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Standardization of *Newbouldia Laevis* Powdered Pulps

Standardisation des écorces de tronc de Newbouldia laevis

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RÉSUMÉ

Introduction. La standardisation, le contrôle de la qualité ainsi que l'intégration appropriée des techniques scientifiques modernes et des connaissances traditionnelles sont importants pour valoriser les remèdes traditionnels pour lutter contre certaines maladies chroniques. Pour une harmonisation mondiale des spécifications, les Directives de l'OMS pour l'évaluation de la sécurité, de l'efficacité et de la qualité des médicaments à base de plantes sont d'une importance primordiale. Différentes techniques impliquant des méthodes macroscopiques, microscopiques, physiques, chimiques et biologiques sont disponibles. **Méthodologie**. Dans le présent travail, des études pharmacognosique et phytochimique de la poudre des écorces de tronc de "Mbikam", scientifiquement connues sous le nom de *Newbouldia laevis, composante d'une recette traditionnellement utilisée pour les teignes,* ont été réalisées conformément aux spécifications de la Pharmacopée Européenne au chapitre 6.0 de la section 2.8. L'évaluation phytochimique et pharmacognosique participe de la standardisation : il s'est agi d'utiliser des procédures spécifiques pour tester la présence de différents métabolites secondaires d'intérêt (*par exemple, alcaloïdes totaux, acides phénoliques totaux, acides triterpéniques totaux, tanins totaux*), établissant ainsi des profils d'empreintes multiples et l'étude qualitative des constituants chimiques importants. **Résultats**. Les résultats obtenus ont donné les observations suivantes: les écorces dures, fibreuses à la fracture et la poudre était de couleur brune avec une odeur aromatique. Le pourcentage en cendres totales était égal à 2,74%, tandis que celui de la perte à la dessiccation était de 5,4%. L'indice d'inflation était de 5,7 mL. L'analyse micrographique a mis en évidence divers tissus à savoir le suber, les fibres, le liber, les cellules scléreuses et les oxalates de calcium. Le criblage phytochimique a révélé la présence d'alcaloïdes, de flavonoïdes, de polyphénols, de mucilages, d'anthocyanines et de saponines. **Conclusion**. Les résultats de la présente étude serviront de base à l'identification des marqueurs et des composés de référence liés à la poudre des écorces de tronc de *Newbouldia laevis*, ainsi qu'à la caractérisation de substances chimiques spécifiques à la poudre brute de l'écorce de cette plante.

ABSTRACT

Objective. Herbal drug technology is used for converting plant materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge are important to valorize traditional remedies for certain chronic diseases. For global harmonization WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines are of utmost importance, with different techniques involved macroscopic, microscopic, physical, chemical and biological methods. **Methods**. In the present work, according to European Pharmacopeia specifications in Chapter 6.0 of section 2.8, pharmacognosy and phytochemical study of *Newbouldia laevis* powdered pulps were performed. Phytochemical evaluation for standardization and where specific procedure are used to preliminary test the presence of different chemical groups of interest (e.g., total alkaloids, total phenolic, total triterpenic acids, total tannins), establishment of fingerprint profiles, multiple marker-based fingerprint profiles and qualitative study of important chemical constituents. For this end, a recipe containing the "Mbikam" pulps, scientifically known as *Newbouldia laevis* were choosen. **Results** obtained gave the following observations: hard pulps, fibrous crack and brown powder with a fragrant odor. The total ashes gave 2.74 %, whereas the lost weight during desiccation is 5.4 %. The inflation index is 5.7 mL. The micrography analysis shows various tissues such as suber, fibers, libers, sclerotic cells and calcium oxalates. The phytochemical screening revealed thatthe presence of alkaloids, flavonoids, polyphenols, mucilages, anthocyanins and saponins. **Conclusion**: Results of the present study will serve as a basis for the identification of the markers and reference compounds, related to powdered pulps of *Newbouldia laevis*, as well as the characterization of chemical specific substances related to the crude powder of the bark of that plant.

INTRODUCTION

Nowadays, there is a great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics [1]. In order to have an acceptable balance between the quality of raw materials, in processes materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modem instrumental method of analysis. Standardization is essential for measuring and ensuring the mastering of quality control processes for herbal drugs [2]. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular plant based medicines. Hence standardization is a tool in the quality control process [3]. It is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also means adjusting the herbal drug preparation to a defined content of constituent or excipients. It can be done also by mixing appropriate known herbal crude drugs or herbal drug preparations [4]. "Evaluation" of a drug means confirmation of its identity and determination of its quality, purity and detection of its nature of adulteration [5]. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Nevertheless, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample [6].

Standardization of herbal products can be done into two ways, firstly, active constituents extract, where biochemical principles are known and have therapeutic values, and secondly, a marker extract, where the active principle is not known and a characteristic compound is used as marker to assess the presence of other therapeutic biochemical compounds [7]. In this approach, World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for analyzing samples, quantification of their biomarkers and/ or chemical markers and the fingerprint profiles. If an active ingredient is known, it is most logical to quantitate it. When active ingredients are identified in plant based preparations, standardization is necessary. When the active ingredients are not identified, a marker which should be specific for the plant can be select for analytical purpose [8]. The authenticity, quality and purity of herbal drugs are established in terms of monographs documents converted into standardized therapeutic and clinical practice with references and numerical value given in pharmacopoeia [9].The aim of the present paper is to insure the valorization of traditional remedies in terms of a phytochemical evaluation for standardization and where specific procedure are used to preliminary test the presence of different chemical groups, quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins), establishment of fingerprint profiles, multiple marker-based fingerprint profiles and quantification of important chemical constituents [10]. For this end, a recipe containing the "Mbikam" pulps, scientifically known as *Newbouldialaevis* (P. Beauv.) Seem. ex Bureau (Bignoniaceae), and traditionally used in the treatment of dermatosis were choosen [11-17].

MATERIAL AND METHODS

Used chemicals

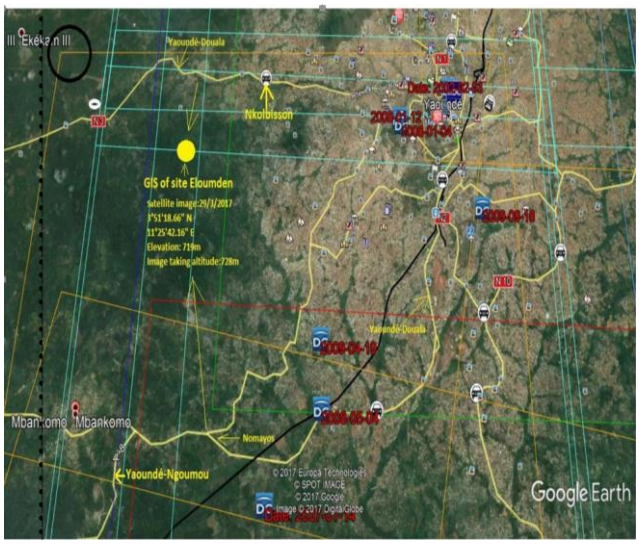
Potash, solution of soda, aqueous chlorhydric acid, picric acid, pure ethanol, alcoholic solution of ferric chloride, distilled water, concentrated sulfuric acid, chloroform, solution of iron chloride, acetic anhydride, and concentrated sulfuric acid.

The locality details of the plant material

The plant material used is the *Newbouldia laevis* truck pulps harvested of February 1, 2016, at Eloundem in the vicinity of Yaoundé. To have a possibility to access, the geographical site coordinates, given by satellite image taken on the 29th March 2017, was specified as follows:3°51 18.66"N;11°25 42.13"E; altitude is 719 m. A card of localization of the site is given in Presentation I.

Plant material

The plant material used is the *Newbouldia laevis* truck pulps harvested of February 1, 2016, at Eloundem in the vicinity of Yaoundé, Cameroon. Subsequently, the specimens were compared to the specimen 29469/HNC at the Cameroonian National Herbarium and properly identified



Presentation I: GIS location of the site Eloundem. Source: Google Earth, Satellite image 29/3/2017.

Hydro alcoholic extracts preparation

The *Newbouldia laevis* pulps were dry at ambient temperature for a week, subsequently, they were smashed and grinded into big granular. A part of the powder was used to performed macroscopic and microscopic characterization according to the WHO [18].Prescription for medicinal plants quality control, the Blond et Al [19] method was used for the microscopic. 250 g of the powdered pulps of the plants were weighted. An extract was performed using a Soxhlet using as a solvent a mixture of ethanol-water in a ratio volume of 70/30. Those extracts used in the phytochemical screening test.

RESULTS

Microscopic characterization

Investigation of foreign elements

Using the European pharmacopeia technique [20], 400g, of *Newbouldia laevis*, powdered pulps was spread on the thin film. No foreign elements were observed on the powder.

Powder description

It was of light-brown color, with an aromatic odor, we also notice the presence of fiber.

Microscopic analysis

The microscopic analysis of the *Newbouldia laevis* powdered pulps of the truck, showed the subers, libers, pericyclic fibers, clustered. These observations were optimal when potash was used [20, 21].

Phytochemical analysis

The weight loss due to desiccation of the vegetal drug s as well as the total content of ashes was estimated using the 6the edition of the European Pharmacopeia [20]. The loss due to desiccation was 5.4 %for the *Newbouldia laevis*. Additionally, the total percentage of ashes for the same plant was 2.74 %.

Inflation indices

The inflation index of the *Newbouldia laevis* powdered pulps was 5.7 mL, determined using the Guedouari method [22].

Phytochemical screening

Search for flavonoids

2 mL of extract was mixed with 2 mL of a solution of soda [23].Adeep yellow colorationwas observed.

Search for alkaloids

0.5 g of extract was added to 0.5 g of steam aqueous chlorhydric acid. Then, wereadded 3 to 5 drops of a solution of picric acid [24]. Turbidity was observed.

Search for polyphenols

0.1 g of vegetal extract in a water-ethanol mixture (1 mL of distilled water + 1 mL of pure ethanol)was dissolved in a test tube. Andthen added a drop of a 2 per cent alcoholic solution of ferric chloride [25]. A change of coloration to green or yellow-greenish was observed

Search for coumarins

0.1 g of extract was dissolved in 2 mL of distilled water, then added 2mL [23]. An orange coloration was observed.

Search for anthocyanins

0.1 g of extract was dissolved in 2 mL of distilled water in a test tube, we then added 2mL of concentrated sulfuric acid [23]. A red-orangish coloration that turns into purple bluewas observed.

Search for saponins

In a test tube with 5 ml of distilled water, 5 mg of extract was dissolved; the mixture was boiled for 5 minutes. After cooling, the content of each test tube was agitated for 15 seconds in the vertical direction, then we left the mixture at rest [26]. The apparition of persistent foam of thickness greater than a centimeterwas observed.

Search for terpenes

To 0.5 g of extract, added 2 mL of chloroform. Then added 3 mL of a concentrated solution of sulfuric acid. They were a formation of a layer [26]. A formation of a brown-reddish ringwas also observed.

Search for tannins

To 10 mL of distilled water, added 0.5 g of extract and boiled the mixture. Then, added few drops of a solution of iron chloride at 0.1 % [26]. A brown colorationwas observed.

Search for mucilages

4 mL of alcohol at 90°C was added to 2 mL of extract. Then agitated the mixture as described on [24], the development of air bubbleswere observed.

Search for steroids

2 mL of chloroform was added to 2 mL of extract. To that mixture, 1 ml of acetic anhydride as well as 2 drops of concentrated sulfuric acid [27]. A ring colored orangewas observed.

INTERPRETATION

Table 1: Macroscopic stem bark of the *Newbouldialaevis*pulps

Pulps from the	Sur	Frac	Pulps aspect
Newbouldialaevis	Hardface	Fibrousture	Greenish on the outer surface and reddish on the inner surface

Figure 1: Image of *Newbouldialaevis*(Left: outer surface, Right: inner surface)

Table 2: Microscopy of the *Newbouldialaevis*powder

Pulverized pulps	Color	Strength of the odor	Odor	Foreign elements
Newbouldialaevis	Brown	Distinct	Aromatic	No foreign element detected. Presence of an average quantity of fibers

The *Newbouldia laevis* powdered pulps were dark-brown (Table II). The fibers were observed with the naked eyes (Table II). This was later confirmed by the microscopic observation of the powder on figures3-5. According to the 6th edition of the European pharmacopoeia [20], the foreign elements were partially or totally made of foreign parts (The element originating from the mother-plants, but not a drug's constituent) or foreign matters (foreign element to the mother-plant from the vegetal and animal origin). No foreign element was observed to the naked eyes (Table II). This has demonstrated the good quality of the plant drug used.

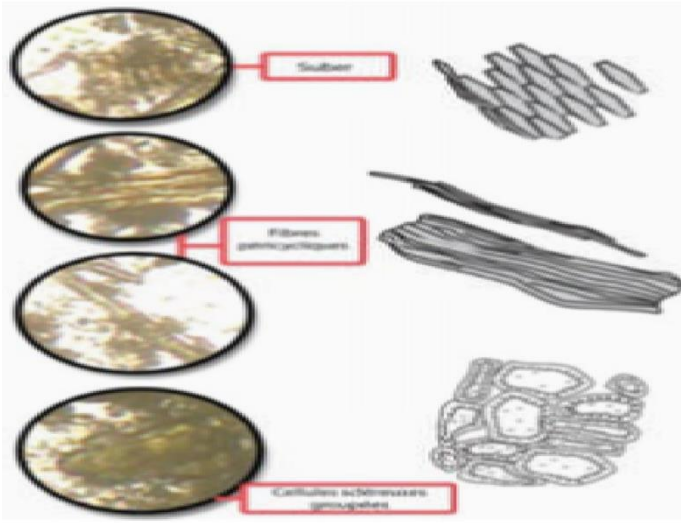


Figure 2: Micrography presenting from top to bottom (the suber, pericyclics fibers, and clustered sclerotic cells)

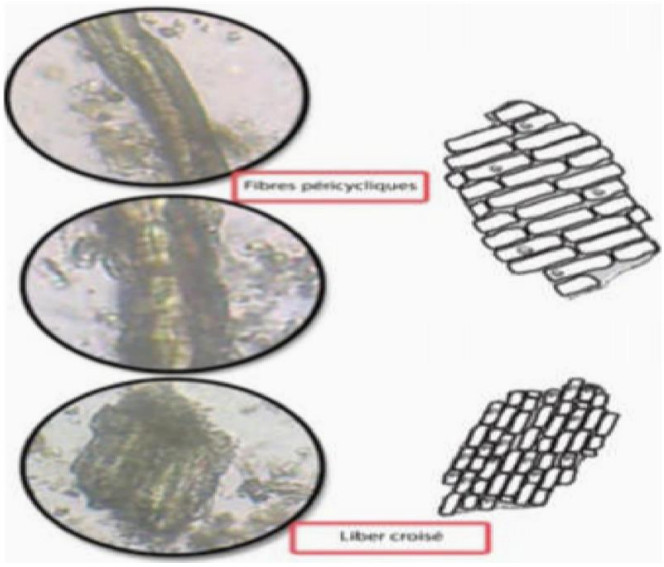


Figure 3: Micrography presenting from top to bottom pericyclic fibers and crossed liber

The microscopic analysis of *Newbouldia laevis* powder shows the crystal of calcium oxalate, the pericyclic fibers, spiral wooden vessels, the clusters of sclerotic cells and the suber (Figures 3-5). The predominant observation of fibers under a microscope confirms the previous observation made by the naked eyes. The observation was optimal when done using potash.

The total percentage of ashes in the *Newbouldia laevis* powder was 2.74 per cent, whereas the lost due to desiccation was 5.4 per cent. The inflation index of the plantpowdered pulps was obtained to be 5.7 mL. Table III shows the phytochemical composition of the hydro-alcoholic extract.

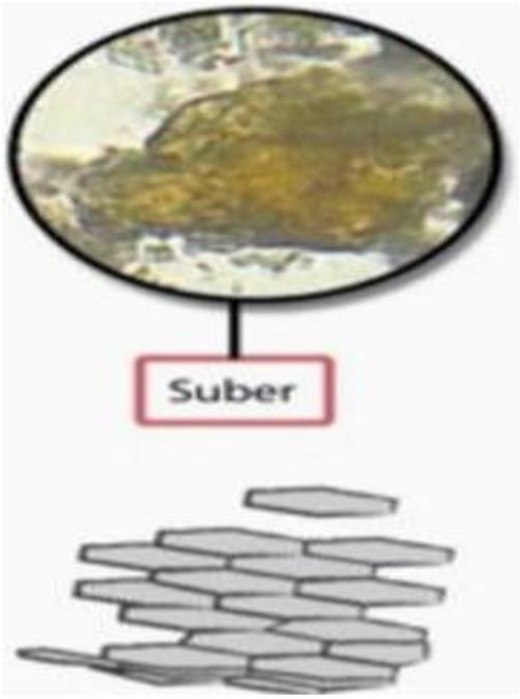


Figure 4: Micrography presenting a suber

Table 3: Phytochemical composition of pure extract

Phytochemical class	Hydro ethanolic extract
Alkaloids	+
Polyphenols	+
Flavonoids	+
ant ho cyanins	+
Saponins	-
Terpenes	++
Mucilages	+

+: Weakly positive reaction;
++: Averagely positive reaction; -: Negative reaction.

Studying the *Newbouldia laevis* hydro-alcoholic extracts, the presence of alkaloids, polyphenols, mucilages, flavonoids, anthocyanins showed in Table 3. The absence of saponins in those hydro-alcoholic extracts could be justified by the type of solvent used. According to Drut-Grevoz [28-30], saponins are soluble in water but not in alcohol. In fact, while using the 70/30 in a volume ratio of ethanol/water as a solvent during the extraction, there is a net reduction of polarity on the oxygen by induction effect, this affects the saponins solubility in the hydro-alcoholic mixture, hence, the absence of saponins in the hydro-alcoholic extract.

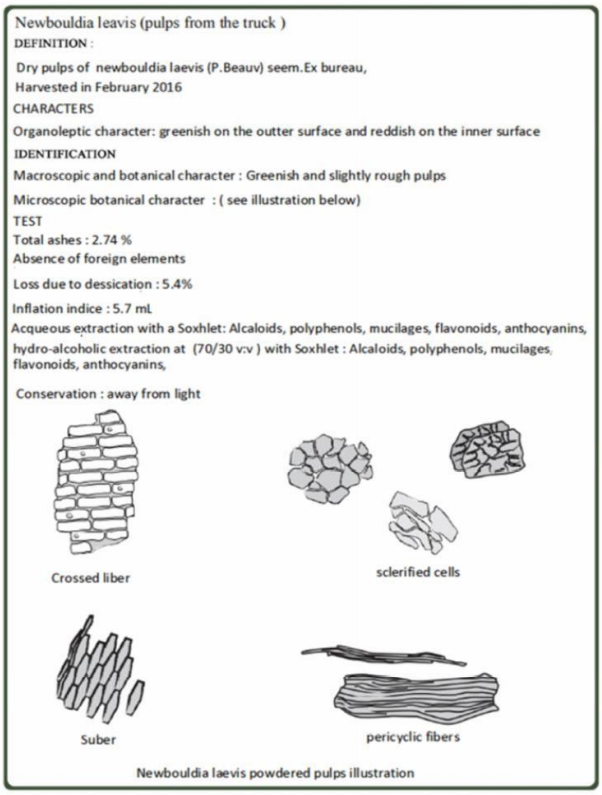


Figure 5: Monographyof*Newbouldialaevis*

CONCLUSION

The presentpaper presents applications of more advanced techniques of standardization. These applications were used as a rapid and specific tool in *Newbouldia laevis* researches. Methods taken into consideration are applied to set quality standards and specifications and to ensure the quality, safety, efficacy and all premises and practices employed the manufacturing and distribution of these product comply with Good Manufacturing Practice (GMP). Furthermore, the quality of the drug is improved. For this end, the aims of the present work are to perform a Pharmacognosy and phytochemical standardization of the *Newbouldia laevis* powdered pulps. The parameters obtained, contributed the elaboration of the powdered pulps of plantdata base for their identification. These data will be used for quality control. Using the makers of the powder, the data obtained will be used for quality control. That would lead to a decrease of fraud or contraband on the medicinal drugs such as *Newbouldia laevis.*

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