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Physicochemical variables and real time stability of the herbal substance of Niprd-AM1®- an antimalarial developed from the root of Nauclea latifolia S.M. (Rubiaceae).

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Abstract

The evidence for using the root of *Nauclea latifolia* S.M. (Rubiaceae) in treating malaria in Africa; and the development of Niprd AM1, as an antimalarial phytomedicine from it, are reviewed. This study aimed at paving the way for the registration and controlled production of Niprd-AM1, which was developed by National Institute for Pharmaceutical Research and development (NIPRD), Abuja, Nigeria.
The study applied official procedures to authenticate and evaluate the changes that occur during normal storage in the physicochemical variables of the herb and extract.
The herb contained alkaloids, saponins, terpenoids, but cardiac and cyanogenic glycosides and anthraquinones were not detected. Heavy metal presence was well below the limits allowed. Most of the quality variables evaluated, including TLCs, remained largely unchanged up to the 39 month of storage, but a few began to decline as from the 21st month.
The results are consistent with NIPRD's intention to regularize the production and use of Niprd-AM1 according to good pharmaceutical practice (GXP), and suggest that the dried herb and extract are stable under tropical room conditions for over a year in sealed glass containers.

Keywords: Niprd-AM1; *Nauclea latifolia;* Antimalarial; Physicochemical; Stability; Good pharmaceutical practice (GXP).

Introduction

Malaria is a pernicious and pervasive disease prevalent in over 100 countries - mainly in Africa, Asia and Latin America, but was previously rampant in Europe and North America, and had been the subject of two Nobel Prizes in Medicine: Ross, in 1902 for identifying the vector; and Leveran, in 1907 for identifying the parasite. Each year over 350 million cases are diagnosed, and over 1.5 million die of malaria. Three factors account for the threat of this scourge especially in developing countries. First, the increasing resistance of mosquitoes to available insecticides. Second, the increasing resistance of parasites to available antimalarials [1]. Third, the high cost of effective antimalarials. Like in most climes where malaria is endemic, its history in Nigeria is both interesting and typical, and is here recounted as an illustration.

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In 1983, malarial mortality in Nigeria was over 1.5 per 100, 000. In 1987, it was nearly 2 per 100, 000, then fell steadily to about 1 per 100, 000 in 1989; and then rose dramatically to about 2 per 100, 000 in 1990 [2]. It was against this background that the Federal Ministry of Health in 1987 set up the National Malaria Technical Committee, led by Professor Ekanem – a renowned malariologist**,** to review the situation and initiate moves for the control of the disease in Nigeria. The Committee came up with nine conclusions that included the following four: *Plasmodium falciparum* was responsible for 90% of malarial cases in Nigeria; malaria in Nigeria was transmitted by two of the most efficient vectors worldwide - *Anopheles gambiae* and *Anopheles funestus*; malaria in Nigeria, is consistently the most prevalent disease, and among the top three causes of death; and malaria eradication in Nigeria is not feasible in the foreseeable future. Although the report commented on antimalarial therapies, it unfortunately, made no mention of traditional herbal remedies, in spite of their wide-spread use and claim of effectiveness; and in spite of the fact that today's most celebrated antimalarial – artemisinin, resulted from research on *Artemisia annua* - an antimalarial in Chinese herbology.

As is well known, traditional antimalarials abound in West Africa, once called the "White man's grave" because of the toll of malaria on the early colonialists. Prominent among these is the water extract of the roots of *Nauclea latifolia* – also called African peach. *Nauclea latifolia* S.M. (Rubiaceae) is a perennial tree, widespread in most parts of Nigeria. Its medicinal uses, especially for malaria, are well known in West Africa – for example in Nigeria [3] and Cote d'Ivoire [4]. Traditional indications of the plant nearly always include malaria, along with other infective illnesses. For example, Onyeyili et al [5] reported its use as an anthelmintic. The tree bears a unique flower consisting of a large reddish brown ball with long projecting stamens – for which reason it is also called pin cushion tree. It fruits late in the rainy season in most of Nigeria. Other names by which the plant is known include: *Nauclea esculenta, Sarcocephalus esculentus,* and *Sarcocephalus latifolius*. Most notably, Benoit-Vical and others [4] showed that the aqueous extract of the stem or root of *Nauclea latifolia* was active against a Colombian, chloroquine-resistant strain; and a Nigerian, chloroquine-sensitive strain of *Plasmodium falciparum*. The extract had IC_{50} values similar to those of other antimalarial plants - *Azadirachta indica* and *Artemisia annua*. Also notable, is the discovery that strictosamide, which is present in the root of *Sarcocephalus latifolius* is active against *Plasmodium falciparum* [6].

When the National Institute for Pharmaceutical Research and Development (NIPRD) became operational in 1989, one of its earliest drives was to develop an antimalarial medicine, which led to Niprd-AM1- a phytomedicine developed from the root of *Nauclea latifolia*. A comparative randomized clinical trial of Niprd-AM1, supported by WHO TDR, was conducted in human volunteers, and the phytomedicine was found to be superior to a sulphadoxine/ pyrimethamine combination, and chloroquine, in combating uncomplicated malaria [7].

Experimental

Treatment and sampling of material

The roots of ***Nauclea latifolia*** (Voucher Specimen Number: NIPRD/H/5579) obtained during the months of July and October from the Institute's botanical garden and it's environ were air-dried in a well-ventilated shade designed for drying medicinal plant materials. The materials were subsequently comminuted to coarse powder, by first chopping them into bits, followed by grinding with mechanized equipment. The procedure of WHO [8] for sampling as per was followed.

Phytochemical tests

The following tests as described by Harborne [9] and Onwukaeme and coworkers [10] were carried out on the herb or aqueous extract.

Dragendorff's test for alkaloids

Dragendorff's reagent consisting of two solutions: Solution A **-** 1.7 g basic bismuth nitrate

in 100 ml water/acetic acid (4:1), and Solution B - 40.0 g potassium iodide in 100 ml of water, was prepared. The two solutions were mixed as follows to yield 100 ml of Dragendorff's reagent: 5 ml of Solution A + 5 ml of Solution B + 20 ml of acetic acid + 70 ml water. The test was carried as follows: About 20 mg of the air-dried herb was extracted with 20 ml of methanol by shaking and heating over a boiling water bath. The extract was subsequently filtered and allowed to cool. Each 2 ml of the filtrate in a test-tube was treated with 2 ml of Dragendorff's reagent. The development of an orange-brown precipitate presumptively indicated the presence of alkaloids.

Frothing test for saponins

A pinch of the aqueous extract was added to 5 ml of water and warmed until dissolved. The solution was subsequently shaken vigorously to generate froth, and then allowed to stand. A rich froth persisting for 10 minutes indicates the presence of saponins.

Salkowaski's Test for Terpenoids

To 0.5 g of the herb or 10 mg of the extract was added 3 ml of chloroform, mixed thoroughly, and filtered. To 1 ml of the filtrate, 3 ml of concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids

Keller-Killani's Test for Cardiac glycosides

To 0.5 g of the herb or 10 mg of the extract in a tube were added 5 ml of water and 2 ml of glacial acetic acid containing one drop of 15 % ferric chloride solution. The contents were thoroughly mixed, and filtered. Subsequently, 2 ml of the filtrate was gently underlayed with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Sodium Picrate Paper Test Cyanogenic Glycosides

An aqueous solution of 0.05M picric acid, was neutralized with sodium bicarbonate, and filtered. Subsequently, a piece of filter paper (5.0 X 1.5cm) was soaked in the mixture. The paper (yellow in color) was dried at room temperature. Detection of cyanogenic glycosides: About 0.5 g of herb or 10 mg of extract in a tube was thoroughly mixed with 5 ml of water and 1 ml of chloroform Fresh plant material was cut into small pieces and placed in a test tube with The tube was stoppered with a cork containing a strip of picrate impregnated paper hanging down from the stopper, and incubated in hot bath for up to 2 hours. A change of color from yellow to brown-red, indicated the release of HCN – hence the presence of cyanogenic glycoside. If there was no release of HCN within 2 h, indicating a negative test, the tube was left at ambient temperature for 24 and 48 h, so that it could be re-examined. No color change after 48 h indicated that the test was negative for cyanogenic glycoside

Borntrager's test for anthraquinone derivatives

About 100 mg of air-dried herb was extracted with 5 ml of chloroform by shaking and warming over a water bath. To about 2 ml of the supernatant, 1ml of dilute 10 %v/v ammonia solution was added, followed by shaking. A pink or red colour in the aqueous layer indicates the presence of fully oxidized anthraquinone derivatives.

Physicochemical tests

The following tests, briefly described, were carried out on the extracts as per WHO [8]. Unless otherwise stated the results are expressed as a %w/w of the air-dried sample, and as X ± SD (mean ± standard deviation).

Loss on drying

Drying to constant weight was effected in a Lindberg/Blue M gravity-convention oven maintained at 105-110 ^{0}C.

Total ash and Acid insoluble ash

The materials were placed in a furnace (Vecstar Furnace) and heated gradually heated to the ignition temperature of 650 - 700 ^{0}C.

Evaluation of water extractive value

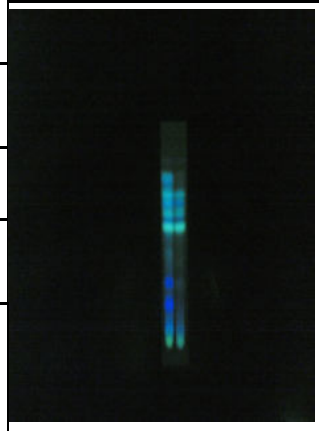
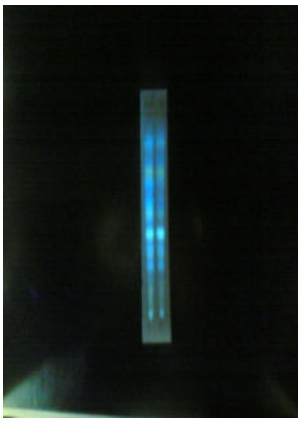
The test utilized ~ 4 g of air-dried and coarsely powdered sample, accurately transferred into a glass-stoppered, 250-ml reflux conical flask, and followed by the addition of 100 ml of water.

366 nm). The resulting chromatogram was photographed as shown in the **Results**.

Assay for metals by atomic absorption spectroscopy

The materials and method for atomic absorption spectroscopy were as per the British Pharmacopoeia as described in detail elsewhere [11].

Table 1: Phytochemical and TLC Profiles of the herb and extract of *Nauclea latifolia*

Phytochemical constituent	Herb	Extract	Normal phase TLC	Reverse phase TLC
Saponins	+	+		
Terpenoids	+	+		
Alkaloids	+	+		
Flavonoids	+	+		
Cyanogenic glycoside	?	?	Herb (L) and extract (R) in ethanol developed with Hexane: Ethyl acetate (4:1) on K2 normal phase TLC, and viewed at 366 nm. The herb yielded 9, while the extract yielded only 5 principal spots	Herb (L) and extract (R) in ethanol developed with Methanol: Water (4:1) on KC20 reverse phase TLC, and viewed at 366 nm. Both the herb and the extract yielded 9 principal spots.
Anthraquinones	?	?		
Cardiac glycoside	?	?		

Footnote to Table 1: (+): Indicates present. (?): Indicates absent or below detection

Thin layer chromatography (TLC)

Florescent, precoated plates were used for both the normal and reverse phase TLC. The normal phase utilized silica K6, and hexane: ethylacetate: methanol (4:4:1) as mobile phase while the reverse phase utilized KC18 plate, and methanol: water (80:20). The air-dried plate was visualized using a viewing cabinet (Cammag) and a UV-lamp (Cammag – equipped to emit light at 254 or

Determination of bitterness value

The bitterness of the herb was determined by the method described by WHO [8] which compare the threshold bitter concentration (TBC) of an extract of the herb with the TBC of a dilute solution of quinine hydrochloride. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1 g of quinine hydrochloride in 2000 ml. Thus, the bitterness value of the solution (1 g of quinine

hydrochloride in 2000 ml of drinking water) is set at 2×10^5 units.

Determination of Foaming Index

Decoctions of plant materials foam due to the presence of saponins. This ability is measured as foaming index, and is an important quality control parameter. The test was performed by the procedure of WHO [8]. The results are expressed as a quantity [Q] per ml or as $[Q]ml^{-1}$.

Light absorption and pH of preparations

Preparations of the plant in water or in methanol were appropriately diluted to obtain absorbance readings of at least 0.050 at the wavelength showing maximum absorption (λmax), using 1-cm quartz or glass cells and a Jenway or Shimadzu UV-VIS Spectrophotometer. In each determination, both the dilution factor and the absorbance were noted for comparative purposes. The acidity or alkalinity of preparations was tested by means of a Jenway pH meter, calibrated with standard buffer tablets of pH 4, 7 and 10.

Results

Results on phytochemical and physicochemical tests on herb and extract of *N. latifolia*

The phytochemical and physicochemical results, including TLC and light absorption, are shown in Tables 1 and 2. The results provide useful criteria for authentication and characterization of the herb and the extract. Normal phase TLC indicated 9 and 5 principal spots for the herb and extract respectively; while the reverse phase TLC indicated 9 principal spots for both.

Table 2: Results of some physicochemical tests on the herb and extract of *Nauclea latifolia*

Parameter (Mean ± SD)	Air-dried Herb	Water Extract
Description	Yellow wood/ brown bark; odourless/ faintly aromatic; slightly bitter.	Yellow-brown granules; odourless/ faintly aromatic; hygroscopic; bitter.
Loss on drying (LOD: % w/w)	9.79 ± 2.00 (30)	6.50 ± 1.22 (8)
Total ash (TA: % w/w)	3.09 ± 1.28 (57)	9.88 ± 2.60 (15)
Acid insoluble ash	Below detection	Below detection
Water Extractability (% w/w)	16.56 ± 3.93 (29)	-
Water Solubility (ml/g)	-	90 ± 5 (6)
pH of 5 % w/v in water	6.4 ± 0.2	6.6 ± 0.2
Bitterness value (male subjects)	630 ± 50	2820 ± 280 (6)
Foaming Index (FI: as ml^{-1})	670 ± 20	1430 ± 70 (6)
A 1%1cm at λ225 nm in water	50.6	278

Footnote to Table 2: Footnote to Table 2: The herb consists of the bark and woody parts of the root of *Nauclea latifolia*. The extracts were prepared by hot extraction, and subsequently evaporated to dryness. The results represent Mean ± SD. The TA of the extract was ~ 3x that of the herbal drug, but acid insoluble matter was below detection in both, suggesting that the herb may have relatively high contents of water soluble minerals. The spectra revealed 3 peaks (at: 688, 454 and 225) and 3 valleys (at: 697, 548 and 391). The absorption in the visible region (400-600 nm) was consistent with the golden yellow color of the extract and solution.

Results of metallic contents determinations of the herb and extract of *N. latifolia*

Table 3 shows that both herb and extract contain metallic trace elements, including lead and cadmium, but their levels were well below the limits allowed in food and drugs, which are: 1.0 mg/100g for lead; and 0.03 mg/100g for cadmium (WHO, 1998).

Table 3: Metallic contents of the herb and extract of *Nauclea latifolia*

Metallic Element (mg/100g)	Na	K	Ca	Mg	Mn	Fe	Cu	Zn	Cd	Pb	Se	Cr
Herb	3.2	6.4	200	297	0.81	0.37	1.80	0.79	< 0.01	< 0.1	4.1	0.16
Extract	6.1	2.3	54	260	0.79	0.10	1.18	0.65	< 0.01	< 0.1	2.4	0.47

Footnote to Table 3: The results are consistent with the inference that the TAs of the samples are mainly physiological ash, because: even though the TA of the herb (3.09 ± 1.28) was lower than that of the extract (9.88 ± 2.60), the extract generally contained less of each metal per unit weight. Apart from Na and Cr whose levels were higher in the extract, the level of every other metal was higher in the herb, suggesting that only a fraction of their salts were extracted with water. The TA results for the herb are, of course, independent of extractability. Apart from lead oxide, the oxides of all the other metals above are soluble in the 2M HCl or (70g/l) used in the determination of acid insoluble ash. The levels of Cd and Pb were the lowest - occurring below the allowed limits of 0.3mg/100g for Cd; and10 mg/100g for Pb. The levels of Ca and Mg, on the other hand, were the highest, ranging from 53.9 to 297.3 mg/100g.

Effect of Storage on Quality Variables of the Herb and Extract of *Nauclea latifolia*

Effect of storage on moisture content and appearance of herb and extract

Table 4 shows that the moisture content and the appearance of the samples changed significantly with storage, for up to 39th month.

Table 4: Effect of storage on moisture content and appearance of herb and extract

Months of Storage	Herb Mean ± SD		Extract Mean ± SD	
	Moisture (%w/w)	Appearance	Moisture (%w/w)	Appearance
0	9.17 ± 0.70	Yellow-brown particles of wood/ cork tissues	7.87 ± 0.90[b]	Yellow-brown granules
3	7.69 ± 0.59[a]	Unchanged from above	8.83 ± 1.62[b]	Unchanged from above
9	9.04 ± 0.66[a]	Unchanged from above	10.02 ± 1.20[b]	Unchanged from above
21	8.61 ± 0.86[a]	Unchanged from above	11.42 ± 1.78[b]	Unchanged from above
39	9.22 ± 0.40[a]	Unchanged from above	9.12 ± 1.21[b]	Unchanged from above

Footnote to Table 4: The results represent (Mean ± SD; n=5-7). Both ([a]) and ([b]) indicate that the results did not differ significantly from those of the 0[th] month (P > 0.05).

Effect of storage on extractability/ solubility/ appearance/ pH of herb and extract

The Table 5 shows that the extractability of the herb, and the appearance and pH of the resulting aqueous solution, did not change significantly with storage. Similarly, the solubility of the dry extract, and the appearance and pH of the resulting aqueous solution did not change significantly with storage, for up to 39th month.

Table 5: Effect of storage on extractability/ solubility/ appearance/ pH of herb and extract

Months of storage	Herb			Extract		
	Extractability	Appearance	pH	Solubility (ml/g)	Appearance	pH
0	17.22 ± 1.84	Clear, yellow	6.4 ± 0.2	90 ± 5	Clear, yellow	6.6 ± 0.2
3	15.74 ± 1.04[a]	Clear, yellow	6.6 ± 0.2[b]	95 ± 10[c]	Clear, yellow	6.4 ± 0.2[d]

9	16.37 ± 1.62 [a]	Clear, yellow	6.2 ± 0.2 [b]	95 ± 10 [c]	Clear, yellow	6.3 ± 0.2 [d]
21	17.11 ± 1.38 [a]	Clear, yellow	6.4 ± 0.2 [b]	95 ± 5 [c]	Clear, yellow	6.4 ± 0.2 [d]
39	16.84 ± 1.51 [a]	Clear, yellow	6.5 ± 0.2 [b]	90 ± 10 [c]	Clear, yellow	6.3 ± 0.2 [d]

Footnote to Table 5: The results represent (Mean ± SD; n=5-7). The notation ([a]), ([b]), ([c]) and ([d]) indicate that the results did not differ statistically from those of 0[th] month (P > 0.05).

Effect of storage on light absorption and TLC features of the herb and extract

Table 6 shows that storage had no significant effect on light absorption and the TLC characteristics of the samples. But it is remarkable that the reverse phase TLC of the extract revealed 5 principal spots, while of that of the herb revealed 9 spots. This suggests that the extract may be deficient in some constituents – owing to their being less water soluble.

Table 6: Effect of storage on light absorption and TLC features of the herb and extract

Months of storage	**Herb**			**Extract**		
	Absorbance at λ 225 nm	Nos. TLC spots		Absorbance at λ 225 nm	Nos. TLC spots	
		Nor-mal	Rev-erse		Nor mal	Rev erse
0	2.048 ± 0.028	9	9	2.017 ± 0.044 [b]	5	9
3	2.081 ± 0.020 [a]	9	9	2.031 ± 0.024	5	9
9	2.127 ± 0.029 [a]	9	9	2.041 ± 0.034	5	9
21	2.065 ± 0.024 [a]	9	9	2.065 ± 0.024 [b]	5	9
39	2.087 ± 0.024 [a]	9	9	2.077 ± 0.044 [b]	5	9

Footnote to Table 6: The solutions of herb and extract were made by thoroughly mixing 1 part of solute and with 100 parts of solvent (MeOH: H_2O [50:50, v/v]), filtering, and diluting the filtrates by 25x (herb) or 125 x (extract) with the same solvent. Absorbencies were measured at λ225 nm, using the solvent as the blank. The results represent (Mean ± SD; n=5-7). Both ([a]) and ([b]) denote that the results did not differ significantly from those of the 0[th] month (P > 0.05). Both the normal and reverse TLCs of the herb gave 9 spots, but the corresponding TLCs for the extract were 5 and 9.

Effect of storage on the bitterness values and foaming indices of herb and extract

Table 7 shows that the bitterness value and foaming index of the herb did not change significantly with storage up to 39th month. By contrast, the corresponding results for the extract decreased significantly from the 21[st] week of storage. These results in combination with the TLC results above may suggest the superiority of the dry herb over the dry water extract.

Table 7: Effect of storage on the bitterness values and foaming indices of herb and extract

Months of storage	**Bitterness value**		**Foaming index**	
	Herb x 10^3	Extract x 10^3	Herb x 10^3	Extract x 10^3
0	0.63 ± 0.05 (7)	2.82 ± 0.28 (6)	0.67 ± 0.02 (7)	1.43 ± 0.07 (6)
3	0.57 ± 0.07 [a] (6)	3.07 ± 0.37 (7)	0. 65 ± 0.02 [c] (6)	1.34 ± 0.07 (7)
9	0.58 ± 0.07 [a] (7)	3.08 ± 0.20 (5)	0.71 ± 0.03 [c] (6)	1.33 ± 0.06 (5)
21	0.51 ± 0.05 [a] (6)	1.88 ± 0.22 [b] (7)	0.65 ± 0.02 [c] (7)	1.05 ± 0.05 [d] (7)
39	0.55 ± 0.05 [a] (5)	2.11 ± 0.18 [b] (5)	0.67 ± 0.03 [c] (5)	1.18 ± 0.07 [d] (5)

Footnote to Table 7: The results represent (Mean ± SD). The notations ([a]) and ([c]) imply that the results did not differ significant from those of the 0th month ($P > 0.05$). By contrast ([b]) and ([d]) imply that the results for the 21st / 39th months were significantly lower than those of preceding months ($P < 0.05$).

Discussion

Studies at NIPRD on *Nauclea latifolia* and Niprd AM1 since the 1990's had confirmed the efficacy and safety of Niprd AM1. Under Professor Gamaniel, the department of Pharmacology had confirmed the efficacy and safety profile of Niprd AM 1 in mice infected with *Plasmodium berghei*; found the LD50 in mice and rats to be over 2000mg/kg per oral; and confirmed the efficacy and superiority of Niprd AM1 over standard antimalarial therapies in human clinical trials [7]. The drug Niprd AM1, administered as capsules in the trial, was prepared in the department of Pharmaceutical Technology, under Professors Nasipuri and Kunle, who confirmed that the herbal substance had the following features: bulk density, 0.1435 g/ml; tapped density, 0.1786 g/ml; true density, 3.0345 g/ml; and hydration value, 3.8833 units. However, GMP production requires also that the keeping qualities of the herb and extract be assured. Thus, this study was prompted by the need to assess the real time stability of these materials.

The herbal substance of Niprd AM1 is here defined as the dry water extract of the coarsely comminuted fragments of the root of *Nauclea latifolia,* which consists of a thick, dark brown bark and a yellow woody core. The parts are odourless or faintly aromatic. The taste is excitingly bitter – more like the taste niacinamide rather than quinine. Botanical studies in NIPRD's department of Medicinal Plant Research and Traditional Medicine revealed the following, on the root of *N. latifolia*: the bark consists of exfoliated, slightly lignified, roughly striated, thin walled polygonal cork cells. The cortex contains layers of oblong parenchymatous cells, oil globules, and sclereids occurring singly or in groups, and possessing a constricted lumen. The phloem consists of lignified fibres, interrupted by radially packed medullary rays and starch-laden parenchymatous cells. The xylem consists of large and small vessels composed of tubular cells bearing yellowish grains. A key step in a controlled production of Niprd AM1 would ensure that the vegetal raw material to be used has the above botanical features. Thus, in consonance with good manufacturing practice (GMP), any portion of the root system lacking these features, and those described in Tables 1-3, are to be excluded in the production of the Niprd AM1.

Another important consideration in GMP production is the need for assurance that the vegetal matter used in production has adequate keeping qualities, since the herb is mostly not used immediately. Indeed, the practice among traditional healers is to have the dry herb in their apothecary in readiness for when decoctions are required. Usually the healers have the well dried herb neatly wrapped up in cellophane bags, or stored away in covered plastic containers. The materials may remain in this condition some times for over a year or season before they are consumed or discarded. The question that arises is: For how long can the dried herb or the extract be kept without significant deterioration? The results in Tables 4-7 provide an attempt to answer this important question, both from the point of view of traditional usage and the intention by NIPRD to optimize that usage. The results collectively prove that when the dry herb and the dry water extract of the root of *Nauclea latifolia* are stored away from light under tropical room conditions, the quality variables evaluated do not change significantly for up to 39 months for the herb and 21 months extract. For extract (not the herb) the bitterness value and foaming index fell slightly but significantly as from the 21st month of storage. This defect and the fact the normal phase TLC of the extract yielded only 5 instead of the 9 principal spots yielded by the herb, should be taken note of in further development work on Niprd AM1.

Conclusion

The results of this study establish that the herb and the dry water extract of the root of *Nauclea latifolia* are stable for at least a year and a half

even in tropical conditions. The results are consistent with the practice of traditional healers in West Africa who use the dry herb for antimalarial therapy even after a year or two of storage in their apothecaries.

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