64)	2(8)	1(4)	4(1)	1(4)	16(1)	2(2)	2(32)	**80**
	MIC/4	1(4)	4(4)	1(16)	2(8)	1(4)	4(1)	2(2)	16(1)	4(1)	8(8)	**70**
Ofloxacin	0	2	2	2	16	4	2	2	16	4	1	
	MIC/2	1(2)	2(1)	0.25(8)	16(1)	0.125(32)	4(0.5)	0.5(4)	8(2)	2(2)	1(1)	60
	MIC/4	1(2)	2(1)	0.25(8)	16(1)	0.25(16)	4(0.5)	0.5(4)	16(1)	4(1)	1(1)	40
Flucloxacillin	0	16	2	32	4	32	8	2	32	4	32	
	MIC/2	2(8)	4(0.5)	4(8)	1(4)	4(8)	32(0.25)	8(0.25)	64(0.5)	2(2)	4(8)	60
	MIC/4	8(2)	4(0.5)	32(1)	1(4)	4(8)	32(0.25)	8(0.25)	64(0.5)	4(1)	16(2)	40

The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5] IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination 0: MICs values of antibiotics tested alone PBS : percentage of bacterial susceptibility *E. coli: Escherichia coli* *E. aerogenes: Enterobacter aerogenes* *K. pneumoniae: Klebsiella pneumoniae* *P. aeruginosa: Pseudomonas aeruginosa* *P. stuartii: Providencia stuartii* The MIC of extract sample is those showed in Table 4

Table 8. MICs of antibiotics associated with *Termitomyces titanicus*

Antibiotics	MICs of plant extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	ATCC29916	NAE16	PA01	PA124	
Oxacillin	0	32	32	64	64	64	8	64	64	64	32	
	MIC/2	8(4)	32(1)	16(4)	32(2)	32(2)	8(1)	16(4)	16(4)	32(2)	8(4)	**80**
	MIC/4	16(2)	32(1)	32(2)	32(2)	32(2)	8(1)	32(2)	16(4)	32(2)	8(4)	**80**
Thiamphenicol	0	2	2	4	4	32	16	16	8	2	32	
	MIC/2	0.125(16)	1(2)	2(2)	4(1)	32(1)	4(4)	32(0.5)	0.25(32)	2(1)	4(8)	60
	MIC/4	0.25(8)	1(2)	2(2)	4(1)	32(1)	8(2)	32(0.5)	0.25(32)	2(1)	8(4)	60
Erythromycin	0	1	2	16	16	16	8	16	16	16	2	
	MIC/2	2(0.5)	.0.125(16)	16(1)	16(1)	64(0.25)	4(2)	0.125(128)	1(16)	64(0.25)	1(2)	50
	MIC/4	2(0.5)	0.125(16)	16(1)	16(1)	64(0.25)	8(1)	0.25(64)	2(8)	64(0.25)	1(2)	40
Gentamicin	0	2	1	16	8	16	8	16	8	16	4	
	MIC/2	1(2)	0.25(4)	0.5(32)	4(2)	8(2)	2(4)	16(1)	16(0.5)	8(2)	0.25(16)	**80**
	MIC/4	1(2)	0.25(4)	1(16)	4(2)	16(1)	2(4)	16(1)	16(0.5)	16(1)	0.25(16)	60
Ciprofloxacin	0	1	2	1	4	8	8	16	16	8	32	
	MIC/2	0.125(8)	0.125(16)	0.25(4)	2(2)	1(8)	0.5(16)	8(2)	0.25(64)	1(8)	2(16)	**100**
	MIC/4	0.2(4)	0.25(8)	0.25(4)	2(2)	8(1)	0.5(16)	8(2)	0.25(64)	2(4)	2(16)	**90**
Doxycycline	0	2	8	2	2	4	8	2	16	4	16	
	MIC/2	0.125(16)	4(2)	0.25(8)	2(1)	0.25(16)	1(8)	0.125(16)	4(4)	0.25(16)	2(8)	**90**
	MIC/4	0.25(8)	4(2)	0.25(8)	2(1)	0.25(16)	2(4)	0.25(8)	16(1)	0.25(16)	4(4)	**80**
Azithromycin	0	4	16	16	16	4	4	4	16	4	64	
	MIC/2	2(2)	16(1)	8(2)	16(1)	2(2)	8(0.5)	4(1)	16(1)	2(2)	2(32)	50
	MIC/4	2(2)	16(1)	8(2)	16(1)	2(2)	8(0.5)	4(1)	16(1)	2(2)	2(32)	50
Ofloxacin	0	2	2	2	16	4	2	2	16	4	1	
	MIC/2	0.5(4)	0.5(4)	0.5(4)	16(1)	2(2)	2(1)	0.25(8)	16(1)	2(2)	4(0.25)	60
	MIC/4	0.5(4)	2(1)	0.5(4)	16(1)	2(2)	2(1)	0.25(8)	16(1)	2(2)	4(0.25)	50
Flucloxacillin	0	16	2	32	4	32	8	2	32	4	32	
	MIC/2	16(1)	1(2)	32(1)	2(2)	4(8)	4(2)	2(1)	32(1)	0.5(8)	64(0.5)	50
	MIC/4	16(1)	1(2)	32(1)	2(2)	4(8)	4(2)	2(1)	32(1)	0.5(8)	64(0.5)	50

The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5] IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination 0: MICs values of antibiotics tested alone PBS : percentage of bacterial susceptibility *E. coli: Escherichia coli* *E. aerogenes: Enterobacter aerogenes* *K. pneumoniae: Klebsiella pneumoniae* *P. aeruginosa: Pseudomonas aeruginosa* *P. stuartii: Providencia stuartii* The MIC of extract sample is those showed in Table 4

Table 9. MICs of antibiotics associated with *Phoenix dactylifera*

Antibiotics	**MICs of plant extract**	**Bacterial strains and concentrations of antibiotics**										**PBS (%)**
		E. coli		***E. aerogenes***		***K. pneumoniae***		***P. stuartii***		***P. aeruginosa***		
		ATCC8739	**AG102**	**ATCC13048**	**CM64**	**ATCC11296**	**KP55**	**ATCC29916**	**NAE16**	**PA01**	**PA124**	
Oxacillin	0	32	32	64	64	64	8	64	64	64	32	
	MIC/2	0.25(128)	0.25(128)	32(2)	32(2)	16(4)	4(2)	64(1)	64(1)	8(8)	8(4)	**80**
	MIC/4	0.25(128)	0.5(64)	32(2)	32(2)	32(2)	4(2)	64(1)	64(1)	8(8)	8(4)	**70**
Thiamphenicol	0	2	2	4	4	32	16	16	8	2	32	
	MIC/2	0.5(4)	0.25(8)	4(1)	4(1)	32(1)	8(2)	32(0.5)	16(0.5)	0.5(4)	16(2)	50
	MIC/4	0.5(4)	0.25(8)	4(1)	4(1)	32(1)	16(1)	32(0.5)	16(0.5)	1(2)	16(2)	40
Erythromycin	0	1	2	16	16	16	8	16	16	16	2	
	MIC/2	2(0.5)	1(2)	32(0.5)	32(0.5)	0.5(32)	8(1)	32(0.5)	16(1)	16(1)	2(1)	20
	MIC/4	2(0.5)	1(2)	32(0.5)	32(0.5)	2(8)	8(1)	32(0.5)	16(1)	16(1)	2(1)	20
Gentamicin	0	2	1	16	8	16	8	16	8	16	4	
	MIC/2	0.25(8)	0.25(4)	4(2)	8(1)	0.5(32)	0.125(64)	16(1)	4(2)	16(1)	4(1)	60
	MIC/4	0.25(8)	1(1)	4(2)	8(1)	2(8)	0.25(32)	16(1)	8(1)	16(1)	4(1)	40
Ciprofloxacin	0	1	2	1	4	8	8	16	16	8	32	
	MIC/2	0.125(8)	0.25(8)	1(1)	2(2)	8(1)	0.125(64)	16(1)	16(1)	8(1)	64(0.5)	40
	MIC/4	0.125(8)	0.25(8)	1(1)	4(1)	8(1)	0.5(16)	16(1)	16(1)	8(1)	64(0.5)	30
Doxycycline	0	2	8	2	2	4	8	2	16	4	16	
	MIC/2	0.5(4)	0.25(32)	0.25(8)	1(2)	4(4)	0.25(32)	2(1)	16(1)	4(1)	2(8)	**70**
	MIC/4	0.5(4)	2(4)	0.25(8)	1(2)	8(2)	1(8)	2(1)	16(1)	4(1)	2(8)	**70**
Azithromycin	0	4	16	16	16	4	4	4	16	4	64	
	MIC/2	4(1)	8(2)	2(8)	32(0.5)	4(1)	1(4)	4(1)	16(1)	1(4)	64(1)	40
	MIC/4	4(1)	16(1)	2(8)	32(0.5)	4(1)	2(2)	4(1)	16(1)	2(2)	64(1)	30
Ofloxacin	0	2	2	2	16	4	2	2	16	4	1	
	MIC/2	0.5(4)	1(2)	0.5(4)	16(1)	0.5(16)	2(1)	0.25(8)	16(1)	4(1)	0.5(2)	60
	MIC/4	0.5(4)	1(2)	0.5(4)	16(1)	4(2)	2(1)	0.5(4)	16(1)	4(1)	1(1)	50
Flucloxacillin	0	16	2	32	4	32	8	2	32	4	32	
	MIC/2	16(1)	1(2)	16(2)	2(2)	8(4)	8(1)	0.5(4)	32(1)	8(0.5)	4(8)	60
	MIC/4	16(1)	2(1)	16(2)	4(1)	8(4)	8(1)	0.5(4)	32(1)	8(0.5)	16(2)	40

The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5] IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination 0: MICs values of antibiotics tested alone PBS : percentage of bacterial susceptibility *E. coli: Escherichia coli* *E. aerogenes: Enterobacter aerogenes* *K. pneumoniae: Klebsiella pneumoniae* *P. aeruginosa: Pseudomonas aeruginosa* *P. stuartii: Providencia stuartii* The MIC of extract sample is those showed in Table 4

Conclusion

The overall results presented here showed that *G. lucida*, *T. cacao* and *S. macrocarpon* extracts inhibited many studied bacteria with strong and significant antibacterial activities. Extract from *G. lucida* was most efficient. These three extracts highly enhanced the antibacterial activity of almost 70% of used antibiotics against the majority of studied bacteria indicating synergistic effects between these antibiotics and bioactive compounds of these plants on the one hand, and suggesting that these plants could contain substances acting as efflux pumps inhibitors on the other hand. Bacterial growth kinetic study showed that *G. lucida* extract inhibited the growth of *E. coli* ATCC8739 strain at the exponential-phase. Furthermore, the same plant extract exhibited an inhibitory effect of the H^+-ATPase-mediated proton pumps of this bacterial strain. This work provides additional files for the antimicrobial activity of used plants and their potential benefit in the fight against infectious diseases caused by MDR bacteria phenotype overexpressing efflux pumps.

Additional file

Table 1. MICs of antibiotics associated with plant samples against Pseudomonas aeruginosa PA124. Available online at: https://www.investchempharma.com/imcp49-ngaffo-et-al-supplementary-file/

Abbreviations

ATCC :	American Type Culture Collection
MIC :	Minimal inhibitory concentration
MBC :	Minimal bactericidal concentration
DMSO :	Dimethylsulfoxide
INT :	p-Iodonitrotetrazolium chloride
SFR/CAM :	Society of forest reserve of Cameroon
NHC :	National herbarium of Cameroon
MHA :	Mueller Hinton agar
MHB :	Mueller Hinton broth
OD :	Optical density
RND :	Resistance-nodulation-cell division
EPI :	Efflux pumps inhibitor
IAF :	Improvement activity factor
EY :	Extractive yield
OXA :	Oxacillin
THI :	Thiamphenicol
ERY :	Erythromycin
GEN :	Gentamicin
CIP :	Ciprofloxacin
DOX :	Doxocyclin
AZI :	Azithromycin
OFL :	Ofloxacin
FLU :	Flucloxacillin
PSBS :	percentage of susceptible bacteria to substances
PBS :	percentage of bacterial susceptibility

Authors' Contribution

CMNN realized antibacterial activities of samples alone and in combination with antibiotics. phytochemical screening was done by MGGF. Mechanisms of action of the most active sample were carried out by BENW, INB and PN. The manuscript was written by SBT and the work was supervised by VK and ATM.

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Conflict of interest

The authors declare no conflict of interest

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References

1. WHO G. 2013. WHO methods and data sources for global burden of diseases estimates 2000-2011. Geneva: Department of Health Statistics and Information Systems.
2. Khameneh B,Iranshahy M, Soheii V, Bazzaz FSB. 2019. Review on plant antimicrobial: a mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*, 8:118
3. Odonkor TS, Addo KK. 2011. Bacteria resistance to antibiotics: recent trends and challenge. *International Journal of Biology and Medical Research*, 2(4):1204-1210.
4. Soro D, Koné MW, Kamanzi AK. 2010. Evaluation de l'activité antibactérienne et anti-radicale libres de quelques taxons bioactifs de Côte d'Ivoire. *European Journal of Scientific Research*, 40: 307-317.
5. Oussou KR, Yolou SF, Tue Bi B, Kanko C, Boti JB, Ahibo C, Casanova J. 2010. Etude chimique bio-guidée de l'huile essentielle de *Ocimum gratissimum* (Lamiaceae). *European Journal of Scientific Research,* 40 (1): 50-59.
6. Guardabassi L, Courvalin P. 2006. Modes of antimicrobial action and mechanisms of bacterial resistance. In: Aarestrup F.M. (Ed.), Antimicrobial resistance in bacteria of animal origin. *American Society for Microbiology Press*: Washington. p 1-18
7. Cesur S, Demiroz AP. 2013. Antibiotics and the mechanisms of resistance to antibiotics. *Medical Journal of Islamic World Academy of Sciences*, 21(4): 138-142
8. Alekshun MN, Levy SB. 2007. Molecular mechanism of antibacterial multidrug resistance. *Cell*, 128: 1037-1050
9. Al-Asmari AK, Abbasmanthiri R, Abdo Osman NM, Siddiqui Y, Al-Bannah FA, Al-Rawi AM, Al-Asmari SA. 2015. Assessment of the antimicrobial activity of few Saudi Arabian snake venoms. *The Open Microbiology Journal*, 9:18-25
10. Guinoiseau E. 2010. Molécules antibactériennes issues d'huiles essentielles : séparation, identification et mode d'action. Sciences du Vivant [q-bio]. Thèse de Doctorat, Université de Corse, France. p 5-99
11. Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AHL, Kuete V. 2013. Antibacterial activities of selected edible plant extracts against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine, 13:164
12. Seukep JA, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, Noumdem JAK, Nkuete LAH, Kuete V. 2013. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. *SpringerPlus*, 2:363
13. Tankeo SB, Lacmata ST, Noumedem JAK, Dzoyem JP, Kuiate JR, Kuete V. 2014. Antibacterial and antibiotic-potentiation activities of some Cameroonian food plants against multi-drug resistant Gram-negative bacteria. *Chin J Integr Med*, 20(7): 546-554
14. Badawe G, Fankam AG, Nayim P, Wamba BEN, Mbaveng AT, Kuete V. 2018. Antistaphylococcal activity and antibiotic-modulating effect of *Olax subscorpioidea*, *Piper guineense, Scorodophloeus zenkeri, Fagara leprieurii*, and *Monodora myristica* against resistant phenotypes. *Investigational Medicinal Chemistry and Pharmacology*, 1(2):17
15. Manekeng TH, Mbaveng TA, Nguenang SG, Seukep AJ, Wamba NBE, Nayim P, Yinkfu NR, Fankam AG, Kueta V. 2018. Anti-staphylococcal and antibiotic-potentiating activities of seven Cameroonian edible plants against resistant phenotypes. *Investigational Medicinal Chemistry and Pharmacology*, 1(1): 7
16. Harbone JB. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. London, p 302.
17. Eloff JN. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64: 711-713.

18. Kuete, V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. 2009. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of *Ficus ovata (Moraceae)*. *Journal of Ethnopharmacoly,* 124:556–561.
19. Tamokou JDD, Mbaveng TA, Kuete V. 2017. Chapter 8- Antimicrobial activities of African medicinal spices and vegetables. In: Medicinal spices and vegetables from Africa (Eds). Kuete V.: *Academic Press*; London, 207-237.
20. Coutinho HD, Vasconcellos A, Freire-Pessoa HL, Gadelha CA, Gadelha TS, Almeida-Filho GG. 2010. Natural products from the termite *Nasutitermes corniger* lower aminoglycoside minimum inhibitory concentrations. *Pharmacognosy Magazine*, 6:1-4.
21. Cox SD, Mann CM, Markham MJL, Bell HC, Gustafson JE, Warmington NJR, Wyllie SG. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of Applied Microbiology,* 88 : 170-175.
22. Manavathu EK, Dimmock JR, Sarvesh CV, Chandrasekar PH. 2001. Inhibition of H^+-ATPase-mediated proton pumping in *Cryptococcus neoformans* by a novel conjugated styryl ketone. *Journal of antimicrobial chemotherapy*, 47: 491-494.
23. Piéboji GJ, Eze N, Djintchui NA, Ngameni B, Tsabang N, Pegnyemb ED, Biyiti L, Ngassam P, Shiro KS, Galleni M. 2009. The *in-vitro* antimicrobial activity of some traditionally used medicinal plants against bêta-lactam-resistant bacteria. *Journal of Infection Developing Countries,* 3(9):671-680.
24. Momo IJ, Kuete V, Dufat H, Sylvie M, Wandji J. 2011. Antimicrobial activity of the methanolic extract and compounds from the stem bark of *Garcinia lucida* Vesque (Clusiaceae). *International Journal of Pharmaceutical Sciences*, 3(3): 215-217.
25. Lacmata ST, Kuete V, Dzoyem JP, Tankeo SB, Teke NG, Kuiate JR, Pages JM. 2012. Antibacterial activities of selected Cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. *Evidence-Based Complementary and Alternative Medicine*, Vol 2012, ID 623723, doi:10.1155/2012/623723
26. Ilodibia CV, Akachukwu EE, Chukwum, MU, Igboabuchi NA, Adimonyemma RN, Okeke NF. 2016. Proximate, Phytochemical and Antimicrobial Studies on *Solanum macrocarpon* L. *Journal of Advances in Biology & Biotechnology*, 9(2): 1-7.
27. Famuwagun AA, Taiwo KA, Gbadamosi SO, Oyedele DJ, Aluko RE, Adebooye OC. 2017. Extraction optimization ant antioxidant properties of African eggplant (*Solanum macrocarpon*) leaf polyphenols, *Journal of Food Quality*, vol 2017, ID 2159183, https;//doi.org/10.1155/2017 /2159183
28. Hong H, Lee JH, Kim SK. 2018. Phytochemical and antioxidant capacity of some tropical edible plants. *Asian-Australasian Journal of Animal Science*, 31(10):1677-1684
29. Santos RX, Oliveira DA, Sodré GA, Gosmann G, Brenda M, Pungartnik C. 2014. Antimicrobial activity of fermented *Theobroma cacao* pod husk extract. *Genetics and Molecular Research*, 13(3):7725-7735
30. Adi-Dako O, Ofori-Kwakye K, Manso FS, El Boakye-Gyasi M, Sasu C, Pobee M. 2016. Physicochemical and antimicrobial properties of Cacoa pod huck pectin intended as a versatile pharmaceutical excipient and nutraceutical, *Journal of Pharmaceutic*, vol 2016, article ID 7608693, https;//doi.org/10.1155/2016/7608693.
31. Metoui M, Essid A, Bouzoumita A, Ferchichi A. 2018. Chemical composition, antioxidant and antibacterial activity of Tunisian date palm seed. *Journal Polish of Environmental,* 28: 267-274.
32. Nayim P, Mbaveng TA, Wamba NEB, Fankam GA, Dzotam KJ, Kuete V. 2018. Antibacterial and antibiotic potentiating activities of thirteen Cameroonian edible plants against Gram-negative resistant phenotypes. *The Scientific World Journal,* 14.
33. Hasanuddin A, Anwar K, Mappatoba M, Hafsah 2019. Antibacterial and antioxidant activities of ethanol extracts of Cocoa Huck (*Theobroma cacao* L.) with Maltodextrine in various concentration. *Earth and Environmental Science*, 255 (2019) 012017 doi.10.1088/1755-1315/255/1/012017
34. Al-daihan S, Bhat Shafi R. 2012. Antibacterial activities of extracts of leaf, fruit, seed and bark of *Phoenix dactylifera. African Journal of Biotechnology*, 11(42): 10021-10025.
35. El Sohaimy SA, Abdelwahab AE, Brennan CS, Aboul-enein AM. 2015. Phenolic contents, antioxidant and antimicrobial activities of Egyptian date palm (*phoenix dactylifera* L.) fruits. Australian Journal of Basic and Applied Sciences, 9(1):141-147
36. Samad AM, Hashim HS, Simarani K, Yaacob SJ. 2016. Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (*Phoenix dactylifera*) cultivars. *Molecules,* 21: 419.
37. Sani NM, Abdulkadir F, Mujahi NS. 2017. Antimicrobial activity of *phoenix dactylifera* (date palm) on some selected members of *Enterobacteriaceae*. *Bayero Journal of Pure and Applied Sciences*, 10(1): 36-39
38. Hsieh HM, Yu-Ming J. 2018. Medicinal components in *Termitomyces* mushroom. *Applied Microbiology and Biotechnology*, 102:4987-4994
39. Reuben KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO. 2008. Phytochemical screening and *In Vitro* antimicrobial investigation of the methanolic extract of *Croton Zambesicus* Muell ARG. Stem bark; *European Journal of Scientific Research*, 23(1): 134-140
40. Kuete V. 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Medica*, **76**(14): 1479–1491
41. Chinedu SN, Olasumbo AC, Eboji OK, Emiloju OC, Arinola OK, Dania DI. 2011. Proximate and phytochemical analyses of *Solanum aethiopicum* L. and *Solanum macrocarpon* L. Fruits. *Research Journal Chemical Science*, 1(3): 63-71.
42. Santos RX, Oliveira DA, Sodré GA, Gosmann G, Brendel M, Pungartnik C. 2014. Antimicrobial activity of fermented *Theobroma cacao* pod husk extract. *Genetics and molecular research*, 13: 7725-7735.
43. Fotie J, Bohle DS, Olivier M, Gomez MA, Nzimiro S. 2007. Trypanocidal and antileishmanial dihydrochelerythrine derivatives from *Garcinia lucida*. *Journal of Natural Products*, 70(10): 1650-1653.
44. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. 2011. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. *BMC Complementary and Alternative Medicine*, 11: 104
45. Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocaru D, Dzoyem JP, Kuete V. 2013. *In vitro* antibacterial and antibiotic-potentiation activities of four edible plants against multidrug-resistant gram-negative species. *BMC Complementary and Alternative Medicine*, 13: 190
46. Tankeo SB., Damen F, Sandjo LP, Celik I, Tane P, Kuete V. 2016. Antibacterial activities of the methanol extracts, fractions and compounds from *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae). *Journal of Ethnopharmacology*, 190: 100–105
47. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Charton-Souza E, Nascimiento AMA. 2005. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Canadian Journal of Microbiology*, 51: 541-547
48. Stermitz FR, Lorenz P, Tawara JN, Lauren A, Zenewicz L, Lewis K. 2000. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'- methoxy hydnocarpin, a multidrug pump inhibitor. *Proceedings of the National Academy of Sciences of the USA*, 97: 1433-1437
49. Singh SB, Barrett JF 2006. Empirical antibacterial drug discovery product foundation in natural. *Biochemistry and Pharmacology*, 71: 1006-1015
50. Adwan G, Mhanna M. 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from specimens. *Middle-East Journal of Scientific Research*, **3**(3): 134-139
51. Delhalle L, Daube G, Adolphe Y, Crevecoeur S, Clinquart A. 2012. Les modèles de croissance en microbiologie prévisionnelle pour la maitrise de la sécurité des aliments (synthèse bibliographique). *Biotechnology and Agronomic Society of Environment*, 16(3): 369-381
52. Sezonov G, Joseleau-Petit D, D'Ari R. 2007. *Escherichia coli* physiology in Luria-Bertani broth. *Journal of Bacteriology*, 189:8746-8749
53. Buchanan RE. 1918. Life's phase in bacterial culture. *Journal of Infectious Disease*, 23: 109-125
54. Kobayashi H. 1985. A proton-translocating ATPase regulates pH of the bacterial cytoplasm. *Journal of Biological Chemistry*, 260: 72-76
55. Kakinuma Y.1998. Inorganic cation transport and energy transduction in *Enterococcus hirae* and other *Streptococci*. *Microbiology and molecular Biology Reviews*, 62(4): 1021-1045
56. Bavishi C, DuPont HL. 2011. Systematic review: the use of proton pumps inhibitors and increased susceptibility to enteric infection. *Alimentary Pharmacology and Therapeutics*, 34: 1269–1281
57. Youmbi LM, Atontsa BC, Tankeo SB, Wamba NEB, Nayim P, Nganou KB, Bitchagno GTM, Simo KI, Mpetga JDS, Penlap VB, Kuete V. 2020. Antibacterial potential and mechanism of action of botanicals and phytochemicals from *Stachytarpheta cayennensis* (Verbenaceae) against Gram-negative multidrug-resistant phenotypes expressing efflux pumps. *Investigational Medicinal Chemistry and Pharmacology*, 3(1):35
58. Guedje NM, Fankap R. 2001. Utilisations traditionnelles de *Garcinia lucida* et *Garcinia kola* (Clusiaceae) au Cameroun. *Nationale Plantentuin van België,* 71:747-75.
59. Nadkarni KM. 1976. Indian Materia Medica Vol 1. Mumbai: Bombay popular prakashan Pvt (Ed). Ltd.
60. Morton J. 1987. Fruits of warm climates. Miami, Florida, 5–11.
61. Barh D, Mazumdar BC. 2008. Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date *(Phoenix sylvestris* Roxb*)* sap in treatment of anemia. *Research Journal of Medicine and Medical Sciences,* 3: 173-176.
62. Bello SO, Muhammed BY, Gammaniel KS, Abdu-Aguye I, Ahmed H, Njoku CH, Pindiga UH, Salka AM. 2005. Preliminary evaluation of the toxicity and some pharmacological properties of the aqueous crude extract of *Solanum melongena. Research Journal of Agriculture and Biological Sciences,*1(1): 1-9.
63. Trognitz B, Scheldeman X, Hansel-Hohl K, Kuant A, Grebe H, Herman M. 2011. Genetic population structure of cacao plantings within a young production area in Nicaragua. *PLo Currents,* 6: 16056.
64. Jayanthi A, Nidhi S, Shreyan D, Abhirup D, Chowdhury, Akash R. 2015. Antimicrobial activity and cytotoxicity of *Theobroma cacao* extracts. *Der Pharmacia Lettre,* 7:287-294.
65. Nidhi S, Shreyan D, Abhirup D, Akash RC, Jayanth A. 2015. Antimicrobial activity and cytotoxicity of *Theobroma cacao* extracts. *Scholars Research Library*, 22: 287-294.
66. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Albert-Franco S, Ngadjui BT, Pagès J-M. 2010. Efflux pumps are involved in the defense of Gram-negative bacteria against the natural products Isobavachalcone and diospyrone. *Antimicrobial Agents and Chemotherapy*, 54: 1749–1752.
67. Lorenzi V, Muselli A, Bernadini AF, Berti L, Pagès J-M. 2009. Geraniol restores antibiotic activities against multidrugs resistant isolates from Gram-negatives species. *Antimicrobial Agents and Chemotherapy*, 53: 2209-2211.
68. Chevalier J, Pagès J-M, Eyraud A, Malléa M. 2000. Membrane permeability modifications are involved in antibiotic resistance in *Klebsiella pneumoniae*. *Biochemical and Biophysical Resource Common*, 274: 496-499.
69. Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. 1992. Quinone chemistry and toxicity. *Toxicological Applied in Pharmacology*, 112: 2–16.
70. Bagliomi P, Liberatori S, Pallini V, Marri L. 2003. Proteome analysis of *Escherichia coli* W3110 expressing an heterogenous sigma factor. *Proteomic*, 3: 1060-1065.

71. Ghisalberti D, Masi M, Pagès J-M, Chevalier J. 2005. Chloramphenicol and expression of multidrug efflux pump in *Enterobacter aerogenes*. *Biochemical and Biophysical Resource Common*, 328: 1113-1118.
72. Malléa M, Chevalier J, Bornet C, Eyraud A, Pagès J-M, Davin-Régli A. 1998. Porin alteration and active efflux: two in vivo drug resistance strategies used by *Enterobacter aerogenes*. *Microbiology*, 144: 3003–3009.
73. Pradel E, Pagès J-M. 2002. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen Enterobacter aerogenes. *Antimicrobial Agents and Chemotherapy*, 46: 2640-2643.
74. Fredrickson J, Zachara J, Balkwill D. 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford site, Washington State. *Applied Environmental Microbiology*, 70: 4230 – 4241.