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Evaluation of microbial bioburden of some Oral Liquid Herbal Medicines (*Agbo*) vended in Ilorin, Kwara State, North-Central Nigeria

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ABSTRACT: Frequent use of herbal medicinal products for the treatment of different ailments is common in Nigeria. Herbal preparations produced and vended in the Ilorin metropolis require an urgent microbiological evaluation for the quality and safety assurance of their products. This study aims to determine the microbiological quality of oral liquid herbal medicines vended in the Ilorin metropolis, Kwara State, North-Central Nigeria. A total of 102 oral liquid herbal medicines were collected from 34 vendors randomly selected in the Ilorin metropolis and evaluated for microbiological quality. Eighty-eight samples (86.27%) had a total fungal count in the range of 1.0×10^2 to 4.1×10^3 cfu/ml, twenty-eight (27.45%) had a total bacterial count in the range of 1.2×10^3 to 8.1×10^4 cfu/ml. *Scedosporium* species colonies were fast growing, conidia were cylindrical to slightly flask-shaped and measured about 5 – 14 × 2 – 5 µm. *Candida albicans* and *Candida krusei* were both round and cream. Indole, methyl red, mortality and catalase tests were positive which indicates probable bacteria *Escherichia coli*. The fungal isolates were most sensitive to fluconazole and ketoconazole and had no susceptibility to griseofulvin. For bacteria, the isolates were most susceptible to gentamycin and ciprofloxacin followed by ciprofloxacin. The least active antibiotics on the bacterial isolates were streptomycin and amoxicillin/clavulanic acid. This study showed that the microbial bioburden of these herbal products is high compared to WHO standards and could pose a great health challenge to the community. It is recommended that vendors should adhere to good hygiene and manufacturing practices in the production of their herbal medicine for the safety of consumers in Ilorin metropolis.

Keywords: Bacterial count, fungal count, herbal medicines, microbial bioburden, microbiological evaluation.

INTRODUCTION

Herbal medicines are plant products or plant-derived substrates which are commonly used to treat different diseases causing by either bacteria, fungi, parasites or viruses (Kira *et al*., 2021). World Health Organization (WHO, 1998) has describes herbal medicinal product also known as phytomedicine which refers to the use of potential medicinal products such as plants, barks, leaves, seed, flowers, roots or berries, herbal preparation and herbal substrates for the prevention or treatment of diseases (Tilburt and Kaptchuk, 2008). Recently, in Nigeria, there has been a notable increase in usage of herbal products for the treatment or prevention of different diseases (Oladosu *et al*., 2020). Pathogenic bacteria such as *Proteus* species, *Escherichia coli*, *Mycobacterium* species, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Campylobacter* species, *Bacillus* species, *Clostridium* species and some fungi commonly isolated from herbal medicinal products may cause serious health risk to consumers (Ngari *et al*., 2013; Braide *et al*., 2013). Poor personal hygiene and manufacturing practices and exposition of medicinal products in contaminated or polluted environment during processing, storage and marketing strategies may be sources of microbial contamination. Herbal medicinal products use has

remarkably increased in both developing and developed countries and in high demand for health care purposes (Turkson *et al*., 2020). The European Medicine Evaluation Agency (EMEA) defines herbal products as medicinal substance containing exclusively herbal drug products or herbal medicine as an active ingredient (Abel and Busia, 2005). Any isolated substrates or combination with chemically defined active substances is not considered as herbal medicinal products (Ampofo *et al*., 2012). Without compromising patient's safety, frequent use of herbal products has called for the evaluation and assurance of quality and safety of herbal products. This is likely to promote sustainability of the products and ensure their continued utilization in rural communities. However, there is paucity of research to guarantee the safety of herbal medicinal products in Nigeria. Most of the herbal preparations are not subject to standard methods during various stages of preparation, packaging, storage and transportation as demanded by regulatory norms of National Agency for Food and Drug Administration and Control (NAFDAC) which target at achieving high standard of quality of food and drugs including herbal medicinal products in Nigeria (Chitrarekha *et al*., 2010; Kigen *et al*., 2013). Some vendors of herbal products in Nigeria have limited knowledge to perform quality control on their herbal medicinal products. Many people in the country rely mostly on the use of herbal medicine for the treatment of diseases (Oladosu *et al*., 2020). Therefore, herbal medicinal products must be free from microbial contaminants, must be of good quality and must meets approved manufacturing and WHO standard (WHO, 2003). It is important for health care workers and vendors of herbal medicines to have adequate knowledge about the microbiological safety of the herbal medicinal products.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Ilorin metropolis from July 2021 to July 2022. Ilorin is the capital of Kwara State in Nigeria, West Africa. Ilorin is located on the globe which lies between latitude 8°30`N and longitude 4°33`E. Ilorin has a tropical savannah climate and estimated as 777,667 in population (National Population Commission of Nigeria, 2011). Ilorin's central location with an area of 765 Km^2 and density of 1,188/Km^2 makes it easily accessible from all parts of the country. The inhabitants usually receive health care services from public government owned health care facilities, private owned health care facilities, non-governmental based organization facilities and herbal medicine vendors. One hundred and two samples were obtained randomly from 34 vendors in the purposively selected areas in Ilorin metropolis based on the number of herbal medicine product vendors established during preliminary visits. The vendors were blinded on the actual purposes for buying their medicinal products in order to facilitate cooperation and avoid bias.

Study design

A cross-sectional descriptive approach was used where herbal medicinal products were obtained from vendors including the information regarding water used for the preparation, handling practices, state of herbal medicinal products obtained using a pre-validated checklist and packaging. Valid information and demography regarding the training of the vendors on the herbal medicinal products safety were also obtained.

Sample collection

Approximately 500 ml of each medicinal product was collected aseptically into sterile plastic bottles. All samples were labelled appropriately stored in a cool box with ice packs and transported to the laboratory for microbiological analyses on the collection day.

Microbial analysis

In the laboratory, 10 ml of herbal medicinal samples were serially diluted for up to 10 dilutions. The dilutions were plated out in duplicate on different media plates to suit the type of organism being counted. Microbial quality was evaluated by standard plate count method as described by Okunlola *et al*. (2007). Total fungal count, total bacterial count, *Salmonella* count and total coliform count were done. Saboraud dextrose agar was used for total fungal count, Nutrient agar was used to count for total bacteria, Xylose Lysine Deoxycholate agar was used for *Salmonellae* count, MacConkey agar was used for total coliform count and Eosine Methylene Blue agar was used for fecal indicator *E. coli* count. For fungal count, the plates were incubated at 27°C for up to 72 hours and plates for bacterial were incubated at 37°C for 24 hours.

Isolation and Identification of fungal Isolates

Pure culture of the fungal isolates was obtained and identified based on moulds and yeasts characterization. Moulds were identified using macroscopic characterization, slide culture, wet mount and microscopic characterization using atlas of clinical fungi (de Hoog and Guarro, 1995). The yeasts were identified using wet mount, direct mount, sugar assimilation, urease test and germ tube test.

Isolation and Identification of bacterial Isolates

Pure cultures of bacterial isolates were obtained, identified and characterized based on cellular morphology, colonial morphology, biochemical characteristics such as sugar fermentation, indole, motility, methyl red, spore stain, Voges-Proskeur, oxidase, citrate utilization, coagulase, catalase and urease tests.

Antibiotic susceptibility test

Fungi isolated from the herbal formulations were screened for their susceptibility to the selected antibiotics, according to clinical and laboratory standard institute (2002a) methods. Pure cultures of the fungal isolates were emulsified in peptone water and the suspensions were matched against McFarland standard. About 0.2 ml of the standardized suspension was inoculated on the agar plate by spreading on the agar plate, after which wells of 7 mm was dug on the agar plates. 0.2 ml of different concentrations of antifungal dilution was placed into each well according to clinical and laboratory standard institute, 2006 techniques. The antifungal drugs used were fluconazole, clotrimazoles, nystatin, ketoconazole and griseofulvin. The plates were incubated at 27°C for 120 hours and the zones of inhibitions measured and recorded accordingly. Antimicrobial sensitivity test of bacteria was one using the disc diffusion method on Mueller Hinton Agar. Pure cultures of the bacteria isolates were emulsified in 2 mL of peptone water and the turbidity of the suspension was matched against McFarland standard according to the clinical and laboratory standard institute, 2002b methods. 0.2 ml of the suspension was spread on the agar plate. Commercial antibiotic discs were aseptically placed on the inoculated plates and incubated at 35°C for 24 hours. The zones of inhibition were measured and recorded. The antibiotics disc used were rifampicin, amoxicillin, ofloxacin, streptomycin, ciprofloxacin, sulfamethozole/trimethoprim, ampicillin/ cloxacillin, chloramphenicol, erythromycin, ciprofloxacin, cefuroxime, sparfloxacin, amoxicillin/clavulanic acid, and gentamicin. Agar well diffusion technique on Mueller Hinton Agar was employed for antibiotic sensitivity of fungi.

RESULTS

A total of 102 oral liquid herbal medicines were collected from 34 vendors randomly selected within Ilorin metropolis. Information about socio-demographic characteristics, therapeutic claims, preservative additives, mode of preparation, and uses were obtained from vendors using a concise interview. And their socio-demographic characteristics were as indicated in Table 1. Unhygienic processing, handling practices and low level of knowledge on safety of the herbal medicines was observed across the vendors. The microbial bioburden testing shows that majority of the oral liquid herbal products were grossly contaminated by fungal and bacterial contaminations. In Table 2, the analysis of the findings shows that eight samples (7.84%) of the oral liquid herbal medicines were free from microbial contamination. Fourteen samples (13.73%) had no fungal growth. Twenty samples (19.61%) had no bacterial growth. All samples had no *Salmonellae* growth. Twenty-six samples (25.49%) had no coliforms. Forty-three samples (42.17%) had no *E. coli* growth. Eighty-eight samples (86.27%) had total fungal count in the range of 1.0×10^2 to 4.1×10^3 cfu/ml, these complied with the WHO limits for microbial loads (WHO Guideline, 2007). Twenty-eight samples (27.45%) had total bacterial count in the range of 1.2×10^3 to 8.1×10^4 cfu/ml, these complied with the WHO standard for bacterial loads. Fifty-four samples (52.94%) had total bacterial count in the range of 3.2×10^5 to 7.8×10^8 cfu/ml which does not comply with the WHO limits for bacterial loads. *Phialophora* species, *Penicillium* species, *Scedosporium* species, *Aspergillus* species, *Candida species*, *Staphylococcus* species, *Escherichia coli*, *Providencia* species, *Bacillus* species, *Enterobacter* species and *Acinetobacter* species were the fungal and bacterial species isolated from the oral liquid herbal medicines. Table 3 is the microscopic and colonial characteristics of mold species. The result shows that *Phialophora* species colonies were slow growing, conidia were cylindrical to sausage-shaped and measured about 2.5 – 5.8 × 1 – 2 µm. *Scedosporium* species colonies were fast growing, conidia were cylindrical to slightly flask-shaped and measured about 5 – 14 × 2 – 5 µm. Biochemical and microscopic characteristics of yeasts isolated results as shows in Table 4 revealed that *Candida* species were both round and cream. Germ tube, mannitol, glucose and maltose tests were positive and that indicates probable yeast *Candida* species. Urease and glucose tests were positive and that indicates probable yeast *Candida* species. Table 5 is the biochemical and morphological characteristics of bacteria species isolated from the herbal products. The results show that indole, methyl red, mortality and catalase tests were positive and that indicates probable bacteria *Escherichia coli*. Indole, methyl red, urease, mortality, citrate and catalase were positive and that indicates probable bacteria *Providencia* species. Gram reaction, methyl red, mortality, citrate and catalase were positive and that indicates probable bacteria *Bacillus* species. Gram reaction, catalase and coagulase tests were positive and that indicates probable bacteria *Staphylococcus* species. For the fungal isolates in Table 6 shows that the fungal isolates were most sensitive to fluconazole and ketoconazole and had no effect to griseofulvin. *Phialophora* species was sensitive to fluconazole, clotrimazole, nystatin and ketoconazole. *Penicillium* species was sensitive to fluconazole, nystatin

Table 1. Socio-demographic characteristics of vendors of oral liquid herbal products.

Characteristics Evaluated	Category	Number of respondents (%)
Gender	Male	4 (11.8)
	Female	30 (88.2)
Age	20 – 29	5 (14.7)
	30 – 39	14 (41.2)
	40 – 49	7 (20.5)
	50 – 59	4 (11.8)
	60 – 69	2 (5.9)
	70 Above	2 (5.9)
Level of Education	No Formal Education	18 (52.9)
	Primary Education	6 (17.7)
	Secondary Education	6 (17.7)
	Tertiary Education	4 (11.8)
Attending Training on Safety of Herbal Medicine	Yes	4 (11.8)
	No	30 (88.2)

Table 2. Microbial count of oral liquid herbal medicines (cfu/ml).

S/N	Product code	Fungi	Bacteria	*Salmonellae*	Coliforms	*E. coli*
1	A1	2.4×10^3	5.2×10^7	-	2.2×10^4	2.2×10^3
2	A2	2.2×10^4	2.0×10^6	-	-	1.1×10^3
3	A3	2.0×10^2	3.1×10^5	-	1.3×10^4	2.1×10^2
4	B1	2.0×10^2	3.1×10^4	-	6.6×10^4	-
5	B2	-	8.6×10^6	-	2.1×10^4	2.2×10^3
6	B3	1.6×10^3	6.0×10^5	-	-	-
7	C1	1.7×10^2	2.1×10^3	-	1.7×10^4	-
8	C2	2.0×10^2	1.1×10^4	-	4.2×10^3	-
9	C3	1.1×10^3	4.8×10^7	-	1.2×10^5	1.1×10^4
10	D1	-	-	-	-	-
11	D2	2.0×10^3	3.2×10^5	-	2.2×10^3	1.2×10^3
12	D3	2.0×10^4	4.0×10^6	-	-	-
13	E1	1.0×10^4	-	-	3.3×10^4	-
14	E2	4.0×10^3	-	-	3.6×10^3	-
15	E3	3.1×10^4	-	-	-	-
16	F1	1.6×10^3	5.0×10^5	-	1.1×10^3	1.0×10^2
17	F2	1.0×10^2	3.1×10^6	-	5.7×10^4	1.2×10^2
18	F3	2.3×10^4	8.1×10^4	-	2.2×10^3	3.2×10^3
19	G1	1.1×10^3	6.8×10^6	-	-	4.1×10^2
20	G2	2.0×10^4	3.1×10^6	-	3.6×10^3	6.1×10^3
21	G3	2.3×10^4	6.2×10^3	-	3.2×10^2	4.2×10^5
22	H1	-	-	-	-	-
23	H2	2.7×10^3	4.1×10^5	-	5.3×10^2	2.1×10^3
24	H3	1.0×10^3	-	-	6.0×10^4	6.1×10^3
25	I1	1.6×10^3	7.6×10^6	-	1.1×10^5	-
26	I2	2.6×10^2	6.5×10^7	-	-	-
27	I3	1.1×10^3	4.1×10^8	-	3.7×10^2	2.0×10^2

Table 2. Contd.

28	J1	3.0×10^3	1.4×10^4	-	4.2×10^4	4.2×10^4
29	J2	-	-	-	-	-
30	J3	-	7.2×10^4	-	6.2×10^5	3.1×10^4
31	K1	2.3×10^4	1.2×10^3	-	4.2×10^3	1.1×10^2
32	K2	1.0×10^2	4.2×10^7	-	-	-
33	K3	1.3×10^4	6.1×10^6	-	5.3×10^3	-
34	L1	2.0×10^4	7.3×10^8	-	3.4×10^2	6.1×10^2
35	L2	2.1×10^3	2.6×10^5	-	6.1×10^4	3.4×10^2
36	L3	-	-	-	-	-
37	M1	2.2×10^2	4.1×10^4	-	3.7×10^2	1.1×10^3
38	M2	1.3×10^3	8.2×10^5	-	2.1×10^6	6.2×10^5
39	M3	2.1×10^3	7.8×10^8	-	1.2×10^2	-
40	N1	1.0×10^4	4.1×10^4	-	-	-
41	N2	1.4×10^4	3.2×10^3	-	5.2×10^3	1.2×10^2
42	N3	2.0×10^2	4.6×10^3	-	-	2.1×10^2
43	O1	1.0×10^3	5.1×10^4	-	2.3×10^3	4.1×10^5
44	O2	2.1×10^3	-	-	5.6×10^3	2.1×10^3
45	O3	4.1×10^3	-	-	2.2×10^3	-
46	P1	1.0×10^2	6.1×10^3	-	4.1×10^3	-
47	P2	2.7×10^4	1.1×10^4	-	1.2×10^3	-
48	P3	2.1×10^3	6.1×10^4	-	-	4.2×10^2
49	Q1	3.1×10^2	4.0×10^7	-	1.2×10^5	4.1×10^2
50	Q2	2.2×10^4	3.1×10^5	-	5.6×10^4	6.1×10^2
51	Q3	1.0×10^4	3.0×10^5	-	-	2.2×10^3
52	R1	1.0×10^3	6.0×10^6	-	4.1×10^4	4.1×10^4
53	R2	2.0×10^4	-	-	4.3×10^3	-
54	R3	4.1×10^3	5.1×10^4	-	3.1×10^4	-
55	S1	2.1×10^4	3.1×10^6	-	4.1×10^4	1.2×10^3
56	S2	1.1×10^2	8.0×10^5	-	-	6.0×10^3
57	S3	2.0×10^4	3.3×10^6	-	5.0×10^4	4.2×10^2
58	T1	-	-	-	-	-
59	T2	-	6.0×10^6	-	4.2×10^3	-
60	T3	-	3.0×10^6	-	5.6×10^4	-
61	U1	1.3×10^2	7.2×10^3	-	3.0×10^2	4.2×10^5
62	U2	1.0×10^2	2.4×10^3	-	2.2×10^3	3.0×10^3
63	U3	1.7×10^3	3.1×10^4	-	-	3.1×10^3
64	V1	1.1×10^4	3.7×10^2	-	2.0×10^4	3.1×10^4
65	V2	-	-	-	-	-
66	V3	2.1×10^3	6.6×10^3	-	4.3×10^2	-
67	W1	2.1×10^3	6.1×10^8	-	3.0×10^2	-
68	W2	3.1×10^3	1.1×10^5	-	-	1.2×10^3
69	W3	2.5×10^4	4.8×10^6	-	3.0×10^4	3.5×10^4
70	X1	2.0×10^2	7.0×10^4	-	4.2×10^3	1.1×10^4
71	X2	1.3×10^4	-	-	4.0×10^3	-
72	X3	-	3.2×10^7	-	4.3×10^4	-
73	Y1	2.3×10^4	6.3×10^5	-	4.3×10^3	-
74	Y2	2.1×10^4	7.1×10^8	-	-	3.1×10^2
75	Y3	1.1×10^2	2.0×10^5	-	3.1×10^4	6.4×10^4
76	Z1	2.1×10^3	4.2×10^6	-	1.0×10^5	1.0×10^2
77	Z2	3.2×10^2	4.0×10^5	-	4.7×10^2	4.1×10^4

Table 2. Contd.

78	Z3	1.0×10^4	-	-	2.0×10^3	-
79	AA1	1.1×10^4	7.2×10^6	-	2.2×10^2	-
80	AA2	2.0×10^3	8.1×10^3	-	-	3.1×10^3
81	AA3	1.0×10^4	3.2×10^5	-	2.2×10^4	4.2×10^5
82	BB1	1.0×10^3	4.0×10^6	-	5.1×10^3	1.0×10^5
83	BB2	2.0×10^4	3.1×10^6	-	1.3×10^4	2.1×10^3
84	BB3	-	-	-	-	-
85	CC1	2.1×10^4	3.6×10^6	-	2.1×10^4	2.1×10^2
86	CC2	1.1×10^2	5.0×10^5	-	5.1×10^4	1.0×10^2
87	CC3	2.0×10^4	3.1×10^6	-	1.7×10^4	-
88	DD1	2.1×10^2	-	-	4.2×10^3	-
89	DD2	2.1×10^3	6.8×10^6	-	1.2×10^5	1.5×10^3
90	DD3	2.1×10^4	3.1×10^6	-	6.6×10^3	3.1×10^4
91	EE1	-	-	-	-	-
92	EE2	1.0×10^2	2.5×10^3	-	6.1×10^4	4.1×10^3
93	EE3	1.7×10^3	4.1×10^5	-	3.3×10^4	-
94	FF1	1.1×10^4	3.9×10^2	-	3.6×10^3	-
95	FF2	3.6×10^3	-	-	5.1×10^3	3.4×10^2
96	FF3	2.1×10^3	6.5×10^7	-	-	2.0×10^3
97	GG1	2.1×10^3	4.1×10^8	-	5.7×10^4	1.1×10^3
98	GG2	-	1.4×10^4	-	2.2×10^3	-
99	GG3	2.5×10^4	6.8×10^6	-	4.2×10^4	-
100	HH1	2.0×10^2	7.2×10^4	-	-	1.1×10^5
101	HH2	1.3×10^4	-	-	3.2×10^2	-
102	HH3	1.1×10^3	4.2×10^7	-	5.2×10^3	2.1×10^2

Table 3. Microscopic and colonial features of mold from the oral liquid herbal medicines.

Microscopic view	Colony features	Probable mold
Conidia were balls, thin-walled, hyaline, cylindrical to sausage-shaped, measured about 2.5 – 5.8 × 1 – 2 µm. Hyphae hyaline were brown with rough-walled. Phialides were brown, thick-walled, slender, acular to cylindrical towards the tip measured 14 – 52 µm long, small funnel-shaped collarettes.	Cultures were thriving slowly, whitish-grey to olivaceous-grey pigmentation, suede-like with radial furrows.	*Phialophora* species
Septatehypae were smooth-walled, each bearing phialides contain terminal verticils and conidia appeared rough-walled with brownish pigment.	Colonies were grayish white and downy with soluble red pigment that diffuses into the agar and making the reverse side appears red or pink.	*Penicillium* species
Conidia were cylindrical to slightly flask-shaped, smooth-walled, producing slimy heads of singled-celled, obovoid and subhyaline measured 5 – 14 × 2 – 5 µm.	Colonies were thriving quickly, and producing a light yellow diffusible pigment when incubated in few days.	*Scedosporium* species
Conidia were dark brown with rough-edged conidia propagules and brown conidiophore.	Colonies were white and becomes black quikly with conidial production. After incubation at between 25°C to 37°C, produced slightly brown colonies, and smooth-walled colonies of conidia.	*Aspergillus* species

Table 4. Biochemical and microscopic features of yeasts isolated from the oral liquid herbal medicines.

Isolates	A1	A2
Shape	Round	Round
Colour	Cream	Cream
Lactose	-	-
Germ Tube	+	-
Mannitol	+	-
Urease	-	+
Raffinose	-	-
Glucose	+	+
Maltose	+	-
Sucrose	-	-
Probable Yeast	*Candida albicans*	*Candida krusei*

Keys: - = Negative, **+** = Positive.

Table 5. Biochemical and morphological features of bacterial species isolated from the oral liquid herbal medicines.

Morphology	Coccus	Rods	Rods	Rods	Rods	Rods
Glucose	0	AG	AG	AG	AG	AG
Sucrose	0	AG	-	-	A	AG
Maltose	0	A	-	-	A	A
Raffinose	0	-	-	-	A	-
Mannitol	0	-	A	-	A	-
Lactose	0	AG	A	-	A	-
Gram Reaction	+	-	-	+	-	-
Indole	0	+	+	-	-	-
Methyl Red	0	+	+	+	+	+
VogesProkeur	0	-	-	-	-	+
Urease	0	-	+	-	+	+
Mortality	0	+	+	+	-	-
Citerate	0	-	+	+	+	+
Oxidase	0	-	-	-	-	-
Catalase	+	+	+	+	-	+
Coagulase	+					
Probable Bacteria	*Staphylococcus* species	*Escherichia coli*	*Providencia* species	*Bacillus* species	*Enterobacter* species	*Acinetobacter* species

Keys: A = Acid, G = Gas = Negative, + = Positive, 0 = No Test.

Table 6. Antifungal sensitivity of the fungal species isolated from the Oral Liquid Herbal Medicine.

Yeast Isolates	Zone of inhibition produced by different antibiotics in approximate millimeter					
	***Phialophora* species**	***Penicillium* species**	***Scedosporium* species**	***Aspergillus* species**	***Candida albicans***	***Candida krusei***
Fluconazole (10µg)	25	24	20	20	25	20
Clotrimazole (5µg)	25	-	16	19	13	-
Nystatin (5µg)	15	10	12	12	-	20
Ketoconazole (10µg)	20	22	20	20	19	25
Griseofulvin (10µg)	-	-	-	-	-	-

Key: - = No Effect.

Table 7. Antibiotic sensitivity of the bacterial isolates.

Bacterial Isolates	Zone of inhibition produced by the different antibiotics in approximate millimeter					
	***Staphylococcus* species**	***Escherichia coli***	***Providencia* species**	***Bacillus* species**	***Enterobacter* species**	***Acinetobacte* species**
Rifampicin (25µg)	15	0	0	15	0	0
Amoxicillin (30µg)	0	-	20	0	15	17
Ofloxacin (30µg)	0	15	20	0	17	20
Streptomycin (30µg)	-	-	-	-	-	-
Perfloxacin (10µg)	15	-	20	11	20	17
Sulfamethozole/Trimethoprim (30µg)	20	-	-	20	-	15
Ampicillin/cloxacillin (30µg)	-	0	0	-	0	0
Chloramphenicol (30µg)	0	-	15	0	15	20
Erythromycin (10µg)	10	0	0	-	0	0
Ciprofloxacin(10µg)	28	-	20	20	20	17
Cefuroxime (20µg)	20	0	0	15	0	0
Sparfloxacin (10µg)	0	17	10	0	11	15
Amoxicillin (30µg)	-	0	0	30	0	0
Amoxicillin/Clavulanic Acid (30µg)	0	-	-	0	-	-
Gentamicin (10µg)	9	20	20	11	20	22

Keys: - = No Effect, 0 = No Test.

and ketoconazole. *Scedosporium* species was sensitive to fluconazole, clotrimazole, nystatin and ketoconazole. *Aspergillus* species was sensitive to fluconazole, clotrimazole, nystatin and ketoconazole. *Candida* species was sensitive to fluconazole, clotrimazole and ketoconazole. *Candida* species was sensitive to fluconazole, nystatin and ketoconazole. Antibiotic sensitivity tests result as show in Table 7 for bacteria reveals that the organisms were most susceptible to gentamicin and ciprofloxacin with the least active on the organisms being streptomycin and amoxicillin. *Staphylococcus* species was sensitive to sulfamethozole/ trimethoprim and ciprofloxacin. *Escherichia coli* was sensitive to gentamicin. *Providencia* species was sensitive to amoxicillin, ofloxacin, ciprofloxacin and gentamicin. *Bacillus* species was sensitive to sulfamethozole/ trimethoprim, ciprofloxacin and amoxicillin. *Enterobacter* species was sensitive to ciprofloxacin and gentamicin. *Acinetobacter* species was sensitive to ofloxacin, chloramphenicol and gentamicin.

DISCUSSION

This study evaluated high levels of microbial bioburden of some oral liquid herbal medicines (*Agbo*) vended in Ilorin metropolis, Kwara State, North-Central Nigeria. The presence of microbes in the herbal products could be due to numerous factors such as use of untreated water, harvest of the medicinal products from contaminated environment, exposition of products in a polluted domicile, use of contaminated containers, poor handling practices of the medicinal products and packaging materials. Most of the vendors were not registered by NAFDAC and hence not regulated. Most of the vendors (52.9%) had no formal education and (11.8%) attended training on safety of herbal medicine (Table 1) which is similar to the studies of Kira *et al*. (2021). This study suggests that there should be urgent need for training of vendors to safeguard consumers of these herbal medicines. The medicinal products were contaminated to varying degrees with fungi and bacteria which are consistent with previous studies on microbiological assessment carried out by Archibong *et al*. (2017). Eight of the 102 (7.84%) evaluated were free from microbial contamination. This may be due to antimicrobial agents of chemical substances such as organic extracts, oil, liquid and peptides contained by certain medicinal plants which exert typical inhibitory compounds on microbial growth and stability as reported by Tiwari *et al*. (2009). None of the samples evaluated had *Salmonella* growth. Fourteen samples (13.73%) had no fungal growth and twenty samples (19.61%) had no bacterial growth which is consistent with the findings of Idu *et al*. (2011). According to WHO Guidelines (2007) standards value, microbial limits should be accepted if the total count is less than 10^5/g or ml for total aerobic bacteria. *Escherichia coli* and *Salmonella* should totally be absent, 10^3/ml for molds and yeast and 10^3/ml for *Enterobacteria*. Eighty-eight

samples (86.27%) of fungal count and twenty-eight samples (27.45%) of bacterial count complied with the WHO standard value. This observation is consistent to the findings of Onyambu *et al*. (2013) who reported that the herbal products were contaminated beyond limits. The features of the isolates in this study are in agreement with the reports on characterization by Willey *et al*. (2008). This study found that 52.94% had total bacterial count in the range of 3.2×10^5 to 7.8×10^8 cfu/ml which does not comply with the WHO limits for bacterial loads (Table 2) and is consistent to the findings of Kira *et al*. (2021) that reported higher bacterial levels than what is acceptable by WHO standard. This implies that most of the herbal medicines vended in the Ilorin metropolis were not safe for human consumption. The presence of *Escherichia coli* in some herbal products may indicate faecal contamination due to poor handling practices and poor personal and environmental hygiene by vendors. WHO reported that no coliform organism is acceptable in any product intended for human use (WHO Guidelines, 2007). Presence of *Staphylococcus* species has been reported to occur in vended herbal medicines in various countries such as Kenya, Nigeria, Tanzania and India (Kira *et al*., 2021). *Staphylococcus aureus* is an important pathogen that caused food poisoning following ingestion of preformed heat-resistant toxins that leads to severe gastroenteritis (Kira *et al*., 2021). These fungal and bacterial isolates in vended herbal products is highly associated to poor personal and handling practices and use of unhygienic materials by vendors which may be due to the inadequate knowledge on good manufacturing practices (Rocha *et al*., 2011). All the fungal and bacterial isolates evaluated from the indigenous herbal products in this study have been observed in previous studies on transmissible diseases and gastroenteritis (Pearce *et al*., 2004). Czech *et al*. (2001) reported that *Escherichia coli* and *Enterobacteria* reflect the situation of fecal contamination, although *Enterobacteria* can be commonly found in nature and this family possesses some indicative potential towards fecal contamination. This can be noted as an indicator for unacceptable hygiene practices. Studies reported that the episodes of diarrhea infective etiology are about 27% which resulted to a high mortality rates and complications (Wylie and Norwick, 2005). This can produce proteins that can damage vital tissues and impair the immune system and release exotoxins which cause gastroenteritis (pearce *et al*., 2004). Studies reported that *Enterobacter asburiae* as an opportunistic pathogen associated with diarrhea in children in nosocomial and extra-intestinal infections (Rajapandiyan *et al*., 2013). Therefore, the high recovery rates of these pathogens from indigenous herbal medicines could be of clinical important. Kunle *et al*. (2012) revealed that *Providencia* species are coliforms that cause urinary tract and eye infections in humans. They are commonly found in human gut and known to cause a number of opportunistic infections in human. Currently, it has emerged as nosocomial pathogen of clinical relevance and common cause of traveler's diarrhea. *Providencia* species isolates in this study is consistent with other findings reported by Kunle *et al*. (2012). Idu *et al*. (2011) reported that *Acinetobacter* species is becoming increasingly relevant in nosocomial infections as opportunistic pathogen and commonly known to be multi-drug resistant. Rajapandiyan *et al*. (2013) revealed that *Bacillus subtilis* is the commonly isolated from herbal medicines. *Bacillus* species are commonly known to cause gastrointestinal infection which is categorized by diarrhea. Numerous pathogens evaluated can be directly used and the scientists have generally used indicator organisms by human pathogens as an index of possible water contamination by human pathogens. Forest (2004) revealed that the significance of fecal coliforms is that other harmful organisms will be present if these specific bacteria are present microorganisms such as *Salmomella*. *Candida*, *Penicillium* and *Aspergillus* species are of medical important. *Aspergillus* and *Penicillium* are responsible for infections especially in immune-compromised individuals and are associated with food poisoning. Molds are commonly responsible for bio-deterioration of a number of raw substrates and potentially decreases the herbal medicines efficacy (Kumar *et al*., 2009). *Penicillium* and *Aspergillus* species in herbal medicines could indicates that there was some growth of these isolates established even before the complete of the herbal preparation. In order to inhibit such growth and production of toxic metabolites, *Aspergillus* is capable of thriving at low water content. Adequate care should be observed to dehydrate the water content quickly before molds can have potential to establish any significant growth before preparation of the herbal medicines. Busse (2000) reported that most members of the genera *Aspergillus* and *Penicillium* are producing the widest range of carcinogenic and nephrotoxic mycotoxins such as aflatoxin and ochratoxin A. Idu *et al*. (2011) revealed that *Phialophora parasiticum* can cause arthritis, mycotic keratitis, subcutaneous infection, endocarditis and mycetoma. The high level of microbial bioburden evaluated in this study may be attributed to the methods used for the preparation of the herbal products as reported by Rocha *et al*. (2011) in their studies. The storage methods, handling practices, soil, drying and harvesting can influence the microbial quality of the herbal products (Okunlola *et al*., 2007). High level of microbial agents in non-sterile medicinal products can inactivate or decrease the therapeutic activity of the medicines and can leads to serious adverse effects on consumers. Antibiotic susceptibility evaluation on the bacterial and fungal contamination of the herbal medicines showed that the microorganisms were susceptible and resistant to some of the antibiotics tested. Alwakeel (2008) reported that the close uniformity of antibiotic susceptibility of the pathogens could be attributed to their environmental origins.

Escherichia coli was resistant to most antibiotics used in this study and that indicated a great threat to consumers.

Conclusion

This study evaluated the presence of microbial bioburden above the WHO acceptable limits in oral liquid herbal medicines used by residents and vended in Ilorin metropolis. These findings demonstrate important health risks for consumers associated with the use of herbal products and the urgent need for establishment of stricter control procedures and surveillance in the preparation and marketing of these herbal products to guarantee their safety and quality. These control measures will help to decrease the additional risks such as food-borne infections to the consumers. There is urgent need to extend national regulation program to herbal medicines to ensure that their processing, production, handling and packaging comply with good manufacturing practices, so as to reduce the health risks to consumers.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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