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Full Length Research Paper

Some basic requirements for preparing an antisickling herbal medicine -NIPRISAN®

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NIPRISAN®, an antisickling phytomedicine for the management of sickle cell crises, was developed at the National Institute for Pharmaceutical Research and Development (NIPRD) from a traditional medicine used among the Yoruba people of Nigeria, for the treatment of "abiku" or "ogbanje" – a condition characterized by high frequency of ill health, painful crises, jaundice and childhood death. The product is prepared from the seeds of *Piper guineense*; flower buds of *Eugenia caryophyllata*; stem parts of *Pterocarpus osun*; leaf stalk of *Sorghum bicolor*; and trona - a solid mineral. The five starting materials are normally sourced from their natural habitats, and procured as such, from local food stalls or herbal medicine dealers. This paper describes and quantifies as per WHO (1998) and BP (2004), the most striking physicochemical characteristics of these materials; and demonstrates that all, except *E. caryophyllata*, exist in more than one variety, differing significantly in either loss on drying alone, or in both total ash and water extractable matter. The results, including the occasional presence of lead in the trona samples, are discussed in the context of production according to good manufacturing practice (GMP).

Key words: NIPRISAN®, starting materials, characteristics, antisickling, herbal medicine, chemical-manufacturing-control.

INTRODUCTION

Since the Alma-Ata Declaration of 1978 (WHO, 1978), by the World Health Assembly, promoting Traditional Medicine worldwide, herbal and other traditional pharmacologic therapies have steadily gained ground throughout the globe. Such was the state of affairs in the late 1990's when Niprisan was developed from a traditional recipe by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Niprisan is a multicomponent product of four herbal substances and a solid mineral. An herbal substance is defined as material derived from plant by extraction, mechanical manipulation, or some other process (WHO, 2005b). In herbological parlance each herbal substance in its entirety is regarded as the active substance, even though the constituents may be a group of chemically defined entities acting cooperatively to achieve the pharmacological attribute of the medicinal plant (Bandaranayake, 2006).

Chemical- manufacturing-control considerations for phy-tomedicines or herbal products, defined as herbal mate-rial administered to clinical subjects (WHO, 2005a), such as Niprisan; stem from, and focus on the fact that herbal substances are prone to contaminations by herbicides, pesticides, mycotoxins and others; and are subject to profound variations in physicochemical characteristics, such as loss on drying, extractability and others. Such considerations are even more acute for mu-lticomponent products like Niprisan. The identity, geography, culti-vation, harvest, contaminations, process history and physicochemical characteristic of the herbal substances are critical to CMC considerations, if good manufacturing practice (GMP) is to be applied in the production of the phytomedicine of interest.

The traditional recipe, from which Niprisan was deve-

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loped, is an infusion of the seeds of *Piper guineense*; flower buds of *Eugenia caryophyllata*; stem parts of *Pterocarpus osun*; and the leaf stalk of *Sorghum bicolor*, in a local gin, "ogogoro", containing trona. The recipe has been in use for ages in the treatment of sickle cell crises among the Yoruba people of Nigeria. Niprisan, whose profile has been on the rise, is patented and currently been produced by Xechem Pharmaceuticals Limited, under license from NIPRD, to meet increasing global demand by sufferers of sickle cell disease (SCD). The aim of this study is to describe and quantify the salient physicochemical characteristics of the five starting materials procured from established local sources. These characteristics are considered most relevant to achieving a batch to batch uniformity as required in GMP production.

Sickle cell crises are the manifestations of SCD, an inherited red blood cell disorder that has an interesting but alarming statistics. About 89% of sicklers worldwide are in Africa, of which Nigeria alone constitutes 25%. Europe has the least, 0.1%, while the US, Asia and the Mediterranean have 3.5% each (Awosiyan, 2006). It is estimated that about 5% of the world population are carriers of a trait gene for sickle-cell disease or thalassemia; and that the percentage of people who are carriers of the gene is as high as 25% in some regions (WHO, 2006). SCD crises are characterized by pains and injuries to internal organs, leading to frequent infections (Steinberg, 1999). In the US, where there were about 75,000 SCD sufferers in 2003, the disease was associated with a cost of about $475 million (Pandey, 2003). With such figures for the US alone, it is evident that the cost of the disease globally is immense. Many types of treatments have been advocated for SCD (Steinberg, 1999), but have not been cost effective. However, the availability of Niprisan in the late 1990's brought a great deal of hope for SCD sufferers in Nigeria and Overseas.

The biochemistry and pharmacology of Niprisan have already been described (Awodogan et al., 1996; Gamaniel et al., 1998). The product inhibits sickling and reduces the frequency and severity of SCD crises in about 70% of patients in phases II and III clinical trials (Wambebe et al. 2001a, b; Cordeiro and Oniyangi, 2006). Niprisan had in various publications been called NIPRD 94/002/1- 0 or Nix-0699 by workers in the US (Iyamu et al., 2003; Gillette et al., 2004). The product is currently in high demand in Nigeria, India and the US, where it was granted Orphan Drug status by the US Food and Drug Administration (Pandey, 2003). In 2005, the European Medicine Evaluation Agency also approved Orphan Drug status for Niprisan (Waknine, 2005). The demand for Niprisan has been on the rise ever since, hence the need to develop specifications for the materials for its production. In the present study some of the more decisive specifications relating to foreign matter (FM), loss on drying (LOD), total ash (TA), water extractable matter (WEM) and others are determined.

EXPERIMENTAL

Sampling of Materials

Sampling, carried out as per WHO (1998) consisted of the following steps:

Three (3) original samples from each container were drawn from the top, middle and bottom.
The 3 original samples were combined into a pooled sample and mixed carefully.
The average sample is obtained by "quatering" the pooled sample.

Quatering consists of the following steps:

Each pooled sample above was mixed thoroughly, and constituted into a square-shaped heap.
The heap above was divided diagonally into 4 equal parts. Any 2 diagonally opposite parts were taken and mixed carefully.
The above step was repeated as necessary until the required quantity of sample was obtained.
Any material remaining was returned to the batch.

Final samples and treatment of samples

Final samples were obtained from an average sample by quatering, as described above. This means that an average sample gave rise to 4 final samples. Each final sample was divided into 2 portions. One portion was retained as reference material, while the other was tested. Thus, for each determination carried out on an average sample, there were 4 test results.

The plant materials

Consignments of the seeds of *P. guineense* (L.), Schum., & Thonn., (Piperaceae) and the flower buds of *E. caryophyllata* (L.),Thunb., (Myrtaceae) obtained locally from their natural habitats, were purchased from food stalls in the market, while the stem parts of *P. osun* (L.) Craib. (Fabaceae) and the leaf stalk of *S. bicolor* (L.) Moench, (Poaceae) also obtained locally from their natural habitats, were purchased from herbal medicine dealers. All the materials were identified and confirmed by the Institute's Ethnobotanist, Mallam Ibrahim Muazzam of the Department of Medicinal Plant Research. The materials were sampled and tested for foreign matter (FM) content, loss on drying (LOD), total ash (TA) and water-extractable matter (WEM) as prescribed by WHO (1998). Representative samples were archived in the Institute's Herbarium. The Herbarium numbers of the plant materials are: *P. guineense* – NIPRD/H/5386; *E. caryophyllata* – NIPRD/H/5371; NIPRD/H/5366; *P. osun* – NIPRD/H/3672.

The solid mineral

All consignments of trona obtained locally from their natural habitats, were purchased from food stuffs in the market. They were identified based on appearance, solubility, taste and reaction with dilute acids, and sampled as stated for the herbal components. Some cationic constituents (magnesium, manganese, zinc, copper and lead) were identified and quantified as per BP (2004), with necessary modifications. Representative samples were archived as described for the herbal materials.

Computation of results and statistical analyses

The results are expressed as mean ± standard deviation or as a

Table 1. Macroscopic and organoleptic characteristics of the five starting materials of Niprisan®.

Component	Description
P. guineense	Seeds; dry, dark brown and spherical, 3 – 8 mm diameter; 20 – 60 mg in weight. The milled sample is gritty and slightly lachrymatory; Odour, aromatic and characteristic
E. caryophyllata	Flower buds; dry, brown and nail shaped, 10 – 15 mm in height; 60 – 110 mg in weight. The milled material is gritty and slightly lachrymatory; Odour, aromatic.
P. osun	Stem parts; red and woody material, odorless; usually procured milled. The finely milled material is unctuous, and readily yields a red mixture with water.
S. bicolor	Leaf stalk, brownish and fibrous; odorless. The finely milled material is unctuous, and yields with water a red mixture, which when heated to near boiling, flocculates and assumes the appearance of fresh blood.
Trona	Off-white, granular mass; odourless, readily soluble in water, yielding a strongly alkaline solution. The pH of 6% and 10% solutions are 9.98 ± 0.04 and 9.90 ± 0.05 respectively.

range in %w/w. In establishing statistical significance, the Student's *t* Distribution was used as a test of the null hypothesis. The number of samples/ determinations is denoted by "n". Each determination per sample per consignment was carried out in quadruplets. The levels of significance are indicated in the footnote to the results.
The foreign matters mostly consist of extraneous parts of the plant material. Foreign grains and inorganic particles occur only occasionally. The test is not applicable to trona, and was not carried out on *P. osun*, because it was mostly procured in the milled form.

It is essential to spot the differences above in order to determine the correct quantities of material to dispense.

It is to be noted that in most cases, or in cases where the most extreme results are excluded, the range is well within the mean ± 3SD, which is the limit prescribed by WHO (1998) for plant materials intended for production of herbal medicines.

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The sampling procedure and the number of samples/ determinations (n) were as described in the section on Experimental

DISCUSSION

In many countries, the regulatory requirements for herbal products are no less stringent than those for regular pharmaceuticals. Thus, if Niprisan® is to be mass produced to meet rising demand, its production must conform to GMP. Such conformity must take into cognizance the inherent variations of biological materials. Accordingly, the following actions must be considered:

a) Limits must be set for the starting materials.
b) The manufacturing process must be chosen, such that mechanical efficiency and biochemical compatibility are simultaneously attained.
c) The manufacturing process must be observable and reproducible.
d) The finished product must pass relevant tests, including, where possible, one directly related to the disease condition of interest. To attend to these steps methodically, is to develop a system for assuring the quality of Niprisan. This study is an attempt to address the first of these actions in applying GMP to Niprisan®.

In Nigeria, trona, is commonly called "potash", which is a misnomer, since the mineral is a sesquicarbonate of sodium (Okere and Obimah, 1998). Trona is *kanwa* in Hausa, and other Nigerian languages. It is used in cooking as a tenderizer. *P. guineense*, called *okwa ose* in Igbo, is used as a soup condiment. *E. caryophyllata,* called clove, is *kanunfari* in Hausa, and is a popular spice. *P. osun* stem, called *osun* in Yoruba, is a hard red wood obtained mainly from south west Nigeria, where it is included in some oral recipes. *S. bicolor* leaf stalk is a well known constituent of folk medicines used in Nigeria. The need to develop specifications for the starting materials of herbal products is a well documented imperative (Obodozie, 2000), anticipated by the WHO (1998) in its publication of a manual on methods for quality control of medicinal plant materials. The data presented here derive from the application of those methods to the locally sourced materials, during a span of about 5 years.

A brief description of the materials is given in Table 1. The results of the quantitative determinations, expressed as means ± SD's, are given in Tables 2 to 6, revealing the following key trends:

a) The FM contents varied erratically in all the materials, but were in all such cases below 10%w/w (Table 2).
b) Aside from *E. caryophyllata*, which appeared to exist as one variety, all the other components appeared to exist in two varieties, based either on LOD alone or on both TA and WEM.

Table 2. Foreign matter (FM) contents of some of the starting materials of Niprisan®.

Foreign matter %w/w	*P. guineense*	*E. caryophyllata*	*S. bicolor*
Range	2.23 – 6.50	0.05 – 5.54	0.58– 5.20
Mean ± SD	4.23 ± 1.11(n=16)	1.91 ± 1.71 (n=16)	2.24± 1.48 (n=16)
Mean ± SD	4.22 ± 0.88(n=14)	1.78 ± 1.44 (n=14)	2.21± 1.28 (n=14)
Mean ± 3SD	4.22 ± 2.64	1.78 ± 4.32	2.21± 3.84

Table 3. Loss on drying (LOD) characteristics of the five starting materials of Niprisan.

Loss drying %w/w	*P. guineense*	*E. caryophyllata*	*P. osun*	*S. bicolor*	Trona
Range	6.38 – 8.80	7.67 – 9.82	4.02 – 6.96	7.15– 8.17	20.44 – 28.35
Mean ± SD	7.84 ± 0.66 (n=20)	8.68 ± 0.59 (n=20)	5.74±0.54(n=28)	7.85± 0.47(n=7)[a]	22.22 ± 2.12 (n=12)[b]
Mean ± SD	7.87 ± 0.55 (n=18)	8.67±0.50(n=18)	5.76±0.36(n=27)	7.85± 0.47(n=7)	21.80 ± 0.88 (n=10)
Mean ± 3SD	7.87 ± 1.65	8.67 ± 1.50	5.76 ± 1.08	7.85± 1.41	21.80 ± 2.64
Range				3.20– 4.42	16.90 – 18.50
Mean ± SD	-	-	-	3.62± 0.34(n=16)[a]	17.38 ± 0.49 (n=8)[b]
Mean ± SD	-	-	-	3.59± 0.27(n=11)	17.38 ± 0.49 (n=8)
Mean ± 3SD	-	-	-	3.59± 0.81	17.38 ± 1.47

a, The level of significant difference (P< 0.001) in the LODs suggests the existence of at least two varieties of S. bicolor , one with LOD = 7.85 ± 0.47 and the other 3.62 ± 0.34.
b, The level of significant difference (P< 0.001) in the LODs suggests the existence of at least two varieties of Trona, one with LOD = 22.22 ± 2.12 and the other 17.38 ± 0.49.

Table 4. Total ash (TA) characteristics of the four herbal materials of Niprisan.

Total ash %w/w	*P. guineense*	*E. caryophyllata*	*P. osun*	*S. bicolor*
Range	9.37 -17.93	3.98 - 6.29	0.92 – 1.80	4.88 – 9.84
Mean ± SD	14.22 ± 3.09 (n=4)[a]	5.47 ± 0.63 (n=20)	1.48 ± 0.34 (n=4)[b]	6.91 ± 0.87 (n=19)
Mean ± SD	15.83 ± 1.84 (n=3)	5.51 ± 0.52 (n=18)	1.48 ± 0.34 (n=4)	6.86 ± 0.22 (n=17)
Mean ± 3SD	15.83 ± 5.52	5.51 ± 1.56	1.48 ± 1.02	6.86 ± 0.66
Range	4.57 – 6.21	-	2.81 – 4.37	-
Mean ± SD	5.15 ± 0.41 (n=16)[a]	-	3.66 ± 0.52 (n=15)[b]	-
Mean ± SD	5.15 ± 0.41 (n=16)	-	3.67 ± 0.47 (n=13)	-
Mean ± 3SD	5.15 ± 1.23		3.67 ± 1.41	

[a], The level of significant difference (P< 0.001) in the TAs suggest the existence of at least two varieties of *P. guineense*, one with TA = 14.22 ± 3.09 and the other 5.15 ± 0.41.
[b], The level of significant difference (P< 0.001) in the TAs of the samples suggests the existence of at least two varieties of *P. osun*, one with TA = 1.48 ± 0.34 and the other 3.66 ± 0.52.

c) *S. bicolor* and trona exhibited two varieties differing significantly in LOD (Table 3).
d) *P. guineense* and *P. osun* differed significantly in TA (Table 4) and WEM (Table 5).

The results on trona in Table 6 show some of its cationic constituents, revealing the occasional presence of lead, a known toxicant. The results are useful for diagnoses, and especially, to exclude samples containing detectable quantities of lead. No attempt however, was made to establish any relationship between the concentrations of cations and the LOD results of trona.

The TA results (Table 4) may be useful for purposes of delimitation; and as a means of gauging inorganic impurities, such as sand; but their relevance to quantities to be dispensed in production was not determined. By contrast, the LOD results (Table 3) are clearly essential in determining the quantities of materials to be dispensed. The WEM results (Table 5) are also critical to production. Their importance stems from the fact that, in the absence of a delineated assay, WEM is the only quantitative variable around which posological projections can be tested. For instance, if the WEM of an herbal drug is 1.5%w/w, it may be presumed that 1 g of the crude drug

Table 5. Water extractable matter (WEM) characteristics of the four herbal materials of Niprisan.

Water extractable matter %w/w	*P. guineense*	*E. caryophyllata*	*P. osun*	*S. bicolor*
Range	13.18 – 15.80	21.68 – 29.14	2.17– 3.19	5.59 – 10.47
Mean ± SD	14.45 ± 0.94 (n=4)[a]	25.56± 2.22 (n=28)	2.84± 0.40 (n=4)[b]	7.54 ± 1.44(n=20)
Mean ± SD	14.45 ± 0.94 (n=4)	25.57 ± 2.05(n=26)	2.84± 0.40 (n=4)	7.65 ± 1.04(n=16)
Mean ± 3SD	14.45 ± 1.32	25.57 ± 6.15	2.84± 1.20	7.65 ± 3.12
Range	26.21 – 33.38	-	4.06– 6.57	-
Mean ± SD	29.32 ± 2.13 (n=8)[a]	-	4.83± 0.78 (n=15)[b]	-
Mean ± SD	29.32 ± 2.13 (n=8)	-	4.76± 0.63 (n=13)	-
Mean ± 3SD	29.32 ± 6.39	-	4.76± 1.89	-

[a], The level of significant difference (P< 0.001) in the WEMs suggests the existence of at least two varieties of *P. guineense*, one with WEM = 14.45 ± 0.94 and the other 29.32 ± 2.13

[b], The level of significant difference (P< 0.001) in the WEMs suggests the existence of at least two varieties of *P. osun*, one with WEM = 2.84 ± 0.40 and the other, 4.83 ± 0.78

Table 6. Some cationic constituents of trona used in the production of Niprisan®.

Cation concentration	Copper %w/w X 10^{-4}	Lead %w/w X 10^{-4}	Magnesium %w/w X 10^{-4}	Manganese %w/w X 10^{-4}	Zinc %w/w X 10^{-4}
Range	6 – 17	0 – 4	120 - 170	40 – 80	3 - 15
Number (n) of samples/ determinations	23	23	23	23	23
Mean ± SD	16 ± 10	2 ± 2	142 ± 21	61 ± 15	9 ± 6

is equivalent to 15 mg of the WEM. This type of statistic is vital in dosage development and production. Since the results suggested two varieties of *P. guineense* and *P. osun* with clearly divergent extractabilities, it is essential to differentiate them, to be able to determine the quantities to be dispensed. In the production of Niprisan®, more of the varieties with lower WEMs, as compared to those with the higher WEMs, would be dispensed.

Conclusion

Most of the samples encountered in this study are deemed to have passed the tests of uniformity of the physicochemical characteristics examined, given the suggestion of the WHO (1998) that samples varying predictably, with values within the mean ± 3SD, may be considered for use in the production of herbal preparations. This favourable outcome is however not unexpected, since all the items were obtained from reliable sources, patronized by the public for various culinary or medicinal uses. The results underscore the need to procure raw materials from established sources. Owing to the occasional presence of lead in trona, all samples of the material should be analyzed for heavy metals as per BP, 2004.

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