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Quality control and standardization Of faca ® syrup

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Abstract

Sickle cell disease is a major public health problem. It is the first genetic disease in the world. FACA syrup offers an alternative treatment. It is a dry powder preparation of two components, the roots barks of *Zanthoxylum zanthoxyloides* Lam. (Rutaceae) Zepernick, Timler and *Calotropis procera*. Ait. R.B.r. (Asclepiadaceae). The product was developed at Institute for Research in Health Sciences (IRSS) from a traditional recipe used in Burkina Faso for treatment of sickle cell crises. This study aimed to establish physical-chemical, pharmaco technical and microbiological control parameters essential for the standardization of the phytomedicine. This valuation concerned specifications of moisture content, pH, the fingerprint by thin layer chromatography, pesticide residues, heavy metal content, microbial quality, and total ash. These charcteristics were determined by the methods prescribed by the World Health Organization (1998) and the European Pharmacopoeia 6th edition. The results have shown that dry syrups and reconstituted syrups were sweet, slightly spicy with a bitter after taste, a white room color and a faint odor. The density at the preparation was 0.985 and the pH was 5.93. After 2 months of storage in the laboratory, the organoleptic parameters of the reconstituted syrups have not changed. They were mold free, the density remained around 1 and the pH between 5 and 4. These parameters have shown that the quality of plants powders and this medicine comply with the recommendations of the European pharmacopoeia. Faca syrup may contribute to the better management of sickle cell disease in children.

Keywords: FACA syrup, sickle cell disease, plant powder, medicinal plant, quality control.

Introduction

Sickle cell disease is a major public health problem. It is the first genetic disease in the world [1] and affects about 4% of the world's population. In Africa, prevalence is estimated between 5% and 7% with highest frequency in sub-Saharan [2]. In the United States, the disease affects more than 70,000 African Americans. In Central Africa and Madagascar, 20 to 30% of the populations are healthy carriers of the sickle cell disease gene [3]. Each year, nearly 2% of newborns are affected by the disease and 50-75% die before the age of 5 [4, 5]. Optimizing treatment is a major challenge for any health program [6,7]. Thus, using an approach based on ethnopharmacology, the Department of Traditional Medicine and Pharmacopoeia of the Institute of Research in Health Sciences has demonstrated the antifalcemic properties of the combination of two plants *Zanthoxylum zanthoxyloides* and *Calotropis procera* with clinical confirmation in humans [8, 9]. This phytomedicine in the form of capsules obtained its marketing authorization in 2010 and is listed in the list of essential drugs in 2011 in Burkina Faso [2, 10]. Through the Phytomedicine Production Unit (U-PHARMA) this pharmaceutical form is marketed in many countries around the world. It has earned several innovation awards including the first prize of the International Forum of Inventions and Technological Innovations in 2011 in Niger and the 10th National Forum for Research and Technological Innovation in 2012 in Burkina Faso Faso. However the administration of capsules by infants being difficult or impossible, a standardization study of the root bark powder of *Zanthoxylum zanthoxyloides* for the production of pediatric anti- sickle cell phytomedicine was carried out [11, 12]. To ensure uniformity of all batches of the drug in syrup form. It is necessary to establish an appropriate standard for identity, purity, content, behavior and other characteristics. It is the strict observance of these standards that makes it possible to obtain the desired quality. This study aimed at galenic formulation and quality control in view of the standardization of FACA syrup.

Materials and Methods

Raw materials and syrups

Raw materials were root bark powders from *Zanthoxylum zanthoxyloides* (code: Ts) and *Calotropis procera* (code : Cp). Three (03) batch of dry syrup made from a mixture of the two powders were used for this study (code : Fs1; Fs2; Fs3). The powders were

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collected in the Pharmaceutical Production and Marketing Unit (U-PHARMA) of the Institute for Research in Health Sciences (IRSS) in Burkina Faso.

Chemicals, excipients, reagents and instruments

All chemicals, excipients and reagents used in the study were obtained from a standard supplier and was of good quality.

Quality control of the mixture of raw materials

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

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. 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).

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).

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 percentage.

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

Heavy metal content: The heavy metals sought (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).

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

Galenic formulation

Several formulations were made and the dry syrups were reconstituted by adding distilled water and left under observation. The stability studies allowed to retain a single formula qualitative and quantitative composition. The samples were prepared in triplicate using the mixture of *Zanthoxylum zanthoxyloides* and *Calotropis procera*, Tween 80 as a wetting agent, Xanthan gum as a viscosifying and stabilizing agent, Sucrose as a sweetener, and Sodium benzoate as a preservative.

Quality control of syrups

The syrups were checked from the dates of preparation. The control parameters are physical and organoleptic characteristics (color, odor, and flavor) of dry syrup, residual moisture content, pH, density and other parameters such as fermentation, stability during storage and fingerprint by thin layer chromatography (TLC).

The density of syrup is expressed by the ratio of its mass and volume. We took 10 mL of syrup from each syrup vial 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 the naked eye.

Chemical Identification by Thin Layer Chromatography (TLC)

The MeOH extracts were obtained by mixing 3 g of powder of each sample with 45 mL of methanol, followed by maceration with stirring from time to time for 1 hour. The extracts were then filtered through cotton and dried using a rotary evaporator. Each dry extract was solubilized in methanol and deposited on a HPTLC Silica gel 60F254 10 10 cm plate for the development of the chromatogram. Chromatography was developed over a 8 cm course in the solvent system Toluene-ethyl formate-formic acid (4: 3: 1); The revelation was made under ultraviolet light at λ = 365 nm.

The stability of syrup results in the constant over time of different starting parameters. Stability was controlled by tasting and observation with the naked eye. The choice of the packaging material for the syrup was made by conducting: - 3 batches of dry syrup that are packaged in 3 different types of packaging (amber glass bottle, amber plastic bottle, transparent plastic bottle) and kept for three (03) years. - 3 batches of syrup reconstituted by adding water and packaged in 3 different types of packaging (amber glass vial, amber plastic bottle, transparent plastic bottle), kept for seventy

(70) days. The chemical quality control of the syrups was carried out by thin-layer chromatography.

Results and Discussion

Quality control of the mixture of raw materials

Table 1 shows the physico-chemical characteristics of the powder of the mixture of *Zanthoxylum zanthoxyloides* and *Calotropis procera*.

Table 1: Physico-chemical characteristics of the powder of the mixture

Designation	Macroscopic and organoleptic characteristics	pH (1%)	Residual moisture content (%)	pesticide residues (mg/kg)	Total ashes (%)	Microbiological Control (CFU / g)		
						Thermo-tolerant Coliforms	Total flora	Salmonella / 25g
Mixture	whitish and brown particles, little odoriferous, bitter flavor with a sensation of tingling and anesthesia on the tongue.	5,4 ± 0,06	8,01 ± 0,06	< 0,02	5,5 ± 0,35	Absence	$3,2.10^4$	Absence

The organoleptic and macroscopic characteristics found in the powder of the mixture will be useful for the quality control and the identification of the raw material. The water content, less than 10%, is good for conservation of vegetable powders. This means the extracts are adequately dry and could be kept for a long time without the development of mold or yeast [13]. The residual levels of pesticides in the powders of trunk barks (Table 1) were lower than the limit values set by European Pharmacopoeia 6.0, European Community Directives 76/895 and 90/642 [14] , including their Appendices. The microbial quality (Table 1) of the powders was in accordance with the recommendations of the European Pharmacopoeia 6th edition of natural raw materials administered by oral route. The absence of specific pathogenic germs such as Salmonella and the low presence of total flora confirms a good microbial quality of the vegetal powders [15].

Heavy metal content

Table 2: Heavy metal content.

	Content in ppm (RSD (%))			
Samples	Arsenic	Mercury	Cadmium	Lead
FACA® Poudre	0,85(3,4)	≤ LD	0,22(1,9)	0,255(1,9)

The heavy metal content (Table 2) were 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 that are at risk of releases [16].

Granulometric of powders

The granulometry determined by the screening method of the 6th edition of the European Pharmacopoeia has results presented in the table 3.

Table 3: Particle size of the mixture

Sieve mesh sizes (µm)	Receptacle 0	100	200	315	400	500	630	700	800
Mass of powder retained by sieve meshes	21,35	24,5	18,53	16,24	12,11	4,1	1,17	1,3	0,7
Cumulative Frequency (%)	21,35	45,85	64,38	80,62	92,73	96,83	98	99,3	100

Quality control of syrups

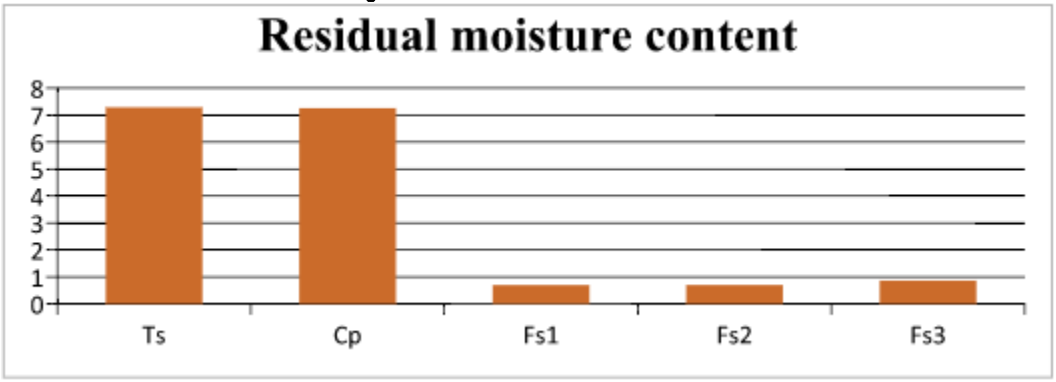
Dry syrup powders are amorphous, yellowish-white in color, with a faint odor and a sweet taste. The batch of dry syrup packaged in an amber glass bottle was retained for the rest of the study because no bottle experienced any change in the characteristics of the powder under the preservation conditions tested.

The reconstituted syrups had good appearance without deposit, sweet taste, slightly spiced with a bitter aftertaste, a white room color and a faint odor. The density at the preparation was 0.985 and the pH was 5.93.

Residual moisture content

The residual moisture content, less than 10%, is good for preservation of dry syrups.

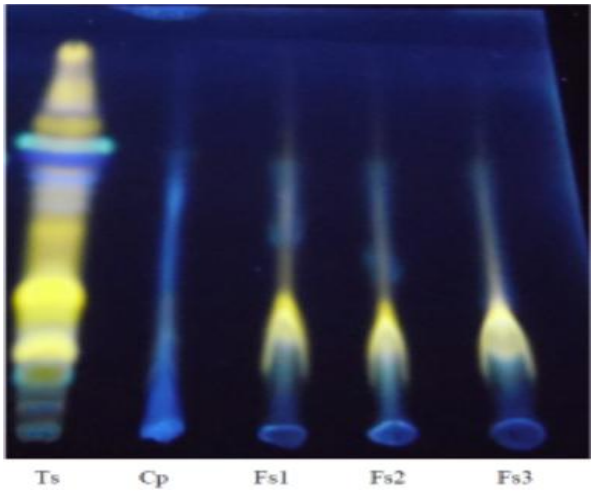
Figure 1 : Residual moisture content.



Chemical Identification by Thin Layer Chromatography (TLC)

The fingerprints of methanol extracts by thin-layer chromatography of raw material powders and syrups are shown in figure 2

Figure 2: Fingerprints of methanolic extracts by TLC; Eluent: Toluene-ethyl formate-formic acid (4: 3: 1); Revelation under ultraviolet lamp at λ = 365 nm



The chromatographic profiles shows that the spots of the different batches of Faca syrup are similar. This indicates that the batches have homogeneous composition.

Stability study

After seventy (70) days of storage the syrups have retained their taste and odor. There was no mold formation. The density remained constant around 1 and the pH between 5 and 4 (table 4).

Table 4: Stability of syrups

Batch	Day 1		Day 10		Day 30		Day 50		Day 60		Day 70	
	pH	Densité	pH	Densité	pH	Densité	pH	Densité	pH	Densité	pH	Densité
Fs1	5,88	0,991	4,60	1,065	4,28	1,077	4,01	1,067	3,91	1,083	3,83	1,073
Fs2	5,92	0,964	5,20	1,059	4,52	1,082	4,42	1,085	4,10	1,071	3,95	1,067
Fs3	6,00	1,00	5.63	1,066	4,31	1,074	4,00	1,058	4,02	1,065	4,01	1,073
Average	5,93	0,985	5,14	1,063	4,37	1,077	4,14	1,070	4,01	1,073	3,93	1,071
Standard deviation	0,040	0,014	0,3	0,002	0,090	0,002	0,180	0,010	0,060	0,006	0,060	0,002

Conclusion

FACA is currently used in the treatment of sickle cell disease in capsule form. Syrup has a therapeutic interest in the treatment for sickle cell disease in infants. This study made it possible to ensure the good quality of the syrups manufactured. The parameters studied will be used routinely as elements of the evaluation and the guarantee of quality. Production of pilot lots of syrups is ongoing. These lots will be used to evaluate the tolerance and efficacy of syrups before it is granted marketing authorization.

Acknowledgments

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