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Formulation and Evaluation of a Syrup Based on *Balanites aegyptiaca* L. Delile

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors Ouédraogo Sylvin, TA and KF designed the study. Authors Ouedraogo Salfo, TS, YJ, TTK and LM conducted the study. All the authors performed the analyzes and wrote the manuscript. They all contributed substantially to the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The kernel powder of the fruit of *Balanites aegyptiaca* (L.) Del is mixed with sorghum flour for the treatment of intestinal worms in Burkina Faso. Previous preclinical studies have demonstrated the efficacy and safety of the medicine.
Objective: The objective of this study is to execute galenic formulations based on extracts of the kernel of the fruit of *Balanites aegyptiaca* (L.) with good pharmaceutical quality.
Methods: The quality control was carried out for the freeze-dried extracts of kernels and the syrups were prepared thereon. Thin layer chromatography has been used to characterize the chemical constituents. Syrups were prepared from lyophilized aqueous extracts. Fifteen (15) test formulationswere prepared and the quality control of these samples helped to sort out only one best among them.
Results and Discussion: The results show that the syrups had a good appearance without

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sedimentation, it was easy to disperse and, it showed absence of *Salmonella* and coliforms. Stability studies showed that after two years' of storage, the syrups kept the same colors, taste and were free of mold. The chemical composition of the syrup was similar during the studied storage time. Assessments of bacterial contamination of syrups showed an absence of *Salmonella* and coliforms. After two years of storage they have maintained a good microbiological quality.
Conclusion: This study made it possible to ensure good quality of the syrups prepared. Parameters studied can be used routinely as elements of evaluation and quality control.

Keywords: Balanites aegyptiaca; formulation for worms; helminths; quality control; syrups.

1. INTRODUCTION

Intestinal parasites exist in all countries of the world,predominantly in the tropical zone [1]. Intestinal parasitosis caused by protozoa and helminths is a major public health problem in developing countries [2-5]. They are one of the leading causes of child morbidity and mortality worldwide [6]. It is estimated that about 3.5 billion people are infected with intestinal parasites worldwide, and 450 million of them are sick, the majority being children [7]. These parasitic intestinal infections mainly affect school-aged children in the poorest communities [8]. They affect the health and development of children and slow their growth, while reducing the productivity and work capacity of adults [9]. Intestinal parasites, especially helminths, also aggravate anemia in pregnant women [10]. However any intervention strategy that would be effective should be based on an understanding of the epidemiology of these parasitic diseases. Numerous studies already carried out in parasite pathology in Burkina Faso were based on the direct search for parasites in the stool, blood, urine, skin and also using immunological techniques. The results recorded had made it possible to describe the epidemiological profile of some parasitosis and their mode of treatment. Most of the drugs used in these treatments are based on active ingredients derived from chemical synthesis, often resulting in several side effects and often inaccessible. This leads a large part of the populations of underdeveloped countries to use traditional medicine as an alternative cure with satisfaction [11]. As a result of this unsatisfactory management, research is carried out in many laboratories to elucidate traditional recipe mechanisms using ethnopharmacological approaches to offer alternative or complementary herbal therapeutics. For example, in Burkina Faso, ethnopharmacological, toxicological, phytochemical and pharmacological studies have revealed anthelmintic properties of *Balanites aegyptiaca* (L.) Del. (Balanitaceae) [12,13]. This constitutes an interesting potential from the point of view of the treatment of digestive parasitoses. These preliminary studies have opened perspectives for the development of prototypes of galenic forms of phytomedicines for a clinical assessment of therapeutic activity. However, scaling up prototype phytomedicines requires a stable dosage formulation to ensure quality monitoring parameters during manufacturing. This study aimed galenic formulation and quality control of BALEG syrup in view of the clinical evaluation of the therapeutic activity.

2. MATERIALS AND METHODS

2.1 Materials

Fruits of *Balanites aegyptiaca* (L.) Del was harvested in November 2015 in Ouagadougou (Burkina Faso). The drupes were dried dust-free and then crushed to extract the kernels which were also dried before being pulverized by a blade mill. The powders was macerated with distilled water for 24 hours and then lyophilized and defatted. The extracts were kept in the Pharmaceutical Production and Marketing Unit (U-PHARMA) of the Health Sciences Research Institute (IRSS) in Burkina Faso. All chemicals, excipients and reagents used in the study were obtained from a standard supplier (FARGON, Belgium; PROLAB, France; MERK, Germany) and were of good pharmaceutical quality.

2.2 Methods

2.2.1 Quality control of extracts

Macroscopic and organoleptic characteristics: The organoleptic characteristics (taste and smell) were determined by tasting and sniffing the powder.

Determination of pH: The pH was determined by putting the pH-meter electrode (Eutech, Singapore) in 1% (w/v) aqueous solutions of each vegetable material (thrice).The test was

performed in triplicate and the mean and standard deviation were calculated (m ± standard deviation, n = 3).

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) gram of powder. The mean and standard deviation were calculated (n = 3, mean, standard deviation).

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 content was expressed as percentage.

Microbiological quality: The microbial load assayed were total microbial flora, *Salmonella* and thermo-tolerant coliforms. Total microbial 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).

Chemical Identification by Thin Layer Chromatography (TLC): TLC was performed to establish a fingerprint for the extract, syrups prepared instantly and stored after two years. A test portion of 2 g of each sample (powder, syrups) was dissolved in 15 mL of water and then partitioned with (10 mL x 3) of *n*-hexane and *n*-butanol and then concentrated in an oven at a temperature of 40°C. for 24 hours. 50 µL of each fraction is deposited on a TLC plate for the development of the chromatogram. The chromatography was developed over an 8 cm course in the solvent system *n*-hexane - ethyl acetate - methanol 7: 2 : 1. The revelation was made under ultraviolet light at λ = 365 nm.

Granulometry of powders: The granulometry was determined by the sieving method of the European Pharmacopoeia 6th edition [14].

2.2.2 Preparation and evaluation of syrup

Preparation of syrup: Fifteen (15) pré-formulations of syrups were made and the dry syrups were reconstituted by adding distilled water and left under observation (Table 1). The quality control studies allowed to retain a single formula qualitative and quantitative composition. The samples were prepared in triplicate using the mixture of the freeze-dried kernel extract of *B. aegyptiaca*, tween 60 as a wetting agent, xanthan gum as a viscosifying and stabilizing agent, sucrose as a sweetener, and sodium benzoate as a preservative. The formulations were stored at 25°C in the laboratory for two weeks.

Packaging of syrups: The choice of the packaging material for the syrup was made with 3 batches of the formulation retained in 3 different types of packaging (amber glass vial, transparent plastic bottle, amber plastic bottle) and kept for two (02) years.

Evaluation of syrup formulation: The syrups were checked from the dates of preparation. The controlled parameters were physical and organoleptic characteristics (color, odor, and flavor), pH, density and other parameters such as fermentation, the microbial quality control and fingerprint by thin layer chromatography (TLC).

Density of a syrup is expressed by the ratio of its mass and volume. Ten (10) mL of syrup from each syrup vial was used to fill a previously weighed 10 mL volumetric flask and average mass was calculated. The difference between this weight and the empty weight of the volumetric flask allowed the average mass of syrup to be given. The mass of the syrup was related to the volume of the syrup, which made it possible to calculate the density.

The fermentation of the syrup is recognized by the formation and the proliferation of molds on the surface of the syrup. The observation was made with naked eyes.

Stability studies: Stability was checked from the dates of preparation and were carried out with optimized formulation (F2) according to the International Conference on Harmonization (ICH) guidelines [15]. The samples were stored in closed molded Amber Glass Bottle Type III bottles and kept for two (02) years 1 month (Fig. 7). Its were removed and evaluated at intervals of 1, 10, 30, 50, 60, 70 days and then every 3 months up to 2 years and 1 month. The control parameters were physical and organoleptic characteristics (color, odor, and flavor), pH, density and other parameters such as microbiological quality, fermentation, stability during storage, the absence of agglutination over time and the fingerprint by thin layer chromatography (TLC).

Table 1. Qualitative and quantitative compositions of excipients

Ingrédients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Tween 60	0.05%	0.05%	0.05%	0.05%	0.05%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%
Gomme xanthane	0.1%	0.2%	0.3%	0.4%	0.5%	0.1%	0.2%	0.3%	0.4%	0.5%	0.1%	0.2%	0.3%	0.4%	0.5%
Saccharose	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%	45%
Benzoate de Na	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Water (Qsf)	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL

Qsf: In sufficient quantity to make

3. RESULTS AND DISCUSSION

3.1 Quality Control of Raw Materials

The macroscopic and organoleptic characteristics study of the freeze-dried kernel powder of *Balanites aegyptiaca* revealed red particles (Fig. 1), mild odor with a sour taste. The organoleptic and macroscopic characteristics found in the extract can be useful for the quality control and the identification of the raw material [16]. The residual moisture content was 7.32 ± 0.08 meaning the extracts were adequately dry and could be kept for a long time without the development of mold or yeast [17]. The microbial quality control showed absence of *Salmonella* and thermotolerant coliforms in sample extract. The absence of specific pathogenic germs such as *Salmonella* and the low presence of total microbial flora confirmed a good microbial quality of extracts [18]. This quality is in accordance with the recommendations of the European Pharmacopoeia 9th edition of natural raw materials administered by oral route [19].

Fig. 1. Freeze-dried kernel extract of *Balanites aegyptiaca*

3.2 Granulometry of Powders

The granulometry determined by the screening method of the 6th edition of the European Pharmacopoeia showed results ad presented in the Fig. 2.

The powders obtained with the 1 mm to 0.1 mm sieves were used for the preparation of the syrups.

3.3 Evaluation of Syrups

Syrups of formulation F2 had the best appearance without deposit, yellowish in color, with a faint odor and a sweet taste (Fig. 3). The density at the preparation was 1.067 and the pH was 4.9. The formulation F1 had compact sediments and was difficult to disperse. Formulations F3 to F15 showed agglutination after one week of storage.

Three lots of the selected F2 formulation were packaged in 3 differents types of packaging (lot 1 with amber glass bottle, lot with 2 clear plastic bottles, lot 3 with amber plastic bottle). The results of the pH measurements showed a slight variation in the pH of the 3 batches during the first 2 months of storage (Fig. 4). After this time the pH remained without significants variations until 2 years 1 month. The results of measurements of the density of lot 1 and 2 showed variations during the preservation period (Fig. 5). Only lot 3 did not experience significant variation up to 2 years 1 month. Its average density remained 1. 06 during the entire period of conservation.

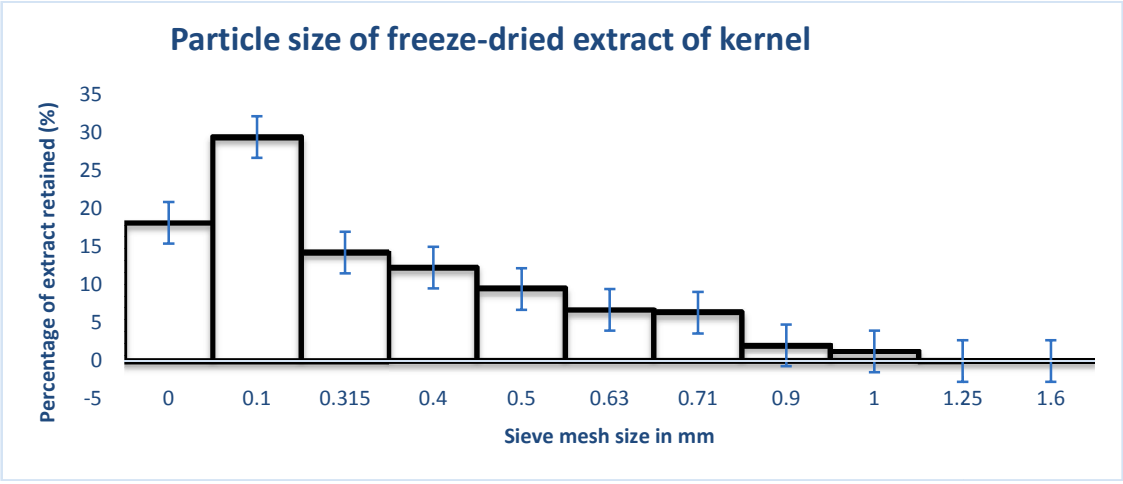

Fig. 2. Particle size distribution of the extracts

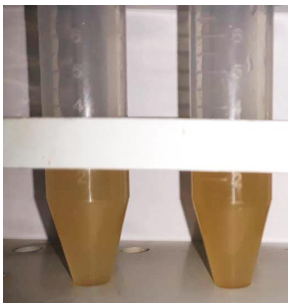

Fig. 3. Formulation F2

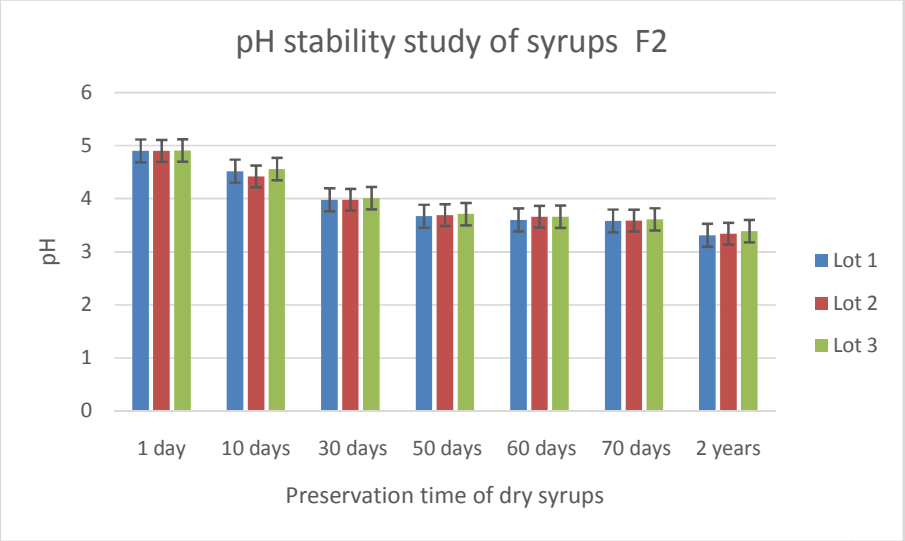

Fig. 4. pH variation of syrups as a function of time

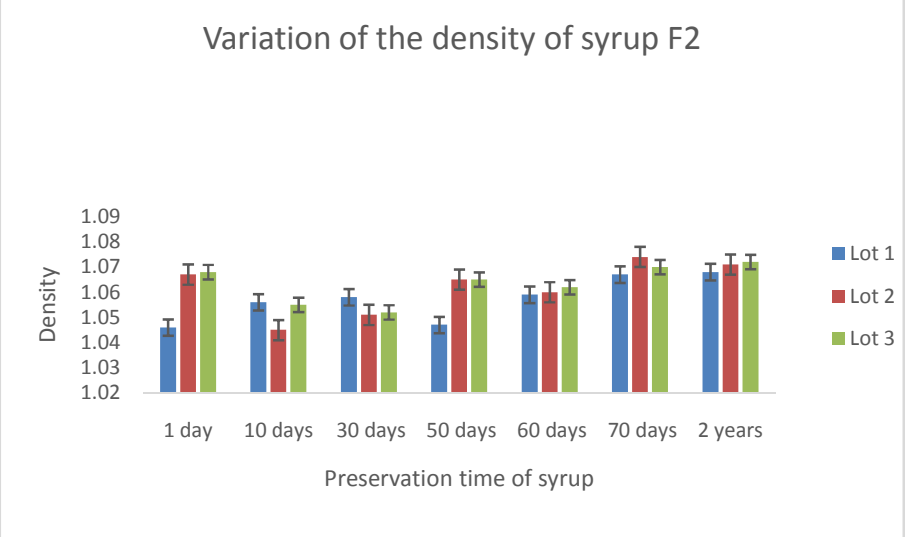

Fig. 5. Density variation of syrups as a function of time

3.4 Chemical Fringerprinting by Thin Layer Chromatography (TLC)

The fringerprint analyzes carried out by TLC of the lyophilized extracts (lot 1), syrups at the date of manufacture (lot 2) and after storage for 2 years 1 month (lot 3) revealed the chromatographic fingerprints (Fig. 6) which will serve as an identity for the quality control. According to data from the literature, results obtained by TLC can be used for routine analyzes of pharmaceutical [20]. The chromatographic profiles show similar spots between the lyophilized extracts used as the active ingredient and the syrups at the date of manufacture. This indicates that there is no incompatibility between the selected excipients and the active ingredient so as to destroy certain chemical groups. Indeed excipients must be inert in respect of the active ingredient, packaging materials and the human body [21].

3.5 Stability Study

After two years of storage, the syrups kept the same colors, the same tastes and were free of mold. The average pH of the syrups measured in distilled water at 25°C was 3.346 ± 0.028. The density remained constant around 1, Its average value was 1.0703 ± 0.0015. These analyses showed insignificant physicochemical alteration in the storage conditions used. Assessments of bacterial contamination of syrups showed absence of *Salmonella* and coliforms. These results are in line with the recommendations of the European Pharmacopoeia 6.0 for oral preparations containing naturally occurring raw materials [14,22]. This indicates that the syrups have maintained a good microbiological quality during the shelf life and under the conditions studied. The chromatographic profiles show similar spots between the syrups at the date of manufacture and the syrups kept for two years (Fig. 6). This means that the chemical composition of the syrup after two years of storage under the conditions studied was always similar and therefore homogeneous composition. The syrup could be stored for two years under the same conditions.

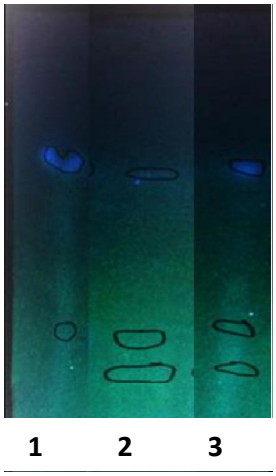

Fig. 6. Fingerprints of *n*-hexanic extracts of the active ingredient, syrup at the date of manufacture and syrup after two years of storage by TLC; Eluent: *n*-Hexane - ethyl acetate - méthanol 7:2:1

Revelation under ultraviolet lamp at λ = 365 nm

Fig. 7. Syrups stored in closed molded Amber Glass Bottle Type III bottles

4. CONCLUSION

This study was carried out with the aim of offering a therapeutic alternative for the management of parasitic diseases. The results showed that the syrups made had a good appearance without sediment formation and easy to disperse. they had good microbial quality. After two years of storage the phisicochemical characteristics (pH, Density), and the chemical composition on thin layer chromatography was without significant variations. The syrups were of good pharmaceutical quality and could be stored for two years under the conditions used during the clinical confirmation study in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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