

## Research Article

# Quality Control Tests on *Andrographis paniculata* Nees (Family: Acanthaceae) – an Indian ‘Wonder’ Plant Grown in Nigeria

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## Abstract

**Purpose:** To characterize the aerial parts of *Andrographis paniculata*, a bitter Indian herb grown in Nigeria, for the purpose of quality control.

**Methods:** The determination of bitterness value and of various physicochemical characteristics; tests for key phytochemicals; and thin layer chromatography (TLC) of the air-dried herb, were carried out as prescribed in standard texts.

**Results:** The mean bitterness value of the herb for both men and women was  $2.86 \pm 1.74 \times 10^3$  units per g. The male value ( $2.07 \pm 1.42 \times 10^3$ ) appeared to be lower than the female's ( $3.52 \pm 1.82 \times 10^3$ ) but the difference was not statistically significant. The results (% w/w) of loss on drying ( $10.64 \pm 0.36$ ), total ash ( $14.10 \pm 4.49$ ), water extractive value ( $30.37 \pm 2.63$ ) and acid insoluble ash ( $1.00 \pm 0.06$ ) were similar to those reported for the Asian plant. The phytochemical tests revealed the presence of glycosides, saponins, tannins and alkaloids, but not of anthraquinones. Normal phase TLC of the drug yielded 5 spots as against 6 spots yielded by reverse TLC.

**Conclusion:** The results provide useful quantitative and descriptive data that are essential for identifying and characterizing the Nigerian grown herb for the purpose of quality control; and confirm key similarities between the Nigerian and the Asian plant.

**Keywords:** *Andrographis paniculata*, Quality control, Bitterness value, Physicochemical, Phytochemical, Chromatography.

Received: 12 January 2010

Revised accepted: 11 June 2010

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## INTRODUCTION

The World Health Organization (WHO) [1] prescribed a number of quality control tests that medicinal plant materials should undergo. These can be classified into four broad areas: botanical, physicochemical, pharmacological and toxicological. The botanical embraces sensory evaluation, foreign matter, microscopy and histochemistry while the physicochemical includes loss on drying, ash values, extractive values, volatile oils and chromatography – TLC and HPLC. The pharmacological includes bitterness value, haemolytic activity, astringency or tannin content, and swelling and foaming indices. The toxicological embraces a variety of studies including: tests for arsenic and heavy metals, pesticides, aflatoxins, radioactivity, microbial contamination, and tests for specific organisms such as *Salmonella*.

In this study, we have determined the bitterness value of *Andrographis paniculata* as a key pharmacological parameter; substituted microscopy and histochemistry for classical phytochemical screening; and carried out the following physicochemical tests: loss on drying, ash, extractability and TLC. The cultivation of *Andrographis paniculata* in NIPRD's botanical garden commenced in the 1990's with seeds obtained from India. In this study we examine the dry aerial parts of the plant obtained in the Institute, using the methods prescribed by WHO [1]. The results obtained are discussed within the context of the Institute's aim of developing a suitable dosage form from *Andrographis paniculata* – a bitter wonder plant reputed for its immunostimulant and other properties in humans [2-7].

## EXPERIMENTAL

### Sampling and treatment of material

The aerial parts of *Andrographis paniculata*, obtained during the months of September and October 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 [1] for sampling was as follows: 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.

### Determination of bitterness value

The bitterness of herbs was determined by the method described by WHO [1] which 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. Thus, the bitterness value of the solution (1 g of quinine hydrochloride in 2000 ml of drinking water) is set at  $2 \times 10^5$  units. The method is identical to that described in the European Pharmacopoeia [8], and used by Meyer et al [9]. The following provisos, based on WHO stipulation [1] were applied:

1. Safe drinking-water was used for extracting the aerial parts of the herb, for mouth-wash after each tasting, and for dissolution of the quinine.

2. In order to obtain an informed consent of the volunteers consisting of staff, interns and students, they were first given a seminar on the taste physiology, bitterness and the WHO method for determining bitterness. Subsequently a date for the test was agreed. It was further agreed that participants will refrain from food, drinks and medicaments an hour before the test.
3. Since sensitivity to bitterness varies greatly between persons and at different times in the same person, each participant in the test tasted both the herbal extract and the quinine solution within a short space of time.
4. All participants were first required to taste the drinking water to be used in the test and a solution of 0.058 mg of quinine hydrochloride in 10 ml of that water. Only those who sensed no bitterness in the water, but sensed bitterness in the quinine solution were included in the test.
5. The tasting each series of dilutions of the extract or quinine solution must commence with the lowest concentration in order to retain sufficient sensitivity of the taste buds.
6. All the solutions and the drinking-water for mouth washing should be at 20-25°C.
7. Since the calculation of bitterness value is based on the procedure here described, this must be followed with strictly as follows:

#### **Preparation of standard quinine solution**

Exactly 0.1 g of quinine hydrochloride was dissolved in sufficient drinking-water to produce 100 ml. Subsequently, 5 ml of this solution was diluted to 500 ml with safe drinking-water to give the stock standard solution of quinine hydrochloride labeled  $S_q$ , and contained 0.01 mg of the quinine standard/ml. Nine test-tubes labeled 1 to 9 were set up to contain 4.2, 4.4, 4.6, 4.8, 5.0,

5.2, 5.4, 5.6 and 5.8 ml of ( $S_q$ ), respectively. Into tubes 1 to 9 were then added 5.8, 5.6, 5.4, 5.2, 5.0, 4.8, 4.6, 4.4 and 4.2 ml of drinking-water, respectively. This meant tubes 1 to 9 contained 0.042, 0.044, 0.046, 0.048, 0.050, 0.052, 0.054, 0.056 and 0.058 mg of quinine hydrochloride, respectively.

#### **Preparation and dilutions of herbal extract (test) stock solution**

Exactly 1 g of the herb was extracted with drinking-water to produce 1000 ml of aqueous extract. Subsequently, 5 ml of the extract was diluted to 500 ml with drinking-water. This solution was labelled the stock extract ( $S_h$ ). It contained 0.01 mg of the herb /ml. Ten tubes labeled 1 to 10 were set up to contain 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of  $S_h$ , respectively. Into tubes 1 to 10 were then added 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0 ml of drinking-water, respectively.

#### **Procedure for the test**

First, the participant rinsed his or her mouth with drinking-water, and then tasted 10 ml of the most dilute solution by swirling it in the mouth for 30 s, noting whether or not the solution tasted bitter. He or she held the solution in the mouth for another 30 s, and noted whether or not there was a loss of bitterness. Subsequently, the solution was spat out, and the mouth rinsed with drinking-water. The participant waited for 10 min before the next higher concentration was tasted. The procedure above was repeated until the dilution with TBC (that is the lowest concentration at which a solution continues to taste bitter after 30 seconds) was attained by the participant. After the first series of tasting (either with the quinine solution or the herbal extract), the mouth was rinsed thoroughly with drinking-water until no bitter sensation remained. A waiting time of 10 minutes must elapse before carrying out the second series of tasting. Bitterness value (units/g) was computed from Eq 1 [1].

$$\text{Bitterness value (units/g)} = (2000 \times C) / (A \times B) \dots\dots (1)$$

where  $A$  = the concentration of the herbal stock solution ( $S_h$ ) in (mg/ml),  $B$  = the volume of  $S_h$  (in ml) in the tube with the threshold bitter concentration, and  $C$  = the quantity of quinine hydrochloride (in mg) in the tube with the threshold bitter concentration.

### **Physicochemical tests**

The following tests, briefly described, were carried out on the extracts as per WHO [1].

#### **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-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  $\pm$  standard deviation.

#### **Total ash (TA) and Acid insoluble ash (AIA)**

These values were determined using a minimum of 0.5 – 1.0 g of material in a furnace (Vecstar Furnace) heated gradually to the ignition temperature of 650 - 700 °C. The process was repeated until at least two consecutive constant weights were obtained. The results are expressed as range or mean value  $\pm$  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  $W_1$ . 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  $W_2$ , and then readjusted to  $W_1$

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.

### **Phytochemical tests**

The following tests as described by Harborne [10] and Onwukaeme and coworkers [11] were carried out on the herb or aqueous extract.

#### **Fehling's test for reducing sugars**

To about 10 mg of the extract in test-tube was added 2 ml of water, followed by 0.2 ml of 0.1 M HCl, to effect hydrolysis of the glycosides. For the control, 0.2 ml of water was used instead of the acid. The mixture was heated in a boiling water bath to accelerate dissolution, and then left further in the bath for 5 min. Subsequently, 1 ml each of Fehling's solutions A and B were added while continuing to shake the mixture in the bath for 10 min. A brick-red precipitate indicated the presence of reducing sugars, formed from the hydrolysis of glycosides.

#### **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 min indicates the presence of saponins.

#### **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 indicated the presence of fully oxidized anthraquinone derivatives.

### **Test for tannins**

About 10 mg of the aqueous extract was vigorously shaken with 3 ml of warm water until dissolved. This was followed by the addition of 1 ml of 15 % ferric chloride test solution. A blue-green coloration indicated the presence of tannins.

### **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 Solution A + 5 ml Solution B + 20 ml 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.

### **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 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.

## **RESULTS**

Results from the determination of bitterness value are shown in Table 1, while those from the physicochemical determinations are shown in Table 2. The phytochemical profile of the herb is shown in Table 3, while the attempt to provide TLC fingerprints for the herb is indicated in Figure 1. Table 1 shows that that the males were more sensitive than females to the bitterness of quinine hydrochloride; the difference was significant at ( $p < 0.05$ ): Although the males appeared to be less sensitive to the bitterness of *Andrographis paniculata*, in that their mean bitterness value was lower than those of the female, the difference was not significant ( $p = 0.05$ ; two-tail, 10 degrees of freedom). Notably, the mean bitterness value for both sexes was  $2.86 \pm 1.74 \times 10^3$ . The volunteer denoted (\*), aged 42, had cold at the time of the experiment. If the result of this volunteer ( $1.30 \times 10^3$ ) is set aside, the female mean value becomes  $3.97 \pm 1.63^c \times 10^3$ . Similarly, if the most extreme male result ( $4.60 \times 10^3$ ) is set aside, the male mean value becomes  $1.44 \pm 0.09^c \times 10^3$ . In that scenario the sex difference denoted by  $^c$  becomes statistically significant at  $p < 0.05$  (two-tail, 8 degrees of freedom). Notably, results for loss in drying, ash value and extractable value obtained in

this study compared well with those obtained for the Asian plant [12] (see Table 2). Table 3 shows that acid hydrolysis of the test extract gave rise to reducing sugars, implying the presence of glycosides in the herb. The presence of saponins, as revealed in Table 3, is attested to by the foaming produced by the aqueous extract. Table 3 also suggest the presence of some tannins and alkaloids, but not of anthraquinones. Figure 1 shows that the normal phase TLC of the herb or its aqueous extract yielded 5 spots as against 6 spots by reverse TLC. Although the image is not a 'TLC fingerprints' in a true sense, it does, however, could be useful in identifying herb extract.

**Table 1:** Bitterness value of *Andrographis paniculata* (derived from Eq 1)

Volunteer code	C (mg)	B (ml)	Bitterness value (units/g)
SJA (m)	0.046	6.0	$1.53 \times 10^3$
MJS (m)	> 0.058 (limit)	-	-
NKO (m)	0.052	> 10 (limit)	-
OOD (m)	0.044	6.0	$1.47 \times 10^3$
ATA (m)	0.050	7.0	$1.43 \times 10^3$
DAE (m)	0.046	2.0	$4.60 \times 10^3$
CHS (m)	0.046	7.0	$1.31 \times 10^3$
<b>Mean <math>\pm</math> SD</b>	<b>0.043 <math>\pm</math> 0.003<sup>a</sup></b>	-	<b>2.07 <math>\pm</math> 1.42<sup>b</sup> <math>\times 10^3</math></b>
MOI (f)	0.046	5.0	$1.84 \times 10^3$
OBS (f)	0.044	4.0	$2.60 \times 10^3$
CSO (f)	0.048	2.0	$4.80 \times 10^3$
RHB (f)	0.054	2.0	$5.40 \times 10^3$
EOO* (f)	0.052	8.0	$1.30 \times 10^3$
BSA (f)	0.052	2.0	$5.20 \times 10^3$
<b>Mean <math>\pm</math> SD</b>	<b>0.049 <math>\pm</math> 0.004<sup>a</sup></b>	-	<b>3.52 <math>\pm</math> 1.82<sup>b</sup> <math>\times 10^3</math></b>

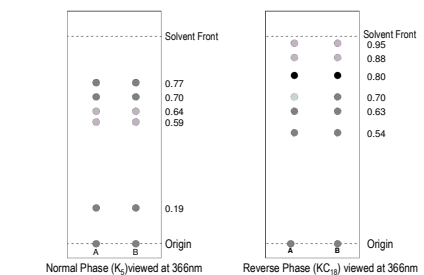
**Note:** (<sup>a</sup>): indicates a statistically significant difference ( $p < 0.05$ ); (<sup>b</sup>): indicates a statistically insignificant difference ( $p = 0.05$ ; two-tail, 10 degrees of freedom). (<sup>c</sup>) becomes statistically significant at  $P < 0.05$  (two-tail, 8 degrees of freedom)

**Table 2:** Physicochemical characteristics of aerial parts of *Andrographis paniculata*

Parameter	Present study	WHO report[12]
Description	Dry, dark green aerial parts; practically odorless or faint and characteristic aroma.	-
Loss on drying (%w/w)	$10.64 \pm 0.36$	$\leq 10$
Total ash (%w/w)	$14.10 \pm 4.49$	-
Water extractive value (%w/w)	$30.37 \pm 2.63$	$\geq 18$
Acid insoluble ash (%w/w)	$1.00 \pm 0.06$	$\leq 2$

**Table 3:** Inference from tests for phytochemical constituents of *Andrographis paniculata*

Test	Observation	Inference
Fehling's test	Red ppt.	The presence reducing sugars, following acid hydrolysis, suggest the sample contained glycosides
Frothing test	Copious froth	Saponins present
Borntrager's test	No change	Anthraquinones Absent
FeCl <sub>3</sub> test	Blue-black ppt.	Tannins present
Dragendorff's test	Orange-brown ppt.	Alkaloids present



**Figure 1:** Illustration of the TLC of the ethanol extract of *Andrographis paniculata*. **Note:** A = ethanol extract; B = aqueous extract dissolved in ethanol. The figures on the right of each plate are the Rf values of the spots

## DISCUSSION

As stated earlier, *Andrographis* is called a wonder plant because it is used to treat several conditions including fever, acquired immune deficiency syndrome, herpes, influenza, cancer [2].

Determination of bitterness by sensation is subjective and cumbersome, and does not readily appeal to every analyst. However, determination of bitterness by taste, as in the present study, remains an appropriate method. It is well known that medicinal plant materials called "bitters" are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice [16]. As indicated under Table 1, the mean bitterness value for men and women was  $2.86 \pm 1.74 \times 10^3$  units per g. This translates to about  $1.43 \pm 0.87$  %w/w of the bitterness value of quinine hydrochloride. The wide range of the value is not unusual. For example, Meyer *et al* [9] found bitterness values of  $58.1 \pm 110 \times 10^5$  and  $51.6 \pm 156 \times 10^5$  for praziquantel and (*R*)-praziquantel, respectively. The chief bitter agent in *Andrographis* is thought to be andrographolide, which constitutes about 2.4 %w/w of the dry herb [18]. Figure 1 is, without doubt, useful; and in combination with the physicochemical measurements shown in Table 2, represents a step in characterizing the Nigerian grown *Andrographis*. Table 3 shows that the herb contains reducing sugars (including probably, glycosides), saponins, tannins and alkaloids.

Figure 1 shows that normal phase TLC of the herb or its aqueous extract yielded 5 spots as against 6 spots yielded by reverse TLC, but the chemical character of the spots were not investigated. Either of these chromatograms may be useful in identifying the aqueous or ethanol extract of *Andrographis paniculata*.

## CONCLUSION

The findings confirm some key similarities between the Nigerian and the Asian herb, as well as provide quantitative and descriptive data essential for identifying and characterizing the Nigerian grown *Andrographis paniculata* - an essential step in drug development.

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