Hygienic Status of the Stem Bark of *Sarcocephalus latifolius* (Smith) E.A. Bruce (Rubiaceae) Stored and Sold at Medicinal Plant Market (Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RNAK, CKJ and KKB carried out the microbiology study. Authors RNAK, KB and DMUD carried out the ethnobotanical survey. Authors RNAK and KB wrote the manuscript. Authors KMW and DM conceived and designed the experiments and supervised the work. All authors approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The present study relates to the hygienic status of medicinal plants sold on markets of the district of Abidjan. This paper focused on *Sarcocephalus latifolius* used to treat various diseases such as Malaria.
Place and Duration of Study: The ethnobotanical survey was conducted during November 2017, on the Siaka Koné market in Abobo. The microbiology study was carried out at "InstitutPasteur de Côte d'Ivoire".

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Methodology: A semi-structured interview was used during the survey. Germs were isolated and microbial load counted from aqueous extracts (maceration) of collected samples of stem bark using standard bacteriology methods. Investigations were also made on control sample collected in the Savannah at Lamto reserve (Toumodi).

Results: 86% of the plants sold on this market are not well maintained. In fact, 53% of plant organs are stored outdoors on pieces of brick exposed to dust, air humidity and car exhaust. While 20% are under black tarpaulins or stored in dilapidated stores away from light and 13% in bags in open air. To confirm our survey, *Sarcocepha luslatifolius* was selected from the highest frequency of citations for performing microbiological tests. The number of total coliforms ranged from $1.3×10^3$ to $9.2×10^7$ CFU/g plant, the mean value of total coliforms was $4.7×10^5$ CFU/g, that of mesophilic aerobic germs from $8.1×10^3$ to $5.1×10^5$ CFU/g of plants, the average value of mesophilic aerobic germs was $1.2×10^5$ CFU/g. The presence of *Streptococcus*, *Pseudomonas* and *Escherichia coli* was observed respectively on 93.33%, 16.67% and 3.33% of the samples collected.

Conclusion: Medicinal plants sold and stored under current market conditions are potentially dangerous to health.

1. INTRODUCTION

Plants undeniably remain an important source of medication. According to WHO in 2000 [1], in Africa 85% of people use traditional medicine to meet their primary health needs. This high use could be linked to cultural behavior, and poverty in several West African countries. This situation could explain a strong rush of populations towards the medicinal plants to cure various affections.

However, the use of plant-based medicines is far from devoid of any risk to the patients [2]. Plants may contain toxic molecules at relatively high doses for both animal and human organisms [3,4]. Also, external contamination may be of a chemical, radioactive and/or microbiological nature [3].

Microbiological contaminations may be due to the maltreatment of plants from harvest to transport and their poor conditions on the market. This could lead to the deterioration of the chemical composition in the plant. Such a condition gives rise to low quality of herbal products with little or no therapeutic efficacy. This endangers the health of the consumers [5] by ingestion of toxins or worsening of health by a lack of activity on the targets of the remedy. According to WHO, the number of reports of harmful effects of traditional medicines on the health of populations has increased along with their use. The investigations and studies carried out on this subject show that there are several reasons for these problems [6]. One of the main causes of adverse effects due to the use of herbal medicines is directly related to the poor conditions of the raw materials used in their manufacture.

Hence the interest of carrying out a quality control on these raw materials which can be of various natures (organs, juices, flowers, etc.). The present study concentrates on stem bark of *Sarcocephal uslatifolius* (Smith) E. A. Bruce. Studies on hygienic quality of the products of traditional medicine are scarce in Côte d'Ivoire [7]. The main objective of this work was to evaluate the conditions of treatment and storage of medicinal plants on markets from Côte d'Ivoire.

Sarcocepha luslatifolius is one of those medicinal plants whose parts are sold on various markets of Abidjan (Côte d'Ivoire). It is widely used by people to treat various diseases, including typhoid fever and malaria [8]. Subsequently the microbiological quality of the stem bark of this plant was accessed.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted on the Siaka Koné market in Abobo (Abidjan), one of the major supplies and distribution centers for medicinal plants in the District of Abidjan [7]. With regard to the medicinal plant trade, herbalists expose their goods in the open air, very often on the ground, sometimes close to garbage and on the edge of the railroad. At the outset, plant organs are often contaminated with dust and mildew, Fig. 1.

(A) Exposure of medicinal plants at the Siaka Koné market of Abobo

(B) Exposure of medicinal plant barks and roots to the Siaka Koné market of Abobo

(C) Exposure of medicinal plants on the Siaka Koné market of Abobo

(D) Exposure of dried bark and leaves of medicinal plants near the railroad on the Siaka Koné market of Abobo

Fig. 1. Conditions of exposure of medicinal plants on the Siaka Koné market of Abobo

2.2 Survey on Markets

A survey was conducted on the Siaka Koné market in Abobo, with herbalists for collecting data on the origin, conditions of transport and storage of medicinal plants. It was carried out for three weeks during November 2017. During these interviews, the indirect method, with the semi-structured approach was used. This approach consisted of formulating questions based on responses to existing questions on an interview guide. The citation frequencies were calculated according to the formule1:

$$Cf = \frac{n}{N} \times 100 \quad (1)$$

Cf: citation frequency; n: Number of quotes of the plant species;

N: Total number of people surveyed

2.3 Collection of Samples

The samples were collected during November 2017, from 30 herbalists. Also control sample of the plant was collected in the savannah of Lamto reserve (Toumodi). During the collection, sterile gloves, a charlotte and a gown were used to prevent contamination of the collected samples.

Plant organs were labeled and put in sterile stomacher bag. A cooler containing ice pack was used to transport all samples to the laboratory at "Institut Pasteur de Côte d'Ivoire", for microbiological analyzes. Once at the laboratory, some of the samples were immediately subjected to analysis without prior storage and preservation.

The other part was dried in an air-conditioned room at 18°C. The organs were then crushed to obtain powders.

2.4 Preparation of Stock Solutions and Inoculums

For each sample, a stock solution was prepared. Briefly, 3 g of fragmented stem bark were added to 30 mL of a buffered peptone water solution (BPW), then the whole was homogenized and allowed to macerate for 24 h at 37°C. Dilutions of the obtained extract were prepared as following: 1 mL of the macerate was transferred to a test tube containing 9 mL tryptone salt (TS); for obtaining the dilution 10^{-1}. 1 mL of this dilution was added to 9 mL of TS. The different dilutions prepared ranged from 10^{-1} to 10^{-3}.

2.5 Microbiological Studies

2.5.1 Total coliforms

The initial inoculum and dilutions (1 mL) were cultured on Violet Red Bile Lactose Agar (VRBL) pre-cast in 90 mm Petri dish for searching total coliforms. The plates were incubated at 30°C for 24 h (NF ISO 4831). The Petri dish with 30-300 colonies were considered for enumeration (Fig. 2A).

2.5.2 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 NF V08-051 (Fig. 2B).

2.5.3 Escherichia coli

For enrichment of *E. coli*, 1 ml of the inoculum was added to 9 ml of Buffered Peptone Nutrient Broth (EPT). The whole was incubated at 44°C for 24 h. After the incubation, a portion of the bacterial suspension was seeded on Rapid E. coli 2 (REC2) agar and incubated at 44°C for 24 h (NF V 08-060).

On the medium, *E. coli* normally appears as purple to pink colonies. For the identification of the bacteria, the reduced Leminor rack technique was applied with the following medium: KiglerHajnar, Citrate and Urea. Using a sterile platinum loop, germs suspended in the urea-indole medium (previously prepared suspension) were seededand incubated at 37°C for 24 h.

2.5.4 Streptococci

The Streptococci were identified on Bile EsculineAzide agar (BEA). The different Petri dishes were incubated at 37°C for 24 hours. All colonies on Petri dish (15-150 colonies) were counted 24 h post-incubation (NF ISO 21528-2). On the BEA medium, streptococci appear as small colonies surrounded by a black halo (Fig. 2C). Suspicious colonies were subcultured on standard agar and incubated at 37°C for 24 h. Confirmation of Streptococci was carried out with catalase and oxidase tests.

2.5.5 Pseudomonas

For enrichment of Pseudomonas, 1 ml of inoculum was added to 9 ml of buffered peptone nutrient broth (BPN). The whole was incubated at 37°C for 24 h. After incubation, to isolate the bacteria, a portion of the bacterial suspension was seeded on the cetrimide agar and incubated at 37°C for 24 h. On this medium, *Pseudomonas* appear as colonies with characteristic blue or blue-green pigmentation. The Petri dish with 15-150 colonies were considered for enumeration.

These colonies were then cultured on agar plate and incubated at 37°C for 24 h. For confirmation, the oxidase test and the seeding of suspicious colonies on the King A and King B rack were performed.

2.6 Data Analysis

The number of colonies (N) present was determined using the following counting formula 2:

$$\mathbf{N} = \frac{\Sigma C}{V(n1 + 0.1n2)d} \qquad (2)$$

Σ c: expressed in Colony Forming Units (CFU), refers to the sum of the colonies counted on the selected Petri dishes;

n1: number of Petri dishes of the lowest dilution where the colonies could be counted;

v: volume of inoculum.

n2: number of Petri dishes of the dilution that follows the previous one;

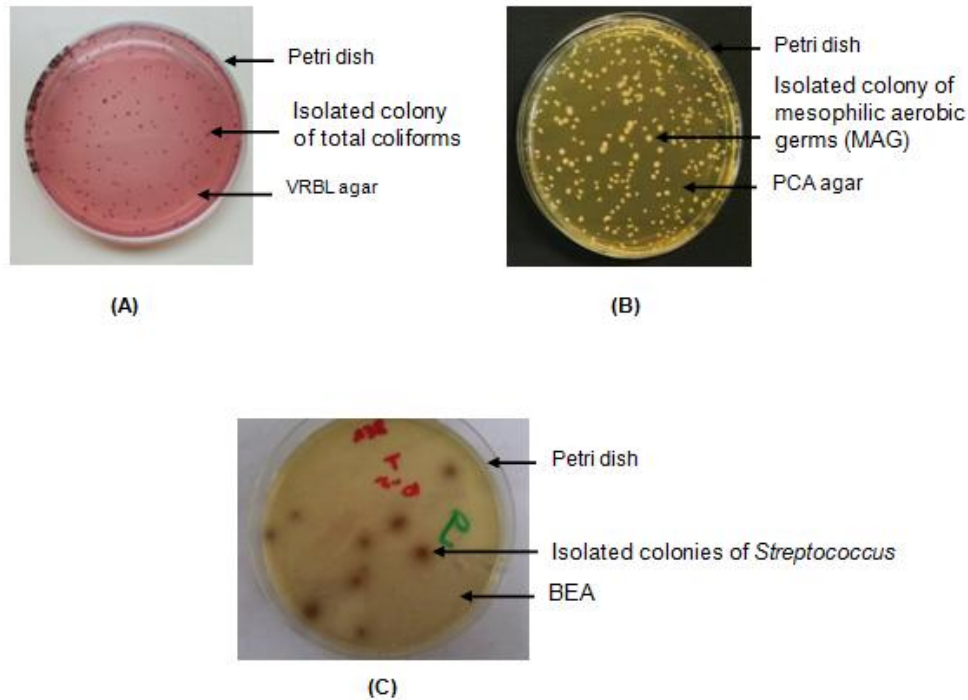d: dilution ratio corresponding to the very first dilution retained;

Fig. 2. Images showing the enumeration of colonies on the different types of agars. (A) Total coliform colonies on Violet Red Bile Lactose Agar (VRBL), (B) Colonies of aerobic mesophilic germs on Plate Count Agar plates (PCA), (C) *Streptococcus* colonies on Bile EsculineAzide (BEA) agar

The statistical analysis was performed using software R version 3.3.1 for comparing the hygienic quality of the samples related to their microbial load. This analysis was carried out using unidirectional analysis of variances (ANOVA ONE WAY), followed by the Dunnett test (glm, family = fish) for the classification of samples. The level of significance was at a threshold α = 5%. Excel 2016 software was used to build graphics.

3. RESULTS

3.1 Packaging of Plants sold

According to the traders (herbalists) surveyed, the plants sold on the Siaka Koné market are collected in the Banco forest (Abidjan), Agboville and Alépé in Southern Côte d'Ivoire and in bush from Northern or Central Côte d'Ivoire. Some plants are imported from bordering countries such Burkina Faso and Mali.

Means of transportation are cars such as foodstuffs or public transport (taxi, bus). In the markets, these plants undergo various transformations: drying (usually in the open air) and crushing of the organs. Plastic bags were mostly used for packaging. In some cases, the samples are directly conditioned without prior treatment.

At their point of sale, 53% of medicinal plants were stored outdoors on pieces of brick, 20% under black tarpaulins or stored in dilapidated stores away from light and 13% in bags. In addition, some herbalists keep the organs of different plants on tables covered with black tarpaulins.

3.2 Plant Species Sold on Markets

The survey identified the most represented species on the market and the most used by populations for treating diseases (Fig. 3). *S. latifolius* was selected on the basis of its high frequency of citations. According to the herbalists, this species is widely used against various diseases including malaria, typhoid fever, stomachache, etc.

3.3 Germs on Samples

3.3.1 Total coliforms and aerobic mesophilic germs (AMG)

Total coliforms were isolated on all samples collected with an average of $4.7×10^5$ CFU/g of plant (Table 1). The load ranged from $1.3×10^3$ to $9.2×10^7$ CFU/g of plant. Of the 30 samples, 25 samples (83.33%) had a number of germs greater than 10^3 CFU/g.

The presence of AMG was observed on all samples collected with an average of $1.2×10^5$ CFU/g of plant. The microbial load ranged from $8.1×10^3$ to $5.1×10^5$ CFU/g of plant. Of the 30 samples, 15 samples (46.67%) were contaminated with a number of AMG greater than 10^5 CFU/g. Control sample (D1) was collected in the savannah of Lamto Scientific Reserve (Toumodi). The samples D2-D30

were collected from 30 different herbalists (Table 1a).

3.3.2 Contamination with *Escherichia coli*, *Pseudomonas* and *Streptococci*

The presence of *E. coli* germs was observed only in one sample (3.33%) of the 30 analyzed. For the genus *Pseudomonas*, five samples (16.67%) were contaminated. *Streptococci* were found on 93.33% of the samples (Fig. 4).

3.4 Comparison of Microbial Loads of Samples

The number of total coliforms varied significantly between samples (glm, family = fish, deviance = 4900.4, df = 29, *P*< .001). However, all samples were contaminated. The rate of AMG also varied (glm, family = fish, deviance = 2898.6, df = 29, *P*< .001).
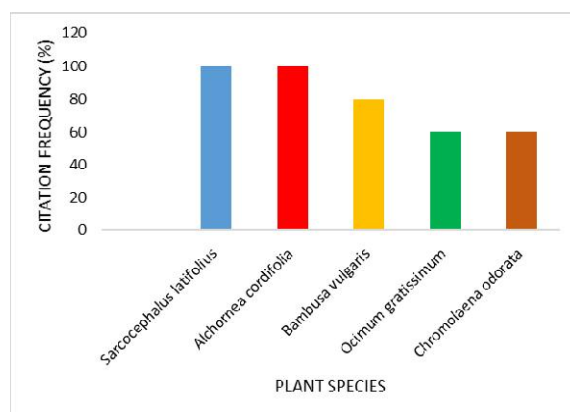

Fig. 3. Histogram showing the most cited plant species in the treatment of malaria

Table 1. Microbial load found on collected samples

Samples	Total coliforms (CFU/g)	WHO[9] limit, 2007 (CFU/g)	AMG(CFU/g)	WHO[9] limit, 2007 (CFU/g)
D1	$3.3×10^4$	$≤ 10^3$	$1.8×10^5$	$≤ 10^5$
D2	$4×10^5$	$≤ 10^3$	$3.9×10^4$	$≤ 10^5$
D3	$1.9×10^5$	$≤ 10^3$	$1.2×10^5$	$≤ 10^5$
D4	$4.7×10^4$	$≤ 10^3$	$1.1×10^5$	$≤ 10^5$
D5	$4.5×10^3$	$≤ 10^3$	$3.8×10^5$	$≤ 10^5$
D6	$3.2×10^4$	$≤ 10^3$	$5.1×10^5$	$≤ 10^5$
D7	$2.3×10^4$	$≤ 10^3$	$1.4×10^5$	$≤ 10^5$
D8	$2.5×10^5$	$≤ 10^3$	$2.8×10^5$	$≤ 10^5$
D9	$2×10^4$	$≤ 10^3$	$2.8×10^5$	$≤ 10^5$
D10	$2.7×10^4$	$≤ 10^3$	$1.3×10^4$	$≤ 10^5$
D11	$2.5×10^4$	$≤ 10^3$	$1.7×10^5$	$≤ 10^5$
D12	$2.3×10^5$	$≤ 10^3$	$1.7×10^4$	$≤ 10^5$
D13	$5×10^4$	$≤ 10^3$	$1.8×10^5$	$≤ 10^5$
D14	$1.6×10^4$	$≤ 10^3$	$2×10^4$	$≤ 10^5$
D15	$1.4×10^4$	$≤ 10^3$	$8×10^4$	$≤ 10^5$

AMG = Aerobic mesophilic germs; D = Samples collected; CFU = Colony forming units

Table 1(a). Microbial load found on collected samples (Continued)

Samples	Total coliforms (CFU/g)	WHO limit, 2007 (CFU/g)	AMG(CFU/g)	WHO limit, 2007 (CFU/g)
D16	2.7×10^4	$\leq 10^3$	2.7×10^4	$\leq 10^5$
D17	9×10^3	$\leq 10^3$	8.6×10^3	$\leq 10^5$
D18	9×10^3	$\leq 10^3$	1.7×10^5	$\leq 10^5$
D19	1.4×10^4	$\leq 10^3$	1.2×10^5	$\leq 10^5$
D20	1.5×10^4	$\leq 10^3$	1.4×10^4	$\leq 10^5$
D21	1.5×10^3	$\leq 10^3$	1.4×10^5	$\leq 10^5$
D22	1.4×10^3	$\leq 10^3$	1.7×10^5	$\leq 10^5$
D23	5×10^4	$\leq 10^3$	8.1×10^3	$\leq 10^5$
D24	5×10^4	$\leq 10^3$	2.8×10^4	$\leq 10^5$
D25	9.2×10^7	$\leq 10^3$	6.3×10^4	$\leq 10^5$
D26	7.9×10^5	$\leq 10^3$	1×10^5	$\leq 10^5$
D27	3.9×10^7	$\leq 10^3$	2.4×10^4	$\leq 10^5$
D28	3.8×10^6	$\leq 10^3$	6.2×10^4	$\leq 10^5$
D29	4.7×10^6	$\leq 10^3$	8.9×10^4	$\leq 10^5$
D30	4.8×10^5	$\leq 10^3$	8.2×10^4	$\leq 10^5$
Average	4.7×10^5	$\leq 10^3$	1.2×10^5	$\leq 10^5$

AMG = Aerobic mesophilic germs; D = Samples collected; CFU = Colony forming units

The number of germs varied significantly in some samples compared to the control according to the Anova test (Table 2). For the coliforms, samples D2, D10, D19 and D20 gave values close to the control D1. The other samples showed lower values than the control. For AMG, samples D5, D6, D11 showed values substantially equal to control D1. This result shows that samples from markets were more contaminated than those from the wild. Also, the plants were handled in poor hygienic conditions from transport to storage on markets.

4. DISCUSSION

This study aimed at evaluating the conditions of treatment and storage of medicinal plants and the microbiological quality of 30 samples of *Sarcocephalus latifolius* stem bark sold at the Siaka Koné market in Abobo.

The results showed that 86% of the plants sold on this market are not well maintained. In fact, 53% of plant organs are stored outdoors on pieces of brick exposed to dust, air humidity and car exhaust. While 20% are under black tarpaulins or stored in dilapidated stores away from light and 13% in bags in open air. According to FAO [10], environmental dust and car exhaust gases deposit all kind of contaminants on foodstuffs. Medicinal plants can be contaminated because of storage in the same markets as foods.

The humidity of air may favor the proliferation of molds [11]. These microorganisms have a natural tropism for water environments. On Siaka Koné market, mud and puddles were recorded. These biotopes may favor contamination of plant parts sold. These microbial contaminants such as *Aspergillus spp.* could produce mycotoxins such as highly carcinogenic aflatoxins. Therefore, it is important to identify microbial contaminants in products of traditional medicine as indicators of safety and quality [11].

The results of microbiology investigations revealed the presence of germs such as total coliforms, aerobic mesophilic germs, *Escherichia coli*, *Pseudomonas spp* and *Streptococcus spp*. Some of these microorganisms are agents causing infections, allergies and/or producing endotoxins of health risks [12]. The high bacterial load found in the samples may reflect plants were exposed to bad handling, processing methods and storage conditions. Coliforms are one of the most important groups of bacteria for evaluating the safety and quality of biological materials.

The presence of total coliforms showed a possible contamination and inadequate hygienic conditions during storage. The load of germs ranged from 13×10^2 to 92×10^6 CFU/g of plant. For 33.33% of the samples studied, the load was greater than 10^3 CFU/g as recommended by WHO. The high bacterial load may reflect bad handling of samples. The Coliforms were

Table 2. Comparison of the samples studied

Total coliforms control vs samples	P value	AMG control vs samples	P value
D1 vs D2	P = .47	D1 vs D2	P< .001
D1 vs D3	P< .001	D1 vs D3	P< .001
D1 vs D4	P< .001	D1 vs D4	P< .001
D1 vs D5	P< .001	D1 vs D5	P = .59
D1 vs D6	P< .001	D1 vs D6	P = .11
D1 vs D7	P< .001	D1 vs D7	P< .001
D1 vs D8	P< .001	D1 vs D8	P< .001
D1 vs D9	P< .001	D1 vs D9	P< .0011
D1 vs D10	P = .09	D1 vs D10	P< .001
D1 vs D11	P< .001	D1 vs D11	P = .17
D1 vs D12	P< .001	D1 vs D12	P< .001
D1 vs D13	P < 0.001	D1 vs D13	P = .74
D1 vs D14	P< .001	D1 vs D14	P< .001
D1 vs D15	P< .001	D1 vs D15	P< .001
D1 vs D16	P = .09	D1 vs D16	P< .001
D1 vs D17	P< .001	D1 vs D17	P< .001
D1 vs D18	P< .001	D1 vs D18	P = .17
D1 vs D19	P = .09	D1 vs D19	P< .001
D1 vs D20	P = .78	D1 vs D20	P< .001
D1 vs D21	P< .001	D1 vs D21	P< .001
D1 vs D22	P< .001	D1 vs D22	P = .27
D1 vs D23	P< .001	D1 vs D23	P< .001
D1 vs D24	P< .001	D1 vs D24	P< .001
D1 vs D25	P< .001	D1 vs D25	P< .001
D1 vs D26	P< .001	D1 vs D26	P< .001
D1 vs D27	P< .001	D1 vs D27	P< .001
D1 vs D28	P< .001	D1 vs D28	P< .001
D1 vs D29	P< .001	D1 vs D29	P< .001
D1 vs D30	P < .001	D1 vs D30	P< .001

AMG = Aerobic mesophilic germs; P < .001 Significant; D = Samples collected
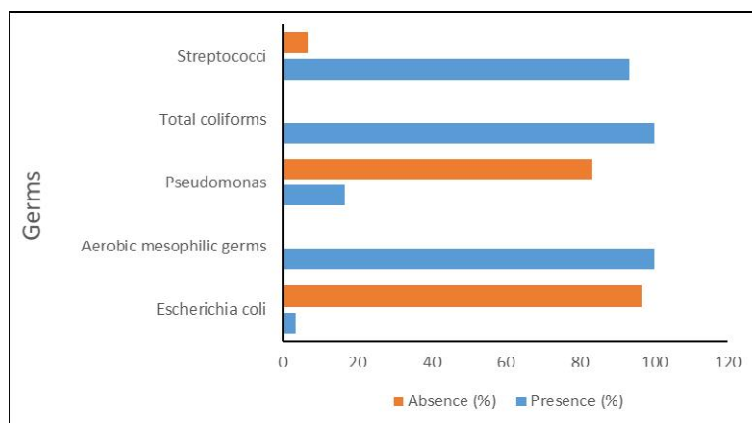

Fig. 4. Histogram showing the germs found in samples of *Sarcocephaluslatifolius* (Rubiaceae) collected in the municipality of Abobo

reported as microbial contaminants in Pakistan [13] also in the city of Peshawar the presence of pathogenic organisms was observed in available commercially dried medicinal plants. For Egyptian spices and medicinal plants, the results of Abou-Donia [14] revealed a relatively low load of coliforms (10^1--10^3). The presence of coliforms is linked to a possible faecal contamination and inadequate hygienic conditions during the growth of plants.

For aerobic mesophilic germs, the load ranged from 8.1×10^3 to 5.1×10^5 CFU/g. Of the 30 plant samples, 73.33% showed a load greater than 10^3 CFU/g as recommended by WHO. The high bacterial load may be due to unsuitable treatment methods and storage conditions. In contrast, Idu et al [15] reported relatively low bacterial counts (10^2-10^4 CFU/g) in samples of medicinal plant. The total number of aerobic bacteria may vary according to plant types, geographical distributions, environmental factors, treatment techniques and storage conditions [16].

For *Escherichia coli*, 3.33% of the samples were contaminated. Contamination due to *E. coli*, may be due to harvest near houses or sanitation pits. *Escherichia coli* was isolated from *Ficusglomerata* [14] and the strain *E. coli* O157 from the root system of a lettuce [17]. Human health is at risk in case of food contamination with these bacteria.

In total, 16.67% of the samples were contaminated by *Pseudomonas spp*. This growth of this bacteria may be favor by moisture or water in samples. Nandna et al. [5] reported an absence of *Pseudomonas aeruginosa* in commercially samples studied. While Rajapandiyan et al. [18] found the presence of *P. aeruginosa* in 31 commercial medicinal herbs. This could be due to the conditions of conservation or hygiene of these different places. *Streptococci* contaminated 93.33% of the samples. The presence of these germs is an indication of faecal contamination due to the poor state of the premises and the presence of mud and puddles. These germs are responsible for several diseases such as meningitis and pneumonia [19].

The studied samples had a load far above the limits of WHO. The poor treatment and storage of the medicinal plants sold by herbalists could have negative impact on their safety.

5. CONCLUSION

A significant load of total coliforms, aerobic mesophilic germs, *streptococci* and *E. coli* was observed on samples of the stem bark of *Sarcocepha luslatifolius* collected on the market Siaka Koné in the district of Abidjan. These results showed that the plants sold on the markets are potentially contaminated and may be hazardous to the health of consumers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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