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Microbial and Heavy Metal Contaminants in Herbal Preparations Sold in Maseru, Lesotho

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Abstract: The majority of the populations, both from developed and developing countries, use herbal preparations for primary healthcare purposes. In particular, the use of herbal preparations in Lesotho is high due to inadequate healthcare facilities and inaccessibility of healthcare services. Herbal preparations are inexpensive, easily accessible and culturally accepted than conventional medicines. Although herbal preparations are popularly used, they could be contaminated with pathogenic microbes, toxic heavy metals and non-metals, agrochemical residues, mycotoxins and endotoxins and, thus World Health Organization (WHO) recommends that herbal preparations should be evaluated for safety, efficacy and potency so as to protect the consumers. This study was, therefore, designed to evaluate heavy metals and microbial contaminants in some of the commercially available herbal preparations in Maseru, Lesotho. A total of five herbal preparations were randomly purchased from different areas of Maseru at market price and were subjected to toxic heavy metals and microbial load analysis in accordance to International pharmacopeia and European pharmacopeia. Antimicrobial sensitivity test was performed to the isolated microorganisms. Our results revealed that all of the five herbal preparations were found to be contaminated with fungi beyond WHO limit, 10^3 CFU/ml. *Pseudomonas aeruginosa* was also isolated in all the five herbal preparations. The isolated *P. aeruginosa* was found to be susceptible to ciprofloxacin and ceftriaxone, clinically used antibiotics. There was no growth of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* from all the five herbal preparations. Again, total coliform count in three samples exceeded 10^3 CFU/ml, WHO safety limits. Finally, all herbal preparations complied with the limit test for chlorides; however, only two herbal preparations complied with the limit tests for total heavy metals, less than 20ppm. Therefore, this study reports and concludes that herbal preparations sold in Maseru could be contaminated with pathogenic microorganisms, acid radical's impurities and toxic heavy metal metals. The testing of herbal preparations for microbial and heavy metal contaminants is highly recommended and, may become mandatory.

Keywords: Microbial Contaminants, Heavy Metal Contaminants, Herbal Preparations, Antimicrobial Sensitivity, Lesotho

1. Introduction

The majority of the populations, both from developed and developing countries, use herbal preparations for primary healthcare purposes [1–3]. The use of herbal preparations is mainly through self-medication [4] and they are available as medicinal preparations, nutraceuticals and cosmetics [5]. It is also reported that 88% of World Health Organization (WHO) Member States use traditional and complementary medicines [6]. It is estimated that 70–80% of the population in developing countries, relay on herbal preparations because they are inexpensive, easily accessible, culturally acceptable and sustainable than conventional medicines [5, 7, 8]. Herbal preparations in Lesotho are still viewed as important because of lack of adequate healthcare facilities and inaccessibility of available healthcare services [9]. In rural areas of Lesotho, medicinal plants are the most accessible and affordable form of therapy [10].

Although herbal preparations are considered to be safe [1] and cause no side effects, there have been reports of acute and chronic toxicity resulting from their use [8]. The safety of herbal preparations is still a concern due to contamination by pathogenic microbes, toxic heavy metals and non-metals, agrochemical residues, mycotoxins and endotoxins [11, 12].

The contaminants in herbal preparations turn to be carried along from soil where the medicinal plants were grown [5]. The microorganisms adhere to leaves, stems, flowers, seeds, and roots of the medicinal plants used to prepare the herbal products [13]. The microbial contamination of herbal preparations can be caused by unsafe collection, transportation, drying, preparation, improper cleaning procedures or storage [13]. Other sources of contaminants could include the use of unsterile water, handling of preparations with contaminated hands or using contaminated packaging materials [5, 14]. Some herbalists sell their herbal products by the road side thus exposure to dust could be the possible source of fungal contaminants [5, 15]. Toxic heavy metals in herbal preparations could be from contaminated water, agricultural methods, manufacturing processes and polluted environment in which the medicinal plants are grown [16, 8]. Sometimes the use of heavy metals in herbal preparations is intentional, as some of these heavy metals are believed to be beneficial to the human body [7]. Non-essential heavy metals are toxic even in trace amounts [17].

Unlike conventional drugs, herbal preparations are not governed by stringent legislative regulations [5, 7] and therefore could pose health problems to consumers especially if contaminated with heavy metals and pathogenic microbes [1]. Herbal preparations are sometimes classified under dietary supplements and as a result manufacturers sell their herbal preparations without any evidence based scientific study regarding their safety and efficacy [7]. WHO recommends that herbal preparations should be evaluated for efficacy, safety and potency so as to protect the consumers [6]. In Lesotho, several herbal preparations are commercially available but knowledge of their safety with reference to microbial and heavy metal contamination is limited. This study was, therefore, designed to evaluate microbial and heavy metal contamination in some of commercially available herbal preparations in Maseru and the results are communicated in this paper.

2. Materials and Methods

The study received the ethical approval from the National University of Lesotho (NUL), Research and Ethics Committee (ID97-2021). The reagents used were of high quality purchased from Prestige laboratory supplies (PTY) Ltd in South Africa and used directly.

2.1. Study Area and Sample Collection

A total of five herbal preparations were randomly purchased from herbal medicine retailers in different areas in Maseru at market price on 15 January 2021 and transported to pharmacy laboratory at the National University of Lesotho (NUL) in the same day. The samples purchased were in their original packaging and required no further processing. The purchased herbal preparations were not beyond their expiry date and were intended for oral administration. The samples were assigned unique codes that were used in laboratory analysis and presentation of results [18]. Table 1 summarizes the information of the five herbal preparations.

2.2. Determination of pH

The pH of herbal preparations was determined by using microprocessor pH meter (model pH50+DSH®purchased in Italy) by following the reported procedure with some modifications [7]. The measurement was done in triplicates and the average pH was calculated.

Table 1. *Composition of the five herbal preparations (HPs), their daily adult dose as indicated on the label and the measured pH.*

Sample ID	Sample volume	Uses of the preparation as indicated on the label of finished product	Daily adult dose (70 Kg body weight)	pH± SD
TNHP01	1000ml	Blood pressure, Arthritis, Cancer, Diabetes, Genital Herpes, Wound infection, and Sexually transmitted infections (STIs).	About 50ml administered two times per day	5.85±0.01
TNHP02	500ml	Influenza, Respiratory infections and Cold related illnesses.	About 80ml administered three times per day (as hot drink)	5.86±0.02
TNHP03	1000ml	Period pains, Herpes Zoster, Diabetes, Generalized body pains, Haemorrhoids, Low, Kidney problems, and Chest pains.	About 30ml administered two times per day	3.84±0.01
TNHP04	1000ml	Joint pains, Arthritis, Fatigue, Inflammation, Myalgia, Period pains, Blood pressure and Persistent headaches.	About 30ml administered two times per day	5.39±0.06
TNHP05	1000ml	Wound infections, Blood pressure, Diabetes, Dizziness, Myalgia, Muscle pains, Muscle pulls, Boils, Tuberculosis (TB), and Arthritis,.	About 50ml administered two times per day	4.64±0.02

2.3. Determination of Heavy Metals and Non-metals Impurities

2.3.1. Limit Test for Heavy Metals

The 25 ml of the herbal preparation pH was adjusted to between 3 and 4 using dilute acetic acid or dilute ammonia solution as reported [19]. The solution was then diluted to 35 ml with distilled water. Freshly prepared 10 ml of hydrogen sulphide solution was added, and the solution was diluted with distilled water to 50 ml. The solution was allowed to stand for 5 minutes and thereafter was viewed downwards over a white surface and the colors were recorded. Simultaneously, the same procedure was carried out using 20 ppm standard lead solution. If color produced in sample solution was less than the standard solution (less than 20 ppm), the sample was considered to have passed the limit test of heavy metals. The metallic impurities in substances are expressed as parts of lead per million parts of the substance [19] because lead is one of the most toxic among toxic heavy metals [8]. Metals that respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum [19].

2.3.2. Limit Tests for Acid Radical Impurities

i. Limit test for chlorides

To 1 ml of herbal preparation was added 10 ml of dilute nitric acid and the solution was diluted to 50 ml with distilled water. One milliliter of silver nitrate solution (5% w/v) was added and the solution was stirred immediately with the glass rod. The solution was allowed to stand for 5 minutes and thereafter the opalescence was observed. Simultaneously, the same procedure was carried out using 0.05845% w/v solution of sodium chloride as the standard solution [20]. If opalescence produced in sample solution was less than the standard solution, the sample was considered to have passed the limit test of chlorides [20, 21].

ii. Limit test for sulfates

Two milliliters of dilute hydrochloric acid was added to 1 ml of herbal preparation and the solution was diluted to 45 ml with distilled water. Five milliliters of barium sulphate reagent was added and the solution was allowed to stand for 5 minutes and thereafter the turbidity was observed. Simultaneously, the same procedure was carried out using 0.1089% w/v solution of potassium sulphates as the standard solution [19]. If turbidity produced in sample solution was less than the standard solution, the sample was considered to have passed the limit test of sulphates [19, 22].

2.4. Microbiological Analyses

2.4.1. Media Preparation and Sample Pre-treatment

The media powder was weighed, reconstituted with distilled water and heated until the media had completely dissolved [12]. The manufacturer's instructions were followed for proper reconstitution of the media [11]. The media was then sterilized by autoclaving at 115 kPa, 121°C for 15 min [12]. About 15-20 ml the of sterile media was poured into the petri dishes at not more than 45°C and allowed to solidify at room temperature [12]. The media was then sealed with parafilm, labelled and stored at between 2-8°C. The sterility of the prepared media was checked by randomly incubating selected agar plates at 37°C for 24 hrs [11].

One millilitres of the sample was dissolved in 9 ml buffered sodium chloride-peptone solution pH 7.0 which was then followed by other tenfold serial dilutions [23] until 10^{-6} dilution was achieved.

2.4.2. Microbial Enumeration Test

i. Total Aerobic Microbial Count

The sample was vortexed to ensure uniform distribution of microorganisms if any. One millilitres of the pre-treated sample was spread into the two nutrient agar and incubated at 32°C for 48 hrs [1]. Simultaneously, the nutrient agar without an inoculum was incubated as the negative control [23]. The number of colonies formed was counted and the number of cfu per ml of the sample was calculated.

ii. Total Coliform Count

The sample was vortexed to ensure uniform distribution of microorganisms if any. One millilitres of the pre-treated sample was spread into two MacConkey agar and incubated at 37°C for 24 hrs [1]. Simultaneously, the MacConkey agar without an inoculum was incubated as the negative control [23]. The number of colonies formed was counted and the number of cfu per ml of the sample was calculated.

iii. Total Combined yeasts/moulds Count

One milliliters [1] of the pre-treated sample was spread over the surface of Sabouraud-dextrose agar of two petri dishes and incubated at 25°C for 5 days [24]. Simultaneously, the Sabouraud-dextrose agar without an inoculum was incubated as the negative control [23]. The number of colonies formed was counted and the number of cfu per ml of the sample was calculated.

2.4.3. Test for Specific Microorganisms

i. Pseudomonas aeruginosa

One millilitres of the sample was dissolved in 9 ml buffered sodium chloride-peptone solution pH 7.0 [23] and this 10 ml was inoculated in 100 ml casein soya bean digest broth, homogenized and incubated at 35°C for 24 hrs. Then subculture on a plate of cetrimide agar and incubated at 35°C for 24 hrs [25, 26]. Simultaneously, the cetrimide agar without an inoculum was incubated as the negative control [26]. The growth of colonies was considered to be the presence of *Pseudomonas aeruginosa* which required to be confirmed by identification tests [25].

ii. Staphylococcus aureus

One millilitres of the sample was dissolved in 9 ml buffered sodium chloride-peptone solution pH 7.0 [23] and this 10 ml was inoculated in 100 ml casein soya bean digest broth, homogenized and incubated at 35°C for 24 hrs. Then subculture on a plate of mannitol salt agar and incubated at 35°C for 24 hrs [25]. Simultaneously, the mannitol salt agar without an inoculum was incubated as the negative control [26]. The growth of yellow/white colonies surrounded by a yellow zone was considered to be the presence of *Staphylococcus aureus* which required to beconfirmed by identification tests [25].

iii. Escherichia coli

One millilitres of the sample was dissolved in 9 ml buffered sodium chloride-peptone solution pH 7.0 [23] and this 10 ml was inoculated in 100 ml casein soya bean digest broth, homogenized and incubated at 35°C for 24 hrs [25]. The container was shaken and 1 mL of incubated casein soya bean digest broth was transferred to 100 mL of MacConkey broth and incubated at 44°C for 24 hrs. Then subculture on a plate of MacConkey agar at 35°C for 24 hrs [25, 26]. Simultaneously, the MacConkey agar without an inoculum was incubated as the negative control [26]. The growth of colonies was considered to be the presence of *Escherichia coli* which required to be confirmed by identification tests [25].

iv. Klebsiella pneumonia

One millilitres of the pre-treated sample was spread into nutrient agar and incubated at 32°C for 48 hrs [1]. Simultaneously, the nutrient agar without an inoculum was incubated as the negative control [23]. A pure culture from nutrient agar was inoculated into MacConkey agar and incubated at 33°C for 72 hrs [5]. Simultaneously, the

MacConkey agar without an inoculum was incubated as the negative control [23]. The growth of round mucoid colonies with colourless edges was considered to be the presence of *Klebsiella pneumonia* which required to beconfirmed by identification tests [5].

2.5. Disk Diffusion Method for Antibiotic Susceptibility Test

2.5.1. Preparation of 0.5 McFarland Standard

A 0.5 McFarland standard was prepared by adding 0.05 ml of $BaCl_2 \cdot 2H_2O$ (1.175% w/v) to 9.95 ml of H_2SO_4 (1% v/v) with constant stirring. The absorbance of prepared 0.5 McFarland standard was measured at 625 nm to verify the correct turbidity. The McFarland standard was tightly sealed in the test tube and stored in the dark at room temperature. The McFarland standard was vigorously agitated with the vortex mixer before use [27–29].

2.5.2. Kirby-Bauer Test Procedure

Using a sterile inoculating loop, four to five isolated colonies of the organism to be tested was suspended in 2 ml of sterile saline. The saline tube was vortexed to create a smooth microbial suspension and this suspension was compared with 0.5 McFarland standard and adjusted by adding more microorganisms or adding more sterile saline until both 0.5 McFarland standard and microbial suspension had the same turbidity. The plates of Mueller-Hinton agar (MHA) were inoculated by dipping the sterile swabs into the inoculum and streaking the swabs over the surface of MHA three times while rotating the plate through a 60° angle after each application. The inoculum was left to dry for five minutes at room temperature with the lid closed [27–29].

The antibiotic discs were placed on the inoculated plates using sterile forceps and the plates were incubated at 35°C for 18 hours [27–29]. Thereafter, the zones of inhibition were measured using digital vernier calipers and the results were recorded and interpreted as per British Society for Antimicrobial Chemotherapy guidelines on Antimicrobial susceptibility testing [30] and the Clinical & Laboratory Standards Institute Standards for Antimicrobial Susceptibility Testing [31].

3. Results

3.1. Measurement of pH and Organoleptic Evaluation

The pH analysis indicates that the herbal preparations had the pH range of 3.84±0.01 to 5.86±0.02. Four (80%) of the samples had no color change after 39 days from the day of purchases while one sample (TNHP04) showed color change. All the samples were redispersible with small amount of agitation.

3.2. Heavy Metals and Non-metals Impurities

Table 2 shows the results for heavy metals and non-metals analysis. All the samples have passed the limits test for chlorides and sulphates except one sample (TNHP03) which failed the limit test for sulphates. Out of the five herbal preparations, only two (TNHP02 and TNHP05) had total heavy metals less than 20 ppm.

Table 2. *Limit tests for total heavy metals and acid radical impurities.*

Sample ID	Limit tests for acid radical impurities		Limit tests for metallic impurities
	Chlorides	Sulphates	Total Heavy metals in parts per million (ppm)
TNHP01	Pass	Pass	> 20
TNHP02	Pass	Pass	< 20
TNHP03	Pass	Fail	> 20
TNHP04	Pass	Pass	>20
TNHP05	Pass	Pass	< 20

3.3. Microbial Analysis

Table 3 shows the results for microbial enumeration tests. Three (60%) herbal preparations exceeded the total aerobic microbial count safety limits, one (20%) herbal preparation (TNHP03) was at marginal level and one (20%) herbal preparation (TNHP05) was within the safety limits (table 3 and table 4). Total coliform count was found in three samples (TNHP01, TNHP02 and TNHP04) where they exceeded the safety limit (table 3 and table 4). All the five herbal preparations exceed the safety limit for total combined yeasts/moulds count as reflected in (table 3 and table 4).

Table 3. *Microbial enumeration tests for herbal preparations.*

Sample ID	Total Aerobic Microbial Count (CFU/ml)	Total Coliform Count (CFU/ml)	Total Combined yeasts/moulds Count (CFU/ml)
TNHP01	2.7×10^6	1.72×10^6	3×10^6
TNHP02	1.49×10^6	7×10^5	3×10^6
TNHP03	4.04×10^5	Negative	6×10^8
TNHP04	3.6×10^8	9.1×10^5	4.9×10^5
TNHP05	5.6×10^4	Negative	2.66×10^6

CFU=Colony Forming Unit

Table 4. *AHPA Recommended Microbial Limits for 'Finished' Botanical Preparations.*

Organization	**EP category C**	**WHO**
Product	**Herbal medicinal products that have failed category B (CFU/ml)**	**Plant materials for internal use (CFU/ml)**
Total aerobic microbial count	10^5	10^5
Total combined yeast & mold count	10^4	10^3
Bile-tolerant Gram- negative bacteria	10^4	10^3 except *E. coli*
Escherichia coli	Absence in 1 ml	10 in 1 ml
Pseudomonas aeruginosa	Not Assigned	Absent
Staphylococcus aureus	Not Assigned	Absent

AHPA – American Herbal Preparations Association Guidance, 8630 Fenton St. #918, Silver Spring, MD 20910; 301-588-1171
EP: European Pharmacopoeia Edition 8.0, 5.1.8, 2013.
WHO – World Health Organization, Quality control methods for medicinal plant materials, Geneva, 1998

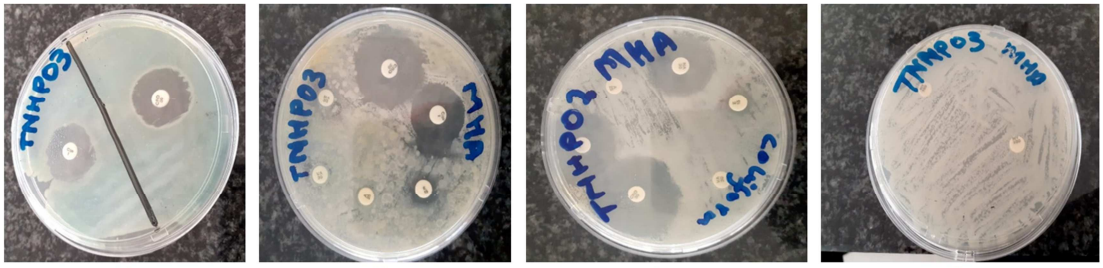
Table 5 shows the results for tests for specified microorganism in herbal preparations. As reflected in table 5, there was no growth of *Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* in all herbal preparations. In contrast, all the five herbal preparations had *Pseudomonas aeruginosa.*

Table 5. *Tests for specified microorganism in herbal preparations.*

Sample ID	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Klebsiella pneumonia***
TNHP01	Negative	Positive	Negative	Negative
TNHP02	Negative	Positive	Negative	Negative
TNHP03	Negative	Positive	Negative	Negative
TNHP04	Negative	Positive	Negative	Negative
TNHP05	Negative	Positive	Negative	Negative

3.4. Disk Diffusion Method for Antibiotic Susceptibility Test

Figure 1 shows some of the results for drug susceptibility tests of *Pseudomonas aeruginosa*, unidentified aerobic microorganisms, unidentified coliforms and unidentified yeasts/moulds. Only *Pseudomonas aeruginosa* was isolated from all the five herbal preparations. There were zones of inhibition in *Pseudomonas aeruginosa*, unidentified aerobic microorganisms and unidentified coliforms where ciprofloxacin and ceftriaxone were used. Some of the unidentified aerobic microorganisms and unidentified coliforms showed no zones of inhibition against test antibiotics as summarized in table 6. Table 7 shows the drug sensitivity pattern of microorganisms against the different antibiotics. All the five herbal preparations contained unidentified yeasts/moulds which showed no zone of inhibition on nystatin. There were also no zones of inhibition for Amoxicillin 25µg, Erythromycin 15µg, Vancomycin 30µg and Nalidixic acid 30 µg in some of the unidentified aerobic microorganisms and unidentified coliforms.



(a) (b) (c) (d)

Figure 1. *Drug susceptibility tests of Pseudomonas aeruginosa and unidentified microorganisms. (a) Observation of diameter zones of inhibition for Pseudomonas aeruginosa in TNHP03, (b) diameter zones of inhibition for unidentified aerobic microorganism in TNHP03, (c) diameter zones of inhibition for unidentified coliforms TNHP02 and (d) diameter zones of inhibition for unidentified yeast/mold count in TNHP03.*

Table 6. *Antimicrobial susceptibility of microorganisms.*

Sample ID	**Microorganism from each sample**	**Diameter of zones of inhibition (mm)**						
		A25	**E15**	**VA30**	**CIP1**	**CRO30**	**NA30**	**NY100**
TNHP01	*Pseudomonas aeruginosa*	-	-	-	30.65	32.30	-	-
	Unidentified aerobic microorganisms	Negative	10.17	Negative	32.42	29.64	Negative	-
	Unidentified coliforms	Negative	Negative	Negative	29.98	32.44	24.98	-
	Unidentified yeasts/moulds	-	-	-	-	-	-	Negative
TNHP02	*Pseudomonas aeruginosa*	-	-	-	30.30	30.18	-	-
	Unidentified aerobic microorganisms	20.21	11.83	Negative	20.59	26.36	Negative	-
	Unidentified coliforms	Negative	Negative	Negative	26.35	28.34	22.52	-
	Unidentified yeasts/moulds	-	-	-	-	-	-	Negative

Sample ID	Microorganism from each sample	Diameter of zones of inhibition (mm)						
		A25	E15	VA30	CIP1	CRO30	NA30	NY100
TNHP03	*Pseudomonas aeruginosa*	-	-	-	21.10	22.28	-	-
	Unidentified aerobic microorganisms	Negative	10.39	Negative	28.26	21.25	Negative	-
	Unidentified yeasts/moulds	-	-	-	-	-	-	Negative
TNHP04	*Pseudomonas aeruginosa*	-	-	-	31.70	27.07	-	-
	Unidentified aerobic microorganisms	Negative	Negative	Negative	34.06	31.80	26.54	-
	Unidentified coliforms	Negative	Negative	Negative	-	21.4	22.68	-
	Unidentified yeasts/moulds	-	-	-	-	-	-	Negative
TNHP05	*Pseudomonas aeruginosa*	-	-	-	27.14	30.95	-	-
	Unidentified aerobic microorganisms	28.15	19.46	34.48	23.00	25.74	Negative	-
	Unidentified yeasts/moulds	-	-	-	-	-	-	Negative

Key: CIP1=Ciprofloxacin 1µg; CRO30=Ceftriaxone 30 µg; A25=Amoxicillin 25µg; E15=Erythromycin 15µg; VA30=Vancomycin 30µg; NA30=Nalidixic acid 30 µg; NY100=Nystatin100 units
-=Test not done/Not studied

Table 7. *Drug sensitivity pattern of microorganisms against the antibiotics.*

Sample ID	Microorganism from each sample	Sensitivity test	No zones of inhibition observed*	zones of inhibition observed**
		Susceptible		
TNHP01	*Pseudomonas aeruginosa*	CIP1, CRO30	-	-
	Unidentified aerobic microorganisms	-	A25, VA30, NA30	E15, CIP1, CRO30
	Unidentified coliforms	-	A25, E15, VA30	CIP1, CRO30, NA30
	Unidentified yeasts/moulds	-	NY100	-
TNHP02	*Pseudomonas aeruginosa*	CIP1, CRO30	-	-
	Unidentified aerobic microorganisms	-	VA30, NA30	A25, E15, CIP1, CRO30
	Unidentified coliforms	-	A25, E15, VA30	CIP1, CRO30, NA30
	Unidentified yeasts/moulds	-	NY100	-
TNHP03	*Pseudomonas aeruginosa*	CIP1, CRO30	-	-
	Unidentified aerobic microorganisms	-	A25, VA30, NA30	E15, CIP1, CRO30
	Unidentified yeasts/moulds	-	NY100	-
TNHP04	*Pseudomonas aeruginosa*	CIP1, CRO30	-	-
	Unidentified aerobic microorganisms	-	A25, E15, VA30	CIP1, CRO30, NA30
	Unidentified coliforms	-	A25, E15, VA30	CRO30, NA30
	Unidentified yeasts/moulds	-	NY100	-
TNHP05	*Pseudomonas aeruginosa*	CIP1, CRO30	-	-
	Unidentified aerobic microorganisms	-	NA30	A25, E15, VA30, CIP1, CRO30, NA30
	Unidentified yeasts/moulds	-	NY100	-

Key: CIP1=Ciprofloxacin 1µg; CRO30=Ceftriaxone 30 µg; A25=Amoxicillin 25µg; E15=Erythromycin 15µg; VA30=Vancomycin 30µg; NA30=Nalidixic acid 30 µg; NY100=Nystatin100 units
*There was no diameter zone of inhibition on a particular antibiotic but since the microorganism was unidentified, results do not tell if the unidentified microorganisms were resistant or the antibiotic was not suitable for those unidentified microorganisms.
**There was diameter zone of inhibition on a particular antibiotic but since the microorganism was unidentified, the results to do not tell if those microorganisms are susceptible, intermediate or resistant to antibiotic
-=Test not done/Not studied

4. Discussion

The pH analysis indicates that three samples were within the required pH range for oral medicines of 5 to 8 [7]. One sample showed color change after 39 days of purchases which is an evidence of physical instability. A color change in oral suspension may indicate chemical degradation or microbial contamination [32]. All the suspensions were redispersible with small amount of agitation and this ensures uniform concentration of solutes upon administration.

The results obtained elucidate that three herbal preparations had higher content of heavy metals. The general limit for heavy metals in the United States Pharmacopeia (USP) and European Pharmacopeia (EP) is 10 ppm or 20 ppm [33, 34]. Metals that respond to limit test for heavy metals are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum [19]**.** However, the limit test is not sensitive enough to detect low concentrations of heavy metals and does not reveal which heavy metal is above the recommended limit. Therefore, these herbal preparations can potentially cause heavy metal toxicity to consumers [1, 8]. Although two herbal preparations were found to be within permissible limits for total heavy metals, the safety of these herbal preparations cannot be guaranteed as permissible daily exposures for the heavy metals could be exceeded because the consumption of the heavy metals is directly proportional to the dose of the herbal preparations [8].

The results show that all the five herbal preparations comply with requirements for limit test for chlorides [20, 21] as all the samples were less opalescent than the prepared sodium chloride standard solution. One herbal preparation was more turbid than the prepared standard potassium sulphate solution thus indicating that it did not pass the limit test for sulphates [19, 22]. Chlorides and sulphates are acid radical impurities and generally arise from the use of tap water

in manufacturing processes [20]. Chlorides could get into surface water from several sources including; rocks as they contain chlorides, agricultural run-off, waste water from industries, oil well wastes, and effluent waste water from waste water treatment plants [35]. Sulphates find way into water possibly due to natural sources as well as anthropogenic sources [36]. The presence of impurities such as chlorides and sulphates affect the efficacy and safety of herbal products.

Microbial analyses revealed that total coliform count was beyond limits in three samples. Coliforms indicate faecal contamination [13] which means the herbal preparations may have been directly or indirectly contaminated by human or animal faecal matter [38]. The detection of coliforms may also indicate the presence of pathogenic bacteria such as *Salmonella* spp [5, 39]. Coliforms are the major cause of waterborne and foodborne diseases which may cause intestinal tract infections to consumers [13, 37]. The herbal preparations which contain coliforms are therefore not suitable for human consumption [38].

The detection of pathogenic gram negative bacteria, *P. aeruginosa,* in all the five samples was of concern. *P. aeruginosa* is expected to be absent in herbal preparations [5] and its presence indicates health risk associated with the use of these products. *P. aeruginosa* was also detected in one study that evaluated microbial contaminants of herbal preparations [38]. The presence of both total aerobic microbial count beyond limits and *P. aeruginosa* could indicate poor hygiene conditions in the preparation or storage of these herbal preparations [5, 13]. The fungi that exceed WHO recommended limits suggested that the herbal preparations might also contained mycotoxins [15], which when ingested may cause illness or human death [40].

The microbial contaminants in these herbal preparations were probably caused by unsafe collection, transportation, drying, preparation, improper cleaning procedures or storage [13]. Other sources of contamination could be the use of unsterile water, handling of preparations with contaminated hands or using contaminated packaging materials [5, 14]. Some herbalists sell their preparations by the road side thus exposure to dust might be the possible source of fungal contaminations [5, 15]. One or some of these sources of herbal preparation contaminations could have affected the microbial quality of the final finished product. This study is in agreement with previous studies [1, 11] in that both studies did not detect *Klebsiella pneumonia, Escherichia coli* and *Staphylococcus aureus* in herbal preparations.

Antibiotic susceptibility studies on the herbal preparations indicated that the isolated *P. aeruginosa* was susceptible to ciprofloxacin and ceftriaxone. However, the herbal preparations contained unidentified yeast/moulds, unidentified aerobic bacteria and unidentified coliforms upon which there were no zones of inhibition to some antibiotics tested. Although there were no zones of inhibition on amoxicillin, erythromycin, nalidaxic acid, nystatin and vancomycin, it could not be concluded as to whether the unidentified microorganisms were resistant or the antibiotic was not suitable for those unidentified microorganisms. On the other hand, no zone of inhibition on amoxicillin, erythromycin, nalidaxic acid and vancomycin may suggest emerging multidrug resistance. One study recovered multidrug resistant bacteria from herbal preparations in southern Nigerian [41]. The study also showed that there was diameter zone of inhibition on some antibiotics but since the microorganism was unidentified, the results could not tell whether those microorganisms are susceptible or intermediate or resistant to antibiotics tested. The herbal preparations in this study are commercially sold to children less than five years, healthy and sick people including cancer, HIV/AIDS and patients on immunosuppressants hence the use of contaminated herbal preparations can cause serious health hazard.

5. Recommendations

The future studies should focus on quantitative analysis of heavy metals in herbal preparation so as to determine daily intake limit of heavy metals. Again, studies to isolate and characterize microorganisms from herbal preparations and determine their resistance patterns to antibiotics are recommended. The manufacturers are advised to adhere to WHO guidelines on good manufacturing practices (GMP) for herbal medicines or subject their finished herbal products to standardization of herbal medicines so as to maintain correct quality, safety and efficacy of the final herbal preparations.

6. Conclusion

This study concludes and reports that herbal preparations sold in Maseru could be contaminated with microorganisms, some of which are pathogenic. The study also showed that the herbal preparations could contain acid radicals and heavy metal impurities, or maybe of pH that is outside the required pH range. Based on the results, there is a need to enforce standardization of herbal preparations before consumption. The manufacture of herbal preparations for commercial use should be monitored from beginning to final products to ensure that they are produced in accordance to WHO guidelines on good manufacturing practices for herbal medicines and thus reduce the risks to consumers. Therefore, testing of herbal preparations for microbial and heavy metal contaminants is highly recommended and, may become mandatory.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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