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PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF *NAUCLEA LATIFOLIA* AND *PILIOSTIGMA THONNINGII* MACERATES USED IN TRADITIONAL MEDICINE FOR THE TREATMENT OF MALARIA

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ABSTRACT

Macerates of *Nauclea latifolia* and *Piliostigma thonningii* are used by the population of Korhogo for their antimalarial properties. However, the risk of microbial proliferation in the macerate is significant. The overall objective was to determine the physicochemical and microbiological parameters of *Nauclea latifolia* and *Piliostigma thonningii* macerates. For the preparation of the macerates, tap water was added to the roots of *Nauclea latifolia* and the bark of *Piliostigma thonningii* on day D_0. The evolution of the microbial flora and physicochemical parameters were monitored from D_0 to D_{12}. All analyses were carried out according to international standards. The loads of Aerobic Focal Mesophilic Microorganisms, total and thermotolerant coliforms, *Escherichia coli*, *S. aureus*, and molds increased from 3.15 to 8.89 $\log_{10}$ CFU/mL and 3.34 to 8.51 $\log_{10}$ CFU/mL; 2.31 to 7.87 $\log_{10}$ CFU/mL and 2.39 to 7.8 $\log_{10}$ CFU/mL; 2.0 to 7.94 $\log_{10}$ CFU/mL and 2.18 to 7.63 $\log_{10}$ CFU/mL; 1.74 to 6.6 $\log_{10}$ CFU/mL and 1.96 to 6.71 $\log_{10}$ CFU/mL; 2.35 to 6.92 $\log_{10}$ CFU/mL and 1.72 to 6.83 $\log_{10}$ CFU/mL; and 1.64 to 7.17 $\log_{10}$ CFU/mL and 1.98 to 7.42 $\log_{10}$ CFU/mL from D_0 to D_{12}, respectively, for the *N. latifolia* and *P. thonningii* macerates. These contamination levels exceeded the values established by standards for pharmacopeia products. The pH of the macerates was acidic (4.92 to 6.5). Temperatures ranged from 25.7 to 32.7°C. Dissolved oxygen decreased from 3.6 to 1.2 mg/L. The physicochemical parameters influenced the proliferation of microorganisms. The microbiological quality of macerates is important, especially when used by young, elderly, or disease-weakened individuals.

Keywords: *Nauclea latifolia, Piliostigma thonningii*, Macerate, Microbiological Quality, Côte d'Ivoire

1 INTRODUCTION

For the management of diseases in developing countries, many populations resort to traditional medicine and the use of plants. Traditional medicine is part of the socio-cultural practices of many peoples [1]. Moreover, in the face of the ineffectiveness and numerous contraindications of synthetic drugs, the use of medicinal plants is on the rise even in industrialized countries, with the integration of modern techniques [2].

Plants contain a wide variety of phytochemical constituents. They therefore offer significant potential in the treatment of several human and animal diseases [3,4]. They also constitute a promising source of medicines. Phytochemical compounds of therapeutic interest can come from the bark, leaves, flowers, roots, fruits, seeds, etc. [5]. Research efforts are underway in many countries to evaluate the quality, safety, and efficacy of medicines derived from plants. Thus, biologically active compounds can be isolated from the plant using modern physicochemical and biological techniques, such as guided fractionation, isolation, and purification [5]. However, populations continue to use traditional processes, namely decoction, infusion, maceration, etc. [3].

The use of antimalarial plants exhibits significant variability across regions and localities. *Nauclea latifolia* and *Piliostigma thonningii* are well known among Côte d'Ivoire populations, particularly those in Korhogo, for their antimalarial properties. The potential of these plants (*Nauclea latifolia* and *Piliostigma thonningii*) to reduce malaria parasite load has been highlighted in numerous studies [6,7]. However, practises often differ from one region to another. They are generally influenced by culture, traditional knowledge, and the experience of practitioners [8,9]. Herbal vendors propose several combinations of these plants. These combinations consist of multiple plants, often in fresh or dried form. Decoction, infusions, and maceration are the most frequent preparation methods [9,10].

The safety of these products is important to ensure their harmlessness or their potential to interact negatively with other medications, especially for young, elderly, or disease-weakened individuals. In addition, there are risks of microbiological contamination. Indeed, bark and roots in water provide organic matter that is susceptible to microbial proliferation [11].

In Côte d'Ivoire, traditional medicine products largely escape quality control by health authorities. This weak quality control feeds user concern, especially in urban areas. Regardless of the form of use, a minimum level of control is necessary. This study focused on the health risks associated with the consumption of macerates. Maceration is a process of partial dissolution and extraction using a given solvent, generally water. It consists of keeping the plant material cold and in contact with this solvent for several hours or even weeks. The mixture (raw material and solvent) must be shaken at regular intervals. The resulting product is a macerate [5]. The macerate is drunk by patients at least twice a day throughout the duration of a treatment. Given the maceration time, storage conditions, and solvent quality, the risk of developing significant microbial flora in the macerate cannot be ruled out. Furthermore, previous studies have demonstrated the presence of pathogenic microorganisms in various medicinal plant preparations [11,12].

This study aims to contribute to improving the quality of medicinal plant macerates. Its overall objective is to determine the physicochemical and microbiological parameters of *Nauclea latifolia* and *Piliostigma thonningii* macerates used in the treatment of malaria in Korhogo.

2 MATERIAL AND METHODS

2.1 Material

2.1.1 Biological Matrix

The biological material consisted of macerates from the roots of *Nauclea latifolia* and the bark of *Piliostigma thonningii* (**Figure 1**).

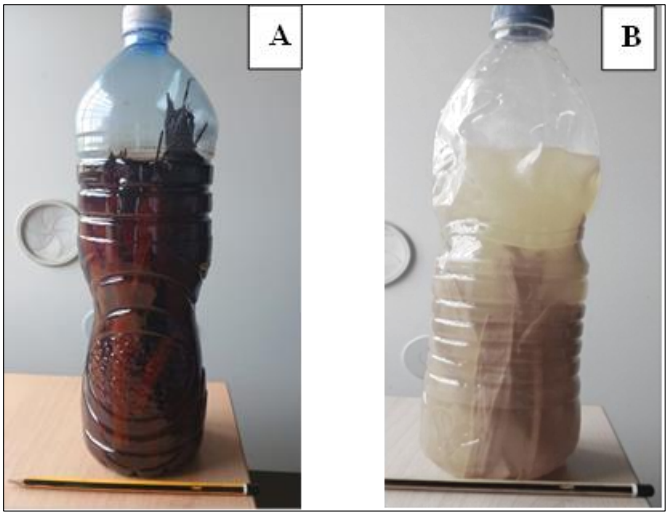


Figure 1: Photographs of the macerates (A: bark of *Piliostigma thonningii*; B: roots of *Nauclea latifolia*).

2.1.2 Material used

The equipment used to measure the physicochemical parameters consisted of two portable devices. A multi-parameter instrument from HANNA (model HI991301) was used to measure temperature, pH, conductivity, total dissolved solids (TDS) and redox potential. The other device used to measure dissolved oxygen was an oximeter, Lutron DO-5519. Like the multi-parameter instrument, the oximeter also measures temperature. Both devices are equipped with a measuring probe connected to the instrument by a cable.

In addition to the usual equipment found in a microbiology laboratory, the following culture media were used:

- Baird-Parker agar (BP OXOID CM0275) enriched with tellurite for the enumeration of *Staphylococcus aureus*;
- Violet Red Bile Lactose agar (VRBL BIO RAD) for the enumeration of coliforms (total and thermotolerant);
- Plate Count Agar (PCA BIO RAD) for the enumeration of Total Mesophilic Aerobic Flora (TMAF);
- Tryptone Bile X-glucuronide agar (TBX BIO RAD) for the enumeration of *Escherichia coli*;
- Oxytetracycline Glucose Yeast Extract Agar (OGYE Neogen® a) for the enumeration of molds.

The media were prepared according to the manufacturers' instructions provided in the manuals and were poured into Petri dishes.

2.2 Methods

2.2.1 Sample Collection

A preliminary survey conducted among traditional healers and sellers of medicinal plants in the markets of Korhogo made it possible to select the plants studied. These were *Nauclea latifolia* and *Piliostigma thonningii*. These plants are known for their medicinal properties. In the treatment of malaria, maceration is the main method of use for these plants. Bundles consisting solely of the roots of *Nauclea latifolia* and the bark of *Piliostigma thonningii* were purchased in the main market of Korhogo. The roots and bark were placed in sterile plastic containers of 1.5 litres. Tap water was added to the bottles on day J_0, corresponding to the start of the analyses. During the two weeks of analyses, the water contained in the bottles (macerate) was not renewed.

2.2.2 Measurement of phyisco-chemical parameters

Before each measurement, the device was switched on and calibrated. Twenty-five (25 mL) of the macerate was placed in a plastic jar, and the probe of the device was immersed in it. The value of the selected parameter automatically appeared on the screen and was recorded. Before proceeding to the measurement of the next extract, the probe was rinsed with distilled water to avoid any contamination that could compromise the reliability of subsequent measurements.

2.2.3 Microbial load of the macerates

2.2.3.1 Preparation of working solutions

From the macerate solution (mother suspension), a series of decimal dilutions was carried out according to ISO 6887/2017. In sterile tubes, 9 mL of sterile physiological water was placed. A sterile pipette was used to collect 1 mL of the macerate, which was then transferred into the first tube $N^0 1$ containing the diluent. The mixture at a concentration of 10^{-1} was homogenized using a vortex. One (1) mL of the 10^{-1} dilution was taken and added to the 9 mL of physiological water in the second tube. The homogenized mixture corresponded to the 10^{-2} dilution. For dilutions from 10^{-3} to 10^{-7}, the same approach was followed.

2.2.3.2 Enumeration of total mesophilic aerobic flora according to NF ISO 4833:2013

Approximately 15 mL of PCA agar, prepared and maintained in a supercooled state in a water bath, was poured into petri dish containing 1 mL of the selected working dilution. The contents of the dish was gently homogenized by circular movements. Three dishes were inoculated per dilution. After solidification of the inoculated agar, the dishes were inverted and incubated at 37°C for 72 hours. The dishes from successive dilutions with colonies ranging between 30 and 300 were counted.

2.2.3.3 Enumeration of *E. coli* according to NF ISO 16649-1:2018

TBX agar is a selective chromogenic medium intended for the enumeration of *Escherichia coli* β-D-glucuronidase positive in food products. A volume of 1 mL of the suspension and decimal dilutions were placed in sterile petri dishes, then approximately 15 mL of medium already prepared and maintained at 45°C was added to the dishes. After homogenization and solidification, the dishes were incubated at 44 ± 1°C for 24 hours. The characteristic colonies, blue to blue-green, were counted.

2.2.3.4 Enumeration of total and thermotolerant coliforms according to NF ISO 4832:2006

The same pour-plate inoculation procedure was carried out as previously described, but this time using VRBL agar. The dishes were incubated at 37°C and 44°C for 24 hours, respectively for total coliforms and thermotolerant coliforms. The dishes containing colonies of violet-pink color ranging between 15 and 150 were counted.

2.2.3.5 Search and enumeration of *Staphylococcus aureus* according to NF-V08-057-1:2004

Baird-Parker medium enriched with egg yolk and potassium tellurite was prepared and poured into petri dishes. The surface of the agar was inoculated with 0.1 mL of the working dilution. For each dilution, three dishes were inoculated and then incubated in an oven at 37°C for 24 hours. Petri dishes with colonies characteristic of *Staphylococcus* (black, shiny, entire, convex colonies surrounded by clear zones) with counts ranging between 15 and 150 were enumerated.

2.2.3.6 Enumeration and identification of yeasts and molds according to ISO 6611:1996

Petri dishes containing OGYE agar previously poured were inoculated with 0.1 mL of each of the selected dilutions. The dishes were then incubated at 30°C for 3 to 5 days. Mold colonies, often pigmented, with a velvety appearance and varying sizes, were counted.

For identification, a well-isolated strain was collected and sub-cultured in the centre of a freshly poured dish. After 48 to 72 hours, macroscopic and microscopic observations were carried out. Macroscopic observation focused on growth rate, colour, texture and colony morphology. Microscopic observation of the colonies was performed using a light microscope at magnifications of ×40 and ×100. For this, a well-isolated mold was collected and placed on a slide moistened with distilled water. The slide was then carefully covered with a coverslip. The characters observed were the mycelium, its septa, the presence of sporangia, and spores. The identification keys proposed by Talaiekhozani and Ponraj [13] were used for identification.

2.2.4 Calculation of the microbiological load according to ISO 7218:2007

The contamination level of the macerate samples was determined using formula (**1**). It is expressed in Colony Forming Units per milliliter (CFU/mL).

$$N = \frac{\sum C}{V \times d \times (n1 + 0.1n2)} \qquad (1)$$

- **ΣC** : sum of colonies on all plates from the two successive dilutions;
- **V:** volume of the inoculum applied to each plate in millilitres;
- **n1** et **n2:** number of plates respectively for the first and second dilution considered;

- **d** : dilution rate of the first plate that produced countable colonies (lowest dilution)

2.2.5 Statistical treatment of the data

IBM SPSS Statistics software, version 20, was used for the statistical processing of microbiological and physico-chemical data. Comparison of distributions between groups for physico-chemical and microbiological parameters were performed using the Kruskal–Wallis one-way ANOVA test.

3 RESULTS AND DISCUSSION

In the present study, the physicochemical parameters and microbial loads of the macerates were evaluated over time.

3.1 Physicochemical parameters of macerates

Regarding the physicochemical parameters, temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), conductivity and oxidation-reduction potential (ORP) of the macerates were determined during this study.

The values of the measured physicochemical parameters of the root macerate of *Nauclea latifolia* (Macerate M1) are recorded in **table 1**. The pH values were acidic, ranging from 4.97 to 6.5. Total dissolved solids (TDS) data were low, ranging between 0.25 and 0.48 mg/L. The

lowest value was measured on Day 0 (D_0) and the highest was on Day 3 (D_3). Conductivity values ranged between 0.52 mS (D_0) and 0.95 mS (D_3). The redox potential of the macerate evolved over time, increasing from 71 mV at D_0 to 206 mV at D_{12}. Dissolved oxygen values decreased over the days, dropping from 3.3 mg/L at D_0 to 1.3 mg/L (D_{12}). Temperature values ranged from 25.7°C to 32.60°C.

The values of the physicochemical parameters of the bark macerate of *Piliostigma thonningii* (Macerate M_2) are summarized in **table 2**. The pH values were also acidic, progressively decreasing from 6.5 (D_0) to 4.92 (D_{12}). The TDS values were higher than those of macerate M_1, increasing over time from 0.69 (D_0) to 1.23 (D_{12}). Conductivity values ranged from 1.32 to 2.39 mS, with the lowest value obtained on day 0 (D_0) and the highest value at D_6. The redox potential increased over time, measuring 27 mV at D_0 and 211 mV on the final day (D_{12}). For dissolved oxygen, the highest value was measured on D_0 (3.6 mg/L) and the lowest on D_{12} (1.2 mg/L). The temperature ranged between 25.6°C (D_3) and 32.7°C (D_9).

Table 1: Physicochemical parameters of the root macerate of *Nauclea latifolia* (M_1)

	Physico-chemical parameters					
Day	**pH**	**TDS (PPT)**	**Cond (mS)**	**Redox (mv)**	**DO (mg /L)**	**T (°C)**
J_0	5.73 ± 0.02^{a}	0.25 ± 0.01^{a}	0.52 ± 0.01^{a}	71 ± 1.73^{a}	3.3 ± 0.1^{d}	25.73 ± 0.15^{a}
J_3	4.97 ± 0.04^{b}	0.48 ± 0.005^{c}	0.95 ± 0.02^{c}	100 ± 2^{b}	2.8 ± 0.1^{c}	27.6 ± 0.1^{b}
J_6	6.5 ± 0.1^{c}	0.467 ± 0.01^{bc}	0.93 ± 0.02^{bc}	118.67 ± 2.08^{c}	1.77 ± 0.11^{b}	32.5 ± 0.1^{c}
J_9	5.3 ± 0.1^{d}	0.46 ± 0.005^{bc}	0.89 ± 0.02^{bc}	125 ± 2.64^{d}	1.63 ± 0.05^{b}	32.63 ± 0.05^{c}
J_{12}	6.1 ± 0.1^{f}	0.42 ± 0.01^{b}	0.87 ± 0.01^{b}	206.33 ± 2.51^{e}	1.3 ± 0.1^{a}	32.6 ± 0.1^{c}

Table 2: Physicochemical Parameters of the Macerate from the Bark of *Piliostigma thonningii* (M_2)

	Physico-chemical parameters					
Day	**pH**	**TDS (PPT)**	**Cond (mS)**	**Redox (mv)**	**DO (mg /L)**	**T (°C)**
J_0	6.5 ± 0.01^{a}	0.69 ± 0.03^{a}	1.32 ± 0.02^{a}	27 ± 1^{a}	3.6 ± 0.1^{a}	26.23 ± 011^{b}
J_3	5.48 ± 0.01^{b}	1.15 ± 0.01^{b}	2.3 ± 0.02^{a}	84.33 ± 3.05^{b}	1.8 ± 0.1^{b}	25.6 ± 0.1^{a}
J_6	5.22 ± 0.01^{c}	1.16 ± 0.01^{bc}	2.39 ± 0.01^{a}	106.67 ± 2.51^{c}	1.57 ± 0.05^{c}	31.3 ± 0.17^{c}
J_9	5.12 ± 0.01^{d}	1.22 ± 0.04^{c}	2.38 ± 0.01^{a}	113 ± 2^{d}	2.33 ± 0.11^{d}	32.7 ± 0.1^{d}
J_{12}	4.92 ± 0.12^{e}	1.23 ± 0.02^{bc}	2.09 ± 0.58^{a}	211 ± 2^{e}	1.2 ± 0.1^{e}	32.37 ± 0.25^{d}

Within the same column, means assigned the same letter are not significantly different at the 5% level Macerate temperatures fluctuated between 25°C and 32°C. These values correspond to the ambient temperatures of the city of Korhogo, as the macerates were stored at room temperature. Such temperatures are optimal for the proliferation of mesophilic microorganisms, notably coliforms, *S. aureus*, and certain molds [14]. The pH of the macerates was slightly acidic (4.9–6.5), a range corresponding to the optimal growth pH for the microorganisms isolated in this study. The pH measures the chemical activity of hydrogen ions in solution and is an essential parameter for assessing water quality; it is also a major factor influencing microbial growth [14,15]. The measured electrical conductivity was high, exceeding 1 mS/cm; however, values remained within the WHO acceptability limit (1.2 mS/cm at 25°C). Conductivity allows for the evaluation of dissolved ion concentrations in water [16]. Total dissolved solids (TDS) values were low. TDS provides an assessment of suspended particles and primarily impacts acceptability and taste [16]. The physicochemical parameters serve as indicators of water quality and can also influence the microbiological quality of water [17].

3.2 Evolution of the Microbial Load of the Macerates

The results of the microbiological analyses are presented in **table 3** for the *Nauclea latifolia* macerate (M_1) and in **table 4** for the *Piliostigma thonningii* macerate (M_2).

For both macerates, contamination levels were high and increased over the storage period. From Day 0 to Day 12, microbial counts for the *N. latifolia* and *P. thonningii* macerates, respectively, increased from 3.15 to 8.89 $\log_{10}$ CFU/mL and 3.34 to 8.51 $\log_{10}$ CFU/mL for aerobic mesophilic flora (AMF); 2.31 to 7.87 $\log_{10}$ CFU/mL and 2.39 to 7.80 $\log_{10}$ CFU/mL for total coliforms; 2.00 to 7.94 $\log_{10}$ CFU/mL and 2.18 to 7.63 $\log_{10}$ CFU/mL for thermotolerant coliforms; 1.74 to 6.60 $\log_{10}$ CFU/mL and 1.96 to 6.71 $\log_{10}$ CFU/mL for *Escherichia coli*; 2.35 to 6.92 $\log_{10}$ CFU/mL and 1.72 to 6.83 $\log_{10}$ CFU/mL for *Staphylococcus aureus*; and 1.64 to 7.17 $\log_{10}$ CFU/mL and 1.98 to 7.42 $\log_{10}$ CFU/mL for molds. Across the sampling intervals, a statistically significant difference ($p < 0.05$) was observed for each enumerated microorganism.

Table 3: Microbiological loads of the *Nauclea latifolia* root macerate (M_1)

	Microbial loads ($\log_{10}$FCU/mL))					
Day	**TAMF**	**TC**	**Th C.**	***E. coli***	**Stap**	**Molds**
J_0	3.15±0.05^a	2.31±0.06^a	2.09±0.12^a	1.74±0.06^a	2.35±0.11^a	1.64±0.2^a
J_3	5.26±0.03^b	4.24±0.04^b	3.83±0.15^b	3.55±0.38^b	3.25±0.07^b	3.75±0.07^b
J_6	6.22±0.05^c	5.25±0.05^c	5.13±0.004^c	4.84±0.12^c	4.5±0.68^c	4.43±0.13^c
J_9	8.057±0.09^d	6.07±0.06^d	5.24±0.06^d	5.04±0.05^d	6.03±0.08^d	6.36±0.1^d
J_{12}	8.89±0.33^e	7.87±0.02^e	7.94±0.03^e	6.69±0.11^e	6.92±0.03^e	7.17±0.04^e
CM	**5 – 6**	**3 - 4**	**1 - 3**	**-**	**-**	**3 – 4**

Table 4: Microbiological loads of the *Piliostigma thonningii* bark macerate (M_2)

	Microbial loads ($\log_{10}$FCU/mL)					
Day	**TAMF**	**TC**	**Th C.**	***E. coli***	**Stap**	**Molds**
J_0	3.34±0.05^a	2.39±0.08^a	2.18±0.14^a	1.96±0.12^a	1.72±0.28^a	1.98±0.09^a
J_3	5.28±0.06^b	4.21±0.09^b	4.1±0.18^b	3.58±0,76^b	2.7±0.13^b	3.61±0.46^b
J_6	6.55±0.14^c	5.21±0.18^c	5.13±0.15^c	4.74±0.21^c	4.23±0.1^c	4.35±0.12^c
J_9	8.01±0.01^d	6.87±0.13^d	5.67±0.11^d	5.56±0.17^d	6.62±0.03^d	6.029±0.04^d
J_{12}	8.51±0.1^e	7.78±0.14^e	7.63±0.14^e	6.71±0.2^e	6.83±0.15^e	7.42±0.15^e
CM	**5 – 6**	**3 - 4**	**1 - 3**	**-**	**-**	**3 – 4**

TAMF: Total Aerobic Mesophilic Flora; **TC:** Total Coliforms; **ThC:** Thermotolerant Coliforms; **Staph:** *Staphylococcus*; **Molds:** Molds; **MC:** Microbiological Criterion

In the same column, means sharing the same superscript letter are not significantly different at the 5% level ($p > 0.05$).

The isolated microorganisms included *Staphylococcus aureus*, coliforms (total coliforms, thermotolerant coliforms), *Escherichia coli*, and molds. Contamination levels exceeded the limits set by standards to ensure consumer safety. The presence of potentially pathogenic microbes at loads exceeding standard limits in the macerates could be attributed to primary plant contamination, poor hygienic conditions, and the quality of the water used. Previous studies evaluating bacterial loads in raw materials and herbal preparations have similarly indicated health risks associated with consuming plant-based medicinal remedies [18,19,20], in which *Escherichia coli*, *Salmonella* spp., and *Pseudomonas aeruginosa* were detected. According to these authors, the presence of coliforms confirms poor hygienic practices and unsuitable storage conditions. The presence of *S. aureus* poses a risk of staphylococcal gastroenteritis, as *S. aureus* is capable of secreting heat- and acid-stable enterotoxins that, upon ingestion, can cause nausea, vomiting, abdominal pain, and diarrhea [21]. In their study on the microbiological quality of medicinal plants, Opuni *et al*., [22] also confirmed contamination by *E. coli*, *Salmonella* spp., and *S. aureus*, attributing these contaminations to unhygienic practices spanning from harvest to distribution. Furthermore, the quality of water used in preparing medicinal plants may explain the high bacterial contamination observed in artisanal preparations. Potable water must be free of pathogenic microorganisms and bacterial indicators of fecal contamination [23].

3.3 Fungal profile of the macerates

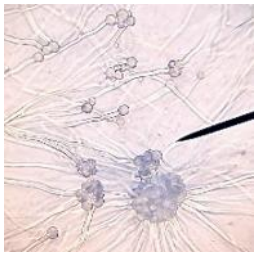
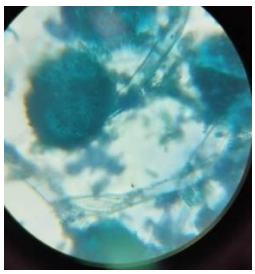
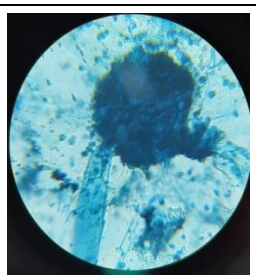
Two fungal species (*Aspergillus niger* and *Rhizopus* sp.) belonging to two genera were isolated from macerate M_1. In macerate M_2, *Acremonium* sp. and *Aspergillus niger* were isolated. Identification was performed using the identification keys proposed by Talaiekhozani and Ponraj [13] based on macroscopic and microscopic observations (**Table 5**).

Aspergillus niger colonies exhibited an olive-green color with lighter zones at the periphery, and their surface was velvety or granular. Under the microscope, aspergillar heads, vesicles, and conidiophores were observed.

Rhizopus sp. colonies were black to dark brown with a granular or powdery texture. Microscopically, numerous long, thin and branched filaments (hyphae) were observed, collectively forming the mycelium; spores were also noted.

Acremonium sp. colonies ranged from dark olive-green to blackish-gray at the centre, with a white peripheral border and a velvety to powdery texture. Microscopic observation revealed conidia clustered in globose heads at the apex of thin, elongated phialides.

Table 5: Phenotypic identification of molds isolated from the macerates

Macroscopic aspect (Front)	Microscopic aspect (GX : 40 ; 100)	Species
		*Acremonium* sp.
		*Rhizopus* sp.
		*Aspergillus niger*

Identification of the isolated molds confirmed contamination by *Aspergillus niger*, *Rhizopus* spp., and *Acremonium* spp. These fungal species are capable of secreting toxins harmful to consumer health. Similar studies have demonstrated the presence of fungal species known to produce mycotoxins, such as *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus* and *Aspergillus parasiticus* [24]. These species present a health risk when present in products intended for oral administration, as is the case for the macerates analyzed in this study.

4 CONCLUSION

Macerates of *Nauclea latifolia* and *Piliostigma thonningii* are used by the populations of Korhogo for their antimalarial properties. In this study, microbiological and physicochemical analyses were performed on macerates of these plants.

The results of the microbiological analyses confirm contamination by total and thermotolerant coliforms, *Escherichia coli*, *Staphylococcus aureus*, and molds such as *Aspergillus niger*, *Rhizopus* spp., and *Acremonium* spp. Contamination levels increased over time and exceeded established regulatory limits. *S. aureus*, *Aspergillus niger*, *Rhizopus* spp., and *Acremonium* spp. are potentially pathogenic and capable of secreting toxins harmful to consumer health.

The physicochemical parameters of the analyzed macerates varied throughout the storage period, with the values obtained influencing the development of the microbial flora and indicating a degradation of the water quality within the macerates. Ensuring the safety of these macerates is essential, particularly when consumed by young, elderly, or immunocompromised individuals.

This study provides a foundation for future research. Envisioned perspectives include standardizing storage parameters for these macerates and screening for mycotoxins. Furthermore, future work could expand to other types of medicinal plant preparations, such as decoctions and infusions.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Author Contributions

TRAORE Moumouny: Conceptualization, Formal Analysis, Writing original draft, Responsibility for the integrity of the published work **KOUAME N´zebo Desire**: Analysis and interpretation of data, writing the article; **SANOGO Seydou**: Investigation, Formal analysis. **YORO Thierry Dezay**: Investigation ; proofreading and editing the manuscript; **TOURE Abdoulaye**: Approval of the final version.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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