Research Article

Standardization of Extracts from Trunks's Barks of *Lannea microcarpa* engl. and K. Krause (Anacardiaceae) and *Anogeissus leiocarpus* (DC) Guill. and Perr. (Combretaceae) for the Formulation of Antihypertensive Herbal Medicines

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ABSTRACT

Ethnopharmacological surveys carried out in Burkina Faso have shown that the trunks barks of *Lannea microcarpa* and *Anogeissus leiocarpus* are used as decoctate in the treatment of arterial hypertension. Previous preclinical studies have demonstrated the efficacy and safety of freeze-dried aqueous extracts. This work has been undertaken with the aim of standardizing the extracts in order to ensure the reproducibility of the extraction process during the manufacture. The parameters studied were the physicochemical, microbiological and pharmacotechnical characteristics of the extracts of the two plants. The results obtained show that the optimum extraction yield is 33.46 ± 0.36%, after a decoction of 40 minutes and then a freeze-drying of 120 hours for *Lannea microcarpa*. For *Anogeissus leiocarpus*, It is 26.77 ± 2.86% after a decoction of 30 minutes and then freeze-drying for 120 hours. The freeze-dried extract of *Anogeissus leiocarpus* is brown in color, with an astringent, slightly sweet taste and *Lannea microcarpa* is reddish in color with a slightly bitter taste. Both extracts are very hygroscopic, readily soluble in water, very little soluble in ethanol 96 ° and absolute, practically insoluble in chloroform and readily soluble in buffers (pH 1.2 and pH 6.8). They have a mediocre flow according to the European Pharmacopoeia and can be kept for six months without alterations of the physicochemical characteristics. These extracts can thus be used for the formulation of phytomedicines while ensuring reproducibility from one batch to another in the amount of the selected tracer.

Keywords: *Lannea microcarpa* - *Anogeissus leiocarpus* - arterial hypertension- standardizing- extracts – phytomedicines.

INTRODUCTION

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being[1]. The use of plants by man is confused with the history of mankind, both for food, therapeutic purposes, etc. Indeed Plants with their biosynthetic intermediates display a variety of biological activities of interest to the pharmaceutical, cosmetic and food sectors[2]. In the last decade, traditional medicine has become very popular in sub-Saharan Africa, partly due to the precarious economic situation in the countries[3]. Some of these ancient uses are now verified by scientific studies which have led to the isolation of new active ingredients and / or the placing on the market of herbal medicines or standardized extracts. This has prompted an intensification of research on medicinal plants against pathological or infectious pathologies such as AIDS or malaria and especially against noncommunicable diseases including hypertension, diabetes and cancer are the first representatives. Among these plants are *Spondias mombin, Ziziphus mauritiana, Catharanthus roseus, Cassia occidentalis*, etc [4,5]. These numerous studies do not always end with physicochemical, microbiological and pharmacotechnical data essential for the quality control and the standardization of the raw materials before their manufacture as drugs. Many traditional medicinal herbs from Burkina Faso are used to treat arterial hypertension (HTA). Among them, *Anogeissus leiocarpus* and *lannea microcarpa* which are well known and widely used[6]. Toxicological, phytochemical and pharmacological studies have demonstrated the antihypertensive properties of the trunk bark of *Anogeissus leiocarpus* (Combretacaea) and *Lannea microcarpa* (Anacardiaceae)[7,8]. This is an interesting potential for the treatment of hypertension. These preliminary studies have opened up prospects for the development of prototypes of pharmaceutical formulations of phyto-medicines for a clinical evaluation of therapeutic activity. This valuation requires the standardization of raw materials. The aim of this study was to determine the parameters of standardization of freeze-dried aqueous extracts of the trunk's barks of *Anogeissus leiocarpus* (Combretacaea) and *Lannea microcarpa* (Anacardiaceae).

MATERIALS AND METHODS

Plants materials

Fresh trunks's barks of *Lannea microcarpa* (Anacardiaceae) and *Anogeissus leiocarpus* (Combretacaea) were harvested at *Loumbila commune* (Burkina Faso) in February 2015 and identified by a botanist from the Ecology Laboratory of the University of Ouaga 1 Pr Joseph KI-ZERBO in reference to the herbarium of each of them (N ° 1544 and N ° 361 respectively for *Anogeissus leiocarpus* and *Lannea microcarpa*). The barks of the harvested trunks were dried and crushed to powder.

Preparation of aqueous decocts

Ten grams (10 g) of each vegetable powder was decoctioned separately with 20, 30, 40, 50, 75, 100 and 150 mL sterile distilled water while different times from 20, 30, 40, 50 and 60 minutes for each mixture.

Preparation of freeze-dried aqueous extracts

The extracts obtained were frozen for 24 hours and then lyophilized at different times of 48, 72, 96, 120, 144 and 168 hours. Each test was performed three times, finally the mean value and the standard deviation were calculated (m ± standard deviation, n = 3). These freeze-dried extracts were used for the following studies.

Analysis

Residual moisture content and extraction yield

The residual moisture content of the extracts 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 extract. The mean and standard deviation were calculated (n = 3, mean, standard deviation). The extraction yield expressed the percentage of dry extract obtained after lyophilization with respect to 100 grams of dry powder of the trunk's barks.

Macroscopic and organoleptic characteristics

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

Microbiological quality

The germs sought 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 sdandars. Colony counts were performed for calculations of the number of colony forming units per gram (CFU / g).

Determination of pH

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

Phytochimical analysis

The chemical groups were characterized by the establishment of their chromatographic profiles by thin layer chromatography (TLC). A test sample of 2.00 grams of each vegetable powder was dispersed in 40.0 mL of distilled water and then introduced into an ultra sonic bath for homogenization. The solution obtained 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 solvent systems Hexane - Ethyl acetate - methanol (7 :3 : 1). Observation of TLC plates was carried out in daylight, under UV lamp at λ = 365 nm, under UV lamp at λ = 254 nm and after revelation anisaldehyde sulfuric.

Dosage of phenolic compounds

Previous studies have shown that lyophilized aqueous extracts of the trunk bark of both plants contained abundant phenolic compounds[9]. These compounds are known for their positive effects against hypertension[10]. In view of this, the phenolic compounds are retained as tracers in this study. The phenolic content was determined according to the method of Singleton[11] by using Folin-Ciocalteu as reactive reagent. The reaction mixture consisted of 1 mL of extract, 1 mL of 2N FCR and 2 mL of a 20% sodium carbonate solution. A control solution referred to as white identical to the reaction mixture except that the extract was replaced with distilled water was used. The solutions were allowed to stand at room temperature for 40 min and then the absorbance was measured at 760 nm using the Visible UV spectrophotometer. A standard curve was plotted with gallic acid (1-5 µg / ml). The trials were carried out in triplicate. The results are expressed in microgram gallic acid equivalents / mg extract (µGAE / mg) with reference to the gallic acid calibration curve.

Granulometric of powders

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

Flow test

The flow test was determined by measuring with a stopwatch the time that 100 g of extract to flows completely through a funnel of defined size in the European Pharmacopoeia.

Compressibility Index

The compressibility index was determined by measuring the unfilled bulk volume and then the final volume after compaction of extract until a constant volume was obtained in a test piece according to the method described in European Pharmacopoeia 6.0.

Hygroscopicity

Hygroscopicity was determined using 1 g of extract according to the method described in European Pharmacopoeia 6.0. The extract was introduced into a suitable desiccator containing a saturated solution of ammonium chloride at 25 ° C. for 24 hours. The increase in mass allowed the calculation of the ratios expressed as a percentage.

Solubility

The solubility of the extracts was carried out in distilled water and in buffer media pH 1.2 and pH 6.8 according to the method described in European Pharmacopoeia 6.0.

Stability test

The stability study was carried out for six (06) months at 25° C temperature in the laboratory. The samples were packaged in food sachets. The color, the taste, the pH and the phenolic compounds were determined according to the methods described above.

RESULTS ET DISCUSSION

Macroscopic and organoleptic characteristics

The analyzes revealed a brown color, an astringent and slightly sweet taste for the freeze-dried aqueous extract of *Anogeissus leiocarpus*. *Lannea microcarpa* was red, without odor with a slightly bitter taste.

Residual moisture content

The results of the residual moisture contents of the freeze-dried aqueous extracts are shown in figure 1. This figure indicates the percentages of water contained in the extracts as a function of the lyophilization time.

They vary between 11.2% after 48 hours of freeze-drying and 6.13% after 168 hours of freeze-drying for *Lannea microcarpa*. For *Anogeissus leiocarpus*, 10.72% after 48 hours of freeze-drying and 6.52% after 168 hours of freeze-drying.

After 72 hours of freeze-drying, all values were less than 10. This means the extracts are sufficiently dry and can be kept for a long time without the development of mold or yeast[12]. The analyzes revealed that loss of desiccation gradually decreased with lyophilization time up to 120 hours, from which time THR did not differ significantly ($p > 0.05$) up to 168 hours. This can be explained by the principle of primary desiccation in which the free water contained in the products becomes practically zero[13,14].

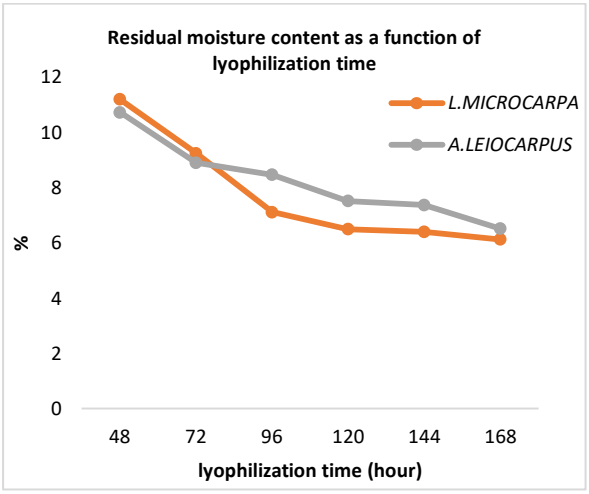

Figure 1: Residual moisture content as a function of lyophilization time of *Lannea microcarpa* and *Anogeissus leiocarpus* (n = 3).

From these results it could be observed that the optimum lyophilization time for the two freeze-dried extracts was 120 hours.

Extraction yield

The yields of extractions are represented in histograms in figure 2 and 3. Optimal extraction yields are on average 33.46 ± 0.36% for *lannea microcarpa* in figure 2 and 26.77 ± 2.86% on average for *anogeissus leiocarpus* in figure 3. However, the decoction after 40 minutes seems to be the best time of decoction for *Lannea microcarpa* and after 30 minutes for *Anogeissus leiocarpus.* The analyzes revealed that extraction yields vary according to the extraction volume and are better with high volumes. The abundance of polar compounds in the two powders of plants could explain this phenomenon. Indeed, the literature reports that these two plants contain many polar secondary metabolites such as compounds phenolic[9].

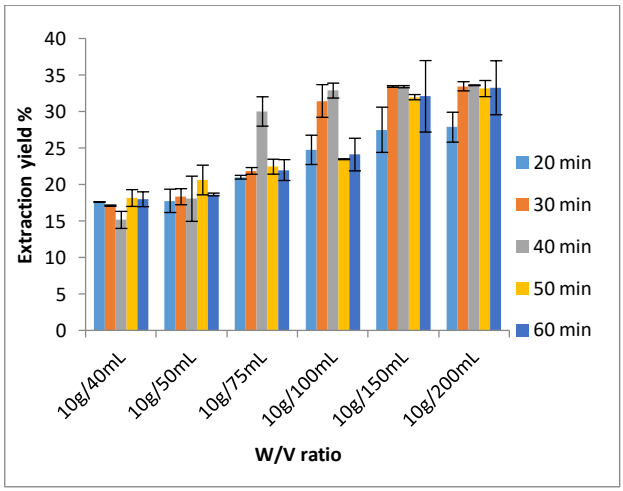

Figure 2: Extraction yield as a function of the mass / volume ratio (g / ml) and the decoction time (minutes = min) of *Lannea microcarpa*. (n = 3)

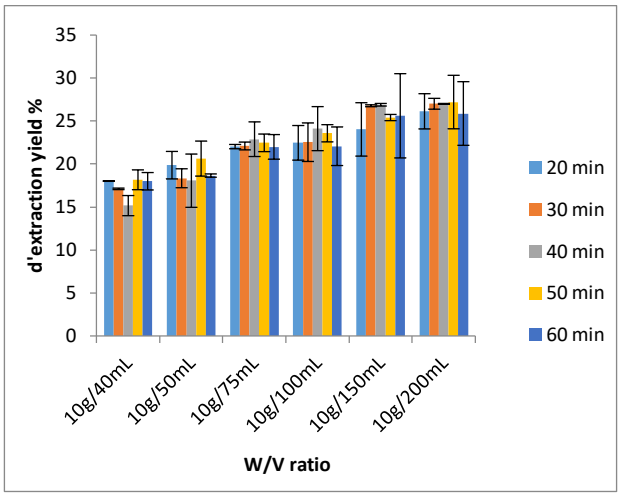

Figure 3: Extraction yield as a function of the mass / volume ratio (g / mL) and the decoction time (minutes = min) of *Anogeissus leiocarpus* (n = 3).

Microbiological quality

The assessments of the bacteria contamination of freeze-dried aqueous extracts of *Lannea microcarpa* were $1.1.10^4$ (CFU / g) total flora and $5.7.10^4$ (CFU/g) for freeze-dried aqueous extracts of *Anogeissus leiocarpus*. Thermo-tolerant coliforms and salmonella were not detected in any samples. These were in accordance with the recommendations of the European Pharmacopoeia 6.0. This indicates that the process used did not result in microbial contamination to the point of altering the quality of the extracts.

Chromatographic profile of extracts

The chemical analyzes carried out by TLC of the lyophilized extracts revealed the chromatographic fingerprints (Figure 4) which will serve as an identity for the lyophilized extracts. According to data from the literature, results obtained by TLC can be used for routine analyzes of freeze-dried extracts of *Lannea microcarpa* and *Anogeissus leiocarpus* in future preparations to control their quality[15].

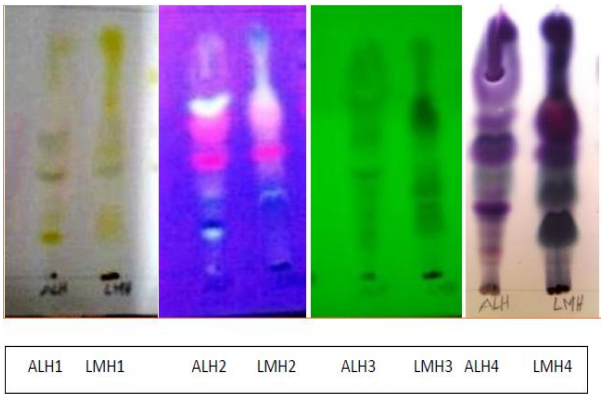

Figure 4: Photographic images of the fingerprints of the hexane fractions of *Lannea Microcarpa*. (LM) and *Anogeissus Leiocarpus*. (AL). ALH1 and LMH1: observation in daylight. ALH2 and LMH2: Observation under UV lamp at λ = 365 nm. ALH3 and LMH3: observation under UV lamp at λ = 254 nm. ALH4 and LMH4: after revelation anisaldehyde sulfuric.

pH

The pH in distilled water at 25 ° C. of *Lannea microcarpa* extracts was 7.04 ± 017 and 7.26 ± 038 for aqueous extracts of *Anogeissus leiocarpus*. A change in pH indicates that certain properties of the extracts are affected.

Dosage of phenolic compounds

Phenolic compounds Content of Lyophilized Extracts were 1.72 ± 0.04 (µGAE / mg) for *Lannea microcarpa* and 0.93 ± 0.02 (µGAE / mg) *Anogeissus leiocarpus*. These values are expressed as gallic acid equivalent (GAE) / mg of extract. In the specific case of cardiovascular disease treatment, numerous scientific studies have revealed a number of properties such as vasorelaxation from polyphenols[16, 17].

The calibration curve of the gallic acid (Figure 5) obtained has the equation: y = 29.07x + 0.020 (R^2 = 0.999). This curve was used for the determination of gallic acid in the samples.

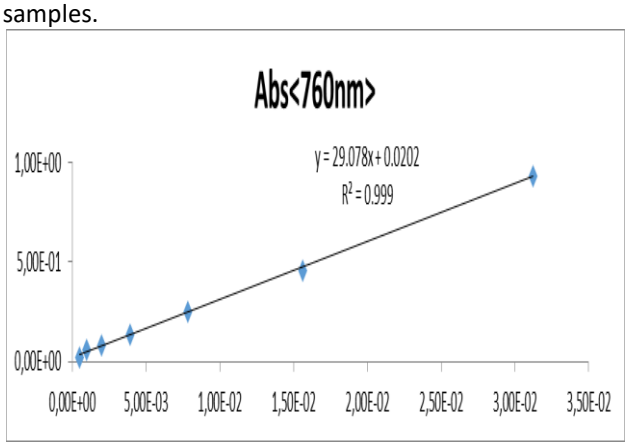

Figure 5: Standard line obtained with gallic acid

Pharmacotechnical characteristics

The results of the pharmacotechnical characteristics of the freeze-dried aqueous extracts of the two powders were interpreted according to the European pharmacopoeia and represented by Table 1.

Hygroscopicity of extracts

The moisture contents are all above 15%, indicating that the extracts studied of *Anogeissus leiocarpus* and *Lannea microcarpa* are microporous and very hygroscopic, the rehydration of which is almost instantaneous according to the European pharmacopoeia 6.0. The extracts can thus absorb the humidity of the air, by absorption or by adsorption during the manipulations at ambient temperature.

Flow rate

The flow times of the extracts are greater than 30 seconds and according to European Pharmacopoeia 6.0, they have a poor flow. The rheological properties orientate towards the choice of processes improving the

speed of flow of the extracts during the manufacturing process because they are likely to adhere to the walls of the equipment[18, 19]. These are techniques of improvement, such as the manufacture of pellets.

Table 1: Pharmacotechnical characteristics, n=3

Lyophilized extract	hygroscopicity (Percentage of moisture)	Flow (Time, seconds)	Compressibility Index	Solubility		pH in water, 25 ° C,
A. Leiocarpus	15.95 %	> 30	37.6 %	Easily soluble in water, very slightly soluble in ethanol 96 ° and absolute ethanol, practically insoluble in chloroform	Easily soluble In the buffers (pH 1.2 and pH 6.8)	7.04±017
L. microcarpa	17.42 %	> 30	50.76 %			7.26±038

Solubility

The two extracts are readily soluble in water, very slightly soluble in ethanol 96 ° and absolute ethanol and practically insoluble in chloroform. At the various pHs of 1.2 and 6.8, the extracts are readily soluble according to the classification of the European Pharmacopoeia 6.0. This study was necessary in order to define the conditions for the development of the in vitro dissolution method[20]. Indeed, the low solubility of an active principle in water can be problematic for obtaining in vitro results close to those in vivo.

Granulometry

The granulometric distribution of the extracts is represented by figure 6. The particle size intervenes in the physical and functional properties of a powder (flow, density, solubility, wettability, etc.)[21]. It determines the choice (granulometry) of the excipients to be used, as well as the rheological characteristics of the future formulation[22].

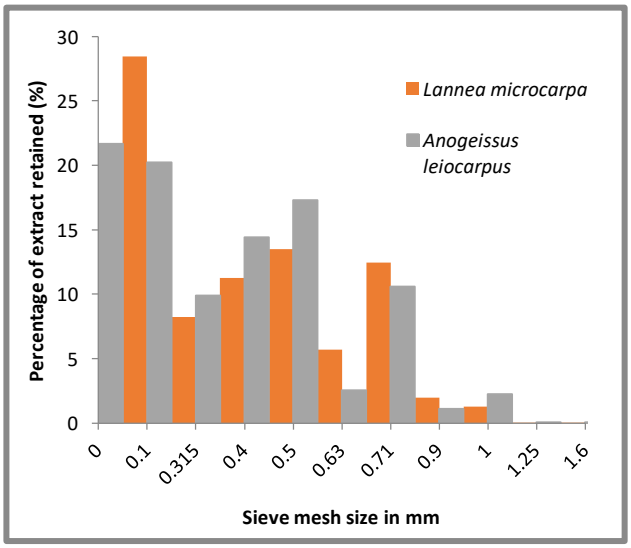

Figure 6: Particle size distribution of the extracts

Stability study

After six (06) months of storage at room temperature in the laboratory, the extracts retained the same colors, the same tastes and were free of mold. The pH of the extracts measured in distilled water at 25 ° C. remains around 7.

The contents of phenolic compounds are shown in Table 2. These results indicate a variation of approximately 4% within six (06) months of storage. These analyzes show a non-significant (p> 0.05) absence or presence of physicochemical alteration under the preservation conditions used. The tracer contents remain in the 95-105% range. This stability study has carried out in compliance with conditions[23].

Table 2: Phenolic Compounds Content After 6 Months of Storage Preservation at Laboratory Temperature (n = 3).

Designation	Extract *A. leiocarpus* (µGAE / mg)	Extract *L. microcarpa* (µGAE / mg)
Day 0	0.93164 ± 0.0219	1.71899 ± 0.0426
1 month	0.9164±0.036	1.70089±0.0611
3 months	0.9172±0.013	1.65135±0.0098
6 months	0.91005±0.095	1.63103±0.0324

CONCLUSION

The quality of a plant extract medicinal product depends on the extraction process because it determines the composition and the effectiveness of the finished products. This study gives the conditions necessary to obtain an optimum extraction yield for each extract. It makes it possible to produce extracts with identical processes, giving rise to homogeneous compositions, and therefore to equal results. The microbiological analyzes carried out show that the extracts are free from pathogenic flora. However, the physico-chemical and pharmacotechnical parameters indicate that both extracts are highly hygroscopic, readily soluble in water, very slightly soluble in 96 ° ethanol and absolute ethanol, practically insoluble in chloroform and readily soluble in buffers (pH 1.2 and pH 6.8). They have a poor flow according to the Eur Pharmacopoeia. These parameters of characterization and quality control make it possible to choose the dosage form, the choice of the manufacturing process thus and the excipients to be used.

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