Original Research Article

Comparative study of the antibacterial and cytotoxic activity of leaves, trunk and roots of *Schumanniophyton magnificum*

ABSTRACT

Aims: *Schumanniophyton magnificum* is a plant reputed traditionally to treat numerous urogenital infections linked to several germs, notably: *Escherichia coli, Staphylococcus aureus, Neisseria gonhorhea, Treponema palidum, Candida albican.* This study aims to evaluating the *to evaluate and compare the in vitro antibacterial, and cytotoxic activities of the leaves, trunk and roots of S. magnificum in order to determine the parts displaying the best activity.*

Study design: This is an experimental study.

Location and duration of the study: The work was conducted at the Pharmacochemistry and Natural Substances Laboratories of the Faculty of Medicine and Pharmaceutical Sciences, University of Douala for the extraction and at the Medical Analysis Laboratory of the Douala General Hospital for antibacterial test as well as Biomedical Chemistry Research Center of Rhodes University in South Africa for cytotoxic essay. All the experiments were carried out from the 15th November 2020 to the 31th May 2021.

Methodology: The *in vitro* Antibacterial activity assay was conducted on two strains, one Gram+ bacteria: *Staphylococcus aureus* and one Gram- bacteria: *Escherichia coli*, while cytotoxicity was evaluated on HeLa cell line.

Results: The root wood extract and the root bark extract presented a good antibacterial activity (MIC = 195.31 µg/ml) and average activity (MIC = 390.63 µg/mL) respectively with *E. coli.* The different extracts were found to be bactericidal against the tested micro-organisms. At the concentration of 20 µg/mL, all extracts tested did not significantly reduce HeLa cells, with an inhibition rate ranging from 13.5 to 37.6%, suggesting a low cytotoxicity of *S. magnificum* extracts towards these human cells line.

Conclusion: This is the first comparative report on the antibacterial and cytotoxic study conducted on the different parts of *S. magnificum.* It appears from this study that these results could justify the use of the stem bark of *S. magnificum* in the traditional pharmacopeia for the treatment of urogenital infections. However, the roots wood having presented the best antibacterial activity (MIC = 195.31 µg/mL), it would be beneficial to suggest this plant part to the local populations for the treatment of bacterial diseases specific to *E. coli* and *S. aureus*.

Keywords: Schumanniophyton magnificum, antibacterial activity, cytotoxicity, medicinal plant.

1. INTRODUCTION

Infectious diseases are a public health problem in both developing and industrialised countries, both in terms of their frequency and severity [1]. They are responsible for 17 million deaths per year worldwide, 43% of which occur in underdeveloped countries [2].

Their management relies on the use of antimicrobials [3]. *Escherichia coli*, a major cause of urinary tract infections has also become resistant to fluoroquinolone antibiotics in many countries around the world [4]. It's the same case with *Staphylococcus aureus*, a common cause of severe infections in health facilities which has also developed antibiotic resistance [4]. The use of antimicrobial treatments in the clinic is hampered by the natural phenomenon of microbial resistance, which is accentuated by the irrational use of available molecules [5]. In 2016, the World Health Organization (WHO) reported that *Escherichia coli* was resistant to fluroquinolones, the most widely used class of drugs against this germ in urinary tract infections [6]. antimicrobial resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 [7].

In addition to this worrying situation, there is the problem of misuse, abuse and availability of the drug. This justifies the need to search for new effective anti-infective molecules that can be used as a therapeutic reserve. The use of the antimicrobial properties of plants dates back to ancient times [8]. Due to the emergence of drug-resistance, there is a permanent need for the discovery of new treatment for bacterial infections and medicinal plants remain a good target for this research [9]. In 1998, the WHO recommended the use of plants in the search for new therapeutic opportunities. In developing countries, 80% of the population uses herbal preparations for their healthneeds at least once a year [10]. These preparations, used without prior scientific evaluation of their toxicological or therapeutic properties, are at risk of intoxication to humans cells or inefficient regarding the target.

In our environment, *Schumanniophyton magnificum*, a plant of the Rubiaceae family, is reputed on one hand to traditionally treat malaria, anaemia and numerous urogenital infections linked to several germs, notably : *Escherichia coli, Staphylococcus aureus, Neisseria gonhorhea, Treponema palidum, Candida albican* [11;12] and on the other hand, many researchers have evaluated activities such as: antimalarial [13], antimicrobial [14;15] antivenomous [16] of the stem bark; anticonvulsant [17] and antiviral against Acquired Immunodeficiency Virus and Herpes Simplex Virus infection [18] of the root bark. In addition, some claim that the plant is used to treat cancer [11]. The choice of this plant was made on the basis of an ethnobotanical survey and previous *in vitro* and *in vivo* studies yet reported. In order to scientifically justify the claims of the herbalists, we proposed to carry out an experimental study to evaluate and compare the *in vitro* antibacterial, and cytotoxic activities of the leaves, trunk and roots of *S. magnificum* in order to determine the parts displaying the best activity.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves, trunk (bark and wood) and roots (bark and wood) of *S. magnificum* were collected on October (raining season in Cameroon) 2019 in Okon, a village located in centre region of Cameroon. The botanical identification was carried out at the National Herbarium of Cameroon by Mr. Nana Albert by comparison to a samples having the Voucher number 52128/HNC.

2.2. Preparation of extracts

The different parts of the plant were dried and powdered (1.3 kg of leaves, 1.5 kg of stem bark, 1.2 kg of trunk wood, 1.2 kg of root bark and 1.2 kg of root wood). The obtained powders were macerated with 96% methanol (15 L, 12 L, 12 L, 12 L and 12 L respectively) at room temperature for 72 h. The macerates were filtered on Whattman paper and evaporated under reduced pressure at 40°C at 60 rpm.

UNDER PEER REVIEW

The extraction yields were calculated according to the formula below:

$$yield = \frac{\text{weight of the dried crude extract}}{\text{weight of the powder}} \times 100$$

2.3. Antibacterial activity

Antibacterial activity assay was conducted on two strains, one Gram+ bacteria: *Staphylococcus aureus* 050402052773271 and one Gram- bacteria: *Escherichia coli* 0405610540006601. The bacterial strains were provided by the Medical Analysis Laboratory of the Douala General Hospital. They were clinical isolates from urine and vaginal samples of hospitalized patients. Cyprofloxacine was used as the refence drug.
Mueller Hinton medium (liquid and solid) from LIOFILCHEM laboratories enriched with 0.005% red phenol for bacteria; Chapman medium, selective for staphylococci; EMB medium (methylene blue eosin); RPMI 1640 containing 2 mM L-glutamine and 25 mM Hepes (Lonza) supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2-4%. The culture media were prepared and autoclaved according to the manufacturer's recommendations for testing.

2.3.1. Sterility test

The purpose of this test is to verify if different extracts are free of bacteria. Sterility will be checked by plating the different extracts on Mueller Hinton agar followed by incubation at 37˚C for 2 days with a reading every 24 hours. Extracts will be declared sterile, if no colonies are visible on the agar plate. Antibacterial activities were evaluated following the micro method dilution on wells in liquid media.

2.3.2. Evaluation of the antibacterial activity of methanolic extracts of *S. magnificum*

A method used for the evaluation of the antibacterial activity of the different extracts was the dilution method in liquid medium (micro-dilution), for the determination of the parameters of inhibition of the bacterial grow was the Minimum Inhibitory Concentration (MIC) which is the smallest concentration of extract that inhibits all microbial growth visible to the naked eye ; the Minimum Bactericidal Concentration (MBC) which is the lowest concentration of extract that kills 99.99% of bacteria compared to the growth control or conversely, it is the lowest concentration that leaves a 0.01% survival compared to the growth control.

Using a platinum loop, an isolated colony of microorganisms is picked and streaked onto Mueller Hinton Agar medium poured into Petri dishes. The plates are incubated at 37°C for 24 hours [19;20].

For each microorganism to be tested, bacterial colonies from a pure culture of less than 24 hours inoculated onto Mueller Hinton Agar medium and incubated at 37°C were suspended in test tubes containing 5 mL of sterile physiological water, until a turbidity similar to that of the 0.5 point of the McFarland scale, corresponding to the concentration of 1.5 x 108 Colony Forming Units/mL (CFU/mL), was achieved. The inoculum thus prepared was diluted 10 times.

2.3.3. Micro-dilution method in liquid media on wells

This test is used to determine the inhibition parameters of plant extracts, including the Minimum Inhibitory Concentration (MIC) and the Minimum Microbicidal Concentration

UNDER PEER REVIEW

(MMC). It is based on the visual observation of the pellet formed by the deposition of bacteria and/or the change in colour of the colour indicator (red phenol), which changes either from red to yellow due to the release of acidic metabolites into the medium, or from red to pink due to the release of basic metabolites into the culture broth.

Preparation of extracts (S1): Dissolve 600 mg of extract in 800 µL of 10% DMSO and leave in contact for 3 h; Vortex and add 2700 µL of Mueller Hinton liquid medium enriched with 1% glucose and 0.005% red phenol for bacteria and yeast respectively; The final volume is 3000 µL of solution for a concentration of 200 mg/mL.

Preparation of stock solution (S2): For a total volume of 5000 µL solution; 500 µL of DMSO was mixed with 4500 µL of Mueller Hinton enriched with 1% glucose and 0.005% red phenol respectively. The inoculum thus prepared was diluted 100 times; Inoculation: The 96-well U-bottom microplates were used for MIC determination as previously described by Odeh et al. 2014 [21].

Procedure: Identify the plate with the name of the strain tested, the reading direction, the positive and negative control, the date of the manipulation, the initials of the manipulator; take 100 µL of S1 with a multichannel pipette and pour into the first 3 wells of the first column; Then dispense 50 µL of S2 medium into each well of the first 3 rows of the plate from the 2nd to the 11th column; Remove 50 µL of S2 and pour into the first 3 wells of the 12th column; Begin dilutions by taking 50 µL of the mixture from the 1st to the 11th well in a geometric progression of reason 2 ; Then take 50 µL of the mixture from the 12th column wells and pour into the first 3 wells; Add 50 µL of inoculum to the first 2 rows of the plate from the 2nd to the 12th column; Close and incubate the plate aerobically at 37 ± 1°C for 24 and 48 hours. At the end we determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration. The MIC is obtained for the lowest concentration of extract showing no visible growth, i.e. the change in colour from red to yellow of the medium due to the formation of acid metabolites corresponding to microbial growth. The MIC value is considered in the well just before which there was no change in colour.

One loop of the test mixture was removed from each MIC that showed no growth, inoculated onto MHA, incubated (37°C, 24 h) and inspected for the presence of colonies indicating growth. The MBC/MIC ratio was calculated to determine whether the microbial inhibition of the plant extract is bactericidal or bacteriostatic. Therefore, when MBC/MIC < 4, the activity is considered to be bactericidal; when MBC/MIC ≥ 4, the inhibition is considered bacteriostatic of the test substances [22].

2.4 Cytotoxic Activity

The cytotoxicity of the extracts was assessed on HeLa (human cervix adenocarcinoma) cells using the protocol previously described by our team [23-25]. Briefly, 100 µl containing $2x10^4$ HeLa cell in Dulbecco's Modified Eagle's Medium (DMEM) with 5 mM L-glutamine (Lonza), supplemented with 10% foetal bovine serum (FBS) and antibiotics (penicillin/streptomycin/fungizone - PSF) were dispensed into wells of the flat bottom 96 well plate. The plates were preincubated for 24 hours in a 5% CO_2 humidified incubator. This allowed the cell to adhere to the bottom of the well. The culture medium (supernatant containing death cells) was removed afterward and replaced with 200 µl of the same fresh culture medium containing plants extracts at the maximum concentration of 50 µg/ml. the plates were designed in such a way that each concentration of each plant's extracts is prepared in duplicate. The prepared plate was incubated in the same condition for 48 hours. The cell viability was monitored by checking the mitochondria functionality using the resazurin based tests. The test is based on the reduction of the oxidized non-fluorescent blue resazurin to a red fluorescent dye (resorufin) by the mitochondrial respiratory chain in live cells. The resorufin was read in the fluorescent mode with Excitation/Emission (560 nm/590 nm). The intensity of the fluorescence is directly proportional to the number of living cells. The following formula was used to calculate the percentage of living cells.

UNDER PEER REVIEW

2.6. Data Analysis and Statistical Parameters

Excel software was used to record the data and GraphPad Prism 8.0.1. software allowed us to plot the curves.

3. RESULTS

3.1. Extraction

Extraction of the different parts of *S. magnificum* allowed us to have the concentrations of the crude extracts, their colour and appearance (Table I).

Table 1. Extraction yield of the methanolic extracts of the different parts of *Schumanniophyton magnificum*

Plant material	Powder (g)	Crude extract (g)	Yield (%)	Aspect	Colour
Root wood	1200	22	1.83	Pasty/oily	Yellow
Root bark	1200	25.1	2.05	Pasty/oily	Brown
Trunk wood	1200	30	2.5	Pasty/oily	Yellow
Stem bark	1500	12	0.8	Sticker	Green
Leaves	1300	60	4.6	Pasty	Black

The leaves extract of *S. magnificum* has the highest yield (4.6%) followed by trunk wood extract (2.5%), root bark extract (2.05%), root wood extract (1.83%) and trunk bark extract (0.8%).

3.2. Antibacterial activity

The various total extracts (SMMwt, SMMwr, SMMbt, SMMwt, SMMle) were subjected to antibacterial tests by the micro-dilution method on plates for the determination of MIC, MBC. The antimicrobial activity estimation scale given by Kuete in 2010 was used as a reference [26].
Crude extracts from the leaves, trunk and roots of *S. magnificum* were subjected to antibacterial tests. The concentrations of the extracts ranged from 200 mg/mL in the 1st well to 0.19 mg/mL in the 11th well for MICs and 1562.50 to 12500 µg/mL for MBCs. The results obtained are grouped in Table II.

Table 2. Values of MIC, MBC and MBC/MIC ratios of extracts.

Extracts	Parameters (µg/mL)	Bacterials isolates	
		E. coli	*S. aureus*
Stem bark (SMMsb)	MIC	3125.00	6250.00
	MBC	3215.00	12500
	MBC/MIC	1	2
Root bark (SMMrw)	MIC	390.63	781.25
	MBC	1562.50	1562.50
	MBC/MIC	3.99	2
Trunk wood	MIC	1562.50	3125.00

UNDER PEER REVIEW

(SMMtw)	MBC	1562.50	6250.00
	MBC/MIC	1	2
Root wood (SMMrw)	MIC	193.31	781.25
	MBC	ND	ND
	MBC/MIC	0	0
Leaves (SMMle)	MIC	3125.00	3125.00
	MBC	6250	6250
	MBC/MIC	2	2
Ciprofloxacin	MIC	2	4
	MBC	2	8
	MBC/MIC	1	3

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bacteriostatic Concentration, MBC/MIC = MBC/MIC ratio, ND = Not Determined; Ciprofloxacin¨: Positive Control, EC: Escherishia coli, SA: Staphylococcus aureus, SMMrw: S. magnificumroot wood, SMMrb: S. magnificum root bark, SMMwt: S. magnificum stem wood, SMMsb: S. magnificum stem bark, SMMle: S. magnificum leaves

The micro-dilution method in liquid media allowed the determination of the inhibition parameters of the micro-organisms. Table II above illustrates the results obtained. The inhibitory activities of the different extracts were observed at concentrations between (195.31 and 6250 µg/mL) for MICs and (12500 to 1562.50 µg/mL) for MBC. We observe that: The methanolic extract of the stem bark (SMMsb) gave a MIC of 3125.00 towards *E. coli* and 6250.00 towards *S. aureus* and a MBC of 3125.00 towards *E. coli* and 12500.00 towards *S. aureus* ; Methanolic root bark extract (SMMrb) gave 390.63 MIC against *E. coli* and 781.25 MIC against *S. aureus,* with a MBC of 1562.50 against the two germs tested ; The methanolic extract of trunk wood (SMMtw) gave a MIC of 1562.50 for *E. coli* and 3125.00 for *S. aureus* inhibition against a MBC of 1562.50 for *E. coli* inhibition and 6250.00 for *S. aureus* ; The methanolic extract of root wood (SMMrw) gave a MIC of 195.31 against *E. coli* versus 781.25 against *S. aureus* and an undetermined MBC against both germs ; The methanolic leaves extract (SMMle) gave a MIC of 3125.00 for both germs and a MBC of 6250.00 for both germs.

3.3. Cytotoxic activity

The five extracts (SMMrw, SMMrb, SMMtw, SMMsb, SMMle) were subjected to cytotoxicity tests against HeLa cells. However, no significant cytotoxicity was observed on the viability of HeLa cells. Figure 1 shows the percentage viability values of the different extracts and their standard deviation.

UNDER PEER REVIEW

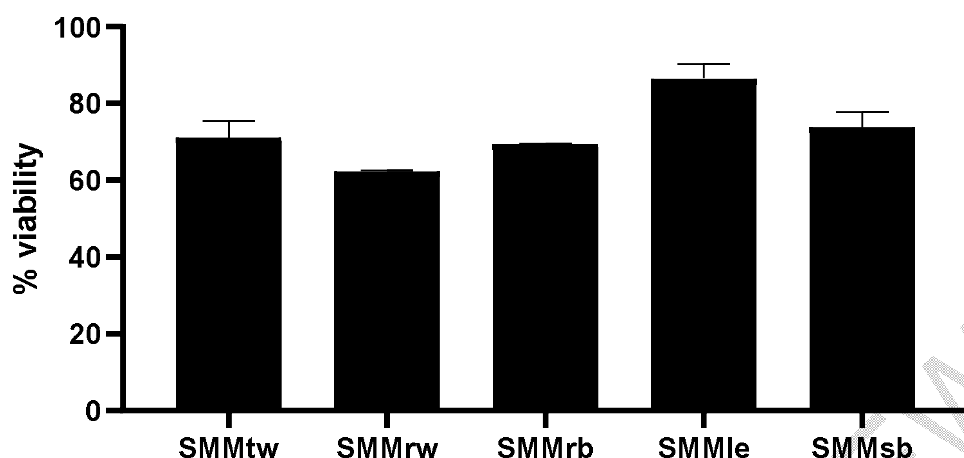

Figure 1: Percentage viability of HeLa cells.
Legende : %. Viability : percentage viability, SMMrw: Schumanniophyton magnificum methanolic extract of root wood, SMMrb: Schumanniophyton magnificum methanolic extract of root bark, SMMtw: Schumanniophyton magnificum methanolic extract of trunk wood, SMMsb: Schumanniophyton magnificum methanolic extract of stem bark, SMMle: Schumanniophyton magnificum methanolic extract of leaves

Figure 1 show that *S. magnificum* extracts did not significantly reduce the viability of HeLa cells with viability percentages of 71.1% for SMMtw, 62.4% for SMMrw, 69.5% for SMMrb, 86.5% for SMMle and 73.8% for SMMsb.

4. DISCUSSION

In the present work, we aimed to valorise the use of *S. magnificum* by traditional therapists, more specifically to evaluate the antibacterial and cytotoxic activities of the leaves, trunk and roots.

4.1. Extraction

The extractions of the *S. magnificum* organs were carried out in a single step by maceration with methanol. The solvent was chosen on the one hand for its low boiling temperature which is arount 65°C and on the other hand for its capacity to dissolve a large proportion of polar and non-polar compounds. We obtained low yields ranging from 0.9 to 4.6% for the different extracts. These results do not corroborate those of Ben in 2018 that made an aqueous decoction of *S. magnificum* leaves with a yield of 11.8% [27]. This difference could be explained on the one hand by the difference in the solvent, the themperature of extraction and on the other hand by the extraction method, the place of harvesting and the temperature of the environment; Ben Emma harvested during the month of March (dry season) in southern Cameroon [27], Unlike us who harvested in October (raining season) in the central region of Cameroon.

4.2. Antibacterial activity

Antibacterial activity was evaluated by the micro-dilution method on wells in liquid medium following the protocol of Odeh et ***al.*** 2014 [21]. The results showed that extracts of different parts exhibited variable levels of antibacterial activity (Table 2). According to Kuete in 2023, for plant extracts we can have outstanding activity when MIC ≤ 8 µg/mL; excellent activity

UNDER PEER REVIEW

when 8 < MIC ≤ 64 µg/mL; very good activity when 64 < MIC ≤ 128 µg/mL; good activity when 128 < MIC ≤ 256 µg/mL, average activity when 256 < MIC ≤ 512 µg/mL, weak activity when 512 < MIC ≤ 1024 µg/mL, and not active MIC > 1024 µg/mL [26]. The results ranged from 195.31 to 6250 µg/ml for MIC values and 3125 to 12500 ug/ml for BMCs against *E. coli* and *S. aureus*. From this classification, it can be seen that the methanolic extract of stem bark (SMMsb) showed a weak activity against *E. coli* (MIC = 3125.00 µg/mL) and *S. aureus* (MIC = 6250.00 µg/mL) ; Methanolic root bark extract (SMMrb) displayed a average activity against *E. coli* (MIC = 390.63 µg/mL) and a weak activity against *S. aureus* (MIC = 781.25 µg/mL); The methanolic extract of trunk wood (SMMtw) exhibit not activity against *E. coli* (MIC = 1562.50 µg/mL) and *S. aureus* (MIC = 3125.00 µg/mL) ; The methanolic extract of root wood (SMMrw) showed a good activity against *E. coli* (MIC = 195.31 µg/mL) and a weak activity against *S. aureus* (MIC = 781.25 µg/mL); The methanolic leaves extract (SMMle) gave not activity against the both germs (MICs = 3125.00 µg/mL). These results are comparable with those obtained with those of Bayebec *et al.* in 2017 who found a low antibacterial activity of the ethyl acetate fraction of methanolic extracts of *Schumanniophyton magnificum* root with an MIC between 1250 and 2500 µg/mL due to the presence of flavonoids [15]. According to Marmonier's scale in 1990, when the BMC/MIC ratio is less than 4, the extract has bactericidal activity and when it is greater than or equal to 4 the activity is bacteriostatic (MBC/MIC < 4) [22]. On this basis, it could be said that the crude extracts of *S. magnificum* have a bactericidal inhibition towards the tested micro-organisms. These results also contributed to validate the use of leaves and barks of *S. magnificum* by local population in Cameroon to fight against urogenital flora infections in traditional medicine. The result showed that root wood and root bark could also be used.

4.3. Cytotoxic activity

The cytotoxicity of the extracts did not show significant cytotoxicity on HeLa cells as the percentages of cell viability remained high (> 50%) for all tested extracts, their IC_{50} values were not assessed. Hence, extracts of leaves, trunk and roots of *S. magnificum* were considered non-cytotoxic. This observation might be an indicator of their selectivity as drugs used in pharmacopeia.Moreover, a specis of the same family has demonstrated a very high cytotoxic activity of its leaves: *Psychotria forsteriana* on human leukemia (molt4) as well as on rat hepatoma cell lines (FAZA). The following alkaloids: isopsychotridine C, quadrigemins A and B, psychotridine showed high toxicity relative to vincristine, a bisindole alkaloid used in anti-tumour chemotherapy on rat hepatoma in cell culture [28]. Also, Awondiran *et al.* in 2009, evaluated cytotoxic and genotoxic properties of the stem bark of *Morinda lucida* Benth, a plant of the same family, in mice; to provide information as an antimalarial plant. The 70% ethanol extract of *M. lucida* was administered orally (125, 250 and 750 mg/kg/day, for 10 days; cyclophosphamide and distilled water were used as positive and negative controls, respectively; mice were injected with 0.04% colchicine intraperitoneally 24 h after administration of the extracts and bone marrow was harvested. Slides of bone marrow cells were stained with giemsa and assessed microscopically for mitotic index (MI) and scored for chromosomal aberrations, the lower the MI the higher the cytotoxicity. The activity of *M. lucida* was (p^0.05) lower than that of cyclophosphamide (1.81). *M. lucida* did not show significantly different cytotoxicity from the negative control. This study concluded that *M. lucida* exerts only a genotoxic effect [29].

The initial evaluation of the cytotoxicity testing, the corresponding cell culture were incubated with tests sample at a fixed concentration of 50 µg/ml in triplicate. The percentages of growth inhibition were calculated relative to untreated control cultures using the upstated formulas. The validation of the tests was done using a positive control at 10 µM emetine (for cell apoptosis). The extracts were tested in a range of concentration ranging from 50 to 0.046 µg/ml using a 3-fold serial dilution. IC_{50} values for cytotoxicity were not determined due to the low inhibition observed in the single concentration screening.

UNDER PEER REVIEW

4. CONCLUSION

In continuation of the quest for discovery of effective antibacterial drugs, the *in vitro* antibacterial and cytotoxicity activities were assessed for different extracts parts of *Schumanniophyton magnificum*. The root wood extract and the root bark extract displayed a good antibacterial activity with MIC value of 195.31 µg/mL and average activity with MIC value of 390.63 µg/mL respectively with *E. coli,* while the other extracts (leaves, bark wood and trunk wood) showed not activities towards *E. coli* and *S. aureus* with MIC values ranging from 1562.50 to 6250.00 µg/mL). The ratio of MBC/MIC lower than 4 against *E. coli* and *S. aureus* allowed us to deduce that the methanolic extracts of *S. magnificum* parts are bactericidal. These results could justify the use of the stem bark to treat urogenital flora infections in traditional medicine. Additionally, the root wood would be more appropriate than the stem bark already used. Regarding cytotoxic activity, 20 µg/mL concentration extracts did not significantly reduce HeLa cells, with an inhibition rate ranging from 13.5 to 37.6%, suggesting a low toxicity of *S. magnificum* extracts towards human cells. The roots were the part with the best activity. However, additional work need to be done (toxicity, activity of fractions and *in vivo* study) on the root wood and the root bark extracts to confirm these preliminary observations in order to validate the use of this plant in pharmacopeia and lead to a drug preparation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors declare that 'ethical clearance was obtained from the Institutional Ethics Committee of the University of Douala for the conduct of this study and for the publication of this article'. The protocols used were developed according to the techniques generally used in the Department of Pharmaceutical Sciences of the University of Douala and in agreement with the Ethics Committee of the University of Douala (2208CEI-UDo/07/2020/T). All experiments were reviewed and approved. A copy of the ethical clearance is available for review by the editorial office/editor/editorial board members of this journal.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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