Microbial Contamination of Herbal Medicines in Africa, 2000-2024: A Systematic Review

Authors: Ahiabor, Wisdom K, Darkwah, Samuel, and Donkor, Eric S

Source: Environmental Health Insights, 18(2)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/11786302241293345

Microbial Contamination of Herbal Medicines in Africa, 2000-2024: A Systematic Review

Environmental Health Insights
Volume 18: 1–18
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11786302241293345

Wisdom K Ahiabor, Samuel Darkwah and Eric S Donkor

Department of Medical Microbiology, University of Ghana Medical School, Korle Bu, Accra, Ghana.

ABSTRACT

INTRODUCTION: Herbal medicine has been a cornerstone of healthcare for centuries, with an estimated 80% of the world's population relying on it. In Africa, herbal medicine is the backbone of rural healthcare, serving 80% to 90% of the population. Despite its widespread use, the safety of herbal medicine raises a significant concern considering the lack of regulation and testing, particularly in Africa. Microbial contamination is a primary safety risk threatening consumer health. In this systematic review, we aimed to synthesise evidence on microbial contamination in herbal medicines across Africa, provide a clear understanding of the problem, and inform effective public health interventions regarding microbial contamination of herbal medicines in Africa.

METHOD: The systematic review was conducted in accordance with the PRISMA guidelines. A literature search was conducted across PubMed, Web of Science, Science Direct, Scopus, and Google Scholar using appropriate search terms. Eligible studies were selected based on predetermined criteria, and data were extracted and analysed.

RESULTS: The review included fifty eligible studies in Africa, with a combined sample size of 1996, of which 1791 showed microbial contamination. Bacterial contaminants were reported in 98% of studies, with *Escherichia coli* (62%) being the most reported bacteria, followed by *Staphylococcus aureus* (57%), and *Bacillus* spp. (55%). Fungal contaminants were reported in 70% of studies, with *Aspergillus* spp. (40%) being the most reported, followed by *Penicillium* spp. (27%) and *Candida* spp. (26%). Parasitic contaminants were reported in 2% of the studies reviewed. A total of 70 bacterial species, 37 fungal species, and 6 parasite species were identified in this review.

CONCLUSION: Herbal medicines in Africa pose significant health threats to consumers due to the high prevalence of diverse microbial contaminants and clinically significant pathogens. This emphasises the need for stricter regulations and quality control measures in the production, sale and use of herbal medicines.

KEYWORDS: Herbal medicine, herbal medicine safety, herbal products, microbial contamination, safety assessment, Africa, medicinal plants, public health

RECEIVED: August 6, 2024. **ACCEPTED:** October 4, 2024.

TYPE: Review

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by the National Institutes of Health, USA, through the "Application of Data Science to Build Research Capacity in Zoonoses and Food-Borne Infections in West Africa (DS-ZOOFOOD) Training Programme" hosted at the Department of Medical Microbiology, University of Ghana Medical School (Grant Number: UE5TW012566-01). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Eric S Donkor, Department of Medical Microbiology, University of Ghana Medical School, P. O. Box KB 4236, Korle Bu, Accra, Ghana.
Email: esampane-donkor@ug.edu.gh

Introduction

Herbal medicine has been an integral resource for health in communities globally for centuries. It is estimated that 80% of the world's population use herbal medicines.[1-4] In Africa, herbal medicine is the backbone of rural healthcare, providing essential support to a significant number of the population (an estimated 80%-90%).[5] The patronage and use of herbal medicines have increased due to their availability, accessibility, and affordability.[6-8] They provide a practical alternative to healthcare services in the rural communities of developing nations.[9]

Considering the expanding market for herbal remedies across African countries, it is important to address all safety concerns associated with their use.[10] Several herbal products used in Africa remain untested and unregulated,[1,4,11,12] posing significant health risks to consumers. According to a survey conducted by the World Health Organisation (WHO), only 43% of African member states currently have regulations in place for herbal medicines.[4] The lack of effective regulation and monitoring make consumers vulnerable to diseases.[13] Typically, the safety risks associated with herbal medicines include contamination by microbiological agents (such as bacteria and fungi), and chemical agents (such as metals, pesticides, residual solvents, and mycotoxins).[14] Microbial agents are however, the most implicated contaminants in herbal medicines.[11,12,15] The presence of pathogenic microbial contaminants in herbal medicines has generated increased apprehension, as they can lead to the development of serious infections.[16]

Across Africa, research on the microbial safety of herbal medicines is only largely conducted within individual countries. Thus, the fragmented nature of the relevant research hinders the development of continent–wide, comprehensive herbal safety guidelines and public health policies. It is therefore important to curate evidence that reflects the extent and diversity of microbial contamination in herbal medicines throughout the African region. To the best of our knowledge, no published review specifically collates and synthesises the evidence on microbial contamination in herbal medicines across Africa. While a previous review by Opuni et al.[15] examined

various contaminants in herbal medicines across low– and middle–income countries, it did not address parasitic contaminants, limiting our understanding of the full spectrum of microbial risks associated with herbal medicines. Similarly, the review by Walusansa et al.[12] only examined bacterial contaminants in herbal medicines from East Africa, overlooking other microbial contaminants. Our systematic review aimed to provide a holistic and up–to–date analysis of the microbial contaminations associated with herbal medicines in Africa, integrating findings that have emerged since the publication of previous reviews. This study assessed original research articles that explored the presence and diversity of microbial contaminants in herbal medicines across African countries, spanning from 2000 to 2024. By examining this body of research, we sought to identify emerging trends and challenges concerning microbial contamination in herbal medicines in the 21st century. This knowledge is necessary to guide the development of relevant public health interventions and offer direction for future research on herbal medicine safety in Africa.

Method

Search strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta–Analyses (PRISMA) guidelines.[17] Literature search was conducted between June 7th and 15th, 2024, across PubMed, Web of Science, Scopus, Science Direct, and Google Scholar, to identify articles related to the microbial contamination of herbal medicines in Africa, from year 2000 to 2024. The primary search strategy incorporated both Medical Subject Headings (MeSH terms) and keywords such as 'Herbal Medicine'[Mesh] OR herbs OR 'Plant medicine' OR 'Plants, Medicinal'[Mesh] OR 'Plant Preparations'[Mesh] AND 'Microbiology'[Mesh] OR microbes OR 'Bacteria'[Mesh] OR 'Fungi'[Mesh] OR 'Viruses'[Mesh] OR 'Colony Count, Microbial'[Mesh] AND 'Africa'[Mesh]. The citations and references of the identified articles were carefully reviewed to include all relevant studies. The full electronic search strategy for all the databases used is shown in Supplemental Table 1.

Eligibility criteria

The review exclusively examined studies conducted from 2000 to 2024, which presented evidence of microbial contamination in herbal medicines across Africa. These studies were required to identify the specific microbial contaminants isolated from herbal medicines and determine the prevalence and/or load of these microbes. To ensure the reliability of the findings, only peer-reviewed articles that employed standardised laboratory methods for assessing microbial contamination and were published in English were included. Studies that investigated contaminants and adulterants other than microbes were excluded. Additionally, studies conducted outside Africa, review articles, and studies that did not specify microbial contaminants or provide sufficient information on contamination levels in herbal medicines were excluded from the review.

Study selection

The selection of studies for this review involved a three–phase screening process to retrieve articles of interest. In the initial phase, duplicates were identified and manually eliminated using the systematic review tool 'Rayyan QCRI'.[18] Two researchers then independently screened the remaining articles by reading the titles, abstracts, and keywords to identify relevant studies. Finally, the full texts of the remaining articles after the second screening phase were thoroughly reviewed to determine which studies met the inclusion criteria and were ultimately included in the review. The PRISMA flow diagram below (Figure 1) illustrates the article selection process.

Quality assessment

To evaluate the quality of the included articles, we employed the modified Oxman and Guyatt score,[19] an analytical tool adapted from previous systematic reviews on herbal medicines.[15,20,21] This tool assessed the study methodology, country of origin, and specific microbial contaminants reported, with 1 point allocated per dimension for a maximum score of 10. Two authors independently assessed and scored the articles, and discrepancies were resolved through consensus among all 3 authors. Articles with total scores ranging from 8 to 10 were considered to be of good quality, those scoring from 5 to 7 as fair, and those scoring from 0 to 4 as poor quality. The scoring system for quality appraisal and the assessment of included studies are presented in Supplemental Tables 2 and 3, respectively.

Data extraction and analysis

Two independent researchers extracted data from 50 articles that met the inclusion criteria. Each researcher entered the extracted data into spreadsheets, documenting various attributes, including author(s) name, year of publication, country, sample size, number of contaminated samples, identification methods, type of microbial contaminant, microbial loads, and specific microbial contaminants isolated. The geographical distribution of the included articles was visualised using a map, while bar charts and tables were used to visualise the distribution of study characteristics and findings. Bar graphs illustrating microbial contaminants and their occurrence levels in the included studies were created using Microsoft Excel. The data for these graphs were sourced from the individual studies included in this review.

Results

From an initial pool of 8005 search results, 50 research articles were selected for inclusion after the three–phase screening process. The quality of the selected articles ranged from fair to good based on the quality assessment parameters used.

Table 1. Characteristics of the included studies in this review.

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Hassan et al.[22]	2021	Kenya	86	72	Conventional culture method	NA	NA	Bacterial	*Escherichia coli* *Salmonella typhi* *Salmonella paratyphi* Enterobacteriaceae
Abba et al.[23]	2009	Nigeria	150	131	Conventional culture method	0 to 2.25×10^8 cfu/mL	NA	Bacterial	*Salmonella typhi* *Shigella* spp. *Escherichia coli* *Staphylococcus aureus*
Archibong et al.[24]	2017	Nigeria	60	57	Conventional culture method	Registered samples 1.2×10^3 cfu/mL to 2.1×10^6 cfu/mL Unregistered samples 3.6×10^3 cfu/mL to 2.42×10^8 cfu/mL	Registered samples 1.0×10^2 cfu/mL to 1.4×10^5 cfu/mL Unregistered samples 2.0×10^2 cfu/mL to 2.0×10^6 cfu/mL	Bacterial Fungal	*Providencia rettgeri* *Enterobacter asburiae* *Acinetobacter baumannii* *Escherichia coli* *Bacillus* spp. *Staphylococcus* spp. *Candida albicans* *Candida krusei* *Scedosporium aurantiacum* *Penicillium marneffei* *Aspergillus niger* *Phaeoacremonium parasiticum*
Brooks and Takim[25]	2014	Nigeria	28	20	Conventional culture method	Solid samples 2.05×10^4 cfu/g to 5.6×10^4 cfu/g Liquid samples 3.8×10^4 cfu/mL to 6.8×10^4 cfu/mL	NA	Bacterial Fungal	*Salmonella* spp. *Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Bacillus subtilis* *Klebsiella* spp. *Enterobacter* spp. *Aspergillus flavus* *Aspergillus niger* *Rhizopus* spp. *Mucor* spp. *Fusarium* spp. *Candida tropicalis*
Govender et al.[26]	2006	South Africa	15	15	Conventional culture method	1.2×10^3 cfu/mL or g to 1.19×10^9 cfu/mL or g	0 to 2.5×10^6 cfu/mL	Bacterial Fungal	*Bacillus* spp. *Pantoea* spp. *Rahnella aquatilis* *Acinetobacter baumannii* *Pseudomonas* spp. *Chryseomonas* spp. *Flavimonas* spp. *Stenotrophomonas maltophilia* *Ewingella americana* *Salmonella* spp. *Klebsiella pneumoniae* *Bordetella* spp. *Pasteurella pneumolytica* *Serratia* spp. *Penicillium* spp. *Mucor* spp. *Aspergillus* spp
Mautsoe et al.[27]	2021	Lesotho	5	5	Conventional culture method	5.6×10^4 cfu/mL to 3.6×10^8 cfu/mL	3.0×10^5 cfu/mL to 6.0×10^8 cfu/mL	Bacterial Fungal	*Pseudomonas aeruginosa* Coliforms Yeast and moulds

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Nwankwo and Olime[28]	2019	Nigeria	60	60	Conventional culture method	Liquid 3.10×10^2 cfu/mL to 2.56×10^3 cfu/mL Powder 9.0×10^1 cfu/g to 1.5×10^2 cfu/mL	Liquid 2.0×10^1 cfu/mL to 1.9×10^2 cfu/mL Powder 1.0×10^1 cfu/mL to 1.0×10^2 cfu/mL	Bacterial Fungal	*Bacillus* spp. *Bacillus subtilis* *Bacillus polymyxa* *Bacillus cereus* *Bacillus licheniformis* *Aspergillus* spp. *Penicillium* spp.
Odonkor et al.[29]	2011	Ghana	10	8	Conventional culture method	2.2×10^3 cfu/mL to 6.2×10^3 cfu/mL	6.2×10^3 cfu/mL	Bacterial Fungal	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Bacillus* spp. Fungi
Kalumbi et al.[30]	2020	Malawi	29	20	Conventional culture method	NA	NA	Bacterial	*Citrobacter* spp. *Bacillus* spp. Coagulase negative *Staphylococcus* *Klebsiella* spp. *Enterobacter* spp.
Walusansa et al.[31]	2022	Uganda	140	140	Conventional culture method	Liquid 0.0 to 1.42×10^7 cfu/mL Solid 1.8×10^3 cfu/g to 1.67×10^7 cfu/g	NA	Bacterial	*Klebsiella pneumoniae* *Escherichia coli* *Staphylococcus aureus* *Klebsiella oxytoca* *Bacillus cereus* *Pseudomonas aeruginosa* *Enterobacter* spp.
Ezekwesili-ofili et al.[32]	2014	Nigeria	210	210	Conventional culture method	NA	NA	Bacterial Fungal	*Escherichia coli (EPEC, EHEC)* *Bacillus* spp. *Salmonella* spp. *Enterococcus faecalis* *Pseudomonas* spp. *Klebsiella* spp. *Aeromonas* spp. Coliforms *Aspergillus flavus* *Cladosporium* spp. *Rhizopus* spp. *Penicillium* spp. *Mucor* spp. *Aspergillus niger* *Curvularia* spp. *Candida* spp. *Geotrichum* spp. *Aspergillus fumigatus*
Tatfeng et al.[33]	2010	Nigeria	6	6	Conventional culture method	0.2×10^2 cfu/mL to 4.7×10^7 cfu/mL	0.2×10^2 cfu/mL to 4.7×10^7 cfu/mL	Bacterial Fungal	*Enterococcus faecalis* *Staphylococcus aureus* *Escherichia coli* *Bacillus* spp. *Staphylococcus epidermidis* *Pseudomonas aeruginosa* *Proteus mirabilis* *Mucor* spp. *Serratia marcescens* *Aspergillus niger*

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Walther et al.[34]	2016	Tanzania	109	89	Conventional culture method	10^2 to 10^4 cfu/mL	NA	Bacterial	*Klebsiella pneumonia* *Enterobacter aerogenes*
Kaume et al.[35]	2012	Kenya	24	24	Conventional culture method	APC counts 1.5×10^1 cfu/g to 7.1×10^8 cfu/g	<10 cfu/g to 9.0×10^4 cfu/g	Bacterial Fungal	Coliforms *Escherichia coli* *Staphylococcus aureus* Yeast Mould
Van-Vuuren et al.[8]	2014	South Africa	75	75	Conventional culture method	3.03×10^4 cfu/g to 4.22×10^5 cfu/g	NA	Bacterial	*Acinetobacter baumannii* *Acinetobacter lwoffii* *Bacillus amyloliquefaciens* *Bacillus lentus* *Bacillus megaterium* *Bacillus subtilis* *Bacillus vallismortis* *Enterobacter cloacae* *Klebsiella oxytoca* *Leclercia adecarboxylata* *Pantoea* spp. *Pseudomonas oryzihabitans* *Sphingomonas paucimobilis* *Streptococcus mitis* *Staphylococcus hominis*
Igbeneghu and Lamikanra[36]	2016	Nigeria	50	49	Conventional culture method	0 to 2.94×10^{12} cfu/mL	0 to 3.54×10^{12} cfu/mL	Bacterial Fungal	*Bacillus cereus* *Citrobacter* spp. *Enterobacter* spp. *Escherichia coli* *Klebsiella* spp. *Pantoea agglomerans* *Proteus* spp. *Pseudomonas fluorescens* *Pseudomonas* spp. *Salmonella* spp. *Staphylococcus* spp.
Kanu et al.[37]	2015	Sierra Leone	20	20	Conventional culture method	30 cfu /mL to 9.37×10^9 cfu/mL	30 cfu/mL to 1.60×10^9 cfu/mL	Bacterial Fungal	*Staphylococcus aureus* *Bacillus* spp. *Escherichia coli* *Staphylococcus epidermidis* *Salmonella* spp. *Candida albicans* *Aspergillus flavus* *Aspergillus niger* *Cryptococcus neoformans* *Trichoderma harzanium* *Aspergillus nidulans*

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Darkwah et al.[10]	2022	Ghana	30	30	Conventional culture method	Coliform count 3.1×10^1 cfu/mL to 1.7×10^5 cfu/mL	NA	Bacterial Fungal	*Escherichia coli* *Pseudomonas aeruginosa* *Bacillus* spp. *Citrobacter divergens* *Citrobacter* spp. *Staphylococcus aureus* *Staphylococcus* spp. *Enterobacter* spp. *Shigella sonnei* *Moraxella catarrhalis* *Serratia marcescens* *Candida* spp.
Oladosu et al.[38]	2020	Nigeria	20	10	Conventional culture method	Liquid 7.22×10^4 cfu/mL Powder 1.35×10^4 cfu/mL to 2.53×10^4 cfu/mL	NA	Bacterial Fungal	*Bacillus subtilis* *Shigella* spp. *Klebsiella pneumoniae* *Staphylococcus aureus* *Proteus* spp. *Pseudomonas aeruginosa* *Enterococcus feacalis* *Escherichia coli* *Alterneria* spp. *Aspergillus niger* *Aspergillus flavus* *Aspergillus fumigatus* *Cladosporium cladosporioides* *Mucor* spp. *Rhizopus arrhizus*
Anie et al.[39]	2022	Nigeria	7	7	Conventional culture method	1.8×10^6 cfu/mL to 7.5×10^6 cfu/mL	NA	Bacterial Fungal	*Staphylococcus aureus* *Proteus* spp. *Pseudomonas* spp. *Streptococcus* spp. *Candida* spp. *Aspergillus niger* *Aspergillus flavus*
Ideh and Ogunkunle[40]	2019	Nigeria	12	10	Conventional culture method	1.0×10^5 cfu/mL to 1.34×10^7 cfu/mL	1.0×10^5 cfu/mL to 1.5×10^7 cfu/mL	Bacterial Fungal	*Streptococcus* spp. *Staphylococcus* spp. *Salmonella* spp. Enterobacteria Yeasts Moulds
Oshoma and Dijeh[41]	2017	Nigeria	10	10	Conventional culture method	9.5×10^3 cfu/mL to 2.9×10^4 cfu/mL	6.0×10^3 cfu/mL to 1.8×10^4 cfu/mL	Bacterial Fungal	*Bacillus licheniforms* *Bacillus subtilis* *Pseudomonas aeruginosa* *Escherichia coli* *Staphylococcus aureus* *Staphylococcus epidermidis* *Penicillium* spp. *Aspergillus flavus* *Aspergillus niger* *Rhizopus* spp. *Mucor* spp.

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Turkson et al.[42]	2020	Ghana	4	4	Conventional culture method	1.21×10^3 cfu/mL to 2.23×10^3 cfu/mL	1.01×10^3 cfu/mL to 2.43×10^3 cfu/mL	Bacterial Fungal	Aerobic bacteria Yeasts and moulds
Chinakwe et al.[43]	2023	Nigeria	30	30	Conventional culture method	1.0×10^6 cfu/mL to 7.8×10^7 cfu/mL	3.0×10^3 cfu/mL to 1.3×10^8 cfu/mL	Bacterial Fungal	*Bacillus* spp. *Corynebacterium* spp. *Micrococcus* spp. *Enterococcus* spp. *Staphylococcus* spp. *Mucor* spp. *Saccharomyces* spp. *Penicillium* spp.
Abubakar et al.[44]	2018	Nigeria	8	8	Conventional culture method	1.0×10^7 cfu/mL to 1.8×10^8 cfu/mL	NA	Bacterial	*Staphylococcus aureus* *Escherichia coli*
Osei-Adjei et al.[45]	2013	Ghana	16	16	Conventional culture method	1.0×10^2 cfu/mL to 1.0×10^9 cfu/mL	3.2×10^5 cfu/mL	Bacterial Fungal	*Bacillus subtilis* *Bacillus coagulans* *Bacillus licheniforms* *Enterobacter aerogenes* *Klebsiella oxytoca* *Serratia odorifera* *Cladosporium herbarum.* *Penicillium digitatum* *Aspergillus ustus* *Aspergillus oryzae* *Aspergillus sulphureus*, *Mycelia sterilia* *Trichosporon mucoides* *Saccharomyces kluyverii* *Rhodotorulla minuta* *Candida membranifasciens* *Sporobolomyces salmonicolor*
Ampofo et al.[46]	2012	Ghana	31	26	Conventional culture method	9.4×10 cfu/mL to 2.32×10^3 cfu/mL	NA	Bacterial Fungal	*Clostridium* spp. *Pseudomonas* spp. *Bacillus* spp. *Salmonella* spp. Faecal coliform Heterotrophic bacteria Mould
Akande et al.[47]	2013	Nigeria	15	15	Conventional culture method	1.0×10^0 cfu/mL to 9.0×10^5 cfu/mL	1.0×10^0 cfu/mL to 8.0×10^5 cfu/mL	Bacterial Fungal	*Escherichia coli* *Salmonella* spp. *Klebsiella* spp. *Moraxella* spp. *Enterococcus* spp. *Pseudomonas* spp. *Bacillus* spp. *Staphylococcus* spp. *Streptococcus pneumoniae* *Alternaria* spp. *Rhizopus* spp. *Fusarium* spp. *Penicillium* spp. *Mucor* spp. *Candida* spp.

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Famewo et al.[48]	2016	South Africa	9	9	Molecular technique	NA	NA	Bacterial	*Raoultella ornithinolytica* *Rahnella aquatilis*, *Bacillus anthracis* *Bacillus cereus* *Salmonella enteric* *Enterobacter cloacae* *Klebsiella oxytoca* *Klebsiella pneumonia* *Enterobacter asburiae*, *Paenibacillus polymyxa*, *Pantoea rwandensis*, *Klebsiella variicola* *Pseudomonas* spp.
Sebiawu et al.[49]	2020	Ghana	15	12	Conventional culture method	$1.0 \pm 0.02 \times 10^1$ cfu/mLto $2.3 \pm 0.30 \times 10^6$ cfu/mL	NA	Bacterial	*Staphylococcus aureus* *Salmonella* spp. Coliforms
Ayansina and Akinsola[50]	2020	Nigeria	21	21	Conventional culture and molecular techniques	NA	NA	Bacterial Fungal	*Providencia* spp. *Pantoea* spp. *Citrobacter* spp. *Serratia* spp. *Proteus* spp. *Klebsiella* spp. *Kluyvera* spp. *Enterobacter* spp. *Brenneria* spp. *Escherichia coli* *Edwardsiella* spp. *Salmonella* spp. *Cedecea* spp. *Pseudomonas* spp. *Yersinia* spp. *Aspergillus niger* *Mucor* spp. *Rhizopus stolonifera* *Candida stolonifera* *Aspergillus nidulans*
Ngemenya et al.[51]	2019	Cameroon	8	8	Conventional culture method	NA	NA	Bacterial	*Citrobacter freundii* *Citrobacter youngae* *Citrobacter* spp. *Enterobacter cloacae* *Escherichia coli* *Proteus mirabilis* *Proteus vulgaris* *Providencia rettgeri* *Salmonella typhi* *Salmonella* spp.
Odo et al.[52]	2023	Nigeria	8	8	Conventional culture method	1.8×10^3 cfu/mL to 9.3×10^3 cfu/mL	1.3×10^3 cfu/mL to 2.5×10^3 cfu/mL	Bacterial Fungal	*Bacillus* spp. *Escherichia coli* *Staphylococcus aureus* *Enterobacter* spp. *Aspergillus niger* *Penicillium* spp. *Scedosporium* spp. *Phialophora parasiticum*

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Idu et al.[53]	2010	Nigeria	17	17	Conventional culture method	1.3×10^5 cfu/g to 6.7×10^6 cfu/g	0 to 7.1×10^6 cfu/g	Bacterial Fungal	*Citrobacter* spp. *Klebsiella aerogenes* *Bacillus subtilis* *Diphtheroids* *Arizona* spp. *Staphylococcus epidermidis* *Serratia marcescens* *Pseudomonas aeruginosa* *Escherichia coli* *Proteus* spp. *Acinetobacter* spp. *Staphylococcus aureus* *Streptococcus* spp. *Aspergillus fumigatus* *Absidia* spp. *Mucor* spp. *Penicillium* spp. *Aspergillus niger* *Aspergillus ochraceus* *Saccharomyces cerevisiae* *Rhizopus nigricans*
Omoruyi et al.[54]	2023	Nigeria	50	20	Conventional culture method	2.8×10^4 cfu/mL to 12.6×10^8 cfu/mL	NA	Bacterial	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Proteus mirabilis* *Enterobacter* spp. *Citrobacter* spp.
Bello et al.[55]	2019	Nigeria	10	8	Conventional culture method	2.5×10^2 cfu/mL to 4.4×10^6 cfu/mL	NA	Bacterial	*Bacillus subtilis* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Staphylococcus aureus* *Enterobacter cloacae* *Enterobacter gergoviae* *Serratia marcescens*
Ngari et al.[56]	2013	Kenya	22	3	Conventional culture method	3.0×10^3 cfu/mL to 2.6×10^6 cfu/mL	NA	Bacterial Fungal	*Escherichia coli* *Pseudomonas aeruginosa* *Salmonella typhi* *Candida albicans*
Adounkpe et al.[57]	2017	Benin	13	13	Conventional culture method	$9.15 \pm 2.32 \times 10^7$ cfu/mL to $3.65 \pm 0.87 \times 10^9$ cfu/mL	$1.6 \pm 0.5 \times 10^5$ cfu/mL to $3.5 \pm 1.1 \times 10^6$ cfu/mL	Bacterial Fungal	Total coliforms *Escherichia coli* *Staphylococcus aureus* *Salmonella typhi* *Aspergillus flavus* *Aspergillus niger* *Penicillium expansum* *Fusarium solani*
Bernadin et al.[58]	2018	Cote d'Ivoire	188	188	Conventional culture method	1.0×10^3 cfu/g to 4×10^8 cfu/g	2.0×10^4 cfu/g to 4.4×10^7 cfu/g	Bacterial Fungal	Aerobic mesophilic bacteria Thermotolerant coliforms *Escherichia coli* *Staphylococcus aureus* *Enterococci* spp. *Pseudomonas* spp. Yeasts and moulds

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Osei-Asare et al.[59]	2023	Ghana	15	10	Conventional culture method	Less than 1.0×10 to TNC	Less than 1.0×10 to TNC	Bacterial Fungal	*Escherichia coli* *Staphylococcus aureus* *Salmonella typhi* Fungi
Usanga et al.[69]	2023	Nigeria	50	50	Conventional culture method	NA	NA	Fungal	*Aspergillus niger* *Aspergillus flavus*
Bashir et al.[60]	2017	Nigeria	12	12	Conventional culture method	3.1×10^5 cfu/mL to 1.85×10^6 cfu/mL	3.1×10^5 cfu/mL to 1.85×10^6 cfu/mL	Bacterial Fungal	*Staphylococcus aureus* *Bacillus* spp. *Escherichia coli* *Salmonella typhi* *Aspergillus* spp. *Penicillium* spp.
Onyambu et al.[61]	2013	Kenya	30	30	Conventional culture method	6.0×10^5 cfu/mL to 1.50×10^{10} cfu/mL	5.0×10^5 cfu/mL to 1.56×10^9 cfu/mL	Bacterial Fungal	*Klebsiella pneumoniae* *Klebsiella oxytoca* *Enterobacter cloacae* *Bacillus flexus* *Bacillus safensis* *Bacillus subtilis* *Bacillus pumilus* *Staphylococcus aureus* *Escherichia coli* *Salmonella* spp. *Enterobacter aerogenes* *Chryseomonas luteola* *Shigella* spp. *Flavobacterium* spp. *Enterobacter agglomerurans* *Serratia marcescens* *Kocuria rosea* *Rhizobium* spp. *Pseudomonas aeruginosa* *Aspergillus* spp. *Fusarium* spp. *Candida* spp. *Penicillium* spp. *Torula* spp. *Rhizopus* spp.
Kira et al.[62]	2021	Tanzania	50	44	Conventional culture method	9.09×10^4 to 1.64×10^8 cfu/g per mL	NA	Bacterial	*Staphylococcus aureus* *Escherichia coli* *Enterobacter* spp. *Bacillus* spp. *Staphylococcus epidermidis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa*
Dabo et al.[63]	2024	Nigeria	30	30	Conventional culture method	NA	NA	Bacterial Fungal	*Salmonella* spp. *Escherichia coli* *Klebsiella* spp. *Proteus* spp. *Staphylococcus* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus ochraceus* *Rhizopus stolonifera* *Trichosporon mucoides*

(Continued)

Table 1. (Continued)

REFERENCE	YEAR	COUNTRY	SAMPLE SIZE	SAMPLES CONTAMINATED	IDENTIFICATION TECHNIQUE	MICROBIAL LOAD		CONTAMINANT GROUP	SPECIFIC ORGANISMS ISOLATED
						BACTERIAL LOAD	FUNGAL LOAD		
Omoruyi et al.[64]	2024	Nigeria	50	20	Conventional culture method	NA	NA	Bacterial	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Proteus mirabilis* *Enterobacter* spp. *Citrobacter* spp.
Osei et al.[65]	2024	Ghana	3	3	Conventional culture method	$3.6 \pm 0.03 \times 10^3$ cfu/mL to $4.1 \pm 0.19 \times 10^3$ cfu/mL	$1.2 \pm 0.19 \times 10^3 \pm 0.19$ cfu/mL to 1.6 ± 0.30 cfu/mL $\times 10^3 \pm 0.30$ cfu/mL	Bacterial Fungal	Aerobic bacteria Yeast and mould
Onyemelukwe et al.[66]	2019	Nigeria	80	80	Conventional culture method	2.1×10^3 cfu/mL to 9.0×10^6 cfu/mL	1.1×10^3 cfu/mL to 8.0×10^5 cfu/mL	Bacterial Fungal Parasitic	*Bacillus* spp. *Pseudomonas aeruginosa* *Escherichia coli* *Enterobacter* spp. *Staphylococcus aureus* *Klebsiella* spp. *Salmonella* spp. *Aspergillus flavus* *Rhizopus* spp. *Geotrichum candidum* *Aspergillus niger* *Trichophyton rentagrophytes* *Microsporium canis* *Penicillium* spp. *Mucor* spp. *Syncephilastrum racemosus* *Ascaris lumbricoides* *Hookworm* *Toxocora canis* *Entamoeba coli* *Giardia infestinalis* *Entamoeba histolytica/dispar*
Addotey and Nyansah[67]	2016	Ghana	11	11	Conventional culture methods	NA	1.1×10^2 cfu/mL to 1.6×10^4 cfu/ml	Bacterial Fungal	*Staphylococcus aureus* Moulds and yeasts
Udeogu et al.[68]	2020	Nigeria	44	27	Conventional culture methods	7.0×10^5 cfu/mL to 8.9×10^6 cfu/mL	NA	Bacterial	*Klebsiella pneumoniae* *Enterococcus faecalis* *Staphylococcus aureus* *Escherichia coli* *Proteus* spp. *Salmonella* spp.

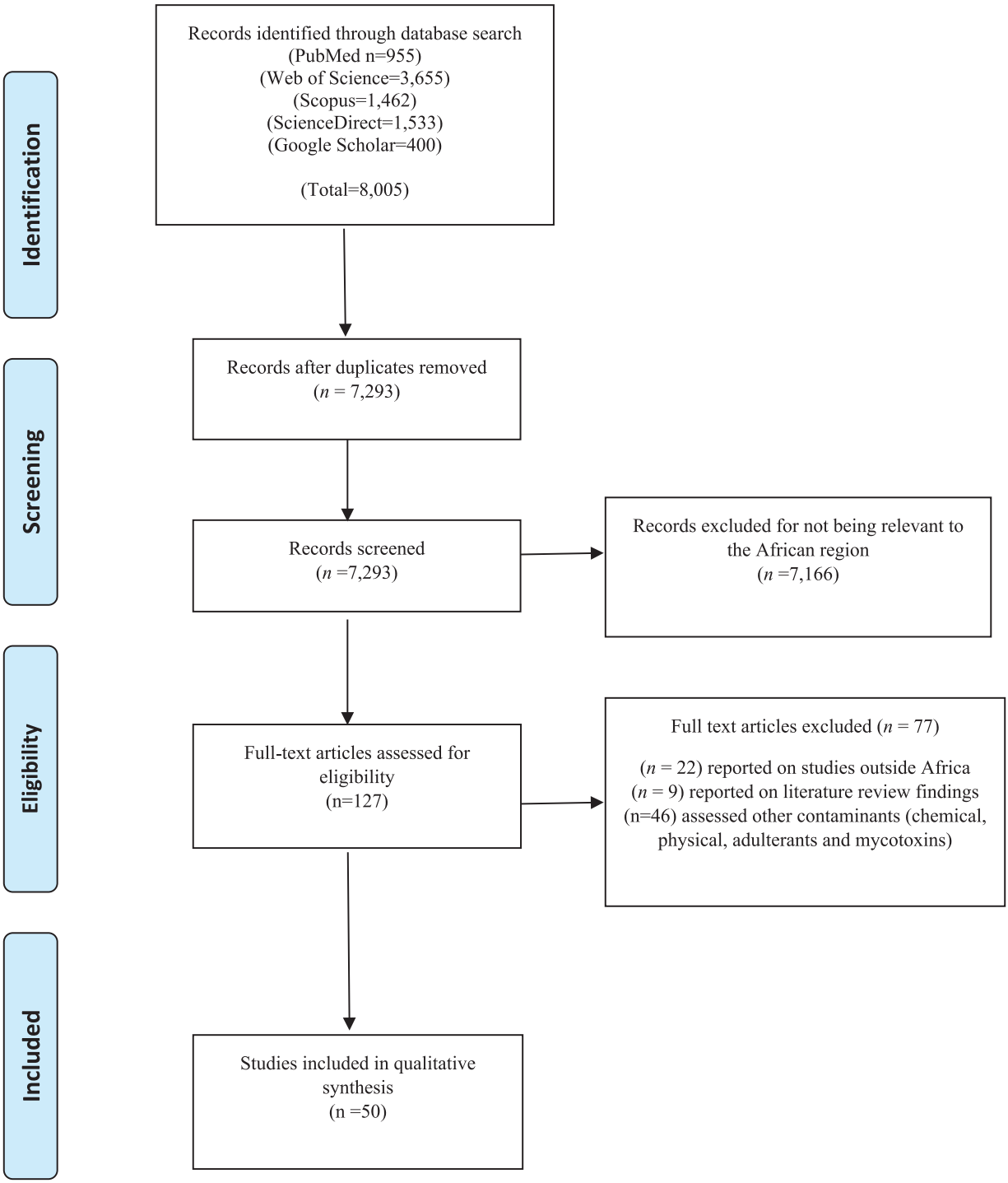

Figure 1. PRISMA flow diagram providing a detailed representation of the article selection process.

Characteristics of the eligible studies

Table 1 shows a summary of the characteristics of the 50 studies included in this review. The highest number of eligible studies came from Nigeria, 25 (50%), followed by Ghana with 9 (18%), Kenya with 4 (8%), South Africa with 3 (6%), and Tanzania with 2 (4%). Côte d'Ivoire, Malawi, Cameroon, Sierra Leone, Benin, Uganda, and Lesotho, contributed 1 study each (2%). The distribution of these studies across different regions is shown in Figure 2.

Of the 50 studies reviewed, 49 (98%) reported on bacterial contaminants, 35 (70%) reported on fungal contaminants, and only 1 (2%) study reported on parasitic contaminants in herbal medicines. Some studies examined multiple types of contaminants, resulting in a combined total that exceeds the total number of individual studies. Conventional culture and identification methods, encompassing gram staining, biochemical reactions, and physiological techniques, were employed in 96% of the studies to identify bacterial, fungal, and parasitic contaminants in herbal medicines. Molecular techniques for isolation and

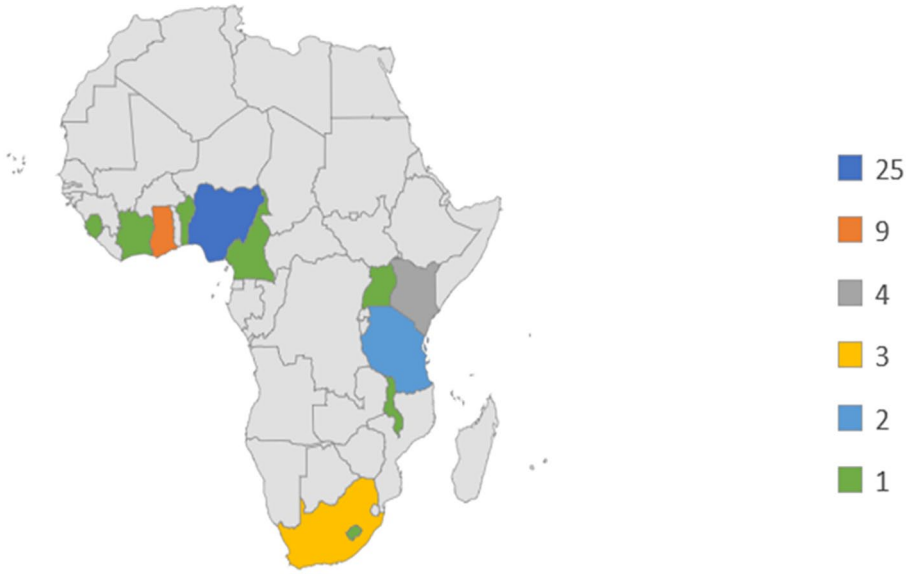

Figure 2. Geographical distribution of the included articles.

identification were used in only 4% of the studies. The prevalence of microbial contamination in herbal medicines varied widely, ranging from 14% to 100%.

Collectively, the included studies examined 1996 herbal medicine samples, with 1791 of the samples harbouring microbial contamination. This equates to an overall contamination prevalence of 90% in herbal medicines across Africa. Sixty-two percent (62%) of the reviewed studies reported a 100% prevalence of microbial contamination. The majority of studies included in this review, 39 (78%), were published from 2014 to 2024, while 11 (22%) were published between 2000 and 2013.

Bacterial contaminants of herbal preparations in Africa

A significant number of studies (98%) reported diverse bacterial contaminants in herbal medicines.[8,10,22–68] Across these studies, 70 bacteria from 37 different genera were isolated. *Escherichia coli* emerged as the most frequently identified bacteria, reported in 62% of the studies. Other commonly reported bacterial contaminants include *Staphylococcus aureus* (60%), *Bacillus* spp. (54%), *Pseudomonas* spp. (46%), *Salmonella* spp. (44%), *Klebsiella* spp. (44%), *Enterobacter* spp. (38%), *Proteus* spp. (22%), *Serratia* spp. (16%), *Citrobacter* spp. (16%), *Enterococcus* spp. (12%), *Streptococcus* spp. (10%), *Pantoea* spp. (10%), *Shigella* spp. (8%), *Acinetobacter* spp. (8%), *Providencia* spp. (6%), *Rahnella* spp. (4%), *Chryseomonas* spp. (4%), and *Moraxella* spp. (4%). Each of the following bacterial contaminants was reported in 2% of the included studies: *Edwardsiella* spp., *Cedecea* spp., *Flavimonas* spp., *Stenotrophomonas* spp., *Ewingella* spp., *Bordetella* spp., *Pasteurella* spp., *Aeromonas* spp., *Arizona* spp., *Kocuria* spp., *Rhizobium* spp., *Leclecia* spp., *Sphingomonas* spp., *Raoultella* spp., *Paenibacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., and *Yersinia* spp. Figure 3 illustrates the percentage distribution of the common bacterial isolates identified in this review.

From the studies included in this review, reports on the bacterial loads of the various herbal medicines analysed, revealed varying levels of contamination across different countries. The bacterial loads documented in this review generally ranged from 0 cfu/mL to 3.54×10^{12} cfu/mL. The highest bacterial load recorded (3.54×10^{12} cfu/mL) was reported in Nigeria by Igbeneghu and Lamikanra.[36] The samples in this study were sourced from unregulated herbal medicines on the market. Similarly, another study by Nwankwo and Olime,[28] which investigated microbial contamination in registered herbal preparations on the Nigerian market, reported bacterial loads ranging from 3.10×10^2 cfu/mL to 2.56×10^3 cfu/mL in liquid formulations, and 9.0×10^1 cfu/g to 1.5 cfu/g $\times 10^2$ cfu/g in powdered herbal preparations.

In a study conducted in Nigeria by Tatfeng et al.,[33] it was noted that 'schnapps' and palm wine–based preparations were mostly contaminated with *Bacillus* spp. (aerobic spore bearers), while water-based preparations had several bacterial isolates, including *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli* 0157, *Proteus mirabilis*, *Enterococcus faecalis, Serratia marcescens*, *Staphylococcus aureus*, and *Bacillus* spp.

Also in the studies outlined, Brooks and Takim[25] reported a Total Viable Bacterial Count (TVBC) of 2.2×10^4 cfu/g to 5.6×10^4 cfu/g for solid dosage forms and 3.8×10^4 cfu/mL to 6.8×10^4 cfu/mL for liquid forms of herbal medicines sold in Calabar, Nigeria. Nwankwo and Olime[28] reported a Total Heterotrophic Bacterial Count (THBC) of 3.1×10^2 cfu/mL to 2.65×10^3 cfu/mL for liquid preparations and 1.1×10^2 cfu/g to 1.5×10^2 cfu/g for powdered preparations. Walusansa et al[31] in a study conducted in Uganda reported a mean viable load of 126.407×10^4 cfu/mL or g across 140 samples. Kaume et al.[35]

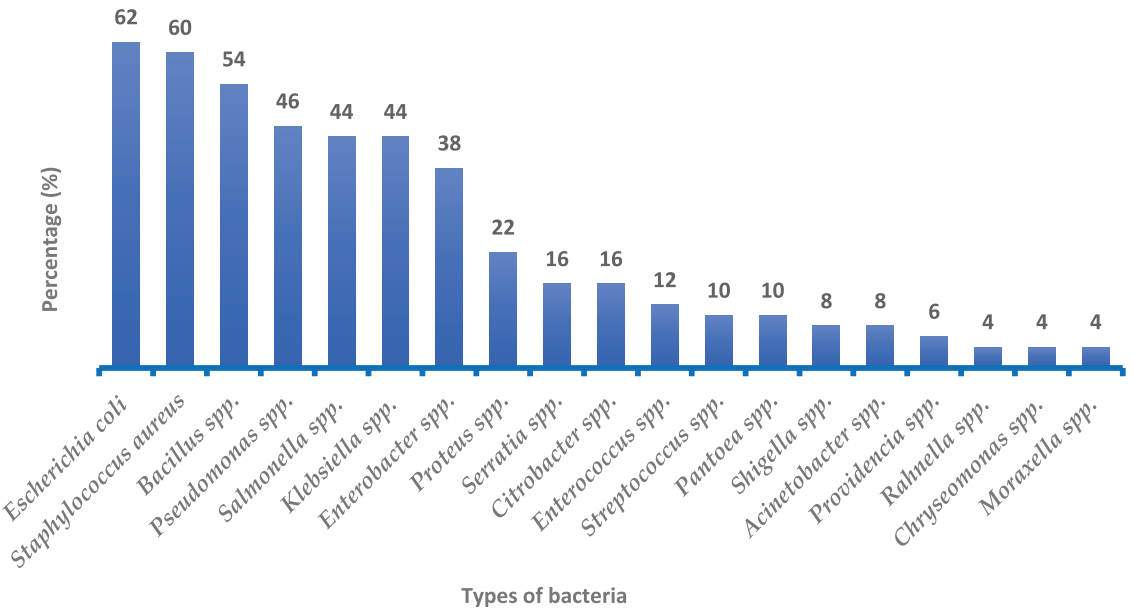

Figure 3. Percentage frequency of bacterial isolates identified in the included studies.

reported bacterial loads ranging from 3.03×10^4 cfu/mL to 4.22×10^5 cfu/mL in some herbal medicines in Kenya.

Omoruyi et al.[54] in Nigeria found microbial counts ranging from 2.8×10^4 cfu/mL to 3.1×10^4 cfu/mL for regulated products and 3.8×10^4 cfu/mL to 12.6×10^3 cfu/mL for unregulated products. A study conducted by Onyambu et al.[61] on regulated and unregulated herbal medicines in Kenya reported a bacterial load count of 1.50×10^{10} cfu/mL in unregulated herbal medicines and counts below 100 cfu/mL in registered herbal products. Adounkpe et al.[57] reported bacterial loads ranging from 9.15×10^7 cfu/mL to 3.65×10^9 cfu/mL in herbal medicines from Benin. Osei-Adjei et al.[45] reported bacterial loads ranging from 1.0×10^2 cfu/mL to 1.0×10^9 cfu/mL in herbal medicines from Ghana. Kira et al.[62] reported a mean bacterial load of 1.64×10^8 cfu/mL in herbal medicines from Tanzania.

Fungal contaminants of herbal medicines in Africa

Fungal contaminants were reported in 35 (70%) studies.[10,24-29,32,33,35-43,45-47,50,52,53,56-61,63,65-67,69] Forty (40) fungal species from 24 different genera were identified (Table 1). *Aspergillus* spp. was the most commonly reported fungal species, appearing in 40% of the studies. This was followed by *Penicillium* spp. (28%), *Candida* spp. (24%), *Mucor* spp. (20%), *Rhizopus* spp. (20%), *Fusarium* spp. (8%), *Cladosporium* spp. (6%), *Saccharomyces* spp. (6%), *Trichosporon* spp. (4%), *Scedosporium* spp. (4%), and *Geotrichum* spp. (4%). Other fungal contaminants reported include *Phaeoacremonium* spp., *Curvularia* spp., *Cryptococcus* spp., *Trichoderma* spp., *Alternaria* spp., *Mycelia* spp., *Rhodotorula* spp., *Sporobolomyces* spp., *Phialophora* spp., *Torula* spp., *Trichophyton* spp., *Microsporium* spp., and *Syncephilastrum* spp., each reported in 2% of the reviewed studies.

Several studies included in this review also documented a wide range of fungal loads in herbal medicines. The total fungal loads reported across the studies ranged from 0 cfu/mL to 3.54×10^{12} cfu/mL. Some of the highest fungal loads reported in this review were: 3.54×10^{12} cfu/mL recorded in Nigeria,[36] 1.60×10^9 cfu/mL from Sierra Leone,[37] 6.0×10^8 cfu/mL from Lesotho,[27] 1.3×10^8 cfu/mL from Nigeria,[43] 4.4×10^7 cfu/g recorded in Cote d'Ivoire,[58] 4.7×10^7 cfu/mL[33] and 1.5×10^7 cfu/mL both recorded in Nigeria. Akande et al.[47] reported total fungal counts of 1.0×10^5 cfu/mL to 8.0×10^5 cfu/mL in herbal medicines from Nigeria. The fungal counts as presented by the studies included are shown in Table 1. Figure 4 shows the percentage frequency of the most reported fungal isolates.

Parasitic contaminants of herbal medicines in Africa

Only 1 study (2%) reported parasite contamination in herbal medicines in the reviewed studies. The study by Onyemelukwe et al.[66] reported a 53% occurrence of parasites in 80 herbal medicine samples from Nigeria. *Ascaris lumbricoides*, was the most prevalent parasite in that study, detected in 53.7% of the samples, followed by hookworm ova (19.5%), and *Toxocara canis* (12.2%). The least prevalent parasites were *Entamoeba coli*, *Giardia infestinalis*, and *Entamoeba histolytica/dispar* each found in 4.9% of the samples.

Discussion

The increasing use of herbal medicines and other crude concoctions in Africa raises concerns about their safety to consumers, particularly relating to their microbial quality. This study analysed fifty studies that investigated the prevalence and loads of microbial contaminants in herbal medicines across Africa; Nigeria, Ghana, Kenya, South Africa, Tanzania, Cote d'Ivoire,

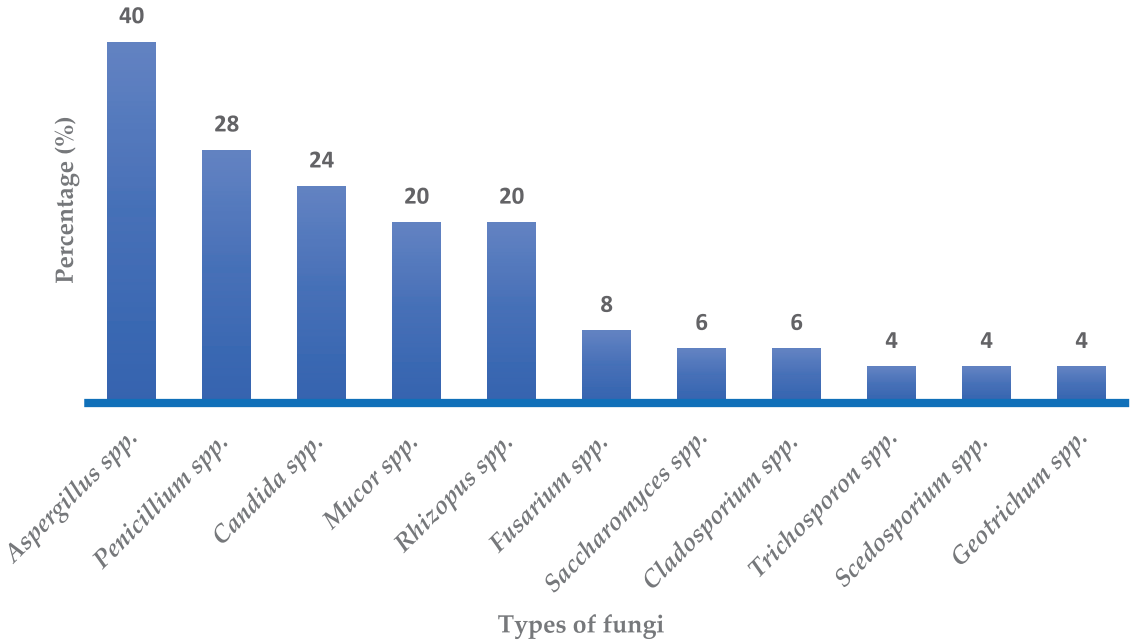

Figure 4. Percentage frequency of fungal isolates identified in the included studies.

Malawi, Cameroon, Sierra Leone, Benin, Lesotho, and Uganda. Most (78%) of these studies were conducted in the recent decade (2014-2024). This trend is supported by other findings,[12] indicating a significant increase in research addressing the microbial contamination of herbal medicines in Africa.

This review reported on bacterial, fungal, and parasitic contaminants in herbal medicines across the African region. The majority of included studies (98%) reported on bacterial contaminants. *Escherichia coli* was the most reported bacterial pathogen in herbal medicines across the African region. This finding is consistent with the report from the study conducted by Walusansa et al, which identified *Escherichia coli* as the most prevalent bacterial contaminant in herbal medicines.[12] Findings from another study conducted by Opuni et al.,[15] also reported *Escherichia coli* as the most reported bacterial contaminant found in herbal medicines across low–and middle– income countries. The presence of this pathogen in herbal medicines suggests possible faecal contamination, raising concerns about the potential for direct or indirect exposure to human or animal waste during preparation.[27,31] According to the World Health Organisation, (WHO),[70] the presence of *E. coli* not only indicates faecal contamination but also raises concerns about the potential presence of more virulent strains, such as shiga toxin-producing *E. coli*. These strains are implicated in life–threatening diseases such as haemolytic uraemic syndrome, particularly in vulnerable populations like young children, the elderly, and HIV/AIDS patients.[15,70] Notably, some studies included in this review[26,35] investigated herbal medicines marketed to HIV/AIDS patients. These studies revealed alarming levels of bacterial contamination exceeding acceptable limits. As reported in these studies, liquid formulations recorded bacterial counts as high as 1.19×10^9 cfu/mL,[26] while solid dosage forms recorded 7.1×10^8 cfu/g,[35] exceeding the acceptable limits of 10^5 cfu/mL for liquid samples and 10^7 cfu/mL for solid samples.[71] This poses a significant health threat to an already immunocompromised population.

This review also identified *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas* spp., and *Salmonella* spp. as commonly reported bacterial pathogens (60%, 54%, 46%, and 44% of included studies respectively) from herbal medicines in Africa. These findings are consistent with findings from low–and middle–income countries in other regions. Studies conducted by Opuni et al.[15] and De Souza Lima et al.[11] identified *Salmonella* spp., *Bacillus* spp., *Pseudomonas aeruginosa* and *Staphylococcus* spp. as common bacterial pathogens in herbal medicines. These organisms which are also indications of faecal contamination, reveal poor hygiene conditions in the preparation and storage of these herbal medicines, thus making them unsafe for consumption.[15,72] In this review, Govender et al.[26] identified diarrhoeal toxins produced by *Bacillus cereus* in herbal medicines from South Africa. Additionally, other studies[26,73,74] highlight the potential health risks posed by toxins when consumed. The potential for severe infectious diseases among the African population due to contaminated herbal remedies is a serious concern, given the presence of numerous medically important pathogens. *Staphylococcus aureus*, for example, which was reported in 60% of studies included in this review causes staphylococcal gastroenteritis, scalded–skin syndrome, toxic shock syndrome, endocarditis, lung infection, folliculitis, among other diseases.[75-77] These diseases are life–threatening in older people and immunocompromised adults.[76]

According to the World Health Organisation (WHO), '*Salmonella* and *Shigella* species must not be present in herbal medicines intended for internal use, at any stage'.[71] Contrary to this guideline, *Salmonella* spp., and *Shigella* spp. were reported

in 44% and 8% of the studies respectively. These organisms have the potential to cause large disease outbreaks due to their low infectious dose.[78] They are responsible for a significant disease burden worldwide, causing diarrhoea and a spectrum of associated symptoms, from mild to life–threatening.[79] The CDC estimates that *Salmonella* spp. causes approximately 1.4 million infections, 26,500 hospitalisations, and over 400 deaths annually in the United States.[80] This poses a significant threat to public health in Africa, where many people rely on herbal remedies and may lack access to adequate medical care. Similar to our findings in Africa, gram–negative bacteria such as *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Shigella* spp., and *Salmonella* spp. in addition to several species of *Staphylococcus* have been reported as major contaminants in herbal medicines from other continents, particularly Asia.[81-83]

Another popular finding across multiple studies included in this review is the presence of fungal isolates from the genera *Aspergillus*, *Penicillium*, *Candida*, *Mucor*, *Rhizopus*, *Fusarium, Cladosporium*, and *Scedosporium* in herbal medicines across the African region. These fungal species have been identified in herbal medicines across various regions globally as evidenced in studies conducted by De Souza et al., Kneifel et al., Lee & Yoon, Opuni et al., and Zheng et al.[15,72,84-86] The study by Kneifel et al.[84] revealed that fungal isolates in herbal medicines can degrade active ingredients reducing their effectiveness, and potentially produce mycotoxins. These toxins are mainly produced by fungi from the genera *Aspergillus, Penicillium* and *Fusarium*.[87] Exposure to these toxins can have devastating effects on human health, potentially leading to liver cancers, weakened immunity, altered protein metabolism, seizures, and respiratory problems among other health complications.[88-90]

Herbal medicines to a large extent are mostly contaminated with bacterial and fungal elements.[15] However, one study included in this review reported the contamination of herbal products from Nigeria with parasite forms such as helminths and protozoans. The study by Onyemelukwe et al.,[66] reported the presence of helminth eggs and protozoan cysts in herbal preparations at a staggering 53% occurrence. The parasites found in these herbal preparations included *Ascaris lumbricoides*, hookworm, *Toxocara canis*, *Entamoeba coli*, *Entamoeba histolytica/dispar* and *Giardia intestinalis*.[66] Data from other regions such as Asia supports the occurrence of parasitic contaminants in herbal medicines. A study conducted by Posadzki et al.,[21] found parasitic contaminants similar to those identified in the study from Nigeria in herbal medicines.

The problem of microbial contamination of herbal products in Africa is exacerbated by widespread environmental pollution and unsanitary conditions[66,91] which is common in Africa. Several studies included in this review[8,10,22,24-27,34,44,56] attributed the high prevalence of microbial contamination in herbal medicines to a combination of factors, including lack of regulation, and pollution throughout the production chain, from harvesting raw materials, to handling, processing, storage, and transportation. According to Onyemelukwe et al., the trees and plants from which medicinal preparations are made could have microorganisms adhered to their stems, barks, leaves, flowers, fruits, and roots eventually leading to contamination of the product.[66] Other factors contributing to the high prevalence of microbial contamination in herbal medicines as reported in the reviewed studies include the use of untreated water supply, poor quality of packaging materials, use of contaminated containers, working from polluted faecal environments, and poor personal hygiene behaviours during handling.[29,31-33,59]

A survey conducted by the World Health Organisation (WHO) in 2019, indicated that 43% of African member states regulate herbal medicines, compared to 26% in 2005.[4] However, despite the progress in regulatory efforts, this study found a significant 90% overall prevalence of microbial contamination in herbal medicines, highlighting the need for stricter regulations in the African region. The prevalence of microbial contamination in herbal medicines is a public health concern in Africa. To address this challenge, it is important that existing regulations are enforced and novel regulations adopted in countries where they are lacking. Also, producers of herbal medicines should ensure strict quality control measures and Good Manufacturing Practices (GMP) are followed throughout the production and distribution processes to minimise the proliferation of microorganisms in these products. Failure to address this issue could lead to widespread health problems in Africa. Research on the microbiological safety of herbal medicines in Africa must expand beyond fungal and bacterial contaminants to include parasites for a comprehensive understanding of the unique challenges associated with these remedies.

Limitations of the Study

While this systematic review provides valuable insights, it is subject to some key limitations. Firstly, the literature search was restricted to peer-reviewed studies published in English language, potentially excluding grey literature and other relevant studies not published in English. Secondly, the studies captured in this review were mainly from the western, eastern, and southern parts of Africa, limiting its generalisability.

Conclusion

This systematic review provided a comprehensive overview of the microbial contaminants reported in herbal medicines across Africa, revealing a disturbingly wide range of bacterial, fungal, and parasitic species with varying degrees of contamination. The presence of pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Klebsiella* spp., *Shigella* spp., *Aspergillus* spp., *Penicillium* spp., *Candida* spp., *Mucor* spp. and *Entamoeba histolytica* among others, poses a significant risk to consumer safety. The findings of this review underscore the urgent need for stricter regulations and quality control measures to ensure the safety of herbal medicine products in Africa, ultimately protecting the health and well-being of consumers.

Author Contributions

Conceptualisation, ESD; methodology, WKA, SD, and ESD; validation, SD, and ESD; formal analysis, WKA, and SD; resources, ESD; data curation, WKA; writing—original draft preparation, WKA, SD and ESD; writing—review and editing, WKA, SD, and ESD; visualisation, WKA, and SD; supervision, ESD.

ORCID iDs

Wisdom K Ahiabor https://orcid.org/0009-0006-1860-6248
Samuel Darkwah https://orcid.org/0000-0003-0868-1798
Eric S Donkor https://orcid.org/0000-0002-5179-546X

Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4:177. doi:10.3389/fphar.2013.00177.
2. Umair M, Altaf M, Abbasi AM. An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan. *PLoS One*. 2017;12:e0177912.
3. WHO traditional medicine strategy: 2014-2023 [Internet]. [cited 2024 Feb 15]. Available from: https://www.who.int/publications-detail-redirect/9789241506096
4. World Health Organization. WHO global report on traditional and complementary medicine 2019. [Internet]. World Health Organization; 2019:226. https://iris.who.int/handle/10665/312342
5. Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA. Toxicity and safety implications of herbal medicines used in Africa. In: Builders F ed. *Herbal Medicine*. [Internet]. IntechOpen; 2019;63(5):1992-0849. https://www.intechopen.com/books/herbal-medicine/toxicity-and-safety-implications-of-herbal-medicines-used-in-africa
6. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *J Ethnobiol Ethnomed*. 2013;9:31.
7. Truter I. African traditional healers: cultural and religious beliefs intertwined in a holistic way. *South Afr Pharm J*. 2007;74:56-60.
8. Van Vuuren S, Williams VL, Sooka A, Burger A, Van der Haar L. Microbial contamination of traditional medicinal plants sold at the Faraday muthi market, Johannesburg, South Africa. *S Afr J Bot*. 2014;94:95-100.
9. Hayta S, Polat R, Selvi S. Traditional uses of medicinal plants in Elazığ (Turkey). *J Ethnopharmacol*. 2014;154:613-623.
10. Darkwah S, Agbettor D, Codjoe F, Donkor ES. Microbial contamination of herbal preparations on the Ghanaian market, Accra. *Microbiol Insights*. 2022;15:11786361221139602.
11. de Sousa Lima CM, Fujishima MAT, de Paula Lima B, et al. Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. *BMC Complement -med Ther*. 2020;20:17.
12. Walusansa A, Asiimwe S, Kafeero HM, et al. Prevalence and dynamics of clinically significant bacterial contaminants in herbal medicines sold in East Africa from 2000 to 2020: a systematic review and meta-analysis. *Trop Med Health*. 2021;49:10.
13. Ventola CL. Current issues regarding complementary and Alternative Medicine (CAM) in the United States: Part 2: Regulatory and safety concerns and proposed governmental policy changes with respect to dietary supplements. *P T*. 2010;35:514-522.
14. Kosalec I, Cvek J, Tomić S. Contaminants of Medicinal Herbs and Herbal Products. *Arch Ind Hyg Toxicol*. 2009;60:485-501.
15. Opuni KFM, Kretchy JP, Agyabeng K, et al. Contamination of herbal medicinal products in low-and-middle-income countries: a systematic review. *Heliyon*. 2023;9:e19370.
16. ContagionLive. Infectious diseases dominate WHO's List of 2019 health threats. [Internet].2019.https://www.contagionlive.com/view/infectious-diseases-dominate-whos-list-of-2019-health-threats
17. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *Ann Intern Med*. 2009;151:264-NaN9, W64.
18. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. *Syst Rev*. 2016;5:210.
19. Oxman AD, Guyatt GH. Validation of an index of the quality of review articles. *J Clin Epidemiol*. 1991;44:1271-1278.
20. James PB, Wardle J, Steel A, Adams J. Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. *BMJ Glob Health*. 2018;3:e000895.
21. Posadzki P, Watson L, Ernst E. Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews. *Eur J Clin Pharmacol*. 2013;69:295-307.
22. Hassan KM, Njogu PM, Njuguna NM, Ndwigah SN. Microbiological contamination of herbal medicinal products marketed in Kenya for chronic diseases: A case study of Nairobi metropolis. *J Herb Med*. 2021;29:100475.
23. Abba D, Inabo H, Yakubu S, Olonitola O. Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. *Afr J Tradit Complement Altern Med*. 2009;6:70–77. https://www.ajol.info/index.php/ajtcam/article/view/57076
24. Archibong J, Igboeli CN, Okoro NC, Obika I. Microbiological assessment of some liquid herbal medications sold in Awka Metropolis, Anambra State. *Bioeng Biosci*. 2017;5:37-46.
25. Brooks A, Takim O. Assessment of microbiological status of some herbal medicines sold in Calabar, Nigeria. 2014. https://agris.fao.org/search/en/providers/122436/records/64747a6479cbb2c2c1b5d117
26. Govender S, Du Plessis-Stoman D, Downing TG, Van de Venter M. Traditional herbal medicines: microbial contamination, consumer safety and the need for standards. *S Afr J Sci*. 2006;102:253-255.
27. Mautsoe R, Noko T, Hlokoane O. Microbial and heavy metal contaminants in herbal preparations sold in Maseru, Lesotho. *Int J Res Pharm Chem*. 2021;7:71-79.
28. Nwankwo CC, Olime T. Microbial quality of herbal preparations sold in some parts of Nigeria. *GSC Biol and Pharm Sci*. 2019;6:076-084.
29. Odonkor S, Osei J, Anim A, Laar C, Okyere M. Microbiological quality of some herbal medicinal products sold in Accra, Ghana. *Elixir Pharmacy*. 2011;40:5499-5503.
30. Kalumbi MH, Likongwe MC, Mponda J, et al. Bacterial and heavy metal contamination in selected commonly sold herbal medicine in Blantyre, Malawi. *Malawi Med J*. 2020;32:153-159.
31. Walusansa A, Nakavuma JL, Asiimwe S, et al. Medically important bacteria isolated from commercial herbal medicines in Kampala city indicate the need to enhance safety frameworks. *Sci Rep*. 2022;12:16647.
32. Ezekwesili-Ofili J, Onyemelukwe N, Agwaga P, Orji I. The bioload and aflatoxin content of herbal medicines from selected states in Nigeria. *Afr J Tradit Complement Altern Med*. 2014;11:143-147.
33. Tatfeng YM, Olama EH, Ojo TO. Microbial burden of some herbal antimalarials marketed at Elele, Rivers state. *Afr J Tradit Complement Altern Med*. 2010;7:149-152. https://www.ajol.info/index.php/ajtcam/article/view/50875 Available from.
34. Walther C, Marwa KJ, Seni J, et al. Microbial contamination of traditional liquid herbal medicinal products marketed in Mwanza city: magnitude and risk factors. *Pan Afr Med J*. 2016;23:65. https://www.panafrican-med-journal.com//content/article/23/65/full
35. Kaume L, Foote JC, Gbur EE. Microbial contamination of herbs marketed to HIV-infected people in Nairobi (Kenya). *S Afr J Sci*. 2012;108:1-4.
36. Igbeneghu O, Lamikanra A. Assessment of the microbial quality of some oral liquid herbal medicines marketed in Ile-Ife, South-western Nigeria. *AJMR*. 2016;10:1618-1624.
37. Kanu A, Igbeneghu O, Orafidiya L. The organoleptic and microbial quality of some herbal medicinal products marketed in Freetown, Sierra Leone. Afr J *Tradit Complement Altern Med*. 2015;12:1-8.
38. Oladosu O, Mohammad F, Aboh M, Olatunji K, Izebe K. Microbiological Quality Assessment of herbal products produced and marketed in Gombe Metropolis, North-East Nigeria—PDF free download. [Internet]. 2020. https://docplayer.net/196028982-Microbiological-quality-assessment-of-herbal-products-produced-and-marketed-in-gombe-metropolis-north-east-nigeria.html
39. Anie OC, Egbon OT, Enemchukwu CM, Adushoke EL. The microbial quality of herbal products. *J Drug Deliv Therapeut*. 2022;12:64-69.
40. Ideh JE, Ogunkunle ATJ. User frequency and microbial contaminants of traditional oral powdered herbal formulations in Ogbomoso, Nigeria. *J Med Plants Econ Dev*. 2019;3:1-9.
41. Oshoma C, Dijeh E. Microbiological evaluation of locally processed herbal drugs sold in benin city. *Afr Sci*. 2017;18:135-142.
42. Turkson BK, Mensah MLK, Sam GH, et al. Evaluation of the microbial load and heavy metal content of two polyherbal antimalarial products on the Ghanaian market. *Evid Based Complement Alternat Med*. 2020;2020:1014273.
43. Chinakwe EC, Ngumah JC, Kenechukwudozie OQ, et al. Microbial Quality and public health risks of selected herbal remedies sold in open markets in Owerri Metropolis, South Eastern, Nigeria. *Int J Microbiol Res*. 2023;33:24-31.
44. Abubakar ZA, Abubakar U, Tal KM. Contamination of locally produced herbal medicine sold in Gombe main market with selected pathogenic bacteria. *J Mol Stud Med Res*. 2018;3:120-127.

45. Osei-Adjei G, Hackman HK, Mills-Robertson FC, Tay SC. Quality assessment of aqueous herbal/medicinal products sold on the Ghanaian market. *Food Sci Qual Manage*. 2013;20:13-19. https://www.iiste.org/Journals/index.php/FSQM/article/view/8020
46. Ampofo JA, Andoh A, Tetteh W, Bello M. Microbiological profile of some Ghanaian herbal preparations—safety issues and implications for the health professions. *OJMM*. 2012;2:121-130.
47. Akande T, Agbulu C, Oche M. Microbial contamination of herbal mixtures (local concoctions) used in the treatment of typhoid fever, Malaria Fever, and Dysentery in Makurdi Metropolis. [Internet]. Cenres in Journals. 2013. https://www.cenresinjournals.com/2020/02/25/microbial-contamination-of-herbal-mixtures-local-concoctions-used-in-the-treatment-of-typhoid-fever-malaria-fever-and-dysentery-in-makurdi-metropolis-akande-t-agbulu-c-o-and-oche-m-dep/
48. Famewo EB, Clarke AM, Afolayan AJ. Identification of bacterial contaminants in polyherbal medicines used for the treatment of tuberculosis in Amatole District of the Eastern Cape Province, South Africa, using rapid 16S rRNA technique. *J Health Popul Nutr*. 2016;35:27.
49. Sebiawu G, Antwi-Akomeah S, Mensah N, Abana D. Heavy metal and bacteriological contamination of herbal medicines sold over the counter in the municipality of WA of the Upper West Region-Ghana. 2020;15–23.
50. Ayansina ADV, Akinsola AO. Microbial quality and antimicrobial evaluation of some herbal concoctions in a rural town in Nigeria. *World J Bio Pharm Heal Sci*. 2020;4:048-058.
51. Ngemenya MN, Djeukem GGR, Nyongbela KD, et al. Microbial, phytochemical, toxicity analyses and antibacterial activity against multidrug resistant bacteria of some traditional remedies sold in Buea Southwest Cameroon. *BMC Complement Altern Med*. 2019;19:150.
52. Odo J, Aerman P, Sar T, Nweke O. Microbiological quality of herbal formulation used for the treatment of typhoid fever sold in Makurdi Metropolis, Central Nigeria. *Asian J Heal Sci Asian J Heal Sci*. 2023;2:15-26. https://ajhsjournal.ph/index.php/gp/article/view/24
53. Idu M, Omonigho SE, Erhabor J, Efijuemue HM. Microbial load of some medicinal plants sold in some local markets in Abeokuta, Nigeria. *Trop J Pharm Res*. 2010;9:251-256. https://www.ajol.info/index.php/tjpr/article/view/56285
54. Omoruyi Z, Okundaye M, Tunde E. Evaluation of the microbial quality and safety of regulated and unregulated liquid herbal preparations in Benin City, Nigeria. *Int J Adv Res Biol Sci*. 2023;10:59-71.
55. Bello R, David M, Olutayo A, et al. Bacteriology of some liquid herbal products sold in Ilorin-Kwara State Nigeria. *J Sci Pract Pharm*. 2019;6:324-330.
56. Ngari F, Gikonyo N, Wanjau R, Njagi E. Investigation of selected pathogenic microorganisms and toxic elements in herbal materials used in management of oral health in Nairobi County, Kenya. *J Appl Environ Biol Sci*. 2013;3:1-7.
57. Adounkpe F, Allabi A, Baba F, et al. Microbiological quality assessment of aqueous herbal teas sold in Cotonou, Benin. *Int J Herb Med*. 2017;5:121-126.
58. Bernadin K, Witabouna KM, Julien CK, Mireille D. Microbial contamination of the stem bark of Mitragyna Ciliata, a commercially available medicinal plant in the district of Abidjan (Cote d'Ivoire). *ISEE Conference Abstracts*. 2018;404-415.
59. Osei Asare C, Akuffo Owusu FW, Apenteng JA, et al. Evaluation of the microbial quality of commercial liquid herbal preparations on the Ghanaian market. *INNOSC Theranost Pharmacol Sci*. 2023;6:0425.
60. Bashir M, Mustapha S, Tawfiq UA. Microbiological quality evaluation of typhoid and diarrheal herbal formulations sold in Yola and Environs. *UMYU J Microbiol Res*. 2017;2:94-98.
61. Onyambu M, Chepkwony H, Thoithi G, Ouya G, Osanjo G. Microbial quality of unregulated herbal medicinal products in Kenya. *Afr J Pharmacol Ther*. 2013;2:70.
62. Kira J, Mkupasi E, Katakweba A, Ngowi H. Assessment of bacterial contamination in herbal medicine products vended in Morogoro municipality, Tanzania. *East Central Afr J Pharm Sci*. 2021;24:21-28.
63. Dabo DA, Zechariah KI, Pauline G, et al. Assessment of microbial contamination of Solid Herbal Medicine sold in Makurdi Metropolis. *World J Adv Res Rev*. 2024;21:496-505.
64. Omoruyi Z, Okundaye MI, Egunjobi TO. Microbiological indices of unregulated herbal drinks in benin City, Nigeria: a potential health hazard to consumers. *Environ Dis*. 2024;9:29-35.
65. Osei PK, Asante-Kwatia E, Turkson BK, et al. Quality assessment and evaluation of the aphrodisiac property and toxicity profile of a Ghanaian herbal male vitality booster. *Med Plus*. 2024;4:100503.
66. Onyemelukwe N, Chijioke O, Dozie-Nwakile O, Ogboi S. Microbiological, parasitological and lead contamination of herbal medicines consumed in Enugu, Nigeria. *Biomed Res*. 2019;30:828-833.
67. Addotey J, Nyansah M. Quality assessment of some topical polyherbal preparations on the Ghanaian market. *J Pharm Pharm Sci*. 2016;5:461-472.
68. Udeogu CV, Agbakoba NR, Chukwuma GO. Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria. *Afr J Clin Exp Microbiol*. 2020;21:354-359.
69. Usanga VU, Agbo NL, Kalu ME, Ude UA, Azi SO. Microbial contamination, antimicrobial activities of dissotis rotundifolia leaf: a common ethnomedicine for ocular diseases. *Niger J Pharm Res*. 2023;19:71-77.
70. E. coli. [Internet]. https://www.who.int/news-room/fact-sheets/detail/e-coli
71. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. 2007;118. https://www.who.int/publications/i/item/9789241594448
72. de Souza C, Ameyapoh Y, Karou SD, et al. Assessing market-sold remedies in Lomé (Togo) for Hygienic Quality. *Biotechnol Res Int*. 2011;2011:1-5.
73. Jessberger N, Kranzler M, Da Riol C, et al. Assessing the toxic potential of enteropathogenic Bacillus cereus. *Food Microbiol*. 2019;84:103276.
74. Organji SR, Abulreesh HH, Elbanna K, Osman GEH, Khider M. Occurrence and characterization of toxigenic bacillus cereus in food and infant feces. *Asian Pac J Trop Biomed*. 2015;5:515-520.
75. Gnanamani A, Hariharan P, Paul-Satyaseela M, et al. Staphylococcus aureus: overview of bacteriology, clinical diseases, epidemiology, antibiotic resistance and therapeutic approach. *Frontiers in Staphylococcus Aureus*. [Internet]. IntechOpen; 2017;4:10-5772. https://www.intechopen.com/chapters/54154
76. Pal M, Yonas Shuramo M, Tewari A, Priyadarshini Srivastava J, Steinmetz CHD. Staphylococcus aureus from a commensal to zoonotic pathogen: a critical appraisal. *Int J Clin Exp Med*. 2023;7:220-228.
77. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Vg Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28:603-661.
78. Cetinkaya F, Cibik R, Ece Soyutemiz G, et al. Shigella and Salmonella contamination in various foodstuffs in Turkey. *Food Control*. 2008;19:1059-1063.
79. Dekker JP, Frank KM. Salmonella, Shigella, and Yersinia. *Clin Lab Med*. 2015;35:225-246.
80. Salmonella Homepage. CDC. [Internet]. 2024. https://www.cdc.gov/salmonella/index.html
81. Alharbi SF, Althbah AI, Mohammed AH, et al. Microbial and heavy metal contamination in herbal medicine: a prospective study in the central region of Saudi Arabia. *BMC Complement Med Ther*. 2024;24:2.
82. Ting A, Chow Y, Tan W. Microbial and heavy metal contamination in commonly consumed traditional Chinese herbal medicines. *J Tradit Chin Med*. 2013;33:119-124.
83. Alwakeel SS. Microbial and heavy metals contamination of herbal medicines. *Res J Microbiol*. 2008;3:683-691.
84. Kneifel W, Czech E, Kopp B. Microbial contamination of medicinal plants—a review. *Planta Med*. 2002;68:5-15.
85. Lee JS, Yoon YS. Studies on bacterial and fungal contamination in the herbal medicines. *J Korea Acad Ind Coop Soc*. 2010;11:4826-4832.
86. Zheng RS, Wang WL, Tan J, et al. An investigation of fungal contamination on the surface of medicinal herbs in China. *Chin Med*. 2017;12:2.
87. Rizzo I, Vedoya G, Maurutto S, Haidukowski M, Varsavsky E. Assessment of toxigenic fungi on Argentinean medicinal herbs. *Microbiol Res*. 2004;159:113-120.
88. Lizárraga-Paulín E, Moreno-Martínez E, Miranda-Castro S. Aflatoxins and their impact on human and animal health: an emerging problem. *Biochem Mol Biol*. 2011;13:255-262.
89. Shephard GS. Impact of mycotoxins on human health in developing countries. *Food Addit Contam Part A*. 2008;25:146-151.
90. Zain ME. Impact of mycotoxins on humans and animals. *J Saudi Chem Soc*. 2011;15:129-144.
91. Ovuru K, Izah S, Yasmin H, et al. Microbial contaminants of herbal remedies: health risks and Sustainable Quality Control Strategies. In: Izah S, Ogwu M, Akram M, eds. *Herbal Medicine Phytochemistry: Applications and Trends*. Springer International Publishing; 2023;1-30.