Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 32-36
Received: 09-03-2020
Accepted: 11-04-2020

Joseph Bertin Alexis Zoa Bindzi
Faculty of Medicine and Biomedical Sciences, The University of Yaoundé 1, Yaoundé, Cameroon

Rose Ngono Mballa Abondo
National Drug Quality Control and Evaluation Laboratory, Yaoundé, Cameroon

Joseph Ngoupayo
Department of Therapeutical Chemistry and Pharmacognosy, Faculty of Medicine and Biomedical Sciences, Yaoundé, Cameroon

Beyeme Fridoline Victorine
Cameroonian Higher Council for Traditional Medicine, Yaoundé, Cameroon

Corresponding Author:
Joseph Bertin Alexis Zoa Bindzi
Faculty of Medicine and Biomedical Sciences, The University of Yaoundé 1, Yaoundé, Cameroon

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 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* trunk's 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 anthelmintic, 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].

Micrography

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].

Fig 1: Stem bark; 1.a) *Persea americana*; 1.b) *Bridelia micrantha*

Sterols and polyterpenes test (Liebermann reaction)

0.5 g of each extract is dissolved hot in 2 ml of acetic anhydride; 1 mL of concentrated sulfuric acid is added to the mixture. The appearance, at the interphase, of a purple or purple ring, turning blue then green, indicates a positive reaction [10].

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 H_2 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 M_0 and dried at 105ºC (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]:

$DM = \frac{(M2-M0)}{M2} x100$; With M_0 (mass in g of the empty capsule) and M_2 (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 105ºC (M_2) were placed in a muffle oven set at 550ºC, 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 (M_3). The ash content (AC) per 100g of dry matter (DM) was calculated by the formula [13]:

$AC = \frac{(M3-M0)}{M2} x100$; With M_0, 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

	Bridelia micrantha	***Persea americana***
External color	dark gray	orange-brown
Internal color	gray-brown	Brown
Flavor	slightly bitter	slightly acidic
Fissure plan	longitudinally, striated and dotted with many lenticels	longitudinally, striated and dotted with many lenticels
Smell of the powder	Weak	Weak

Table 2: qualitative phytochemical composition

Phytochemical class	**Aqueous extract *Persea americana***	**Aqueous extract *Bridelia micrantha***	**Characteristic color**
Alkaloids	-	++	Yellow precipitate
Anthraquinons	-	-	no reaction
Flavonoids	+	+++	Orange
Sterols and polyterpenes	++	-	purple green
Saponosides	+	+	

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

Table 3: physico-chemical tests

Stem bark	**DM (%)**	**Water content (%)**	**Ash rate (%)**
Persea americana	92.17	7.83±1	3.36±1
Bridelia micrantha	89.83	10.17±1	4.25±1

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 retusa 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.

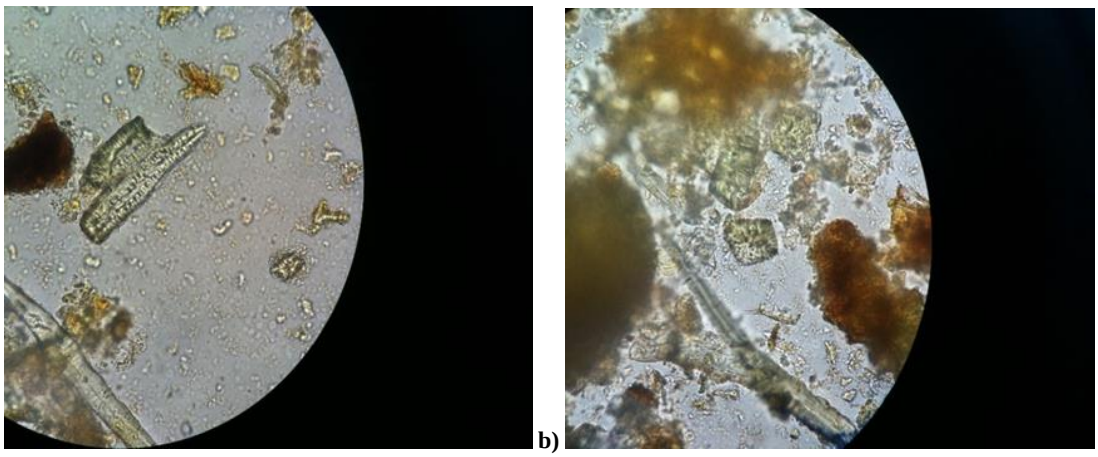

Fig 2: *Persea americana* micrography; 2.a) fibers and bases of cells containing calcium oxalate crystals (x40); 2.b) cluster of parenchymal cells (x40)

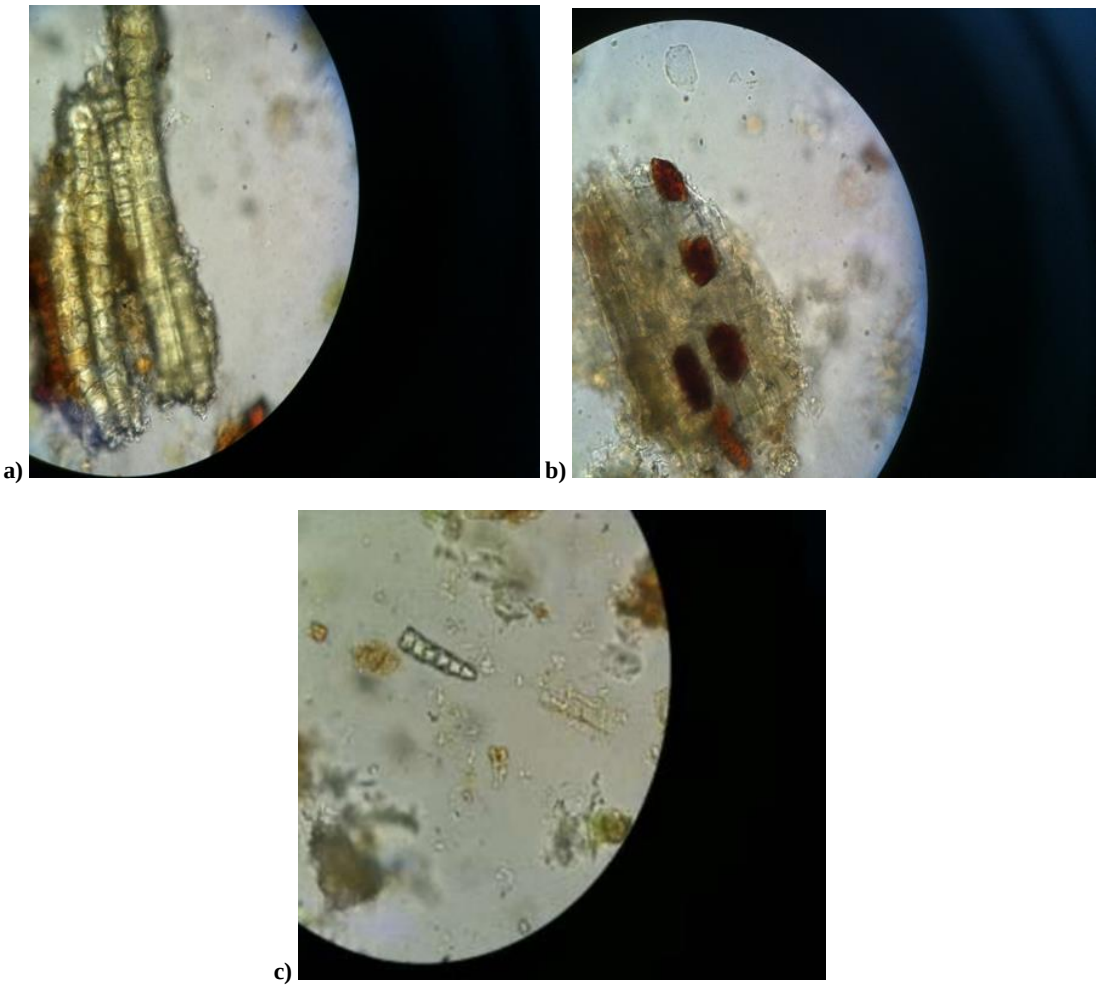

Fig 1: *Bridelia micrantha* micrography; 3.a) layers of cells containing calcium oxalate crystals (x40); 3.b) red cell parenchyma (x40); 3.c) wooden vessel (x10)

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.

Funding
Not applicable.

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.

Acknowledgments
We would like to thank Dr Alexis ZOA and Dr. Rose NGONO MBALLA for the supervision and contribution in the development of this publication. Our thanks also go to Pr Joseph NGOUPAYO and Victorine BEYENE for the participation and follow-up in this study. We will not fail to thank the Laboratory of Therapeutical Chemistry and Pharmacognosy of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 and the Institute of Medical Research and Medicinal Plant Study (IMPM) for the technical platform.

References

1. Yasir M, Das S, Kharya MD. The phytochemical and pharmacological profile of Persea americana Mill. Pharmacogn Rev. 2010; 4(7):77-84.
2. Ngueyem TA, Brusotti G, Caccialanza G, Finzi PV. The genus Bridelia: a phytochemical and ethnopharmacological review. J Ethnopharmacol. 2009; 124(3):339-49.
3. Maroyi A. Ethnopharmacology and Therapeutic Value of Bridelia micrantha (Hochst.) Baill. in Tropical Africa: A Comprehensive Review. Molecules. 2017; 22(9).
4. Tchindjang M. cartographie des acteurs de la médecine traditionnelle au Cameroun : cas de la région du centre. Ethnopharmacologia. 2015; n°53:56-63.
5. Prashant Yadav KM, Ashok Kumar. Standardization and Evaluation of Herbal Drug Formulations. Journal of Advanced Laboratory Research in Biology. 2011; 2(4):6.
6. EDQM. Guide for the preparation of plant drug monographs and preparations based on plant drugs. In: Pharmacopeia E, editor. 2007, 23.
7. Blond SB, Cachet X, Cottet K, Genta-Jouve G, Grougnet R, Kritsanida M, F.-H. Porée. Pharmacognosie: Guide De Travaux Pratiques 3. In: Descartes Up, editor. Fascicule de TP-Pharmacognosie. 2014, 22. www.pharmacognosie-parisdescartes.fr.
8. Sofowora A. Research on medicinal plants and traditional medicine in Africa. J Altern Complement Med. 1996; 2(3):365-72.
9. Evans W. Trease and Evans' Pharmacognosy 15th Edition. In: Elservier, editor. 2002; 616.
10. Yves-Alain BÉKRO JAMB, Boua B, BOUA, Fézan H, TRABI, Ehouan E, ÉHILÉ. Étude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. et Zarucchi (Caesalpiniaceae) Sciences & Nature. 2007; 4(2):9.
11. Ciulei I. Pratical Manuals On The Industrial Utilization Of Medicinal And Aromatic Plants I. Methodology For Analysis Of Vegetable Drugs. In: Bucarest FoP, editor. 1982; 67.
12. Abdul Wadood MG, Syed Babar Jamal, Muhammad Naeem, Ajmal Khan, Rukhsana Ghaffar, Asnad. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochemistry & Analytical Biochemistry. 2013; 2(4):4.
13. AFNOR. Recueil des normes françaises, contrôle de la qualité des produits alimentaires, céréales et produits céréaliers. 2e édition. 1982 ; 325.
14. Bohn PR. Contribution à l'étude du rôle de l'oxalate de calcium chez les végétaux. Bulletin de la Société Botanique de France. 1935; 82(1):15-7.
15. Kumar saurab SkK. Pharmacognostic and preliminary phytochemical investigation on bark of Bridelia retusa Spreng. International Journal of Pharmaceutical and Clinical Research. 2009; 1(1):5.
16. Naomi Waiganjo HO, Dorcas Yole. Phytochemical analysis of the selected five plant extracts Chemistry and Materials Research. 2016; 3(9):7.
17. Arukwe A, Duru, Agomuo, Adindu, Odika, Lele, Egejuru, Anudike. CHEMICAL COMPOSITION OF PERSEA AMERICANA LEAF, FRUIT AND SEED. International Journal of Research and Reviews in Applied Sciences. 29; 11(2):4.