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Antimicrobial Activities of Some Medicinal Plants Against Multiresistants Microorganisms Causing Urogenital Tract Infections in Cameroon

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Abstract: Urogenital tract infections remain a serious global treat especially in women. The control of these infections is increasingly complicated due to development of resistance against available drugs. Therefore a perpetual search of new antimicrobial molecules is needed to face the challenge of microbial resistance. This study was initiated to screen the antimicrobial activities of methanol extracts of nine Cameroonian medicinal plants, used for the treatment of infectious diseases, against multiresistant pathogens isolated from urogenital infected patients. The plants included: *Cussonia arborea, Dissotis longipetala*, *Lonchocarpus sepium*, *Nauclea pobeguinii*, *Picralima nitida*, *Rumex abyssinicus*, *Rumex berqueatii*, *Sapium ellypticum*, *Psorospermum febrifigum*, They were tested on seven strains including six clinical isolates (*Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa*, S*taphylococcus saprophiticus*, *Candida krusei*) and one reference strain *(E. coli JM109).* Clinical strains were checked for their multiresistance using disc diffusion method. The antimicrobial activities were determined by agar well diffusion method. MICs were determined using microdilution assay. The phytochemical screening of plants was also done. All the bacteria strains were found to be multidrug-resistant (MDR) against at least 7 of the 12 antibiotics tested. Antimicrobial activities demonstrated that 6/9 (SE, DL, RA, RB, PN, LS) plants were active on at least six microorganisms. MICs ranged from 0.125 to 128mg/ml for crude extracts, from 0.5 to 0.16µg/ml for gentamicin and was 0,002mg/ml for fluconazole. One extract (NP) which showed limited results on agar, inhibited the growth of all the strains with MICs ranging from 1to 16mg/ml showing a limited activity of this extract on agar. We found that four extracts (SE, NP, RA, DL) have significant activities since they presented MICs ≤ 8mg/ml on at least 5 tested microorganism individually. Findings from phytochemical screening showed that most active extracts contain tannins, alkaloids and saponins which could be responsible of these activities.

Keywords: Urogenital Infections, *Sapium, Nauclea, Dissotis, Rumex, Picralima*, Antimicrobial Activities

1. Introduction

Urogenital tract infections (UGI) remain a serious global health threat. Despite the continuous efforts, World Health Organization (WHO) estimated the incidence in 2013 at about 500 million new cases each day worldwide [1]. These infections can have serious consequences including damages of reproductive organs leading to infertility, social rejection and death. They are also the first infections involved in neonatal deaths and complications related to pregnancy and childbirth [2]. Furthermore, the resistance of pathogens against antimicrobial therapeutics is increasing all over the world and remains a great challenge in the eradication of pathogens. Hence, the fight against infectious diseases in general and urogenital tract infections in particular has been seriously compromised by the ineffectiveness of most available and cheaper treatments due to microbial resistance, and by the unavailability and non-affordability of many treatments [3]. This has recently led to the screening of hundreds of plants derived products, in order to discover novel antimicrobial agents which could be the potential solution to pathogens resistance, considering the wide range of secondary metabolites they usually contains [4]. *Cussonia arborea* (CA), *Dissotis longipetala* (DL), *Lonchocarpus sepium* (LS), *Nauclea pobeguinii* (NP), *Picralima nitida* (PN), *Rumex abyssinicus* (RA), *Rumex berqueatii* (RB), *Sapium ellypticum* (SE), *Psorospermum febrifigum* (PF) are nine (09) medicinal plants of the Cameroonian pharmacopeia used traditionally for the treatment of a wide range of infectious diseases including urogenital tract infections; they may therefore possess antimicrobial activities. It is in this view that this work was initiated aiming to assess the antimicrobial activities of these nine Cameroonian pharmacopeia plants commonly used against infectious diseases.

2. Material and Methods

2.1. Plant Material

The different plant parts were harvested in West and North West regions of Cameroon. The various plants were identified at the National Herbarium of Cameroon (NHC), where voucher specimens were deposited with voucher number. Detailed information on each medicinal plant is given in table 1.

2.2. Preparation of Extracts

Fresh plant materials were collected and dried at room temperature in an aerated laboratory and pulverized. Five (05) kg of the powder of each plant were then macerated in 13.5 L of methanol for 48 hours to give about 7.5 L of extract after filtration. The filtrate was evaporated on a Buchi rotary evaporator and the extraction yields of different crude extracts were determined (table 1). An aliquot of each extract was used for antimicrobial studies while the remaining portion was kept for further studies.

2.3. Phytochemical Screening

Qualitative phytochemical tests were performed for methanolic extracts of all the plants to identify the various classes of phyto-constituents, according to the protocols described by [5].

2.4. Antimicrobial Activities

2.4.1. Microbial Strains

The plants were tested on seven microbial strains including six clinical strains (*Escherichia coli, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophiticus,* and *Candida krusei*) and one reference strain (E. coli JM109). While clinical strains were obtained from two laboratories, the Laquintinie Hospital – Douala (Littoral region – Cameroon) and Solidarity Clinic, Buea, South-West region Cameroon. They were all from urinary or genital tracts specimens (urine, vaginal smear, and sperm) from symptomatic patients presenting urogenital infections (for which the consulting physician requested for an antibiogram). Reference strain was obtained from BEI resources, NIAID, NIH, Manassas USA.

2.4.2. Characterization of Microbial Strains

Clinical isolates were characterized and identified with microscopic, cultural and biochemical methods [6].

After the gram stain, bacteria isolates were grown on various media. Gram negative bacteria were cultured on MacConkey and Cled agar, while Gram positive were cultured on Mannitol Salt Agar.

Gram negative bacteria alongside with the reference strain *E. coli* JM109 were characterized biochemically using the following test:

- The Kliger iron agar test, was carried out to detect the ability of the microorganism to ferment glucose and /or lactose with/without gas production, it also showed the ability of the organism to produce SH2 gas. After incubation, red/yellow slant indicated negative/positive lactose, while a red/yellow bottom indicated a negative/positive glucose. Gas production was manifested by the presence of bubbles in the tube, while the sulfide production was manifested by a back deposit at to bottom of the tube.
- Citrate test, which permitted us to determine the ability of the microorganism to metabolize only citrate as it sole carbon source. The change in color media from green to blue showed a citrate positive.
- Urea test: it was used to determine the capacity of the bacteria to hydrolyze urea using the enzyme urease. The change in color of the broth from orange to pink indicated a urease positive.
- Indole test: it detects the capacity of the bacteria to produce the enzyme tryptophanase, which convert the amino acid, tryptophane to indole. Indole reacts with added Kovac's reagent to form rosindole dye which is

red in color (indole +)

- Gram positive bacteria alongside with the reference strain *S. aureus RN4220* was characterized biochemically using the following tests:
- Catalase test: This test was used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. The bubbles resulting from production of oxygen gas clearly indicated a catalase positive result.
- Coagulase test: Coagulase is an enzyme that clots blood plasma. The test was performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus.* This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species (coagulase negative Staphylococcus).
- For Candida: The sample was culture on Saboureaud chloramphenicol agar, and the isolate was tested for the evidence of production of germ tube in human serum, the test kit Integral System YEASTS (Liofilchem) permitted the identification of *Candida krusei*.

Tables 2 and 3 summarized the results obtained.

Table 1. *Detailed information of medicinal plants used in this work.*

Scientific name of the plant	Family of the plant	Part used	Extraction yield (%)	Voucher number
Cussonia arborea (CA)	*Araliaceae*	Bark	13.43	39978/HNC
Dissotis longipetala (DL)	*Melastomataceae*	Bark	9.00	40925/HNC
Lonchocarpus sepium (LS)	*Fabaceae*	Bark	8.00	76230/HNC
Nauclea pobeguinii (NP)	*Rubiaceae*	Bark	10.98	504710/HNC
P*icralima nitida* (PN)	*Apocynaceae*	Bark	14.08	565411/HNC
Rumex abyssinicus (RA)	*Polygonaceae*	Bulbs	17	27239/SRF Cam
Rumex berqueatii De Wild (RB)	*Polygonaceae*	Bulbs	14	7665/SRF Cam.
Sapium ellypticum (SE)	*Euphorbiaceae*	Stem bark	16	47266/HNC
Psorospermum febrifigum (PF)	*Guttiferae*	Stem bark	10.40	36617/ HNC

Table 2. *Biochemical characterization results of Gram negative bacteria.*

Tested strains	Fermentation Glucose	Fermentation Lactose	Production SH2	Production gaz	Urease	Indol	citrate	Presumptive bacteria species
E coli JM109	+	+	_	+	_	+	_	
MCK1	+	+	_	+	_	+	_	*E. coli*
MCK2	+	_	+	_	+	+	_	*Proteus vulgaris*
MCK9	+	_	_	+	_	+	+	*Providencia stuartii*
CLED2	_	_	_	_	_	+	+	*Pseudomonas aeruginosa*

Table 3. *Results of biochemical characterization of gram Positive bacteria.*

Tested strains	Catalase	Coagulase	Yellow colonies on MSA	Presumptive bacteria specie
S. aureus RN4220	+	+	+	
MSA1	+	-	+	*Coagulase negative Staphylococcus (CNS) S. saprophiticus*

2.4.3. Antibiogram

The antibiogram of clinical isolates was carried out in order to select resistant strains. The antibiotics were selected from a list provided by two medical doctors. They were those currently prescribed to patients suffering from urogenital tract infections. The prices were attributed to the antibiotics and only the cheaper once were selected for this study. The following antibiotics were selected: Ciprofloxacin, Norfloxacin, Erytromycin, Cefotaxime, Doxycycline, Bactrim, Metronidazole, Amoxicillin + clavunilic acid and Chloramphenicol for bacteria species; Nystatin, Fluconazole, Griseofluvin and Ketoconazole for candida specie.

The antibiogram of clinical isolates was then performed using disc diffusion method describe by [7]. Briefly, an inoculum of approximately 10^8CFU/ml was swabbed on dried poured plate. The commercial antibiotics discs from the above list were delicately dropped on the surface of plates and allowed at room temperature for pre-diffusion for 15 minutes, and then the plates were incubated at 37°C for 24 hours for bacteria and for 48 hours for *Candida*. After incubation, the diameters of inhibition zone were measured and interpreted using Bauer chart on antibiogram result [7] and the strains were classified as resistant (R) intermediate (I) or sensitive (S) against each antibiotics (see table 5 below).

2.4.4. Antimicrobial Tests

(a) Inhibition zone diameters

The susceptibility tests were performed using the agar well diffusion method described by [4] with slight modifications. Stock solutions of test samples were prepared in 10% v/v dimethylsulfoxide (DMSO) solution at concentrations of 50 mg/ml (for crude extract) and 40µg/ml for gentamicin and fluconazole used as positive control. The inocula were prepared by dissolving 3 to 5 colonies in sterile saline (0.9% NaCl) from a 24 hours culture plates for bacteria and 48 hours for *Candida*, and the turbidity was then adjusted to match 0,5 Mc Farland standard turbidity. 20 ml of Muller Hinton Agar was poured into each of the 90 mm petri dishes and inoculated using a swap tip. The medium was punched with six millimeters diameter wells and filled with 80µl of the test sample. Aqueous DMSO 10% v/v was tested as negative control. After a pre-diffusion of 1 hour, the plates

were incubated at 37±1°C for 24 hours for bacteria species and for 48 hours for *Candida* and inhibition zones were measured. All tests were done in triplicate and the results were recorded as the mean of diameter of zones of inhibition.

(b) Minimum Inhibitory Concentration (MIC)

MICs were determined by broth microdilution technique using 96-well plates as previously described by [8]. Culture media (nutrient broth) were supplemented with 0.2% of glucose and 0.005% phenol red end point indicator. The wells were filled with 100µL of broth, and 100µL of extract was added in triplicate to the first column previously prepared in DMSO to make a final concentration of 512 mg/ml. Successive dilutions were done by transferring 100µl of the mixture from the first well to the eleventh well. An aliquot (100µl) was discarded from the eleventh well. The twelfth well served as control since no sample (extract, or reference antibiotics) was added in it. Finally, 100µl of a standardize inoculum at 10^6 CFU/ml was added in each test well for Gram negative bacteria and 10^5 CFU/ml for Gram positive and yeasts. The final concentration of the extracts used to evaluate the antimicrobial activity ranged from 128 to 0.125 mg/ml and from 128 to 0.125 µg/ml for the reference drugs. Tests were incubated aerobically at 37±1°C for 24 and 48 hours for bacteria and Candida species respectively. The end point was revealed by a color change of the indicator from red to pink or to yellow by comparing the 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). The results were recorded as mean of MICs.

(c) Minimum Microbicidal concentration [9]

A loopful of the test mixture was removed from each MIC well that showed no growth, inoculated onto free Mueller-Hinton agar plate, incubated (37°C, 24-48h), and inspected for presence of colonies indicating growth. The minimal microbicidal concentration was the lowest concentration of extract that showed no microbial (bacterial of fungi) growth.

(d) MMC/MIC ratio

The MMC/MIC ratio was calculated to find out if the microbial inhibition of plant extract was microbicidal or micro-static. Therefore, when MMC/MIC ≤ 4, the inhibition was considered microbicidal, and when MMC/MIC ≥4, the inhibition was considered micro-static [10].

2.5. Statistical Analysis

Excel was used to process data and statistical analysis were done using SPSS version 20 for window. Univariate Analysis of Variance test was used to compare means of diameter of inhibition.

3. Results and Discussion

3.1. Extraction Yield

The table 3 below shows the yields of extraction of the various plants. They vary between 8% and 17%. The methanol was used to extract all the plants. The differences observed in the results show that the yield of extraction is proportional to the solubility of the plant's components in the extracting solvent. Therefore, the yield of extraction increases alongside with the solubility of plant components in the solvent.

3.2. Phytochemical Screening Results

The phytochemical screening results (table 4) showed that except PN, PF and CA, the rest of the studied plants were found to be very rich in tannins. This could explain the antimicrobial activities observed. The results also showed that these previous tree plants (PN, PF and CA) are found not to have most of the screened phytochemicals; this observation could also explain the poor antimicrobial activity displayed by these extracts. Indeed, the antimicrobial activity of extracts depends on the concentration the active components (secondary metabolites) it may contain [11].

Table 4. *Phytochemical test screening results of methanolic extracts of plants.*

	Steroids	Alkaloids	Saponins	Tannins	Terpernoids	Flavonoids
CA	-	-	-	-	+	-
DL	-	+	+	+++++	-	+
LS	-	+	+	+++++++++	-	-
NP	++++	+	++	+	-	-
PF	-	-	-	-	+	+
PN	-	-	-	-	-	-
RA	-	++	++	+++++	+	-
RB	-	++	+	++	+	-
SE	-	+	+++	+++++++++	+	-

(+) present and the number of (+) increases with the intensity of the coloration (-) absent

3.3. Antibiogram

Table 5 presents the antibiogram (ATB) results. It shows that all the five clinical bacteria strains were resistant to at list five of the twelve antibiotics tested, showing the multiresistant character of those strains. *P. stuartii* was the most resistant strain followed by *P. aeruginosa* being resistant to eleven and to nine of the twelve tested antibiotics respectively. Concerning *C. krusei*, it was resistant to two of the 4 antifungal tested. This resistant character of clinical isolates could be related to the inappropriate use of antibiotics by patients, by non-respecting either dose or the duration of the treatment, the too frequent prescription of "broad-spectrum antibiotics", in place of a better targeted

antibiotic this through more precise diagnosis or the unnecessary prescription of antibiotics for viral infection against which they have no effect [12].

Table 5. *Antibiogram results: inhibition zone diameter (mm) of antibiotic discs on tested microorganism.*

Antibiotic/Antifungi	*E. coli*		*P. aeruginosa*		*P. stuartii*		*P. vulgaris*		*S. saprophiticus*		*C. krusei*	
AMC 30	12.8±2.5	R	0.0±0.0	R	0.0±0.0	R	28.3±2.9	S	19.0±1.4	S	-	-
C: R≤12; I:13-17; S≥18	23.0±0.0	S	10.0±0.0	R	0.0±0.0	R	0.0±0.0	R	10.0±0.0	R	-	-
CIP 30: R≤15; I:16-20; S≥21	0.0±0.0	R	30.0±0.0	S	20.0±0.7	I	25.5±2.8	S	10.0±0.0	R	-	-
CN 120: R≤12; I:13-14; S≥15	21.0±0.0	S	23.0±0.0	S	18.0±0.0	S	20.0±0.0	S	20.0±0.0	S	-	-
CRO 30 R≤14; I:15-22; S≥23	20.0±0.0	I	0.0±0.0	R	0.0±0.0	R	26.0±0.0	S	0.0±0.0	R	-	-
CTX 30: R≤14; I:15-22; S≥23	21.0±1.4	I	0.0±0.0	R	11.0±1.4	R	31.3±1.2	S	12.5±1.7	R	-	-
D0 30: R≤14; I:15-18; S≥19	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	18.7±2.3	I	-	-
E 15: R≤13; I:14-22; S≥23	10.8±1.9	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	21.0±1.4	I	-	-
MET 50	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	-	-
NOR 10: R≤15; I:16-20; S≥21	0.0±0.0	R	12.7±0.6	R	9.5±0.7	R	13.3±1.1	R	0.0±0.0	R	-	-
TE 30: R≤14; I:15-18; S≥19	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	17.3±3.1	I	-	-
TMP 5: R≤10; I:11-15; S≥16	19.0±1.4	S	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	0.0±0.0	R	-	-
FLU 100	-	-	-	-	-	-	-	-	-	-	23.5±2.1	S
GRS 10	-	-	-	-	-	-	-	-	-	-	0.0±0.0	R
KTC 10	-	-	-	-	-	-	-	-	-	-	31.0±1.4	S
NY 100	-	-	-	-	-	-	-	-	-	-	15.5±0.7	R
12/4	7R		10R		10R		7R		7R			

Values are mean ±SD. (-) = not tested, CIP 30: Ciprogloxacin, NOR 10: Norfloxacine, CRO 30: Ceftriaxone, CTX 30: Cefotaxime, DO 30: Doxycycline, TE 30: Tétracycline, TMP 5: E 15: Erytromycin, MET 50: Metronidazole, AMC 30: Amoxicilin + acide clavunilique, CN120: Gentamicine, C: Chloramphénicole NY 100: Nystatine, FLU 100: Fluconazole, GRS 10: Grisefluvin, KTC 10: Kétoconazole.

3.4. Antimicrobial Activities

3.4.1. Inhibition Zone Diameters

Table 6, below present inhibition zones diameters (mm), a bacterium or a yeast strain was considered susceptible to an extract when the tested extract exhibited on it an inhibition zone diameter equal to 7 mm and to 10 mm respectively [13]. The results show that at a concentration of 50mg/ml, only two extracts of the tested plants PF, CA showed no activity on all the pathogens. While the rest of the plant extracts SE, NP, DL, PN, RB, and RA were active on at least 50% of tested strains with inhibition zone diameters ranging from 7.67±0.58 to 24.33±1.15 mm. only *P. nitida, could* inhibit the growth of *E. coli* on agar media. *P. stuartii* was resistant to gentamicin, the reference drug, confirming its natural resistance against gentamicin [14]. Comparing the activity of extracts with reference antimicrobial agents at the tested concentrations using diffusion method, *PN* has an activity comparable to that of fluconazole ($p<0.05$). The same observation was made between gentamicin and the following extracts *RA*, *RB, DL* on *S. saprophiticus* ($p<0.05$) as well as between gentamicin and *DL* on *P. vulgaris*. However, the activity of most extracts was higher than that of gentamicin on the reference strain *E. Coli JM109* and that of *SE* was higher than that of gentamicin on *E. Coli JM109, P. stuartii,* and *S. saprophiticus.*

Table 6. *Inhibition zone diameters (mm) of methanolic plant extracts on tested microorganisms.*

Substance tested	*C. krusei*	*E. coli*	*E. Coli JM109*	*P. aeruginosa*	*P. stuartii*	*P. vulgaris*	*S. saprophiticus*
CA	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
DL	15.00±0.00b	0.00±0.00a	24.33±1.15e	15.00±0.00d	14.67±0.58c	17.33±1.15d,e	16.33±0.58d
LS	10.00±0.00b	0.00±0.00a	22.00±2.00d,e	10.00±0.00c	0.00±0.00a	13.33±1.15b,c	11.00±1.00b
NP	14,67±1,53b	0.00±0.00a	7.67±0.58b	10.00±0.00c	0.00±0.00a	0.00±0.00a	13.33±1.15b,c
PF	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
PN	20.33±1.53c	12.00±0.00b	19.00±1.73c,d	9,00±0,00b	0.00±0.00a	0.00±0.00a	20.00±0.00e
RA	15.00±1.00b	0.00±0.00a	21.67±1.53d,e	15.33±0.58d	10.67±0.58b	13.67±1.53b,c	16.00±0.00c,d
RB	14.00±0.00b	0.00±0.00a	16.33±1.53c	10.00±0.00c	13.00±1.00c	12.33±1.15b	14.33±0.58c,d
SE	16.33±1.53b	0.00±0.00a	24.00±1.00e	15.00±0.00d	14.00±1.00c	15.33±0.58c,d	19.33±1.15e
GENTA*	NT	23.00±1.00c	11.33±1.15b	20.00±0.00e	0.00±0.00a	20.00±0.00e	16.00±1.00c,d
FLUCO*	21,67±1,53c	NT	NT	NT	NT	NT	NT
DMSO	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Values are mean ± SD; DMSO: diluting solvent, NT (not tested) *Cussonia arborea (CA), Dissotis longipetala (DL Lonchocarpus sepium (LS), Nauclea pobeguinii (NP), Picralima nitida (PN), Rumex albissinicus (RA), Rumex berqueatii (RB), Sapium ellypticum (SE), Psorospermum febrifigum (PF)*, *: reference drugs. Plant extract not carrying the same letter in a column are statistically different ($p>0.05$), and plant extract carrying the same letter in a column are statistically identical ($p<0.05$). Activities of extracts carrying letter a<b<c<d<e

3.4.2. Microdilution Tests: Inhibition Parameters (MIC, MMC, and MMC/MIC Ratio)

Table 7 resumes minimum inhibitory concentrations (MIC), minimum microbicidal concentrations (MMC) and MMC/MIC ratios of tested extracts on pathogens. MICs results showed that MICs ranged from 0.125 to 128mg/ml for

plant extracts, from 0.0005 to 0.016mg/ml for gentamicin and that of fluconazole on *C. krusei* was 0.002mg/ml. Authors are not unanimous about standard cut-off points describing antimicrobial activities of crude extracts. Nevertheless, according to [15], an activity is considered to be significant if MIC values are below 0.1mg/ml for crude extract and moderate when 0.1< MIC < 0.625 mg/ml, considering this stringent classification, the activity of *S. ellipticum, R. abyssinicus*, and *D. longipetala* can be considered moderate since they show a MIC ≤ 0.25mg/ml on two resistant strains *S. aureus* and *C. krusei*. An alternative criterion has been described by [16], which considers extracts having MIC values below 8 mg/ml to have noteworthy antimicrobial activity. Based on these previous reports, and considering the differences in methods used, as well as the level of resistance of microorganisms involved in the studies, the antimicrobial potential of the various extracts in this study has been categorized, as follow: MIC<1mg/ml the plant extract was very active, 1≥MIC≤8mg/ml, the plant extract was moderately active, 8>MIC≤64mg/ml were considered less active or with activity negligible, MIC>64mg/ml was not active. The table 7 below summarizes the categorization of the crude extracts of plants.

Table 7I. *Categorization of the activity of plant extracts.*

Substance tested	Inactive MIC>64mg/ml	Less active 8>MIC≤64mg/ml	Moderately Active 1≥MIC≤8mg/ml	Very active MIC<1mg/ml	total
CA	4	3	0	0	7
DL	0	2	4	1	7
LS	0	2	4	1	7
NP	0	0	7	0	7
PF	3	4	0	0	7
PN	0	5	2	0	7
RA	0	2	3	2	7
RB	0	5	1	1	7
SE	0	0	5	2	7
Total of plant	7	23	26	7	63

Cussonia arborea (CA), Dissotis longipetala (DL Lonchocarpus sepium (LS), Nauclea pobeguinii (NP), Picralima nitida (PN), Rumex albissinicus (RA), Rumex berqueatii (RB), Sapium ellypticum (SE), Psorospermum febrifigum (PF)

Sapium ellipticum showed the highest inhibitory activity. It was very active on two strains *S. saprophiticus* and *C. krusei* (MIC< 1mg/ml), and moderately active on the rest of the strains with (MICs ranging from 1mg/ml to 8mg/ml). The growth inhibition was bactericidal MMC/MIC ≤4 on all the pathogens [10] (see Table 8) making it a good candidate for drug preparation. The phytochemical screening results showed that it contents alkaloids, saponins, tannins, and terpenoids which could explain the observed antimicrobial activities. 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 [17]. Some of characteristics of saponins include formation of foam in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [18]. The antimicrobial mechanism of action of the alkaloids may be through DNA intercalation and inhibition of DNA synthesis through topoisomerase inhibition [18]. Some studies have been done on the isolation and characterization of biological active components of this specie [19] but very few studies have reported the antimicrobial activities of this plant. The methanolic extract has been found to be hepato-protective [20]. Anti-fungal activities of dichloromethane and aqueous bark extracts of *Sapium ellipticum* have been carried out in Tanzania [19]. But, to the best of our knowledge, antibacterial activities of methanolic extracts of this plant are reported here for the first time.

Nauclea pobeguinii was moderately active on all the seven tested pathogens with MICs comprised between 1mg/ml and 8mg/ml, and the inhibition was bactericidal on all these strains. The plant was found to contain steroids, saponins and tannins which could explain the activities observed [18, 21]. Antimicrobial activity of the bark extract of this plant, using the microdilution method, presented better results than well diffusion method. This could be explained by a limited diffusion of the extract in the agar. To the best of our knowledge, antibacterial and antifungal activities of this plant are reported here for the first time. However, previous studies demonstrated a moderate antiplasmodial activity [22]; Moreover antioxidant activities of methanol bark extract of *N. pobeguinii* has been reported [23].

Dissotis longipetala was very active on *S. saprophiticus*, (MIC=0.25mg/ml) and the inhibition was bactericidal. It was moderately active on *C. krusei, P. stuartii, P. vulgaris* and *E. coli, (*MIC ≤8mg/ml); and less active on *P. aeruginosa,* and *E. coli JM109* The phytochemical screening of this plant showed that it contains tannins, alkaloids and saponins, hence the antimicrobial properties demonstrated by this plant could be attributed to some of these biological active components [17-18]. This plant was reported to have very important antioxidant and reducing power [23] but the antimicrobial properties of the plants are reported here for the first time.

R. abyssinicus was very active on *S. saprophiticus* and *C. krusei*, MIC = 0.25mg/ml, and the inhibition was bactericidal on *S. aureus* (MBC/MIC=1) and bacteriostatic on *C. krusei* (MMC/MIB = 8) [10]. The extract was moderately active (MIC≤8mg/ml) on *P. stuartii, P. vulgaris* and *E. coli JM109,* while less active on *E. coli, P. aeruginosa*, this activities could be attributed to tannins and alkaloids contained by the

plant [17-18]. Some studies have been reported on biological properties of this plant. Antimicrobial activities of this plant has previously been demonstrated on *Salmonella typhymurium, Listeria monocytogens, Escherichia coli and Staphylococcus saprophiticus* [24] an against *streptococcus pyogenes, S. aureus*, *Coxsackie virus B3* and *Influenza A virus* [25]. Our results are slightly different from those of [24], but this difference in susceptibility could be attributed to the inherent resistance factors of the test organisms [26], the strains we used were by demonstrated to be multidrug resistant (see table V above). Moreover, the difference could also be explained by the test procedure which was different [24]. Except these properties, this plant has been shown to have antioxidant properties [24], anticancer activities [24, 27] antihelmentic activities [28], diuretic and analgesic effects [29], antiplasmodial properties [30].

Picralima nitida was moderately active on two strains (*S. saprophiticus* and *C. krusei*) and less active on the rest of five strains. A good number of reports on antimicrobial activities of this plant and its derivatives have been published [31-34] and our results corroborate those of the literature [35-36].

Antimicrobial activities of the crude extracts of *R. berquaetii*, *P. febrifugum* and *Cussonia arborea* corroborate those of literature [24], however, on the best of our knowledge, the antimicrobial activities of the crude extracts of *Lonchocarpus sepium* are reported here for the first time and have shown moderate activities on four strains (MIC≤8mg/ml).

Regarding the phytochemical screening results, all the extracts (*CA, PF*, *PN*,) which show poor activity on the tested microorganisms were found to contain none or very few classes (in traces) of secondary metabolites tested during this work (see table 4). This remark sustains the hypothesis where by the antimicrobial activity of an extract is attributed to the secondary metabolites it contains [10, 17-18, 21]. On the same note, most extract which show good activities in this study were found to abundantly contain tannins. Therefore the antimicrobial activities of the extracts demonstrated here could be attributed to tannins.

The overall results of the MICs showed that apart from the strain which the MIC could not be determined, the MICs of gentamicin and fluconazole are lower compare to that of the various extracts, this could be explained by the fact the extract till contain some impurities which can hinder its activity in comparison with reference antimicrobial agent which are pure: biological active compounds.

4. Conclusion

The results of the present study provided evidence for the antibacterial and antifungal activities of the studied plant extracts and brought supportive data for future investigations that will lead to their use in urinary and genital tracts infections therapy, and other infections cause by these pathogens. The categorization of extract activities permitted us to select four of the plants as having important activities (*Sapium ellipticum, Nauclea pobeguinii, Dissotis longipetala* and *Rumex abyssinicus*) having demonstrated a MIC ≤ 8mg/ml on at least five of the eight tested pathogens. They were therefore kept for further investigations. This study also revealed the presence of various phytochemical groups in various plants, but tannins have been shown as responsible of the observed activities.

Table 8. *Minimal Inhibitory Concentrations (MIC), (mg/ml) Minimal Microbicidal Concentrations (MMC) and the ratios MMC/MIC of plant extracts and reference drugs on tested pathogens.*

EXTRACTS	*C. Krussei*			*E. coli*			*E. Coli JM109*			*P. aeruginosa*		
	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC
CA	64	128	2	64	ND	ND	128	ND	ND	128	ND	ND
DL	1	1	1	8	128	16	16	32	2	16	32	2
LS	4	4	1	16	64	4	32	64	2	64	64	1
NP	1	8	8	1	8	8	8	8	1	8	16	2
PF	16	128	8	64	ND	ND	128	128	1	128	128	1
PN	8	16	2	16	32	2	32	64	2	64	64	1
RA	0.25	2	8	32	64	2	8	4	2	64	64	1
RB	0.25	2	8	32	128	4	32	64	2	64	64	1
SE	0.25	0.25	1	8	32	4	8	16	2	4	8	2
GENTA	NT	NT	NT	0.0005	0.0005	1	ND	ND	ND	0.002	0.002	1
FLUCO	0.002	4.00	2.00	NT	NT	NT	NT	NT	NT	NT	NT	NT
DMSO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 8. *Continue.*

EXTRACTS	*P. stuartii*			*P. vulgaris*			*S. saprophiticus*		
	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC
CA	128	ND	ND	64	ND	ND	128	ND	1
DL	4	32	8	8	32	8	0.25	0.5	2
LS	16	32	2	8	32	4	8	16	2
NP	4	8	2	4	16	4	1	4	4
PF	64	ND	ND	64	ND	ND	128	128	1
PN	32	64	2	32	64	2	2	4	2
RA	8	32	4	8	128	16	0.25	0.25	1

EXTRACTS	*P. stuartii*			*P. vulgaris*			*S. saprophiticus*		
	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC	MIC	MMC	MMC/MIC
RB	32	64	2	16	64	4	2	4	2
SE	1	2	2	2	2	1	0.12	0.25	2
GENTA	0.016	0.032	2	0.005	0.0005	1	0.0025	0.0025	1
FLUCO	NT	NT	NT	NT	NT	NT	NT	NT	NT
DMSO	ND	ND	ND	ND	ND	ND	ND	ND	ND

Values are mean; ND: not determined NT (not tested) (the value is higher than the highest concentration tesded) -: Untested *Cussonia arborea* (CA)*, Dissotis longipetala* (DL), *Lonchocarpus sepium* (LS), *Nauclea pobeguinii* (NP), *Picralima nitida* (PN), *Rumex albissinicus* (RA), *Rumex berqueatii* (RB), *Sapium ellypticum* (SE), *Psorospermum febrifigum* (PF), *: reference drugs, DMSO: diluting solvent

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