Antibacterial Screening of Fifteen Cameroonian Medicinal Plants against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*

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Abstract: Multi-drug resistant microbial infections caused by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* represent a critical public health issue worldwide. Plant-derived agents are credible sources for search and development of alternative antimicrobials to fight these infections. This study describes the *in-vitro* antibacterial activity of plants extracts from 15 medicinal plants of the Cameroonian pharmacopeia against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Plants selected based on their ethnopharmacological uses were extracted by either maceration or decoction using distilled water, ethanol, methanol and ethylacetate. The afforded extracts were phytochemically screened for bioactive secondary metabolites. The microdilution method was used to assess the antibacterial activity. The two promising extracts were tested in combination using the checkboard technique. Eighty one extracts showed antibacterial activity with eight having minimum inhibitory concentrations below 2.5 mg/mL. Results from combination assays with extracts from *Terminalia catappa* and *Gnidia glauca* leaves showed synergistic effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* and additive effect against *Staphylococcus aureus*. These extracts mainly contained saponins, tannins, glucosides, phenols, triterpenes and anthraquinones. These results support the ethnobotanical claims and indicate further directions for the investigation of plants extracts to develop alternative drugs against multi-resistant bacteria.

Key words: Antibacterial activity, *Terminalia catappa*, *Gnidia glauca*, combination, phytochemical screening.

1. Introduction

Multi-drug resistant microbial infections caused by Gram-positive bacteria (such as *Staphylococcus aureus*) and Gram-negative (such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) represent an exponentially growing health problem affecting communities worldwide [1-3]. In recent years, they emerged as an important nosocomial pathogen due to multiple drug resistant mechanisms [4]. They cause a variety of infections that include pneumonia, wound, urinary tract, bloodstream and intra-abdominal

infections [5]. The Gram-positive *Staphylococcus aureus*, when pathogenic, is a common cause of skin infections (e.g., boils), respiratory disease (e.g., sinusitis) and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *Staphylococcus aureus* (e.g., MRSA (methicillin-resistant *Staphylococcus aureus*)) is a worldwide problem in clinical medicine. The following Gram-negative bacteria also exact a heavy toll. Virulent strains of *Escherichia coli* can cause gastroenteritis, urinary tract infections and neonatal

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meningitis. In Cameroon, these infections are increasing with children being the most affected. In rarer cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia. *Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora and most man-made environments throughout the world. As an opportunistic, nosocomial pathogen of immunocompromised individuals, *Pseudomonas aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections. *Salmonella* bacteria are zoonotic and can be transferred between humans and other animals. Many infections are due to ingestion of contaminated food. *Salmonella*, such as *Salmonella enterica* subsp. *enterica* serovar *typhi*, can cause serious illness. *Salmonella typhi* is adapted to humans and does not occur in other animals [6-9]. The used synthetic antibiotics are costly and are out of range from the patient belonging to developing countries, the microorganism develop resistance against antibiotics and the antibiotics may be associated with adverse effects on the host, including hypersensitivity, immune suppression and also allergic reactions. Due to the side effects and the resistance built by pathogens against antibiotics, much attention has been paid to extract and bioactive compounds from plants used in herbal medicine [5, 10]. Cameroon has a rich biodiversity and established tradition in the use of herbal medicine for the treatment of various diseases [11]. The screening of plant extracts and phytochemicals for antimicrobial activity has shown that higher plants, especially their secondary metabolites, are a source of structurally diverse bioactive compounds with a wide range of pharmacological activities [12, 13].

In a search for alternative medicines against bacterial infections, this study was designed to investigate the *in-vitro* antibacterial activity of extracts from 15 medicinal plants of the Cameroonian

pharmacopeia and their combination against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

2. Material and Methods

2.1 Collection and Extraction of Plant Materials

Plant materials were harvested and identified as *Azadirachta indica*, *Monodora tenuifolia*, *Terminalia catappa*, *Polyalthia suaveolens*, *Gnidia glauca*, *Uvaria muricata*, *Uvaria angolensis*, *Uvariadendron calophyllum*, *Monodora miristica*, *Hypericum lanceolatum*, *Piptostigma calophyllum*, *Sizygium aromaticum*, *Xanthozylum xanthozoloides*, *Gnidia glauca* and *Viscum album* at the National Herbarium of Cameroon, Yaoundé, where voucher specimens were deposited under specific reference numbers. Plants reference numbers, traditional uses, place of collection and previous studies are summarized in Table 1.

100 g of ground plant material from each dried powder of *Azadirachta indica*, *Monodora tenuifolia*, *Terminalia catappa*, *Viscum album*, *Gnidia glauca*, *Uvaria muricata*, *Uvaria angolensis*, *Uvariadendron calophyllum*, *Monodora miristica*, *Hypericum lanceolatum*, *Polyalthia suaveolens* and *Piptostigma calophyllum* were macerated in 1 L capacity glass bottle using distilled water, 95% ethanol, 70% ethanol and methanol. *Sizygium aromaticum*, *Xanthozylum xanthozoloides* and *Gnidia glauca* were extracted by decoction. *Viscum album* was also macerated in ethyl acetate. The samples were periodically stirred in the mornings and evenings per day using a mechanical shaker. The extracts were filtered using filter paper (Whatman paper No. 1), and concentrated under vacuum using a rotary evaporator (Rotavapor BUCHI 071). Water extract were dried under ventilation. The extracts obtained were stored at 4 °C in the refrigerator until bioassay.

2.2 Phytochemical Screening

The extracts with promising activity were subjected to phytochemical screening to detect the presence of

Table 1 Summary of plants, used parts and extraction.

Plants and reference number	Family	Local name	Collection site	Traditional use	Previous studies	Used parts	Solvent	Method	Crude extracts code
<i>Azadirachta indica</i> 19223SRFK	Meliaceae	Neem tree	Yaoundé	Malaria, bacterial infections; Fungal infections and viral infections; Skin diseases	Antibacterial activities	Stembark; Stem; Leaf	Water; Ethanol; Water-ethanol	Maceration; Maceration; Maceration	AlIH ₂ O; AlIH ₂ O/EtOH; AlsbEtOH; AlsbH ₂ O; AlstEtOH
<i>Gnidia glauca</i> 60120HNC	Thymelaeaceae	Tamil; Nacchi-nar	Dschang	Cancers, sore throat, abdominal pain, wounds, burns and snake bites. It is also used as molluscicidal, insecticidal, piscicidal and as arrow poisons [14].	Antioxidant [15] antidiabetic [16] and toxicity [17]	Leaf; Bark; Twigs	Hot water; Water	Decoction; Maceration	GGldecoc; GGbdecoc; GGH ₂ O; GGtwH ₂ O
<i>Hypericum lanceolatum</i> 14330SRFCAM	Hypericaceae		Dschang	Skin infections, epilepsies and tumours, while the roots are boiled in water and used to treat venereal diseases, gastrointestinal disorders and infertility [18].	Antimalarial activity [19]	Leaf; Flower; Twig	Hot water; Methanol	Decoction; Maceration	HLficedoc; HLIH ₂ O; HLJWMeOH; HLJNWMeOH; HLtw+stWMeOH; HLtw+st NWMeOH
<i>Monodora mirisica</i> 1927/SRFK	Annonaceae	African nutmeg, yellow-flowered nutmeg	Yaoundé	Witchcraft	Antimalarial activity [20]	Twigs	Water	Maceration	MMtw
<i>Monodora tenuifolia</i> 30549/HNC	Annonaceae	Ebo osé; Lakosin	Mount Kalla	Fever, dyscentery [21]; Used as spices, in the treatment of skin diseases	Antifungal activity; Antidiarrhea activities [22]; Antioxidant [23]	Seed; Pericarp; Pulp; Branch; Leaf	Water; Ethanol; Water-ethanol	Maceration; Maceration; Maceration	MTbr; MTbrEtOH; MTbrH ₂ O; MTbrH ₂ O/EtOH; MTI; MTIH ₂ O/EtOH; MTIEtOH; MTIH ₂ O; MTpe; MTpeH ₂ O/EtOH; MTpu; MTpu H ₂ O/EtOH; MTpuEtOH; MTpuH ₂ O; MTse; MTse H ₂ O; MTseH ₂ O/EtOH; MTseEtOH; MTtw; MTtwH ₂ O/EtOH; MTtwEtOH; MTtwH ₂ O

(Table 1 continued)

Plants and reference number	Family	Local name	Collection site	Traditional use	Previous studies	Used parts	Solvent	Method	Crude extracts code
<i>Piptostigma calophyllum</i> 38333HNC	Annonaceae		Mount Kalla		Antifungal and antioxidant activity [24]	Leaf	Ethanol	Maceration	PCIEOH
<i>Polyalthia suaveolens</i> 1227/SRF/CAM	Annonaceae	Afoumengen	Mount Kalla	Dysmenorrhea	Antimalarial activity [25]	Leaf Stem bark Trunk	Ethanol	Maceration	PSIEtOH; PSSbEtOH; PStrEtOH
<i>Syzgium aromaticum</i> 506167HNC	Myrtaceae	Clove	Yaoundé	Used as spices, toothache, wounds, cystitis, kidney, infection of the gastrointestinal tract [26]	Antifungal activity [27, 28]	Grains	Water	Maceration	SAse
<i>Terminalia catappa</i> 51244/HNC	Combretaceae	Badamier; Cocoma	Yaoundé	Diarrhoea, Amygdalites, skin diseases [29]	Antibacterial, antioxidant and Antifungal [30-34]	Leaf; Stem bark; Stem	Water; Ethanol; Water-ethanol	Maceration; Maceration; Maceration	TCIH ₂ O; TCIH ₂ O; TCIH ₂ O/EtOH; TCSbH ₂ O; TCSbH ₂ O; TCSbH ₂ O/EtOH; TCtEtOH; TCSH ₂ O/EtOH
<i>Uvaria angolensis</i> HNC6415/SRF/CA M	Annonaceae		Mount Kalla	Dysentery, fatigue, sénescence, abdominal pains	Antifungal activity [35]; Antioxidant and antiplasmodial activities [20]	Leaf; Twigs; Stem bark	Ethanol	Maceration	UAEtOH; UALI; UALI; UAsEtOH; UAstI; UAwEtOH; UAwI
<i>Uvariadendron calophyllum</i> 28734/SFR/CAM	Annonaceae	Obom ossoé (Ewondo)	Mount Kalla		Antifungal and antioxidant activity [24]; Antiplasmodial activities [20]	Leaf Twigs Stem bark	Water Ethanol	Maceration; Maceration	UCIEtOH; UCIH ₂ O; UCsbH ₂ O; UCstH ₂ O; UCtwEtOH; UCtwH ₂ O
<i>Uvariadendron molundense</i> 41685HNC	Annonaceae	Ojobilobe	Mount Kalla		Antifungal and Antioxidant activity [24]	Twigs	Ethanol	Maceration	UMtwEtOH
<i>Uvaria muricata</i> HNC6415/SRF/CA M	Annonaceae		Mount Kalla	Dysentery, fatigue, sénescence, abdominal pains	Antifungal activity [35]	Leaf; Twigs; Stem bark	Ethanol	Maceration	UMulEtOH; UMustEtOH; UMustI; UMutwEtOH; UMutwI

(Table 1 continued)

[illegible]

saponins, tannins, flavonoids, glucosides, phenols, alkaloids, triterpenes, anthocyanins, anthraquinones and steroids using protocols described in Refs. [37, 38].

2.3 Antibacterial Assay

2.3.1 Preparation of Plant Extracts Stock Solution and Antibiotic

200 mg of different extracts was dissolved in 2 mL of DMSO 10% to reach a final concentration of 100 mg/mL. 250 mg of Chloramphenicol (Mission pharma, Pharmaceutical Co. Ltd.) was dissolved in 2.5 mL of DMSO 10% to reach 100 mg/mL. After preparation, the different stock solutions were sterilized by heating at 60 °C for 30 min.

2.3.2 Microbial Cultures and Inoculum Preparation

Gram-positive bacteria used for the antibacterial assay included *Staphylococcus aureus* CIP 7625 and Gram-negatives, *Pseudomonas aeruginosa* CIP 76110, *Salmonella typhi* and *Escherichia coli* ATCC 25922 obtained from patients unresponsive to antibiotic treatment of the Essos Hospital Centre, Yaoundé (Cameroon). They were grown and maintained on nutrient agar slants at 35 °C until used. They were then stored under aerobic conditions.

The inoculum was prepared from colonies collected from 18 h old bacterial culture with inoculating loop and suspended in sterile saline (0.9% NaCl) and homogenize. The turbidity of the solution were compared to a 2-McFarland standard and adjusted to a final density of 10^8 CFU/mL by dilution corresponding to 0.5-McFarland standards.

2.3.3 Determination of MIC (Minimal Inhibitory Concentration)

The MIC was determined according to CLSI (Clinical Laboratory Standards Institute) M07-A9 microdilution method, using 96 wells microtitre plates. 148 µL of Nutrient Broth “E” (Lab M Limited Topley House) was introduced in the wells of Column 1 (1A-1H), and 100 µL in the remaining wells. Later on, 52 µL of stock solution of plants extracts at

100 mg/mL were added to the first well. The medium and sample in the first well were mixed thoroughly and serially diluted by transferring 100 µL from wells of Column 1 to wells of Column 2, and so until Column 11. Plant extracts concentration range from 20 mg/mL to 0.01953 mg/mL. Chloramphenicol was used as positive control in each microtitre plate with the concentration ranging from 10 mg/mL to 0.09765 mg/mL. Thereafter, 20 µL of the inoculums were introduced each well containing the test substances except the blank consisting of wells in Column 12. After an incubation period at 37 °C for 24 h, turbidity was observed as indication of growth. Thus, the lowest concentration inhibiting the growth of bacteria was recorded as the MIC. Each experiment was performed in duplicates.

2.3.4 Effect of Increase Inoculums Size on MIC (Minimal Inhibitory Concentration)

The extracts with MIC of less than or equal to 5 mg/mL were used to explore the effect of inoculum size two times greater than that use in the first MIC determination (2×10^8 CFU/mL) using previously describe procedure.

2.4 Evaluation of Synergy between the Two More Active Plant Extracts

Decoction of *G. glauca* leaf and aqueous extract of *T. catappa* leaf active against the four strains were tested in combination. A two-dimensional, two-agent broth microdilution checkerboard technique was used to study the interaction between the two extracts. The FIC (fractional inhibitory concentration) was derived from the lowest concentration of extracts in combination permitting no visible growth of the test organisms. FICI (FIC index) is calculated as the sum of the FICs of each agent. A FICI of ≤ 0.5 indicates synergistic interaction; > 0.5 but ≤ 1 , additive interaction; > 1 but ≤ 4 , indifferent interaction; and > 4 , antagonistic interaction. The FIC of each agent is calculated as the MIC of the agent in combination divided by the MIC of the agent [39].

3. Results

The different results were statistically analysed using the software SPSS 17.0 for windows and variance analysis by ANOVA coupled with Turkey test, where $p < 0.05$ was considered as statistically significant.

3.1 Minimal Inhibitory Concentration of Extracts

The MIC ranged from 0.625 to 20 mg/mL (Table 2) and vary with plants materials, plants part and extraction procedure. The most active extract from these plant are ethanolic extract of *A. indica* stem, aqueous extract of *T. catappa* leaves and decoction of *G. glauca* leaves. This activity could be due to the nature of secondary metabolites present in the plant part and the solvent used for extraction. In fact, the capacity of extraction and solubilisation of solvent is different towards the secondary metabolites.

Twenty extracts inhibited the growth of the four strains at $\text{MIC} \leq 5\text{mg/mL}$. Out of 81 extracts tested eight (AIsbEtOH, AIstEtOH, GGldcoc, TCI water, TCH₂O/EtOH, TCIEtOH, TCsbEtOH and TCtwH₂O/EtOH) with MIC less or equal to 2.5 mg/mL on four strains were selected to determine the effect of double size inoculum (Table 3).

The MIC of the eight tested extracts ranged from 2.5 to 10 mg/mL (Table 3). Seven extracts inhibited the growth of the four strains with $\leq 5\text{ mg/mL}$ and one extract inhibited with 10 mg/mL on three strains. The extracts with $\text{MIC} \leq 2.5\text{ mg/mL}$ on the four strains were selected and tested for combination consisting of GGldcoc (decoction of *G. glauca* leaf) and TCIH₂O (aqueous extract of *T. catappa* leaf).

3.2 MIC (Minimal Inhibitory Concentration) of Extract Combination Tests

The checkerboard assay was conducted using the decoction of *G. glauca* leaf and aqueous extract of *T. catappa* leaf against bacteria strains (Table 4). This method supports evidence for synergy, competition or antagonism for selected compounds as it is reflected by

the FICI. A FICI of ≤ 0.5 indicates synergistic interaction. These plant extracts increased their respective antibacterial activity against the tested strains. Synergism effect was observed on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and additive effect on *Staphylococcus aureus* with the two plant extracts. The mean FIC indices ranged from 0.33 to 0.68 on the four bacteria strains (Table 4).

3.3 Phytochemical Composition of the Two Extracts

Phytochemical screening revealed the presence of saponins, tannins, glucosides, phenols, triterpenes and anthraquinones in both extracts. Alkaloids and steroids were present only in the aqueous extract of *T. catappa* leaf and flavonoids in the decoction of *G. glauca* leaf (Table 5).

4. Discussion

This study was carried out on various plant extracts chosen on the ethnopharmacological basis where their efficacy for century's usage was verified in many cases by scientific studies (Table 1). The antibacterial evaluation of 81 extracts from 15 plants revealed a significant potency against four bacterial strains which varied with plants, plant parts, extraction procedures and bacterial strains. Although it is evident that many constituents within plants interact, it should also be noted that other interactions may occur between groups of molecules from plant part or between different plants species. Besides, traditional healers in the world particularly in Africa often combine various plant part or plant species in order to enhance their efficacy by possible synergistic interaction between different groups of bioactive compounds.

T. catappa and *G. glauca* combination was tested against bacteria and the FICI values showed synergistic and additive effect on the four bacterial strains. These interactions (synergistic and additive) result from the combined effect of active constituents from each extract. Apart from flavonoids found in the aqueous extract of *T. catappa*, both extracts used in combination

Table 2 MIC (minimal inhibitory concentration) values of extracts against four bacteria (units in mg/mL).

Extracts	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
VAlAcet	20.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00	10.00 ± 0.00
VAstAcet	10.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
VAstEtOH	20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
VAstH ₂ O	10.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
VAstMeOH	20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
VAtrAcet	> 20.00 ± 0.00	> 20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
VAtrEtOH(50%)	> 20.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
VAtrEtOH(70%)	10.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
VAtrH ₂ O	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
VAtrMeOH	20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
AlIH ₂ O	10.00 ± 0.00	2.50 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
AlIH ₂ O/EtOH	10.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
AlsbEtOH	2.50 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	1.25 ± 0.00
AlsbH ₂ O	2.50 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	2.50 ± 0.00
AlstEtOH	2.50 ± 0.00	5.00 ± 0.00	1.25 ± 0.00	1.25 ± 0.00
GGbadecoc	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
GGIH ₂ O	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
GGldecoc	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
GGtwH ₂ O	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00
HLfdecoc	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
HLIH ₂ O	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
HLIWMeOH	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
HLINWMeOH	10.00 ± 0.00	20.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00
HLtw+stWMeOH	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
HyLtw+stSWMeOH	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
MMtwdecoc	20.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
MTbr	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
MTbrEtOH	10.00 ± 0.00	5.00 ± 0.00	1.25 ± 0.00	20.00 ± 0.00
MTbrH ₂ O	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00
MTbrH ₂ O/EtOH	10.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
MTI	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
MTIH ₂ O/EtOH	10.00 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	20.00 ± 0.00
MTIEtOH	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
MTIH ₂ O	5.00 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
MTpe	10.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
MTpeH ₂ O/EtOH	10.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
MTpu	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00
MTpuH ₂ O/EtOH	10.00 ± 0.00	> 20.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00
MTpuEtOH	> 20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	> 20.00 ± 0.00
MTpuH ₂ O	20.00 ± 0.00	20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
MTse	10.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
MTseH ₂ O	10.00 ± 0.00	20.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
MTseH ₂ O/EtOH	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
MTseEtOH	10.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
MTtw	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
MTtwH ₂ O/EtOH	5.00 ± 0.00	2.50 ± 0.00	5.00 ± 0.00	2.50 ± 0.00
MTtwEtOH	> 20.00 ± 0.00	> 20.00 ± 0.00	20.00 ± 0.00	> 20.00 ± 0.00
MTtwH ₂ O	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00
PCIEtOH	10.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	10.00 ± 0.00
PSIEtOH	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
PSsbEtOH	20.00 ± 0.00	20.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00
PStrEtOH	> 20.00 ± 0.00	20.00 ± 0.00	> 20.00 ± 0.00	20.00 ± 0.00
SAse	2.50 ± 0.00	1.25 ± 0.00	2.50 ± 0.00	10.00 ± 0.00
TCIH ₂ O	5.00 ± 0.00	1.25 ± 0.00	0.625 ± 0.00	2.50 ± 0.00

(Table 2 continued)

Extracts	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
TCIH ₂ O	1.25 ± 0.00	2.50 ± 0.00	1.25 ± 0.00	2.50 ± 0.00
TCIH ₂ O/EtOH	1.25 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
TCsbH ₂ O	2.50 ± 0.00	2.50 ± 0.00	1.25 ± 0.00	1.25 ± 0.00
TCsbH ₂ O	2.50 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
TCsbH ₂ O/EtOH	10.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00	5.00 ± 0.00
TCstEtOH	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	2.50 ± 0.00
TCstH ₂ O/EtOH	5.00 ± 0.00	1.25 ± 0.00	1.25 ± 0.00	2.50 ± 0.00
UCIEtOH	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
UCIH ₂ O	10.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
UCsbH ₂ O	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
UCstH ₂ O	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
UCtwEtOH	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	5.00 ± 0.00
UCtwH ₂ O	5.00 ± 0.00	2.50 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
UMtwEtOH	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
UMuLEtOH	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
UMustEtOH	10.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
UMustI	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00
UMutwEtOH	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00	> 20.00 ± 0.00
UMutwI	20.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
UALEtOH	10.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	10.00 ± 0.00
UALI	10.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
UALII	2.50 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
UAstEtOH	20.00 ± 0.00	5.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00
UAstI	5.00 ± 0.00	10.00 ± 0.00	5.00 ± 0.00	20.00 ± 0.00
UAtwEtOH	10.00 ± 0.00	10.00 ± 0.00	2.50 ± 0.00	> 20.00 ± 0.00
UAtwI	10.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	10.00 ± 0.00
ZXse	5.00 ± 0.00	2.50 ± 0.00	5.00 ± 0.00	10.00 ± 0.00
Chloramphenicol	2.50 ± 0.00	> 10.00 ± 0.00	> 10.00 ± 0.00	5.00 ± 0.00

Results are expressed as mean ± standard deviation; AIIH₂O: aqueous extract of *A. indica* leaf; AIIH₂O/EtOH: hydroethanolic extract of *A. indica* leaf; AIsbEtOH: ethanolic extract of *A. indica* stem bark; AIsbH₂O: aqueous extract of *A. indica* stem bark; AIsEtOH: ethanolic extract of *A. indica* stem; GGIdecoc: decoction of *G. glauca* leaf; GGBadecoc: decoction of *G. glauca* bark; GGIH₂O: aqueous extract of *G. glauca* leaf; GGtwh₂O: aqueous extract of *G. glauca* twig; HLfIdecoc: decoction of *H. lanceolatum* flower; HLH₂O: aqueous extract of *H. lanceolatum* leaf; HLWMeOH: methanolic extract *H. lanceolatum* leaf west; HLNWMeOH: methanolic extract *H. lanceolatum* leaf northwest; HLTw+stWMeOH: methanolic extract *H. lanceolatum* twig+stem west; HLTw+stNWMeOH: methanolic extract *H. lanceolatum* twig+stem northwest; MMTw: decoction of *M. miristica* twig; MTbr: *M. tenuifolia* branch; MTbrEtOH: ethanolic extract of *M. tenuifolia* branch; MTbrH₂O: aqueous extract of *M. tenuifolia* branch; MTbrH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* branch; MTI: *M. tenuifolia* leaf; MTIH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* leaf; MTIEtOH: ethanolic extract of *M. tenuifolia* leaf; MTIH₂O: aqueous extract of *M. tenuifolia* leaf; MTpe: *M. tenuifolia* pericarp; MTpeH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* pericarp; MTpu: *M. tenuifolia* pulpe; MTpuH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* pulp; MTpuEtOH: ethanolic extract of *M. tenuifolia* pulp; MTpuH₂O: aqueous extract of *M. tenuifolia* pulp; MTse: *M. tenuifolia* seed; MTseH₂O: aqueous extract of *M. tenuifolia* seed; MTseH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* seed; MTseEtOH: ethanolic extract of *M. tenuifolia* seed; MTtw: *M. tenuifolia* twig; MTtwH₂O/EtOH: hydroethanolic extract of *M. tenuifolia* twigs; MTtwEtOH: ethanolic extract of *M. tenuifolia* twigs; MTtwH₂O: aqueous extract of *M. tenuifolia* twigs; PCIEtOH: ethanolic extract of *P. calophyllum* leaf; PSIEtOH: ethanolic extract of *P. suaveolens* leaf; PSsbEtOH: ethanolic extract of *P. suaveolens* stem bark; PStrEtOH: ethanolic extract of *P. suaveolens* trunk; SAsH₂O: aqueous extract of *S. aromaticum* seed; TCIH₂O: aqueous extract of *T. catappa* leaf; TCIH₂O: aqueous extract of *T. catappa* leaf; TCIH₂O/EtOH: hydroethanolic extract of *T. catappa* leaf; TCsbH₂O: aqueous extract of *T. catappa* stem bark; TCsbEtOH: ethanolic extract of *T. catappa* stem bark; TCsbH₂O/EtOH: hydroethanolic extract of *T. catappa* stem bark; TCstEtOH: ethanolic extract of *T. catappa* stem; TCstH₂O/EtOH: hydroethanolic extract of *T. catappa* stem bark; UAIEtOH: ethanolic extract of *U. angolensis* leaf; UAI: aqueous fraction of *U. angolensis* leaf; UAIII: hexanic fraction of *U. angolensis* leaf; UAstEtOH: ethanolic extract of *U. angolensis* stem; UAstI: aqueous fraction of *U. angolensis* stem; UAtwEtOH: ethanolic extract of *U. angolensis* twigs; UAtwI: aqueous fraction of *U. angolensis* twigs; UCIEtOH: ethanolic extract of *U. calophyllum* leaf; UCIH₂O: aqueous extract of *U. calophyllum* leaf; UCsbH₂O: aqueous extract of *U. calophyllum* stem

bark; UCstH₂O: aqueous extract of *U. calophyllum* stem; UCtwEtOH: ethanolic extract of *U. calophyllum* twigs; UCtwH₂O: aqueous extract of *U. calophyllum* twigs; UMTwEtOH: ethanolic extract of *U. Molundense* twigs; UMEtOH: ethanolic extract of *U. muricata* leaf; UMEtOH: ethanolic extract of *U. muricata* stem; UMEtOH: aqueous fraction of *U. muricata* stem; UMEtOH: ethanolic extract of *U. muricata* twigs; UMEtOH: aqueous fraction of *U. muricata* twigs; VAlAcet: ethyl acetate extract of *V. album* leaf; VAlAcet: ethyl acetate extract of *V. album* stem; VAlEtOH: ethanolic extract of *V. album* stem; VAlH₂O: aqueous extract of *V. album* stem; VAlMeOH: methanolic extract of *V. album* stem; VAlAcet: ethyl acetate extract of *V. album* trunk; VAlEtOH(50%): hydroethanolic extract of *V. album* trunk (v/v); VAlEtOH(70%): hydroethanolic extract of *V. album* trunk; VAlH₂O: aqueous extract of *V. album* trunk; VAlMeOH: methanolic extract of *V. album* trunk; ZXse: aqueous extract of *Z. xanthoxoloides* seed.

Table 3 MIC values of the eight extracts on double inoculum size of the four bacteria strains.

Extracts	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
AlsbEtOH	5.00 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	5.00 ± 0.00
AlstEtOH	5.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
GGLdecoc	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
TCIH ₂ O	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
TCIH ₂ O/EtOH	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	2.50 ± 0.00
TCIEtOH	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
TCsbEtOH	5.00 ± 0.00	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
TCstH ₂ O/EtOH	5.00 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00

Results are expressed as mean ± standard deviation; TCIH₂O: aqueous extract of *T. catappa* leaf; TCIH₂O/EtOH: hydroethanolic extract of *T. catappa* leaf; TCsbEtOH: ethanolic extract of *T. catappa* stem bark; TCstH₂O/EtOH: hydroethanolic extract of *T. catappa* stem bark; AlsbEtOH: ethanolic extract of *A. indicastem* bark; AlstEtOH: ethanolic extract of *A. indicastem*; GGLdecoc: decoction of *G. glauca* leaf.

Table 4 FICI of GGLdecoc/TCIH₂O combination against four bacteria strains.

Four bacteria strains					
<i>Staphylococcus aureus</i>	FIC GGLdecoc	0.25 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
	FIC TCIH ₂ O	0.50 ± 0.00	0.25 ± 0.00	0.13 ± 0.00	1.00 ± 0.00
	FICI (ΣFIC)	0.75 ± 0.00	0.75 ± 0.00	0.63 ± 0.00	1.50 ± 0.00
	Average of FICI (interpretation)	0.9062 (additive)			
<i>Escherichia coli</i>	FIC GGLdecoc	0.063 ± 0.00	0.063 ± 0.00	0.13 ± 0.00	-
	FIC TCIH ₂ O	0.50 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	-
	FICI (ΣFIC)	0.56 ± 0.00	0.18 ± 0.00	0.25 ± 0.00	-
	Average of FICI (interpretation)	0.3333 (synergy)			
<i>Pseudomonas aeruginosa</i>	FIC GGLdecoc	0.03±0.00	0.25 ± 0.00	0.25 ± 0.00	-
	FIC TCIH ₂ O	0.50±0.00	0.25 ± 0.00	0.13 ± 0.00	-
	FICI (ΣFIC)	0.53±0.00	0.50 ± 0.00	0.37 ± 0.00	-
	Average of FICI (interpretation)	0.4687 (synergy)			
<i>Salmonella typhi</i>	FIC GGLdecoc	0.13 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	-
	FIC TCIH ₂ O	0.50 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	-
	FICI (ΣFIC)	0.63 ± 0.00	0.38 ± 0.00	0.38 ± 0.00	-
	Average of FICI (interpretation)	0.4583 (synergy)			

FIC TCIH₂O: fractional inhibitory concentration of aqueous extract of *T. catappa* leaves; FIC GGLdecoc: fractional inhibitory concentration of decoction of *G. glauca* leaf.

Table 5 Phytochemical composition of the decoction of GGLdecoc (*G. glauca* leaf) and aqueous extract of TCIH₂O (*T. catappa* leaf).

Secondary metabolites	Alkaloids	Flavonoids	Saponins	Tanins	Glucosides	Phenols	Steroids	Triterpens	Anthocyanins	Anthraquinones
TeCIH ₂ O	+	-	+	+	+	+	+	+	-	+
GnGGLdecoc	-	+	+	+	+	+	-	+	-	+

“+”: present; “-”: absent; TCIH₂O: aqueous extract of *T. catappa* leaf; GGLdecoc: decoction of *G. glauca* leaf.

revealed the presence of phenols, tannins, saponins, triterpenes, and glycosides known to impact the growth and metabolism of microorganisms [40]. Though the detection of such metabolites does not automatically predict the antimicrobial activity of a plant extract, it has clearly been demonstrated that several compounds belonging to the investigated classes of metabolites showed antibacterial activities [40-42]. Tannins exert its antimicrobial activity by binding with proteins and adhesins, inhibiting enzymes, complexation with the cell wall and metal ions, or disruption of the plasmatic membrane [42]. Flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophilic amino acids. This complex often leads to inactivation of the protein and loss of its function [43]. The mode of action of saponins against bacteria is due to its ability to cause leakage of proteins and certain enzymes from the cell [44]. The sensitivity of steroids and the membrane lipids indicate their specific association that causes leakage from liposomes. It seems that both active compounds from extracts directly or indirectly attach to the different site on bacterial cell. Mechanism of synergy is still insufficiently researched [45].

Synergy effects of the mixture of bioactive constituents contained in plant extracts are claimed to be responsible for the improved efficiency of many extracts. For a long time, the mechanisms underlying these synergy effects remained unexplained. With the exact knowledge of these mechanisms, it will be possible to develop a new generation of standardized, effect-optimized mono- and multi-extract preparations, which not only fulfill today's standards for quality, safety and efficacy of medicinal drugs but can ideally be used for the treatment of diseases that have been treated previously with chemosynthetics or antibiotics [46]. Synergy research in phytomedicine has established itself as a new key activity in recent years. It is one main aim of this research to find a scientific rational for the therapeutic superiority of many herbal drug extracts derived from traditional medicine as compared with

single constituents thereof. For this reasons, the combinations of plant extracts could be a significant basis for the development of new approach to fight against resistance. Then the extracts contain mixtures of different bioactive compounds, which make microbial adaptability very difficult comparing to single-constituent antibiotics [47, 48].

5. Conclusions

The antibacterial activity exhibited by the crude extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* that are associated with various infectious diseases, has provided scientific justification in the use of these plants in Cameroonian folk medicine and could lead new phytomedicine. However, further isolation of active constituents from such plants, determination of their safety and pharmacokinetics properties are required.

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