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## Microbial Contamination of the Stem Bark of *Mitragyna Ciliata*, A Commercially Available Medicinal Plant in the District of Abidjan (Cote d'Ivoire)

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### ABSTRACT

Raw plant material sold on markets may constitute hazardous risk to population due to storage conditions. This study aimed at assessing the microbial quality of *Mitragyna ciliata* an important medicinal plant being used in Cote d'Ivoire for treating malaria and other diseases. A total of 188 samples of stem barks of *M. ciliata* were purchased from markets in 3 settings (Abobo, Adjamé, Yopougon) over a period of 3 months. The samples were analyzed using standard microbiological methods. Also, some physicochemical parameters were measured.

The moisture of the samples varied from 5.3 to 61.18%, pH was 4.88-6.35 and temperature 24.5-31°C. The samples showed high rate of contamination to mesophilic aerobic bacteria (100%), total coliforms (99.50%), thermotolerant coliforms (99.50%), *Escherichia coli* (10%), *Staphylococcus aureus* (98.93%), Enterococci (85.56%), *Pseudomonas* (61%), yeasts and moulds (93%). Any *Salmonella* was found in all samples. Average loads of microorganisms ranged from 2.6 x10<sup>3</sup> to 8.7 x10<sup>7</sup> CFU/g for mesophiles, 4.0 x10<sup>3</sup> to 3.4 x10<sup>7</sup> CFU/g for total coliforms, 1 x10<sup>3</sup> to 6.6 x10<sup>6</sup> CFU/g for thermotolerant coliforms, 8.0 x10<sup>4</sup> to 4 x10<sup>8</sup> CFU/g for *Staphylococcus aureus*, from 3.2 x10<sup>3</sup> to 2.2 x10<sup>8</sup> CFU/g for Enterococci, and 2.0 x10<sup>4</sup> to 4.4 x10<sup>7</sup> CFU/g for yeasts and moulds. Most of samples had unsatisfactory microbiological quality compared to WHO standards. This study demonstrates population exposure to health risk and the great need to carry out microbiological tests frequently in these medicinal plants marketed in Abidjan.

**Keyword:** Medicinal plants; markets; storage; microbiology; quality; Cote d'Ivoire

### INTRODUCTION

As in South Africa [1, 2], the use of herbal remedies is unlikely to change to any considerable degree in years to come in West

Africa. Products of traditional medicine are still widely used in many countries such as Cote d'Ivoire. The products include plants, herbal materials, herbal preparations and finished

herbal products which contain as active ingredients parts of plants or other plant materials or a combination of both [3]. The trade of medicinal plants is expanding rapidly and generates significant incomes. In Cote d'Ivoire, medicinal plants sold on the markets are not usually inspected and studied to ensure their safety [4]. Once on the market, these plants are exposed to climate and storage hazards that may affect their hygienic, chemical and pharmacological qualities [5]. Due to poor storage conditions and handling, these materials may constitute a risk to consumers. Food-borne diseases are currently a huge public health problem worldwide. Their impact on health and global economy is increasingly recognized [6].

The vulnerable populations are infants, elderly people, pregnant women and immunocompromised [7].

Foodborne diseases are transmitted to humans through the consumption of contaminated food. The dominant germs found in foodstuffs consumed in Côte d'Ivoire are Salmonella, Anaerobic Sulfite-Reducers, Clostridium, Staphylococci, Coliforms, Yeasts and moulds [8]. Medicinal plants are stored in the same conditions as foodstuffs on markets. Consequently, this raw material could be contaminated by many of these microorganisms which may lead to serious health conditions such as fever, vomiting, diarrhea, fatal kidney failure, and death in some cases [9]. Mycotoxins produced by fungi generally have haemorrhagic, immunotoxic, hepatotoxic, nephrotoxic, neurotoxic, estrogenic, teratogenic and mutagenic and carcinogenic effects [10].

For reducing this negative impact on health, the quality of commercially available medicinal plants is being studied in many countries. The leaves of *Moringa oleifera* sold in Accra markets were found to be contaminated by broad spectrum of microorganisms such as coliforms, moulds and yeasts, *Escherichia coli* and *Pseudomonas*. The amount of these microorganisms is over national and international standards [11]. The herbal remedies sold to people infected with HIV in Kenya, showed rates of microbial contamination above the thresholds allowed by WHO [12].

Pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus* were isolated from five types of tea marketed in Nigeria [13]. Similar results were obtained for India [14] and Pakistan [15].

Most of these microorganisms are very harmful to humans [16-17] and consumers may be at risk. In Cote d'Ivoire, despite the number of studies on the biological activities of medicinal plants, no work concentrates on their microbiological quality. On the other hand, the lack of trade regulation on medicinal plants from Cote d'Ivoire, regardless to collection, processing and storage does not provide security. These plants are stored in conditions that may alter their quality and effectiveness. The assessment of the microbiological risk of medicinal plants is an urgent need to ensure the safety and health of consumers. *M. ciliata* is one of these medicinal plants that are sold on the markets of the district of Abidjan. The stem bark and leaves of this plant are widely used in traditional medicine for treating dysentery, fever, malaria and gonococcal disease [18].

The aim of this work was to evaluate the microbiological quality of the stem bark of *M. ciliata* stored and sold on markets of the district of Abidjan.

## MATERIALS AND METHODS

### Sampling

The samples were collected on markets of three settings (Abobo, Adjame and Yopougon), that are the main entrances and supply points of medicinal plants in the district of Abidjan. The sampling was performed as following

- Batch 1: arrival of plants on the wholesale markets,
- Batch 2: one month later in the saleswomen's shop,
- Batch 3: two months later (same stock),
- Batch 4: three months (same stock).

The samples (Figure 1) were collected under aseptic conditions, packed in sterile stomacher bags and placed in coolers containing icepack (+ 4 °C). A total of 188 samples (47/month) were collected for laboratory analyses.



**Fig. 1:** Stem bark of *Mitragyna ciliata*

## PHYSICOCHEMICAL ANALYSIS OF SAMPLES

### Determination of moisture

Each sample was dried at  $105 \pm 2$  °C until a constant weight was obtained [19]. The rate of humidity was calculated using the formula:

$$H = [(P_0 - P_1) / P_0] \times 100,$$

**H:** humidity (%); **P<sub>0</sub>:** weight of the sample before drying; **P<sub>1</sub>:** weight of the sample after drying.

### Measurement of pH and temperature

For this experiment, 3 g of sample were added to 30 mL of boiled distilled water [20]. After cooling under continuous stirring, the pH and temperature were measured using a multiparameter type HANNA.

## MICROBIOLOGICAL ANALYSIS

### Preparation of the initial suspension

From each sample, 25 g of powder were mixed with 225 mL of peptone water. The mixture was homogenized and kept at room temperature for 10 min. Then three serial dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ) were made from this initial suspension [21].

### Enumeration of microorganisms

The control of microbial contamination was performed using standard methods, NF V08-051 (aerobic microorganisms at 30 °C), NF V08-050 (total coliforms), NF V08-060 (thermotolerant coliforms), ISO 16649-2 (*Escherichia coli*), NF EN ISO 6888-1 (*Staphylococcus aureus*), NF ISO 21528-2 (Enterococci), NF EN ISO 6579 (Salmonella), NF EN ISO 13720 (*Pseudomonas*) and NF V08059 for yeasts and moulds. For each

microorganism, the numbers of Colony Forming Unit per gram of sample (CFU/g) were calculated taking in account the dilutions. Each seeding was repeated three times.

### Aerobic mesophilic bacteria (total flora)

The initial suspension and dilutions (1 mL) were cultured on Plate Count Agar (PCA) and incubated at 30 °C for 72 h. Then all colonies on Petri dish (30-300) were enumerated according the standard method [22].

### Total coliforms, thermotolerant coliforms and *Escherichia coli*

The initial inoculum and dilutions (1 mL) were cultured on Violet Red Bile Lactose Agar (VRBL) for searching coliforms. The Trypone Bile X Glucuronide medium (TBX) was used for *E. coli*. The plates were incubated at 30 °C for total coliforms [23], 44 °C for thermotolerant coliforms and *E. coli* [24]. The Petri dish with 15-150 colonies were considered for enumeration (coliforms thermotolerant) and 30-300 colonies for total coliforms.

### *Staphylococcus aureus*

The inoculum (0.1 mL) was seeded on complete Baird Parker medium in duplicate. The Petri dishes were incubated at 37 °C for 48 h. The suspicious colonies were counted and cultured on Chapman medium followed by Gram coloration. Then Deoxyribonucleic Acid (DNA) agar was used for confirmation. The Petri dish with 15-150 colonies were considered for enumeration [25].

### Enterococci

The Enterococci were identified on Bile Esculine Azide agar (BEA). All colonies on Petri dish (15-150 colonies) were counted 24 h post-incubation at 37 °C [26].

### Pseudomonas

For identification of Pseudomonas, cetrimide medium was used for culture and media King A and King B for confirmation [27]. After 24 h of incubation at 37 °C, the Petri dish with 15-150 colonies were considered for enumeration.

### Salmonella

For control of Salmonella contamination, the culture was performed in Buffered peptone water and Rappaport-Vassiliadis broth. Then isolation was carried out on Xylose-Lysine-Deoxycholate (XLD) and Hektoen agar plates and suspicious colonies cultured on a non-selective medium for biochemical characteristics [28].

### Yeasts and moulds

Yeast and moulds were isolated on Chloramphenicol Sabouraud agar. After 5 days post-incubation at 25 °C, all colonies on Petri dish (15-150 colonies) were counted [29].

### Comparison to WHO standards

The load of each microorganism analyzed was compared to the WHO standards for microbial contamination in medicinal plants [30]. The tolerance thresholds in medicinal plants are 10<sup>5</sup> CFU/g for aerobic mesophilic germs, 10<sup>3</sup> CFU/g for other Enterobacteria, yeasts and moulds. The

absence in 1 g of sample is required for Salmonella and *Escherichia coli*.

### Statistical Analysis

The results were analyzed using a model of variance analysis (one factor) performed with STATISTICA 7.1 software. The comparison of the means was carried out with the test of the Least significant difference (LSD) for classification of microbial loads. The differences were significant ( $P < 0.05$ ) and highly significant ( $P < 0.01$ ).

## RESULTS

### Physicochemical parameters of samples

For all samples collected in the same setting, the average rate of moisture decreased from batch 1 to batch 4 (Table 1). The highest value was recorded for samples of Abobo (batch 1 = 61.18%) and the lowest for samples of Adjamé (batch 4 = 5.35%).

In this study, all the samples had a pH < 7 (minimum = 4.88, maximum = 6.35) corresponding to relative low acidity (Table 1). The highest values (pH = 6.35) were obtained for batches 4 from Adjamé and Yopougon. The low value was recorded for batch 2 from Abobo (pH = 4.88).

The temperatures recorded for all the samples ranged from 24.5 to 31 °C (Table 1). The high values (30-31°C) were obtained for batches 1 (Adjamé, Yopougon), batch 2 (Adjamé) and batches 3 (Adjamé, Yopougon). The low values (24-25°C) were obtained for batches 4 of all settings.

**Table 1:** Physicochemical parameters of the stem bark of *Mitragyna ciliata*

Parameters	Batches	Settings		
		Abobo	Adjamé	Yopougon
pH	1	5.6±0.26	5.16±0.16	5.14±1.05
	2	4.88±0.22	5.48±0.18	5.76±0.68
	3	5.22±2.02	6.33±1.02	5.4±0.45
	4	5.87±0.06	6.35±0.07	6.35±0.16
Humidity (%)	1	61.18±0.52	48.09±0.65	48.61±0.38
	2	44.32±0.75	44.57±1.7	33.35±0.52
	3	11.54±0.45	40.57±0.6	15.01±1.02
	4	9.82±1.5	5.35±0.23	10.66±0.6
Temperature (°C)	1	29±1.6	31±2.4	30.7±0.23
	2	28.4±2.02	30.3±0.05	29.7±0.78
	3	29.9±2.07	30.08±1.4	30.5±0.56
	4	24.5±0.68	24.6±1.56	24.8±0.18

**Microbiological characteristics of samples**

A large proportion of the samples analyzed was contaminated by microflora including aerobic mesophilic germs, total coliforms, thermotolerant coliforms, *Escherichia coli*, *Staphylococcus aureus*, Enterococci, Pseudomonas, yeasts and moulds (Table 2). No Salmonella was recorded in samples. The percentages of samples contaminated (Figure 2) by this microflora was 100% for aerobic mesophilic bacteria, total coliforms (99.50%), thermotolerant coliforms (99.50%), *Escherichia coli* (10%), *Staphylococcus aureus* (98.93%), Enterococci (85.56%), Pseudomonas (61%), yeasts and moulds (93%).

The analysis of variance revealed no significant difference between the average loads of thermotolerant coliforms and Enterococci in samples of the three settings visited. However, high significant difference was noted for mesophiles. All the results are presented in Table 2. The average loads of microorganisms in samples ranged from  $2.6 \times 10^3$  to  $8.7 \times 10^7$  CFU/g for aerobic mesophilic germs, from  $4.0 \times 10^3$  to  $3.4 \times 10^7$  CFU/g for total coliforms and from  $1 \times 10^3$  to  $6.6 \times 10^6$  CFU/g for thermotolerant coliforms and from  $8.0 \times 10^4$  to  $4 \times 10^8$  CFU/g for *Staphylococcus aureus*. For faecal Enterococci, yeasts and moulds, the values were respectively  $3.2 \times 10^4$ - $2.2 \times 10^8$  CFU/g and  $2.0 \times 10^4$ - $4.4 \times 10^7$  CFU/g.

**Table 2:** Microbial contamination of the stem bark of *Mitragyna ciliata*

Microorganisms	Settings	Means $\pm$ SD (CFU/g)	Statistical parameters	Contamination percentage
Aerobic mesophilic bacteria	Abobo	$4.40 \pm 0.68 \times 10^{6b}$	$P < 0.001$	100%
	Adjamé	$9.44 \pm 1.17 \times 10^{6c}$		
	Yopougou	$8.46 \pm 0.52 \times 10^{5a}$		
Total coliforms	Abobo	$5.95 \pm 0.04 \times 10^{5a}$	$P < 0.001$	99.50%
	Adjamé	$2.09 \pm 0.47 \times 10^{6b}$		
	Yopougou	$3.13 \pm 0.86 \times 10^{6b}$		
Thermotolerant coliforms	Abobo	$5.37 \pm 0.28 \times 10^{5a}$	$P = 0.456$	99.50%
	Adjamé	$7.60 \pm 0.07 \times 10^{5a}$		
	Yopougou	$1.05 \pm 0.51 \times 10^{6a}$		
<i>Staphylococcus aureus</i>	Abobo	$4.12 \pm 2.64 \times 10^{6a}$	$P < 0.001$	98.50%
	Adjamé	$1.95 \pm 0.01 \times 10^{7b}$		
	Yopougou	$2.53 \pm 0.24 \times 10^{7b}$		
Enterococci	Abobo	$1.88 \pm 0.18 \times 10^{6a}$	$P = 0.063$	85.56%
	Adjamé	$1.57 \pm 0.69 \times 10^{6a}$		
	Yopougou	$1.07 \pm 0.61 \times 10^{6a}$		
Yeasts and moulds	Abobo	$3.25 \pm 1.64 \times 10^{6b}$	$P < 0.001$	93%
	Adjamé	$4.26 \pm 0.34 \times 10^{6b}$		
	Yopougou	$1.09 \pm 0.61 \times 10^{6a}$		

CFU = Colony forming unit



**Fig. 2:** Petri dish with colonies of yeast and moulds on chloramphenicol Sabouraud agar

#### Average loads of the microorganisms counted by batches

The average loads of microflora in the 188 samples are reported in Table 3. The analysis of variance showed no significant difference between the four batches for aerobic mesophilic germs. For total coliforms, a high significant difference was observed between batch 1 and batches 2, 3 and 4. The highest microbial load was obtained for batch 1 ( $4.33 \times 10^6$  CFU/g). For thermotolerant coliforms, there was high significant difference between batch 1, and batches 2, 3 and 4, with the highest microbial load ( $1.13 \times 10^6$  CFU/g) for batch 1. The difference between average loads of

*Staphylococcus aureus* in batches 1, 2 and batches 3, 4 was highly significant, with the highest value for batch 2 ( $3.19 \times 10^7$  CFU/g). The control of Enterococci contamination showed high significant difference between the four batches, with the highest load in batch 3 ( $1.65 \times 10^7$  CFU/g). Also, there was high significant difference between the average loads of yeasts and moulds for the four batches with the highest value obtained for batch 1 ( $4.16 \times 10^6$  CFU/g) (Table 3). The flora of alteration and germs indicating contamination in the stem bark of *M. ciliata* varied according the different periods of sampling.

**Table 3:** Average loads of microorganisms isolated from the contaminated stem bark of *Mitragyna ciliata*

Microorganisms	Batches	Means $\pm$ SD (CFU/g)	Statistical parameters
Mesophilic aerobic germs	1	$2.27 \pm 0.64 \times 10^6$ <sup>a</sup>	$P = 0.46$
	2	$3.07 \pm 0.67 \times 10^6$ <sup>a</sup>	
	3	$1.06 \pm 0.36 \times 10^7$ <sup>a</sup>	
	4	$5.95 \pm 1.14 \times 10^6$ <sup>a</sup>	
Total coliforms	1	$4.33 \pm 1.16 \times 10^6$ <sup>b</sup>	$P < 0.001$
	2	$9.56 \pm 0.02 \times 10^5$ <sup>a</sup>	
	3	$8.80 \pm 0.03 \times 10^5$ <sup>a</sup>	
	4	$5.32 \pm 0.02 \times 10^5$ <sup>a</sup>	
Thermotolerant coliforms	1	$1.13 \pm 0.2 \times 10^6$ <sup>b</sup>	$P < 0.001$
	2	$5.54 \pm 0.02 \times 10^5$ <sup>a</sup>	
	3	$4.66 \pm 0.02 \times 10^5$ <sup>a</sup>	
	4	$7.64 \pm 0.02 \times 10^5$ <sup>a</sup>	

<i>Staphylococcus aureus</i>	1	1.69±0.41 x 10 <sup>7b</sup>	<i>P</i> < 0.001
	2	3.19±0.44 x 10 <sup>7b</sup>	
	3	3.76±1.16 x 10 <sup>6a</sup>	
	4	4.30±0.85 x 10 <sup>6a</sup>	
Enterococci	1	1.99±0.67 x 10 <sup>6b</sup>	<i>P</i> < 0.001
	2	5.45±0.02 x 10 <sup>5a</sup>	
	3	1.65±0.29 x 10 <sup>7c</sup>	
	4	7.80±0.19x 10 <sup>6b</sup>	
Yeasts and moulds	1	4.16±1.17 x 10 <sup>6b</sup>	<i>P</i> < 0.001
	2	3.46±0.98 x 10 <sup>6ab</sup>	
	3	3.32±0.82 x 10 <sup>6ab</sup>	
	4	1.68±0.32 x 10 <sup>6a</sup>	

CFU = Colony forming unit

#### Hygienic quality of stem bark of *Mitragyna ciliata*

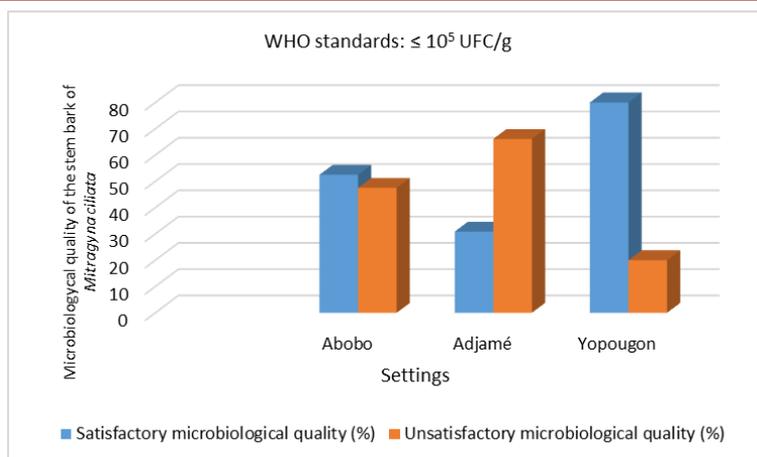
The percentage of samples with a satisfactory microbiological quality compared to WHO standards was low. For most of the microorganisms, loads were higher than WHO thresholds (Table 4). For example, approximate

100% samples had unsatisfactory quality for total coliforms, *Staphylococcus aureus* and thermotolerant coliforms (Figures 3, 4 and 5). Interestingly, all samples (100%) showed satisfactory microbiological quality for salmonella.

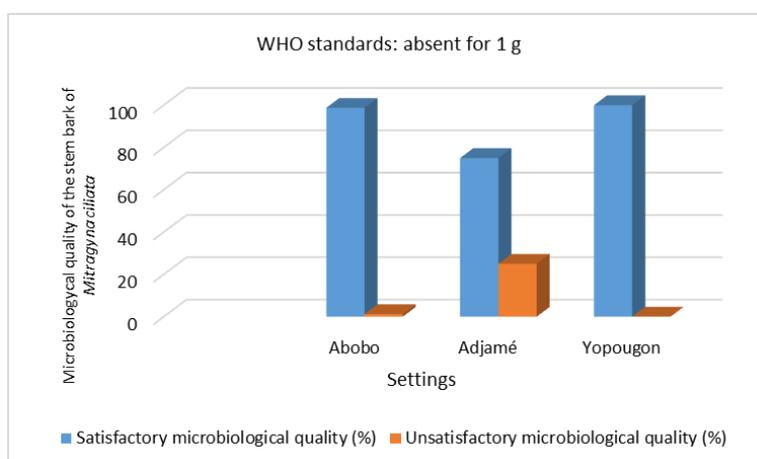
**Table 4:** Microbiological quality of the marketed stem bark of *Mitragyna ciliata* according WHO standards

Microorganisms	Settings	WHO standards	Satisfactory (%)	Unsatisfactory (%)
Total coliforms	Abobo	≤ 10 <sup>3</sup> CFU/g	0	100
	Adjamé		0	100
	Yopougou		5	95
Thermotolerant coliforms	Abobo	≤ 10 <sup>3</sup> CFU/g	2.5	97.5
	Adjamé		0	100
	Yopougou		7.5	92.5
<i>Salmonella</i>	Abobo	Absent for 1 g	100	0
	Adjamé		100	0
	Yopougou		100	0
Enterococci	Abobo	≤ 10 <sup>3</sup> CFU/g	8.75	91.3
	Adjamé		20.6	79.4
	Yopougou		25	75
Yeasts and moulds	Abobo	≤ 10 <sup>3</sup> CFU /g	2.5	97.5
	Adjamé		10.3	89.7
	Yopougou		10	90

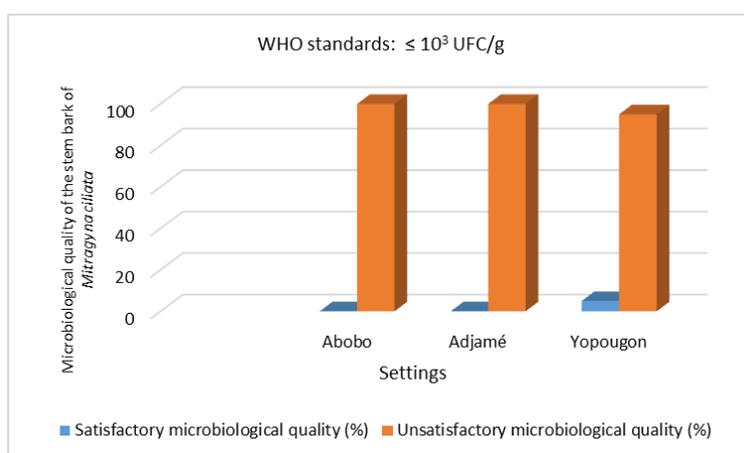
CFU = Colony forming unit



**Fig. 3:** Aerobic mesophilic bacteria contamination of the stem bark of *Mitragyna ciliata* according WHO standards



**Fig. 4:** *Escherichia coli* contamination of the stem bark of *Mitragyna ciliata* according WHO standards



**Fig. 5:** *Staphylococcus aureus* contamination of the stem bark of *Mitragyna ciliata* according WHO standards

## DISCUSSION

This study was conducted to assess the microbiological quality of the stem bark of *M. ciliata* stored and sold in the markets of the district of Abidjan. A total of 188 samples were collected and analyzed. The physicochemical

parameters showed that humidity rates of samples varied from 5.35% to 61.18%. The pH values were comprised between 4.88 and 6.35 while the temperature ranged from 24.5 °C to 31 °C. Such results indicate that many bacteria, including spoilage germs, could easily grow in

the samples due to good growth conditions [21, 31].

In the present study, all samples (100%) were contaminated by mesophilic aerobic germs with total number of  $2.6 \times 10^3$  to  $8.7 \times 10^7$  CFU/g. The percentage of samples with an unsatisfactory microbiological quality was 44.5%. This is the first report on microbial contamination of raw material of medicinal plants from Cote d'Ivoire. These results are similar to those obtained for other plant-based materials [32, 33]. In general, a great amount of mesophiles in plants is considered as a sign of sanitation and a quality parameter [34]. High bacterial load in samples may indicate that plants have been exposed to improper handling, inadequate processing methods and inappropriate storage conditions. The average loads had remained equal from batch 1 to batch 4. Such results showed that the storage conditions did not vary from the first harvest until the fourth harvest.

A high proportion of samples was contaminated by total and thermotolerant coliforms. High presence of coliforms was reported for plant species sold on markets in Ghana [11]. In this previous study, 97.5% of samples were contaminated by loads of coliforms above WHO standards. This high number of coliforms is an indication of bad hygiene and lack of sanitary measures. Contamination may result from several sources, including inadequate cleaning procedures and open-air drying of plant material causing contamination by soil and dust [35]. The strong contamination of the stem bark by coliforms may constitute a public health concerns. Coliforms could be transferred contamination [21] which sometimes become very pathogenic. Another health risk associated with coliforms is the production of histamine, a biogenic amine resistant to heat and toxic to humans [21]. Based on the sampling period, average load of coliforms in the batch 1 is higher than the number in batch 3, suggesting that storage tends to decrease the number of coliforms in the sample.

*Escherichia coli* contaminated 1.25% of the samples from Abobo and 25% of stem bark collected in Adjame. This bacteria was not observed in samples from Yopougon. These results showed that some of the samples were unsatisfactory for consumption. Abou-Donia [33] and Khanzadi [14] also reported *Escherichia coli* contamination of several plant species marketed.

The detection of *E. coli* in the samples confirms fecal contamination, which is directly associated with unhealthy conditions. *E. coli* is exclusively present in the intestines of humans and animals.

*Staphylococcus aureus* was found in 98.93% of samples with loads ranging from  $8.0 \times 10^4$  to  $4 \times 10^8$  CFU/g. The percentage of samples with an unsatisfactory microbiological quality was 98.33%. Studies in Nigeria and South Africa reported high rates of *S. aureus* contamination in marketed plants [35]. The presence of *S. aureus* in food and herbal products is associated with none hygienic handling [11]. This bacteria is harbored by mucous membranes of nose, throat, purulent wounds and skin. Then it can contaminate food or plants when coughing, sneezing or in contact with infected skin. *Staphylococcus aureus* produces a violent toxin that is resistant to heat and provokes a food poisoning [36]. That is more worrying as some plants are often used under maceration.

Interestingly Salmonella were absent from all samples. This result is in full agreement with the findings of Abou-Donia [33] revealing that Egyptian spices and medicinal plants are free from Salmonella spp. However, high number of Salmonella was reported in Thai medicinal herbs [32].

Enterococci were detected in 85.56% of the analyzed samples ( $3.2 \times 10^3$  to  $2.2 \times 10^8$  CFU/g). The percentage of stem bark with an unsatisfactory microbiological quality was 81.9%. Most Enterococcus species are part of the intestinal flora of many animals and their concentration in faeces ranged from  $10^5$  to  $10^7$  CFU/g [37]. The loads observed in the studied samples in the present study were close to these values and higher than that reported for soybeans [38] and olives ( $2.2 \times 10^3$  CFU/g) [39]. This high Enterococci contamination of the stem bark of *M. ciliata* indicated a possible faecal pollution and poor hygienic conditions.

For Pseudomonas, 61% of studied samples were contaminated. In Benin, Omogbai *et al.* [12] noted the presence of Pseudomonas in several marketed plant samples. This was not the case in India where total absence of Pseudomonas in several medicinal plants commercialized in different markets was reported by Nandna *et al.* [15].

Fungal contamination of herbal products occurs mainly during slow or insufficient drying, post-

harvest storage if the relative humidity is high and temperatures are favorable [11]. In this study, 93% of the samples were contaminated with fungi at various loads ( $2.0 \times 10^4$  to  $4.4 \times 10^7$  CFU/g) from batch 1 to batch 4. The percentage of stem bark with an unsatisfactory microbiological quality was 92.4%. Such contaminated products may cause fungal infections or other serious health conditions due to the accumulation of mycotoxins produced by fungi such as *Aspergillus parasiticus* and *Aspergillus flavus* [12]. The results also showed a gradual decrease in the average load of yeasts and moulds over the three months. This could be explained by the decrease of moisture in samples during this experiment, as most molds need high rates of humidity for growth [40].

### CONCLUSION

The present study revealed that the stem bark of *M. ciliata* stored and sold in the markets of the district of Abidjan are contaminated by a microflora composed of aerobic mesophilic bacteria, total coliforms, thermotolerant coliforms, *Escherichia coli*, *Staphylococcus aureus*, Enterococci, Pseudomonas, yeasts and moulds before and during storage. This contamination significantly increased during storage due to the poor conditions observed in the different markets. The microbiological quality of a great part of the samples did not satisfy the WHO requirements on medicinal plants. So, population may be at risk by consuming such raw plant material as these microorganisms could cause foodborne diseases, serious health problems and death.

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### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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