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Investigation of Selected Pathogenic Microorganisms and Toxic Elements in Herbal Materials Used in Management of Oral Health in Nairobi County, Kenya

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ABSTRACT

Traditional medical practitioners in Kenya have used herbal materials to treat oral diseases for several years. However data on contamination of the herbal medicine with microbial pathogens and toxic metals is lacking. The aim of this study was to investigate presence of selected pathogenic microorganisms and selected elemental levels of herbal materials used in management of oral health in Nairobi. Herbal materials were purchased from Nairobi County and taken to the laboratory for analysis. Microbial contaminants were analyzed by inoculating the herbal samples in selective media followed by macroscopic, microscopic and biochemical studies to identify the microorganisms. Mineral elements were investigated by Total Reflection X-Ray Fluorescence (TXFR). Results indicate that herbal materials are contaminated with, *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* The herbal materials are also contaminated with aluminium, chromium, manganese, iron, copper, zinc and lead at various concentrations. The study concludes that some herbal materials in the market are not safe for use by humans orally.

KEY WORDS; microbial contaminants, toxic metals, herbal materials

INTRODUCTION

Oral health care facilities have not been adequate for majority of African population [1]. In Kenya, the oral health facilities and infrastructure in most health centers do not have sufficient resources [2] and therefore majority of people will continue to use herbal materials for their oral health care.

Herbal medicines are widely perceived as being natural and free from side effects. Nevertheless it is now well established that a number of these agents have potential to produce minor or major safety problems [3]. Some reported adverse effects following the use of herbal medicine have been associated with contamination with microorganisms and heavy metals [4]. The commonly used herbal materials include chewing sticks, herbal pastes, powders, herbal mixtures and suspensions [5]. Most of these herbal materials are prepared and sold under unhygienic conditions. A number of oral health care materials are hawked when not packaged and this raises the possibility of contamination. Most of these materials are used directly without further processing (for example chewing sticks) thereby increasing the risk of disease transmission. Herbal preparations are used in different forms and may carry a large number of microbes originating from soil usually adhering to various parts of herb [6]. The contaminants that present serious health hazard are pathogenic bacteria such as *Salmonella, Escherichia coli, Staphylococcus aureus, Shigella* species and other Gram positive and Gram negative strains of bacteria [7].

Cases of poisoning with toxic heavy metals from herbal products are well documented [8]. Medicinal plants are normally contaminated with toxic metals during growth, development and processing [9]. Indeed the issues of safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. This could be due to lack of standards for herbal products. To maximize the potential of African traditional medicines as a source of healthcare, the safety, efficacy and quality need to be assessed. The study aimed at evaluating elemental and microbial properties of herbal products sold in Nairobi County used for management of oral health.

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Objective

To investigate the presence of selected pathogenic microbes and elements

MATERIALS AND METHODS

Microbial contaminants

Herbal products were randomly purchased from traditional medical practitioners in various parts of Nairobi, County. The herbal products were chosen on the basis of their commercial availability and popularity of use. The collected samples were given sample codes (MPP- powders, HS- herbal suspension, HP- herbal paste) and the following were recorded; manufacturing and expiry date, indications, mode of application, and whether the product had Kenya Bureau of Standards (KEBS) mark of quality. The samples were stored in a refrigerator at 4 ºC pending analysis.

Media and isolation of pathogenic microorganisms

All media used were prepared according to the manufacturer's instructions. The required amount of medium was weighed and dissolved in distilled water, autoclaved at 121 ºC and the solid medium dispensed in Petri dishes and slants.

Isolation of indicator microorganisms

The microorganisms *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Candida albicans* were investigated as described by various authors [10, 11] with slight modification. To determine the presence of bacterial contaminants, 10 grams of a herbal powder was dispersed in the medium and made to 100mls while for liquid samples, 10 ml was diluted to 100 mls by adding soybean casein digest medium and then incubated.

For isolation of the *S. aureus,* a portion of the enrichment culture medium was spread on the surface of the Vogel-Johnson agar medium and manitol-salt agar which were freshly prepared according to manufacturer's instructions. The plates were incubated at 37 ºC for twenty four hours under aerobic conditions. Colonies showing golden yellow color or colorless were considered to be *S. aureus.* The pure isolates were further subjected to gram staining for microscopy, biochemical test such catalase as well as tube and slide coagulase to confirm the identity of *S. aureus*. A portion of the enrichment culture medium was streaked on centrimide agar medium for detection *P. aeruginosa.*

To detect *E. coli*, fluid lactose medium was added to 10 mls of the sample to make 100 mls. Fluid lactose enrichment was streaked onto differential MacConkey agar plates for *E.coli* and incubated at 44 ºC for twenty four hours. Inoculum from *E. coli* medium was then streaked onto the surface of Eosin methylene blue (EMB) agar and incubated for 24 hours. Dark colonies with metallic sheen indicated the presence of lactose fermenters. The colonies were further subjected to indole, motility, Voges Proskaur and citrate biochemical test as described by Prescott et al [12].

For detection of *Salmonella* species, 1ml of sample was transferred into 9 mls fluid selenite-cystine and fluid tetrationate respectively. The cultures were incubated at 35±2 ºC for 12- 24 hours and were sub-cultured further on the surface of brilliant green agar and bismuth sulfite agar media respectively. The appearance of typical black and green colonies was regarded as positive for *Salmonella* species. The colonies were further streaked on the nutrient agar slants for further biochemical identification using Triple Sugar Iron (The butt-slant).

For detection of *C. albicans,* 10 grams of each sample was added to Sabrounds dextrose broth to make 100 mls. The latter was incubated at 20-25 ºC for seven days. The incubated samples were examined and cultured on Sabrounds dextrose agar. Where microbial growth was observed, the colonies were identified by germ tube method. About 0.5 mls of human serum was placed in small test tube. Using a sterile wire loop, the serum was inoculated with a yeast colony. The inoculated serum was incubated at 37 ºC for three hours. A drop of the serum yeast culture was transferred to a glass slide using a Pasteur pipette. The preparations were examined using magnification of X10 and X40 objectives for sprouting yeast cells.

Determination of viable counts

The herbal samples were subjected to the following examinations: total aerobic viable count (TAVC), viable counts of *S. aureus, E. coli, P. aeruginosa,* fungi and *Salmonella spp* by pour plate method based on the method by Okunlola et al [7] and Enayatifard et al [11] with slight modification. Ten grams of a herbal powder was dispersed in the sterile physiological saline and made to 100mls while for liquid samples, 10 ml was diluted to 100 mls.

Serial dilutions of the samples were used so that the number of colony forming units (CFU) in petri dishes wound be less than 300. One ml aliquots in duplicates of each dilution sample were pipetted onto separate sterile petri dishes (9 cm in diameter). For total viable counts 20 ml of nutrient medium for cultivation of bacteria and Sabourauds agar for fungi was added while appropriate selective medium was used for pathogenic bacteria under investigation. After solidification of the soft agar the petri dishes were incubated in duplicates at 37 ºC for 48-72 and at 44 ºC for *E.coli* hours for bacterial counts and at 25 ºC for five days for fungal counts [13]. After incubation the number of colony forming units was recorded for each plate.

Elemental analysis

Herbal powders were digested using standard procedure. However, triplicate known weights (1.0g) of each paste was placed in a clean vial and 10 mls of double distilled water added and shaken for homogenization. A volume of 20 µl of 1000 µg/g Gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2 µg/g Ga in each sample. Aliquots of 10 µl of each sample in triplicates were pipetted onto clean quartz carrier using a micro-pipette. The carriers were then dried in an oven to evaporate the solvent. Each sample carrier was irradiated for 300 seconds using an S2 PICOFOX TXRF Spectrometer which was operated at 50kV and a current of 1000µA. Evaluation of the measured spectra was done using S2 PICOFOX software on the basis of the chosen elements. The concentrations were calculated based on the net intensities of the analyte peak elements and that of the internal standard.

DATA ANALYSIS

The number of microorganisms in each sample was evaluated by multiplying the average number of colonies per plate with the dilution factor. The counts were expressed as colony forming unit per gram or one ml (CFU/ml). Elemental concentration was expressed as mean ±Standard deviation

RESULTS

Microbial contaminants

Twenty two herbal products, 7 powders, 10 herbal suspension and 5 herbal pastes were analyzed. The study revealed microbial contamination in some of the herbal products traded in Nairobi. *Escherichia coli* and *Salmonella typhi* were detected in three herbal products (Table 1). Nineteen herbal products had no microbial contaminants being investigated in this study. All liquid samples did not have pathogenic microbes, while 1 out of 5 pastes and 2 out 7 powders were contaminated. Product MPP-4 was the most contaminated. Whereas *E. coli. S. typhi, P. aeruginosa* and fungi were found in herbal materials, *S. aureas* was not detected in any sample.

Table 1. Microbial contaminants in herbal products used in management of oral health in Nairobi County. (Where MPP: herbal powder and HP:-herbal paste).

Product code	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhi*	Fungal
MPP-1	+	-	-	+	-
MPP-4	+	-	+	+	+
HP-5	+	-	-	+	-

Viable counts

Table 2 shows viable counts in various products. The total viable counts ranged from $3.0x10^3$ to $2.60x10^6$. Twelve of the investigated products lacked viable counts. Although total viable counts for MPP-2, MPP-5, MPP-6, HS-8, HS-9, HS-10 and HP-2 were high, there were no viable counts for pathogenic microbes under investigation. Whereas *S. typhi* and *P. aeruginosa* were detected in some herbal products (Table 1), they had no viable counts in the samples (Table 2).

Table 2. Viable counts of microbial contaminants in herbal products used for oral health in Nairobi County. (Where MPP: herbal powder, HS: herbal suspension and HP: herbal paste).

	TVC	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhi*	Fungi
MPP-1	$2.10X10^5$	$1.0x\ 10^4$	0	0	0	0
MPP-2	$6.90X10^4$	0	0	0	0	0
MPP-4	$4.70X10^4$	$2.80x10^2$	0	0	$2.0X10^3$	$3.40x10^4$
MPP-5	$1.74X10^5$	0	0	0	0	0
MPP-6	$1.14\ x10^5$	0	0	0	0	0
HS-8	$3.0X10^3$	0	0	0	0	0
HS-9	$4.0X10^3$	0	0	0	0	0
HS-10	$1.13X10^5$	0	0	0	0	0
HP- 2	$3.10X10^4$	0	0	0	0	0
HP-5	$2.60\ X10^6$	$2.5x10^4$	0	0	$2.40x10^4$	0

Elemental profile

Aluminium was detectable in 5 products ranging from 392.03 ppm to 582.86 ppm (Table 3) while it was below the instruments detection limit of 16 ppm for the other samples. Chromium concentration ranged from 0.02 ppm to 7.31 ppm while all other samples had concentrations below detectable levels <0.01 ppm. The highest amount of manganese detected was 233.84 ppm and that of iron was 1984.2 ppm. Copper levels were less than 10 ppm in the samples analysed. Lead was detected in all the samples with highest amount in product HP-1 at 22.08 ppm.

Table 3. Average amount of elements (ppm ± SD) in herbal materials used in management of oral health in Nairobi County. (Where MPP: herbal powder, HS: herbal suspension and HP: herbal paste).

Product code	Aluminium	Chromium	Manganese	Iron	Copper	Zinc	Lead
MPP-1	<16.00	<0.01	21.54±0.59	288.40±10.97	8.89±0.07	12.63±0.02	3.74±0.45
MPP-2	482.08±106.70	4.00±1.37	56.19±1.39	1204.90±27.20	3.83±0.08	13.85±0.04	5.00±0.64
MPP-4	<16.00	<0.01	60.58±2.60	446.82±17.30	4.47±0.08	8.46±0.20	3.44±0.78
MPP-5	440.30±62.52	<0.01	54.94 ±1.33	561.28±20.60	2.99±0.46	15.36±0.66	1.98±1.33
MPP-6	<16.00	<0.01	46.58±1.57	247.66±11.15	2.77±0.20	7.72±0.67	6.20±0.25
MPP-7	<16.00	<0.01	51.59±2.92	437.20±23.50	3.51±0.20	11.69±0.10	4.71±2.48
MPP-8	<16.00	<0.01	82.09±2.96	356.74±12.73	3.98±0.71	10.69±0.34	3.42±0.41
HS- 1	<16.00	0.01	0.05±0.01	0.75±0.12	0.01±0.00	0.05±0.00	0.02±0.00
HS- 2	<16.00	< 0.01	0.05±0.01	0.21±0.05	0.02±0.00	0.05±0.02	0.11±0.03
HS- 3	<16.00	<0.01	0.79±0.00	1.14±0.01	0.03±0.00	0.18±0.00	0.02±0.01
HS- 4	<16.00	<0.01	0.08±0.01	0.55±0.02	0.013±00	0.09±0.00	0.05±0.01
HS- 5	<16.00	0.02±0.00	0.09±0.00	0.48±0.01	0.01±0.00	0.06±0.00	0.05±0.00
HS- 6	<16.00	<0.01	0.74±0.02	0.96±0.04	0.02±0.00	0.11±0.00	0.02±0.01
HS- 7	<16.00	0.02±0.00	0.74±0.01	4.09±0.12	0.03±0.00	0.050±0.00	0.03±0.01
HS- 8	<16.00	0.02 ±0.00	0.1±0.02	0.60±0.34	0.01±0.00	0.04±0.00	0.01±0.08
HS- 9	392.03±11.24	0.139±0.02	0.86±0.34	2.21±0.06	0.03±0.00	0.20±0.021	0.11±0.01
HS- 10	< 16.00	0.02±0.00	0.34±0.01	1.85±0.05	0.02±0.00	0.07±0.00	0.05±0.01
HP- 1	<16.00	<0.01	11.08±2.65	123.17±7.33	0.04±0.00	0.04±0.00	22.08 ±4.70
HP- 2	<16.00	<0.01	5.99±0.40	52.46±3.50	0.04±0.00	2.89±0.50	10.92±0.63
HP- 3	<16.00	<0.01	2.41±0.69	90.75±6.52	3.24±0.16	26.68±1.53	5.93±0.27
HP- 4	<16.00	<0.01	116.22±2.77	363.40±10.58	5.91±1.08	6.92±0.83	13.75±0.28
HP- 5	582.86±36.3	7.31±0.47	233.84±4.50	1984.2±291.05	5.54±0.52	23.07±0.30	4.91±0.90

DISCUSSION

The presence of microbial contaminants in herbal medicine depends on several environmental factors and these have an impact on the overall quality of herbal products [14]. The microbes isolated and identified in this study such as *E. coli*, *P. aeuruginosa*, *S. typhi* and *C. albicans* cause serious health conditions [6]. Contamination of herbal products with *E. coli* may be as a result of environmental problems [15]. This was evident during the study as most of the products were poorly packaged and traded in unhygienic business premises. Consumers of these products risk infection associated with *E. coli* like diarrhoea especially in children. The levels of *E. coli* reported here ($2.80x10^2$ -$2.5\ x10^4$ are much higher than what is recommended (50 colonies) [16]. The high levels of *E. coli* reflect the poor handling of medicinal plants.

Isolation of a gram negative *P. aeruginosa* from herbal materials raises deep health concerns. Edaphic factors are the probable source of the isolate as the bacteria is primarily a soil bacterium, reflecting poor harvesting and cleaning of herbal materials. Salmonella species detected in some samples are causative agents of various infections like salmonella food poisoning which is a major problem in the world [17].

The presence of the fungal contaminant shows the possibility of poor storage conditions. This is a serious contaminant since some common species of fungi produce toxins like aflatoxins. According to the WHO [17], aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts.

The limits of microbial contamination are total aerobic bacteria 10^5 CFU/g yeast and mould 10^3 CFU/g [18]. However, none of the herbal suspensions exceeded the recommended total aerobic counts. The absence of contaminants may be due to hygienic packing or presence of bacteriostatic substance that would have killed possible microbial contaminants. Investigation of possible antimicrobial adulterants in the herbal suspensions is suggested. The isolation of the pathogens from herbal products in other parts of the world has been reported. Microbial load of some medicinal plants sold in local markets of Benin, Nigeria reported presence of *P. aeruginosa* and *B. subtilis* among others [20]. In Kaduna Nigeria, studies indicated presence of pathogenic *S. typhi* in 65.7% of herbal products analysed and *E. coli* in 58.7% of the samples analysed [10]. Evaluation of microbial quality of plant materials in Belgrade indicated the presence of *E. coli, Bacillus* and *Clostridia* species [13]. When evaluation of microbial and fungal contaminations of herbal products was carried out in Ghana [21], aflatoxins, nitrobacteria and *P. aeruginosa* among others were found to be present. In South Africa, studies have shown that herbal products are heavily contaminated with bacteria [6].

Mineral elements play a vital role in many human physiological activities and their deficiency or excess can affect human health [22]. In the current study, the high levels of aluminum reported (392-582 ppm) pose a health hazard to the consumers. The levels exceed the maximum allowed levels of 0.2 ppm [23]. Aluminium as a metal when present in food, water and soil can induce individuals to suffer from Aluminium toxicity. It is believed that Alzheimer disease is related to Aluminium toxicity. Although the herbal products were to be gurgled in the mouth, the dosage from these medicinal plants should be monitored to avoid toxicity. The presence of aluminium could be attributed to pollution during collection of herbs, processing, storage and trade.

Presence of lead in all the products raises concern on the overall quality of herbal products. All the herbal powders exceeded the recommended daily allowance (RDA) of 3 mg/week indicating possible toxicity [9] leading to malfunctioning of both the brain and kidney [24]. The levels of lead in twelve herbal products ranged from 1.98- 13.75 ppm and are much higher compared to those reported in Nigeria [9, 25]. The recorded difference could be due to different ecological sources of the plants or during processing, storage or trade of the herbs. The presence of lead in herbal materials may also be due to environmental pollution arising from automobile and industrial activities [25] or poor packaging especially using recycled paint containers.

Chromium plays a major role in human body through regulation of insulin [26]. The chromium levels recorded here ranged from 0.02 to 7.31 ppm while those reported in Nigeria [9] ranged from 0.00-2.63 ppm. The concentration of copper in herbal products that ranged from 0.01 ppm to 8.89 ppm is much lower than that reported by Annan et al. [27] of 8.0 to 114.5 ppm. The difference can be explained by sources of samples.

The amounts of iron reported (247.66-1204.9 ppm) is much higher than that reported by Muhammad [28] of 147.91-540.0 ppm and that reported by Samali et al [9] which ranged from 0.00-5.96 ppm.

Zinc is necessary for bone formation and wound healing [9]. The presence of zinc in these products may contribute to the healing properties of oral diseases. The levels reported here are much lower than those reported in Ghana 43.5-495.0 ppm [27].

High levels of manganese reported could be attributed to its abundance in the soil. The maximum safe and adequate daily dietary intake is 11000 ppm [29] meaning that the herbal powders in this study are not safe for human use. The range of manganese content of (21.5 to 87.8 ppm) compares well with levels reported in herbal teas (7.4-86.67 ppm) in Nigeria[8] but the reported levels are much lower than those recorded by Annan et al. [27] of 556 to 1455 ppm.

CONCLUSION

Based on the results of this study, it is concluded that some herbal products are contaminated with microbes and some have high levels of toxic elements. Consumers of these products are at risk of infection with pathogenic microbes and suffering from toxicity from toxic metals.

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