



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2019; 8(6): 1817-1822

Received: 28-09-2019

Accepted: 30-10-2019

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Pilot study of quality control of *Artemisia annua*-based herbal medicine sold in Benin

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Abstract

The quality control of medicines is one of the key factors which determine its efficacy. In Africa, particularly in Benin, a large population often uses traditional medicines. Therefore, controlling their quality becomes a major concern for public health. It is in this context that this study was designated to ensure an adequate system of drugs quality control, especially herbal medicines. We collected three different samples (T1, T2 and T3) of Traditional Medicines (MTA) based on *Artemisia annua* and we controlled their quality. The identification of artemisinin was carried out by Thin Layer Chromatography (TLC) and the quantification by High Performance Liquid Chromatography (HPLC). After analyzing the organoleptic characteristic, the loss of water and the impurities, we found that only T3 was conform to all the specification as MTA. This work opens a new platform of expertise in the field for our team and further a hope for the management of public health in term of the control of MTA.

Keywords: Medicinal plants, traditional medicine, *Artemisia annua*

Introduction

With the emergence of resistance to antimalarials by the *Plasmodium falciparum* parasites, especially chloroquine, it appeared necessary to develop alternative drugs. In the 1990s, WHO (World Health Organization) was already considering the clinical use of artemisinin and its derivatives. Artemisinin was isolated from *Artemisia annua* (Qinghaosu), a plant used in China traditional medicine to treat malaria for more than 2000 years [1].

In 2001, WHO technical report recommended the use of Artemisinin-based Therapeutic Combinations (ACTs) as a response to the increasing resistance of the malaria parasites toward the existing antimalarial drugs [2-3].

Since then, the number of artemisinin-based drugs manufactured worldwide has risen considerably, from 11 million in 2005 to 392 million in 2013. In 2004, Benin adopted the use of ACTs (artemether + lumefantrine) as first line treatment of uncomplicated malaria [3]. Meanwhile, the traditional use of *Artemisia annua* also developed, hence, the number of phytomedicines derived from this plant increased over time.

Several scientific studies have shown that herbal teas based on *A. annua* and (or sometimes also on *Artemisia afra* (*A. afra*)) are efficient and, as based on polytherapy, may decrease the risk of resistance development [4-10].

In Benin, like many African countries, the health system does not have a defined policy to regulate the quality and the use of Traditional Medicines (MTA). This is therefore an area that needs urgent action and decision to ensure the quality and the safety of the herbal medicines used in Benin. In line with this goal, this study aimed to develop a simple quality control protocol that could serve as a model for other phytomedicines using *A. annua*. Herein, we analyzed the quality of *A. annua*-based phytomedicines sold in pharmacies and markets in Cotonou, Benin.

Materials and Methods**Materials**

The samples were collected at three different sites from drug store (T1), the Cotonou market (T2) and a pilot production sample by the School of Pharmacy, Faculty of Health Sciences (T3).

All the reagents glycerol, methanol, ethanol, ethyl acetate, chloral hydrate, acetic acid, anisaldehyde and sulfuric acid were purchased from Scientific Control Center of our country, and were used as solvents for characterization, extraction and quantification. All were of HPLC grade.

Packaging analysis

This was conducted with reference to the guidelines of Good Manufacturing Practices (GMP) and all specifications according to European Pharmacopea (Eur Ph), which requires the full disclosure of components on the packaging, name of company, dosage form, manufacturing number, dates of manufacture and expiration [11-14].

Weight uniformity

This was carried out based on Eur Ph standards. Twenty units were weighed and statistical analysis was done [12, 14].

Organoleptic Characteristics

For all the samples, the taste, color, impurities and water content were checked by visual observation and using specific methods.

For impurities (such as heavy metals, pesticides, mycotoxins, fumigants as well as microbial contamination, including those arising from extraneous sources of the culture area) determination, the powder was weighed and visually examined. The percentage of impurities was calculated with the formula $T (\%) = (I/P) * 100$ (I = weight of the impurities (g); P= weight of herbal drug (g)) [13].

The color (green) and the taste (bitter) were determined by three persons according to the method described by Kataoka *et al.* [15].

The loss of water (water content) was realized by weighing 3 g of powder and drying at 100-105 °C for 5 hours in oven. After 5 hours, the three samples had been weighted (Mh5) and reputed in oven at 100-105 °C for 1 hour. After 1 hour, samples had been weighted (Mh6). This procedure was repeated until the final weight of the powder was obtained when the difference between two successive weight was less than 5 mg [14]. The percentage of water loss was calculated by

the formula $T (\%) = ((M - MhX) * 100) / M$ with M= initial weight (in g) of the sample and m = final weight (in g) of the sample.

Microscopic analysis was done by using several solvents specifically chloral hydrate and glycerol to search for the research of specific characteristic of Artemisinin [12, 14, 16-18].

Identification and dosage of artemisinin

For the identification, Thin Layer Chromatography (TLC) was used with stationary phase of silica gel. 3.5 g of powder was weighed and suspended in 50 mL of ethanol, and kept at 30 °C for 1 h. After filtration, the extract was prepared to a final concentration of 10mg/mL. A standard solution of pure artemisinin was prepared to a final concentration of 1mg/mL. A mixture of ethyl acetate and toluene (9:1 v/v) was used as the solvent system for elution. The TLC plate was revealed by using the mixture of acetic acid/ sulfuric acid/ anisaldehyde (100:2:1 v/v/v) and heating at 100 °C for 5 min [11].

The quantification of artemisinin was done by High Performance Liquid Chromatography (HPLC). The method used was that developed by H.Z. DIAWARA *et al.* [19-20].

Results and Discussion

The results of the packaging analysis are shown in the table 1. Only T3 followed all the GMP and Eur Ph specifications [11, 14, 21]. We can assume that there is no GMP used for the production for T1 and T2. We also noticed that there was no mention of the name of the manufacturer on T2. This is source of counterfeit especially in our context where quality control determination of phytomedicines is limited. The differences between T2 and T3 could due to the herbal drug used. However, the T2 sample except to contain *A. annua* may have *A. afra* as mentioned by the manufacturer.

Table 1: Results of the labeling analysis

	T1	T2	T3
Trade name	Tisane Bio <i>Artemisia</i>	Natur Pure Bio <i>Artemisia annua</i>	Valtramed® Annu
Presentation	Crushed aerial parts	Powder	Powder
Composition (presentation on the label)	Leaves and stems of <i>A. annua</i> and <i>A. afra</i> dried and ground	<i>A. annua</i> plant powder	Per infusette: a minimum dose of 8 to 10 mg of artemisinin
Name /logo of the manufacturer	House of Artemisia Farm Ago-ecological LA Providence	None	Valtramed UFR Pharmacy/ FSS
Ful Adresse of the manufacturer	Yes	None	Yes
Number of units per container	Paper bags 40mg	Aluminum Bag 50 g of powder	Box of 21 bag of <i>d'A. annua</i> 's powder.
Bach number	None	None	D01802-03
Date of manufacture	None	None	03 February 2018
Expiry date	May 2021	August 2021	02 February 2020

The table 2 presents the result of the weight uniformity assay. According to the WHO guidelines standard maximum deviation of 7.5% is accepted when the unit weight is higher than 300 mg [11]. The average weight of T1 is 37.74 g which is near to the declared weight of 40 g. In contrast, T2 sample

displayed an average of 26.91 g which is far from that mentioned on the package (50 g). The average weight of T3 sample was about 4.5 g which is near to the 4 g stated by the production department of the school of Pharmacy.

Table 2: Results of Weight uniformity assay

		Total weight (g)	Empty (g)	Container (g)	Difference (%)
T1 Tisane Bio <i>Artemisia</i>	m ₁	46.5821	7.1209	39.4612	4.56
	m ₂	47.3480	7.1986	40.1494	6.38
	m ₃	40.8057	7.1927	33.6130	10.94
	m ₄	-	-	-	-
	m	44.9119	7.1707	37.7412	-
T2 Natur Pure Bio <i>Artemisia annua</i>	m ₁	35.3473	7.3784	27.9689	3.95
	m ₂	35.1070	7.4719	27.6351	2.71
	m ₃	35.1275	7.4827	27.6448	2.74

	m ₄	31.7403	7.3618	24.3785	9.40
	m	34.3305	7.4237	26.9068	-
T3 Valtramed® Annua	m ₁	5.0679	0.5490	4.5189	0.03
	m ₂	5.0629	0.5425	4.5204	0.33
	m ₃	5.0375	0.5389	4.4986	0.36
	m ₄	5.0518	0.5416	4.5102	0.12
	m	5.0448	0.5406	4.5042	

m: masses's average, empty: the package without product; 1,2...the number of the samples, difference = total weight -container

The n of uniformity test should be 20 but for T1 and T2 it was difficult to access enough sample for all analysis as preliminary test we just used the samples available.

The results of the investigation of impurities (T1 and T3) are shown in table 3. T1 contains more impurities than T3. However, we were unable to confirm the identities of these impurities.

Table 3: Results of the investigation of impurities

P(g)	T1			T3		
	3	3	3	3	3	3
I(g)	0.62	0.57	0.59	0.14	0.15	0.13
T(%)	20.66	19	19.66	4.67	5	4.33
AVERAGE (%)	19.77			4.67		

T The percentage of impurities, I: weight of the impurities; P: weight of herbal drug

The organoleptic characteristics of the different samples are shown in table 4. T1 and T3 characteristics matched with all

the specification described in literature. However, the taste and texture of T2 did not comply with specifications [12].

Table 4: The organoleptic characteristics of the different samples

	T1	T2	T3
Texture and colour	Mixture of crushed aerial parts (leaves and identifiable coarse twigs). color (powder) green-brown	Powder of different grain sizes Green-brown color with small whitish masses	Fine powder Color green-brown
Smell	Characteristic of <i>A. annua</i>	Not characteristic of <i>A. annua</i>	Characteristic of <i>A. annua</i>
Taste	Slightly bitter with a sensation of steam rising from essential oils in the airways	Very bitter with no sensation of essential oil vapor rising into the airways	Slightly bitter with a sensation of steam rising from essential oils in the airways

The loss of water results are shown in table 5. All the samples conformed to the WHO specification (less than 13%) [12]. This

could help to ensure the stability over the time of all the samples.

Table 5: Results of the loss of water

Horus (H)		T1			T2			T3		
H0	m _v (g)	17.13	17.07	17.13	16.56	17.39	16.56	22.83	17.39	17.07
	m _s (g)	3.07	3.03	3.02	3.01	3.01	3.07	3.05	3.01	3.01
	m ₀ (g)	20.19	20.10	20.14	19.57	20.41	19.64	25.87	20.39	20.07
H5	m ₅ (g)	19.87	19.77	19.83	19.31	20.13	19.33	25.52	20.09	19.76
	Δm ₀₅ (mg)	325.90	332	310.1	261	268.8	300.8	363.4	303	305.5
	T ₀₋₅ (%)	10.60	10.97	10.30	8.67	8.60	8.00	11.92	10.08	9.26
H6	m ₆ (g)	19.86	19.76	19.83	19.30	20.13	19.33	25.51	20.09	19.76
	Δm ₅₋₆ (mg)	4.5	5.1	2.9	3.6	2.5	1	4.1	0.9	0.7
	T ₀₋₆ (%)	10.77	11.13	10.38	8.783	9.005	9.811	12.05	10.11	10.20
H7	m ₇ (g)	-	19.7583	-	-	-	-	-	-	-
	Δm ₆₋₇ (mg)	-	2.3	-	-	-	-	-	-	-
	T ₀₋₇ (%)	-	11.21	-	-	-	-	-	-	-
Average		10.77			9.20			10.77		

m_v: empty weight, m_s: weight of sample, Δm: variation of the weight, T: percentage of humidity.

The results of the microscopic analysis of the herbal drugs are represented in the figure 1. The cells of the upper and lower epidermis showed an irregular shape as shown by the narrow in the figure. However, the visual examination could not be

sufficient to ensure the identity of the drugs. There was therefore a need for quantification for further confirmation of the drug materials.

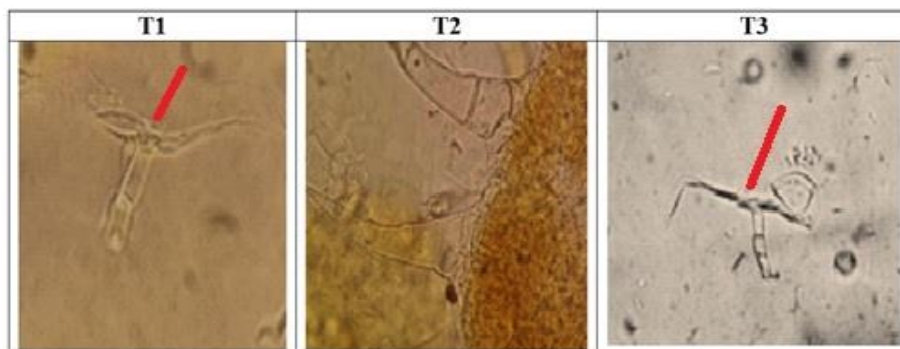


Fig 1: The results of the microscopic analysis (red narrow for the Trichom)

The TLC results are represented in the figure 2. After separation, we can easily notice that T1 and T3 contain artemisinin while T2 does not due to the presence of the spot of artemisinin (red spot) compare to the reference standard (red color spot) which deposit was done in the middle among

the sample. The results of the microscopic analysis of T1 and T3 show trichom, a secretive hair of essential oil that is a microscopic's characteristic of the gender *Artemisia* of Asteraceae's family. T2 doesn't contain that hair.

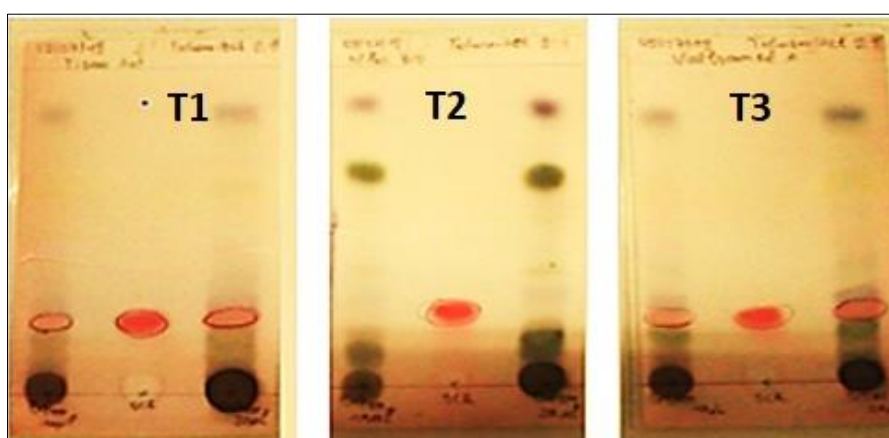


Fig 2: Illustrative photo of thin layer chromatography plate of T1, T2 and T3 plates.

The HPLC analysis (table 7 and figure 3) also confirmed the presence of artemisinin in the samples T1 and T3 [16, 21]. However, the content of artemisinin in these samples (0.15-

0.30 mg) did not meet the required standard of the WHO (0.7 mg in the main plant for manufacturing used) [12, 19].

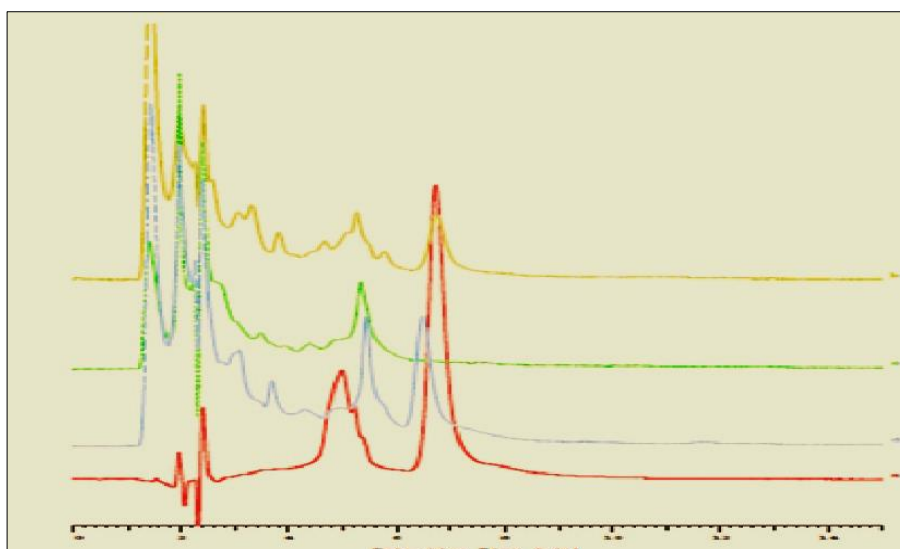


Fig 3: Chromatogram of quantitative analysis by HPLC of Reference substance of Artemisinin in red, of T1 in yellow, of T2 in green and of T3 in blue.

Table 7: Results of artemisinin assays by HPLC

	T1			T2			T3		
	Tisane Bio			Natur Pure Bio			Valtramed®		
	Artemisia			Artemisia annua			Annu		
Extraction	1	2	3	1	2	3	1	2	3
Conc(µg/mL)	2.83	2.76	2.58	–	–	–	3.02	3.02	3.02
m _{ext sec} (µg)	360000	370000	390000	–	–	–	390000	380000	390000
m _{art} (µg)	4076.81	4083.27	4021.96	–	–	–	4711.2	4707.35	4711.2
% _{art} (powder)	0.09	0.09	0.09	–	–	–	0.11	0.11	0.11

Conc: Concentration; m_{ext sec}: weight of dry extract; m_{art}: weight of artemisinin and %_{art}: Artemisinin content (%).

A counterfeit medicine is "a drug that is deliberately and fraudulently labeled with no indication of its identity and / or true source" according to WHO. Therefore, the counterfeit medicine may be a princeps or a generic product, and some of the fake products contain the right ingredients or bad ingredients, or no active ingredients, and there are others where the active ingredient is in insufficient quantity or whose packaging has been falsified ". Sample T2 is a MTA based on plant powder with organoleptic characteristics different from that of *A. annua* with no artemisinin. However, per the information on its package, it is meant to "Fight and prevent malaria", and "Fight against cancer cells through artemisinin". We noticed that there is no indication of the manufacturer or the place of manufacture, only a telephone number (Beninese) was on the package. Ultimately, this sample is a falsified TM [7, 9, 13].

In view of the results of the various analyses (in particular the organoleptic characteristics), several hypotheses are possible. It could be a mixture of leaves and / or stems of bitter plants that have been crushed and packaged as powder. It could also be a species of *Artemisia* that does not contain artemisinin (in particular *A. Afra*) or contains only small quantities (*Artemisia opiacea* and *Artemisia lancea*) [11]. This sample could as well be a powder of *A. annua*, but the herb was not harvested at the indicated time or degraded.

The results of the analyses suggest that samples T1 and T3 contained *A. annua* and various amounts of artemisinin.

The T1 manufacturer should consider all these results to improve its production for future demand of market authorization certificate.

The T3 sample definitely has been prepared according all the Eur Ph specifications and the production respect the GMP in MTA production.

Conclusion

This pilot study demonstrates the need for the establishment of a global quality control policy for phytomedicines and also conditions for their manufacture, importation and sale. This study could also help to ensure the quality of herbal medicine in Benin.

Acknowledgement

We thank the PRD-ARES project (VALTRAMED) and Parfait DOFFON for the technical contribution.

Conflict of Interest

The authors declare there is no conflict of interest

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