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Full Length Research Paper

Characterization and Standardization of Niprisan: A Herbal Remedy for Sickle Cell Disease

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Niprisan is a dry extract preparation of five components: the seeds of *Piper guineense*; flower buds of *Eugenia caryophyllata*; stem parts of *Pterocarpus osun*; leaf stalk of *Sorghum bicolor* and trona. The product was developed in our Institute from a traditional recipe used in Nigeria for treating sickle cell crises. This study aims to provide for the components, the following specifications: foreign matter; loss on drying; total ash; and water extractable matter, using the methods prescribed by the WHO (1998) for quality control of medicinal plant materials. The trona was analyzed with methods adapted from BP (2004). The results suggest the existence of one variety of *E. caryophyllata*; but two varieties of *S. bicolor* and trona, differing in loss on drying; and two varieties of *P. guineense* and *P. osun*, differing in both total ash and water extractable matter. The implications of the results for good manufacturing practice are discussed.

Key words: Standardization, specifications, components, niprisan, phytomedicine, sickle cell disease.

INTRODUCTION

One key problem in medicinal plant research is the wide variety of terms that are often used differently by different authors and journals. This problem is particularly acute in the case of quality control of herbal preparations. Therefore, it is essential in this study that all such terms be clearly defined; and once defined, their usage should be consistent and predictable. In this study the NAFDAC system of definitions is adopted. It defined herbal preparations as "regulated products of plant origin consumed or used by man or on animals, examples are: phytomedicines, herbal medicines, dietary supplements, neutralceuticals and phytocosmetics" (Akunyili, 2002). By this definition, niprisan is a phytomedicine, rather than an herbal drug, for two reasons: it contains a non-herbal component - trona; and its production process involves processes other than cutting and drying. This way of categorizing Niprisan stems from the exclusivity of the following definitions: "Herbal drugs are plants or parts of plants having been transformed into a storable condition by drying" (Gaedcke, 2003). "Herbal drugs are mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, lichen in an unprocessed state, usually in a dried form" (European Pharmacopoeia, 2000).

Sickle cell crises; are the manifestations of sickle cell anemia, an inherited red blood cell disorder that remains a problem in Nigeria and others, including India and the US. The crises are characterized by pains and damage to vital organs, leading to frequent infections (Steinberg, 1999). In the US, where there were about 80,000 sicklers in 2003, the disease was associated with a cost of about $475 million (Pandey, 2003). With such figures for the US alone, and the fact that Nigeria and India, for instance, have more cases, it is evident that the cost of the disease is colossal. Several approaches, including hydrourea (Charache et al., 1995), bone marrow transplant, among others (Piomelli, 1992), have been instituted against the disorder with marginal successes. In Nigeria, prepara-tions of *Fagara zanthoxyloides* (Sofowora, 1979) raised

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Abbreviations: WHO, World health organization; BP, British pharmacopoeia; LOD, Loss on drying; TA, total ash; WEM, water extractable mater; SCD, sickle cell disease; SD, standard deviation; GMP, good manufacturing practice; NAFDAC, national agency for food administration and control.

Table 1. Test for statistical significance in the difference in the LOD of varieties of trona (Trona-$_x$ and Trona-$_y$).

LOD of trona-$_x$	**LOD of trona-$_y$**
20.44	16.90
21.04	17.01
21.17	17.17
21.17	17.31
21.30	17.32
21.40	17.36
21.50	17.43
21.68	18.50
21.92	
23.40	
28.35	
X_x = 22.23	X_y = 17.38
SD_x = 2.12	SD_y = 0.49
n_x = 12	n_y = 8

raised hopes for a while, but interest in them waned with the coming of Niprisan in the late 1990's.

The traditional recipe from which Niprisan was developed, 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, called "ogogoro", to which is added some trona - a solid mineral. That recipe has been in use among the Yoruba's of Nigeria for ages in the treatment of sickle cell crises. The aim of this study is to provide specifications for these components. Such specifications are desirable if good manufacturing practice (GMP) is to be applied to the production of Niprisan.

The biochemical pharmacology of Niprisan has 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., 2001; 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 components used in its production, in compliance with GMP.

EXPERIMENTAL

The herbal components

Consignments of the seeds of *P. guineense* (L.), Schum. and Thonn., (Piperaceae) and the flower buds of *E. caryophyllata* (L.),Thunb., (Myrtaceae) were purchased from food stalls in the open market, while the stem parts of *P. osun* (L.) Craib. (Fabaceae) and the leaf stalk of *S. bicolor* (L.) Moench, (Poaceae) were purchased from herbal medicine dealers. All the herbal components 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 and in our department.

The mineral component

All consignments of trona were purchased from food stalls in the open 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 components.

Computation of results and statistical analyses

The results are expressed as mean ± standard deviation or as a range in %w/w. In establishing statistical significance, the student's *t*-distribution was used as a test of the null hypothesis. An example of the calculations is shown in section 2.3.1. To calculate 3 SD's the results are treated as shown in the example in section 2.3.2. The SD's are calculated using Microsoft excel.
Weighted mean of difference in the SD's

$$(S) = \frac{SD^2{}_x + SD^2{}_y}{2}$$

$$t = \frac{X_x - X_y \quad n_x \times n_y}{S \qquad n_x + n_y}$$

$$S = \frac{4.4944 + 0.2401}{2} = 2.3673 = 1.5386$$

$$t = \frac{22.23 - 17.38}{1.5386}\,4.8 = \frac{4.85}{1.5386} \times 2.1909 = 6.9062$$

The value of "t" from the "t" table at 18 DF (P = 0.001, two tails) = 3.92

Interpretation: The difference in LOD between trona-$_x$ and trona-$_y$ is statistically significant at over the 99.9% confidence limit (that is P < 0.001).Table 1

Calculation of 3 SD's using the LOD results of Trona-$_x$

The SD's are calculated using Microsoft excels, and the results are simply multiplied by 3 to obtain 3 SD's. To calculate 3 SD's, in instances where the results vary widely, the lowest and the highest results may be eliminated as shown in the example below, using the LOD results of Trona-x.

Step 1: Rank the results (n =12) in ascending order, and calculate the mean and SD.
n=12 : 20.44, 21.04, 21.17, 21.17, 21.30, 21.40, 21.50, 21.68, 21.92, 23.37, 23.40 and 28.35.

Table 2. Description of the components 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; odourless. 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.

Mean = 22.23 and SD = 2.12

Step 2: Eliminate the lowest and highest results, and recalculate the mean and SD.
n=10: 21.04, 21.17, 21.17, 21.30, 21.40, 21.50, 21.68, 21.92, 23.37 and 23.40
Mean = 21.88 and SD = 0.88

Thus the Mean ± 3SD's for the closest results (n = 10) is: 21.88 ± 2.64 %w/w

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.

DISCUSSION

In many countries, the regulatory requirements for botanicals are no less stringent than those for regular pharmaceuticals. Thus, if Niprisan is to be mass produced to meet rising demand, conformity with quality assurance practices must be applied to its production. Such con-formity must take into cognizance the inherent variability of biological materials. Accordingly, the following actions must be considered:

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

In Nigeria, trona, the solid mineral component of Niprisan, 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 in several other Nigerian languages. It is commonly used in cooking as a tenderizer. *P. guineense*, called okwa ose in Igbo, is used as a soup condiment. *E. caryophyllata,* commonly called clove, is kanunfari in Hausa, and is used worldwide as a spice (Etkin, 2006). *P. osun* stem, called osun in Yoruba, is a red and woody material obtained mainly, but not exclusively, from the rain forest region of south west Nigeria, where it is included in several medicines for oral use. *S. bicolor* leaf stalk is a well known constituent of folk medicines used in Nigeria and elsewhere (Duke and Wain, 1981). The need to develop specifications for components of herbal preparations is a well documented imperative (Obodozie, 2000), that had been anticipated in the 1998 publication of a manual on methods for quality control of medicinal plant materials (WHO, 1998). The data presented here derive from applications of these methods to the locally sourced materials, during a span of about 5 years.

A brief description of all the components is given in Table 2. The results of the determinations, expressed as means ± SD's, are given in Tables 3-7, revealing the fol-lowing key trends: (a) the FM contents varied erratically in all cases, but were below 10%w/w (Table 3); (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; (c) *S. bicolor* and trona exhibited two varieties dif-fering significantly in LOD (Table 4); (d) *P. guineense* and *P. osun* differed significantly in TA (Table 5) and WEM (Table 6). The results in Table 7 show some of the cations of trona, and the occasional presence of lead. The results are useful for diagnoses, especially to exclude samples containing lead - a toxicant. While the TA results (Table 5) may be useful for diagnoses; and as a means of gauging inorganic impurities, such as send;

Table 3. Variations in foreign matter content of some of the herbal components of Niprisan.

Foreign matter %w/w	*P. guineense*	*E. caryophyllata*	*S. bicolor*
Range	2.23 – 6.50	0.05 – 5.54	0.58 – 5.2
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 4. Variations in the loss on drying (LOD) of the five components 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	20.4428.35
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], Indicates that the difference between the LOD's of the two varieties of *S. bicolor* is statistically significant at over 99.9% Confidence Limit (that is, P< 0.001).
[b], Indicates that the difference between the LOD's of the two varieties of Trona is statistically significant at over 99.9% Confidence Limit (that is, P< 0.001).

Table 5. Variations in the total ash (TA) of the four herbal components 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], Indicates that the difference between the TA's of the two varieties of *P. guineense* is statistically significant at over 99.9% Confidence Limit (that is, P< 0.001).
[b], Indicates that the difference between the TA's of the two varieties of *P. osun* is statistically significant at over 99.9% Confidence Limit (that is, P< 0.001).

their relevance to quantities to be dispensed during pro-duction is not yet determined. By contrast, the LOD results (Table 4) are definitely critical in determining the quantities of materials to be dispensed. For example, since the LOD of one variety of *S. bicolor* is 7.85 ± 0.47 and the other is 3.62 ± 0.34, it is essential to determine the variety in hand at any one time. Evidently, more of the variety with the higher LOD must be dispensed. The WEM results are also critical to production. The impor-tance of WEM in this work stems from the fact that, in the absence of a delineated assay, WEM is the only quanti-tative 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 is equivalent to 15 mg of the WEM. This type of infor-mation is vital in dosage development and production. Since the results suggest 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. For example, the quality of the variety of *P. guineense* (WEM = 14.45 ± 0.94) to be dispensed would be about twice the quantity of the other variety (WEM = 29.32 ± 2.13). These remarks equally apply to *P. osun* for the same reasons.

Table 6. Variations in the water extractable matter (WEM) of the four herbal components 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], Indicates that the difference between the WEM's of the two varieties of *P. guineense* is statistically significant at P< 0.001.

[b], Indicates that the difference between the WEM's of the two varieties of *P. osun* is statistically significant at P< 0.001.

Table 7. Concentrations of 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
No. of samples	23	23	23	23	23
Mean ± SD	16±10	2 ± 2	142 ± 21	61±15	9 ± 6

Conclusions

Most of the samples encountered in this study would be deemed to have passed the tests for uniformity, given the suggestion by the WHO, (1998) that samples with values within the mean ± 3SD's may be considered for use in the production of herbal preparations. The outcome in this study is not unexpected, since all the items were obtained from reliable sources, patronized by the public for various culinary or medicinal uses. This favourable outcome underscores the need to procure raw materials from established sources. Owing to the occasional pre-sence of lead in trona, all samples of this component must, in keeping with GMP, be analyzed for heavy metals.

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