Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Plants- Drug Interactions Against Some Multi-drug Resistant Microorganisms Causing Urogenital Tract Infections in Cameroon

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Abstract

Background: Multi drug resistance is an increasing phenomenon plaguing the use of antibiotics nowadays. This favors the spread of pathogenic microorganisms causing a major public health problem. Antimicrobial compounds from medicinal plants can be used synergistically to enhance the activity of standard drugs when used concurrently. The aim of this work was to evaluate the ability of extracts from *Skirakiopsis elliptica (SE), Rumex abyssinicus (RA), Nauclea pobeguinii (NP) and Picralima nitida (PN)* to potentiate the activity of 7 antibiotics against resistant bacteria from urogenital tract infections.

Methods: Using the micro-dilution and the checkerboard methods, the MIC of various agents and the combination effect of extracts and antibiotics were obtained respectively.

Results: A total of 37 additive interactions with Fractional Inhibitory Concentration index (FICI) comprise between 0.56-0.95 and 35 indifferent interactions FICI comprise between 1.03-1.98. No antagonism (FICI >4) was revealed as well as no synergistic effect (FICI <0.5). However, we could notice a decrease at up to 64 fold in MIC of most antibiotics when combined with these extracts. The concentration of Doxycycline was decreased by *Nauclea pobeguinii* aqueous extracts (NPE) at up to 64-fold on *S. saprophiticus*.; that of Norfloxacin and Doxycycline was reduced by aqueous extract *of Skirakiopsis elliptica (SE*E) on *E.coli* at up to 32 and 16-fold respectively.

Conclusion: The results of this study can be exploited to potentiate the antimicrobial activity of antibiotics involved in this study in the bacterial growth inhibition of resistant germs incriminated here.

Keywords: Antimicrobial chemotherapy; medicinal plants; infectious diseases; microbial resistance; synergism.

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Citation on this article: Tabouguia MO, Zofou D, Njouendou JA, Kouipou MRT, Fon PN, Beng VP, Assob Nguedia JCA. Investigational Medicinal Chemistry and Pharmacology (2018) 1(1):4.

Background

There is a global increase in the rate of therapeutic failure as result of the emergence of multi-drug resistant (MDR) micro-organisms. These infectious agents are found in various organs especially the urogenital tract (UG). The World Health Organization (WHO) reported continuous increase in the incidence of UGI from 1999 to 2013 [1], and this constant growth of infectious diseases has been greatly attributed to the rapid emergence of MDR microorganisms to a variety of conventional antibiotics. Therefore, there was a need of an alternative strategy to fight against such organisms. One of the solutions to this strategy was the search of new active principles from medicinal plants [2-4]. During the last decades, systematic screening of natural products as a source of potential bioactive compounds with antimicrobial activity has been a common language with hope to solve the MDR issue in Cameroon. However, with fear of MDR against natural products, the development of new approaches in order to overcome the problem of antimicrobial resistance remains indispensable. These include, the exploration of antimicrobial plant extracts that could be used in combination with some antibiotics facing resistance in order to obtain an increased susceptibility [5-6]. This approach known as synergistic interaction can be exploited to potentiate the antibiotic effect, and therefore reduce the pharmaceutical and the toxicity as well as side effects [7]. There is hope that this new approach could contribute in solving the problem of bacterial resistance and less susceptible bacteria. A selected number of medicinal used in Cameroon and in other African countries based on their previous antimicrobial (against urogenital tract agents) and anti-oxidative activities including *Skirakiopsis elliptica* (*Euphorbiaceae*) (previously known as *Sapium ellypticum), Rumex abyssinicus (Polygonaceae)*, *Nauclea pobeguinii (Rubiaceae), Picralima nitida* (*Apocynaceae*) were used. Besides, we have investigated the antimicrobial properties of these plants in a previous work against five microorganisms isolated from patients suffering from urogenital infections in Cameroon [2]. It was in order to bring back to shelves those antibiotics that are costly affordable, but abandoned because of this phenomenon of resistance that this work was therefore initiated to evaluate the synergistic potentials of these 4 medicinal plants against 7 antibiotics commonly used in the treatment of urogenital tract infections.

Methods

Plant Material

The different plant parts used in this study were harvested in the West and Centre regions of Cameroon. The various plants were identified at the Cameroon National Herbarium (CNH), where voucher specimens were deposited with voucher identification number. Detailed information on each medicinal plant are given in Table 1.

Preparation of Extracts

Fresh plant materials were collected and dried at room temperature in a ventilated laboratory and pulverized. The powders were macerated in various solvent for 72 hours as described by the Table 1 below and the mixtures were agitated daily. Methanol and Methanol-methylene chloride filtrates were evaporated on a Buchi Germany rotary evaporator while aqueous and hydro-ethanolic extracts were evaporated at 40°C in an oven.

Phytochemical Screenings

All plant extracts were subjected to qualitative phytochemical tests to identify the various classes of phytochemical components, according to the protocols previously described [8].

Tests for steroids: In a 10 mL glass tube, a mixture of 1 ml of extract, 1 mL of chloroform and 1 mL of acetic anhydride was prepared. Then 2 drops of concentrated H2SO4was added from the side of test tube. The development of red, then blue and finally green color was indicative of the presence of steroids.

Test for alkaloids: In a 10 mL glass tube, 5 mg of the extracts were dissolved in 2 mL of methanol and developed on thin layer chromatography (silica gel coated plaque) in hexane-ethyl acetate 20:80 as mobile phase. The plaque was pulverized in a hoot with Dragendorf reagent newly prepared and the presence of orange spot on the plaque showed the presence of alkaloids.

Test for saponins: In a 10 mL glass tube, 2 mg of extract was dissolved into 1.5 mL heated distilled water; the mixture was shaken vigorously for 1 min. The formation persistent foam was indicative of the presence of saponins.

Table 1. Plant extracts information

Plant names family *Voucher number*	Part used	Solvent/ solvent system for extraction	Code	Mass of material	Volume of solvent (mL)
Skirakiopsis elliptica *Euphorbiaceae* (SE) *47266/HNC*	Bark	MeOH 100%	(SEM)	200g	800
		MeOH-CH_2Cl_2 (1:1)	(SEMC)	200g	400:400
		H_2O	(SEE)	200g	800
		H_2O - EtOH (1:1)	(SE1:1)	200g	400:400
		H_2O -EtOH (8:2)	(SE8:2)	200g	600:200
Rumex abyssinicus Polygonaceae (RA) *27239/SRF Cam*	Bark	MeOH100%	(RAM)	1000g	4000
		MeOH-CH_2Cl_2 (1:1)	(RAMC)	100g	500
		H_2O	(RAE)	200g	800
		H_2O - EtOH (1:1)	(RA1:1)	200g	400:400
		H_2O -EtOH (8:2)	(RA8:2)	200g	600:200
Picralima nitida *Apocynaceae* (PN) *565411/HNC*	Bark	MeOH100%	PNM	1000g	4000
		MeOH-CH_2Cl_2 (1:1)	(PNMC)	100g	400
		H_2O	(PNE)	200g	800
		H_2O - EtOH (1:1)	(PN1:1)	200g	400:400
		H_2O -EtOH (8:2)	(PN 8:2)	200g	600:200
Nauclea pobeguinii Rubiaceae (NP) *504710/HNC*	Bark	MeOH 100%	NPM	1000g	4000
		MeOH-CH_2Cl_2 (1:1)	(NPMC)	100g	250:250
		H_2O	(NPE)	100g	500
		H_2O - EtOH (1:1)	(NP1:1)	200g	400:400
		H_2O - EtOH (8:2)	(NP8:2)	100g	400:100

SEM: *Skirakiopsis elliptica,* methanolic extract, **SEMC**: *Skirakiopsis elliptica* methanol-methylene chloride (50-50) extract, **SEE**: *Skirakiopsis elliptica* aqueous extract, **SE1:1** *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;* **SE8:2:** *Skirakiopsis elliptica* Hydro-ethanolic extract (80-20)*;* (**RAM**) *Rumex abyssinicus* methanolic extract; (**RAMC**) *Rumex abyssinicus* methanol-methylene chloride (50-50) extract; (**RAE**) *Rumex abyssinicus* aqueous extract; (**RA1:1)** *Rumex abyssinicus* Hydro-ethanolic extract (50-50); (**RA8:2**) *Rumex abyssinicus* Hydro-ethanolic extract (80-20); **PNM**: *Picralima nitida* methanolic extract; (**PNMC**) : *Picralima nitida* methanol-methylene chloride extract (50-50) ; (**PNE**): *Picralima nitida* aqueous extract; (**PN1:1):** *Picralima nitida* Hydro-ethanolic extract (50-50); (**PN 8:2**): *Picralima nitida* Hydro-ethanolic extract (80-20); **NPM**: *Nauclea pobeguinii* methanolic extract; (**NPMC**): *Nauclea pobeguinii* methanol-methylene chloride (50-50) extract; (**NPE**): *Nauclea pobeguinii* aqueous extract; (**NP1:1**): *Nauclea pobeguinii* Hydro-ethanolic extract (50-50); (**NP8:2**): Hydro-ethanolic extract (80-20); **NP**: *Nauclea pobeguinii* methanol;

Test for tannins: In a 10 mL glass tube, 2 mg of plant material was dissolved in 1mL of distilled water, and 2 mL of FeCl3 was added to the filtrate. A Blue-black precipitate indicated the presence of tannins.

Test for terpenoids: In a 10 mL glass tube, 5 mL of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 mL) was added carefully to form a layer. A reddish brown coloration at the inter face was indicative of the presence of terpenoids.

Test for flavonoids: A little amount of magnesium powder and a few drops of concentrated HCL were added to 3 mL of methanolic extract. A red or intense red coloration indicated the presence of flavonoids.

Interaction studies

Antibiotics

Antibiotics were selected based on their antimicrobial susceptibility patterns from hospital records. The detailed are found in our previous work [2]. The selection of antibiotics was oriented by their susceptibility patterns on urogenital microbial agents. The selected antibiotics were those that displayed the highest resistance profile and these included chloramphenicol, ciprofloxacin, Norfloxacin, doxycycline, amoxicillin, ceftriaxone and erythromycin [2].

Microbial strains

The investigation was carry out on the following microorganism: *Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophiticus,* all isolated from urinary or genital tract of infected patients (positive sample from patients who showed up to the hospital complaining about a vagina discharge coupled or not with dysuria, itching, lower abdominal pains, fever,

sterility and other symptoms related with urinary and/ or genital infection, and whose the physician asked for an antibiogram) excepts the reference strain (*Staphylococcus aureus RN4220* which was from BEI resources, NIAID, NIH, Manassas USA.

Determination of minimal inhibitory concentration (MIC) of samples to be tested

MICs were determined for extracts and antibiotics by broth micro-dilution technique using 96-well plates described by the clinical laboratory standard institute standard (CLSI) operational procedures [9]. The wells were filled with 100 µL culture media (Muller Hinton broth) supplemented with 0.005% phenol red and 1% glucose. Subsequently, 100 µL of extract previously prepared in DMSO was added in triplicate into the first column to make a final concentration of 70.8 mg/mL. Successive 2 fold-dilutions were done by transferring 100 µL of the mixture from the first well to the next up to the eleventh well. An aliquot (100 µL) was discarded from the eleventh well. The twelfth well served as control since no sample (extract, or reference antibiotics) was added in it. Finally, 10 µL of standardized inoculums at 10^6 CFU/mL was added in each test well for Gram negative bacteria and 10^5 CFU/mL for Gram positive [9]. The final concentration of the extracts used to determine their MICs ranged from 32 to 0.031 mg/mL, meanwhile each tested antibiotic has its own concentration range; base on information provided by the CLSI on the Performance Standards For Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement [9]. Table 2 below summarizes all the concentration ranges tested. Tests were incubated aerobically at 37±1°C for 24 hours. The end point was revealed by a color change of the indicator from red to pink or to yellow by comparing test wells to control wells (media, diluted extract and distilled water). The MIC was considered as the lowest concentration of sample that could prevent visible growth of microorganism (no change of the indicator) [2]. Only the extract with the lowest MIC of the plant was selected for combination study with antibiotics.

Table 2. Concentration ranges of various agents

Samples	**Ranges of concentration of all the product tested (Wells) [9]**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Extraits (mg/mL)	32	16	8	4	2	1	0,5	0.25	0.125	0.062	0.031	-
Bactrim(µg/mL)	256	128	64	32	16	8	4	2	1	0.5	0.25	-
Chloramphenicol (µg/mL)	512	256	128	64	32	16	8	4	2	1	0.5	-
Ciprofloxacin (µg/mL)	256	128	64	32	16	8	4	2	1	0,5	0.25	-
Norfloxacin (µg/mL)	128	64	32	16	8	4	2	1	0.5	0,25	0.125	-
Doxycyclin (µg/mL)	128	64	32	16	8	4	2	1	0.5	0,25	0.125	-
Amoxicillin (µg/mL)	512	256	128	64	32	16	8	4	2	1	0.5	-
Ceftriaxone (µg/mL)	128	64	32	16	8	4	2	1	0.5	0.25	0.125	-
Erythromycin (µg/mL)	128	64	32	16	8	4	2	1	0.5	0.25	0.125	-

Determination of Fractional Inhibitory Concentration (FIC) and Fractional Inhibitory Concentration Index (FICI)

The checkerboard method was used to determine the combination effect of extract with antibiotics [7]. Briefly, 50 µL of culture media were introduced in each well of the main micro-plate (test plate). Then, 50µl of product A (antibiotic) of the combination was introduced in the first column of the plate except in well A1. Successive dilutions were done by transferring 50 µL of the mixture from the first to the tenth column, except raw A. An aliquot (50 µL) was discarded from the tenth column. Subsequently, a second plate aimed for the preparation of product B (extract) dilutions was prepared. The plate was filled with 200 µL of the broth culture media, then 200 µL of product B was introduced in the first raw (raw A) of the plate except in well A1, and successive dilutions were done by transferring 200 µL of the mixture from the first line (raw A) to the sixth line (raw F) except column 1. At the end of the preparation of the second plate, 50 µL of product B was therefore transfer from 2 to the corresponding well on the main plate. 10 µL of bacterial suspension at concentration of 10^6 CFU/mL for gram negative and 10^5 CFU/mL for Gram positive was introduced in each test line except line H which served as negative control for product A and column 12 which served as negative control of product B. plates were shaken and incubated aerobically at 37°C for 24 hours. After incubation, raw G gave the MIC of product A alone, column 11 gave the MIC of product B alone, well A1 was the blank and the rest gave the MICs if products A and B in combination. The MIC was the smallest concentration that could prevent visible growth of the

microorganism characterized by the presence of the turbidity as compared with the control wells containing only the culture media and the product to be tested. In vitro interactions between antimicrobial agents were determined and quantified by calculating the fractional inhibitory concentration (FIC) index using the following formula: FIC index = (MIC of plant extract in combination/MIC of plant extract alone) + (MIC of antibiotic in combination/MIC of antibiotic alone). Interpretation of the FIC index (FICI) was as follows: FICI≤0.5= synergy; 0.5 > FICI ≤1= additive; 1 > FICI ≤4= indifference; FICI>4= antagonism [7]. The action of antimicrobial agents was considered to be:- *Synergistic* if their joint effect is far stronger than the sum of effects of the individual agents. - *Additive* if their joint effect produces an effect greater than effect of either drug taken alone. - *Indifferent* if their joint effect is equal to the effect of either individual agent. - *Antagonistic* if their joint effect is weaker than the sum of effects of the individual agents or weaker than the effect of either individual agent.

$$\text{FIC A} = \frac{MIC\ A\ in\ combination\quad with\ B}{MIC\ A\ independently}\quad \text{FIC B} = \frac{MIC\ B\ in\ combination\quad with\ A}{MIC\ B\ independently}\quad \text{and FICI} = \Sigma FIC$$

If the MIC of any agent alone occurred at the highest concentration tested, the FIC index was considered not determinable and the type of interaction could not be assessed. And when the MIC of the agent in combination could not be determined, the FICI index was also not determinable. Where more than one combination resulted in a change in the MIC value of the extract or antibiotic, the FIC was expressed as the average of the FIC values.

Table 3. Phytochemical screening results

Samples	Extrait	Steroids	Alkaloids	Saponins	Tannins	Terpernoids	Flavonoids
SE	SEM	-	+	++	+++++++++	++	+
	SEMC	-	+	-	++++++	-	-
	SE1:1	++	+	-	+++++++++	+	-
	SE8:2	++	+	-	+++	+	-
	SEE	+	+	-	+++	+	-
RA	RAM	-	++	++	++++	+	+++
	RAMC	-	++	-	+++	+	++
	RA1:1	++++	+	-	+++	-	+
	RA8:2	++++++++	+	++	+++	-	+
	RAE	++++++	+	-	+++	-	+
PN	PNM	-	++	++++++	-	++	+
	PNMC	-	+	+	-	-	-
	PN1:1	-	+++	++	-	++	-
	PN8:2	-	+++	++	+	++	-
	PNE	-	+++	++	-	++	-
NP	NP	++++	+	++	+	-	-
	NPM	-	+	+++	+	++	++
	NP1:1	+	++	+	+	+	-
	NP8:2	+	-	+	-	+	-
	NPE	+	-	+	+	+	-

(+): present and the number of (+) increases with the intensity of the coloration (-) absent
SEM: *Skirakiopsis elliptica,* methanolic extract, **SEMC**: *Skirakiopsis elliptica* methanol-methylene chloride (50-50) extract, **SEE**: *Skirakiopsis elliptica* aqueous extract, **SE1:1** *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;* **SE8:2:** *Skirakiopsis elliptica* Hydro-ethanolic extract (80-20)*;* (**RAM**) *Rumex abyssinicus* methanolic extract; (**RAMC**) *Rumex abyssinicus* methanol-methylene chloride (50-50) extract; (**RAE**) *Rumex abyssinicus* aqueous extract; (**RA1:1)** *Rumex abyssinicus* Hydro-ethanolic extract (50-50); (**RA8:2**) *Rumex abyssinicus* Hydro-ethanolic extract (80-20); **PNM**: *Picralima nitida* methanolic extract; (**PNMC**) : *Picralima nitida* methanol-methylene chloride extract (50-50) ; (**PNE**): *Picralima nitida* aqueous extract; (**PN1:1):** *Picralima nitida* Hydro-ethanolic extract (50-50); (**PN 8:2**): *Picralima nitida* Hydro-ethanolic extract (80-20); **NPM**: *Nauclea pobeguinii* methanolic extract; (**NPMC**): *Nauclea pobeguinii* methanol-methylene chloride (50-50) extract; (**NPE**): *Nauclea pobeguinii* aqueous extract; (**NP1:1**): *Nauclea pobeguinii* Hydro-ethanolic extract (50-50); (**NP8:2**): Hydro-ethanolic extract (80-20); **NP**: *Nauclea pobeguinii* methanol

Table 4. Minimal inhibitory concentration of extracts and antibiotics on various tested microorganisms (mg/mL)

Samples	*P. vulgaris*	*P. stuartii*	*P. aeruginosa*	*E. coli*	*S. saprophiticus*	*S.aureus RN4220*
SEM	0.25	0.5	1	2	1	0.5
SEMC	0.25	0.5	2	2	1	0.5
SE1:1	0.125	0.25	1	1	0.125	0.5
SE 8:2	0.25	0.25	1	1	0.25	0.5
SEE	0.25	0.25	0.5	1	0.25	0.5
RAM	1	2	8	32	0.25	0.25
RAMC	2	2	16	32	0.25	0.25
RA1:1	2	2	16	32	0.25	0.5
RA8:2	4	2	16	>32	0.5	1
RAE	2	1	4	2	0.5	1
PNM	16	8	8	8	0.25	0.5
PNMC	16	16	16	8	0.125	0.25
PN 1:1	32	16	32	8	0.125	0.25
PN 8:2	16	8	16	4	0.25	0.5
PNE	32	32	32	16	2	2
NP	16	8	16	32	0.25	2
NPM	16	16	32	32	4	4
NP1:1	32	8	16	32	4	4
NP8:2	8	4	16	8	2	4
NPE	2	2	4	4	4	2
BAT	>0.25	>0.25	64	4	8	128
CHLO	0.032	0.12	0.12	0.002	0.032	0.002
CIP	0.002	0.25	0.001	0.064	0.016	<0.25
NOR	8	>256	16	>256	128	2
DO	0.032	0032	0.032	0.016	<0.125	<0.125
AX	0.001	0.51	>0.51	0.25	<0.25	<0.25
CEFT	<0.12	0.001	0.064	<0.12	0.008	0.002
ERY	nt	nt	nt	nt	0.0005	0.00025

Nt=not tested ; **SEM**: *Skirakiopsis elliptica,* methanolic extract, **SEMC**: *Skirakiopsis elliptica* methanol-methylene chloride (50-50) extract, **SEE**: *Skirakiopsis elliptica* aqueous extract, **SE1:1** *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;* **SE8:2:** *Skirakiopsis elliptica* Hydro-ethanolic extract (80-20)*;* (**RAM**) *Rumex abyssinicus* methanolic extract; (**RAMC**) *Rumex abyssinicus* methanol-methylene chloride (50-50) extract; (**RAE**) *Rumex abyssinicus* aqueous extract; (**RA1:1)** *Rumex abyssinicus* Hydro-ethanolic extract (50-50); (**RA8:2**) *Rumex abyssinicus* Hydro-ethanolic extract (80-20); **PNM**: *Picralima nitida* methanolic extract; (**PNMC**) : *Picralima nitida* methanol-methylene chloride extract (50-50) ; (**PNE**): *Picralima nitida* aqueous extract; (**PN1:1):** *Picralima nitida* Hydro-ethanolic extract (50-50); (**PN 8:2**): *Picralima nitida* Hydro-ethanolic extract (80-20); **NPM**: *Nauclea pobeguinii* methanolic extract; (**NPMC**): *Nauclea pobeguinii* methanol-methylene chloride (50-50) extract; (**NPE**): *Nauclea pobeguinii* aqueous extract; (**NP1:1**): *Nauclea pobeguinii* Hydro-ethanolic extract (50-50); (**NP8:2**): Hydro-ethanolic extract (80-20); **NP**: *Nauclea pobeguinii* methanol ; **BAT**: Bactrim; **CHL** : chloramphenicol; **CIP** : Ciprofloxacin; **NOR**: Norfloxacin; **DO:** Doxycycline; AX**:** Amoxicillin; **CEFT**: Ceftriaxone; **ERY**: Erythromycin

Results and Discussion

Phytochemical screening results (Table 3) show that each plant extract possesses at least two of the screened secondary metabolite. These are known for their ability to fight against microbial invasion in plant and it is the same role involved in the in vitro antimicrobial activity of plant extracts. Results show that all the extracts from *Skirakiopsis elliptica* and *Rumex abyssinicus* were proven to be very rich in tannins while *Nauclea pobeguinii* and *Picralima nitida* contain alkaloids and saponins. All the extracts were found to contain alkaloids except the aqueous and hydro-ethanolic (8:2) extract from *Nauclea pobeguinii.* These secondary metabolites exert their antimicrobial activity through different mechanisms; tannins for example act by iron deprivation, hydrogen bounding or non-specific interactions with vital proteins such as enzymes [10]. Some of characteristics of saponins include formation of foam in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [11]. The antimicrobial mechanism of action of the alkaloids may be through DNA intercalation and inhibition of DNA synthesis through topoisomerase inhibition [11]. However, there is a difference in term of quantity and quality of secondary metabolites from one extract of the same plant to the other. This may be explained by the difference in polarity of various solvent used for the extraction.

Table 5. Fractional inhibitory concentration index (FICI) of each studied combination / minimum fold reduction (MFR) of Minimal inhibitory concentration (MIC) of antibiotics (ATB).

Combination	***E. coli***		***P. stuartii***		***P. aeruginosa***		***S. aureus RN4220***		***S. saprophiticus***	
	FICI	**MIC MFR of the ATB**	**FICI**	**MIC MFR of the ATB**	***FICI***	**MIC MFR of the ATB**	**FICI**	**MIC MFR of the ATB**	***FICI***	**MIC MFR of the ATB**
BAT-SEE	1.09 / ind	2	0.95/add	4	1.22/ind	1	*nt*	/	*nt*	*nt*
CHL-SEE	0.91 :add	8	0.62/add	16	0.68/add	8	0.93/add	4	*1.03/ind*	2
CIP-SEE	1.09/ind	2	1.8/ind	1	ND	/	ND	/	*ND*	/
NOR-SEE	0.89 /add	32	0.70/add	4	1.78/ind	1	1.18/ind	1	*ND*	/
DO-SEE	0.70/add	16	1.07/ind	2	0.61/add	8	ND	/	*nt*	/
AX-SEE	ND	/	0.70/add	4	ND	/	1.08/ind	2	*0.71/add*	4
CEFT-SEE	1.18/ind	1	0.70/add	4	ND	/	1.38/ind	2	*NT*	/
ERY-SEE	*nt*	/	*nt*	/	*nt*	/	1.05/ind	4	*ND*	/
BAT-RAE	1.09/ind	2	ND	/	1.18/ind	1	nt		*1.19/ind*	1
CHL-RAE	0.78/add	2	ND	/	0.99/add	2	0.83/add	4	*1.08/ind*	2
CIP-RAE	1.18/ ind	1	ND	/	ND	/	ND	/	*ND*	/
NOR-RAE	ND	/	0.64/add	16	1.98/ind	-1	1.18/ind	1	*ND*	/
DO-RAE	1.03/ind	4	0.80/add	8	0.66/add	8	ND	/	*ND*	/
AX-RAE	ND	/	0.63/add	16	ND	/	1.10/ind	2	*ND*	/
CEFT-RAE	ND	/	0.66/add	4	ND	/	1.39/ind	2	*ND*	/
ERY-RAE	*nt*	/	*nt*	/	*nt*		1.05/ind	4	*ND*	/
BAT-NPE	1.04/ind	4	ND	/	ND	/	*nt*	/	*1.04*	4
CHL-NPE	0.78/add	2	ND	/	0.94/add	8	*nt*	/	*1.03/ind*	4
CIP-NPE	1.18/ind	1	1.16/ind	1	ND	/	*nt*	/	*ND*	/
NOR-NPE	ND	/	0.93/add	8	1.98/ind	-1	*nt*	/	*0.64/add*	64
DO-NPE	0.78/add	2	1.07/ind	2	0.56/add	8	*nt*	/	*0.93/add*	4
AX-NPE	ND	/	1.16/ind	1	ND	/	*nt*	/	*0.71/add*	8
CEFT-NPE	0.78/add	8	0.78/add	2	ND	/	*nt*	/	*ND*	/
ERY-NP							*nt*	/	*ND*	/
BAT-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	/	*0.79/add*	4
CHL-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*1.08/ind*	*2*	*1.08/ind*	2
CIP-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*0.56/add*	*2*	*ND*	/
NOR-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*0.68/add*	*4*	*0.78/add*	2
DO-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*0.93/add*	*4*	*1.18/ind*	1
AX-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*0.78/add*	*2*	*ND*	/
CEFT-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*0.73/add*	*4*	*1.08/ind*	2
ERY-PNMC	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*1.19/ind*	*1*	*ND*	/

FICI≤0.5**=** synergy; 0.5 > **FICI** ≤1**=** additive; 1 > **FICI** ≤4**=** indifference; **FICI**>4= antagonism. For *S.aureus,* RAE has been replace by RAM and for *S. saprophyticus*; RAE has been replace by RAMC, NPE by NP, and SEE by SE1:1. **Nt** = not tested; **ND** = not determined. **SEM**: *Skirakiopsis elliptica,* methanolic extract, **SEMC**: *Skirakiopsis elliptica* methanol-methylene chloride (50-50) extract*,* **SEE**: *Skirakiopsis elliptica* aqueous extract, **SE1:1** *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;* **SE8:2:** *Skirakiopsis elliptica* Hydro-ethanolic extract (80-20)*;* (**RAM**) *Rumex abyssinicus* methanolic extract; (**RAMC**) *Rumex abyssinicus* methanol-methylene chloride (50-50) extract; (**RAE**) *Rumex abyssinicus* aqueous extract; (**RA1:1)** *Rumex abyssinicus* Hydro-ethanolic extract (50-50); (**RA8:2**) *Rumex abyssinicus* Hydro-ethanolic extract (80-20); **PNM**: *Picralima nitida* methanolic extract; (**PNMC**) : *Picralima nitida* methanol-methylene chloride extract (50-50) ; (**PNE**): *Picralima nitida* aqueous extract; (**PN1:1):** *Picralima nitida* Hydro-ethanolic extract (50-50); (**PN 8:2**): *Picralima nitida* Hydro-ethanolic extract (80-20); **NPM**: *Nauclea pobeguinii* methanolic extract; (**NPMC**): *Nauclea pobeguinii* methanol-methylene chloride (50-50) extract; (**NPE**): *Nauclea pobeguinii* aqueous extract; (**NP1:1**): *Nauclea pobeguinii* Hydro-ethanolic extract (50-50); (**NP8:2**): Hydro-ethanolic extract (80-20); **NP**: *Nauclea pobeguinii* methanol ; **BAT**: Bactrim; **CHL** : chloramphenicol; **CIP** : Ciprofloxacin; **NOR**: Norfloxacin; **DO:** Doxycycline; AX**:** Amoxicillin; **CEFT**: Ceftriaxone; **ERY**: Erythromycin;

Table 6. Various extracts selected for the combination study (best extract activity, lowest MIC)

Micro-organism	*P. vulgaris*	*P. stuartii*	*P. aeruginosa*	*E. coli*	*S. saprophiticus*	*S.aureus RN4220*
Best plant extract activity	SEE, RAE, NPE,	SEE, RAE, NPE,	SEE, RAE, NPE,	SEE, RAE, NPE,	SE1:1, RAMC, NP, PNMC	SEE, NPE, RAM, PNMC

(**PNMC**) : *Picralima nitida* methanol-methylene chloride extract (50-50) ; (**RAMC**) *Rumex abyssinicus* methanol-methylene chloride (50-50) extract; **RAM)** *Rumex abyssinicus* methanolic extract; (RAE) *Rumex abyssinicus* aqueous extract; **SEE**: *Skirakiopsis elliptica* aqueous extract; **SE1:1** *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;* (**NPE**): *Nauclea pobeguinii* aqueous extract; **NP**: *Nauclea pobeguinii* methanol.

Interactions studies results

Minimal inhibitory concentration results

The MICs of the various plant extracts and antibiotics against tested microorganisms are presented in Table 4 below. Based on the antimicrobial activity cut off values of crude extracts as earlier categorized [2], extracts with a MIC value on pathogen less than 1 mg/mL is classified as very active; whereas MIC values between 1 and 8 mg/mL are known as moderately active, and those displaying MIC comprise between 8-64 mg/mL, are considered to be less active or with negligible activity. By referring to this scale, all the extracts from *Skirakiopsis elliptica* (SEM, SEMC, SE1:1, SEE) can be considered to be very active on all the tested microbial agents except on *E.coli* and this justify why these extracts were selected for the combination studies. Moreover, most extracts, displayed a MIC higher than 8 mg/mL, especially on Gram negative microorganisms. But only the extract displaying the best activity (the lowest MIC) on each microorganism was recruited for the combination effect with antibiotics. Table 6 shows the best extract activity recorded on each micro-organism. Concerning MICs values obtained with antibiotics, they were all within the values classified as resistant by the CLSI for the enterobacteria [9]. The phytochemical screening results revealed the presence of steroids, flavonoids, tannins, terpenoids, alkaloids and saponins. These secondary metabolites from extracts are known to have an impact on growth and metabolism of microorganisms [12]. They probably played the major role in the antimicrobial activity of the various extracts observed in this study.

Combining effects of some extracts with antibiotics

In this work, possible joint activity of extracts from *Skirakiopsis elliptica, Rumex abyssinicus, Nauclea pobeguinii and Picralima nitida* extracts and eight antibiotics (Bactrim, chloramphenicol, ciprofloxacin, Norfloxacin, doxycycline, amoxicillin, ceftriaxone and erythromycin), was evaluated. The experiment was done against pathogenic bacteria (*Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa and Staphylococcus saprophiticus* and *Staphylococcus aureus* RN4220) isolated in urogenital tracts which were found to be often involved in other human infections. These bacteria were proven to be clinically resistant to the various antibiotics (*chloramphenicol, ciprofloxacin, Norfloxacin, doxycycline, amoxicillin, ceftriaxone and erythromycin**)*** involved in this study. The checkerboard method used in the evaluation of combination effect, permitted us to establish 37 additive interactions with FIC index comprise between 0.56-0.95 and 35 interactions indifferent FICI comprise between 1.03-1.98 (Table 5). No antagonism (FICI >4) was revealed as well as no synergism FICI <0.5. However, we could notice a decrease in the minimum inhibitory concentration of some antibiotics at up to 64 fold. Indeed, it was found that the presence of sub-inhibitory concentrations (1/2 MIC, 1/4MIC) of the extracts modulated the activity of various antibiotics by reducing the concentration of antibiotics needed to inhibit the growth of bacteria. The concentration of Doxycycline was decreased by *Nauclea pobeguinii* aqueous extracts (NPE) at up to 64-fold on *S.saprophiticus*.; that of Norfloxacin and Doxycycline was reduced by SEE on *E.coli* at up to 32 and 16-fold respectively. ; That of chloramphenicol was reduced at 16 and 8-fold by SEE on *P. stuartii* and *E. coli* respectively. This observation is not to be neglected for synergistic interactions stand on the same principle. Additive interaction, occurring when two or more drugs are combined to produce an effect greater than effect of either drug taken alone, as well as synergistic interaction is a positive interaction. Apart from combination with PNMC (*Picralima nitida* methanol-methylene chloride), most combinations with others extracts on staphylococcus species tested in this study were found to be indifferent. The explanation to this observation may be found in their phytochemical contents which appear to be different compare to other plants. Many studies have shown

that active efflux pomp can be the mechanism of resistance put in place by bacteria against almost all antibiotics [13]. The majority of the efflux systems in bacteria are non-drug specific proteins that can recognize and export a broad range of chemically and structurally unrelated compounds from bacteria without drug alteration or degradation [14]. It seems that both active compounds, from extracts and antibiotics, directly or indirectly attach the same site on bacterial cell. Some authors suggest that phytochemical components disturb cell wall or increase permeability of the cytoplasmic membrane and thereby facilitate the influx of antibiotics, produce efflux pump inhibitors or inhibit penicillin-binding proteins [15].

Conclusions

In this study, we could established 37 additive interactions with FIC index comprise between 0.56-0.95 and 35 interactions indifferent FICI comprise between 1.03-1.98. No antagonism (FICI >4) was revealed as well as no synergism FICI <0.5. However, we could notify a decrease in the minimum inhibitory concentration of some antibiotics at up to 64 fold. In conclusion, the results of this study are encouraging for the findings support the possible use of phyto-compounds together with antibiotics to increase their potency and avoid undesirable side effects. The results could be exploited to solve the problem of bacterial resistance and less susceptible bacteria

Abbreviations

AX: Amoxicillin;
BAT: Bactrim;
CEFT: Ceftriaxone;
CFU: Colonies Forming Unit
CHL : chloramphenicol;
CIP : Ciprofloxacin;
CLSI: Clinical Laboratory Standard Institute
CNH: Cameroon National Herbarium
DMSO: Dimethylsulphoxide
DNA: Deoxyribonucleic acid
DO: Doxycycline;
ERY: Erythromycin;
FIC : Fractional Inhibitory Concentration
FICI : Fractional Inhibitory Concentration Index
MDR: Multi-drug resistant
MIC: Minimal Inhibitory Concentration
ND: Not Determined
NOR: Norfloxacin;
NP: *Nauclea pobeguinii* methanol ;
NP1:1: *Nauclea pobeguinii* Hydro-ethanolic extract (50-50);
NP8:2: Hydro-ethanolic extract (80-20);
NPE: *Nauclea pobeguinii* aqueous extract;
NPM: *Nauclea pobeguinii* methanolic extract;
NPMC: *Nauclea pobeguinii* methanol-methylene chloride (50-50) extract;
NT=Not Tested;
PN 8:2: *Picralima nitida* Hydro-ethanolic extract (80-20);
PN1:1: *Picralima nitida* Hydro-ethanolic extract (50-50);
PNE: *Picralima nitida* aqueous extract;
PNM: *Picralima nitida* methanolic extract;
PNMC : *Picralima nitida* methanol-methylene chloride extract (50-50) ;
RA1:1 *Rumex abyssinicus* Hydro-ethanolic extract (50-50);
RA8:2 *Rumex abyssinicus* Hydro-ethanolic extract (80-20);
RAE *Rumex abyssinicus* aqueous extract;
RAM *Rumex abyssinicus* methanolic extract;
RAMC *Rumex abyssinicus* methanol-methylene chloride (50-50) extract;
SE1:1 *Skirakiopsis elliptica* Hydro-ethanolic extract (50-50) *;*
SE8:2: *Skirakiopsis elliptica* Hydro-ethanolic extract (80-20)*;*
SEE: *Skirakiopsis elliptica* aqueous extract,
SEM: *Skirakiopsis elliptica,* methanolic extract,
SEMC: *Skirakiopsis elliptica* methanol-methylene chloride (50-50) extract*,*
UG: Urogenital tract
WHO: World Health Organization

Authors' Contribution

TMO designed the protocol, executed the laboratory work and drafted the manuscript. NJA contributed to ethnobotanical survey, provided the plant and contributed to draft the manuscript. KMRT contributed to design the protocol, read and substantially revised the manuscript. FPN helped in the acquisition of clinical microbial strains. DZ, JCAN and VPB contributed to monitor the laboratory work and provided required chemicals and laboratory consumables for different analysis. All authors read and approved the manuscript.

Acknowledgments

The authors are also grateful to BEI resources, NIAID, NIH, Manassas USA, for the provision of referenced strain. Also they are grateful to all the Buea Solidarity Clinic laboratory staff, as well as to all the Laboratory staff of Deido Hospital (Douala), to Dr Awah, and to Mr Ebane for their contribution in different aspects of the realization of this work.

Conflict of interest

The author(s) declare that they have no competing interests.

Article history:
Received: 22 April 2018
Received in revised form: 28 April 2018
Accepted: 06 May 2018
Available online: 06 May 2018

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