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Spectrophotometric validation of assay method for selected medicinal plant extracts

Matthew I. Arhewoh* and Augustine O. Okhamafe

Department of Pharmaceutics and Pharmaceutical Technology,
Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria

***Correspondence Info:**
Matthew I. Arhewoh
Department of Pharmaceutics and Pharmaceutical Technology,
Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria
E-mail: arhewoh@uniben.edu

Abstract
Objective: To develop UV spectrophotometric assay validation methods for some selected medicinal plant extracts.
Methods: Dried, powdered leaves of *Annona muricata* (AM) and *Andrographis paniculata* (AP) as well as seeds of *Garcinia kola* (GK) and *Hunteria umbellata* (HU) were separately subjected to maceration using distilled water. Different concentrations of the extracts were scanned spectrophotometrically to obtain wavelengths of maximum absorbance. The different extracts were then subjected to validation studies following international guidelines at the respective wavelengths obtained.
Results: The results showed linearity at peak wavelengths of maximum absorbance of 292, 280, 274 and 230 nm for GK, HU, AM and AP, respectively. The calibration curves for the different concentrations of the extract gave R^2 values ranging from 0.9831 for AM to 0.9996 for AP the inter-day and intra-day precision study showed that the relative standard deviation (%) was ≤ 10% for all the extracts.
Conclusion: The aqueous extracts and isolates of these plants can be assayed and monitored using these wavelengths.
Keywords: Extract, Validation, Assay, *Annona muricata, Andrographis paniculata, Garcinia kola, Hunteria umbellata*

1. Introduction

The renewed interest in phytomedicines coupled with the recent advancement in their delivery systems has resulted in increased risk of the production of adulterated herbal medicines, and this has become a major public health issue the world over[1]. It has been reported that, 80% of people in developing countries rely on alternative medicines. A significant proportion of the populations in these countries are exposed to harm due to impurities/adulteration, including herbal medicines mixed with allopathic drugs and sold over the counter or prescribed by practitioners of alternative medicine[1].

Another major challenge in the production of herbal medicine is the issue of standardization. A herbal product that is meant to be marketed as therapeutic agent needs to undergo scientific validation whether the product really have any positive effect to cure or reduce the severity of the disease[2,3]. Another reason for validation would be to develop a means of establishing the stability profile of the product over the period of storage and use. For phytomedicines, this is significant because of the very wide range of plants available as each one is unique and therefore a protocol specific to that plant has to be developed. Thus, validation needs to be individualized[3,4]. Pharmacognostic parameters have been suggested and used for identification and validation of plant parts and products for many years but as the application and demand for therapeutic herbs increase, additional parameters are required for validation purposes. The use of biomarkers has been proposed and is currently applied in a lot of cases[3]. However, the cost of extraction, isolation and analysis of the biomarker using high-tech analytical devices is an issue to contend with particularly in developing countries where herbal remedies play a major role in healthcare delivery.

The plants selected in this study include *Annona muricata* (AM), *Andrographis paniculata* (AP), *Hunteria umbellata* (HU) and *Garcinia kola* (GK). These plants and their parts have been reported to exhibit several therapeutic activities and are used in most cases either alone or in combination in treating a wide range of diseases such as cough, tuberculosis, hypertension, hepatitis, diabetes, cancers and infectious diseases[6-9]. They have also been reported to contain biomarkers or major components that are believed to be responsible for their therapeutic activities.

Herbal remedies are used, in most cases, in their crude forms and hence the need to develop simple and fast assay methods for them in that crude form with a view to their validation and standardization will be vitally important necessity. This study is aimed at developing spectrophotometric assay methods for the aqueous extracts of *Annona muricata, Andrographis paniculata* leaves as well as seeds of *Garcinia kola* and *Hunteria umbellata*.

2. Materials and Methods

2.1 Materials

Fresh leaves of *Annona muricata* (AM), *Andrographis paniculata* (AP) and seeds of *Hunteria umbellata* (HU) and *Garcinia kola* (GK) were collected in Benin City, Nigeria between April and June. The plant parts were authenticated by Mr. Adewale of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where herbarium samples with voucher numbers FHI107678 (HU); FHI108458 (AP); FHI107852 (AM); FHI39154 (GK) were assigned and samples of the plants deposited.

2.2 Preparation of plant extracts

HU and GK seeds were de-shelled and chopped into small pieces with a knife to facilitate drying. The leaves were dried for one week while the chopped seeds were dried for two weeks in sunlight and milled. The powders obtained were passed through a 1 mm aperture sieve, separately packed in air-tight containers and stored in a refrigerator prior to analysis.

Each powdered sample (10 g) of AM, GK, HU and AP, was separately macerated with 250 mL distilled water at room temperature for 24 h. The filtrate was dried in a vacuum oven at 35^{o}C. The dried extracts were stored at 4^{o}C pending further analysis. In order to minimize interference during UV scan of HU and AM, a purer isolate was obtained by maceration of 40 g of their powders in 100 mL of dilute HCl for 24 h and filtered. The extract was shaken with 80 mL of n-hexane to remove fats, oils, terpenes, waxes, pigments and other unwanted materials. This extract was discarded. The free alkaloids in the aqueous medium were then precipitated by the addition of excess sodium bicarbonate and extracted with chloroform. The chloroform extract was then evaporated to give the alkaloidal isolate[10].

2.3 Validation of extract assay method

This was carried out using a UV/Visible spectrophotometer (PG instrument, Model T70, USA). Serial dilutions of each of the extracts/isolates were prepared and the wavelength of maximum absorbance was obtained by scanning over a wavelength range of 350 to 200 nm. The wavelengths of maximum absorbance obtained were subjected to linearity analysis and calibration plots were obtained.

2.3.1 Preparation of calibration curves

The dried extract of each plant (1 g) was redispersed in 10 mL of distilled water and made up to 100 mL to give a 1 % solution (solution A). Ten millilitres of this solution was mixed with 10 mL of distilled water and the solution thoroughly shaken to make solution B. Solution B was divided into two equal parts of 10 mL each. One part was reserved for UV reading while the other half was further diluted with 10 mL of distilled water (solution C). Further serial dilutions were made until 10 different dilutions were obtained. For each dilution, the corresponding concentration of plant extract was noted. Absorbance values were measured at wavelength of maximum absorbance obtained above and plotted against the corresponding concentration values, and the regression equation and coefficient (R^2) values were obtained. This method was validated for robustness, accuracy and specificity[5].

2.3.2 Robustness and Accuracy

The robustness, accuracy and specificity of the UV assay method were determined based on inter-day and intra-day variation (repeatability) assessment. Five samples of each extracts/isolates were assayed within the limits of quantification daily for three different days. This above procedure was repeated by different operators in different places. The precision and accuracy of the method were expressed as relative standard deviation (RSD). The percentage relative standard deviation (% RSD) values were calculated using the Equation bellow

RSD (%) = (SD/Mean) x 100

2.4 Data Analysis

All experiments were conducted at least in triplicate and the results expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Microsoft Excel, version 2007 ANOVA was performed on the data sets generated and differences were considered significant at p values ≤ 0.05.

3. Results and Discussion

The UV spectra of the extracts are shown in Figure 1. In all cases, the second peak was chosen because it demonstrated better linearity within acceptable range when compared to the first peak. The wavelengths of maximum absorbance obtained for the various extract concentrations are shown in Table 1. A peak wavelength corresponding to the wavelength of maximum absorbance obtained for *Garcenia kola* extract was 292 nm, while those of *Annona muricata*, *Hunteria umbellata* and *Andrographis paniculata* were 274, 280 and 230 nm, respectively.

Figure 1: Representative UV absorbance spectra of aqueous extracts/isolates. (a) *G. kola*; (b) *A. muricata*; (c) *A. paniculata* and (d) *H. umbellata*

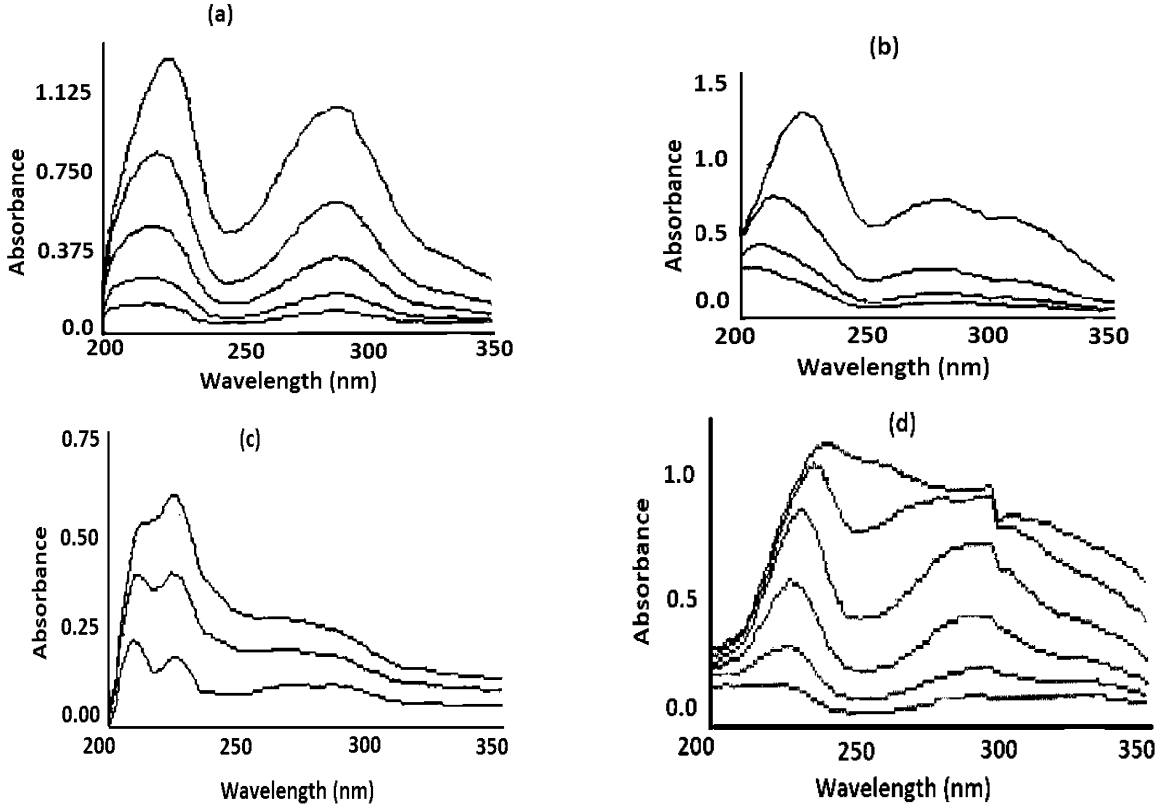

Table 1: Linearity parameters obtained from the calibration curves of the four plants

Parameters	*G. kola*	*H. umbellata*	*A. muricata*	*A. paniculata*
λ max (nm)	292	280	274	230
Beer's law (mg/mL)	0.003 to 0.05	0.015 to 0.125	0.003 to 0.03	0.06 to 1
Adjusted regression equation (y=mx+c)	y=28.704x	y=4.8718x	y=7.83x	y=0.489x
Slope (m)	28.704	4.87	7.83	0.489
Correlation coefficient (R^2)	0.9991	0.9898	0.9831	0.9996
Limit of detection (mg/mL)/absorbance	0.003/0.06	0.015/0.062	0.003/0.023	0.06/0.03
Limit of quantification (mg/mL)/absorbance	0.05/1.439	0.125/0.606	0.03/0.235	1/0.5

Table 2: Inter-day and intra-day precision results

Concentration of extract (mg/mL)	Mean absorbance ± SD		RSD (%)	
G. Kola	**Day 1**	**Day 2**	**Day 1**	**Day 2**
0.00625	0.194±0.007	0.199±0.003	3.608	1.508
0.0125	0.375±0.03	0.359±0.006	8	1.671
0.025	0.702±0.05	0.753±0.07	7.123	9.296
H. umbellata				
0.03125	0.124±0.003	0.199±0.004	2.419	2.01
0.0625	0.391±0.006	0.359±0.005	1.534	1.392
0.125	0.606±0.009	0.753±0.007	1.485	0.929
A. muricata				
0.0156	0.045±0.002	0.033±0.003	4.444	9.09
0.0313	0.091±0.005	0.055±0.001	5.494	1.818
0.0625	0.136±0.010	0.162±0.006	7.194	3.703
A. paniculata				
0.25	0.121±0.002	0.170±0.005	1.652	2.941
0.5	0.252±0.002	0.340±0.008	0.79	2.353
1	0.485±0.008	0.679±0.010	1.649	1.473

The initial spectra obtained from AM and AP scans were not clearly distinct (appeared as plateaus) hence the need for further extraction/isolation of the plant extract for the specific secondary metabolites that they have been reported to produce. In this case their alkaloidal contents were of interest. This simple isolation step resulted in the production of definite spectra characteristic to each plant (Figs 1c and d).

The wavelength of *Garcinia kola* corresponds with that of rutin (a model biflavonoid) and also of the biflavonoid obtained after extraction according to the method of Iwu *et al*[6,11]. Dissolution studies of the dosage forms of *G. kola* have been carried out spectrophotometrically in the range 290 to 294 nm [12] but no report on the validation of the analytical procedures was stated. The selected wavelengths of maximum absorbance of the other plants extracts also corresponded with the types of secondary metabolites they contained such as alkaloids, and flavonoids in AM, AP and HU which contain components like acetogenins, andrographolides and cormines, respectively.

All the calibration curves showed good linearity with R^2 values from 0.9831 to 0.9996 within the absorbance range of interest (Table 1). The inter- and intra-day precision results (Table 2) show specificity and robustness of the method used within the respective optimal concentrations while the relative standard deviation was < 10 %, indicating accuracy of the method used. The linearity parameters obtained (Table 1) suggest that the assay method would be suitable for extract assay. Currently, there are no pharmacopoeia specification for the validation of assay and dissolution of these herbal products. The National Food Administration, Sweden, in a document on the method validation and quality control procedures for pesticide residues analysis in food and feed used a recovery range of 70 to 120 % [13] while other researchers have suggested other limits depending on the nature of material being tested and the purpose of validation[3,4]. The acceptance criterion used in this study was a relative standard deviation (RSD) of ≤ 10 % and recovery of extracts within 80 to 120 %, for precision and repeatability[3,13]. The method was found to be precise in terms of repeatability for the quantification of *Garcinia kola, Annona muricata, Hunteria umbellata and Andrographis paniculata* at 292 nm, 280 nm, 274 nm and 230 nm, respectively, and with a relative standard deviation ≤ 10 %.

4. Conclusion

Investigations using the spectrophotometric method at very low concentrations of plant extracts/isolates reveal that the methods used would be suitable for the assay of the extracts of *Garcinia kola, Annona muricata, Hunteria umbellata and Andrographis paniculata*. This can be further advanced and included in the assay of these plant and their extracts

References

1. The Bulletin of the World Health Organization, August 2004; 82 (8):635
2. Choudhury CR. Traditional and complementary/alternative medicine: where does it stand? (A correspondence). *Nat Med J India* 2003; 16(1): 52.
3. Rolim A, Maciel CP, Kaneko TM, Consiglieri VO, Salgado-Santos IM, Velasco MV. Validation assay for total flavonoids, as rutin equivalents, from *Trichilia catigua* Adr. Juss (Meliaceae), *Ptychopetalum olacoides* Bentham (Olacaceae). commercial extract. *J AOAC Int* 2005; 88(4):1015-9
4. Jain SC, Menghani E, Jain R. Biomarkers as a tool for validation of herbs and spices. *Int J Food Safety* 2007; 9: 1-6
5. Nair VDP, Kanfer I. Development of dissolution tests for the quality control of complementary/alternate and traditional medicines: Application to African potato products. *J Pharm Pharm Sci*. 2008; 11(3): 35-44.
6. Iwu MM, Igoko OA, Okunji CO, Tempesta S. Antidiabetic, aldose reductase activities of biflavonoids of *Garcinia kola*. *J Pharm Pharmacol* 1990a; 42: 290-2.
7. Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC. Cytotoxic diterpenoid constituents from *Andrographis paniculata* Nees leaves. *J Sci. Soc. Thail* 1992; 18: 187–94.

8. Adewole SO, Caxton-Martins EA. Morphological changes, hypoglycemic effects of *Annona muricata* Linn. (Annonaceae). leaf aqueous extract on pancreatic B-cells of *Streptozotocin*-treated diabetic rats. *Afr J Biomed Res* 2006;9: 173-87
9. Adeneye AA, Adeyemi OO. Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata* in normal, glucose- and nicotine-induced hyperglycaemic rats. *International Journal of Applied Research, Nat Prod* 2009;2:9-18
10. Evans WC. Phytochemistry. *In Trease and Evans Pharmacognosy* (15th edn) Elsevier, New Delhi, India. 2005; 135-50.
11. Iwu MM, Igboko OA, Tempesta MS. Biflavonoids constituents of *Garcinia kola* roots. *Fitoterapia* 1990b; 61:178–80.
12. Onunkwo GC, Egeonu HC, Adikwu MU, Ojile JE, Olowosu AK. Some physical properties of tabletted seed of *Garcinia kola* (Heckel). *Chem Pharm Bul* 52:649-53.
13. Ibezim EC, Kenechukwu FC, Odimegwu DC, Builders PF, Kabele-Toge B, Anie C, Igwilo CO, Otuu FC, Onyechukwu C (2011). Antimicrobial efficacy of a syrup formulation from methanol extract of *Garcinia kola* seed. *Afr J Pharm Res Dev*. 3 (1): 22-27
14. SANCO (2009). Method validation, quality control procedures for pesticide residues analysis in food, feed. SANCO/10684 http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en. Assessed 22, February 2012