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Research Article

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PROSPECTS OF PHARMACEUTICAL PRODUCTION OF DOSAGE FORMS FROM NIGERIAN GROWN AYURVEDIC PLANT - *Andrographis paniculata* NEES (FAMILY: ACANTHACEAE)

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ABSTRACT

The intensely bitter Ayurvedic plant - *Andrographis paniculata,* was introduced to the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, in the 1990s, with seeds obtained in India. This study aimed to verify the key quality parameters of the air-dried herb and the aqueous extract of *A paniculata*; and to estimate their shelf lives, for the purpose of local production of the drug, according to good manufacturing practice (GMP). The appearance, moisture content, extractability of the herb or solubility of the extract, pH, light absorption, chromatographic properties, bitterness values and foaming indices of the air-dried herb and extract (stored in capped glass bottles at normal room temperature and humidity) were monitored periodically (at 0, 3, 9, 21 and 39 months) using official procedures. The results showed that the above named features did not change significantly for both the dried herb and extract, for up to 21 months or more, but the herb appeared to have better keeping qualities, as judged by the changes noticed in appearance, extractability, solubility, bitterness values and foaming indices. The results suggest that the dried herb might be more stable than the extract, and that a shelf life of up to a year or more may be considered for both the herb and the extract..

Keywords: Pharmaceutical production, Quality, Stability, *Andrographis paniculata*, Ayurvedic plant.

INTRODUCTION

Seeds of the exceedingly bitter Ayurvedic plant - *Andrographis paniculata,* were introduced from India in the 1990s into the botanical gardens of NIPRD.[1] The Nigerian plant grows erect to a height of up to a meter, but often, the stem has a tendency to slant in a manner that makes the plant appear as if it were creeping. The leaves range in size, but are essentially ovate, pinnate or lanceolate. The flowers have minute white petals bearing purplish spots. The young stem is deep green, with diameter ranging from 2 mm to 6 mm or more. The flowers give rise to oblong capsules bearing numerous, minute brown seeds. In NIPRD, the seed is sown early in the rainy season (March or April), and grows into a perennial herb that begins to flower as from about August or September. Unpublished studies with the Nigerian herb in NIPRD had confirmed the commonly ascribed anti-infective and immunostimulatory properties of the Indian herb.[2,3] Thus, during the late 1990's when there was still a great shortage of affordable intervention in the control of HIV-AIDS in Nigeria, pressure was mounted on NIPRD to meet the challenge head-on. This led inexorably to the development of CONAVIR®, an immunostimulant phytomedicine containing *Andrographis paniculata*.[4,5] A key step in the venture was to domesticate the plant; standardize the dry herb[6]; and determine its keeping qualities. In this paper we present the results of our attempts to evaluate the keeping qualities of the dry herb and the dry aqueous extract stored in firmly capped glass bottles, and maintained at normal room temperature and humidity. We had earlier reported some results on basic phytochemical and quality assessment tests, including heavy metal content.[1,5]

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MATERIALS AND METHODS

Treatment and sampling of material

The aerial parts of *Andrographis paniculata,* obtained during the months of September and October from the botanical garden of 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 powdered aqueous extract of the herb was provided by the Institute's department of Medicinal Plant Research and Traditional Medicine. The procedure for sampling was as per WHO[7] as described previously[1]. Three 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.

Loss on drying (LOD): This was carried out using a minimum of 0.5 – 1.0 g of material. Drying was effected in a Lindberg/Blue M gravity-convention oven maintained at 105-110 0C, 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.

Evaluation of water extractive value*:* 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 water. 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 boiled for 1 h, cooled and weighed again. The weight was recorded as W2, and then readjusted to W1 with water. 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 6 h, cooled in a desiccator for 30 min, and weighed. The water extractable matter was calculated as %w/w of the air-dried sample.

Determination of solubility in water: This was determined at a room temperature of ~25°C, and the result expressed in terms of "parts', representing the number of milliliter (ml) of solvent, in which 1 g of the material is soluble. Vials of appropriate capacities: ~4-ml, ~8-ml, ~12-ml and ~16-ml were used. The mixtures were thoroughly shaken for at least 30 min before they were inspected for any un-dissolved solute. In this method, a series of 10 vials was set up. The vials were arranged in order of increasing capacities so that there would be sufficient space in each vial for shaking. Each vial received 100 mg of sample, and increasing volumes of water (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 ml to the vials. The vials were vigorously shaken up to 30 minutes or more and intermittently examined for any un-dissolved solute. The vial preceding the first vial with un-dissolved was used in the calculation of solubility – defined in this case as the minimum volume of water in which 1 g of the drug is completely soluble under the conditions of the experiment. The following descriptive terms may also be used to qualify solubility as per WHO[6]: very soluble: less than 1 part; freely soluble: 1 – 10 parts; soluble: 10 – 30 parts; sparingly soluble: 30 – 100 parts; slightly soluble: 100 – 1000 parts; very slightly soluble: 1000 – 10, 000 parts; and practically insoluble: more than 10, 000 parts.

Light absorption and pH of preparations: Apparatuses: UV-VIS Spectrophotometer (Jenway or Shimadzu) and pH meter (Jenway).

Procedures: 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 quartz or glass cells for wavelengths below 400 nm or above 400 nm respectively. 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 pH meter.

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). 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 µl 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 is visualized using a viewing cabinet (CAMMAG) and a UV-lamp (CAMMAG – equipped to emit light at 254 or 366 nm). The resulting chromatogram is photographed or drawn to scale.

Pharmacological parameters

Determination of bitterness value: The bitterness of the herb was determined by the method of WHO[7] as previously described in detail[1]. The method compares 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. The method is identical to that described in the European Pharmacopoeia[8] as recently used by Meyer and coworkers[9]. The bitterness value is calculated as follows:

$$Bitterness\ value\ in\ units\ per\ g = \frac{2000 \times C}{A \times B} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Where,

A = concentration of herbal stock solution (S_h) in (mg/ml).

B = volume of S_h (ml) in the tube with the threshold bitter concentration.

C = quantity of quinine hydrochloride (mg) in the tube with the threshold bitter concentration.

Determination of foaming index: Procedure: This was as per WHO[7]**.** The herb or extract was powdered, and 1.0 g was transferred into **a** 250ml 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 100ml volumetric flask, and made up to the mark with water. Subsequently, from the filtrate, portions of 1 ml, 2 ml, 3 ml etc up to 10 ml, were transferred into ten stoppered tubes having the following dimensions: height, 16 cm; diameter, 16 mm. Next, 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. Each tube was allowed to stand for 15 minutes, and then the height of the foam in each tube was measured. The results were assessed as follows: Foaming index (FI) was less than 100, if the height of foam in all the tubes was less than 1 cm. If a height of 1 cm was obtained in any tube, the volume (V) of the decoction in that tube, was used to determine the foaming index, as

$$FI = {}^{1000}/_{V}$$

But if the tube above was the first or the second in the series, an intermediate dilution was prepared to obtain a more precise result. If the height of the foam was more than 1 cm in every tube, the foaming index was considered to be over 1000. To obtain a more precise result, the determination was repeated using a new series of dilutions of the decoction. The tube in which the height of foam was 1 cm was noted, as was the volume (V) of the decoction therein. The foaming index was calculated as FI = 1000/V. Foaming index is expressed as a quantity (Q) per ml or as (Q) ml^{-1}.

RESULTS AND DISCUSSION

The results in Table **1** show that the moisture content of the dried herb and extract did not change significantly during storage in glass bottles for up to 39 months. Table **2** shows that the water extractability of the herb also did not change significantly; neither did the pH; but the greenish brown color of the extract faded slightly by the 21st month of dry storage of the herb. Table **2** also shows that the solubility of the dried extract in water decreased slightly but significantly by the 21st month storage, although the color and the pH did not. The emerging trend in the above is supported by Table **3**, which shows that neither the light absorption at λmax nor the chromatographic characteristics of the materials changed detectably up the 39th month of dry storage of herb or extract. Differing, but only slightly however, are the results in Table **4**, which show that whereas the bitterness values of the herb and extract did not change significantly with storage, the foaming indices of both decreased significantly by the 21st / 39th month of storage.

Although the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) does not explicitly require stability data for registering of herbal remedies, the fact remains that somewhere along the characteristically circuitous process; the need for these data would become pretty obvious. In the European Union (EU) however, stability data are among the four categories of data explicitly required for manufacturing and marketing authorization[10,11]. The three other categories required[11] are: Product information – comprising a summary of product characteristics; Safety data requirements - referring to safety pharmacology, including animal and human studies; and Traditional use evidence - referring to history and prevalence. Actually, stability data are required as a part and parcel of a host of other data, collectively termed "Quality control data", which refers to the set of requirements for production according to good manufacturing practice (GMP). The EU's explicit GMP requirements for herbal manufacturing authorization[11] are:

(i) Production must be in a GMP compliant facility.
(ii) The medicine must be produced with a validated formula.
(iii) There must be a finished product specification.
(iv) The product must be manufactured at least on pilot scale, and three batches used for stability studies.
(v) Stability studies should be carried out on the product packaged in the container proposed for marketing.
(vi) A summary of the stability studies undertaken must be provided.
(vii) From the stability data shelf life and storage precautions should be proposed.
(viii) A quality dossier must be provided for both starting materials and finished product.
(ix) The product must be produced from herbs that have been cultivated and harvested in accordance with Good Agricultural and Collection Practice (GACP).
(x) The raw material must be evaluated for risk of any environmental contamination.

The present study specifically addressed the issues raised in (v), (vi) and (vii) above. To this end therefore, Table **1** shows that the moisture contents of the dried herb and the extract did not change significantly during storage, for up to 39 months. This would indicate that changes in moisture content as a possible cause for microbial spoilage or chemical (hydrolytic) instability would be minimal. As shown in Table **2**, the extractability of the herb also did not change significantly; neither did the pH; but the greenish brown color faded slightly by the 21st month. However, Table **2** does show that the solubility of the dry extract decreased slightly but significantly by the 21st month, although the color and the pH did not. Therefore, the results (Table **2**) collectively suggest that the biochemical integrity of the materials was essentially preserved up to 39 months, for the herb; and 21 months, for the extract. This is supported by the fact that Table **3** shows that neither the light absorption at λmax nor the chromatographic characteristics of the two materials changed detectably up the 39th month of storage. However, a slight modification of this position is called for, by the results shown in Table **4**. Table **4** shows that whereas the bitterness values of the herb and extract did not change significantly with storage, the foaming indices of both the extract and the herb decreased significantly by the 21st and 39th month of storage respectively as compared with 0 month. Therefore, the results on the whole suggest that the herb had better keeping qualities than the extract; but that both can be safely ascribed a shelf life of a year or more.

Table 1. Stability of the dry herb and dry aqueous extract of *Andrographis paniculata* as monitored by loss on drying

Months of storage in capped glass bottles at room temperature and humidity (RTH)	Loss on drying (mean ± SD) %w/w	
	Herb	Extract
0	9.84 ± 0.66[a] (n = 7)	10.07 ± 1.90[b] (n = 7)
3	8.64 ± 0.59[a] (n = 5)	10.84 ± 1.03[b] (n = 5)
9	9.14 ± 0.66[a] (n = 7)	12.14 ± 2.33[b] (n = 5)
21	10.77 ± 0.86[a] (n = 5)	9.84 ± 2.06[b] (n = 5)
39	8.96 ± 0.46[a] (n = 5)	11.44 ± 2.03[b] (n = 5)

**The results show that the moisture contents of the materials did not change significantly (P > 0.05) as denoted by ([a]) for the herb, and ([b]) for the extract, during the storage period of 0 to 39 months. The results suggested that changes in moisture content as a likely contributory factor to moisture induced instability or hydrolytic spoilage would be minimal.*

Table 2. Stability of the dry herb and dry aqueous extract of *Andrographis paniculata* as monitored by extractability/ solubility in water/ appearance/ pH

Storage in capped glass bottles at RTH (months)	Herb			Extract		
	Extractability % Mean ±SD	Appearance of extract	pH	Solubility (ml/g)	Appearance of solution	pH
0	30.80 ± 3.63[a] (n=7)	Clear, greenish brown	7.9	35 ± 5 (n=7)	Clear, greenish brown	8.5
3	33.37 ± 4.77[a] (n=5)	Clear, greenish brown	8.1	40 ± 5 (n=5)	Clear, greenish brown	8.2
9	34.88 ± 5.43[a] (n=5)	Clear, greenish brown	7.9	40 ± 5 (n=5)	Clear, greenish brown	8.2
21	25.08 ± 3.43[a] (n=7)	Clear, pale greenish brown	8.0	45 ± 5[b] (n=7)	Clear, greenish brown	8.3
39	29.02 ± 3.63[a] (n=5)	Clear, pale greenish brown	7.9	45 ± 5[b] (n=5)	Clear, greenish brown	8.3

** The extractability and pH of the herb did not change significantly, but the greenish brown color faded slightly by the 21st month. The solubility of the dry extract decreased significantly by the 21st month, but the color and the pH did not. The values with ([a]) were statistically the same (that is: P > 0.05). The results for the 21st and 39th months marked with ([b]) differed significantly (P < 0.05) from those for 0 – 9 months.*

Table 3. Stability of the dry herb and dry aqueous extract of *Andrographis paniculata* as monitored by light absorption and TLC

Months of storage in capped glass bottles at RTH	Herb			Extract		
	Absorbance at λ225 nm	TLC spots		Absorbance at λ225 nm	TLC spots	
		NP	RP		NP	RP
0	0.083 ± 0.015[a] (n=7)	5	6	0.291 ± 0.037[b] (n=7)	5	6
3	0.107 ± 0.025[a] (n=5)	5	6	0.306 ± 0.031[b] (n=5)	5	6
9	0.115 ± 0.025[a] (n=5)	5	6	0.292 ± 0.031[b] (n=5)	5	6
21	0.078 ± 0.015[a] (n=7)	5	6	0.240 ± 0.031[b] (n=7)	5	6
39	0.091 ± 0.015[a] (n=5)	5	6	0.276 ± 0.031[b] (n=5)	5	6

** Solutions of herb and extract were made by thoroughly mixing 1 part of solute and with 100 parts of solvent (MeOH: H_2O [52:48, v/v]), filtering, and diluting the filtrates by 150X with the same solvent. Absorbencies were measured at λ225 nm, using the solvent as the blank. Both ([a]) and ([b]) denote that any differences seen in the values were not significant (P >0.05). NP and RP mean normal phase and reverse phase respectively.*

Table 4. Stability of the dry herb and dry aqueous extract of *Andrographis paniculata* as monitored by bitterness value and foaming index

Months of storage in capped glass bottles at RTH	Bitterness value		Foaming index	
	Herb x 10^3	Extract x 10^3	Herb x 10^3	Extract x 10^3
0	1.77 ± 0.25[a] (7)	18.65 ± 3.09 (7)	1.56 ± 0.11[a] (7)	8.72 ± 0.32 (7)
3	1.53 ± 0.16[a] (5)	17.31 ± 2.92 (5)	1. 46 ± 0.24[a] (5)	9.31 ± 0.63 (5)
9	2.08 ± 0.21[a] (5)	17.87 ± 3.11 (5)	1.43 ± 0.11[a] (5)	8.68 ± 0.38 (5)
21	1.31 ± 0.18[a] (7)	12.04 ± 2.11[b] (7)	1.38 ± 0.12[a] (7)	6.22 ± 0.34[b] (7)
39	1.43 ± 0.19[a] (5)	12.73 ± 2.08[b] (5)	1. 65 ± 0.11[a] (5)	6.15 ± 0.34[b] (5)

** The results show that both the bitterness value and the foaming index of the herb did not change significantly with storage during 0 – 39 months (P > 0.05), as indicated by ([a]). By contrast, both the bitterness value and the foaming index of the extract decreased significantly with storage as from the 21st/ 39th months of storage (P < 0.05), as indicated by ([b]).*

CONCLUSION

The results of this study established that the herb and the dry water extract of the aerial parts of Nigerian grown *Andrographis paniculata* are stable for at least a year and a half even in tropical conditions, and that the results are of value in GMP production and regulation of the drug produced from the Nigerian grown Ayurvedic plant. It is to be noted that slight variations of this approach to herbal drug development, which is recommended by the World Health Organization[7,12], had been adopted in the development of Niprisan – the sickle cell drug[13], from four tropical herbs, including *Piper guineense*[14].

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