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# Microbial Contamination and Antimicrobial Efficacy of Alternative and Complementary Plant Derived Suspensions Sold by Traditional Practitioners in Yaoundé, Cameroon

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**Abstract:** The rise in the use of herbal products has given rise to various forms of their abuse and adulteration which could lead to fatal effects, such as dysentery, convulsion, renal and liver failures after consumption, making its efficacy doubtful. The aim of this work was to study the safety of commercial plants based medicinal suspensions sold in the city of Yaoundé and to assess their *in vitro* antibacterial and antifungal activities. An experimental study was realised in the Department of Microbiology between September 2021 and May 2022 (9 months). This study was initiated with a survey realised within the localities of Yaoundé and the medicinal suspensions were randomly purchased precisely at the *round-about TKC*, *Mvog-ada* market, *round-about MEEC*, *round-about Nsam*, *Mendong entry* and *EMIA junction*. The microbiological quality of the selected twelve medicinal preparations was evaluated using the spread plate technique on specific culture media like Mac Conkey, Salmonella/Shigella, Cetrimide, Chapman and Sabouraud dextrose+chloramphenicol. Then the sensitivity of three bacteria (two Gram negative and one Gram positive) and one yeast to the medicinal preparations was assessed by the disc diffusion method. Subsequently, the medicinal suspensions that were proven microbiologically safe for consumption were used to evaluate their effect on the kinetics of the microorganisms indicated as target by the traditional practitioners. In general, the male gender is capacitated enough to have this activity at heart with professional secrecy conserved at all cost. In a note shell, the microbiological analysis of medicinal preparations reveals that 75% of the medicinal suspensions were contaminated with concentrations ranging from 0 to 4.2 Log CFU/mL in the first week and from 0 to 8.16 Log CFU/mL in the third week, with *E. coli* dominating in medicinal suspensions, be it in the first or the third week. The antibacterial and antifungal activities with the various techniques used displayed unsuccessful activities conferred by medicinal suspension on the species they aught to deactivate. When compared to the standard curves, the effect of medicinal products on microorganisms with time pointed out a slight difference (between 1.25 to 2.44 Log CFU/mL) to results obtained with the antimicrobial technique. This study proves the absence in safety and efficacy of some traditional products sold by traditional practitioners in the city of *Yaoundé* and raises the need for the authorities to see deeply into this alarming situation and to put in place standardized procedures and laws that will protect the patients.

**Keywords:** Complementary and Alternative Medicine, Microbial Contamination, Plant Derived Suspensions, Herbal Products Safety and Efficacy, Traditional Practitioners

## 1. Introduction

When a Cameroonian patient is taking a plant derivative medicinal product, he expects to benefit from the life force of its ingredients. The use of traditional medicine, in particular medicines of plant origin, has increased worldwide since the

1990s due to its affordability, availability, accessibility and the role in meeting primary healthcare demands in many developing African and Asian countries [1]. The African continent is naturally endowed with various plant species with nutritional and medicinal benefits. Traditional healing plays an integral role in black African culture as it provides primary health care needs for a large majority (about 80%) of the population [2]. Being blessed with enormous biodiversity resources, Africa is estimated to contain between 40,000 and 45,000 species of plant with a potential for development and out of which 5,000 species are used medicinally [3]. Cameroon on its own has about 8,620 species of plants [4], some of which are commonly used in the treatment of several microbial infections and a series of neglected tropical diseases [5]. Despite extensive research studies conducted on the medicinal uses of African plants, the therapeutic potentials of some locally made plant derived medicinal suspensions has remained unexploited. Many studies have been done on the composition and health effect of medicinal plant [5] in Africa and Cameroon but, little scientific data supporting the legendary claims of these herbal mixtures. In fact most of those mixtures are of unclear and secret origin, mainly due to the fact that knowledge is passed through generations by oral means. It is said to be an inheritance passed on by their ancestors hence rendering it safety and efficacy a major public health concern over the years [6]. Nevertheless, a large majority of Cameroonian tilt towards the use of these medicinal suspensions to fight against bacterial and fungal infections which is said to be an increasing cause of morbidity in sub-Saharan Africa [7]. This is partly due to the long unsustainable economic situation in the country, the high cost of pharmaceutical drugs and increase in drug resistance to common diseases like bacterial infections and other sexually transmitted diseases hence causing the therapeutic approach to alternative traditional medicine an option for treatments [8]. Until now and from experience, we still observe that, many of those who consume some of these concoctions of unknown mixture said to be traditional medication end up in the hospital with a more serious condition to seek for proper treatments. It is hence becoming important to assess the therapeutic values of medicinal plant suspensions. Added to this, it is urgent to first assess the chemical and microbiologic safety of these products before their use. Therefore, the aim of this study was to assess the microbial quality of commercial medicinal plant suspensions and their activities against strains which are the causes of infections practitioners claim to treat.

# 2. Materials and Methods

### *2.1. Study Area, Design and Period*

A cross-sectional study was carried out from September 2021 to May 2022 and was initiated with a survey realised within the city of the *Yaoundé*, Cameroon. This study was realised in Yaoundé due to the fact that it is a melting pot for the over 250 ethnic groups found in the country with their different cultural perceptions of illness, diseases and use of plants. To this effect, the survey was carried out using questionnaires. Traditional practitioners were then interviewed and questionnaires filled accordingly. It was done as such because most traditional practitioners could not answer the questions themselves due to their level of education.

### *2.2. Medicinal Preparations Size and Sampling Technique*

In the present study, three (03) products were selected per microorganism incriminated to be the leading cause of a specific disease as simulated in the Table 1. After selecting the traditional practitioners, a random sampling among medicinal preparations exposed was performed to select those needed for the analysis. Sampling was performed within the localities of Yaoundé, precisely at the *round-about TKC*, at *Mvog-ada* market, *round-about MEEC*, *round-about Nsam*, *Mendong entry* and *EMIA junction*. A total of twelve (12) medicinal preparations with oral or topical administration were purchased and transported in their original packaging to the laboratory of Microbiology for microbial analysis. The liquid products were used as obtained and the powdered and solid products were reconstituted with potable water as recommended by the traditional practitioners.

***Table 1.*** *Most incriminated infectious microorganisms according to a set of literature consulted.*

| Isolates/Diseases | Review References of microbial incrimination |
|---|---|
| *Escherichia coli* (Diarrhoeal diseases) | Reported by over 10 books and articles out of which are Onchoa *et al.* [9] and Monica [10], reported *E. coli* to be the leading cause of diarrhoeal diseases. |
| *Salmonella enteritidis* (Toxi-infections) | Reported to be the most common serotype and leading cause of fever and gastroenteritis in Sub-saharan Africa [11], with an infectious dose of $10^3$ microorganisms. |
| *Pseudomonas aeruginosa* (Urinary tract infections) | Maike *et al.* [12] and many other books and articles reveals this pathogen to be the leading cause of opportunistic urinary tract infection, coupled to *E. coli.* |
| *Staphylococcus aureus* (furuncle- Skin infections) | Reported by *Corey et al.* [13] and *Larry et al.*[14] including many others to be the most common cause of furuncle (skin infection) |
| *Candida albicans* (urogenital candidiasis) | Reported by Behzadi [15] and Vincent *et al.* [16] to be leading cause of Urogenital candidiasis |

### *2.3. Antibiotic and Microorganisms*

Ciprofloxacin (CIP), fluconazole (FLU) from Sigma-Aldrich, St Quentin Fallavier, France were used. Microorganisms included in this study for antimicrobial activity were three bacteria (two Gram- and one Gram+) amongst which *Escherichia coli* 555 *(E. coli), Salmonella spp, Staphylococcus aureus SR 222 (S. aureus)* and one

*Candida albicans* isolate. All these strains were kindly offered by the Laboratory of Food Microbiology, University of Bologna (Italy). Strains stored at -80°C were sub-cultured at 37°C for 24 hours twice in nutrient broth before being used in the tests.

### *2.4. Collection of Medicinal Preparations*

The plant material used in this work consisted of twelve medicinal preparations. To this effect, the following medicinal suspensions were purchased from the traditional practitioners (Table 2).

***Table 2.*** *Herbal medicine purchased in different market (Yaoundé, Cameroon).*

| Herbal medicine name | Code | Reported properties by practitioners | Reported dosage by practitioners |
|---|---|---|---|
| Panax Africaine | AE1 | Dark brown and viscous product in a breakable bottle. Broad spectrum natural antibiotic used to treat diarrhea, dysenteries, typhoid, bile problems and vomiting, gastritis and urinary infections. | One tea spoon (5 ml) of product is consumed in the mornings and in the evenings for 3 weeks. |
| Typhoid minus[a] | AE2 | 500mg Powdered product in a degradable polystyrene container to be boiled in 3 liter of potable water. Used to treat gastroenteritis, typhoid and abdominal pains. | One glass (200 ml) of product is consumed in the mornings and in the evenings for 3 weeks. |
| Albat malar | AE3 | Brownish liquid product in a 350 ml plastic bottle. Used to treat gastroenteritis, typhoid, yellow fever and malaria. | Two tea spoons (10 ml) of product is consumed in the mornings and in the evenings for 3 weeks. |
| Gensen Juice | AE4 | Yellowish liquid product in a 300 ml plastic bottle. Used to treat a range of conditions such as typhoid, gastritis, gastroenteritis, fever, malaria, blood purification and respiratory infection. | Two tea spoons (10 ml) of product is consumed in the mornings and in the evenings for 3 weeks. |
| Anti-staph1[a] | AS1 | Powder made up of a collection of bark of trees to be reconstituted by the clients in 5 liter of potable water. Used to treat impetigo, skin rashes and erythema. | One glass (200 ml), to be consumed in the mornings and in the evenings for 2 weeks |
| Anti-staph2 | AS2 | Brownish liquid product in 1L plastic bottle. Used to treat impetigo, skin rashes and erythema. | Applied topically to the infected area of the body just as an ointment. Can also be consumed orally until the infection disappears. |
| Anti-pseudomonas1[a] | AP1 | 500 mg Powdered product in a degradable polystyrene container to be reconstituted and boiled in 3.5L of potable water. Used to treat wound infections, urinary tract infection, skin rashes and ear infections. | Two glass (400 ml) of product is consumed in the mornings and in the evenings for 2 weeks |
| Anti-pseudomonas2[a] | AP2 | Made up of a collection of bark of trees to be reconstituted by the clients in 5L of potable water. Used to treat wound infections, urinary tract infection and skin rashes | One glass (200 ml), to be consumed in the mornings and in the evenings for 2 weeks. |
| Offtra[a] | AP3 | Powder made up of a collection of bark of trees to be reconstituted by the clients in 5L of potable water. Used to treat urinary tract infection, candidiasis, chlamydia, syphilis and genital herpes | One glass (200 ml), to be consumed in the mornings and in the evenings for 2 weeks. |
| NepNep[a] | AC1 | 500mg Powdered product in a degradable polystyrene container to be reconstituted and boiled in 3.5L of potable water. Used to treat thrush, vaginal yeast infections, cutaneous candidiasis. | Two glasses (400 ml) of product is consumed in the mornings and in the evenings for 2 weeks. |
| Anti-candida2[a] | AC2 | Powder made up of a collection of bark of trees to be reconstituted by the clients in 5L of potable water. Used to treat thrush, vaginal yeast infections and cutaneous candidiasis. | One glass (200 ml), to be consumed in the mornings and in the evenings for 2 weeks. |
| Anti-candida3[a] | AC3 | 500mg Powdered product in a degradable polystyrene container to be reconstituted and boiled in 3L of potable water. Used to treat thrush, vaginal yeast infections and cutaneous candidiasis. | One glass (200 ml) of product is consumed in the mornings and in the evenings for 3 weeks. |

[a]Powders were suspended in water as defined by the practitioners before using them in the same way as the liquid suspension.

These suspensions were chosen in accordance with the indications of traditional practitioners relating to the prescriptions giving to their client. They were selected such that 2 to 3 medicinal suspensions had theoretical properties for a particular disease condition.

### *2.5. Evaluation of the Microbiological Quality of Medicinal Suspensionsstudy Area, Design and Period*

Each medicinal suspension was analysed so as to evaluate their microbiological quality. To this effect, for every medicinal suspension selected, Mac Conkey agar was used to isolate *E. coli*, Salmonella/Shigella agar was used to isolate *Salmonella spp* and *Shigella spp*, Cetrimide agar was used to isolate *Pseudomonas aeruginosa*, Chapman was used to isolate *S. aureus* and Sabouraud dextrose + chloramphenicol was used to isolate *C. albicans*. Each sample was evaluated according to the duration of drug consumption as indicated by the traditional practitioner.

In order to evaluate their quality, 100µL of serially diluted medicinal suspension was used to inoculate on each medium using the spread plate technique with the help of a glass spreader. At the end of this process, typical colonies were counted on each specific culture medium and concentration calculated using the formula (1).

$$C=(n*Fd)/(1.1*V) \quad (1)$$

In which C represents the concentration of micro-organism in CFU/mL, n the colonies sum of two successive dilutions, Fd the dilution factor of the smallest dilution, and V the total volume in mL.

The concentrations were used to access the acceptability of the medicinal suspension using the European pharmacopoeia

acceptance criteria for microbiological quality on Table 3 [17].

***Table 3.*** *Acceptance criteria for microbiological quality of non-sterile dosage forms (European Pharmacopoeia [17]).*

| Route of Administration | TAMC (CFU/g or CFU/mL)* | TYMC (CFU/g or CFU/mL)* | Specified microorganism(s) |
|---|---|---|---|
| Non-aqueous preparations for oral use | $10^3$ | $10^2$ | Absence of *Escherichia coli* (1g or 1mL) |
| Aqueous preparations for oral use | $10^2$ | $10^1$ | Absence of *Escherichia coli* (1g or 1mL) |
| Oromucosal use | | | Absence of *Pseudomonas aeruginosa* (1g or 1mL) |
| Gingival use | | | |
| Cutaneous use | $10^2$ | $10^1$ | |
| Nasal use | | | Absence of *Staphylococcus aureus* (1g or 1mL) |
| Auricular use | | | |

TAMC: Total aerobic microbial count, TYMC: Total combined yeasts/molds count, $10^1$ CFU/mL: Maximum acceptance count = 20, $10^2$ CFU/mL: Maximum acceptance count = 200, $10^3$ CFU/mL: Maximum acceptance count = 2000 and so forth.

### *2.6. Microbial Sensitivity to Medicinal Suspensions and Reference Compounds*

The well diffusion method was carried out in accordance with CLSI recommendations [18]. Medicinal suspensions were dissolved in sterilized water then diluted to final concentrations of 150,000 ppm while ciprofloxacin and fluconazole were respectively diluted to 100 ppm and 500 ppm final dilution. Briefly, 1 mL microbial culture (6 Log CFU/mL) were inoculated on nutrient agar and Sabouraud + chloramphenicol agar in a Petri dish. Following this, sterilized filter papers discs of 6 mm diameter which were previously filed with a 10µL of medicinal suspension were deposited on inoculated media. Discs soaked with physiological water were used as negative control. The Petri dishes were then incubated at 37°C for 24 h and 48 hours. The growth inhibition zone diameter (IZ, mm) was measured to the nearest mm. Each experiment was performed in triplicate and the results presented in terms of the concentration that produced the highest inhibition diameter.

### *2.7. Growth Performance of Selected Strains on Medicinal Suspensions*

The highest concentration of the medicinal suspension, not showing inhibition at the level of disc diffusion technique was used for this experiment. Different volumes of the microorganisms at concentration of 6Log CFU/mL were challenged with 750µL of medicinal suspension in 4.25mL of nutrient broth making a final volume of 5ml. The microorganisms were challenged for 0, 1, 2, 6, 8, 10 and 24/48 hours by adding equal volume of medicinal suspension in nutrient broth in a test tube with the required quantity of inoculate added afterword. Microbial load was assessed at different time of incubation up to 24 and 48 hours of growth respectively for bacteria and yeast [19, 20].

### *2.8. Statistical Analysis*

Data were analysed using Excell version 2013 whereas graphics were represented using GraphPad Prism version 8.

## 3. Results and Discussion

### *3.1. Sociodemographic Characteristics of Health Practitioners*

To have tangible information concerning the survey, a total of 51 herbalists were approached and interviewed. Table 4 below represents the sociodemographic characteristics of traditional practitioners interviewed. From this table, it can be observed that, this activity is held by individual whose age range from 28-65 with a mean ± SD age of 46±7.5. Out of the 51 interviewed, 19% of them were female with only about 27% having undergone tertiary education.

***Table 4.*** *Sociodemographic characteristics of the traditional health practitioners (n=51).*

| Characteristics | Stratification | Effective | Frequency (%) |
|---|---|---|---|
| Age group in years | 28-34 | 4 | 7.8 |
| | 34-40 | 14 | 27.5 |
| | 40-46 | 12 | 23.5 |
| | 46-52 | 12 | 23.5 |
| | 52-58 | 4 | 7.8 |
| | 58-64 | 3 | 5.9 |
| | 64-70 | 4 | 7.8 |
| Gender | Male | 41 | 80.4 |
| | Female | 10 | 19.6 |
| Level of Education | Primary | 16 | 31.4 |
| | Secondary | 22 | 43.1 |
| | Tertiary | 14 | 27.5 |
| Training on traditional medicine | Yes | 4 | 7.8 |
| | No | 47 | 92.2 |

The fact that more than two third of traditional healers were older than 40 years implies that the younger generation is less interested in this practice and if no intervention in the promotion of this practice is made, important values could be lost. 7.8% of these healers had received a formal training in traditional medicine, an observation that ties with those of some researchers, who found that, only 18.6% of all participants had received formal training on traditional medicine [21]. A study conducted in Limpopo in South Africa, suggested that, acquired knowledge of traditional medicine have played a great role in the quality of services provided to clients, as well as their efficacy [22]. This implies that, the safety and efficacy of a

tradition medication could be indirectly or directly related to knowledge acquisition of traditional healing.

### *3.2. Shelf Life, Mode of Consumption and Side Effects of Medicinal Suspensions*

Based on the statements of the traditional practitioners from whom we purchased the twelve medicinal preparations, we have highlighted the effect of the physical state and composition on the shelf life of medicinal suspensions. Most (64.7%) of medicinal suspensions sold in *Yaoundé*, have very short life span. Liquid preparations that were kept at room temperature with no natural conservative had shorter life span compared to those that were prepared with limes as natural conservative. Dried products had the highest shelf life.

The short shelf-life of the products could be justified by the fact that, water based preparations have high water activity and are generally pruned to be contaminated and provide rapid growth conditions to microorganisms.

This observation did not however tie with the predicted shelf life for some medicinal suspensions, namely Nibima, Asena, Lippia and NKP 500 capsule which ranged from 21 to 63 months [23]. This difference could be justified by the fact that, plant products used in the later study were purified plant extracts, contrary to those of this study that are water based preparation and may give way to microbial growth if stored for longer period of time.

According to Table 5, 94.1% of these products are consumed orally as indicated by traditional practitioner's posology. To this, 82.4% had no side effect according to information gotten from herbalists owing to the fact that, they are natural products from barks of trees and roots of plants. This is a hazardous over simplification; many different side effects to herbs have been reported and recently reviewed [24-25]. However, 17.6% had side effect recognized by practitioners ranging from mild fever, dizziness to self-limited diarrhea. Traditional practitioners trade their products within an average of 3 weeks interval and cost ranging from 3.35 to 6.72 USD per medication.

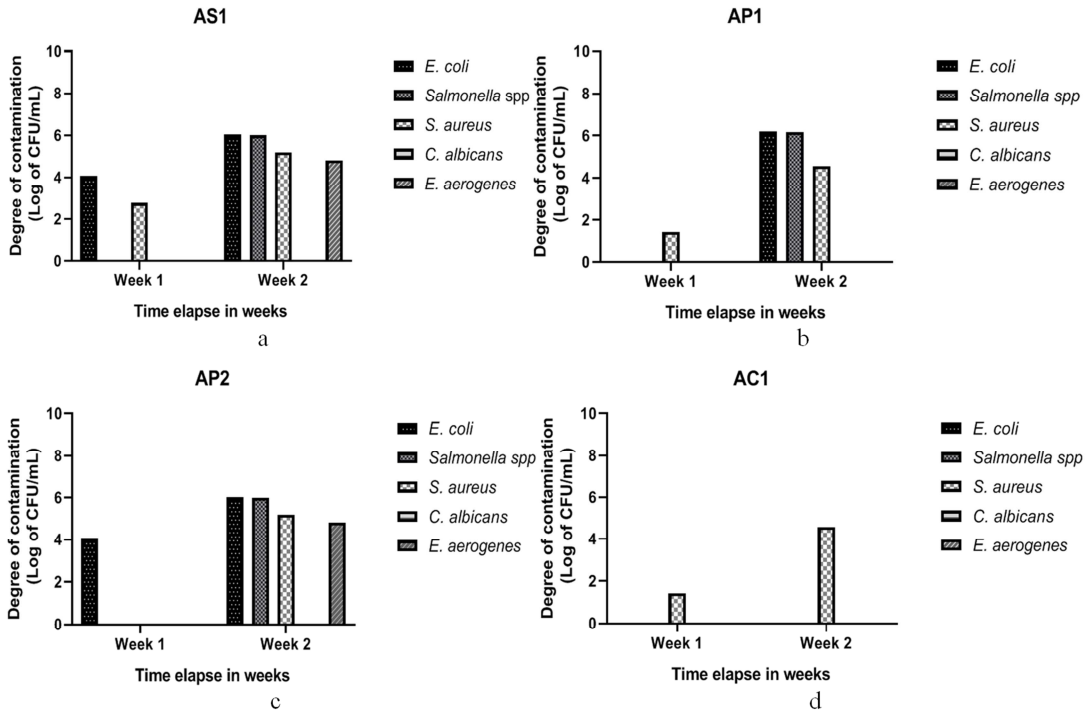
***Table 5.*** *Representation in percentage of the mode of consumption and conservation of medicinal suspensions.*

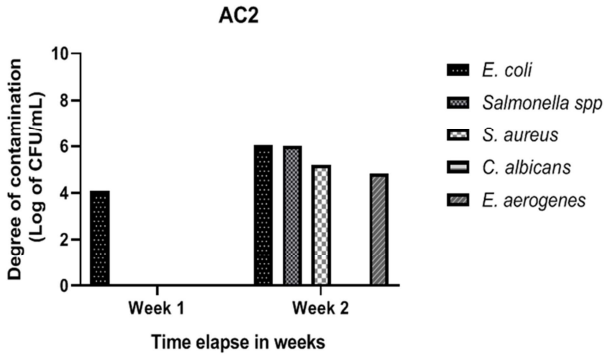
| Characteristics | Stratification | Distribution in% (n) |
|---|---|---|
| Mode of consumption | Oral | 94.1 (48) |
| | Topical | 1.96 (01) |
| | Anal | 1.96 (01) |
| | Inhaled | 1.96 (01) |
| Side effect | Non | 82.4 (42) |
| | Mild side effects | 17.6 (9) |

### *3.3. Microbiological Quality of Medicinal Suspensions*

The presence of certain microorganisms in non-sterile preparations, may have the potentials to reduce or even inactivate the therapeutic potentials of the product and exhibit an undesirable effect on the health of the individual. To this effect, the microbiological quality of selected products was tested during the declared shelf life period of the product as indicated by traditional practitioners. The European pharmacopoeia acceptance criterion for microbiological quality of non-sterile products was the Norm used to verify the conformity of medicinal suspensions [17]. The results of this analysis showed the percentage of non-compliant sample to be high (75%).

The histograms below (Figure 1) represent trend of contamination for products consumed within two weeks whereas, in Figure 2 represents those consumed for three weeks. No results are presented for AS2, AE1, and AE3 because no microbial contamination was observed during storage. They were the only products that complied to the Norm adopted in this work.



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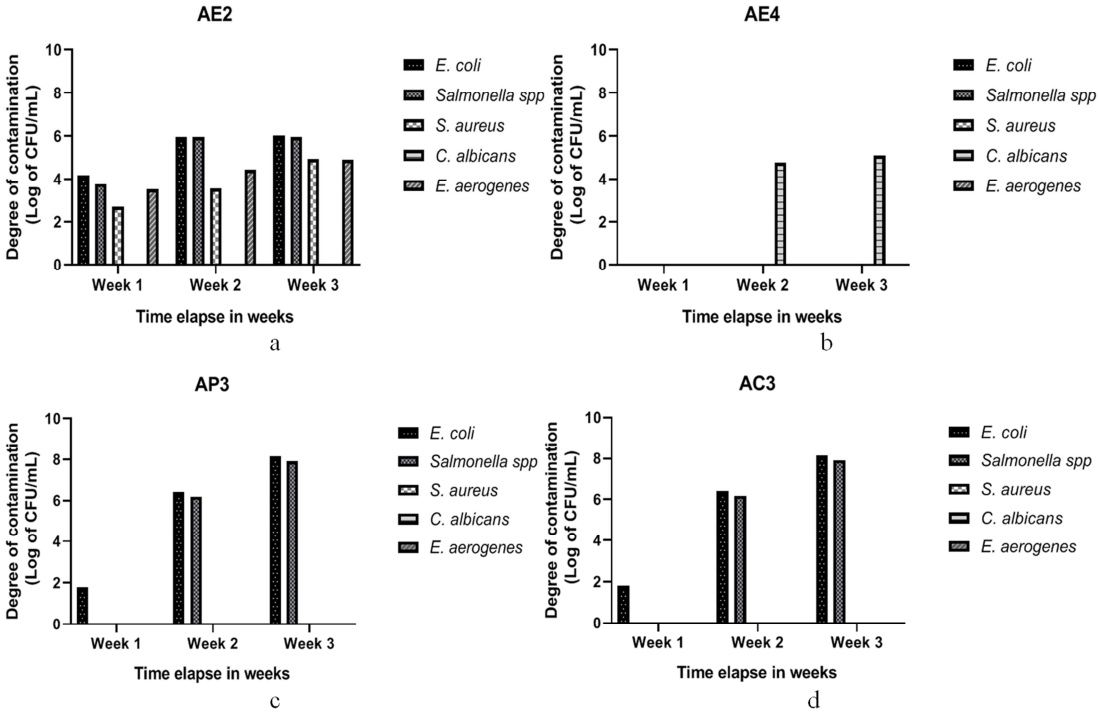
(a) Anti-staph1-AS1, (b) Anti-pseudomonas1-AP1, (c) Anti-pseudomonas2-AP2, (d) Anti-candida1-AC1, (e) Anti-candida2-AC2.

***Figure 1.*** *Histogram demonstrating of contamination of medicinal products within two weeks.*




(a) Typhoid minus-AE2, (b) Gensen Juice-AE4, (c) Anti-pseudomonas3-AP3, (d) Anti-candida3-AC3.

***Figure 2.*** *Histogram representation of contamination of medicinal products consumed within three weeks.*

In Figure 1, we can perceive microbial evolution in products consumed within 2 weeks as indicated by the traditional practitioner. It can be observed that, all contaminated products contained at least three microbial pathogens (*E. coli*, *S. enteritidis, S. aureus*, and *E. aerogenes*) except the product AC1 which contained only *S. aureus*. The degree of contamination ranged from 4.82 Log CFU/mL to 6.2 Log CFU/mL with *E. coli* being the most prevalent amongst the contaminants.

Figure 2 shows the growth and evolution of microbial contaminations in some products consumed within three weeks as indicated by the traditional practitioner. The products were contaminated with concentrations ranging from 4.94 Log CFU/mL to 8.16 Log CFU/mL with *E. coli* being the most prevalent contaminant. This places them in the posture of non-conformity to the European pharmacopoeia criterion.

According to European Pharmacopoeia (EP), the maximum concentration of total aerobic microbe that could be tolerated in a non-sterile medicinal suspension range from 1 Log to 2 Log in CFU/mL. Drugs which do not require sterility regardless of their dosage form and route of administration must conform to the microbiological purity criteria set out in an appropriate edition of the European pharmacopoeia [17]. The results of the microbiological quality of this study, conveyed that, up to 75% of medicinal suspensions did not meet the standards recommended by the EP acceptance criterion. The prominent microbial organism contaminating the medicinal product was *Escherichia coli* for most medicinal products; this result are similar to those who reported *E. coli* to be the most prevalent contaminant followed by *Salmonella* spp. and *Shigella* spp [26]. It was however in discrepancy with those who found *Klebsiella pneumonia* as the most frequent

microorganism among many other microorganisms in herbal concoctions sold at Limpopo province (South Africa) [27]. This could be as a result of the fact that, little or no attention is given to the processes of harvesting, preparation and conservation of these products. This could be the cause of the contamination of end products by microorganism inhabiting the soil, water used and other accessories used during preparation, which intern get to be sold in markets for treatment without any prior quality control of the products. These results however do not confirm works on the quality of non-sterile pharmaceutical products in Poland who reported a 1.87% of non-compliant samples amongst those selected [28]. This difference could emanate from the fact that, the latter study was done in a more developed setting contrary to the former studies which were undertaken in underdeveloped countries, hence the large difference observed with the purity state of the antimicrobial agents.

### *3.4. Antimicrobial Study Using the Disc Diffusion Method*

Four of the medicinal product that abide to the European pharmacopoeia acceptance criteria were used to assess their antibacterial and antifungal properties, where each product was meant to eradicate a particular microorganism causing the disease the product is sold for. Their properties were evaluated with the growth-no-growth approach (Table 6) whereby, the presence of inhibitory zone indicates that, the said medicinal suspension exhibit an antimicrobial property, whereas its absence reflects the contrary.

***Table 6.*** *Inhibition diameters (mm) of medicinal suspensions and reference drugs against test organisms.*

| Products | *Candida albicans* | *Staphylococcus aureus* | *Salmonella enteritidis* | *Escherichia coli* |
|---|---|---|---|---|
| AE1 | NT | NT | NT | 0 |
| AE3 | NT | NT | 0 | NT |
| AS2 | NT | 0 | NT | NT |
| AC1 | 0 | NT | NT | NT |
| Ciprofloxacin | / | 29 | 30 | 29 |
| Fluconazole | 26 | / | / | / |

AE1: Panax Africaine. AE3: Albat matar. AS2: Anti staph 2. AC1: Anti candida 1. NT: Not target microorganism.

According to Table 6, we can deduce that, Panax Africaine (AE1), meant to treat *E. coli* related diseases as indicated by the practitioners did not exhibit any effect against *E. coli* indicated by a 0 mm zone of inhibition. Albat matar (AE3), anti-staph (AS2) and anti-candida (AC1) showed the same trend of antimicrobial activity for *S. enteritidis, S. aureus* and *Candida albicans* respectively.

The presence of certain microorganisms in non-sterile preparations may have the potentials to reduce or even inactivate the therapeutic effects of the product [17]. For this reason, only medicinal suspension that abide to EP criterion were selected for the antimicrobial activity. With regards to the antibiotic and antifungal properties of studied medicinal suspensions, it surges that, out of the 38.5% of medicinal suspensions that conformed to the recommended acceptance criterion by the EP, none of them exhibited an antibacterial or antifungal properties up to a concentration of 150,000 ppm. The inactivity of medicinal suspension could be a leading cause of resistance observed in communities and could be as a result of very low concentrations of active molecule present in the medicinal suspensions [29]. These authors stated that pharmaceutical antibiotics are not the only actors in the emergence and spread of resistant bacteria, most especially in less developed countries.

### *3.5. Effect of Medicinal Suspensions on the Evolution of Microorganisms*

The lack of activity of the suspension at concentration of 150,000 ppm led us to verify if the growth of target pathogens were either delays or not in their growth. In this regard, the growth kinetics of the strains were evaluated in the presence of the medicinal suspensions and compared to their general growth kinetics as predicted from ComBase browser [30]. The conditions used to obtain standard curves are as follows; temperature of 30°C, pH 6.0 and in beef culture medium. The evolution of microorganism with time in the presence of medicinal suspensions and their standard growth curves are presented in Figure 3 and Figure 4 respectively.

From the results obtained in Figure 3, we calculated the increase Log CFU/mL after 24 hours ( 24h) to demonstrate difference of growth. In fact, this value was 1.56 Log CFU/mL for Panax Africaine against *E. coli*, 2.74 Log CFU/mL for Albat matar against *S. enteritidis*, 3.1 Log CFU/mL for anti-staph2 against *S. aureus*, and 2.15 Log CFU/mL for anti-candida1 against *C. albicans*. This indicated that the medicinal suspensions could not prevent the growth of the targeted microorganisms.

From the results obtained in Figure 4, we calculated the increase Log CFU/mL after 24 hours ( 24h) to demonstrate difference of growth. In fact, this value was 4 Log CFU/mL for *E. coli*, 5 Log CFU/mL for *S. enteritidis*, 5 Log CFU/mL for *S. aureus*, and 3.4 Log CFU/mL for *C. albicans*. When comparing the increase Log CFU/mL after 24 hours, it can be observed an approximate difference of 2 Log CFU/mL for strains of *E. coli*, *S. enteritidis*, and *S. aureus* while the difference is 1.25 Log CFU/mL for *C. albicans*. Hence the medicinal suspensions exerted only a delaying effect on the microbial growth. These observations can explain the lack of efficacy of most of the suspensions sold to patients. It is hence urgent that efficacy testing be also done before these products are sold.

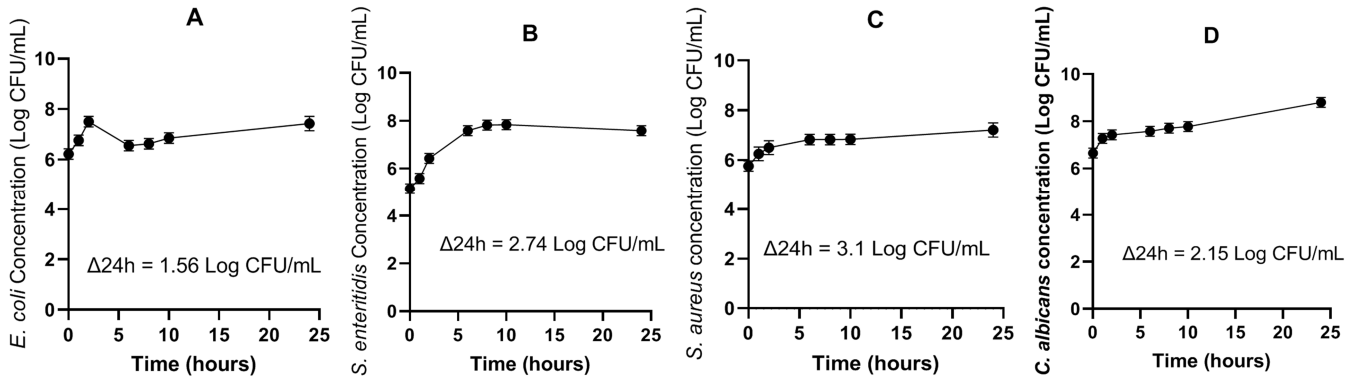
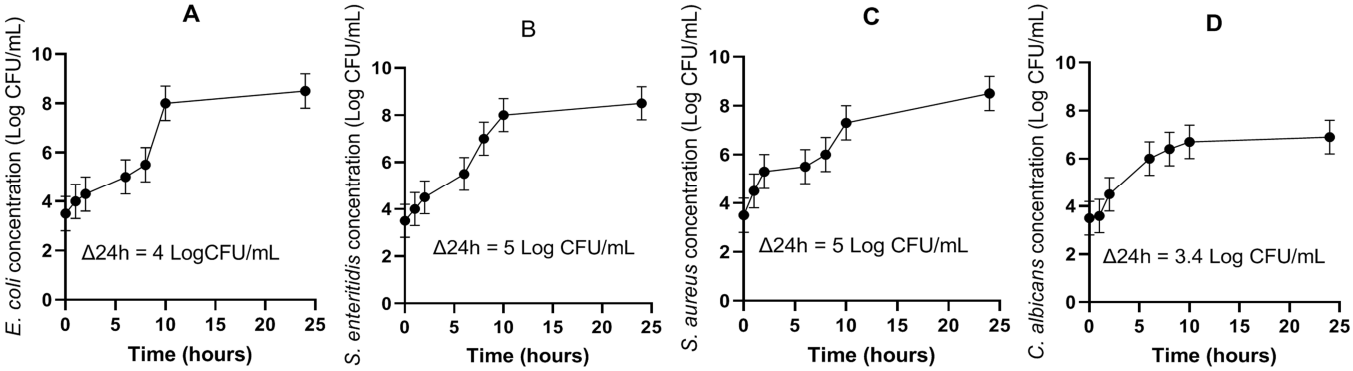



***Figure 3.*** *Effect of medicinal suspensions on the evolution of microorganisms.*

A: Panax Africaine against *Escherichia coli*, B: Albat matar against *Salmonella enteritidis*, C: anti-staph2 against *Staphylococcus aureus*, D: anti-candida1 against *Candida albicans*.




A: *Escherichia coli*, B: *Salmonella enteritidis*, C: *Staphylococcus aureus*, D: *Candida albicans*.

***Figure 4.*** *Standard growth curve of various microorganisms [30].*

# 4. Conclusion

This work has showed that, the male gender are predominantly in the sale of medicinal products and information gotten from these merchants revealed that, most medicinal preparation in liquid form have very short shelf life when compared to pharmaceutical products. More to that, the microbiological quality of 75% of medicinal products sold in the markets of *Yaoundé* does not meet the European Pharmacopoeia acceptance criteria, with *Escherichia coli* being the most prevalent contaminant. The medicinal suspensions analyzed had no activity on target microorganisms when disc diffusion method was used and slightly delayed their growth as compared to enriched commercial growth media. Their antimicrobial failure at this stage should be a reason why more attention be given to what vendors give out to be consumed by their patients, for it may not only revive resistance microorganism but also place the consumers at risk of being infected with additional microorganisms if care not taken. In the future, monitoring will continue on a larger sample and a control protocol will be suggested. Finally, this study proves the absence in safety and efficacy of some traditional products sold by traditional practitioners in the city of *Yaoundé,* hence the need for the authorities to see deep into this alarming situation and to put in place standardized procedures and laws that will protect the patients. For the traditional practitioners to ensure the safety of products given to clients by keeping up the hygienic conditions during preparation of medicinal suspension for it not done, harm to the consumers will be apparent. For the clients to ensure that the product bought is of good quality, well packaged and labelled with expiry dates.

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