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Research Article

Physicochemical and microbiological characteristics of *Calotropis procera* Ait. root bark powders from 20 sites in Burkina Faso for standardization

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Abstract

Calotropis procera is a component of the phytomedicine FACA®, used to manage sickle cell disease in Burkina Faso. This work aimed to standardize plant powders by evaluating the physicochemical characteristics and microbial quality of *Calotropis procera* root bark powders from different sites, with properties similar to those of the reference harvest site, to optimize the production of the phytomedicine. The samples were collected from different geographical areas of Burkina Faso during the dry season. The population, physicochemical characteristics, and microbial quality of each powder were evaluated. Twenty (20) geolocated harvest sites were identified with various soil types (clay, silt, gravel, and sand). The availability of *Calotropis procera* was high in Ziniaré, Ouaga 1, and Boromo; average across 8 sites; and low across 9. Physicochemical analyses of the powders revealed general uniformity in pH and residual moisture content. However, differences were observed in color, bitterness, and hygroscopicity. Total phenolics could serve as tracers, given their content is comparable to that of the reference. All samples complied with the specifications of the European Pharmacopeia 11th edition for total aerobic bacteria, yeasts and molds, and *E. coli*. Pathogens such as *Salmonella* and *Staphylococcus aureus* have been found in some powders. This study identified the optimal sites for collecting *Calotropis procera* samples.

Keywords: Sickle cell disease; *Calotropis procera*; root bark; geographical variability.

INTRODUCTION

The use of medicinal plants for health purposes is a practice deeply rooted in African traditions. According to the World Health Organization (WHO), more than 80% of the sub-Saharan population still depends on this practice for their primary healthcare [1]. In Burkina Faso, this use, combined with socio-cultural factors, is largely facilitated by the richness of floral biodiversity, the affordability of natural remedies, and a strong network of traditional actors [2,3]. In the face of this increasing use, the transition to improved traditional medicines (ITMs) is emerging as a major public health policy strategy to enhance the value of local therapeutic heritage [4].

With this in mind, the WHO recommends that countries in the African region promote, improve, and integrate the use of reliable and effective traditional medicines to enhance the well-being of their populations [5]. However, the transition from raw plant to standardized ITM involves overcoming several complex technical challenges related to safety and efficiency. Indeed, producing an effective and safe ITM necessarily requires producing plant-based raw materials with consistent, controlled quality [6].

Environmental factors, including edaphic and climatic factors, influence the biochemical composition. Plant-derived raw materials are not fixed, as they vary with environmental factors such as soil type, altitude, rainfall, and solar exposure [7-9]. Consequently, plants of the same species, harvested in distinct geographical areas or phytogeographical domains, may exhibit divergent chemical profiles, both qualitatively and quantitatively, which significantly impact their pharmacological activity [10,11].

To ensure the reproducibility of the therapeutic effect and the safety of use, the standardization step is a scientific, industrial, and regulatory imperative [12]. In Burkina Faso, despite efforts to harmonize the African Pharmacopeia and WHO guidelines, many local plant powders still lack rigorous analytical data to assess uniformity across sites [13,14]. In this context, the present study on root bark powders of *Calotropis procera* from 20 sites in Burkina Faso is situated. This plant is one of the components of a phytomedicine called FACA®, which is indicated for the treatment of sickle cell disease [15]. However, no comparative study has been conducted on the physicochemical characteristics of root bark from various harvesting sites, with a view to diversifying the plant raw material's harvesting sites. This study, therefore, aims to identify sites with superior properties and/or potential for plant-based raw materials to optimize the production of the FACA® phytomedicine.

METHODOLOGY

Plant Materials

The plant material consisted of the roots of *Calotropis procera* collected from 20 different sites in Burkina Faso. A botanist and a specimen identified the plant material, which was deposited in the herbarium of the National Center for Scientific and Technological Research (CNRST) of Burkina Faso under number 6139. The harvest was conducted on healthy plants in ecosystems at geolocated sites with a low risk of contamination.

Three (3) adult multi-stemmed plants per site were sampled, with average diameters ranging from 4 to 16 cm and average heights from 0.75 to 5 m. The harvest consisted of locating and collecting a few lateral roots from the plants, depending on the soil type. The harvested root samples were cleaned under running water, then dried at room temperature, away from sunlight, in a well-ventilated room, before being ground into powder and packaged in freezer bags. The powder from the samples in the production unit served as a reference.

Methods

Identification of the species' populations

A survey was conducted across the central, western, southern, and northern parts of the country. The survey focused on the peripheral areas and city centers.

The assessment of the *Calotropis procera* population was conducted through direct observation of land-use patterns, including fields, areas around dwellings, and fallow land, which are considered preferred sites for the species. A four (4) level assessment scale was used according to several criteria and presented in Table 1.

Table 1: Scale for assessing the population size of the species

Scale	Criteria
High availability	Presence of several stands with good regeneration of the species
Medium availability	Colonial vegetation and the local abundance of the species
Low availability	Sporadic presence of individuals of the species
Rare	The species was not observed during the survey.

Physicochemical characteristics

Macroscopic and organoleptic characteristics

The macroscopic and organoleptic characteristics of the powders, namely color, odor, taste, and texture, were determined [16].

pH

The pH was determined using an electrode pH meter. To do this, 110 mg of each powder was weighed in triplicate and placed in Eppendorf tubes to which 11 mL of distilled water at 37 °C was added. After 2 minutes of homogenization and filtration of the mixture, the pH meter electrode was immersed in the filtrate to record the pH meter reading [16].

Residual moisture content (RMC)

The residual moisture content was determined by thermogravimetry. One gram of each powder was weighed in triplicate and placed in a previously dried and tared watch glass. The entire assembly was placed in an oven set at 105 °C for one (1) hours 30 min. After cooling, the assembly was weighed, and the RMC was calculated [16].

Hygroscopicity

The watch glasses (empty mass m_1) containing the samples after loss on desiccation (m_2) were placed in a desiccator at room temperature containing a saturated solution of ammonium chloride. After 24 hours, the entire volume was removed and weighed (m_3). The operation was performed in triplicate. The percentage increase in mass was determined [16].

Thin-Layer Chromatography (TLC) fingerprints

An ultrasonic ethanolic maceration (1/5, m/m) for 30 min was carried out on all powders to create the impression. The TLC fingerprint of the extracts was established according to the method described by Wagner and Balt [17]. A 5 µL volume of each ethanolic extract was deposited onto a chromatoplate (Silica G-60; F254; 10 cm × 5 cm; rigid aluminum support) using the automatic deposition system (Camag). The deposits were eluted over an 8-cm path in a glass tank that had previously contained a mobile phase consisting of ethyl acetate, methanol, and water (20:4:2.8; v/v/v). After elution, the chromatoplates were removed, dried at room temperature in the laboratory, and then in a ventilated oven (40 °C) for 5 min. The dried chromatoplates were observed under UV light at 254 nm and 365 nm.

Assay of phytochemical compounds of interest: Total phenolics

Total phenolic compounds were quantified according to the method of Singleton et al. [18]. One (1) ml of extract was mixed with 1 ml of FCR 2N and 3 ml of a 20% sodium carbonate solution. The mixture was left to rest for 40 minutes at room temperature. Absorbance was measured at 760 nm using a spectrophotometer. Total phenolic content was calculated, and tests were performed in triplicate.

Detection of microbial contaminants

The analysis parameters were enumeration of total viable aerobic germs and detection of specific germs. The samples were analyzed according to the methods described in the European Pharmacopeia, 11th edition [16]. The results obtained were interpreted in accordance with the specifications of the European Pharmacopeia, 11th edition, for herbal medicinal products containing medicinal plants, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (Table 2).

Table 2: Specification of European Pharmacopeia, 11th edition [16]

Target organisms	Specifications
Total viable aerobic bacteria (2.6.12)	$<10^7$CFU/g or CFU/mL
Yeasts and molds (2.6.12)	$<10^5$CFU/g or CFU/mL
Escherichia coli (2.6.31)	$<10^3$CFU/g or CFU/mL
Salmonella (2.6.31)	Absence
Staphylococcus aureus	Absence

Enumeration of total viable aerobic bacteria

The total viable aerobic microorganisms counted in the different powder samples were bacteria, molds, and yeasts.

Stock solutions for 1/10 of the samples were prepared by adding 10 mg of powder to 90 mL of buffered peptone water. The stock solutions were then diluted to 1/100, 1/1000, and 1/10,000. A 1 mL aliquot of the stock solutions and their dilutions was inoculated onto agar plates using the direct incorporation method, according to the target organisms. For aerobic bacteria enumeration, samples were inoculated onto tryptone-casein soy agar (TSA) and incubated at 30 °C for 3 to 5 days. For yeasts and molds, samples were inoculated onto Sabouraud agar and incubated at 25 °C for 5 to 7 days. At the end of the incubation periods, plates with fewer than 250 colonies (for bacteria) and 50 (for yeasts and molds) were selected for counting.

Search for specific germs.

The specified germs sought were *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*.

A 10 g test dose of powder was pre-enriched in 100 mL of tryptone-casein soy broth (TSB). Samples were incubated at 30°C for 24 h for *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*. A second enrichment was carried out in selective media from samples pre-enriched for *Salmonella* spp. and *E. coli*. For the enumeration of *Escherichia coli*, 1 mL of TSB was enriched in 100 mL of MacConkey broth (MCB) at 44 °C for 48 h. For *Salmonella*, 0.1 mL of TSB was enriched in 10 mL of Rappaport-Vassiliadis (RV) at 30 °C for 24 h. The enriched samples were streaked onto media specific to the germs sought (Table 3).

Table 3: Culture media and inoculation conditions

Target organisms	Isolation media	Incubation conditions
Escherichia coli	MacConkey agar	30°C for 24 to 48 hours
Salmonella	Xylose, lysine, deoxycholate (XLD)	30°C for 24 to 48 hours
Staphylococcus aureus	Chapman agar	30°C for 24 to 48 hours

Statistical analysis

The data were entered into Excel 2016. The results were expressed as the mean of three replicates with the standard deviation. They were analyzed using GraphPad Prism 5 software; the one-way ANOVA statistical test was used to compare the powders. Series are considered significant when the probability of error (p) is less than the accepted risk: 0.05 ($p<0.05$).

RESULTS

Identification of the species' populations

The survey identified several sampling areas (Table 3) in the localities of Ouagadougou, Loumbila, and Ziniaré in the central part of the country; Boromo, Houndé, Bobo-Dioulasso, and Banfora in the western part of the country; Kombissiri, Manga, Nobéré, and Saponé in the southern part of the country; and Yako in the northern part of the country. There were twenty (20) geolocated sites (Figure 1, Table 4).

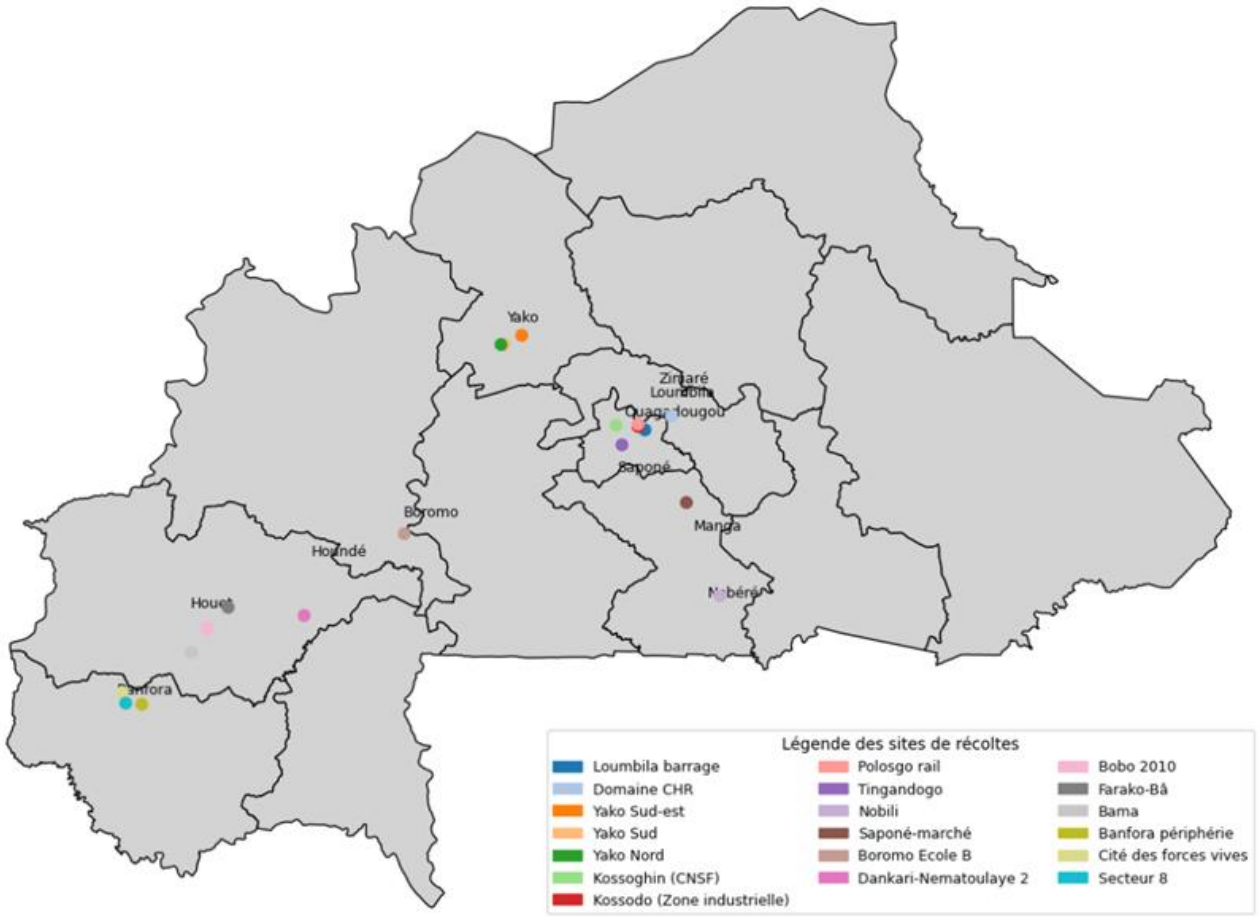

Figure 1: Map of Burkina Faso showing the various *Calotropis procera* harvesting sites

Table 4: Geolocation of sites and assessment of *C. procera* populations

N°	Sampling area	Geolocated sites	GPS coordinates	Availability	Soil texture
1	Yako 1	Yako Southeast	N12°55'40.8''; W002°14'43.0''	Low	Clayey
2	Yako 2	Yako South	N12°55'50.0''; W002°15'22.9''	Low	Clayey
3	Yako 3	Yako North	N12°55'53.9''; W002°15'24.0''	Low	Gravel pit
4	Ouagadougou 1	Kossoghin (CNSF)	N12°24'35.7''; W001°33'24.1''	High	Clay-loam
5	Ouagadougou 2	Kossodo (industrial zone)	N12°26'27.9''; W001°28'34.3''	Average	Clayey-sandy
6	Ouagadougou 3	Pologsorail	N12°26'36.7''; W001°29'58.6''	Average	Gravel pit
7	Ouagadougou 4	Tingandogo	N12°16'24.9''; W001°31'43.2''	Average	Gravel pit
8	Loumbila	Loumbila dam	N12°29'11.4'' ; W001°24'38.1''	Average	Gravel pit
9	Ziniare	CHR domain	N12°35'13.4''; W001°10'41.2''	High	Clayey-sandy

N°	Sampling area	Geolocated sites	GPS coordinates	Availability	Soil texture
10	Manga	Cité Forces vives	N11°41'05.3''; W001°05'26.5''	Low	Gravel pit
11	Nobéré	Nobili	N11°42'49.9'' ; W001°16'45.3''	Low	Clayey
12	Sapone	Saponé-market	N12°04'02.3'' ; W001°33'20.7''	Low	Clayey
13	Boromo	Boromo School B	N11°45'45.8'' ; W002°55'37.8''	High	Silty-clay
14	Hounde	Dankari-Nematoulaye 2	N11°18'30.8'' ; W003°38'39.5''	Low	Gravel pit
15	Bobo-Dioulasso 1	Bobo 2010	N11°13'48.3'' ; W004°18'35.5''	Average	Sandy
16	Bobo-Dioulasso 2	Farako-Bâ	N11°05'29.9'' ; W004°20'51.3''	Low	Clayey
17	Bobo-Dioulasso 3	Bama	N11°22'20.6'' ; W004°22'55.1''	Low	Clayey
18	Banfora 1	Banfora outskirts	N10°41'06.5'' ; W004°43'53.1''	Average	Sandy
19	Banfora 2	City of living forces	N10°40'32.7'' ; W004°43'59.0''	Average	Sandy
20	Banfora 3	Sector 8	N10°38'53.0'' ; W004°45'41.8''	Average	Sandy

*** Ouagadougou : Ouaga, Bobo-Dioulasso: Bobo**

The results show 9 sites with low availability on clay and gravel soils. Plants at these sites were found sporadically in areas such as fallow land, fields, and around dwellings and abandoned houses. There were 8 sites with average availability on sandy, clayey-sandy, and gravelly soils. These sites were characterized by locally abundant colonies of the species. Three (3) sites, namely Ziniaré, Ouaga 1, and Boromo, showed high availability on clayey-sandy, clayey-silty, and silty-clay soils. These sites were distinguished by a high population density (up to 10 individuals/1000 m^2). They show good regeneration in *C. procera*, as evidenced by numerous juveniles.

Physicochemical characteristics

Macroscopic and organoleptic characteristics

The powders were coarse in appearance and had an uncharacteristic odor. The color and taste of the different *Calotropis procera* powders varied by site (Table 5).

Table 5: Color and taste of different *Calotropis procera* powders.

Samples	Color	Taste
Yako 1	Pale brown	Bitter
Yako 2	Pale brown	Very bitter
Yako 3	Pale brown	Bitter
Ouagadougou 1	Pale brown	Slightly bitter
Ouagadougou 2	Brownish white	Bitter
Ouagadougou 3	Brownish white	Bitter
Ouagadougou 4	Pale brown	Slightly bitter
Loumbila	Brownish white	Slightly bitter
Ziniaré	Brownish white	Slightly bitter
Manga	Pale brown	Bitter
Nobéré	Brownish white	Slightly bitter
Saponé	Pale brown	Very bitter
Boromo	Pale brown	Very bitter
Houndé	Brownish white	Very bitter
Bobo-Dioulasso 1	Pale brown	Bitter
Bobo-Dioulasso 2	Pale brown	Very bitter
Bobo-Dioulasso 3	Pale brown	Bitter
Banfora 1	Pale brown	Bitter
Banfora 2	Pale brown	Slightly bitter
Banfora 3	Pale brown	Slightly bitter
Reference	Pale brown	Slightly bitter

Depending on the site, the powders were pale brown for 14 sites and brownish white for 6 sites, namely Ouaga 2, Ouaga 3, Loumbila, Ziniaré, Nobili, and Houndé. The powders from the 14 sites were the same color as the reference, pale brown.

The powders had a bitter taste ranging from slightly to very bitter. The taste was slightly bitter for the powders from the sites of Ouaga 1, Ouaga 4, Loumbila, Ziniaré, Nobéré, Banfora 3, and the reference, bitter for those from the sites of Yako 1, Yako 3, Ouaga 2, Ouaga 3, Manga, Bobo 1, Bobo 3, Banfora 1, Banfora 2, and very bitter for those from the sites of Yako 2, Saponé, Boromo, Houndé, and Bobo 2.

Residual moisture content (RMC)

The residual moisture levels of the different powders are shown in Table 6.

Table 6: RMC of the different powders from the harvesting sites

Samples	RMC (%) of Powders
Yako 1	3.32±0.07 ***
Yako 2	6.42±0.12***
Yako 3	6.06±0.5*
Ouagadougou 1	5.13±0.21 ns
Ouagadougou 2	4.98±0.88 ns
Ouagadougou 3	6.94±0.28***
Ouagadougou 4	6.19±0.19**
Loumbila	6.36±0.09***
Ziniaré	5.61±0.23 ns
Manga	5.24±0.28 ns
Nobéré	6.61±0.23***
Saponé	7.36±0.44***
Boromo	5.62±0.08 ns
Houndé	5.57±0.15 ns
Bobo-Dioulasso 1	5.20±0.20 ns
Bobo-Dioulasso 2	5.54±0.62 ns
Bobo-Dioulasso 3	4.95±0.13 ns
Banfora 1	4.92±0.34 ns
Banfora 2	4.59±0.13 ns
Banfora 3	4.81 ± 0.44 ns
Reference	4.99 ± 0.55

ns: no significant; ***: significant

The residual moisture content (RMC) of the powders ranged from 3.32 ± 0.07% (Yako 1) to 7.36 ± 0.44% (Saponé), with an average of 5.54 ± 0.70%. These results show a slight variation in RMC within the powders. After analysis, the RMC of most sites (12) did not differ significantly from that of the reference, except for Yako 1, Yako 2, Yako 3, Ouaga 3, Ouaga 4, Loumbila, Nobili, and Saponé, which showed significant differences.

pH

The pH values of the powders are presented in Table 7.

Table 7: pH of the different powders from the harvest sites

Samples	pH of Powders
Yako 1	7.07 ±0.01 ns
Yako 2	6.98 ±0.02 ***
Yako 3	6.76 ±0.02 ***
Ouagadougou 1	6.98 ±0.01 ***
Ouagadougou 2	7.01 ±0.02 ***
Ouagadougou 3	6.95 ±0.02 ***
Ouagadougou 4	7.07 ±0.04 ns
Loumbila	7.25 ±0.02 ***
Ziniaré	7.27 ±0.02 ***
Manga	6.92 ±0.01 ***
Nobéré	7.05 ±0.02 ns
Saponé	6.92±0.01***
Boromo	6.69±0.01***
Houndé	6.62±0.03***
Bobo-Dioulasso 1	6.61±0.02***
Bobo-Dioulasso 2	6.48±0.01***
Bobo-Dioulasso 3	6.59±0.04***
Banfora 1	6.48±0.02***
Banfora 2	6.59±0.02***
Banfora 3	6.66±0.04***
Reference	7.1±0.01

ns: no significant; ***: significant

The pH values of the powders from the different sites ranged from 6.48±0.01 (Bobo 2, Banfora 1) to 7.27±0.02 (Ziniaré). The pH levels at the Boromo, Houndé, Bobo 1, 2, and 3, and Banfora 1, 2, and 3 sites ranged from 6.48±0.02 to 6.69±0.01. Except for Yako 3 (6.76±0.02), the pH values of Yako 1 and 2, Ouaga 1, 2, 3, and 4, Manga, Nobili, and Saponé ranged from 6.92±0.01 to 7.07±0.04. The powders from Ziniaré (7.27 ± 0.02) and Loumbila (7.25 ± 0.02) showed similar pH values. No significant difference ($P < 0.05$) was observed in the pH of the Yako 1 (7.07 ± 0.01), Ouaga 4 (7.07 ± 0.04), and Nobili (7.05 ± 0.02) sites compared to the reference pH (7.1 ± 0.01).

Hygroscopicity

The hygroscopicity values of the powders are given in Table 8.

Table 8: Hygroscopicity of powders from different harvesting sites

Samples	Hygroscopicity Value	Hygroscopicity of the powder
Yako 1	14.87±0.45**	Hygroscopic
Yako 2	10.79±0.54***	Hygroscopic
Yako 3	12.12±0.79***	Hygroscopic
Ouagadougou 1	10.05±0.75***	Hygroscopic
Ouagadougou 2	10.61±0.45***	Hygroscopic
Ouagadougou 3	12.39±0.87***	Hygroscopic
Ouagadougou 4	17.89±0.60**	Very hygroscopic
Loumbila	11.75±0.26***	Hygroscopic
Ziniaré	10.73±0.01***	Hygroscopic
Manga	16.21±0.50 ns	Very hygroscopic
Nobéré	13.46±0.35***	Hygroscopic
Saponé	13±0.55***	Hygroscopic
Boromo	13.19±0.50***	Hygroscopic
Houndé	12.68±0.17***	Hygroscopic
Bobo-Dioulasso 1	11.39±0.19***	Hygroscopic
Bobo-Dioulasso 2	10.41±0.38***	Hygroscopic
Bobo-Dioulasso 3	10.86±0.27***	Hygroscopic
Banfora 1	16.56±0.82 ns	Very hygroscopic
Banfora 2	15.04±0.36*	Very hygroscopic
Banfora 3	10.25±0.04***	Hygroscopic
Reference	16.37±0.16	Very hygroscopic

ns: no significant; ***: significant

The evaluation of the hygroscopicity of the different *Calotropis procera* powders showed values ranging from 10.05±0.75 at the Ouaga 1 site to 16.56±0.82 at the Banfora 1 site. These values indicated that the mass increases were less than 15% and greater than 2% for some powders, making them hygroscopic, and greater than or equal to 15% for others, making them very hygroscopic. No significant difference was observed in the hygroscopicity values of the powders from the Manga and Banfora 2 sites compared to the reference.

Chromatographic fingerprint

The results are shown in Figure 2 and Table 9, in the same order as for Yako 1 (reference number 21).

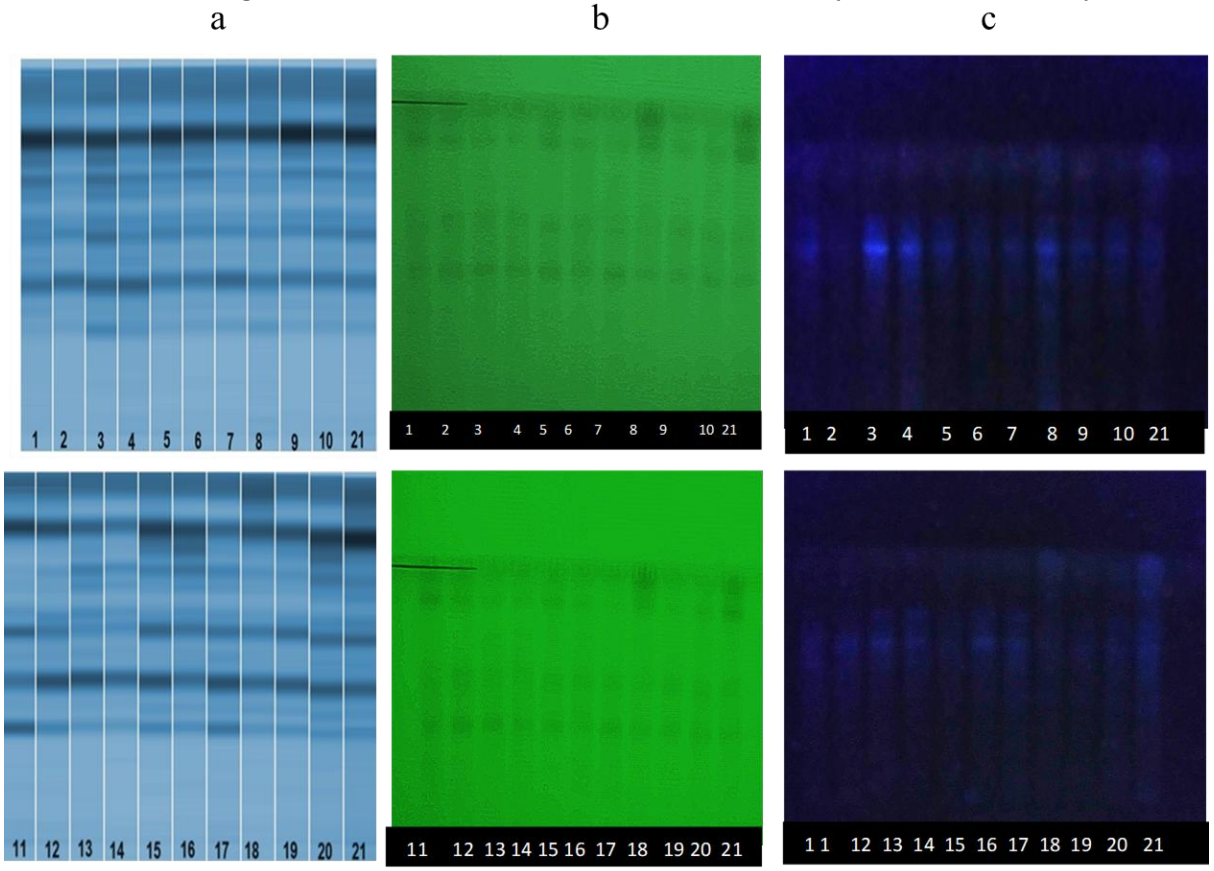

Figure 1: Chromatographic fingerprints of the different powders. a) plates scanned at 225 nm; b) plates observed at 255 nm; c) plates observed at 366 nm.

Table 9: Frontal reference (FR) of major fingerprint spots

Samples	FR at 225nm
Nobili	0.32
Yako 1 Yako 3 Ouaga 1	0.41
Yako 2 Ouaga 2 Loumbila Manga	0.42
Ouaga 3 Ouaga 4 Ziniaré Banfora 3 Référence	0.43
Bobo 3 Banfora 1 Banfora 2	0.45
Nobili Saponé Boromo Bobo 1 Bobo 2	0.46
Référence	0.57
Nobili Bobo 3 Banfora 1	0.59
Saponé Bobo 1 Bobo 2	0.60
Yako 1	0.82
Yako 2 Yako 3	
Ouaga 1 Ouaga 2 Ouaga 3 Loumbila	0.83
Ouaga 4 Ziniaré Manga	0.84
Référence	0.85
Banfora 2	0.87
Saponé Bobo 2 Bobo 3 Banfora 1	0.88
Nobili Boromo Houndé Bobo 1	0.89
Yako 2	0.93

Frontal reference (FR) values range from 0.32 to 0.93. Samples from sites such as Ouaga 3, Ouaga 4, Ziniaré, and Banfora 3 had the same FR = 0.43 as the reference. The powders from the 19 sites, as well as the reference, had FR values between 0.41 and 0.46, and those from 20 sites had Rf values between 0.82 and 0.93. The powders from the Nobili, Bobo 3, Banfora 1, Saponé, Bobo 1, and Bobo 2 sites, as well as the reference, had specific FR values between 0.57 and 0.60.

Total phenolic content

Figure 3 shows the total phenolic content expressed as tannic acid equivalent in the root bark powders of *Calotropis procera*.

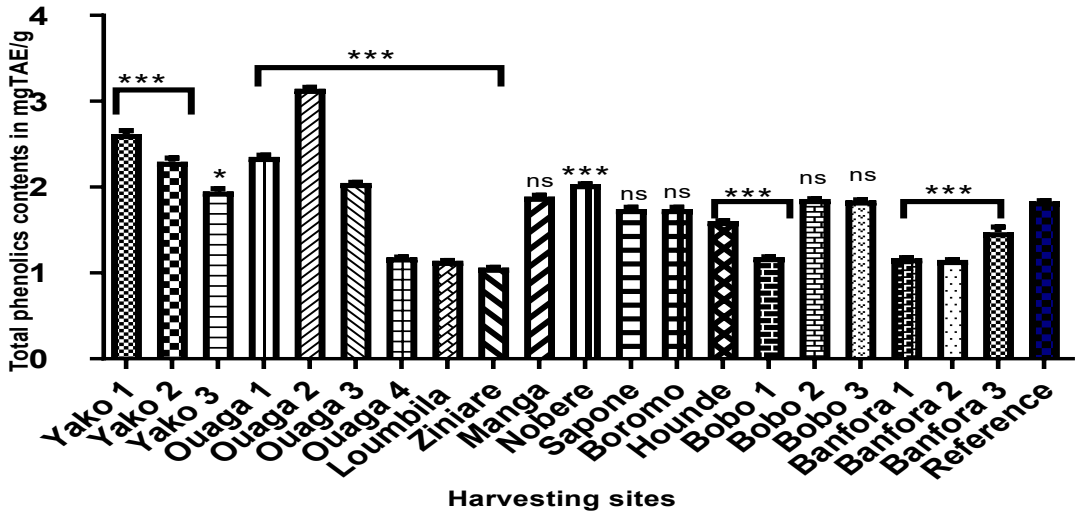

Figure 3: Total phenolic content of *C. procera* root bark powders
ns : no significant ; * and *** : significant compared to the reference

Total phenolic content ranged from 1.06±0.05 mgEAT/g in Ziniaré to 3.14±0.03 mgEAT/g in the Ouaga 2 powder. Statistical analysis showed no significant difference between the reference powders (1.85±0.01 mgEAT/g) and the sites of Manga (1.89±0.03 mgEAT/g), Saponé (1.74±0.02 mgEAT/g), Boromo (1.74±0.03 mgEAT/g), Bobo 2 (1.86±0.01 mgEAT/g), and Bobo 3 (1.85±0.01 mgEAT/g).

Microbiological quality

The results are presented in Table 10, and the specifications are taken from the European Pharmacopoeia, 11th edition.

Table 10: Microbial quality results

Samples	**Total aerobic bacteria**	**Specific germs**			
	Bacteria **Specification ≤10^7 CFU/g** **Max: 50,000,000 CFU/g**	**Yeasts and molds** **Specification: ≤10^5 CFU/g** **Max: 500,000 CFU/g**	***E. coli*** **Specification ≤10^3 UFC /g**	***Staphylococcus aureus,***	***Salmonella***
Yako 1	2270000	15135	-	-	-
Yako 2	4520000	8400	-	-	+
Yako 3	1910000	22450	-	-	-
Ouagadougou 1	2600000	66000	-	-	-
Ouagadougou 2	336400	9700	-	-	+
Ouagadougou 3	744000	1380	-	-	+
Ouagadougou 4	2500000	117100	-	-	-
Loumbila	1418000	170000	-	-	+
Ziniaré	480000	230000	-	-	+
Manga	1809000	55100	-	+	-
Nobéré	748000	23230	-	+	+
Saponé	347000	31000	-	-	-
Boromo	1393000	810	-	-	-
Houndé	2413000	26300	-	-	-
Bobo-Dioulasso 1	375150	70	-	-	-
Bobo-Dioulasso 2	246000	240	-	-	+
Bobo-Dioulasso 3	872200	980	-	-	+
Banfora 1	717000	5080	-	-	-
Banfora 2	921000	2440	-	-	-
Banfora 3	1393000	280	-	-	-
Reference	2200000	2529	-	-	+

+ : present ; - : absent

Aerobic bacteria were present at levels below the specifications of the European Pharmacopoeia 11th edition. The aerobic germ count of the reference powders (2,200,000 CFU/g) was lower than that of the powders from Yako 1, Yako 2, Ouaga 1, Ouaga 4, and Houndé, which ranged from 2,270,000 to 4,520,000 CFU/g.

Yeasts and molds, on the other hand, were present at levels below the European Pharmacopoeia limits at all sites. The reference value (2,529 CFU/g) was lower than those for Yako 2 and 3, Ouaga 1, 2, and 4, Loumbila, Ziniaré, Manga, Saponé, Houndé, and Banfora 1, which ranged from 5,080 to 230,000 CFU/g.

Salmonella was detected at the Yako 2, Ouaga 2 and 3, Loumbila, Ziniaré, Nobili, Bobo 2, Bobo 3, and reference sites. *Staphylococcus aureus* was also detected at the Manga and Nobéré sites. *E. coli* was not identified in any of the samples.

DISCUSSION

Identification of the species' populations

The study showed that *Calotropis procera* is relatively widespread in Burkina Faso, with populations found on various soil types (clay, silt, gravel, sand, clay-silt, and silty clay). Indeed, Calotropis procera can adapt to harsh environmental conditions (drought, high salinity, extreme temperatures, low air humidity, etc.) and grows on several soil types, including dry, sandy, and alkaline soils [19, 20]. This characteristic would be essential for the conservation and sustainable management of the plant as a plant resource for production.

However, the results indicate that its availability remains low in nine (09) areas, particularly in the West of the country (Houndé, Bobo 2, Bobo 3), in the South of the country (Saponé, Nobéré, Manga), and in the North of the country (Yako 1, Yako 2, and Yako 3), especially around roads and in inhabited areas. This low availability could be explained by the soil in these areas, which is predominantly clayey (6 sites) and gravelly (3 sites). Indeed, *Calotropis procera* thrives easily on soils that are very poor in certain nutrients and very dry. In contrast, clay soil is nutrient-rich and retains water strongly, with no drainage outlet, which could explain its low availability on this soil. Furthermore, its frequent use by local populations for medicinal and artisanal purposes could also explain this low availability [21]. Indeed, according to Niang, the *Calotropis procera* species is used by the surrounding populations for health and construction purposes [22].

Its availability was average across the sites, namely Loumbila, Ouaga 2, Ouaga 3, Ouaga 4, Bobo 1, and Banfora 1, 2, and 3. This could be related to the soil types, which are sandy and gravelly. Indeed, *Calotropis procera* grows well in sandy soils [23], which could explain its frequent presence in these soils, according to Gillet and Seignobos [23, 24]. Its low to moderate availability in gravelly soils could be explained by its strong capacity to adapt to different soil types 21.

The study shows a high availability at the sites of Ziniaré, Ouaga 1, and Boromo. These 3 sites were located on clayey-sandy, clayey-silty, and silty-clay soils. This high availability at these 3 sites could be explained by the presence of sand in these soils (depending on its proportion) and by the season (not winter) at harvest. Indeed, it would grow abundantly under xerophytic conditions on a variety of soils, without irrigation or fertilizer, while adapting to road pollutants and contaminated soils [21, 25]. These sites would present favorable advantages for the supply of plant matter, depending on the harvest season (for silty-clay soils). Generally speaking, areas with average availability would serve as an alternative as long as the plant proliferates more on that soil [25]

Physicochemical characteristics

The determination of macroscopic and organoleptic characteristics revealed that all the root bark powders of *Calotropis procera* were white-brown to pale brown in color, with an undefined odor, a bitter taste, and a coarse texture. These results were similar to those obtained by Tripathi et al. in their pharmacognostic study of the plant's roots [26]. However, powders from 14 sites had the same color as the reference powder, namely pale brown. This slight color variation observed in the powders could be related to edaphic conditions and soil types [27]. The brownish-white powders appeared more frequently on sites with gravelly and sandy-clay soils, which, due to their texture and composition, can affect the color of the plants growing there [28]. The pale brown powders appeared on a wider range of soil types. These colors could indicate a soil chemical composition richer in iron oxides [29]. The nature and quality of the soil influence the organoleptic characteristics of plants [28], which could explain this slight variation in color between the different sites.

The powders from 6 sites tasted slightly bitter, as did the reference; those from 9 sites were bitter, and those from 5 sites were very bitter. This bitter taste of the powders could be due to secondary metabolites present in the plant, including tannins, alkaloids, and terpenoids [31], which are known for their bitterness [30, 31]. Differences in phytochemical compound levels could explain the diversity of bitter tastes observed at different sites [32]. Indeed, edaphic conditions such as temperature, latitude, altitude, soil composition, and climate influence the physicochemical characteristics of plants, making them veritable reservoirs of genetic specificities [6, 33].

The highest residual moisture content (RMC) of the powders was 7.36±0.44% (Saponé). Levels below 10% indicate that the powders could be stored with a lower risk of spoilage and microbial growth [34]. The RMC of the reference powder was 4.99 ± 0.55 and was not statistically different from powders from 12 sites.

The pH values of the different *C. procera* powders were between 6.48± 0.02 (Bobo 2, Banfora 1) and 7.27± 0.02 (Ziniaré). The pH values were between 6.48 ± 0.02 and 6.69 ± 0.01 for the powders from the Western part (Boromo to Banfora), and between 6.92 ± 0.01 and 7.07 ± 0.04 for the Northern, Southern, and Central parts (Yako, Ouagadougou, Saponé, and Manga) (including the reference). The powders from Loumbila and Ziniaré had a pH of 7.25 ± 0.02 to 7.27 ± 0.02. This variation in pH observed in the powders may be due to the influence of geographic regions on different soil types rather than to the nature of the soils themselves. Indeed, plant pH depends on soil nutrient availability and on how soil pH influences nutrient absorption by plants [35]. Depending on soil pH, the plant balances its root surface pH to adapt to the environmental conditions in which it grows [35]. This could explain the weak-acid or weak-base character of *Calotropis procera* powders collected in different geographical areas.

All powders were hygroscopic, and data analysis reveals notable variation in hygroscopicity across sites. The

values were from 10.05±0.75% (Ouaga 1) to 16.56±0.82% (Banfora 1). Despite being harvested from different sites, all the powders were hygroscopic. The powders from the Manga and Banfora 1 sites, as well as the reference powder, were very hygroscopic. This could be attributed to environmental factors [36]. Furthermore, this hygroscopic characteristic could be attributed to secondary metabolites with glycosidic rings bearing hydroxyl groups, which can interact with water molecules in the air [20, 22, 38]. Indeed, highly hygroscopic powders are more prone to absorbing ambient moisture, which affects their preservation [37]. These powders require storage under controlled conditions, particularly in hermetically sealed containers [16].

Chromatographic analysis revealed 3 large spots with the reference powder, including 2 spots similar to the spots of the powders from all 20 sites of the study, with reference fronts (RF) between 0.41 and 0.46 and between 0.82 and 0.93. The third spot, with RF values between 0.57 and 0.60, is similar to the spots of the reference powders and to 6 sites (Nobéré, Banfora 1, Saponé, Bobo 1, 2, and 3). This conformity of the RF spots of the powders from different sites to the reference could reflect a similarity among the compounds present in these plant powders [8]. The chromatographic fingerprint is a chemical identity corresponding to the search for constituents present in plant drugs (6), and its analysis would enable their authentication by identifying the chemical groups that characterize them [26].

The choice of total phenolics as compounds of interest (chemo-taxonomic markers) in plant root bark could be explained by quality control and environmental stress tests [38]. The total phenolic content of the powders from the different sites ranged from 1.06±0.05 mgEAT/g to 3.14±0.03 mgEAT/g. There was a statistically significant difference between the reference levels and those of the 15 sites, except at Manga, Saponé, Boromo, and Bobo 2 and 3, where no significant difference was observed. The phenolic compounds identified in the roots of *Calotropis procera* are known for their pharmacological activities [19]. Thus, these compounds could serve as bioactive markers in the standardization of powders from different harvesting sites [39]. Their content could be a determining factor in site selection.

Microbial quality

The microbial quality of *Calotropis procera* powders from different sites shows high variability in contamination levels. The total aerobic bacterial count in *Calotropis procera* powders was below the threshold of 5 x 10^7 CFU/g specified in the 11th edition of the Pharmacopeia [16]. Samples from 5 sites presented microbial loads higher than those of the reference. This high level of contamination could be explained by varying degrees of environmental pollution across regions [40, 41]. Some agricultural or domestic activities may take place near sites with high levels of contamination [42].

All powders from the different sites, including the reference, complied with the maximum threshold set by the European Pharmacopeia, 11th edition, for molds and yeasts, which is 5.105 CFU/g. Powders from 11 sites have values higher than the reference value (2529 CFU/g). The presence of molds and yeasts at varying levels could be linked to high humidity, high temperatures, or other conditions conducive to fungal growth [43].

Escherichia coli was not present in any of the powders from the different sites. This result could be explained by the fact that the collection sites had not been recently exposed to fecal matter. Indeed, the presence of *E. coli* suggests possible recent fecal contamination, with a risk of developing more virulent strains [44].

Salmonella was found in several samples, including those from Yako 2, Ouaga 2, Ziniaré, and the reference sample. The presence of *Salmonella* in these samples could be explained by probable contamination of the sites by contaminated water or by animals [45]. Indeed, these sites, which are often frequented, could be particularly vulnerable to this type of contamination.

Staphylococcus aureus was identified in the Manga and Nobéré samples. The presence of *S. aureus* at both sites in the same area could indicate contamination linked to poor hygiene during harvesting or processing, since this bacterium is naturally present on the skin and mucous membranes (nose, throat) of humans and animals [46,47].

Powders from different sites with varying levels of contamination could be used if pretreatment, including contaminant reduction or removal, is considered. Indeed, these processes enable the reduction or elimination of contaminants in plant powders [48,49].

CONCLUSION

This study enabled evaluation of the availability of *Calotropis procera* at various harvesting sites, as well as its physicochemical characteristics and microbiological quality. Individuals of *Calotropis procera* were poorly available at 9 sites with 2 soil types (gravelly and clayey), moderately available at 8 sites with 3 soil types (gravelly, sandy, and clayey-sandy), and highly available at 3 sites, namely Ziniaré, Ouaga 1, and Boromo, with 3 soil types (clayey-sandy, clayey-silty, and silty-clay). Regarding the physicochemical characteristics, the color of the powders ranged from pale brown to brownish white; the taste ranged from bitter to very bitter; the pH ranged from 6.48 to 7.27; and the RMC was less than 10. The powders ranged from hygroscopic to very hygroscopic. The TLCs showed spots exhibiting Rf values similar to those of the reference—total phenolic content varied from site to site. For microbial control, none of the powders contained *E. coli*. All powder samples from the sites were compliant for total aerobic bacteria, yeasts, and molds; 2 sites had powders positive for *S. aureus*, and 9 sites had powders positive for *Salmonella*. Based on criteria such as availability and physico-chemical and microbiological characteristics of powders from different sites, favorable sites for the supply of *Calotropis procera* have been identified. The sites of Ziniaré, Ouaga 1, and Boromo are distinguished

by their abundant stands of *Calotropis procera* and favorable edaphic conditions (clay-sandy or silty-clay soils rich in essential minerals). In addition, the Loumbila and Ouaga 2 sites offer secondary supply opportunities, although they also require microbial quality monitoring.

Conflicts of interest: The authors declare no conflicts of interest.

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