

Research Article

Isolation and Identification of Microorganisms in Herbal Medicines Sold in Aba Metropolis and Their Resistance to Conventional Antibiotics

Christianah O. Jesubunmi^{1,*}, Nduka C. ThankGod², Charles C. Chigbu², Lawrence E. Okonko²¹ Glorybound International School, Port Harcourt, Rivers State, Nigeria.² Department of Biological Sciences, Clifford University, Owerri, Abia State, Nigeria.* Correspondence: ojesubunmi@yahoo.com<https://doi.org/10.59652/hwzdtk09>

Abstract: Herbal medicines are widely used, but their microbiological quality may be compromised during production, handling, storage, or distribution. This study isolated and identified microorganisms from herbal medicines sold in Aba metropolis, Nigeria, and assessed the susceptibility of bacterial isolates to conventional antibiotics. Eleven herbal medicine samples were subjected to microbiological analysis. Bacterial contamination was reported in 64% of samples and fungal contamination in 91%; 45% of samples were described as highly contaminated with bacteria because colony counts were too numerous to count. Bacterial isolates included *Staphylococcus* spp., *Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Bacillus* spp., *Enterobacter* spp., and *Micrococcus* spp. Fungal isolates included *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp., *Trichosporon* spp., and *Mucor* spp. Antimicrobial susceptibility testing showed that ciprofloxacin and gentamicin had activity against most bacterial isolates, while *Staphylococcus* spp. displayed the greatest overall resistance. These findings indicate substantial microbial contamination of the sampled herbal medicines and support the need for stronger microbiological quality control and good manufacturing practices.

Keywords: Herbal medicines, microbial contamination, antibiotic resistance, antimicrobial susceptibility, Aba, Nigeria

1. Introduction

Naturally occurring compounds derived from plant leaves, barks, roots, seeds, or flowers are used in traditional, local or regional medicine to treat illnesses (1). Complex mixtures of organic chemicals, derived from either raw or processed plant parts, are what make up herbal medicines (2). The practice of using herbs, plant materials, herbal preparations, and completed herbal products that contain active ingredients from plant parts or other plant materials or combinations for the purpose of disease prevention and treatment is known as herbal medicine (3,4).

The active ingredients found in herbal medicine, whether in their processed or raw form, offer protection against damage and diseases to plants while also enhancing their flavor, aroma, and colour (5). Herbal preparations are used to treat a variety of illnesses, including eczema, asthma, chronic fatigue, typhoid, diarrhea, and other skin disorders (6). They can be liquid, dried, capsule, powdered, or chopped form and can be used in various ways including swallowed as pills, powders or tincture, brewed as tea, applied to the skin as gels, lotions or creams and also added to bath water (7).

For their primary medical needs, the majority of people in some parts of the world still rely on their own traditional medicine. According to estimates from the World Health Organization, up to 80% of people in some Asian, African, and Latin American countries receive their primary care from traditional medicine. This is especially true for those who live in rural areas where traditional medicine is the only readily available, reasonably priced, and easily accessible form of healthcare (8). Although 60% of children in Ghana, Mali, Nigeria, and

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Zambia receive high fever treatment with these traditional medicines as their first line of treatment for malaria, 75% of HIV/AIDS patients in San Francisco, London, and South Africa also use traditional medicine. A growing number of studies on natural traditional medicine are being conducted in developing nations like Nigeria, Ghana, India, Madagascar, and Vietnam as a result of the general public's growing interest in using herbal medicines and their preference for natural products. 25% of contemporary medications are thought to be derived from plants that were originally used traditionally (9,10).

Herbal medicine has a very high prevalence in Nigeria. The factors influencing the use of herbal medicines have not been thoroughly studied, despite the fact that vendors of various herbal medicines are practically universal in Nigeria and can be found in traffic jams, highways, bus stops and stations, festivals, and even some traditional health facilities. The herbal medicines that are utilized are either imported, produced locally, refined, certified, or uncertified by the government (11). In Nigeria, the vast majority of people still use traditional medicine practitioners and medicinal plants for their medical needs. Some of the factors contributing to its ongoing use in this decision are a lack of medical facilities, poverty, accessibility, affordability, and an innate trust in the practice. According to data from the World Health Organization, Nigerians prefer to see traditional medicine practitioners over orthodox physicians for their primary medical needs (12).

Owing to the growing popularity of traditional medicine, the World Health Organization is pushing for the inclusion of complementary and alternative medicine, as well as traditional medicine in many countries' national healthcare systems. It is also encouraging the creation of natural policies and regulations pertaining to these medicines within national healthcare systems (13). Herbal medicines can be attacked by microorganisms from the soil, air, or water; these microorganisms may be present and pose a risk to human health. The degree of rainfall, temperature, humidity, handling procedures, and storage conditions of processed medicinal plant materials can all have an impact on the microbial contamination of medicinal herbs (14). Their negative consequences range in intensity and can even be fatal. Microbiological analysis of the product is necessary because microbes cannot be detected by organoleptic methods and microbial contamination in herbal medicine preparations is a significant public health concern (15). Consumers have reported unwelcome adverse reactions as a result of the toxic effects of these herbal medicines. The toxic effects can be caused by harmful substances like tannis, oxalates, and alkaloids; they can also result from improper preparation techniques/use, misidentification of plants and herb interactions when herbal medicine is taken in conjunction with some conventional medications or supplements (16). Certain herbal medicine preparations contain impurities that can lead to various health problems such as liver and kidney issues, gastrointestinal disorders, hyperthyroidism, psychological and neurological disorders, abnormalities of the nervous system, cancer, and lung damage (17).

Quality control and standardization are necessary due to the rising rate of toxicity from the use of herbal medicine. It is important to standardize every step of the production process. The phytochemical evaluation of herbs, which includes aspects like the choice and handling of herbal materials, efficacy, safety and stability assessment of the finished product, documentation of safety and risk based on experience, consumer information about the product, and product promotion, is a necessary part of standardization and quality control in the herbal industry (18). To safeguard consumers' health and safety, strict quality control procedures are necessary (19). Prior to preparing herbal remedies, plants should be identified and detected. Harmful pesticides should not be used on plants (20). Strict adherence to good laboratory and manufacturing practices, as well as good agricultural and collection practices, is necessary to guarantee quality control and standardization (21). Antibiotic resistance spreading quickly is a concern posed by the presence of bacteria in herbal medicine. Multi-drug resistance bacteria are found in herbal medicine in Saudi Arabia (22). Commercial medicinal herbal products in South Africa have been found to contain bacteria that can harm human health, including *Pseudomonas* sp., *Salmonella* sp., *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Staphylococcus* sp. Additionally, tests have shown that *Staphylococcus* sp. is resistant to certain

conventional antibiotics, including methicillin and vancomycin. *Bacillus cereus* diarrheal toxin is one of the bacterial toxins that have also been reported (23).

In Nigeria, including Abia State, the widespread use of herbal medicines raises important safety concerns when products are poorly regulated or prepared under inadequate hygienic conditions. Although herbal medicine has a long history of use, contamination and inappropriate handling can adversely affect product safety. This study was therefore designed to isolate and identify microorganisms contaminating herbal products sold in Aba, Abia State, Nigeria, and to evaluate the susceptibility of bacterial isolates to conventional antibiotics.

2. Materials and Methods

2.1 Study Site/ Sample Collection

The study was conducted in Aba, Abia State, Nigeria. Eleven herbal medicine samples were obtained from selected locations within the metropolis. The sample codes, product names, and collection locations are presented in Table 1.

Table 1: Sample Code and Location

Sample code	Sample name	Location
M1	Haonu Laha	Osisioma Junction
M2	Agbo Atosi	Aba Main Park
M3	Agbo Awoka	Aba Main Park
M4	Agbo-Iba	Aba Main Park
M5	Hajia Alima Powder	Osisioma Junction
M6	Hajia Ayisha Powder	Osisioma Junction
M7	Hajia Alima Powder	Osisioma Junction
M8	Hira Rhumatismes Tisane	Osisioma Junction
M9	Salwa Mu Waraka	Osisioma Junction
M10	Great Mentus	Aba Main Market
M11	Gbogbonise	Obikabia Junction

2.2 Preparation of Media

Nutrient agar, MacConkey agar, and Sabouraud dextrose agar were used for the isolation of bacteria and fungi and were prepared according to the manufacturers' instructions.

2.3 Sterilization/ Serial Dilution

The media and distilled water used for dilution were sterilized by autoclaving at 121 °C for 15 minutes at 15 psi. For liquid and powdered herbal medicines, 1 mL and 1 g, respectively, were added to 9 mL of sterile distilled water, and 10-fold serial dilutions were prepared.

2.4 Inoculation/ Incubation

Dilutions of 10^{-3} , 10^{-4} , and 10^{-5} were inoculated in duplicate using the pour-plate technique. Bacterial cultures were incubated at 37 °C for 24 hours, while fungal cultures were incubated at room temperature for 4-7 days. After incubation, colony morphology was recorded. Nutrient agar was used to subculture bacterial isolates to obtain pure cultures, which were maintained until required for further testing.

2.5 Biochemical Tests

Bacterial isolates were identified according to Cheesbrough (24). Isolates were Gram stained and subjected to biochemical testing, including catalase, oxidase, coagulase, indole, citrate, spore formation, and sugar fermentation tests.

2.6 Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar using the Kirby-Bauer disk-diffusion method with a 0.5 McFarland turbidity standard. Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria cited by the authors (25).

A pure culture of each bacterial isolate was inoculated into peptone broth and incubated at 37 °C for 24 hours until turbidity approximated the 0.5 McFarland standard. A sterile swab was then used to inoculate the standardized suspension onto Mueller-Hinton agar. Antibiotic disks were placed aseptically on the agar and plates were incubated at 37 °C for 24 hours. Inhibition-zone diameters were measured to the nearest millimetre and interpreted using the cited CLSI criteria (25).

The antibiotic disks used were ampicillin (10 µg), meropenem (10 µg), erythromycin (5 µg), tetracycline (30 µg), cotrimoxazole (25 µg), cefuroxime (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amoxicillin-clavulanate (Augmentin; 30 µg), vancomycin (30 µg), ceftazidime (10 µg), and cephalixin (1.5 µg).

3. Result

The morphological characteristics of isolates recovered on nutrient agar and MacConkey agar are presented in Tables 2 and 3. Recorded colony characteristics included shape, elevation, surface, size, and colour.

Table 2: Colonies Morphology of the Organism Isolated on Nutrient Agar

Isolate code	Shape	Elevation	Surface	Size	Colour
M1W1	Round	Flat	Rough	Small	White
M1W2	Round	Flat	Shiny	Small	White
M2M1	Round	Flat	Smooth	Pinpoint	Milky
M2W1	Round	Flat	Dull	Pinpoint	White
M2W2	Round	Hilly	Dry	Pinpoint	White
M3M1	Round	Flat	Slimy	Small	Milky
M3W1	Round	Flat	Brittle	Small	White
M3C1	Round	Flat	Shiny	Pinpoint	Creamy
M4M1	Round	Flat	Smooth	Small	Milky
M4W1	Round	Flat	Dry	Small	White
M5W1	Round	Flat	Rough	Small	White
M5M1	Filamentous	Flat	Rough	Small	Milky
M5M2	Round	Flat	Smooth	Pinpoint	Milky
M6M1	Round	Flat	Smooth	Small	Milky
M6O1	Round	Flat	Smooth	Pinpoint	Milky
M6W1	Round	Flat	Smooth	Small	White
M7M1	Filamentous	Flat	Rough	Small	Milky
M7M2	Round	Hilly	Smooth	Small	Milky
M8M1	Filamentous	Flat	Rough	Small	Milky
M8M2	Round	Flat	Shiny	Pinpoint	Milky

Isolate code	Shape	Elevation	Surface	Size	Colour
M9M1	Round	Flat	Shiny	Small	Milky
M9W1	Round	Hilly	Slimy	Small	White
M10W1	Round	Flat	Rough	Small	White
M10W2	Round	Hilly	Dry	Small	Milky
M10M1	Round	Flat	Shiny	Small	Milky
M11M1	Round	Flat	Dry	Small	Milky

KEY: M1W1= Medicine 1 White 1; M2M1= Medicine 2 Milky 1; M3C1= Medicine 3 Creamy 1; M6O1= Medicine 6 Orange 1.

Table 3: Colonies Morphology of the Organism Isolated on MacConkey Agar

Isolate code	Shape	Elevation	Surface	Size	Colour
M3P1	Round	Flat	Slimy	Small	Pink
M4P1	Round	Flat	Slimy	Large	Pink
M5P1	Round	Flat	Slimy	Small	Pink
M7P1	Round	Flat	Slimy	Small	Pink
M8P1	Round	Flat	Smooth	Large	Pink

KEY: M3P1= Medicine 3 Pink 1

Viable bacterial counts on nutrient agar are presented in Table 4.

Table 4: Viable Total Count on Nutrient Agar

Isolate code	Dilution factor	Bacterial colony count
M1	10 ⁻³	45
M1	10 ⁻⁴	22
M1	10 ⁻⁵	13
M2	10 ⁻³	4
M2	10 ⁻⁴	18
M2	10 ⁻⁵	1
M3	10 ⁻³	TNTC
M3	10 ⁻⁴	TNTC
M3	10 ⁻⁵	164
M4	10 ⁻³	TNTC
M4	10 ⁻⁴	TNTC
M4	10 ⁻⁵	90
M5	10 ⁻³	TNTC
M5	10 ⁻⁴	TNTC
M5	10 ⁻⁵	44
M6	10 ⁻³	150
M6	10 ⁻⁴	106
M6	10 ⁻⁵	106
M7	10 ⁻³	TNTC
M7	10 ⁻⁴	TNTC
M7	10 ⁻⁵	TNTC

Isolate code	Dilution factor	Bacterial colony count
M8	10 ⁻³	TNTC
M8	10 ⁻⁴	TNTC
M8	10 ⁻⁵	TNTC
M9	10 ⁻³	23
M9	10 ⁻⁴	2
M9	10 ⁻⁵	22
M10	10 ⁻³	10
M10	10 ⁻⁴	6
M10	10 ⁻⁵	7
M11	10 ⁻³	20
M11	10 ⁻⁴	11
M11	10 ⁻⁵	3

KEY: M= Medicine; TNTC= Too Numerous To Count

A total of 31 pure cultures were isolated and classified according to their sample sources. The bacterial isolates were identified as *Actinomyces* spp., *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Enterobacter* spp., and *Lactobacillus* spp. The fungal isolates included *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor* spp., *Penicillium* spp., *Trichosporon* spp., and *Aspergillus flavus*. Based on the reported colony counts, samples M8 and M7 showed the greatest contamination, whereas M2 and M10 showed the lowest levels (Figure 1).

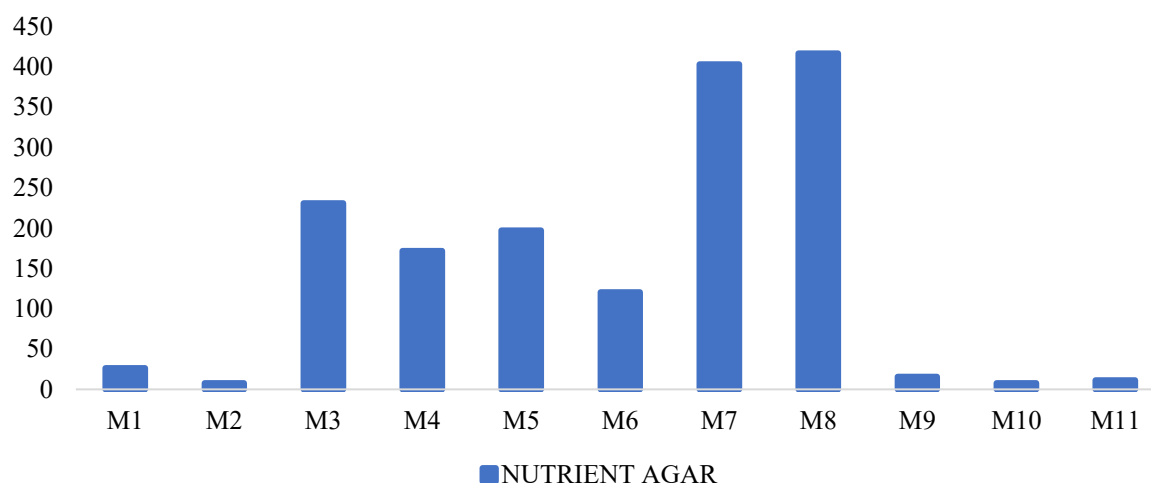


Figure 1: Level of contamination on the herbal products used

The colony morphology of suspected fungal isolates on Sabouraud dextrose agar is presented in Table 5.

Table 5: Colony Morphology of Organisms Isolated on Sabouraud Dextrose Agar

Isolate code	Dilution factor	Slant culture colour	Name of organism
M1	10 ⁻⁴	Black	<i>Aspergillus niger</i>
M2	10 ⁻³	Black	<i>Aspergillus niger</i>
M2	10 ⁻³	White	<i>Mucor</i> sp.
M2	10 ⁻⁴	Black	<i>Aspergillus niger</i>
M2	10 ⁻⁴	White	<i>Mucor</i> sp.
M2	10 ⁻⁵	White	<i>Mucor</i> sp.
M3	10 ⁻⁴	White	<i>Mucor</i> sp.
M3	10 ⁻⁵	White	<i>Mucor</i> sp.
M4	10 ⁻³	Bright green	<i>Aspergillus fumigatus</i>
M4	10 ⁻⁴	White	<i>Mucor</i> sp.
M4	10 ⁻⁴	Bright green	<i>Aspergillus fumigatus</i>
M4	10 ⁻⁴	Grey green	<i>Penicillium</i> sp.
M4	10 ⁻⁵	Creamy	<i>Trichosporon</i> sp.
M5	10 ⁻³	Black	<i>Aspergillus niger</i>
M5	10 ⁻⁴	Black	<i>Aspergillus niger</i>
M6	10 ⁻³	Black	<i>Aspergillus niger</i>
M6	10 ⁻³	White	<i>Mucor</i> sp.
M6	10 ⁻³	Grey green	<i>Penicillium</i> sp.
M7	10 ⁻³	Black	<i>Aspergillus niger</i>
M7	10 ⁻³	White	<i>Mucor</i> sp.
M7	10 ⁻⁵	Black	<i>Aspergillus niger</i>
M8	10 ⁻³	Black	<i>Aspergillus niger</i>
M8	10 ⁻⁴	Black	<i>Aspergillus niger</i>
M8	10 ⁻⁴	Grey green	<i>Penicillium</i> sp.
M8	10 ⁻⁴	Yellowish green	<i>Aspergillus flavus</i>
M8	10 ⁻⁵	Black	<i>Aspergillus niger</i>
M8	10 ⁻⁵	White	<i>Mucor</i> sp.
M10	10 ⁻³	Black	<i>Aspergillus niger</i>
M10	10 ⁻³	White	<i>Mucor</i> sp.

Fungal contamination was detected in 91% of the herbal samples. Among the fungal isolates, *Aspergillus niger* accounted for 41%, *Aspergillus flavus* for 4%, *Aspergillus fumigatus* for 7%, *Penicillium* spp. for 10%, *Mucor* spp. for 34%, and *Trichosporon* spp. for 4% (Figure 2).

were contaminated with *Aspergillus niger*, 4% with *Aspergillus flavus*, 7% with *Aspergillus*

fumigatus, 10% with *Penicillium* sp., 34% with *Mucor* sp., and 4% with *Trichosporon* sp. (Figure

2).

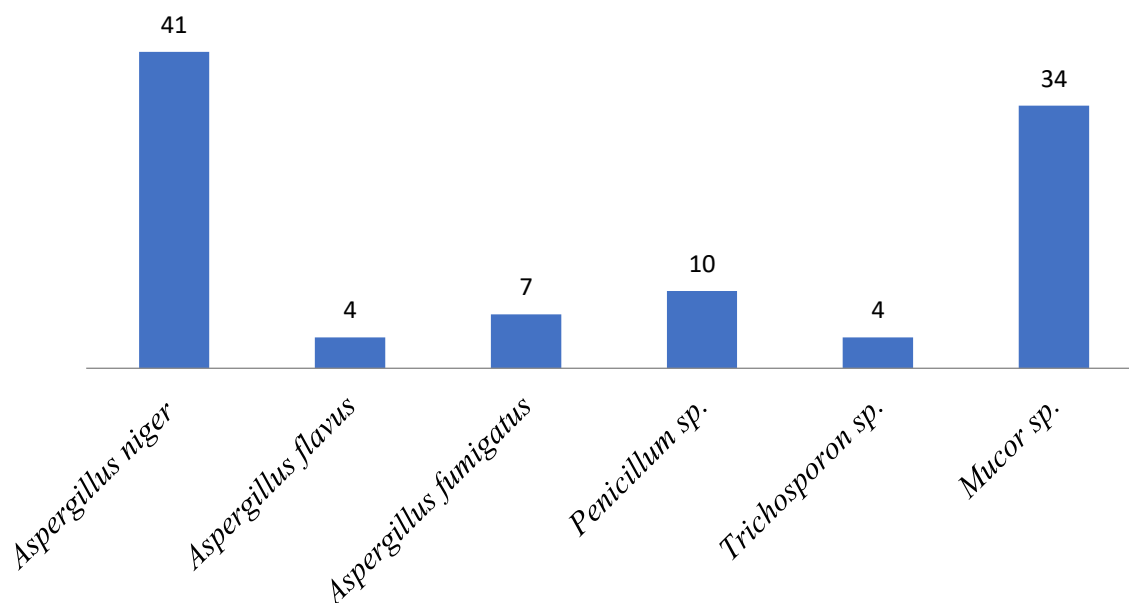


Figure 2: Prevalence rate of fungi isolated from the herbal products

The biochemical test results used for presumptive identification of bacterial isolates are presented in Table 6.

Table 6: Biochemical Test for Bacterial Isolates

Isolate code	Shape	Gram reaction	CAT	COA	OX	IND	CIT	SP	S	B	G	H ₂ S	Possible organism
M1W1	Rod	Positive	-	-	-	-	-	+	+	+	-	-	<i>Actinomyces</i> sp.
M1W2	Cocci	Positive	-	-	-	-	+	+	+	+	-	-	<i>Streptococcus</i> sp.
M2M1	Rod	Positive	-	-	-	-	-	+	+	+	-	-	<i>Actinomyces</i> sp.
M2W1	Cocci	Positive	+	+	-	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M2W2	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M3M1	Cocci	Positive	-	-	-	-	+	-	+	+	+	-	<i>Streptococcus</i> sp.
M3W1	Cocci	Positive	+	-	-	-	+	-	+	+	+	-	<i>Micrococcus</i> sp.
M3C1	Cocci	Positive	+	-	-	-	+	-	+	+	+	-	<i>Staphylococcus</i> sp.
M3P1	Rod	Negative	-	+	-	-	+	+	+	+	+	-	<i>Enterobacter</i> sp.
M4M1	Rod	Positive	+	+	+	-	-	+	+	+	-	-	<i>Bacillus</i> sp.
M4W1	Rod	Positive	-	+	-	-	-	+	+	+	-	-	<i>Bacillus</i> sp.
M4P1	Rod	Negative	-	+	-	-	+	+	+	+	+	-	<i>Enterobacter</i> sp.
M5W1	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M5M1	Cocci	Positive	+	+	+	-	+	+	+	+	-	-	<i>Staphylococcus</i> sp.
M5M2	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M5P1	Rod	Negative	-	+	-	-	+	+	+	+	+	-	<i>Enterobacter</i> sp.
M6M1	Rod	Positive	-	-	+	-	-	+	+	+	-	-	<i>Lactobacillus</i> sp.
M6O1	Rod	Positive	-	-	+	-	+	+	+	+	-	-	<i>Lactobacillus</i> sp.
M6W1	Cocci	Positive	+	-	-	-	-	-	+	+	-	-	<i>Staphylococcus</i> sp.

Isolate code	Shape	Gram reaction	CAT	COA	OX	IND	CIT	SP	S	B	G	H ₂ S	Possible organism
M7P1	Rod	Negative	-	+	-	-	+	-	+	+	+	-	<i>Enterobacter</i> sp.
M7M1	Rod	Positive	+	+	+	-	-	+	+	+	+	-	<i>Bacillus</i> sp.
M7M2	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M8M1	Cocci	Positive	+	+	+	-	+	+	+	+	-	-	<i>Staphylococcus</i> sp.
M8M2	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M8P1	Rod	Negative	-	+	-	-	+	-	+	+	+	-	<i>Enterobacter</i> sp.
M9M1	Rod	Positive	+	+	+	-	-	+	+	+	-	-	<i>Bacillus</i> sp.
M9W1	Cocci	Positive	+	+	+	-	-	-	+	+	+	-	<i>Staphylococcus</i> sp.
M10W1	Cocci	Positive	+	+	+	-	+	+	+	+	-	-	<i>Staphylococcus</i> sp.
M10W2	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
M10M1	Rod	Positive	-	+	-	-	+	+	+	+	-	-	<i>Bacillus</i> sp.
M11M1	Cocci	Positive	+	+	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.

KEY: CAT – Catalase test; COA – Coagulase test; OX – Oxidase test; IND – Indole test; CIT – Citrate test; SP – Spore formation test; Sugar fermentation test: S – Slope; B – Butt; G – Gas; H₂S – Hydrogen Sulphide formation.

Five of the 11 herbal products (45%) were classified as highly contaminated with bacteria because colony growth was too numerous to count. Among the bacterial isolates, 84% were classified by the authors as potentially pathogenic, including *Staphylococcus* spp. (54%), *Bacillus* spp. (19%), *Streptococcus* spp. (8%), and *Enterobacter* spp. (19%). *Lactobacillus* spp. and *Actinomyces* spp. together accounted for 13% of isolates, while *Micrococcus* spp. accounted for 3% (Figure 3).

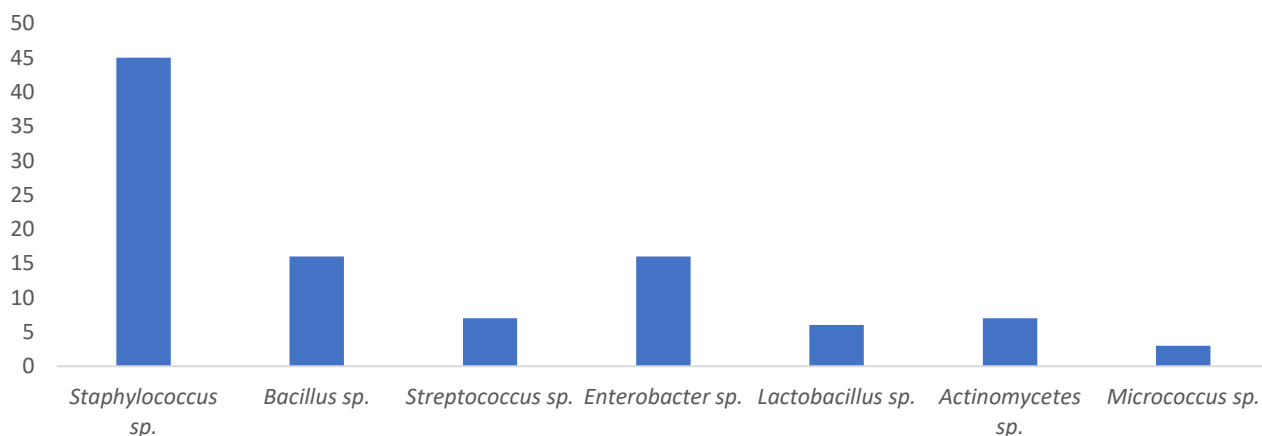


Figure 3: Growth percentage of bacteria Isolated from the herbal products

Antibiotic susceptibility results for the bacterial isolates are presented in Table 7, and the zone-size interpretation chart used by the authors is presented in Table 8.

Table 7: Antibiotic Susceptibility Test Data for Bacterial Isolates

Isolate code	ERY	MEM	AMP	CIP	GEN	CRX	COT	TET	VAN	CPZ	CP	AUG
M1W1	15	0	0	23	18	0	0	17	0	0	13	0
M1W2	0	0	0	22	15	0	0	10	0	0	11	0

Isolate code	ERY	MEM	AMP	CIP	GEN	CRX	COT	TET	VAN	CPZ	CP	AUG
M2M1	24	0	0	25	13	0	0	18	11	0	0	0
M2W1	23	0	0	16	14	0	0	18	10	0	0	0
M2W2	20	10	10	20	15	0	8	12	12	0	0	0
M3M1	16	0	0	30	17	0	21	15	10	0	14	0
M3W1	11	0	12	30	14	0	21	20	0	0	11	0
M3P1	15	0	0	25	18	0	19	20	0	0	15	0
M3C1	25	0	0	28	17	0	10	21	16	10	10	0
M4M1	23	11	0	21	15	0	0	22	14	12	13	0
M4W1	22	0	0	27	20	0	14	23	15	0	15	10
M4P1	12	0	0	29	16	0	15	19	10	0	0	0
M5W1	20	0	8	20	15	0	0	18	13	0	14	0
M5M1	20	0	0	20	15	0	0	19	11	0	11	0
M5M2	22	10	11	28	20	10	19	21	12	13	11	0
M5P1	10	12	9	24	17	0	14	16	0	10	13	10
M6M1	20	0	0	25	14	0	10	20	10	0	12	0
M6O1	25	0	0	20	18	0	0	15	14	10	11	10
M6W1	27	0	0	30	27	0	26	30	19	17	20	20
M7P1	13	0	0	25	15	0	0	16	0	0	10	0
M7M1	23	19	0	21	17	8	23	18	15	10	10	14
M7M2	22	0	0	19	16	10	14	20	15	0	20	0
M8M1	19	0	0	17	12	0	0	15	10	0	11	0
M8M2	19	0	0	21	16	0	12	20	14	8	12	0
M8P1	13	10	0	25	20	0	19	17	10	0	15	15
M9M1	15	0	0	22	15	0	8	17	12	0	12	0
M9W1	0	0	0	20	12	0	9	15	0	0	0	0
M10W1	0	0	0	24	16	0	0	14	0	0	13	0
M10W2	22	0	0	19	15	0	0	13	11	0	13	0
M10M1	20	0	0	21	15	0	10	15	14	0	18	0
M11M1	25	0	0	27	19	0	0	20	12	0	12	0

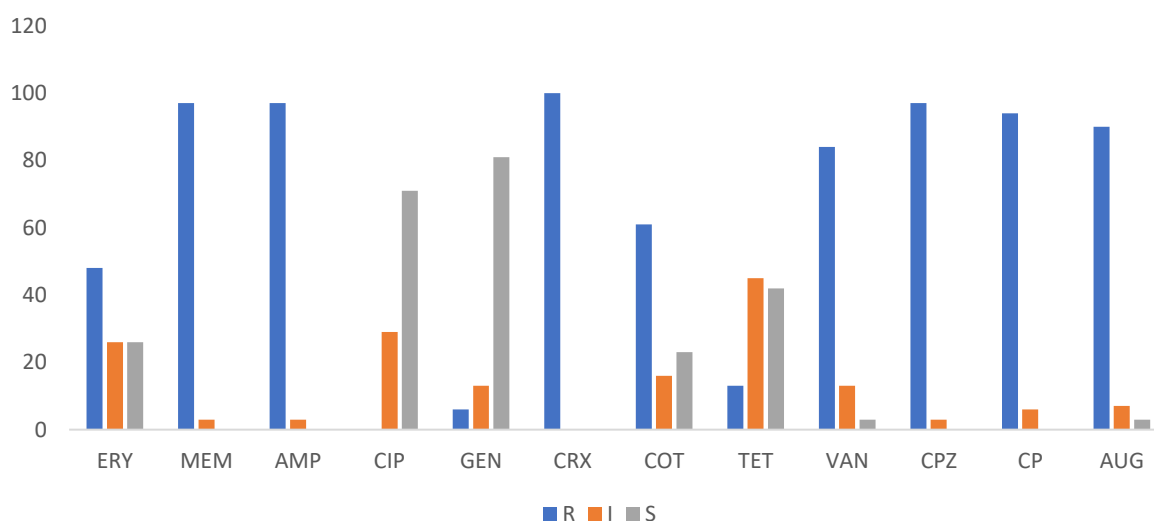
KEY: ERY – Erythromycin; MEM – Meropenem; AMP – Ampicillin; CIP – Ciprofloxacin; GEN – Gentamicin; CRX – Cefuroxime; COT – Cotrimoxazole; TET – Tetracycline; VAN – Vancomycin; CPZ – Ceftazidime; CP – Cephalexin; AUG – Augmentin

Table 8: Zone Size Interpretation Chart for the Susceptibility Test

Antimicrobial agent	Disc code	Resistance mm or less	Intermediate mm range	Sensitive mm or more
Erythromycin	ERY	13	14 – 22	23
Meropenem	MEM	15	16 – 22	23
Ampicillin	AMP	11	12 – 14	15
Ciprofloxacin	CIP	15	16 – 20	21
Gentamicin	GEN	12	13 – 14	15

Antimicrobial agent	Disc code	Resistance mm or less	Intermediate mm range	Sensitive mm or more
Cefuroxime	CRX	14	15 – 17	18
Cotrimoxazole	COT	10	11 – 15	16
Tetracycline	TET	14	15 – 18	19
Vancomycin	VAN	14	15 – 16	17
Ceftazidime	CPZ	14	15 – 17	18
Cephalexin	CP	18	19 – 20	21
Augmentin	AUG	13	14 – 17	18

Ciprofloxacin and gentamicin produced the highest overall inhibition-zone responses and were classified as susceptible against most bacterial isolates. The overall resistance, intermediate, and susceptible profiles are summarized in Figure 4.



KEY: R = Resistance; I = Intermediate; S = Sensitive

Figure 4: Level of antibiotic inhibition degree to bacteria isolates.

4. Discussion

Herbal medicines are widely used in Nigeria because of cultural traditions, accessibility, affordability, and the wide availability of medicinal plants. In settings where access to conventional healthcare is limited, herbal products may provide a readily available alternative or complement to conventional treatment (26).

Of the 11 samples examined, 4 (36%) had no label or stated indication, whereas 7 (64%) included an indication. Nine products (82%) had no stated expiry date and 2 (18%) included an expiry date. These findings are broadly comparable with those of Chiegeiro et al. (27), who also reported deficiencies in product labelling. Nigerian regulatory guidance requires appropriate labelling of regulated herbal products, including relevant registration and expiry information (28).

The bacterial and fungal colony characteristics are shown in Tables 2, 3, and 5, while viable bacterial counts are presented in Table 4. The authors reported bacterial contamination in 64% of the 11 samples and fungal contamination in 91%. Five samples (45%) were described as highly contaminated with bacteria because colony growth was too numerous to

count. Only sample M11 lacked fungal growth. Poor hygiene, contaminated water or equipment, packaging materials, storage conditions, and handling may contribute to microbial contamination of herbal products (27,32).

Aspergillus species, including *A. niger*, *A. fumigatus*, and *A. flavus*, were isolated from the herbal medicines (Table 5 and Figure 2), consistent with previous reports of fungal contamination in herbal products (33). *Trichosporon* spp. were also recovered, while *Mucor* spp. and *Penicillium* spp. were among the other fungal contaminants identified (1,33,34).

The bacterial findings in Table 6 and Figure 3 are also consistent with previous studies reporting *Staphylococcus* spp., *Bacillus* spp., *Enterobacter* spp., *Micrococcus* spp., and other bacterial contaminants in herbal products from Nigeria and elsewhere (1,27,29,31,32,35).

Antibiotic susceptibility testing (Tables 7 and 8; Figure 4) showed variable resistance and susceptibility among the isolates. *Streptococcus* spp. were classified as susceptible to ciprofloxacin and cotrimoxazole, *Actinomycetes* spp. and *Micrococcus* spp. to ciprofloxacin, and *Bacillus* spp. to erythromycin and cotrimoxazole. *Staphylococcus* spp. showed the greatest overall resistance in this dataset. Resistance among staphylococci is clinically important because established mechanisms include beta-lactamase production, altered penicillin-binding proteins, and other acquired resistance determinants (37). Similar resistance patterns have been reported in bacterial contaminants recovered from herbal medicines (22,29,35).

The occurrence of antibiotic-resistant bacteria in herbal products may reflect broader antimicrobial selection pressures in human, animal, agricultural, and environmental settings, together with inadequate hygienic control during production and distribution. Antimicrobial resistance can emerge and persist through multiple mechanisms, including target modification, drug inactivation, reduced permeability, and active efflux (36).

Potentially pathogenic bacteria in herbal products may be introduced through contaminated raw materials, water, equipment, packaging, handling, storage, or the manufacturing environment. Consumption of contaminated products may therefore pose a particular risk to immunocompromised or otherwise vulnerable consumers (23).

Actinomycetes are widely distributed in soil and other environmental habitats and can produce diverse biologically active secondary metabolites (38). *Lactobacillus* spp. and some *actinomycetes* may be associated with beneficial microbial communities or fermentation processes; however, their presence does not in itself establish product quality or therapeutic benefit (39,40). By contrast, fungal contamination by toxigenic *Aspergillus* species is a recognized safety concern because some strains can produce mycotoxins. The health effects of mycotoxin exposure depend on the toxin, dose, duration of exposure, and host factors (41).

5. Conclusions

This study identified substantial bacterial and fungal contamination in herbal medicines sampled in Aba metropolis and demonstrated resistance to several conventional antibiotics among the recovered bacterial isolates. Ciprofloxacin and gentamicin showed the greatest overall activity in the disk-diffusion testing reported by the authors. The findings support stronger microbiological quality control, hygienic production and handling, appropriate product labelling, and adherence to good manufacturing practices for herbal medicines. Continued surveillance is also warranted to better define the public-health significance of antimicrobial-resistant microorganisms in these products.

Conflict of Interest

The authors declare no conflicts of interest.

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