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A Quality Control of Hypoglycemic Herbal Preparations in Nairobi, Kenya

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Research Article

ABSTRACT

Introduction: Methods used by Kenyan herbalists to identify plants and preserve herbal drugs are unclear.

Objective: To assess the accuracy of plant identification, microbial and heavy metal contamination in hypoglycemic herbal preparations.

Method: Four herbalists were identified by purposeful sampling and key informant interviews were carried out. Ethnobotanical walks were used to collect herbs and a botanist checked the accuracy of scientific names. Herbalists were asked to submit formulations. Microbial contamination was evaluated using selective and non-selective cultural media. Levels of heavy metals were evaluated by atomic absorption.

Analysis: Content thematic approach was used to analyze key informant interviews. Degree of agreement between the names assigned by the herbalists and botanists was measured using percentage.

Results: Plant identification relied heavily on macroscopic qualities aided by the plant's geographical location. Both indigenous and botanical names were used. Naming errors using botanical names were recorded. Three formulations were submitted and one of them recorded contamination by *Candida albicans, Escherichia coli* and *Klebsiella pneumoniae*. Heavy metal contamination was not detected.

Conclusion: Plant identification by herbalists by use of botanical names may be inaccurate. Herbalist should be trained on good manufacturing practices.

INTRODUCTION

It is estimated that 80% of Africans use traditional medicine to meet their health needs [1]. Traditional medicine is very diverse and encompasses the use of plant herbal medicines among other therapies. In 1996, the World Health organization (WHO) formulated monographs on selected medicinal plants with an aim to provide scientific data on safety, quality control and efficacy. It also sought to provide models for member states to develop their own monographs [2].

Safety and quality studies of herbal medicines that are widely dispensed by herbalist in Kenya have shown that some herbal drugs are not safe for human consumption [3]. Plant medicines contain many compounds who presence is due to the plant species, geographical zones of growth, time and season of harvesting and preparation methods. This compound may be both desirable and unwanted. In Kenya, most herbal drugs are dispensed to patients without proper evaluation of these compounds. Quality

assurance of herbal medicines comprises aspects such as correct identification, collection and handling of crude materials, safety, efficacy and assessment of the final product for stability [4].

Herbal practitioners are able to assign scientific names to plants from experience. This is part of qualitative evaluation and a key component of quality assurance. Authentication of herbal drugs is essential when considering sources of any drug [5]. Inaccurate identification may lead to collection of wrong plants and reduce safety and efficacy of their medications. Unintentional adulteration and **substitution** may lead to death or poor reproducibility of the medicine. This study was part of a larger study that evaluated the quality assurance practices of herbal practitioners. In the larger study, we found that the practitioners conducted botanical identification without the aid of botanists. In addition, herbalists used excipients to prevent microbial contamination. They also in-cooperated other quality practices to produce safe products. These practices were self-taught. The determination of potentially harmful substances, such as microorganism and heavy metals, is a quality control indice for herbal drugs and is part of toxicological evaluation [4]. Microorganisms may be present in the crude herbs or could be introduced during processing. Heavy metals such as lead and mercury are found naturally in the environment and may accumulate in herbal drugs and causing harm to patients. Good agricultural and manufacturing practices are critical in ridding herbal drugs of such contaminants.

Study Objectives

i) To assess the accuracy of botanical identification as practiced by traditional medicine practitioners.

ii) To identify methods of formulation preservation used by herbalists.

iii) To evaluate microbial and heavy metal contamination of selected hypoglycemic formulations provided by Traditional Medicine Practitioners (TMPs).

METHOD

Ethical Considerations

Permission to carry out the study was obtained from the UoN/KNH Ethics and Research committee at Kenyatta National Hospital (KNH) (reference: KNH-ERC/A/306). This research was carried out in accordance with the basic principles defined in Guidance for Good Clinical Practice and the Principles enunciated in the Declaration of Helsinki [6]. Confidentiality was maintained by using pseudo names in the transcripts and coding of plants and formulations.

Study Design and Area

The study was a descriptive cross-sectional study conducted at the School of Pharmacy, University of Nairobi (UoN) and herbal clinics in Nairobi County and its surroundings.

Data Collection

The study population was herbalists practicing in Nairobi and its environs. Herbalists were included if they had long standing associations with the School of Pharmacy, University of Nairobi. In addition, they gave consent to participate in the study and were willing to provide samples for plant identification.

Four TMPs, considered as experts, were selected using judgmental sampling and interviewed. The sample size was arrived at because is considered satisfactory for an in-depth interview [7,8]. This sampling technique was conducted because it is the most suitable method for documenting secretive practices in detail from knowledgeable information sources [8-10].

An interview guide was used to collect information from the TMPs. Interviews were conducted in English and Kiswahili to elicit local and botanical names of herbs used to make anti-diabetic medications and to identify quality assurance practices. Ethnobotanical walks were carried out for plant collection. A botanist from the Museum of Nairobi was enlisted to aid in plant identification.

The TMPs were then asked to supply formulations used for the management of type 2 diabetes mellitus for evaluation of contamination. Only three herbalists complied. Oral anti-diabetic formulations were supplied and coded as MUI, MWAL and LUC. They were analyzed for microbial contamination and heavy metal poisoning.

Data Analysis

The two study themes from the key informant interviews (KII) were plant identification and product preservation. The data was transcribed and the information was analyzed using content thematic approach. Relevant verbatim quotations were used to present the study findings.

Determination of Microbiological Contamination

Preparation of cultural media for colony counts and herbal formulations

The LUC and MWAL formulations were supplied as powders. The powdered formulations were prepared by boiling as recommended by the TMPs. Tap water was used. Only the MUI formulation was presented to patients as a ready-to-take solution.

The herbalist reported that tap water from Kariminu Water and Sanitation Company was used to prepare his drugs. This water was however not analyzed.

Agar dilution method was used to culture and quantify any microorganisms [11]. Oxoid™ agars were used. The sample solutions were prepared by diluting 10 mL of herbal formulation to make 100 mL and mixing to homogenize the suspension to obtain a test solution (1:10). Further dilution was done to obtain a second test solution (1:100). One mL of each of these two dilutions was poured into two sterile plates. Twenty mL of molten Sabaround Dextrose Agar (SDA) and Tryptone Soya Agar (TSA) were each transferred into the plates. They were solidified and incubate. These plates were prepared in triplicate.

To prepare a negative control, 1 mL of the diluent was transferred into two sterile plates. Twenty mL of SDA and TSA at 45°C were separately poured in and mixed well. To prepare the positive control for yeasts and moulds, 1 mL of the (1:10) test solution were transferred into two petri dishes and 1 mL of *Candida albicans* (50-100 colony forming units/mL) was added. Twenty mL of SDA medium at 45°C was poured into the two plates and mixed well and cooled. After solidification, the plates were incubated. This was repeated with *Straphylococcus aureus* and TSA instead of *Candida albicans* and SDA as positive control for aerobic count.

The fungi were incubated at 20-25°C (SDA) and 37°C for bacteria (TSA) for 3 days and 5 days respectively. The quantity of colonies in each test solution was counted and calculated to colony forming units per mL (Cfu/mL).

Identification of microbiological colonies

Identification of bacterial colonies was done using Vitek®2 (bioMeriux,UK) and morphological characteristics were used for fungus. To identify bacteria, a loopful of formulation was cultured in plate count agar, sheep blood agar, MacConkey agar with sodium chloride, and SDA plates. The plates were incubated at 37°C for up to 48 hours and 30°C for the SDA for 7 days. After 48 hours there was mixed growth on sheep blood agar and lactose fermenters mucoid colonies on the MacConkey agar.

The colonies were sub cultured in TSI (Triple sugar Iron agar) SIM (Sulphur Indole and Motility agar) and Citrate agar and incubated at 37°C for 18 hours. Tube 1; TSI indicated acid butt acid slant with gas. Tube 2; TSI indicated acid butt acid slant. Four drops of SIM were added and red colour formed at the interface. Citrate Agar was positive.

The different colonies were purity plated in SDA and boy plates incubated at 37^0C for 18 hours. Using saline and vitek tubes, a few colonies were emulsified to a concentration of 0.5 McFarland standards. Gram negative cards were used and inserted in the machine. The results were released after 6 hours.

Tests for Heavy Metal on Herbal Formulations and pH Testing

Heavy metal test for lead, copper, cadmium, and arsenic was done using atomic absorption spectroscopy (Analytik Jena, Germany). The powdered herbs were boiled as recommended by the herbalists for patient use in water and allowed to cool. The liquid solution was tested as supplied by the herbalist. Each liquid solution was thoroughly mixed and 10-15 mL of the sample transferred to a digestion vessel. Twenty mL of 5 molar analytical grade nitric acid was added. The digestion vessel was heated until a clear solution was obtained. After cooling, the liquid was transferred into a volumetric flask and diluted with distilled water to 100 mL. A blank sample was prepared alongside. The samples were then run in the atomic absorption machine. Jenway 3540 pH and conductivity meter (Barloworld scientific Ltd) was used to determine pH levels.

RESULTS

Key Informant Interview: Method of Plant Identification Used by Herbalists

The herbs were obtained from both wild and cultivated areas some of which were polluted with waste. The geographical location of the herb assisted in its identity. All herbalists involved in the study attached great importance to previous known sites of plant location. These locations appeared to have been used over long periods of time. If for instance during a field trip, a herb was identified at a location where it had not been found previously by the herbalist, uncertainty as to its identity and medicinal qualities were raised although its observable features were known to them.

Macroscopic and organoleptic qualities of plants were used to identify medicinal herbs. The presences of special observable features of the plants were described by the herbalists. For example; a specific bark was described as *'looking as if numerous ants were walking on it'*. Another was described as *'having a snake like stem'*. Plant that exuded fluid were described as described as *'pouring out with milk' or 'bleeding red'*. During preparation, specific odors were used to confirm identity. For example, *'the powder should smell like biscuits after drying'*.

The herbal practitioners used both botanical names and local indigenous names for plant identification. The indigenous names were complex because the same plant was observed to have different names even within the same ethnic group. Indigenous plants were more difficult to name using binomial nomenclature by all herbalists. The loss of natural habitats and the seasonal nature of most of the herbs also created challenges during the study because the indigenous plants were difficult to locate. Exotic plants were readily named using botanical names by all herbalists. Some of the herbalist showed interest in gaining

more information about the botanical names and actively consulted the botanist. They all however maintained that the plants and geographical regions were trade secrets.

Results of Ethnobotanical Walk and Plants Identified

Only two herbalists agreed to participate in the ethnobotanical walk. The remaining herbalists provided plants for identification. The plants were both indigenous and exotic. A total of 66 plants were collected and registered at the herbarium at the Museum of Nairobi. Of these, 26 plants (39.39%) were identified by the herbalists using botanical names. Six errors (9.09%) were noted in identification using botanical names. Forty plants (60.60%) were identified using local languages **(Table 1).** The level of agreement of some plants between the herbalists and the botanist was assessed in **Table 2.** The herbalist declined to have most plants in the study revealed because they were part of formulations that were awaiting patents.

Table 1. Information provided by interviewed traditional medicine practitioners

Herbalist code	Level of education	Level of use of botanical names	Method of identification (exotic vs. indigenous)	Number of plants identified by the herbalist	Errors noted by the botanist
1	High school	Rarely	Indigenous	40	None. Used local language most of the time
2	College but none medical background	Most of the time	Exotic and indigenous	19	4
3	Medical course at college level	Most of the time	Exotic and indigenous	3	2
4	Medical course at college level	All the time	Exotic and indigenous	4	Declined to participate

Table 2. Comparison of some botanical names between the herbalists and the botanist

Botanical name given by herbalist	Correct botanical name
Senna spectabilis (DC.) H.S. Irwin and Barneby	*Senna septemtrionalis (Viv) H.S. Irwin and Barneby*
Indigofera arrecta Hochst. Ex A. Rich.	*Indigofera spicata Forssk.*
Solanum incanum L.	*Solanum campylacanthum Hochst. Ex A. Rich.*
Cassia species	Senna species

Preservation Techniques used by Herbalist

All the TMPs used sight, smell and taste to determine any spoilage of their herbal products. The packaging of the herbal drugs consisted of plastic bags for the powders and glass bottles for the liquid suspension. Expiry dates or recommended storage conditions were not indicated on any of the products. The MUI formulation contained *Tamarindus indica L.* (tamarind) as a preservative. Tamarind pods had been purchased from a local market by the herbalist.

"The tamarind pods are boiled in 4 ½ liters of water for 5 min. The rest of the herbs are added and the fire is put off 1-2 minutes later. The shelf life of the bottled sealed drug is three months. If opened, refrigerate between use."

Other methods mentioned by herbalist to preserve their herbal products included the use of commercial preservatives such as sodium benzoate, drying and soaking of the herbs in alcohol. Alcohol and the use of chemicals were rarely used because they were considered expensive. Chemicals were also mostly avoided because they were not natural. Drugs that were preserved with alcohol were considered good for 5-7 days. The alcohol of choice was spirit (Vodka® 37.5%) or ethanol (72%).

"Completely infuse 2 tablespoons of dried drug powder into 750 mL of alcohol. Shake frequently for a couple of days. Sieve with a cloth and dilute with 3 parts of boiled cool water. Dispense to the patient."

Results for Microbiological Testing, Heavy Metal Teats and pH of Herbal Formulations

Microbiological testing revealed that the LUC and MWAL formulations had no microbial growths. The MUI formulation that was issued as a suspension was found to be contaminated with 5000×10^6 cfu/mL of bacteria and 4000×10^6 cfu/mL of fungus. Vitek® identified that MUI formulation had heavy growth of *Escherichia coli* and moderate growth of *Klebsiella pneumonia*. The fungus was identified presumptively using morphological features as *Candida albicans* based on its white smooth dome shaped colonies. The pHs of freshly prepared formulations were determined as follows; LUC and MWAL was found to be 6.5; MUI registered a pH of 3.9.

DISCUSSION

In this study, herbalists used both botanical names and local names to identify medicinal herbs. Special features of the plants were considered important in plant identity and the local names reflected these qualities. This use of macroscopic features to identify plants is a standard practice amongst herbalists [12]. Exotic plants were easily and correctly named by the herbalists using botanical names most likely because they had been researched on and information about them was freely available in

various media. These plants are also common. The fact that changes in the geographical locations of indigenous plants resulted in doubt about a plant's identity, reinforces the idea that herbalists should not shy away from seeking advice from botanists.

Errors noted when botanical names were used in plant identity occurred when herbalists were not aware of new changes in naming of some plants. Other errors were due to mis-information. Two of the herbalists were uninterested in knowing about the correct names and preferred to use those familiar to them. Wrong names or outdated names may give rise to errors or confusion [4]. Clear scientific identification is a quality criterial for herbal medicine [13]. Correct identification is important because herbalists were observed to borrow certain plants among themselves. Some of these plants were shared in ground form after the identifying features had been destroyed. Secrecy about the herbs and their locations was thought to contribute negatively to the herbalists' knowledge on plant identity. This certainly impacts on conservation of rare medicinal plants.

Plant materials normally have aerobic spore forming bacteria and fungi from soil which could increase due to faulty handling at different stages of processing [4]. The European Pharmacopoeia provides non-mandatory regulations on acceptable limits [14]. The herbal formulations dispensed as powders in the current study were not contaminated although processing practices employed by all herbalists in this study were rudimentary. They may have contained secondary metabolites such as volatile oils, flavonoids, tannins and saponins with anti-microbial qualities [15]. The powdered drugs were to be prepared daily by patients. The preparation instructions included prolonged boiling for each daily dose. Boiling has been shown to contribute to a reduction in microbial contamination and some heavy metals in herbal drugs [16]. The formulation dispensed as a liquid suspension (MUI) was prepared and packed as multiple doses by the herbalists. Boiling in this case was limited to "1-2 minutes" and was done at the herbalist's clinic. This formulation was found to be contaminated with bacteria and fungi. Microbial contamination of herbal drugs has been reported in several studies [17-19].

The presence of *Escherichia coli* indicated that the drug was contaminated with fecal matter which the European pharmacopoeia states should be absent [4,20]. There should be zero tolerance for the presence of *Escherichia coli* [21]. Contamination with *Klebsiella pneumonia* is also associated with unhygienic preparation. This gram negative bacteria is found in the respiratory, urogenital and intestinal tracts and causes nosocomial infections and urinary tract infections [22,23]. This contamination was almost certainly from the herbalist due to unhygienic handling. The contamination may also have occurred at the wild sources because during field visits, some areas were observed to be highly polluted. Although no heavy metal contamination was found, heavy metals from soil may occur due to pollution and poor agricultural practices [24]. Acceptable limits of heavy metals in herbal drugs have been proposed [25].

Microbial contamination in herbal drugs is a concern. Failure of the preservative in the liquid formulation used could have occurred. This particular formulation (MUI) contained a herbal preservative, *Tamarindus indica L.* (tamarind). Tamarind is known to have broad spectrum anti-microbial qualities against many bacteria [26-28]. In this case, it was not active against the said microorganisms either due to insufficient quantities of the tamarind, heavy bacterial contamination, or resistance of the micro-organisms. The tamarind possibly had a poor yield of essential oils documented to have good anti-microbial properties because the wrong morphological parts were used. In this case, pods were used. The leaves from specific geographical regions have been shown to have higher quantities of these antimicrobial essential oils [26]. The pH of MUI was found to be 3.9 and was done on a freshly prepared solution containing tamarind which is known to be acidic. It is possible that the MUI formulation later degraded to higher pH values causing the observed bacterial growths. It has been reported that bacterial growth occurs at pH 5-8.5 [20].

CONCLUSION

The powdered formulations had no microbial contamination compared to the liquid formulation. Despite collection of plants in polluted areas, no heavy metal contamination was found. Accuracy of plant identification by herbalists was fairly good. Herbalist should enlist the aid of botanists in plant identification. Herbalist should be encouraged to submit samples of their drugs for microbial testing and they should be educated on good manufacturing and good agricultural practices.

Acknowledgements

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