Micrography, phyto-chemical screening and physico-chemical properties of Bridelia micrantha (Hochst.) Baill. and Persea americana L. stem bark

Joseph Bertin Alexis Zoa Bindzi, Rose Ngo Ngono Mballa Abondo, Joseph Ngoupayo and Beyeme Fridoline Victorine

Abstract
Traditional African Medicine in general and Cameroonian in particular use medicinal plant recipes to treat various pathologies. In order to improve the safety and the progressive integration of those plants in our health systems, it is important to set protocol that would permit an easy characterization of medicinal plants. This study is interested in a recipe of Bridelia micrantha and Persea americana from the trunk bark, traditionally used in the center region of Cameroon to treat “hemorrhoids”. Thus, we develop the macrography, the micrography, the phytochemical screening, the water content and the total ash content percentages of the stem bark of each plant. The water contents in P. americana and B. micrantha are 7.82±1% and 10.16±% respectively. And total ash content are 3.36±1% and 4.25±1% for P. americana and B. micrantha respectively. Micrography of truncated P. americana bark showed: cells containing crystals of calcium oxalate and spindle sclerified fibers. That from B. micrantha revealed: fibers and assesses cells containing crystals of calcium oxalate, wooden vessels, and a red parenchyma cells. Phytochemical analysis revealed the presence in our recipe of polyphenolic substances such as flavonoids. The information gathered throughout this study will contribute to the identification and characterization of P. americana and B. micrantha stem bark.

Keywords: Bridelia micrantha, Persea americana, stem bark, identification.

Introduction
Persea americana Mill. (avocado) is a tree, native to central America, cultivated in tropical and subtropical climates around the world, belonging to the family Lauraceae, is widely used in traditional medicine for the treatment of various ailments, such as monorrhagia, hypertension, stomach ache, bronchitis, diarrhea, and diabetes [1]. The genus Bridelia includes approximately 60–70 species found throughout tropical and subtropical regions of the world, particularly Africa and Asia [2]. Bridelia micrantha is traditionally used in tropical Africa to treat a wide range of human and animal diseases including conjunctivitis, painful eyes, constipation, gastric ulcers, cough, headache, rheumatism, painful joints, dysentery, ethno veterinary medicine, malaria, sexually transmitted infections, stomach ache, tape worms and diarrhea. Different plant parts, aqueous and organic extracts exhibited antihelminthic, antimicrobial and β-lactamase inhibitory activities, antidiabetic, antidiarrheal, antioxidant, and hepatoprotective activities [3]. According to the World Health Organization (WHO), 80% of the rural population in Africa use traditional medicine in primary health care, due to its affordability and the local presence of herbal medicines [4]. WHO guidelines on standardization of herbal medicine provide among other things, the macroscopic and microscopic descriptions, phytochemical screening and physico-chemical properties of the parts of the plant to be standardized [5]. After harvesting, the trunk barks were dried, crushed and pulverized. The recipe of the traditional healer recommends that we make a decoction of the mixture of powders obtained in equal proportions. After decoction, the recipe is used to treat “hemorrhoids”. We are interested in this recipe because of its constant use by the population. We already wanted to describe the plants present in this recipe and facilitate their scientific identification.

Materials and methods
As plant material we have Persea americana and Bridelia micrantha stem bark harvested on January 2, 2016, at Etilbibegue, located at almost 33 km from the Yaoundé city center. Following the harvesting, a systematic identification of the specimens was made.
at the National Herbarium of Cameroon by a botanist. The identification was made in comparison with reference numbers 57756/HNC and 19699/HNC for Persea americana et Bridelia micrantha respectively. The macroscopic description of stem bark was made after identification.

Macroscopic description
Whole barks (figure 1) were examined and manipulated to identify the internal and external colors, the flavor, the fissure plan and odour feeling [6].

Preparation of aqueous extracts
The trunk barks were crushed and then dried in an ambient environment for a week. The dry bark was pulverized and part of the powder obtained was used for microscopic investigations following the process described by Blond and collaborators [7]. A powder mass of 25 g was used for the extract, using a Behr Labor-Technik branded Soxhlet to mimic the decoction made by the traditional healer. The extract obtained from this extraction was used for phytochemical screening according to Safowara and collaborators described protocol [8].

Microscopy
Place a drop of 5% potassium hydroxide (KOH) on a slide. With the tip of a spatula, take a small amount of powder and mix it in water, on the blade, until the powder is wet. Cover with a coverslip by pressing lightly with your finger. Observe under the light microscope, starting with the lowest objective. Draw or film the structures observed.

Phytochemical screening
Anthraquinone heterosides test (Borntrager test)
0.5 g of each extract is placed in 5 mL of its extraction solvent for 5 min. The mixture is filtered and an equal volume of 10% ammonia is added to the filtrate. A pink, red or purple color indicates the presence of free anthraquinones [9].

Saponin test
0.5 g of each extract is placed in a test tube. 10 ml of distilled water are added thereto and the mixture is shaken vigorously for 30 seconds and left to stand. The presence of a foam height greater than 1 cm for more than 30 minutes indicates the presence of saponins [8].

Flavonoids test
0.2 g of each extract is placed in two test tubes, 1 to 2 ml of hot 50% methanol are poured into each of the tubes until dissolved; then we filter the mixture. Metallic magnesium was added followed by 4-5 drops of concentrated HCl to the filtrate. The formation of incipient H₂ reacts with the flavonoids to give an orange-red coloration [11].

Alkaloids test
0.2 g of each extract is introduced into a test tube. 3 ml of hexane are added (shake, allow to cool and filter), 5 ml of 2% HCl were poured into the filtrate and the whole was heated so that there was mixing. After mixing, filter; Adding a few drops of picric acid to the above filtrate, causing a yellow precipitate to form, indicates the presence of an alkaloid [12].

Physicochemical testing’s
Water content
A quantity of 2 g of the fresh powder was placed in an empty capsule of mass M₀ and dried at 105°C (221 F) to constant weight in an oven for 24 hours. The total dry residue or dry matter (DM) is expressed as a percentage of fresh matter according to the formula [13]:

\[
DM = \frac{(M₂-M₀)}{M₀} \times 100
\]

With M₀ (mass in g of the empty capsule) and M₂ (mass in g of the capsule containing the powder after baking). The water and volatile matter content noted H is reduced to the dry matter rate by the formula: %H = 100 – DM

Ash rate
The capsules containing the samples from steaming at 105°C (M₃) were placed in a muffle oven set at 550°C, then completely incinerated for 24 hours until white ash was obtained. After incineration, the crucibles were removed from the oven, then cooled in the atmosphere of a desiccator and weighed (M₄). The ash content (AC) per 100g of dry matter (DM) was calculated by the formula [13]:

\[
AC = \frac{(M₄-M₃)}{DM} \times 100
\]
AC = \frac{(M3-M0)}{M2} \times 100; \text{ With } M_0, \text{ mass in g of the empty capsule.}

Results were the average of 02 repetitions

Results
Macroscopic examination
The different observations are mentioned in Table I.

Microscopic examination
Figure 2 (2.a and 2.b) and Figure 3 (3.a, 3.b and 3.c).

Qualitative phytochemical screening
Analysis of the phytochemical composition revealed the presence of alkaloids and flavonoids in the trunk bark of *Bridelia micrantha*, sterols, saponosides and terpenoids in that of *Persea americana*. The different secondary metabolites found are listed in Table II.

Physicochemical testing's
The percent losses of desiccation and total ash measured are shown in Table III below.

Table 1: Macroscopic examination of stem bark

<table>
<thead>
<tr>
<th></th>
<th>Bridelia micrantha</th>
<th>Persea americana</th>
</tr>
</thead>
<tbody>
<tr>
<td>External color</td>
<td>dark gray</td>
<td>orange-brown</td>
</tr>
<tr>
<td>Internal color</td>
<td>gray-brown</td>
<td>brown</td>
</tr>
<tr>
<td>Flavor</td>
<td>slightly bitter</td>
<td>slightly acidic</td>
</tr>
<tr>
<td>Fissure plan</td>
<td>longitudinally, striated and dotted with many lenticels</td>
<td>longitudinally, striated and dotted with many lenticels</td>
</tr>
<tr>
<td>Smell of the powder</td>
<td>Weak</td>
<td>Weak</td>
</tr>
</tbody>
</table>

Table 2: qualitative phytochemical composition

<table>
<thead>
<tr>
<th>Phytochemical class</th>
<th>Aqueous extract <em>Persea americana</em></th>
<th>Aqueous extract <em>Bridelia micrantha</em></th>
<th>Characteristic color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>++</td>
<td>Yellow precipitate</td>
</tr>
<tr>
<td>Anthraquinons</td>
<td>-</td>
<td>-</td>
<td>no reaction</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+++</td>
<td>Orange</td>
</tr>
<tr>
<td>Sterols and polyterpenes</td>
<td>++</td>
<td>-</td>
<td>purple green</td>
</tr>
<tr>
<td>Saponosides</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+: weakly present; ++: medium present; +++: highly present; -: absent

Table 3: physico-chemical tests

<table>
<thead>
<tr>
<th></th>
<th>DM (%)</th>
<th>Water content (%)</th>
<th>Ash rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Persea americana</em></td>
<td>92.17</td>
<td>7.83±1</td>
<td>3.36±1</td>
</tr>
<tr>
<td><em>Bridelia micrantha</em></td>
<td>89.83</td>
<td>10.17±1</td>
<td>4.25±1</td>
</tr>
</tbody>
</table>

Interpretation
We carried out various testing on samples, in order to develop plant identities. The water contents of the trunk bark of *P. americana* and *B. micrantha* were made in order to reassure us on the conservation of the drugs. The values obtained were less than 11% (see Table III), hence a low risk of fermentation and enzymatic oxidation of plant drugs because an excess of water (≥15%) can lead to an alteration of plant substances and microbial proliferation [13]. The percentages in total ash which are 3.36±1% and 4.25±% respectively for the bark of the trunks of *P. americana* and *B. micrantha* tell us about the charge of the mineral elements. Indeed, this would explain the richness in crystals of calcium oxalates observed under microscopy. Oxalic acid in some plants can take the form of fine spicules or rapids, which causes severe irritations of the mucous membranes to the herbivores which would try to eat it [14]. It was observed under an optical microscope in *B. micrantha* (Figure 3): a red cell parenchyma in the form of a spread of contiguous cells impregnated with a red-brown tannoid, wooden vessels; observations somewhat similar to ours were made in India at *Bridelia reusa Spreng* by Banerjee Saurabh et al. in 2009 [15]. In *P. Americana* (Figure 2), we observed simple polygonal parenchymal cells presenting an intracellular space, grayish in color under microscopy and dotted with prisms and calcium oxalate crystals.

![Fig 2: *Persea americana* micrography; 2.a) fibers and bases of cells containing calcium oxalate crystals (x40); 2.b) cluster of parenchymal cells (x40)](image-url)
The presence of alkaloids, flavonoids and saponosides in the aqueous extract of *B. micrantha* corroborates the results of Naomi Waiganjo and collaborators in 2013 [16]. The presence of flavonoids, steroids, and saponins in *P. americana* corroborates with the results of Arukwe et al. in 2012 [17]. The presence in our plants of flavonoids recognized for their anti-inflammatory properties as capable of trapping free radicals and preventing the formation of prostaglandins would justify the use of these plants in a recipe traditionally used against “hemorrhoids”.

**Conclusion**

The aim of this study was to investigate the macroscopy, the microscopy, the qualitative phytochemical screening, the total ash and the water content of the stem bark of *Persea americana* and *Bridelia micrantha* used in a recipe by traditional healer to treat “hemorrhoids”. The macroscopic and microscopic characterization is amongst the most cost efficient used to established and identified vegetal drugs, whereas, the phytochemical analysis help to confirm those finding. The information and data recorded throughout this study will be of capital importance because they allow us to establish a scientific identity card for each of these plants in order to facilitate their integration into the Cameroonian Health System and in the near future for the establishment of a national pharmacopoeia.

**Authors’ contributions**

All the authors read and approved the final version of the manuscript.

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**Availability of data and materials**

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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