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Herbal Drug Regulation Illustrated with Niprifan[®] Antifungal Phytomedicine

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1. Introduction

Quality system is defined as arrangements, procedures, processes and resources; and the systematic actions necessary to ensure that a manufactured product will meet given specifications. On the other hand, quality control is defined as measures taken, including sampling and testing, to ensure that raw materials, intermediates, packaging materials and finished goods conform to given specifications. Quality specification refers to a written procedure and requirements that a raw material, intermediate or finished good must meet for approval. On the other hand, standard operating procedure (SOP) refers a written procedure, giving step-by-step directions on how a particular operation is to be carried out. Quality manual means, a document that describes the various elements of the system used in assuring the quality of results or products generated by a laboratory or factory. The term quality assurance refers to the totality of all the arrangements made with the objective of ensuring that products are of the quality required for their intended use. Good manufacturing practice (GMP), on the other hand, is that aspect of quality control that deals directly with manufacturing and testing of raw materials, intermediates and finished goods to ensure a product of consistent quality. Essentially, GMP involves two types of control - analytical and inspection, and both require: i) clear instructions for every manufacturing process; ii) a means of controlling and recording every manufacturing process; iii) a means of ensuring that the complete history of a batch can be traced; iv) a mechanism for recalling any batch of product from circulation; v) a system for attending to complaints on quality of product or service; and vii) a programme for training operators to carry out and to document procedures. The foregoing definitions and description of GMP conform to those of WHO (2000). It is also clear from the foregoing that GMPs are not prescriptive instructions on how a manufacturer can produce, but are rather a series of principles that must be observed for quality products, services or results to emerge. Invariably, GMPs are approved and enforced by an appropriate National Agency, but the onus of preparing and

executing GMPs rests with the manufacturer. In Nigeria (population ~ 150 million), GMPs are enforced by NAFDAC - established by decree in 1992/93.

The tests carried out for this study were according to official procedures - mostly BP (2004) and WHO (1998). The results are discussed within the context of requirements for herbal drug regulation as per WHO, EMEA and NAFDAC. It is noted that herbal drug regulation in Nigeria (Table 1) as compared that in Europe (Table 2) is paradoxically hampered not by the rigor and "stringency" of rules, but by the fact that the rules are only merely cumbersome, being neither adequate nor enforceable (Table 3), unlike those of EMEA.

S/No.	Regulatory aspect	Requirement
1	Legal status of applicant, who may be: Manufacturer. Marketer. Distributor.	The applicant must be certified by the Corporate Affairs Commission as a registered business in Nigeria. A marketer or distributor must show evidence of Power of Attorney issued by the manufacturer.
2	Analytical status of the product for registration.	The product must : Have a certificate of analysis. Be accompanied by a dossier with information on: Ingredients. Method of analysis. Stability. Dosage. Safety, among others.
3	Pre-registration inspection of premises.	Manufacturing, storage and distribution premises must be GXP compliant. Marketers must provide convincing evidence of GDP and GSP.
4	Post marketing surveillance plan/ report	Applicant may be required to provide a plan for reporting on: The use of the product. Any adverse reactions.

The above information were drawn from NAFDAC's leaflets and website: www.nafdacnigeria.org

Table 1. NAFDAC requirements for registering herbal medicines.

Essentially, the NAFDAC rules seem perhaps affected, or rather made cumbersome without being truly rigorous or "stringent" as actually claimed in the Agency's website. It is further noted that, like in China, where the head of the drug regulatory agency was sentenced to death for corruption (Gross and Minot, 2007), drastic actions, including the wholesale reorganization of NAFDAC management, had to take place in 2000 to straighten things out. The high frequency of confiscation and public destruction of counterfeit products by NAFDAC strongly testifies to the inadequacy of the rules and policies guiding drug regulation in Nigeria. Unfortunately, this worrisome state of affairs is equally true of many countries, as stated in the case of china. There is thus, the need for drug regulatory agencies in these countries to brace up. The aim of this article therefore, is to further an earlier advocacy (Ameh et al., 2010a) that includes alerting and encouraging Drug Regulatory Agencies, Health Ministries, and Parliamentary Health Committees, especially those in developing countries, to enact laws and evolve policies that will better regulate the

S/No.	Regulatory aspect	Requirement
1	Product information: Summary of product characteristics	Name of the product. Strength. Dosage form. Quantity of active ingredient (Example: 25 mg <i>P. guineense</i>). List of excipients (Example: <i>P. guineense</i> , <i>E. caryophyllata</i> etc., etc). Shelf life. Posology and method of administration. Indications. Contraindications/ special warnings. Precautions for use. These data are used as the basis for inserts, packaging, or advertisement. Inserts must pass "readability testing."
2	Quality control data: Refer to GMP requirements for production.	Production must be in a GMP compliant facility Drug must be produced with validated/ reproducible formula method. There must be a finished product specification. The product must be manufactured at least on pilot scale and three batches used for stability studies. Stability studies should be carried out on the product packaged in the container proposed for marketing. A summary of the stability studies undertaken must be provided. From stability data shelf life/ storage precautions should be proposed. A quality dossier must be provided for raw and finished materials The product must be produced from herbs that have been cultivated and harvested in accordance with GACP. 10. The starting material must be evaluated for risk of any environmental contamination.
3	Safety data requirements: Refers to safety pharmacology, including animal and human studies	Published animal or human studies. Review of any potential interactions with other drugs, side effects, and any proposed contraindications/ precautions in the product information. Recognized monographs on the material or product with information on safety. Any information concerning special groups such as children, the elderly or pregnant women. It is interesting to note that in the US, where herbal medicines are regulated as dietary supplements, manufacturers are not required to prove safety or efficacy, but the FDA can withdraw a product from sale if it proves harmful.
4	Traditional use evidence: Refers to history and prevalence.	There is no requirement to prove efficacy. Instead data must provide reference that the product has been in use as medicine for 30 years or more. Of 30 or more years, the last 15 must be in Europe. The data must be presented in a special format, called: <i>Common Technical Document Format</i> .

The Table was drawn based on data gathered from references including (DSHEA 1994; Goldman, 2001; De Smet, 2005; Ann Godsell Regulatory, 2008).

Table 2. EMEA requirements for registering herbal medicines.

S/No.	Extra requirement	Remark
1	Five (5) copies of the product dossier.	Probably unreasonable
2	Three (3) packs of the products samples.	Probably reasonable
3	Notarized original copy of the duly executed Power of Attorney from the product manufacturer.	Clearly unreasonable for all categories of applicants
4	Certificate of Manufacture issued by the competent health or regulatory authority in country of origin and authenticated by the Nigerian Mission in that country. Where there is no Nigerian mission, The British High Commission or an ECOWAS country Mission will authenticate.	Probably unreasonable for all categories of applicants
5	If contract-manufactured, Contract Manufacturing Agreement, properly executed and notarized by a Notary Public in the country of manufacture.	Clearly unreasonable for all categories of applicants
6	Current World Health Organization Good Manufacturing Practice Certificate for the manufacturer, authenticated by the Nigerian Mission.	Clearly unreasonable for all categories of applicants
7	Certificate of Pharmaceutical Products (COOP) duly issued and authenticated.	Clearly unreasonable for all categories of applicants
8	Current Superintendent Pharmacists license to practice issued by the Pharmacists Council of Nigeria (PCN).	Only probably reasonable
9	Premises Registration License from PCN	Only probably reasonable
10	Certificate of Registration of brand name with trademark registry in the Ministry of Commerce here in Nigeria; Letter of invitation from manufacturer to inspect factory abroad, stating full name and location of plant.	Probably unreasonable for all categories of applicants
11	The applicable fee payable only if documents are confirmed to be satisfactory.	Likely to be abused if the amount is high. The fee should be a token amount paid by all applicants
12	Nutraceuticals, medical devices and other regulated drug products have similar requirements, with minor variations. Specific details can be obtained from NAFDAC.	A sketch of the minor variations should be provided in print no matter how brief. Any information provided by NAFDAC should be printable for sake of transparency.

The information on NAFDAC were drawn from leaflets and NAFDAC's website (2010): www.nafdacnigeria.org/ The remarks are informed by current affairs and public perception of NAFDAC's role and activities including the wholesale reorganization of its Management in 2000.

Table 3. NAFDAC's extra requirements for registering herbal medicines.

production, distribution and use of herbal drugs. This is in view of the ever increasing use of herbs notably after the Alma-Ata Declaration (Ameh et al, 2010b) which paved the way for the stupendous growth of herbal drug use worldwide, particularly in North America where that growth had been stymied by the Flexner Report of 1910. That Report, which coincided with Paul Ehrlich's introduction of Salvarsan and the term "chemotherapy" in 1909, had favoured chemical medicine over herbal (Pelletier, 2006). Furthermore, apart from the said

growing use in the West, it is held that some 80 % of the populace in many developing countries still relies predominantly on herbs and other alternative remedies (WHO, 2008). Indeed, in some parts of Africa, for example, Ethiopia, a dependence of up to 90% has been claimed (BBC, 2006).

2. Experimental

The study applied official procedures – mainly WHO (1998) and BP (2004) to: evaluate the quality parameters of the raw materials and their extracts; and the changes in these parameters during dark, dry storage in capped glass bottles under tropical room temperature and humidity (RTH) as obtain in a typical Nigerian Traditional Apothecary (NTA). The parameters evaluated were: appearance, loss on drying, ash values, extractability, solubility, pH, TLC features, light absorption and foaming index. Basic morphological studies were carried out as per WHO (1998). Appropriate phytochemical tests were also conducted by official methods as described elsewhere (Ameh et al., 2010c; 2010d).

2.1 Treatment and sampling of material

The aerial parts of *Mitracarpus scaber* obtained during the months of October and November from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD) were air-dried in a well-ventilated shade, designed for drying medicinal plant materials. The materials were subsequently comminuted to coarse powder with a grinding machine. The procedure for sampling was as per WHO (1998) as had been described in detail earlier (Ameh et al., 2010c). Three (3) original samples from each batch or container were combined into a pooled sample and subsequently used to prepare the average sample. The average sample was prepared by “quartering” the pooled sample as follows: each pooled sample was mixed thoroughly, and constituted into a square-shaped heap. The heap was then divided diagonally into 4 equal parts. Any 2 diagonally opposite parts were taken and mixed carefully. This step was repeated 2 to 4 times to obtain the required quantity of sample. Any material remaining was returned to the batch. The final samples were obtained from an average sample by quartering, 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 in duplicate or triplicate. The samples for stability study were stored at room temperature and humidity (RTH) in capped glass bottles and placed in a shelf protected from light.

2.2 Macroscopic examination and phytochemical tests on the fresh and air-dried materials

The procedures adopted were as per WHO (1998). Shape and size were determined with the aid of a ruler and a pair of calipers. Diffuse day light was used on the untreated sample to determine its colour. The texture and surface/ fracture characteristics of the untreated sample were examined, where necessary, with x10 magnification hand lens to reveal the characteristics of cut surfaces. The material was felt by touch, to determine if it was soft or hard. Or was bent and ruptured, to obtain information on brittleness and appearance of fracture planes – whether it was fibrous, smooth, rough or granular. Odour was determined by placing a pinch in a 50-ml beaker, and then slowly and repeatedly the air above the material was inhaled. If no distinct odour was perceived, the material was crushed between

the thumb and index finger, and inhaled as above. The strength of the odour was determined as: odourless, weak, distinct, or strong. The sensation of the odour was determined as: aromatic, fruity, rancid, etc. etc. When possible, the odour was compared with that of a defined substance, such as menthol, sulphur dioxide, eugenol, etc. etc. Taste: In tasting the material, as recommended by our experience with the material, the following procedure was applied: a pinch of the material was mixed with water and savored, or chewed without swallowing, to determine the strength and the sensation of the taste. The strength is recorded as: tasteless, weak, distinct, or strong; and the sensation, as: sweet, sour, saline, or bitter. Phytochemical tests for tannins, saponins, terpenoids, anthraquinones and alkaloids were carried out on samples by procedures as described in detail elsewhere (Ameh et al., 2010c, d).

2.3 Loss on drying

This was carried out using a minimum of 0.5 – 1.0 g of material. Drying was effected in a Lindberg/Blue M gravity-convection oven maintained at 105-110 °C, for 3 h, after which the sample was allowed to cool to room temperature in a desiccator, and subsequently weighed. The time interval from the oven to point of weighing was usually about 30 minutes. The results are expressed as a range or as mean ± standard deviation.

2.4 Evaluation of extractive matter

About 4 g of accurately weighed coarsely powdered, air-dried sample was transferred into a glass-stoppered, 250-ml reflux conical flask, followed by the addition of 100 ml of solvent. The flask was weighed along with its contents, and recorded as W1. The flask was well shaken, and allowed to stand for 1 h. Subsequently a reflux condenser was attached to the flask, and gently brought to boiling and maintained thereat boiled 20 – 60 minutes depending upon the solvent. The mixture was subsequently cooled and weighed again. The weight was recorded as W2, and then readjusted to W1 with the solvent. The flask was shaken well once again and its contents rapidly filtered through a dry filter paper. By means a pipette, 25 ml of the filtrate was transferred to a previously dried and tarred glass dish and then gently evaporated to dryness on a hot plate. Subsequently, the dish was dried at 105 °C for 1-6 hours, cooled in a desiccator for 30 min, and weighed. The extractable matter was calculated as %w/w of the air-dried sample.

2.5 Determination of solubility of material in a given solvent – Methods I and II

The solubility of a material was determined at room temperature ~ 25°C and expressed in terms of “parts”, representing the number of milliliter of solvent, in which 1 g of the material is soluble. Vials of appropriate sizes: ~4-ml, ~ 12-ml and ~20-ml capacities were used. The mixtures were thoroughly shaken for at least 30 min before inspection for un-dissolved solute. In methods I, each vial received 100 mg of sample and the volume of solvent indicated. In method II, a vial received 100 mg and increasing volumes of solvent. The methods give the same results.

2.6 Light absorption and thin layer chromatography (TLC)

UV-VIS Spectrophotometer (Jenway or Shimadzu) and quartz 1-cm cells were used for the study. Solutions of herb and extract were made by thoroughly mixing 1 part of the solute

and with 100 parts of solvent methanol: water 80:20, v/v filtering, and diluting the filtrates by 150x with the same solvent. Absorbencies were measured at $\lambda 227$ nm, using the solvent as the blank. Florescent, precoated plates were used for normal phase TLC, utilizing silica K6, and hexane: ethylacetate as mobile phase. Solutions of analytes were prepared and applied as follows: To 1 mg of the analyte, 2 drops of ethanol were added and mixed well (~1 %w/v solution). The plates used were 5 cm wide x 20 cm long. With a ruler and a pencil, a distance of 5 mm was measured from the bottom of the plate, and a line of origin was lightly drawn across the plate, without disturbing the adsorbent. The analyte was applied to the origin as a 1 μ l droplet. The spot was allowed to dry. Subsequently, the plate was developed in a developing tank saturated with the vapour of the solvent system to be used as mobile phase. The level of the solvent in the tank was adjusted to a level 2 to 3 mm below the line of origin on the plate. The plate was considered developed when the solvent front reached a predetermined line, not less than 5 mm below the top of the plate. The air-dried plate was visualized using a viewing cabinet (Cammag) and a UV-lamp (Cammag - equipped to emit light at 254 or 366 nm). The resulting chromatogram was photographed and subsequently drawn to scale.

2.7 Determinations of pH of preparations – herb and the dry extract

Determination of pH values was with a Jenway pH Meter. Standard pH solutions: 4, 7 and 10; and freshly distilled water were used for the study.

2.8 Determination of foaming indices of preparations – herb and the dry extract

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 requirements for the test include: conical flasks (500-ml); volumetric flasks (100-ml); test tubes (16cm x 16mm); ruler; and stop-clock. The procedure was as follows: Exactly 1.0 g of powdered material was accurately transferred into a 500-ml conical flask containing 100 ml of boiling water, and maintained at moderate boiling for 30 minutes. The mixture was then cooled and filtered into a 100-ml volumetric flask. The volume was made up to 100 ml with water. Successive portions of 1 ml, 2 ml, 3 ml etc up to 10 ml of the filtrate was poured into ten stoppered tubes having the following dimensions: height, 16 cm; diameter, 16 mm. Subsequently, the volume of each tube was adjusted to 10 ml with water, stoppered and shaken in lengthwise motion for 15 seconds, at 2 shakes per second. The tubes were allowed to stand for 15 minutes, and the height of the foam in each tube was measured. The results were assessed as follows:

Foaming index is ≤ 100 , if the height of foam in all the tubes is less than 1 cm.

If a height of 1 cm is obtained in any tube, the volume [V] of the decoction in that tube, is used to determine the foaming index, as $= 1000/V$.

But if the tube above is the first or the second in the series, prepare an intermediate dilution to obtain a more precise result.

If the height of the foam is > 1 cm in every tube, the foaming index is over 1000.

To obtain a more precise result, repeat the determination using a new series of dilutions of the decoction. Note the tube in which the height of foam is 1 cm, and the volume [V] of the decoction therein, and calculate the foaming index, as $= 1000/V$. Results are expressed as expressed as a quantity [Q] per ml or as $[Q]\text{ml}^{-1}$.

3. Results

3.1 Results of botanical examination / phytochemical tests on the herb and extract

The key botanical and phytochemical characteristics of *Mitracarpus scaber* Zucc (Family: Rubiaceae) with Voucher specimen number: NIPRD/H/4208, preserved in the Institute's Herbarium are indicated in Table 4. The plant grows erect, up to 55 cm high, usually branched; the leaves are lanceolate, 2-4 cm long, with the upper surface bearing minute hairs. The plant manifests dense clusters of inflorescence, 6-14 mm across, with minute white flowers. The fruits are dehiscent capsules, about 0.5-1 mm long. Both the fresh plant and air-dried weed are practically odourless but possess a slight warm taste. Tannins, saponins and anthraquinones were detected in the weed. The extract also contained tannins and anthraquinones but not saponins. Tests for alkaloids were negative for the weed and extract.

Characteristic	Live Sample	Air-dried Sample
General appearance	 <p><i>M. scaber</i> is an annual, with erect stems, up to 55 cm high, and often branched. The leaves are lanceolate, 3-5 cm long, with the upper surface scabrous. The inflorescence consists of clusters of small white flowers. The fruits are dehiscent capsules, up to 1 mm long. The plant is of the Family, Rubiaceae, reproduces by seeds, and is found in the tropics.</p>	 <p>The air-dried sample consists of brownish green twigs and other parts that can readily be ground in a mortar or comminuting machine. The air-drying process takes about a week during the months of October to December, at NIPRD Herbarium, Abuja. The extracts obtained with various solvents yield a black, odorless and sticky mass.</p>
Odor	Odourless	Odourless
Taste	Very slightly warm	Slightly warm
Phytochemicals	Tannins, saponins and anthraquinones were detected. The tests for alkaloids were negative.	Tannins and anthraquinones were detected. The tests for alkaloids and saponins were negative.

The samples described above were obtained from the NIPRD Botanical Gardens at Idu Industrial Area, Idu, Abuja, Federal Capital Territory, Nigeria.

Table 4. Some key characteristics of *Mitracarpus scaber* and its aerial parts.

3.2 Results of physicochemical tests on the herb and extract of *Mitracarpus scaber*

Typical results of loss on drying (LOD as %w/w was 10.29 ± 1.81 for the herb; and 15.86 ± 0.72 for the extract) and total ash (TA as %w/w was 12.44 ± 2.95 for the herb; and 0.40 ± 0.09 for the extract) are shown in Table 5. The Table also shows that a 5%w/v mixture of the herb had pH of 5.7 ± 0.3 , while that of the extract was 6.9 ± 0.3 . The herb in water foamed slightly (that is: Foaming Index [FI] ≤ 100), but the extract did not foam at all (that is: FI = 0). Table 5 further shows that the herb in methanol/water (80/20: v/v) had an A1%1cm of 325.8 ± 15.6 , while that of the extract was 349.5 ± 14.1 . The Table also shows the extractabilities of the herb in various solvents. The extractability results expressed as (%w/w) were as follows: acetone, 6.89 ± 0.89 ; water, 28.37 ± 1.77 ; ethanol, 11.72 ± 0.81 ; ethylacetate, 14.02 ± 1.89 ; hexane, 4.11 ± 0.47 ; and methanol, 15.11 ± 1.07 . The extractability of the air-dried weed was highest in water and least in hexane. Among the organic solvents, extractability was lowest in hexane, and highest in methanol, followed by ethylacetate, ethanol and acetone.

Parameter (Mean \pm SD)	Air-dried Herb	Ethylacetate Extract
Loss on drying (LOD: % w/w)	10.29 ± 1.81 (n=12)	15.86 ± 0.72 (n=12)
Total ash (TA: % w/w)	12.44 ± 2.95 (n=12)	0.40 ± 0.09 (n=11)
pH of 5 % w/v in water	5.7 ± 0.3 (n=5)	6.9 ± 0.3 (n=5)
Foaming Index (FI: as ml ⁻¹)	Slight foam. FI ≤ 100 (n=5)	No foam. FI = 0 (n=5)
A 1%1cm at $\lambda 227$ nm (MeOH/ H ₂ O: 80/20 v/v)	325.8 ± 15.6 (n=5)	349.5 ± 14.1 (n=5)
Extractability (% w/w) in:	Extractive value (n=8-12)	-
Solubility (ml/g) in:	-	Solubility (n=2-6)
Water	28.37 ± 1.77	$>10^3$
Methanol	15.11 ± 1.07	25.0 ± 0.0
Ethylacetate	14.02 ± 1.89	25.0 ± 5.0
Ethanol	11.72 ± 0.81	25.0 ± 0.0
Acetone	6.89 ± 0.89	15.0 ± 0.0
Hexane	4.11 ± 0.47	55.0 ± 5.0

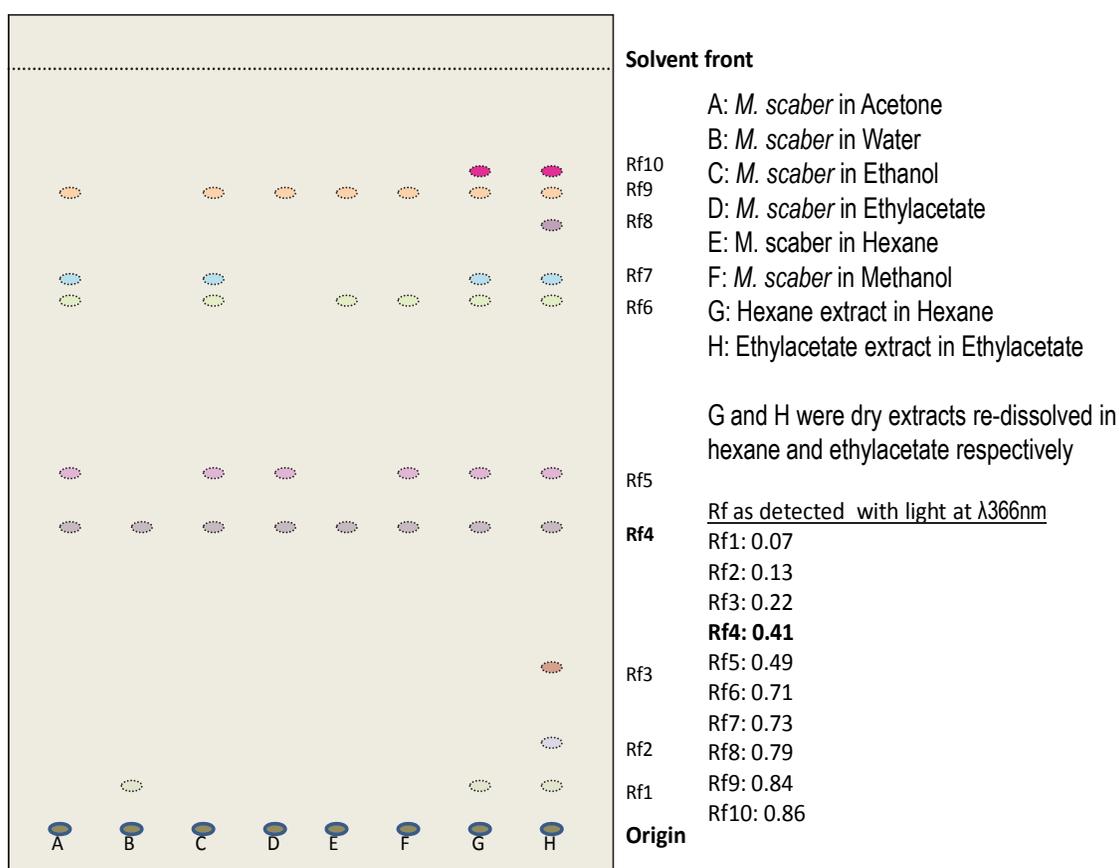
The LOD results were validated by concurrent determination of the LOD of copper sulphate, which result (mean \pm SD) was 36.12 ± 0.19 %. The results prove that the extract was quite hygroscopic. The low TA results for the extract but not the herb probably suggests a high presence of high bio-minerals. The high water extractability result agrees with the high TA of the herb, and the fact that hexane, the least polar solvent, produced the lowest extractability result. Among the organic solvents the solubility of the extract was least in hexane (55 ml/g), but higher in ethanol, ethylacetate and methanol (15-25 ml/g). The extract was practically insoluble in water ($>10^3$ ml/g). The colour of the solution obtained from the herb using different organic solvents was clear and greenish-brown in each case, but that obtained with water was yellowish brown, and slightly cloudy, with no tinge of green.

Table 5. Various physicochemical parameters of herb and extract of *Mitracarpus scaber*.

3.3 Results of thin layer chromatographic (TLC) studies on the herb and extracts of *M. scaber*

Figure 1 is a normal phase TLC of the herb and extract developed with hexane-ethylacetate. The Figure indicates the following: The herb in acetone (A) or ethanol (C) yielded 5 identical

principal spots, while the herb in water (B) yielded only 2 principal spots – Rf1 and Rf4. The herb in ethylacetate (D) or hexane (E) yielded 3 spots, while the herb (F) in methanol yielded 4. The dry hexane extract (G) re-dissolved in hexane yielded 7 spots, while the ethylacetate extract (H) re-dissolved in ethylacetate yielded 10. Notably, Rf4 was present in all the chromatograms, while Rf2 and Rf3 were present only in the H chromatogram. On the other hand Rf1 was present only in the B, G and H chromatograms.



The above diagram is of a normal phase TLC (K5 Silica, using hexane: ethylacetate at 60:40 v/v as mobile phase) of samples of samples of herb and extracts in various solvents as follows. Types of samples/ solvents: A: Herb in Acetone, B: Herb in Water, C: Herb in Ethanol, D: Herb in Ethylacetate, E: Herb in Hexane, F: Herb in Methanol, G: Hexane extract in Hexane, H: Ethylacetate extract in Ethylacetate. The samples in G and H were dry extracts re-dissolved in hexane and ethylacetate respectively. Rf as detected at $\lambda 366\text{nm}$: Rf1: 0.07, Rf2: 0.13, Rf3: 0.22, Rf4: 0.41, Rf5: 0.49, Rf6: 0.71, Rf7: 0.73, Rf8: 0.79, Rf9: 0.84, Rf10: 0.86. Descriptions/interpretations: The herb in acetone (A) or ethanol (C) yielded 5 identical principal spots, while the herb in water (B) yielded only 2 principal spots – Rf1 and Rf4. The herb in ethylacetate (D) or hexane (E) yielded 3 spots, while the herb (F) in methanol yielded 4. The dry hexane extract (G) re-dissolved in hexane yielded 7 spots, while the ethylacetate extract (H) re-dissolved in ethylacetate yielded 10. Notably, Rf4 was present in all the chromatograms, while Rf2 and Rf3 were present only in the H chromatogram. On the other hand Rf1 was present only in the B, G and H chromatograms.

Fig. 1. Diagrammatized normal phase TLC of *M. scaber* extracts showing up to ten principal spots.

Months of storage in capped glass bottles at RTIH	Appearance		Extractability of herb in different solvents			Solubility of extract in different solvents			Loss on drying (mean ± SD) %w/w	
	Herb	Extract	Ethyl ¹ -acetate	n-C ₆ H ₁₄	H ₂ O	Ethyl ¹ -acetate	n-C ₆ H ₁₄	H ₂ O	Herb	Extract
0	Wrinkled, brownish green/ grey leaves and twigs	Sticky mass becoming slightly gritty upon exposure	14.0 ± 1.8	4.2 ± 0.5	36.4 ± 2.2	25.0 ± 5.0	55.0 ± 5.0	>10 ³	10.51 ± 0.74 ^a (7)	14.07 ± 1.73 ^b (7)
3	Practically unchanged from above	Practically unchanged from above	15.3 ± 2.2	4.3 ± 0.5	33.9 ± 2.4	25.0 ± 5.0	45.0 ± 5.0	>10 ³	9.11 ± 0.67 ^a (6)	12.84 ± 1.18 ^b (6)
9	Practically unchanged from above	Practically unchanged from above	14.4 ± 1.9	4.1 ± 0.5	30.2 ± 2.4	25.0 ± 0.0	55.0 ± 0.0	>10 ³	10.44 ± 0.51 ^a (6)	13.14 ± 1.11 ^b (7)
21	Practically unchanged from above	Practically unchanged from above	14.7 ± 1.9	4.1 ± 0.5	30.7 ± 2.2 ^a	25.0 ± 5.0	50.0 ± 5.0	>10 ³	10.87 ± 0.73 ^a (7)	14.84 ± 0.99 ^b (5)
39	Practically unchanged from above	Practically unchanged from above	15.0 ± 2.0	4.3 ± 0.5	27.8 ± 2.0 ^b	25.0 ± 0.0	55.0 ± 0.0	>10 ³	8.96 ± 0.59 ^a (5)	11.44 ± 0.86 ^b (6)

The results show that storage of both the herb and the extract in capped glass bottles at room temperature and humidity for up to 39 months, produced no consistent or statistically significant change in moisture content. Both (a) and (b) indicate that the figures bearing them do not differ statistically from one another ($P > 0.05$).

Table 6. Effect of storage on herb and ethylacetate extract of *M. scaber* as evaluated by appearance, extractability, solubility and loss on drying.

3.4 Effect of storage on herb and ethylacetate extract of *M. scaber* as evaluated by appearance, extractability, solubility and loss on drying

Table 6 shows that the general appearance of the herb as wrinkled, brownish green/ grey leaves and twigs remained essentially unchanged up to the 39th month of storage. However, the extractability of the herb in water fell slightly but significantly as from after the 3rd month of storage. Table 6 also shows that neither the solubility profile nor the appearance of the extract and the solutions made from them in different solvents changed with storage. The same Table 6 shows that storage of the herb and the extract in capped glass bottles at room temperature and humidity (RTH) for up to 39 months produced no consistent or statistically significant changes in moisture content.

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

Table 7 presents the effect of storage on light absorption and TLC features of the herb and extract. It shows the following: that the difference in absorbance between 0th month and the 21st/ 39th months was insignificant for the herb ($P > 0.05$). By contrast, the corresponding difference for the extract was significant ($P < 0.05$). Table 7 also shows that the number of TLC spots observed for the herb and extract at every stage of storage was unchanged up to the 39th month.

Months of storage in capped glass bottles at RTH	Herb							Extract		
	Abs. at $\lambda 227$ nm	Types of solvent/ number of TLC spots						Abs. at $\lambda 227$ nm	TLC spots	
		A	B	C	D	E	F		G	H
0	2.172 \pm 0.104 (5)	5	2	5	3	3	4	2.330 \pm 0.094 (5)	7	10
3	2.221 \pm 0.114 (6)	5	2	5	3	3	4	2.174 \pm 0.107 (5)	7	10
9	2.144 \pm 0.098 (5)	5	2	5	3	3	4	2.104 \pm 0.070 (5)	7	10
21	2.322 \pm 0.117 ^a (5)	5	2	5	3	3	4	2.039 \pm 0.104 ^b (5)	7	10
39	2.233 \pm 0.114 ^a (5)	5	2	5	3	3	4	2.084 \pm 0.111 ^b (6)	7	10

For the herb, the difference in absorbance between 0th month and the 21st/ 39th months, denoted by (a), was insignificant ($P > 0.05$). By contrast, for the extract, the difference in absorbance between the 0th or 3rd month and the 21st or 39th month, denoted by (b), was significant ($P < 0.05$). Notably, the number of TLC spots observed for both the herb and extract remained unchanged up to the 39th month.

Table 7. Effect of storage on light absorption and TLC characteristics of herb and extract of *M. scaber*.

3.6 Effect of storage on pH and foaming indices of herb and ethylacetate extract of *M. scaber*

Table 8 shows that the pH of a 5 %w/v mixture of the herb or extract in water did not change significantly with storage for up to 39 months. However, although the 5 %w/v

mixture of the fresh plant material in water did foam slightly; this property diminished rapidly, and was totally lost after the 3rd month of storage. By contrast, the ethylacetate extract never foamed at any stage of storage.

Months of storage in capped glass bottles at RTH	pH of mixture (5% w/v)		Foaming index	
	Herb in water	Ethyl acetate extract in water	Herb in water	Ethylacetate extract in water
Within 1 st day of harvest or preparation	5.7 ± 0.3 (5)	6.9 ± 0.3 (5)	Foam: Slight ≤ 100 (5)	Foam: Nil (5)
0	5.9 ± 0.2 (6)	6.1 ± 0.3 (5)	Foam: Slight < 100 (5)	Foam: Nil (6)
3	5.6 ± 0.2 (5)	6.2 ± 0.4 (6)	Foam: Slight < 100 (5)	Foam: Nil (5)
9	5.4 ± 0.2 (5)	6.1 ± 0.3 (5)	Foam: Nil (6)	Foam: Nil (5)
21	5.9 ± 0.3 (7)	6.7 ± 0.3 (5)	Foam: Nil (5)	Foam: Nil (5)
39	5.9 ± 0.3 (5)	6.5 ± 0.3 (5)	Foam: Nil (5)	Foam: Nil (5)

Both (a) and (b) indicate that the pH of the 5 %w/v aqueous mixtures at every stage fell within the mean values of $5.8 \pm 0.2a$ and $6.4 \pm 0.4b$ - they indicate that any deviations from these mean values were insignificant ($P > 0.05$). The freshly harvested samples foamed measurably, but the ability was totally lost after the 3rd month of dry storage. In all cases however, the dry ethylacetate extract was virtually insoluble in water, and did not foam at all.

Table 8. Effect of storage on pH and foaming indices of the fresh plant material, the dry herb and the ethylacetate extract of *M. scaber*.

4. Discussions

The aim of this study was to apply official methods of WHO (1998) and BP (2004) to study the key quality attributes of the air-dried weed and the ethylacetate extract of *Mitracarpus scaber*, for the purpose of quality control, GMP production and registration of Niprifan by NAFDAC. WHO had defined "Herbal Substance" as "Material derived from the plant(s) by extraction, mechanical manipulation, or some other process" (WHO, 2005). Thus, either the ethylacetate extract, or even the comminuted, air-dried vegetable matter, may rightly be termed the "Herbal Substance" of Niprifan. Since the advent of the Alma-ata Declaration in 1978, many developing countries opted to adopt the WHO model in developing their National Traditional Medicine, especially phytotherapy (Ameh et al., 2010b). NIPRD's adherence to the WHO model had resulted in the sickle cell drug - Niprisan, developed from Yoruba Traditional Medicine (Wambebe et al., 2001). It is generally held that in most countries, especially in Africa, the populations depend greatly on herbal remedies, up to 90 % in some instances like Ethiopia (BBC, 2006). Such high dependence calls for a system or mechanism for harnessing and optimizing all or most of such plant resources. That means that every effort must be made to obtain maximum benefits from them. One way to do this is to standardize the raw materials used in producing the remedies, by studies such as this

one. Such studies will at least help to minimize waste, and even lead ultimately to conservation of endangered plants. Indeed, efforts at conservation are more likely to succeed when the value of what is to be conserved is proven.

Our immediate interest however, is in the need to entrench the use of these resources by taking appropriate actions, which, in this case is - an application to NAFDAC to consider the registration of Niprifan, based on folkloric use evidence, pertinent literature, and the experimental data provided in this study. These three lines of evidence can be summarized as follows. At the peak of British colonialism in Africa considerable effort was made to harness the continent's wealth in herbal traditions. Thus at as far back as the 1930s a team of British scientists had combed the entire West Africa to research traditional herbal remedies. Thus, for *Mitracarpus scaber*, Hutchinson and Dalziel (1948) reported a number of findings that have subsequently been confirmed by work in NIPRD and elsewhere (Benjamin et al., 1986; Irobi and Daramola, 1994; Cimanga et al., 2004; Abere et al., 2007a, 2007b). These include the following: that *M. scaber* was widely distributed and used topically in all of West Africa for various skin infections; and orally for various internal conditions. Among the traditional indications mentioned, and which have since been confirmed by NIPRD's Ibrahim Muaazzam (ethnobotanist and consultant on TM) are: leprosy, lice, ringworm, eczema and craw-craw. Currently, the plant is used orally for sore throat, for which purpose it is wholly macerated in water.

Among the vernacular names of *M. scaber* are: Hausa (*goga masu*); Fulani (*gadudal*); Yoruba (*irawo-ile*); and Ibo (*obu obwa*). Professor Ogundiani (2005) in his inaugural address at the University in Ile-Ife commented on Niprifan,

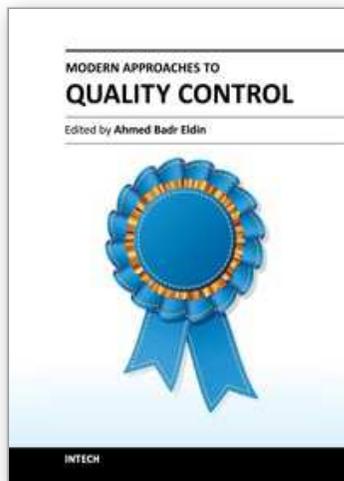
stressing the antimicrobial potency of *M. scaber*. Ogundiani, as stated in the lecture, had been unaware of the NIPRD's work on Niprifan, until shortly before the inaugural, since that work, led by Professors Wambebe, Okogun and Nasipuri, had been unpublished. Therefore, in this paper we elect to present not only these historical antecedents, but also to furnish the results of our evaluation of the key quality variables of the herb and extract of *M. scaber*, with a view to advancing the registration of Niprifan (for skin infections) by NAFDAC. The results here presented probably suffice for quality control and GMP production, particularly if more emphasis is placed on technical requirements than on bureaucracy. It must be remarked at this juncture that NAFDAC only belatedly recognized the sickle cell drug, Niprisan, after the US-FDA and EMEA had granted it orphan status (Pandey, 2003). One may wonder - What a paradox! Why should the US and Europe that need herbal drugs far less than Nigeria be keener in their regulation? Therefore, from the foregoing, it seems that the key to this Nigerian enigma lies not in the technical but in the non-technical differences between NAFDAC and EMEA as depicted in Tables 1-3. The said differences which hinge on NAFDAC's extra requirements (Table 3) suggest that NAFDAC needs to re-strategize for efficient discharge of its Mandate. For example, despite the widespread use of herbal medicines in Nigeria and the Federal Policy on TM (2007), NAFDAC is not known to have "fully registered" a single herbal medicine since its creation in 1992/3, whereas it should. This is the puzzle this article had hoped to address.

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