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Quality control of trunk's barks of *Lannea microcarpa* Engl. and *K. Krause* and *Anogeissus leiocarpus* (DC) Guill. & Perr. for the manufacture of phytomedicines for the treatment of hypertension

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Abstract

Lannea microcarpa (Anacardiaceae) and *Anogeissus leiocarpus* (Combretaceae) are two plants used in the traditional medicine for the treatment of hypertension in Burkina Faso. Previous preclinical studies have demonstrated their efficacy and safety. This study aimed to investigate the quality of trunk barks powder of two plants. The physico-chemical characteristics (macroscopic, organoleptic, water content, ash, heavy metals and pesticides), microbial cleanliness and phytochemical analysis were determined by the methods of the European Pharmacopoeia and / or International standards. The powder of *Anogeissus leiocarpus* trunk's bark was brown with astringent and slightly sweet taste while *Lennea microcarpa* was reddish with a slightly bitter taste. The microbiological parameters (total viable germs, specific microorganisms) and contaminants (heavy metals, pesticides, siliceous substances, etc.) complied with the recommendations of the European pharmacopoeia and ISO 7218 standards. The results of physico-chemical and pharmacotechnical parameters allowed recognition of plant powders and checking of purity level / the degree of cleanliness. Analysis using chromatography of thin layer established the chromatographical print of powders. These parameters shown the quality of powders and can be used as quality control parameters for plant powders when used for the production of herbal medicines.

Keywords: High blood pressure, traditional medicine, plant powder, quality control.

1. Introduction

High blood pressure (hypertension) is currently recognized as a global public health problem because of its frequency and the associated risks of cardiovascular and renal diseases[1]. More than a quarter (26.4%) of the world's adult population suffers from hypertension[2]. Hypertension management is complex and includes chronic treatment, clinical follow-up and biological treatment of patients. Because of poverty and the lack of universal health insurance, the management of hypertension continues to be a concern not only for people who suffer from it, households and governments[3]. A large proportion of the populations of the underdeveloped countries use traditional medicine as an alternative from of health care[4]. Ethnopharmacological, phytochemical and pharmacological investigations confirmed the antihypertensive activity of certain medicinal plants [5-8]. In this line, similar studies in Burkina Faso have demonstrated antihypertensive properties, but also the safety of the trunk bark of *Anogeissus leiocarpus* and *Lannea microcarpa*[9, 10]. These preliminary studies offer perspectives for the development of medicines. plant powders used in the

manufacture of herbal medicines must maintain their effectiveness, safety and quality throughout duration of their intended use [11]. This quality is generally assessed through monitoring parameters such as physical-chemical and microbiological. The inadequacy of these data could lead to therapeutic failure, microbial infection or intoxication for the patient. This study aimed to establish physical-chemical, pharmaco technical and microbiological control parameters for the powders of the trunk's bark of *Anogeissus leiocarpus* and *Lannea microcarpa*.

2. Material and methods

2.1 Plant materials

The plant material was the bark of trunks of *Lannea microcarpa* (Anacardiaceae) and *Anogeissus leiocarpus* (Combretacaea) identified by a botanist of the Ecological Laboratory of the University of Ouaga. 1 Pr Joseph KI-ZERBO in reference to the herbarium of each of (Nos. 1544 and 361 respectively for *Anogeissus leiocarpus* and *Lannea microcarpa*). The collected bark was dried and then reduced to powder. These powders were used for this study.

2.2 Macroscopic and organoleptic characteristics

The macroscopic characteristics (appearance and color) were observed with the naked eye. The organoleptic characteristics were determined by tasting the powder and the odor by sniffing.

2.3 Granulometric of powders

The granulometry was determined by the filter method of the European Pharmacopoeia 6th edition.

2.4 Residual moisture content

The residual moisture content of the powders was determined according to the thermogravimetric method of the European Pharmacopoeia 6th edition in an oven (Memmert, Germany). The assay was performed in triplicate on one (01) g of powder. The mean and standard deviation were calculated (n = 3, mean, standard deviation).

2.5 Total ash content

Total ash levels were determined according to the European Pharmacopoeia 6th edition by calcining one (01) gram of each plant powder in a furnace (Bouvier, Belgium) at a temperature of about 600 ° C. Total ash contents were expressed as a percentage.

2.6 Heavy metal content

The selected heavy metals (total arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb)) were measured by the flame atomic absorption spectrometry (AFAS) using the absorption spectrometer (VARIAN AA 240FS, Belgium).

2.7 Pesticide content

The selected pesticides (organochlorines, carbamates and synthetic pyrethronides) were extracted, purified and analyzed according to the QuEChERS method described in standard NF EN 15662. The essay was performed in triplicate and the mean and standard deviation were calculated (N = 3, mean standard deviation).

2.8 Microbiological quality

The selected germs were total flora, salmonella and thermo-tolerant coliforms. Total flora and salmonella were determined by the method of the European Pharmacopoeia 6th edition. Thermo-tolerant coliforms were determined according to ISO 7218. Colony counts were performed for calculations of the number of colony forming units per gram (CFU / g).

2.9 Determination of pH

The pH was determined by immersing the pH-meter electrode (Eutech, Singapore) in 1% (w / v) aqueous solutions of each plant material. The test was performed in triplicate and the mean and standard deviation were calculated (m ± standard deviation, n = 3).

2.10 Phytochemical analysis

The chemical groups were characterized by identification of their chromatographic profiles by thin layer chromatography (TLC). A test sample of 2.00 g of each vegetable powder was dispersed in 40.0 ml of distilled water and then introduced in to an ultra sonic bath for homogenization. The obtained solution was transferred to a separating funnel of 250 mL. Three series of extraction of the metabolites contained in the aqueous extract were carried out successively with hexane (Prolab, France) (20 mL x 3), dichloromethane (Prolab, France) (60 mL x 3) of acetate Ethyl (SdS, France) (20mLu3) and methanol (Merk, Germany) (20mL x 3). Five (5) µL were deposited on a silica gel coated glass TLC plate (60F254, China).The chromatograms were developed over an 8 cm course in the following solvent systems (Table 1).

Table 1: solvent systems and reagents

Extracts	Solvent systems	Revealing reagent / Observation
Hexane	- Hexane - Ethylacetate - methanol (7 :3 : 1)	Anisaldehydesulfuric / Observation by daylight/at λ = 254 and 365 nm
DCM	-Toluene - ethylacetate – acetic acid (5: 4: 1)	Anisaldehydesulfuric / Observation by daylight
AcOEt	-Toluene - ethylacetate – formic acid (7: 2: 1)	Observation at λ = 254 and 365 nm
MeOH	- Ethylacetate - Methanol - Water (8: 2: 1)	Observation at λ = 254 and 365 nm

3. Results and discussion

The results of powder's characteristics, pH, residual moisture content, total ash and microbiological quality are reported in Table 2.The analyzes revealed a brown color, an astringent and slightly sweet taste for the powder of the trunk bark of *Anogeissus leiocarpus*. *Lannea microcarpa* was red, without odor with a slightly bitter taste. In Mali, Tounkara *et al* found a similar color and taste but an astringent odor for the trunk bark of *Anogeissus leiocarpus*[12]. These characteristics allow immediate recognition of plant powders. These results could also be used to verify the level of purity according to the presence or absence of foreign elements and to detect any adulteration or falsification. The smell, taste and color of the plant powders allow differentiating similar drugs. [13].The pH was 7.16 ± 0.08 for *Lannea microcarpa* and 7.01 ± 0.03 for *Anogeissus leiocarpus* (Table 2).Ph is an important factor that influences the physicochemical properties of the plants and its pharmacological activity as well as its toxicity[14, 15].A variation of these values significantly influences the properties of vegetal powders. This variation may also indicate possible degradation of the powders by the development of microorganisms[13].

Moisture levels were less than 10% (Table 2) and according to the European Pharmacopoeia, powders can be stored over a long period without the growth of molds or yeasts[16].Indeed, water is a favorable environment for the development of germs and also hydrolysis reactions[17]. Knowledge of THR is important in the conservation and flow properties of powders. In addition, the relative humidity influences the repulsion and agglomeration of the powder particles because it has a lubricating role[18].

The total ash contents of the trunk bark powders were 6.27 ± 1.42% for *Lannea microcarpa* and 7.66 ± 2.35 for *Anogeissus leiocarpus* (Table 2). Tounkara[12]found a rate of 12.25% for the trunk bark of *Anogeissus leiocarpus*. This difference may be related to the physicochemical characteristics of the crop site or to the treatment of the plant drugs before analyzes. Indeed, the ash content in plant materials is an indicator of the content of inorganic elements (minerals).It may indicate impurities such as siliceous elements resulting from contamination of plant powders with sand or dust[19].

The microbial quality (Table 2) of the powders was in accordance with the recommendations of the European Pharmacopoeia 6th edition of natural raw materials administered by oral way. The absence of specific pathogenic germs such as Salmonella and the low presence of total flora on firm a good microbial quality of the vegetal powders. Any contamination beyond the norms would lead to a rejection or sterilization of the raw material, resulting in risks of degradation of certain components and an increase in raw material costs[11].

Table 2: Physico-chemical and microbiological analyzes

Designation	Macroscopic and organoleptic characteristics	pH (1%)	Residual moisture content (%)	Total ashes (%)	Microbiological Control (CFU / g)		
					Thermo-tolerant Coliforms	Total flora	Salmonella / 25g
Anogeissus leiocarpus	Brown color with an astringent and slightly sweet taste	7.01 ± 0.03	7.01 ± 0.35	7.66 ± 2.35	Absence	$4.2.10^4$	Absence
Lannea microcarpa	Red color with a slightly bitter taste	7.16 ± 0.08	6.35 ± 0.16	6.27 ± 1.42%	Absence	$0.6.10^4$	Absence

The heavy metal content (Table 3) was lower than the recommendations of the European Pharmacopoeia. This could be explained by the fact that raw materials have been collected at locations far from roads, water drainage ditches, mine wastes, garbage dumps and industries [20].Indeed the accumulation of heavy metals can affect human health[21]. The selected heavy metals were those on the lists of priority contaminants international conventions and regulations because of their frequency[22] and toxicity[23].

Table 3: Heavy metal content of powders

	Content in ppm (RSD (%))			
Samples	**Arsenic**	**Mercury**	**Cadmium**	**Lead**
Anogeissus leiocarpus	0.85 (3.4)	≤ LD	0.22 (1.9)	0.255(1.9)
Lannea microcarpa	0.61 (2.2)	≤ LD	0.1 (1.3)	0.115(13)

LD: Limit of detection(LD Hg = 0.01µg/L) Valeurs limites autorisées: Arsenic ≤ 2ppm; Plomb ≤ 5ppm; Mercure ≤ 1ppm; Cadmium ≤ 1ppm)

The residual levels of pesticides in the powders of trunk barks (Table 4) were acceptable [24].This low pesticide content could be explained by the fact that the raw materials were collected at sites far from the cotton crops areas [11].In fact, reports have shown side effects associated with falsifications and the presence of contaminants (heavy metals, pesticides and microorganisms) [25].

Table 4: Residual pesticide content

Pesticides	LQ(mg/L)	Results(mg/kg)
Organo chlorine		
2,4'-DDT	0.01	< 0.01
Aldrine	0.01	< 0.01
Dieldrine	0.02	< 0.02
Fipronil	0.02	< 0.02
Dicofol	0.02	< 0.02
Beta Endosulfan	0.01	< 0.01
Heptachlore	0.01	< 0.01
Alachlore	0.01	< 0.01
Lindane	0.01	< 0.01
Methidathion	0.01	< 0.01
Carbamates	0.01	< 0.01
Synthetic Pyrethronide	LD** (mg/L)	
Cypermethrine	0.05	< 0.05
Deltamethrine	0.05	< 0.05
Lamda Cyhalothrine	0.05	< 0.05
Permethrine	0.05	< 0.05
Tetramethrine	0.05	< 0.05

** : LQ : Limit of quantitation * * LD: Limit of detection*

Results of phytochemical analysis by thin-layer chromatography (TLC) of the two powders are reported in figure 1 and the frontal references in Table 5. In figure 1, the major spots seen in day light after visualization with sulfuric anisaldehyde have five (05) main bands for *Lannea microcarpa* and four (04) main bands for *Anogeissus leiocarpus*. The major (most intensive) spot is the one that Rf is 0.63 in purplish color for *Lannea microcarpa* and 0.91 in the same color for *Anogeissus leiocarpus*. Under the ultraviolet light at 254 nm, four main bands were observed for both powders and the Rf for the major spots were 0.9125 for *Lannea microcarpa* and 0.65 for *Anogeissus leiocarpus*. These TLC revealed the presence of a wide variety of chemical groups which could be used as chromatographic prints in the quality control in terms of purity. According to data from the literature, the results obtained by the TLC could be used for routine analyzes of powders in future crops in order to verify their quality[26]. The chromatographic profile of metabolites is a tool for qualitative and quantitative evaluation of herbal medicines [27, 28].

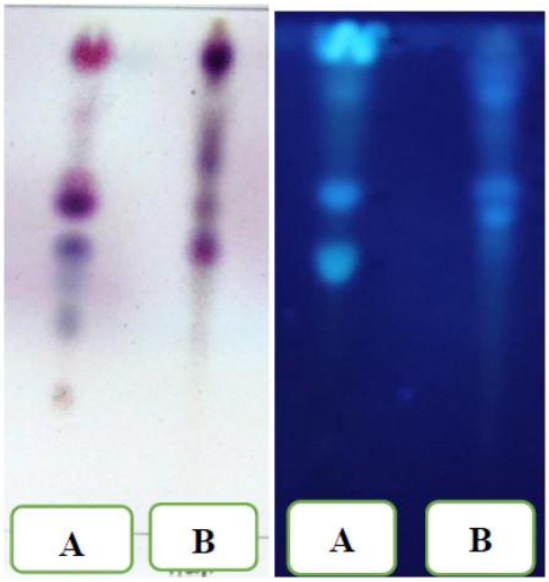

Fig. 1: Chromatographic impressions of the hexane fractions of the powders of the bark of *Lannea microcarpa*(A) and *Anogeissus leiocarpus*(B), after detection with sulfuric anisaldehyde and observation with the UV lamp at 366 nm.

Table 5: Front (RF) ratios of the major chromatographic imprints of the hexane fractions and their coloring

Designation	Rf of major tasks	Color	Observation
Lannea microcarpa	0.9125 0.6375 0.6125 0.525 0.375	Pink Purplish Purplish Blue purplish	In the light of day after revelation with anisaldehyde sulfuric
Anogeissus leiocarpus	0.9125 0.71125 0.6375 0.6125	Purplish Purplish Purplish purplish	
Lannea microcarpa	0.9125 0.8875 0.6375 0.6125	Skyblue Skyblue Skyblue Skyblue	UV lamp at 366nm
Anogeissus leiocarpus	0.9125 0.8875 0.65 0.6375	Skyblue Skyblue Skyblue Skyblue	

Results of granulometric analysis are presented in figure 2. The average particle diameter was 0.7405 ± 0.15 mm for both powders. According to the method of expression of results of the particle size analysis using two filters of the European pharmacopoeia, the powders are classified as coarse powders. This could be explained by the fact that the grinding of the trunk bark was done by the same mill[16]. This particle size distribution of powders is essential because its determines their rheological characteristics[29].

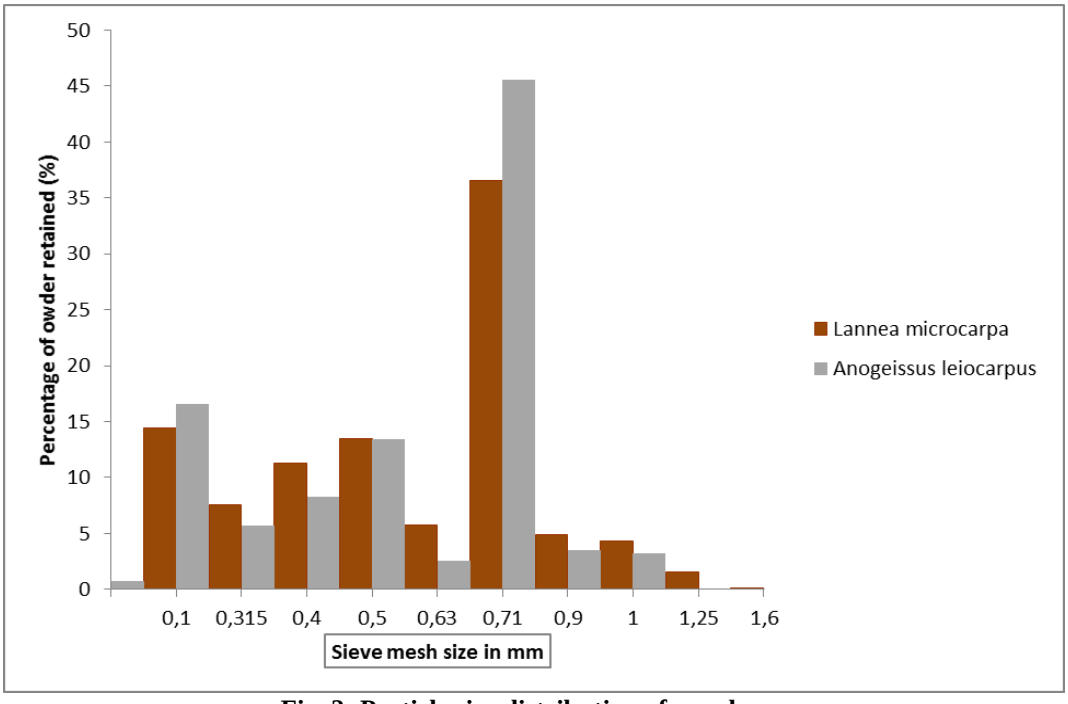

Fig. 2: Particle size distribution of powders.

4. Conclusion

The determination of the physicochemical and microbiological characteristics showed that the powders of *Lannea microcarpa* and *Anogeissus leiocarpus* conformed to the standards of the European Pharmacopoeia related to medicinal plant substances. These parameters make it possible to define quality standards for the control of raw materials and could be used as criteria for quality control of powders

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References

[1]. Zabsonre, P., *et al*., Connaissance et perception des facteurs de risque cardiovasculaire en Afrique subsaharienne. *Archives des maladies du coeur et des vaisseaux*, 2002; 95(1): 23-28.

[2]. OMS, Médecine traditionnelle : Rapport du secrétariat. Conseil exécutif, Cent onzième sessions. . Point 57 *de l'ordre du jour* Vol. EB111/9. 2002.

[3]. Whitworth, J.A., World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens*, 2003; 21(11): 1983-92.

[4]. OMS, Stratégie de l'OMS pour la médecine traditionnelle pour 2014-2023. 2013. 72.

[5]. Kaplan, N.M. and L.H. Opie, Controversies in hypertension. *The Lancet.* 367(9505): 168-176.

[6]. sérin, P., M. Masson, and J. Kedellini, Encyclopédie des plantes médicinales, Identifications, Préparations, Soins. Paris : Edition Larousse/VUEF. 2001. 335.

[7]. Guindo, I., Etude du traitement traditionnel de l'hypertension artérielle au Mali. Bamako: Thèse de pharmacie FMPOS, 2005. 126.

[8]. Ba, S., Etude de la phytochimie et des activités biologiques de Zizyphus mauritiana Lam (Rhamnaceae) utilisée dans le traitement traditionnel du diabète et de l'hypertension artérielle en Mauritanie. 2005, Bamako, Thèse de Pharmacie FMPOS.

[9]. Ouédraogo, S., *et al*., Etude de la toxicité et des propriétés pharmacologiques de l'extrait aqueux de Anogeissus leiocarpus (DC) Guill. et Perr (Combretaceae). Pharmacopée et médecine traditionnelle africaine, 2011. 15: p. 18-22.

[10]. Belemnaba, L., *et al*., Recherche de plantes à potentialités antihypertensives dans la biodiversité du Burkina Faso. *Pharmacopée et médecine traditionnelle africaine*, 2014; 17(1): 8.

[11]. WHO, Réglementation des médicaments à base de plantes: la situation dans le monde. 1998: p. 65.

[12]. Tounkara, A., Etude de l'activité hépatoprotectrice de deux plantes médicinales du Mali : Anogeissus leiocarpus Guill. et Perr.et Terminalia macroptera Guill. et Perr. (Combrétacées). in Faculté de médecine, pharmacie et d'odontostomatologie. . 2006, Université de Bamako, Mali p. 188.

[13]. Lehmann, H., Le médicament à base de plantes en Europe: statut, enregistrement, contrôles. 2013, Université de Strasbourg. p. 341.

[14]. Babich, H. and G. Stotzky, Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: influence of clay minerals and pH. *App and Envi Micro* 1977; 33(5): 1059-1066.

[15]. Babich, H. and G. Stotzky, Sensitivity of various bacteria, including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. *App and Environ Micro*, 1977; 33(3): 681-695.

[16]. Europe, C.D., Pharmacopée européenne 6ème Édition, ed. D.E.d.l.Q.d.M. & and S.d.S. (EDQM). 2007: Strasbourg, France. 1218.

[17]. Aiache, J., S. Aiache, and R. Renoux, Initiation à la connaissance du médicament.-4 ème éd. 2001, Paris: Masson.-338p.

[18]. Dawoodbhai, S. and C.T. Rhodes, The effect of moisture on powder flow and on compaction and physical stability of tablets. *Drug Development and Industrial Pharmacy*, 1989; 15(10): 1577-1600.

[19]. Gras, R., Propriétés physiques des substrats. Les cultures hors sol, 1987: p. 79-126.

[20]. Chiffoleau, J.-F., D. Auger, and E. Chartier, Dosage de certains métaux traces:(Cd, Co, Cu, Fe, Ni, Pb, Zn) dissous dans l'eau de mer par absorption atomique après extraction liquide-liquide. 2002: Editions Quae.

[21]. Wang, Q.-R., *et al*., Soil contamination and plant uptake of heavy metals at polluted sites in China. Journal of Environmental Science and Health, Part A, 2003. 38(5): p. 823-838.

[22]. Calabrese, E.J. and L.A. Baldwin, Hormesis and high-risk groups. *Reg Toxi and Pharm* 2002; 35(3): 414-428.

[23]. Sadiq, M., Toxic metal chemistry in marine environments. 1992: Marcel Dekker, Incorporated. Edition. 390.

[24]. WHO, WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009. 2010: World Health Organization pp 64.

[25]. Sahoo, N., P. Manchikanti, and S. Dey, Herbal drugs: Standards and regulation. *Fitoterapia*, 2010. 81(6): p. 462-471.

[26]. Katekhaye, S.D. and K.K. Bhutani, Standardization of a polyherbal Ayurvedic formulation: Ayaskrti. *Ind Jou of Trad Know* 2011;10(4): 589 – 593.

[27]. Liang, X.M., *et al*., Qualitative and quantitative analysis in quality control of traditional Chinese medicines. *J Chromatogr A*, 2009; 1216(11): 2033-44.

[28]. Giri, L., *et al*., Chromatographic and spectral fingerprinting standardization of traditional medicines: an overview as modern tools. *Research Journal of Phytochemistry*, 2010; 4(4): 234-241.

[29]. Pifferi, G., P. Santoro, and M. Pedrani, Quality and functionality of excipients. *Il Farmaco*, 1999; 54(1–2): 1-14.