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Original Research Article

Antibacterial activity of three Cameroonian medicinal plants traditionally used for the treatment of urinary tract infections and their synergistic effects with amoxicillin and serum.

ABSTRACT

Background: Urinary tract infections (UTIs) are the second most common form of infectious diseases that affect humans after respiratory diseases. It is predicted that 20% of the adult female population is exposed to development of an UTI. The emergence of UTIs is promoted by the development of the resistance to antibiotics, highlighting the research of new antibiotics that can be used to fight effectively and at affordable cost against these infections.

Aim: The present study was designated to evaluate the antibacterial activity of three Cameroonian medicinal plants traditionally used for the treatment of UTIs and their synergistic effects with amoxicillin and serum.

Methods: The plant extracts were prepared by maceration in methanol. The methanol extract was partitioned into hexane, ethyl acetate and residual fractions and phytochemical analysis was carried out using standard methods. The antibacterial activities of the extracts alone and their combinations with amoxicillin and serum were evaluated using the broth microdilution method.

Results: Phytochemical analysis showed the presence of alkaloids, flavonoids, anthraquinones, tanins and polyphenols in the methanol extracts of *Calystegia sepium*, *Dacryodes edulis* and *Carica papaya*. The other classes of compounds (anthocyanines, triterpenes, steroids and saponines) were selectively distributed in the extracts. All the extracts exhibited antibacterial activities (MIC = 256 - 2048 µg/ml) that varied according to the bacterium and extract; confirming the traditional use of these plants in the treatment of UTIs. The fractionation of the

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methanol extract of *D. edulis* leaves did not enhance its activity and unequally distributed the antibacterial activities in different fractions. The interaction of the extracts with serum resulted in a concentration-dependent-increase in the antibacterial activity of the different extracts; suggesting that the chemical constituents of the tested extracts weakly bind to the serum proteins. As a function of tested bacterial species, synergistic, additive and indifference effects between amoxicillin and the MeOH extract of *D. edulis* were observed.

Conclusion: The overall results of this study indicate that the extracts and fractions from the studied plants can be used to treat the UTIs caused by the tested bacteria subject to further toxicological and pre-clinical studies.

Keywords: Medicinal plants, extracts, phytochemical analysis, urinary tract infections, antibacterial, synergy.

1. INTRODUCTION

The urinary tract infection (UTI) is defined as the presence of large numbers of bacteria (> 10^5/ml) in the urinary tract, renal / prostatic parenchyma and/or urine. Most of UTIs are indeed due to germs of digestive origin including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* [1]. The UTI usually has a clinical symptom (cystitis, urethritis, prostatitis, pyelonephritis, renal abscess); however, asymptomatic infection is common and known as asymptomatic bacteriuria. The UTIs are by order of frequency the first of the non-epidemic infectious diseases [2], therefore represent the frequent reason for consultation and the leading cause of Gram-negative sepsis in developed countries, of nosocomial and acute bacterial infections worldwide with an estimated 10% of the population being consulted per year for this reason [3]. UTIs are more common in women of childbearing age than males where they occur at

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older ages [2,4]. About 50% of women develop a symptomatic UTI at least once in their lives [5]. The UTIs represent more than seven million consultations and one million hospitalizations in the United States [2]. The prevalence rates of UTIs among symptomatic patients are 59.8% in Yaoundé-Cameroon [6], 54% in Bamenda-Cameroon [7], 19.3% in Rwanda [8], 10.9% in India [9], and 39.7% in Nigeria [10]. The treatment of these UTIs is always problematic, due to the high cost of antibiotics, the toxicity of antibiotics, and the phenomenon of resistance. The resistance of bacteria to antibiotics has become a real increasing global concern [11,12] that involves all the bacterial species. The resistance of *E. coli* to injectable third-generation cephalosporins (C3G) in community, is progressing and is currently close to 5%, with great variability according to clinical presentation, field and from one region to another [1]. The production of extended-spectrum beta-lactamases (ESBLs) is the primary mechanism of resistance (plasmid), making the bacteria multi-resistant. The ESBLs are defined as β-lactamases capable of hydrolyzing oxymino-cephalosporins and are inhibited by β-lactamase inhibitors [13]. Microorganisms responsible for UTIs such as *Escherichia coli* and *Klebsiella* spp. remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the Enterobacteriaceae family and in certain non-fermenters [14]. The resistance of *E. coli* to fluoroquinolones has increased over the past 10 years from 3% to 25% according to the clinical status and physiological conditions of the patients [1], while the frequency of *Staphylococcus aureus* strains resistant to methicillin increased from 23% in 2000 to 38.7% in 2004 [11]. Facing these issues, the real challenge would be to find new effective antibiotics that are affordable costly and less toxic. As a result, the use of medicinal plants with antibacterial properties is one of the most interesting routes to explore, since they have a variety of secondary metabolites with pharmacological properties [15]. Indeed, according to the World Health Organization (WHO), 80% of rural populations living in developing

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countries depend on traditional medicine for their health care needs. In Cameroon, the use of medicinal plants in the treatment of diseases is an old tradition. Unfortunately, the integration of traditional medicine into the health system is still an issue at stake [16]. However, government health policy has included the organization of the traditional medicine in its agenda, in order to provide the main trends for its development and integration [17]. Thus, several varieties of Cameroonian medicinal plants could constitute a source of antibacterial agents. In the continuation of the strategy of new drug discovery, we evaluated the antibacterial activity of three Cameroonian medicinal plants traditionally used for the treatment of urinary tract infections and their synergistic effects with amoxicillin and serum.

2. MATERIALS AND METHODS

2.1 Plant materials

The medicinal plants used in this study were *Calystegia sepium* (whole plant), *Dacryodes edulis* (stem bark and leafs), *Carica papaya* (root), all collected in Dschang (West Region of Cameroon) in January 2017. They were selected on the basis of their traditional uses (Table 1). These plants were identified and authenticated at the Cameroon National Herbarium, where the voucher specimens were kept under the reference numbers (Table 1). The plant material was washed thoroughly under running water and dried under room temperature. It was crushed to powder using mixer grinder and stored in air tight bottles.

2.2 Preparation of crude extracts

The air-dried and powdered sample from each plant was macerated separately in methanol (Table 1) for 48 h at room temperature with occasional shaking. The extract was filtered through a Whatman no. 1 filter paper. The methanolic filtrate was then evaporated to

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dryness at 65 °C using a rotary evaporator. The extraction yield was calculated (Table 1) and the crude extract was kept at +4 °C until further use.

2.3 Preparation of fractions

2.3.1 Hexane fractions

Part of the methanol extract of *D. edulis* leaves (5 g) was dissolved in 100 ml of hexane and 50 ml of distillated water; the whole was stirred and then left to stand for 1 h. The hexane phase was removed using a separative funnel. The process was repeated with hexane until the solvent phase was as light as possible. Then, hexane was recycled in a rotary evaporator at 68 °C leaving the hexane fraction. The resulting residue was dried in an oven at 50 °C for evaporation of the water.

2.3.2 Ethyl acetate fraction

Ethyl acetate (100 ml) was added to the hexane residue, stirred and allowed to stand for 1 h. The upper phase with ethyl acetate was decanted. The process was repeated several times until the solvent collected was as light as possible. Then, the ethyl acetate phase was evaporated at 78 °C using a rotary evaporator.

2.3.3 Residual fraction

This consisted of the remaining residue after depletion with ethyl acetate. This residue was then dried in an oven at 50 °C for complete evaporation of the solvent.

2.4 Phytochemical screening of extracts

The phytochemical screening of methanol extracts and fractions were carried out according to the standard methods described by Trease and Evans [24]. The plant extract was screened for the

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presence of different classes of compounds including alkaloids, flavonoids, steroids, triterpenes, anthraquinones, tannins, anthocyanins, saponins and polyphenols.

2.5 Microorganisms and *in vitro* antibacterial assays

2.5.1 Bacteria and growth condition

In this study, we investigated the antibacterial properties of plant extracts against Gram-positive (*Bacillus subtilis, Staphylococcus aureus* ATCC25923, methicillin sensitive *S. aureus* MSSA01, methicillin resistant *S. aureus* MRSA03, methicillin resistant *S. aureus* MRSA04) and Gram-negative (*Escherichia coli* S2 (1), *Pseudomonas aeruginosa* PA01, *Shigella flexneri* SDINT, *Pseudomonas aeruginosa*) bacteria. These microorganisms were collected from the Department of Biochemistry, University of Calcutta in India and from the Institute of Medical Mycology**,** Teikyo University in Japan. Nutrient agar was used for preservation and activation of the microorganisms.

2.5.2 Antibacterial assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute [25,26] with slight modifications. Each test sample was dissolved in dimethylsulfoxide (DMSO) and the solution was then added to Mueller Hinton Broth (MHB) to give a final concentration of 8192 µg/ml. Twofold serial dilutions were applied to this solution in MHB to afford concentration range of 8 - 4096 µg/ml. Then, 100 µl of each concentration were added in each well (96-well microplate) containing 95 µl of MHB and 5 µl of inoculums. The final concentrations of the plant extract/fraction ranged from 4 to 2048 µg/ml. The inoculum was standardized at 10^6 CFU/ml by adjusting the optical density to

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0.1 at 600 nm using a JENWAY 6105 UV/Vis spectrophotometer. The final concentration of DMSO in each well was less than 1% [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms]. The negative control wells consisted of 195 µl of MHB and 5 µl of the standard inoculum, whereas dilutions of amoxicillin (Sigma-Aldrich, Steinheim, Germany) and ciprofloxacillin (Sigma-Aldrich, Steinheim, Germany) served as positive controls. The plates were covered with sterile lids, then the well contents were mixed using a plate shaker (Flow Laboratory, Germany) at 300 rpm followed by incubation of the plates at 37 °C for 24 h under shaking. The assay was repeated three times. The MIC values of samples were determined by adding 50 µl of a 0.2 mg/ml *p*-iodonitrotetrazolium (INT) violet solution followed by incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to a pink color. MIC values were defined as the lowest sample concentrations that prevented this change in color indicating a complete inhibition of microbial growth.

For the determination of MBC values, a portion of liquid (10 µl) from each well that showed no growth of microorganism was plated on Mueller Hinton Agar (MHA) and incubated at 37 °C for 24 h . The lowest concentrations of the extracts that yielded no growth after this subculturing were taken as the MBC values.

2.5.3 Combined effect of the MeOH extract of *D. edulis* leaf and amoxicillin.

The interaction between the MeOH extract of *D. edulis* leaf (the most active extract) and amoxicillin was performed by using the broth microdilution method as described above. The antibacterial activities of the MeOH extract of *D. edulis* leaf in the presence of amoxicillin (1/8xMIC and ½xMIC) and that of amoxicillin in the presence of MeOH extract of *D. edulis* leaf (1/8xMIC and ½xMIC) were evaluated as described above. The preliminary tests allow the selection of MIC/8 and MIC/2 as the sub-inhibitory concentrations of the samples. The fractional

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inhibitory concentration (FIC) index for combinations of two antibacterial agents was calculated according to the following equation: FIC index = FIC A + FIC E; where FIC A = MIC of antibiotic in combination / MIC of antibiotic alone; FIC E = MIC of the extract in combination / MIC of the extract alone. The FIC indices were interpreted as follows: ≤ 0.5, synergy; > 0.5 to 1, addition; > 1 and ≤ 4, indifference and > 4, antagonism [27]. All the experiments were performed in triplicate.

2.5.4 Combined effect of extracts and serum.

Two Wistar albino rats fasted for 24 hours were used for serum preparation. The animals were anesthetized with chloroform vapors and sacrificed. The blood was then collected by cardiac puncture, introduced into dry tubes and allowed to stand for 4 hours in the ice. The clothed blood was then centrifuged at 3500 rpm for 15 min. The clear yellowish supernatant was carefully collected and stored in sterile tubes at -20 °C until use.

The combined effect of extracts and serum was performed by using the broth microdilution method. The Mueller Hinton broth (MHB) was prepared, sterilized and cooled to room temperature; then supplemented with rat serum at concentrations of 2.5% and 5%. The supplemented MHB was used to determine the new MIC values of the extracts as previously described.

3. RESULTS

Phytochemical analysis indicated variation of the phyto-constituents in the extracts according to the plant species and the extraction solvent (Table 2). Alkaloids, polyphenols, flavonoids, anthraquinones and triterpenes were found in all the plant extracts. The other classes of

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compounds (anthocyanines, triterpenes, steroids and saponines) were selectively distributed in the extracts. The methanol extract of *D. edulis* leaves contained all classes of secondary metabolites studied which are differently distributed in its fractions.

The antibacterial activity was evaluated by determining the MIC and MBC values of methanol extracts and fractions against multi-drug-resistant pathogenic bacteria (Tables 3 and 4). The analysis of Table 3 showed that the plant extracts displayed distinct antibacterial activities against the tested bacterial strains, with MICs ranging from 256 to 2048 µg/ml for the extracts and 128 to 2048 µg/ml for the fractions. The methanol extract of *D. edulis* leaves (MIC = 256 - 1024 µg/ml) was the most active followed in decreasing order by *C. sepium* (MIC = 512 - 2048 µg/ml), *D. edulis* stem barks (MIC = 512 - > 2048 µg/ml) and *C. papaya* roots (MIC = 1024 - > 2048 µg/ml). The fractionation of the methanol extract of *D. edulis* leaves did not enhance its activity and unequally distributed the antibacterial activities in different fractions. Hence, the hexane fraction is the least active (MIC = 1024 - 2048 µg/ml) whereas the ethyl acetate fraction (MIC = 256 - 1024 µg/ml) and the residual fraction were the most active (MIC = 128-1024 µg/ml). The lowest MIC value (128 µg/ml) corresponding to the highest activity was obtained with the residual fraction against *S. aureus* MRSA03. The most resistant bacteria to all tested samples were *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. flexneri* and *P. aeruginosa* PA01 whereas *S. aureus* MRSA04 was the most sensitive micro-organism. The activities of extracts and fractions were lower than those obtained with the reference antibiotics (amoxicillin and ciprofloxacin).

On the basis of MBC values, the antibacterial activities of the studied plants also varied according to the extracts, fractions and tested microorganisms (Table 4). The MBC values of the extracts and fractions ranged between 128 µg/ml and 2048 µg/ml. The lowest MBCs corresponding to the highest bactericidal activities were obtained with the methanol extract of *D.*

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edulis leaves (256 µg/ml) against *S. aureus* MSSA01 and MSSA04; with the ethyl acetate fraction of *D. edulis* leaves (256 µg/ml) vis-à-vis *S. aureus* MRSA03 and with the residual fraction on *P. aeruginosa* PA01 (256 µg/ml) and *S. aureus* MRSA03 (128 µg/ml). Analysis of Tables 3 and 4 also showed that some extracts/fractions had the same MIC and MBC values with respect to the same bacterial species. This is the case for the MeOH extract of *D. edulis* leaves against *B. subtilis* (512 µg/ml), *E. coli* (512 µg/ml), *P. aeruginosa* (512 µg/ml) and *S. aureus* MRSA04 (256 µg/ml); for the MeOH extract of *C. sepium* against *B. subtilis* (256 µg/ml), *E. coli* (256 µg/ml) and *S. aureus* MRSA04 (1024 µg/ml); for the MeOH extract of *C. papaya* roots on *S. aureus* strains MRSA01 (512 µg/ml), MRSA03 (1024 µg/ml) and MRSA04 (512 µg/ml); for the ethyl acetate fraction of *D. edulis* leaves against *B. subtilis* (1024 µg/ml), *P. aeruginosa* (512 µg/ml), *S. flexneri* (512 µg/ml), *S. aureus* MRSA01 (512 µg/ml), MRSA03 (256 µg/ml) and MRSA04 (512 µg/ml); for the residual fraction on *P. aeruginosa* (1024 µg/ ml), *S. flexneri* (1024 µg/ml), *P. aeruginosa* PA01 (256 µg/ml), *S. aureus* MRSA03 (128 µg/ml) and MRSA04 (1024 µg/ml).

The effect of the interaction between the MeOH extract of *D. edulis* leaves and amoxicillin was studied and the results are depicted in Tables 4-6. With the exception of *S. aureus* MRSA04, the MIC values of the MeOH extract of *D. edulis* in combination with amoxicillin at its ½ and 1/8 of MIC values are smaller than that of the extract used alone; suggesting an increase in the activity of this extract in combination with amoxicillin (Table 5).

In general, the MIC values of amoxicillin in combination with the MeOH extract of *D. edulis* leaves at ½ and 1/8 of MIC are smaller than those of amoxicillin alone; suggesting an increase in the activity of amoxicillin in combination with the extract at ½ and 1/8 of MIC (Table 6).

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The study of association between MeOH extract of *D. edulis* leaves and amoxicillin indicated that addition of amoxicillin exhibited indifference effects against *S. aureus* MRSA04, synergistic effects on *S. flexneri* and *S. aureus* MRSA03 as well as additive effects vis-à-vis *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *P. aeruginosa* PA01 and *S. aureus* MSSA01 (Table 7).

The effect of different concentrations of serum was evaluated on the activity of plant extracts (Table 8). The results obtained show that the MIC values of plant extracts determined in the presence of the serum are generally smaller than those determined in the absence of the serum; suggesting an increase in the antibacterial activity of these extracts in the presence of the serum. In most cases, these activities increased with the concentration of serum in the medium, except for *C. sepium* extracts against *B. subtilis* (at 2.5% of the serum); *D. edulis* (leaves) against *B. subtilis*, *S. aureus* MRSA04 (at 2.5% of the serum), *S. aureus* MRSA01 and MRSA04 (at 5% of the serum); *C. papaya* on *S. aureus* MRSA01 (at 2.5 and 5% of the serum) where the antibacterial activities have decreased. In some cases, the MICs of the extracts remained constant whatever the concentration of the serum in the culture medium.

4. DISCUSSION

The phytochemical analysis of the tested plant extracts was carried out with the aim of correlation the presence of different classes of secondary metabolites to their antibacterial properties. Thus, the differences in chemical composition of the extracts and fractions may be due to the plant species, plant organ and/or extraction solvent used. The methanol extracts from the leaves and stem barks of *D. edulis* contain phenols, alkaloids, flavonoids, triterpenes, tannins, saponins and anthraquinones. These results corroborate those of Okwu and Nnamdi [28] who showed the presence of alkaloids, tannins, phenols, flavonoids and saponins in the extracts of the stems of this plant. The phytochemical analysis of *C. papaya* revealed the presence of alkaloids,

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flavonoids, triterpene and anthraquinones, but also the absence of saponins, steroids, tannins, polyphenols and anthocyanins. Early report demonstrated the presence of carbohydrates, saponins, tannins, flavonoids and terpenoids as well as the absence of alkaloids, glycosides, proteins and amino acids in the chloroform, ethyl acetate, ethanol and methanol extracts of the leaves of this plant [29]. Differences in antibacterial activity were noted between the crude extracts and fractions. These differences may be due to the different classes of secondary metabolites contained in these extracts/fractions. Indeed, according to Reuben et al. [30], the antimicrobial activity of medicinal plants is correlated with the presence in their extracts of one or more classes of bioactive secondary metabolites**.** This would justify the case of the methanol extract of *D. edulis* leaves, which was the most active and contained all other classes of secondary metabolites. These phytochemical constituents grouped into three main classes namely alkaloids, terpenoids and phenolic compounds have been reported for their antibacterial properties [31-33].

The findings of the present study showed that there were differences between the antibacterial activities of the methanol extract of *D. edulis* leaves and those of fractions. This suggests that the methanol extract of *D. edulis* leaves contains several antibacterial principles with different polarities as shown by the phytochemical study. The fractionation of the methanol extract of *D. edulis* leaves did not enhance its activity and unequally distributed the antibacterial activities in different fractions. Hence, the hexane fraction was the least active while the ethyl acetate fraction and the residual fraction were the most active. This indicates that the active principles might be more concentrated in the ethyl acetate and residual fractions and more diluted in the hexane fraction. These results corroborate those obtained by Noumedem et al. [34], who showed that the ethyl acetate and aqueous residual fractions from the methanol extract of *Acalypha manniana* leaves had more antimicrobial activity than the hexane fraction.

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The differences in susceptibility observed between the different bacterial species, which are related to their genetic make-up, indicated not only that these extracts and fractions have limited spectra of activity, but also that bacterial infections must be properly diagnosed given that two different pathogens can cause the same symptoms (diarrhea, vomiting, asthenia) while they are not affected in the same way by antibiotics [35]. This is very important because very often, it is inadequate treatments that are the major source of certain forms of acquired resistance of microorganisms to antibiotics [36].

According to Tamokou et al. [37], a plant extract is considered to be highly active if the MIC < 100 µg/ml; significantly active when 100 ≤ MIC ≤ 512 µg/ml; moderately active when 512 <MIC ≤ 2048 µg/ml; weakly active if MIC > 2048 µg/ml and not active when MIC > 10 mg/ml. Hence, the tested extracts and fractions were significantly active (100 ≤ MIC ≤ 512 µg/ml), moderately active (512 < MIC ≤ 2048 µg/ml) and/or weakly active (MIC > 2048 µg/ml) against the tested bacteria. These observations highlight the traditional use of the tested plant species in the treatment of urinary tract infections, especially those caused by the tested bacteria. The findings of the present study also showed that the MIC values were four times lesser than the MBCs on the corresponding (sensitive) bacteria, confirming the bactericidal effects of the concerned samples [38]. This is interesting in view of the perspective of developing new antibacterial drugs from plant sources.

The antibacterial activities of the methanol extract of *C. papaya* are in agreement with those of the literature [21,29,39]. The antibacterial activity of *D. edulis* (seeds and fruits) has been previously demonstrated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniaes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis*, *Proteus mirabilis* and *Proteus vulgaris* [40,41]. Similarly, the results on the antibacterial activities of *D. edulis* are comparable to those of Obame et al. [42] who reported antibacterial

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activities of the essential oils of the resins from this plant against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria innocua*, *Salmonella enterica*, *Shigella dysenteria*, *Staphylococcus aureus*, *Proteus mirabilis*, *Staphylococcus camorum*, *Candida albicans*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. However, to the best of our knowledge, this is the first report on the antibacterial activities of the methanol extracts/fractions from *C. sepium*, *D. edulis* and *C. papaya* against multi-drug-resistant pathogenic bacteria.

The combinations of the antibiotics can lead to synergistic effects especially during the therapy of bacterial infections. These combinations have been recognized as being able to delay the emergence of resistant strains of microorganisms [43,44]. The synergy effect between plant-derived compounds and antibiotics makes it possible to use antibiotics when their efficacy alone is reduced [31]. These observations could explain the evaluation of the antibacterial activity of the combination between amoxicillin and methanol extract of *D. edulis* leaves. Indeed, in addition to substances having direct antibacterial activity, it has been demonstrated that within the plants other substances can act as adjuvants by modulating the activity of antibacterial agents [45]. The polyphenols, as well as the flavonoids detected in most of these extracts, would be responsible to the potentiating activity observed. Indeed, several studies have shown that polyphenols and flavonoids may improve antibiotic activity against multi-drug-resistant bacterial strains [46,47].

The study of the effect of the serum on the antibacterial activity of an extract could be useful in defining optimal therapy [48]. In this study, we have observed in general a concentration-dependent increase in the antibacterial activity of the plant extracts in the presence of the serum. This result suggests, firstly, a synergistic effect between the constituents of the extracts and those of the serum and secondly that the chemical constituents of these extracts weakly bind to serum proteins. It is generally accepted that antibiotics that bind to serum proteins

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have a reduced antibacterial activity when tested *in vitro* in the presence of serum proteins since only the free drug is available for antibacterial activity [49-51]. *In vitro* synergy between antibiotics and serum components, including antibodies and complement proteins, has been reported [52-54]; and can also be expressed in patients' responses to antimicrobial chemotherapy. Our results corroborate those of Khedmal et al. [55] who showed that the antibacterial activities of cetrimide, NaOCl and chlorhexidine against *Enterococcus faecalis* were improved by the association with bovine serum compared to those of antiseptics used alone.

5. CONCLUSION

The findings of the present study showed that the methanol extracts of *C. sepium*, *D. edulis* and *C. papaya* all contain alkaloids, flavonoids, anthraquinones, tanins and polyphenols. The other classes of compounds (anthocyanines, triterpenes, steroids and saponines) were selectively distributed in these extracts. All the plant extracts exhibited antibacterial activities (MIC = 256 - 2048 µg/ml) that varied according to the bacterium and extract; confirming the traditional use of the studied plants in the treatment of urinary tract infections. The fractionation of the methanol extract of *D. edulis* leaves did not enhance its activity and unequally distributed the antibacterial activities in different fractions. Hence, the hexane fraction was the least active while the ethyl acetate fraction and the residual fraction were the most active. The interaction of extracts with serum resulted in a concentration-dependent-increase in the antibacterial activity of extracts. As a function of tested bacterial species, synergistic, additive and indifference effects between amoxicillin and the MeOH extract of *D. edulis* were observed. The overall results of this study indicate that the studied plants can be used to treat urinary tract infections caused by the tested bacteria, and therefore can be exploited to design toxicological and pre-clinical studies as well as further bio-guided fractionation.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Table 1. Botanical identification, parts used, extraction solvent/yield and traditional therapeutic indications of studied medicinal plants.

Scientific name (Family)	Voucher specimen	Part used; extraction solvent and yield	Traditional therapeutic indications
Calystegia sepium (L.) R. Br. (Convolvulacea)	14735/HNC	Aerial part; methanol; 5.89%	Diuretic, Disorders of bile discharge [18], laxative [19], urinary tract infections.
Carica papaya L. (Caricaceae),	18647/SRF-CAM	root; methanol; 4.05%	Typhoid, urinogenital disorder, Intestinal parasites, chronic skin ulcer, warts, cancer, tumors, nervous pains, hypotension, urinary tract infections, wound, malaria [20,21], gastrointestinal tract disease, oxidization of cholesterol, nausea and morning sickness, weight loss, looting of body immunity, recovery of kidney, dengue fever and menstrual irregularities in women, pest control [22].
Dacryodes edulis (G. Don) H. J. Lam. (Burseraceae)	18258/HNC	Stem bark and leaves; methanol; 5.3% and 9.53%	Malaria, fever, gastrointestinal tract infections, headache, dysentery, anemia, hemorrhoids and urinary tract infections [23].

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Table 2. Main groups of compounds present in the plant extracts and fractions

Phytochemical groups	MeOH extract of *C. sepium* (aerial part)	MeOH extract of *C. papaya* (root)	MeOH extract of *D. edulis* (stem bark)	MeOH extract of *D. edulis* (leaf)	Hexane fraction of *D. edulis* (leaf)	Ethyl acetate fraction of *D. edulis* (leaf)	Residual fraction of *D. edulis* (leaf)
Alcaloids	+	+	+	+	+	+	-
Polyphenols	+	+	+	+	-	+	+
Flavonoids	+	+	+	+	-	+	-
Anthraquinones	+	+	+	+	-	-	+
Anthocyanins	+	-	-	+	+	+	-
Tanins	+	-	+	+	-	-	+
Triterpenes	+	+	+	+	+	-	-
Steroids	+	-	-	+	+	-	-
Saponins	-	-	+	+	-	-	+

(-): Absent ; (+): Present

Table 3. Minimum inhibitory concentrations (in µg/ml) of plant extracts and fractions according to the bacterial species

Bacteria	MeOH extract of C. *sepium* (aerial part)	MeOH extract of *D. edulis* (leaf)	Hexane fraction *D. edulis* (leaf)	Ethyl acetate fraction *D. edulis* (leaf)	Residual fraction *D. edulis* (leaf)	MeOH extract of *D. edulis* (stem bark)	MeOH extract of *C. papaya* (root)	Amoxicillin	Ciprofloxacin
B. subtilis	512	512	2048	1024	512	>2048	>2048	32	1
S. aureus	512	512	2048	512	512	512	>2048	1	4
S. aureus SASM01	1024	256	2048	512	512	>2048	1024	4	1
S. aureus SARM03	2048	1024	2048	256	128	1024	>2048	16	1
S. aureus SARM04	1024	256	1024	512	1024	512	2048	16	2
P. aeruginosa PA01	1024	512	2048	512	256	>2048	>2048	8	1
P. aeruginosa	512	512	2048	512	1024	>2048	>2048	128	2
E. coli	512	512	1024	512	512	>2048	>2048	64	1
S. flexneri	2048	1024	2048	512	1024	>2048	>2048	1	16

Table 4. Minimal bactericidal concentrations (in µg/ml) of plant extracts and fractions according to the bacterial species

Bacteria	MeOH extract of *C. sepium* (aerial part)	MeOH extract of *D. edulis* (leaves)	Hexane fraction *D. edulis* (leaves)	Ethyl acetate fraction *D. edulis* (leaves)	Residual fraction *D. edulis* (leaves)	MeOH extract of *D. edulis* (stem bark)	MeOH extract of *C. papaya* (root)	Amoxicillin	Ciprofloxacin
B. subtilis	512	512	>2048	1024	1024	CMB	>2048	32	128
S. aureus SASM01	1024	256	>2048	512	1024	>2048	>2048	8	2
S. aureus SARM03	>2048	2048	>2048	256	128	1024	>2048	16	64
S. aureus SARM04	1024	256	2048	512	1024	512	>2048	16	64
S. aureus	512	512	>2048	1024	1024	>2048	>2048	1	64
E. coli	512	512	2048	1024	1024	>2048	>2048	64	64
P. aeruginosa PA01	>2048	512	>2048	1024	256	512	>2048	8	1
P. aeruginosa	>2048	512	>2048	512	1024	>2048	>2048	256	256
S. flexneri	>2048	2048	>2048	512	1024	>2048	>2048	4	128

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Table 5. Antibacterial activity of the methanol extract of *D. edulis* in the presence of amoxicillin at ½ and 1/8 of MIC according to the tested bacteria

Bacteria	MeOH extract of *D. edulis* leaf alone	MeOH extract of *D. edulis* leaf with amoxicillin at 1/8 of MIC		MeOH extract of *D. edulis* leaf with amoxicillin at ½ of MIC	
	MIC	MIC	FIC	MIC	FIC
B. subtilis	256	128	0.5	128	0.5
S. aureus	512	256	0.5	256	0.5
S. aureus SASM01	512	256	0.5	256	0.5
S. aureus SARM03	1024	256	0.25	256	0.25
S. aureus SARM04	256	256	1	256	1
E. coli	512	256	0.5	256	0.5
P. aeruginosa	512	256	0.5	256	0.5
P. aeruginosa PA01	512	256	0.5	256	0.5
S. flexneri	1024	512	0.5	128	0.125

MIC: minimum inhibitory concentration in µg / ml; FIC: Fractional Inhibitory Concentration

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Table 6. Antibacterial activity of the amoxicillin in the presence of the MeOH extract of *D. edulis* leaf at ½ and 1/8 of MIC according to the tested bacteria

Bacteria	Amoxicillin alone	Amoxicillin with MeOH extract of *D. edulis* leaf at 1/8 of MIC		Amoxicillin MeOH extract of *D. edulis* leaf at ½ of MIC	
	MIC	MIC	FIC	MIC	FIC
B. subtilis	32	16	0.5	8	0,25
S. aureus	1	0,5	0,5	0,25	0,25
S. aureus SASM01	128	64	0,5	32	0,25
S. aureus SARM03	8	2	0,25	1	0,125
S. aureus SARM04	4	2	0,5	1	0,25
E. coli	64	64	1	16	0,25
P. aeruginosa	128	64	0,5	16	0,125
P. aeruginosa PA01	1	0,5	0,5	0,25	0,25
S. flexneri	128	64	0,5	16	0,125

MIC: minimum inhibitory concentration in µg/ml; FIC: Fractional Inhibitory Concentration

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Table 7. Fractional inhibitory concentration (FIC) indices calculated for the combination amoxicillin and MeOH extract of *D. edulis* leaf with respect to the studied bacteria.

Bacteria	∑ FIC	Interpretation
B. subtilis	0,75	Additive
S. aureus	0,75	Additive
S. aureus SASM01	0,75	Additive
S. aureus SARM03	0,375	Synergy
S. aureus SARM04	1,25	Indifference
E. coli	0,75	Additive
P. aeruginosa	0,625	Additive
P. aeruginosa PA01	0,75	Additive
S. flexneri	0,25	Synergy

Tableau 8. Effect of the various concentrations of the serum on the antibacterial activity of MeOH extracts of studied plants (MIC in µg/ml)

Bacteria	Serum at 0%				Serum at 2.5%				Serum at 5%			
	C. sepium (aerial part)	*D. edulis* (leaf)	*D. edulis* (stem bark)	*C. papaya* (root)	*C. sepium* (aerial part)	*D. edulis* (leaf)	*D. edulis* (stem bark)	*C. papaya* (root)	*C. sepium* (aerial part)	*D. edulis* (leaf)	*D. edulis* (stem bark)	*C. papaya* (root)
B. subtilis	512	512	-	-	1024	1024	256	-	512	512	-	2048
S. aureus	512	512	512	-	512	256	512	2048	512	256	256	-
S. aureus SASM01	1024	256	-	1024	256	512	256	2048	512	1024	128	2048
S. aureus SARM03	2048	1024	1024	-	256	256	512	1024	512	256	128	2048
S. aureus SARM04	1024	256	512	2048	512	256	512	2048	1024	2048	256	2048
E. coli	512	512	-	-	256	256	256	-	256	512	512	2048
P. aeruginosa	512	512	-	-	512	256	1024	-	32	64	16	
P. aeruginosa PA01	1024	512	-	-	256	512	1024	-	128	256	256	2048
S. flexneri	2048	1024	-	-	256	1024	1024	-	512	512	16	1024

- : MIC > 2048 µg/ml