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Assessment of Quality of *Artemisia afra* Powder Used as Antimalarial in Tanzania

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Abstract: The quality of herbal medicine can be affected by environmental conditions where by the medicinal plants are grown or collected, the area where they are dried and processed, storage conditions, transport conditions and manufacturing processes at the point of production. The study aimed to determine the quality of heavy metals, aflatoxins and microbial contaminants of *Artemisia afra* leaves powder used as herbal medicine processed at the Institute of Traditional Medicine of the Muhimbili University of Health and Allied Sciences (MUHAS). *Artemisia afra* leaves powder was obtained from The Institute of Traditional Medicine (ITM) in Tanzania where it is processed and packed as *Fivisia*. Microbial contaminants were analyzed by using plate count method for moulds and *Shigella* spp. while Most Probable Number (MPN) method used for determination of *E. coli*; Heavy metals were analyzed using; Inductively Couple Plasma – Mass Spectrometry (ICP-MS) and aflatoxin analysis was done using High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). The data were tabulated, expressed in mean±S. D and presented in graphs and compared to WHO guidelines. The quantity of molds was between 1.1×10^3 – 1.2×10^3 CFU/g which is below the legal limit according to WHO of maximum of 10^5 CFU/g, none of the samples were contaminated with *E. coli* or *Shigella*s sp. Levels of heavy metals were within the permissible limit for consumed herbal product according to WHO standard guidelines; Chromium 1.69 ± 0.08 ppm, Lead 0.22 ± 0.01, Cadmium 0.08 ± 0.01, Mercury 0.15± 0.08 and Arsenic 0.04 ± 0.02. All samples were found below the detection limit for aflatoxin B1, B2, G1 and G2. The *Artemisa afra* leaves powder processed and packed as *Fivisia* at the Institute of Traditional Medicine; Muhimbili University of Health and Allied Sciences (MUHAS) is safe for human consumption based on the quality parameters that have been studied.

Keywords: *Artemisia afra*, Heavy Metals, Aflatoxin, Microbial Contaminants, Traditional Medicine

1. Introduction

Herbal medicines have existed for generations. They are considered to be safe due to their long time use in health care. The World Health Organization (WHO) estimates that, about 80% of the world's population depends on traditional medicine and traditional treatment for primary health care [1].

In Africa, people use herbal medicine to symbolize a way of life (culture), thus they consider them to be very effective and less harmful than western drugs [2]. Consequently, the World is witnessing increased public interest and acceptance of herbal medicine in both developing and developed countries. The increased use of herbal medicine is associated to toxicity and side effects of

allopathic medicine [3]. The presence of different components / ingredients in the preparation of herbal medicine, makes the assessment of batch to batch to be very important since the variation start from the collection of raw materials [4]. The regulatory Authorities have the responsibilities to ensure purity, safety, potency and efficacy according to the standard guidelines.

The quality of herbal medicine can be affected by different factors, some of which may come from environmental conditions from which the medicinal plants are grown or collected, the area where they are dried and processed, storage conditions, transport condition, and manufacturing processes [5].

Other factors that affect the quality of herbal medicine are seasonal variation, cultivation methods, field collection, substitution, adulteration, and processing [6].

Artemisia afra is the one of the most common plant species for treatment of different diseases like malaria, cough, sore throat, influenza and asthma. The plant is well known for its aromatic nature. The specie also has potential biological activities and different literature have revealed on pharmacology and toxicology [7].

2. Materials and Methods

2.1. Study Design

This study was experimental where by *Artemisia afra* leaves powder was analyzed for heavy metals, aflatoxins and microbial contents.

2.2. Study Area

Processed *Artemisia afra* leaves powder samples were collected at the Institute of Traditional Medicine – MUHAS in April, 2018. The laboratory experiments were conducted at ITM – MUHAS, Tanzania Bureau of Standards (TBS), Dar es Salaam, and Chief Government Chemist Laboratory Agency (CGCLA), Zanzibar.

2.3. Chemicals and Equipment

Chemicals for this study were of analytical grade. These chemicals include:

Nitric acids, Ethanol 95%, 100% methanol (Fischer Scientific, UK), an aflatoxin mixture (AFmix) standard comprising AFB1, AFB2, AFG1, and AFG2 (Roma Lab), acetonitrile (Sigma Aldrich, Germany) Deionized water, Potato Dextrose Agar (Himedia, India), xylose lysine desoxycholate agar, (XLD agar) (Oxoid, England), peptone water broth medium (Himedia, India), Lauryl sulfate tryptose broth (Himedia, India), Durham fermentation tubes, Helium gas (TOL, Tanzania), Inductively Couple Mass Spectrometry (ICP-MS), High Performance Liquid Chromatography – Mass Spectrophotometry (HPLC-MS), Autoclave (CertifClav, Traun Austria) and Oven.

2.4. Collection of the Sample Material

Artemisia afra leaves powdered samples were collected from ITM on April 2018. The samples were transported and stored at room temperatures before analysis [8].

2.5. Determination of Heavy Metals

Presence of heavy metals namely; Cadmium, Lead, Mercury, Arsenic and Chromium were determined**.**

Analysis of the sampled plant material was done on Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) with slight modified optimized conditions [9]. The dried sample was finely powdered and subjected to microwave digestion method. About 0.445 g of *Artemisia afra* leaves powder sample was taken into microwave digester tube; 7ml of 6:1 concentrated nitric acid: water was added and placed in a microwave digestion system ready for digestion. The digested sample was transferred to 50ml polypropylene centrifuge tube and made up to the mark with distilled water, then 2ml of sample was transferred to 14ml polypropylene centrifuge tube and made up to 10ml with distilled water. The final solution was analyzed using inductively coupled plasma mass spectrometry (ICP-MS). Standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid for checking the linearity [9].

2.6. Determination of Aflatoxin

Agilent (HPLC-MS) with a quaternary pump, an auto sampler, a vacuum degreaser and a fluorescence detector (FLD) was used to analyze aflatoxins according to ISO standard No. 16050/2013. The chromatographic separation was performed on a Discovery C18 HPLC column (5 µm particle size, 250 mm× 4.6 mm) protected with a Discovery C18 Supelguard column (5 µm particle size, 20 mm× 4.6 mm). The post-column derivatization of AFs was carried out by using a Kobra cell electrochemical bromine system [10]. About 25 ±0.10 g of tested sample with addition of 70:30 methanol: water extraction solvents were mixed into the flask of 250 mL. The mouth of conical flask was covered with aluminium foil and shaked using gyratory shaker for 30 minutes at 250 rpm. The mixtures were filtered through a Whatman filter paper.4 mL of extract was taken and added with 8 mL of distilled water into teflon tube and vortexed for 1 minute. The second filtrate was quantitatively passed through the immunoaffinity column at flow rate of 1mL/min. The column washed with 10 mL of water. Aflatoxins were eluted with 1 mL of methanol HPLC grade in an amber vial at flow rate of 1 mL/min. The elution step was repeated one more time with 1 mL of water. Then, 100 mL of methanol-water solution was applied to HPLC-FlD system, followed by post derivatization with bromine in Kobra cell [11]**.**

2.7. Determination of Microbial Contaminants

2.7.1. Preparation of Media

All the media for microbiological analysis were prepared according to the manufacture's guidelines and were sterilized in an autoclave (CertifClav, Traun Austria) at 121°C for 15 minutes. The sterile media were dispensed or poured into sterilized Petri dishes. The sterility of the prepared media were confirmed by incubating blindly selected plates at 37°C for

overnight [12].

2.7.2. Isolation of Escherichia Coli

Twenty-five (25) g of each sample (*Artemisia afra* leaves powder) was homogenized in 225 mL Enterobacteria enrichment buffered Peptone water broth medium to make 10^1 dilutions. Then 1milliliter of homogenized sample was sterilely diluted to make 10^1 folds to 10^{-2} and 10^{-3} dilutions. 1 milliliter each dilution was inoculated into 3 tubes containing 10 milliliter Lauryl sulfate tryptose broth with Durham fermentation tube and incubated for 48 hours at 35°C [12].

2.7.3. Isolation of Shigella Spp

Enrichment was done to enhance the growth of pathogenic bacteria. Ten gram of sample (*Artemisia afra* leaves powder) was aseptically transferred in a 150 ml sterile conical flask marked with sample code. The sample was mixed with 90 milliliter amount of buffered peptone water medium to make the final volume reaching to 100 ml and was mixed properly and incubated at 35°C to 24 hours for bacterial enrichment. One milliliter was mixed with nine milliliter of sterile distilled water to make 10^1 fold then diluted to 10^{-2} and 10^{-3} dilutions; herbal water mixture taken and inoculated using sterilized wire loop then sample was streaked onto selective xylose lysine desoxycholate (XLD) agar plate and incubated at 35°C for 24 hours [13].

2.7.4. Qualitative Fungi Counts

Fungi were cultured using potato dextrose agar (PDA) (Hi Media Laboratories Pvt. Ltd, India) after incubation at 30°C for 5 days. The sample preparation was done as described for *E. coli*. At the end of 5-day incubation, the fungal growth were observed (Gavali, 2016). The number of colonies formed was counted, and the CFU/g was calculated using the following equation: CFU/g = (number of colonies x dilution factor) / volume of diluted culture added. Colony-forming unit (CFU) is a measure of possible bacterial or fungal cell contamination. The higher dilutions were prepared to obtain a colony count within countable range [8].

3. Results

3.1. Heavy Metal Determination

The results of heavy metals observed to be below the WHO limit for herbal medicines. Table 1 show the mean level of heavy metals analyzed compared with their WHO limit, shows the mean±S. D of heavy metals in *Artemisia* powder.

Table 1. *Concentration (ppm) of Heavy metals in Artemisia afra leaves powder samples with their WHO limit.*

S/N	Sample	Chromium (Cr) ppm	Lead (Pb) ppm	Cadmium (Cd) ppm	Mercury (Hg) ppm	Arsenic (As) ppm
1	*Artemisia afra*	1.69±0.08	0.22±0.01	0.08±0.01	0.15±0.08	0.04±0.02
2	WHO	2	10	0.3	0.5	5

3.2. Aflatoxin Determination

Aflatoxin were not detected in all *Artemisia afra* leaves powder samples but in spiked sample as expected. This how that; there were no any aflatoxin contamination in *Artemisia afra* leaves powder.

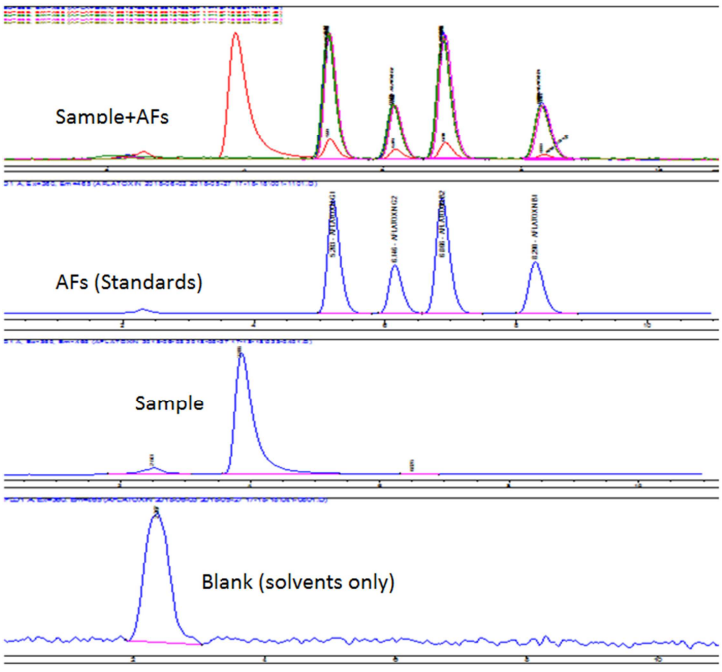

Figure 1. *Shows the aflatoxin peaks detected in the HPLC-MS (level for Artemisia afra samples, standard, spiked and blank (solvent).*

Table 2. *Show the aflatoxin concentrations in Artemisia afra leaves powder and spiked sample.*

Samples ID	B1 (ppb)	B2 (ppb)	G1 (ppb)	G2 (ppb)
S1.1	0	0	0	0
S1.2	0	0	0	0
S1.3	0	0	0	0
S1.4	0	0	0	0
S1.5	0	0	0	0
Spiked sample	5.0809	4.9759	4.9275	5.01609

3.3. Determination of Microbial Contaminants

Determinations of microbial contaminants were analyzed and results were tabulated by comparing with WHO standards. Table 3 below show the results of moulds (Fungus) CFU/g, *E. coli* and *Shigella* spp. with their WHO limit.

Table 3. *Fungus (moulds) (CUF/g), E. coli and Shigella spp. Determination in Artemisia afra leaves powder samples as compared to their WHO standards.*

Sample ID	Fungi (CFU/g)	*Shigela* spp.	*E. coli*
1.1	1.2 x 10^3	0	0
1.2	1.1 x 10^3	0	0
1.3	1.2 x 10^3	0	0
WHO	Maximum 10^5 per gram	Absence 1 per gram	Maximum 10^4 per gram

4. Discussion

Microbial contamination can be introduced during the harvesting time, collection, transportation, processing, packaging as well as during storage [14]. The results of fungal (moulds) contaminants for *Artemisia afra* leaves powder was between 1.1× 10^3 to 1.2× 10^3 CFU/g which is below the legal limit according to WHO which is supposed to be not more than 10^5 CFU/g [15]. On the other hand, *E. coli* and *Shigella*s pp. were not observed in all samples suggesting that, there were no contamination from these two microbes.

Results obtained by HPLC-MS aflatoxin analysis shows that all five samples of *Artemisia afra* leaves powder were found free from aflatoxin B1, B2, G1 and G2 but the spiked samples as expected revealed the presence of the respective aflatoxins. It must be noted that various studies have reported that there is no direct association between toxigenic moulds and aflatoxin determination; meaning that samples can be contaminated with moulds species while aflatoxin may not necessarily be detected. Example different samples of spices contaminated by *Aspergillus*species were analyzed for aflatoxins and were found free of aflatoxins [16]. The factors affecting mycotoxin production in contaminated foods and feeds are physical factors; temperature and humidity, chemical factors; the use of fungicides and fertilizers and biological factors; this is based on the interactions between colonizing fungal species and substrate [17]. So in summary the results obtained in the assessment of herbal medicine, *Artemisia afra* leaves powder, regarding the microbial contaminants as well as heavy metals have indicated that they were below the legal limit set by WHO. However, some other studies like the one done in Kenya by Ngari, *et al*; 2013 observed that Herbal materials used in the management of oral health in Nairobi County, Kenya were contaminated with pathogenic microorganism like *E. coli* as well as heavy metals like chromium and lead at various concentrations [18].

Heavy metal analysis using ICP-MS showed that the average; level of heavy metals detected in *Artemisia afra* leaves powder were 1.69 ± 0.08 ppm for Chromium (Cr), 0.08 ± 0.01 ppm for Cadmium (Cd), 0.22 ± 0.01 ppm for Lead, 0.15 ± 0.08 ppm for Mercury (Hg) and 0.04 ± 0.02 ppm for Arsenic (As). Heavy metals that were detected were within the permissible limits for consumed herbal products according to WHO standard guidelines. This suggests that; there were low level of contaminations of heavy metals in *Artemisia afra* leaves powder. Assessment of heavy metal in Traditional medicine is very important due to the environmental factors like the presence of industrial waste and agricultural activities such as the use of pesticides [19].

5. Conclusion

The quality of herbal medicine is very important in maintaining standardization of phytopharamaceuticals. The quality of *Artemisia afra* leaves powder processed at The Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Tanzania were within the WHO acceptable limit and is safe for human consuption based on the quality of parameters that have been analyzed; heavy metals, microbial contaminants and aflatoxins.

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