

Culture-Based Characterisation of Microbial Contaminants and Microplastic-Associated Biofilms in Locally Prepared Liquid Herbal Medicines (Agbo) Sold in Awka, Anambra State, Nigeria

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Abstract

Locally prepared and sold liquid herbal medicines are widely consumed in Nigeria, but inadequate quality control may expose consumers to microbial contaminants and emerging contaminants such as microplastics and their associated biofilms. This study investigated the occurrence of microbial contaminants, microplastics, and microplastic-associated biofilms in locally prepared and sold liquid herbal medicines in Awka Metropolis, Anambra State. Seven herbal medicine samples marketed for the treatment of infections were purchased from selected locations within Awka and analyzed using culture-based microbiological techniques, biochemical characterization, membrane filtration, light microscopy, and Congo Red Agar screening for biofilm production. Microbial loads were determined on Eosin Methylene Blue, de Man, Rogosa and Sharpe, and Nutrient Agar and expressed as CFU/mL. The highest microbial counts were recorded in Sample A on Nutrient Agar (2.34×10^5 CFU/mL) and EMB agar (2.02×10^5 CFU/mL), while Sample F recorded the highest MRS count (1.87×10^5 CFU/mL). The bacterial isolates recovered were Escherichia coli, Proteus mirabilis, Bacillus spp., and Klebsiella pneumoniae, while Aspergillus spp. and Saccharomyces spp. were recovered among the fungi. Suspected microplastics were detected in all seven samples, giving a total of 2,284 particles comprising 1,910 microfragments, 319 microfibres, and 55 microbeads; Sample A had the highest count (762), while Sample D had the lowest (108). Congo Red Agar demonstrated biofilm production by P. mirabilis and K. pneumoniae, and biofilm-like material associated with microplastic microfragments was observed for these same isolates. The findings demonstrate microbial and suspected microplastic contamination in the examined herbal medicines and highlight the possible role of microplastic particles in supporting microbial attachment. Improved hygiene, appropriate packaging and storage, routine microbiological surveillance, and further studies using polymer-confirmation techniques are recommended.

Keywords: *Herbal medicines; Microbial contaminants; Microplastics; Biofilms*

INTRODUCTION

Herbal medicines are preparations obtained from whole plants or plant parts that are used for the prevention, treatment, or management of diseases and maintenance of health. They may consist of leaves, roots, bark, flowers, seeds, or extracts, either singly or in combinations, and are available in forms such as powders, decoctions, infusions, capsules, tablets, and liquid mixtures. The World Health Organization (WHO) recognizes traditional medicine as an important component of healthcare globally and emphasizes the need for traditional medicine products to be safe, effective, evidence-based, and of appropriate quality (WHO, 2013; WHO, 2025). Despite their widespread use, the quality of herbal medicines can be affected by the quality of raw materials, preparation methods, handling, packaging, transportation, and storage. Plant materials may be exposed to microorganisms and other environmental contaminants before or during processing, making quality control an

important consideration in the production and use of herbal medicines (WHO, 2025). Herbal medicine has long been part of healthcare delivery in Nigeria and remains widely utilized in both rural and urban communities. Affordability, accessibility, cultural beliefs, and confidence in traditional healing practices have contributed to its continued use (WHO, 2019b; Aina *et al.*, 2020). Liquid herbal preparations are commonly used in the management of malaria, typhoid fever, gastrointestinal disorders, hypertension, diabetes, infertility, sexually transmitted infections, and inflammatory conditions. They are often produced by traditional healers, herbal practitioners, and commercial vendors and sold through markets, roadside stalls, motor parks, and shops (Aina *et al.*, 2020; Adigwe *et al.*, 2022; Osayi *et al.*, 2026). Adeoye and Etuk (2023) reported that 21.7% of pregnant women assessed in Ibadan used herbal medicines, demonstrating the continued importance of herbal preparations in healthcare-seeking behaviour. The preparation of locally sold liquid herbal medicines may involve the selection of medicinal plants, drying, grinding, extraction, blending of extracts, and packaging. Water is commonly used for extraction because of its availability and low cost, although other solvents may also be used (Adigwe *et al.*, 2022). Inadequate cleaning of equipment, contaminated water, exposure to dust, and improper handling can introduce contaminants into the preparations. Plastic bottles are also commonly used because they are inexpensive, lightweight, and readily available. In Awka and other urban centres, products may be displayed under ambient conditions without refrigeration or controlled storage facilities, exposing them to dust, heat, sunlight, and repeated handling. Differences in production, packaging, and storage practices, together with limited standardization in some informal settings, may result in variable product quality and raise concerns about consumer safety (Adigwe *et al.*, 2022; WHO, 2018; Roziman *et al.*, 2024; Mponda *et al.*, 2025). Microbial contamination is an important quality and safety concern associated with liquid herbal medicines. Contamination may occur from raw plant materials, water used during processing, equipment, packaging materials, handling, transportation, and storage. Plant materials obtained from farms, forests, and open fields may be exposed to soil microorganisms, animal waste, dust, and wastewater. Untreated or minimally treated water may introduce bacteria and fungi, while inadequately cleaned equipment, repeated manual handling, and improper storage provide opportunities for microorganisms to enter and persist in the products. The high moisture content of liquid herbal preparations can further support microbial survival and growth under favourable conditions. A systematic review of studies conducted across Africa reported microbial contamination in 1,791 of 1,996 herbal medicine samples examined, with bacterial contamination reported in 98% of the studies and fungal contamination in 70% (Ahiabor *et al.*, 2024). Microorganisms associated with herbal medicines include bacteria, fungi, and yeasts. Bacterial contaminants of importance include *Escherichia coli*, *Proteus spp.*, *Klebsiella spp.*, and *Bacillus spp*. *E. coli* is a Gram-negative intestinal bacterium whose presence in herbal medicines is generally regarded as an indicator of faecal or sanitary contamination, although not all strains are pathogenic. *Proteus spp.* and *Klebsiella spp.* are opportunistic pathogens that may cause urinary tract, wound, bloodstream, and other infections (Podschun and Ullmann, 1998), while *Bacillus spp.* are widely distributed in soil, dust, water, and plant materials and produce resistant spores capable of surviving adverse conditions. Their occurrence in herbal preparations may therefore reflect environmental contamination, inadequate handling, or poor processing conditions. Fungi are also important contaminants because their spores are widely distributed in soil, air, water, and decaying organic matter. Moisture, high humidity, and prolonged storage may favour fungal growth. *Aspergillus spp.* are frequently encountered in contaminated herbal medicines and may enter products through plant materials, air, dust, harvesting, drying, storage, and transportation (Ahiabor *et al.*, 2024). Certain species produce mycotoxins, while others may cause opportunistic infections. Other filamentous fungi such as *Penicillium*, *Rhizopus*, and *Mucor* may contribute to spoilage and, in susceptible individuals, opportunistic disease (Cornely *et al.*, 2019). Yeasts such as *Saccharomyces* and *Candida* can also occur in herbal preparations as a result of contaminated raw materials, water, handling, or environmental exposure (Enache-Angoulvant and Hennequin, 2005; Pappas *et al.*, 2018). Thus, the presence of both bacterial and fungal contaminants may compromise the microbiological quality and safety of locally prepared herbal medicines.

In addition to microbial contamination, microplastics represent an emerging concern in consumable products. Microplastics are small plastic particles measuring less than 5 mm and may originate from the fragmentation of larger plastic materials or from plastics manufactured in microscopic sizes for particular applications (Thompson *et al.*, 2004; GESAMP, 2019; Gündoğdu *et al.*, 2024). They are widely distributed in aquatic and terrestrial environments, food, and drinking water. Their persistence results from the resistance of plastic materials to biodegradation, while their small size facilitates movement through water and air and increases opportunities for human exposure (WHO, 2019a). Microplastics may be classified as primary or secondary according to their origin. Primary microplastics are intentionally manufactured at microscopic sizes for industrial and commercial applications and include microbeads, plastic pellets, glitter particles, and some synthetic fibres. Secondary microplastics result from the physical, chemical, and environmental breakdown of larger plastic materials through processes such as sunlight exposure, heat, mechanical abrasion, and weathering (Gündoğdu *et al.*, 2024). Plastic bottles, food packaging, disposable containers, bags, synthetic textiles, tyre wear, industrial activities, wastewater, and degraded plastic waste are among the major sources of secondary microplastics. This distinction is particularly relevant to liquid herbal medicines because plastic containers are commonly used for packaging and may provide a potential source of secondary microplastics. Microplastics vary in size, shape, surface texture, density, and polymer composition. They may occur as fragments, fibres, films, beads, foams, pellets, or irregular particles, while commonly detected polymers include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS),

and polyvinyl chloride (PVC) (Gündoğdu *et al.*, 2024). Smaller particles have a larger surface area relative to their volume, while weathering can produce rough surfaces and cracks that increase available sites for microbial attachment. These characteristics influence their persistence, transport, interaction with contaminants, and ability to support microbial colonization. Human exposure to microplastics occurs mainly through ingestion and inhalation, with food, drinking water, and airborne particles serving as important pathways. Although the health implications of exposure are not yet fully established, experimental evidence indicates that microplastics may contribute to oxidative stress, inflammation, and cellular damage under certain conditions (Prata *et al.*, 2020; Kadac-Czapska *et al.*, 2024; Grari *et al.*, 2026). Their ability to interact with chemical contaminants and support microbial colonization raises additional concerns for products consumed directly. Microplastics have been detected in drinking water and various food and beverage products (Cox *et al.*, 2019; Gündoğdu *et al.*, 2024), although evidence concerning herbal medicines remains limited. Yang *et al.* (2025) reported microplastic release from packaging used for liquid herbal medicines and observed increased particle release following heating. The persistence and surface characteristics of microplastics also allow them to serve as substrates for microbial attachment. Microbial colonization begins when microorganisms encounter a plastic surface and attach to it, initially through weak interactions. Organic materials can form a conditioning layer on the surface, while roughness, cracks, hydrophobicity, nutrient availability, temperature, pH, oxygen, moisture, and polymer characteristics can influence colonization (Amaral-Zettler *et al.*, 2020; Ventura *et al.*, 2024). Continued attachment and microbial multiplication may result in the development of biofilms. Biofilms are structured microbial communities enclosed within an extracellular polymeric substance (EPS) matrix. Their development generally involves initial attachment, irreversible attachment, microcolony formation, maturation, and dispersion. The EPS matrix, consisting mainly of polysaccharides, proteins, lipids, extracellular DNA, and water, anchors microorganisms to surfaces, traps nutrients, and provides protection from environmental stress (Flemming *et al.*, 2016). Microorganisms within biofilms may therefore survive conditions that would normally affect free-living or planktonic cells, including exposure to disinfectants and antimicrobial agents (Ventura *et al.*, 2024). Biofilms can also facilitate interactions and exchange of genetic material between microorganisms. The microbial communities that develop on plastic surfaces are commonly referred to as the plastisphere. Zettler *et al.* (2013) introduced the term to describe microbial communities associated with plastic debris in aquatic environments. Plastisphere communities may contain bacteria, fungi, algae, protozoa, and viruses, with their composition influenced by plastic characteristics and environmental conditions (Jacquin *et al.*, 2019; Ventura *et al.*, 2024). Because microplastics persist and can move between environments, they can transport attached microorganisms and introduce them into new ecological niches. Microplastic-associated biofilms have also attracted attention because microbial interactions within the biofilm may facilitate horizontal gene transfer and the dissemination of antimicrobial resistance genes (Arias-Andres *et al.*, 2018; Stevenson *et al.*, 2024; Goswami *et al.*, 2025; Zhang *et al.*, 2026; Ormsby and Quilliam, 2026). The detection of microbial contaminants commonly involves culture-based methods, in which samples are inoculated onto appropriate media and incubated to recover viable microorganisms. Colony morphology, Gram staining, microscopic examination, and biochemical tests can then support identification. Nutrient Agar supports a broad range of bacteria, Eosin Methylene Blue Agar is useful for enteric Gram-negative bacteria, while Sabouraud Dextrose Agar supports the cultivation of yeasts and molds. Culture-based methods remain useful for routine microbiological quality assessment because they are relatively reliable, inexpensive, and suitable for resource-limited laboratory settings (WHO, 2007; WHO, 2011). Detection of suspected microplastics generally involves separation of particles from the sample matrix followed by microscopic examination and, where available, chemical confirmation. Membrane filtration allows suspended particles in liquid samples to be retained on a filter for examination according to their size, shape, and other visible characteristics. Although techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy can confirm polymer composition, microscopic examination provides a practical preliminary approach where advanced instrumentation is unavailable (WHO, 2019a; WHO, 2022). Although microbial contamination of herbal medicines has been reported in different parts of Nigeria (Ilozue and Okoye, 2024), relatively limited information is available on the occurrence of microplastics and particularly, microplastic-associated biofilms in locally sold liquid herbal medicines in Nigeria. In Awka, previous studies have reported bacterial and fungal contamination of liquid herbal medicines (Archibong *et al.*, 2017), but information on the occurrence of microplastics and their associated biofilms in these products remains limited, creating a knowledge gap. The specific objectives are to isolate and identify microbial contaminants present in locally sold liquid herbal medicines obtained from different locations in Awka and to detect and enumerate suspected microplastic particles and biofilm-like materials present in the herbal medicinal samples. The aim of this study is to investigate the occurrence of microbial contaminants, microplastics, and microplastic-associated biofilms in locally sold liquid herbal medicines in Awka, Anambra State.

MATERIALS AND METHODS

Sample Collection

Seven (7) locally prepared and sold liquid herbal medicines marketed for the treatment of infections were purchased from seven different vendors in Nibo, Amawbia, Eke Awka, and Ifite within the Awka metropolis. Samples were collected in sterile glass containers, appropriately labelled, and transported to the laboratory for microbiological and microplastic analyses.

Sterilization of Materials and Media Preparation

Glassware and other heat-stable materials were washed, rinsed with distilled water, and sterilized in an autoclave at 121°C for 15 minutes under 15 psi pressure. Inoculating

loops and forceps were sterilized by flaming, while work benches were disinfected with 70% ethanol. Nutrient Agar (NA), Eosin Methylene Blue (EMB) agar, Sabouraud Dextrose Agar (SDA), and de Man, Rogosa and Sharpe (MRS) agar were prepared according to the manufacturers' instructions and sterilized at 121°C for 15 minutes. The media were cooled to approximately 45–50°C before being poured into sterile Petri dishes.

Serial Dilution and Inoculation of Samples

Each sample was thoroughly shaken, after which 1 mL was aseptically transferred into 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Serial dilution was continued to 10^{-3}. One millilitre of the appropriate dilution was inoculated into duplicate plates containing NA, EMB agar, SDA, and MRS agar using the pour plate technique. NA, EMB, and MRS plates were incubated at 37°C for 24 hours, while SDA plates were incubated at 28°C for 3–5 days. NA, EMB, and MRS were used for microbial enumeration, while SDA was used for fungal isolation. Colony counts were recorded and expressed as CFU/mL.

Purification and Identification of Isolates

Distinct bacterial colonies from EMB and MRS agar were subcultured onto fresh media and incubated at 37°C for 24 hours to obtain pure cultures. Fungal colonies from SDA were similarly subcultured and incubated at 28–30°C for 3–5 days. The isolates were characterized using cultural and microscopic characteristics. Bacterial identification was supported by Gram staining and biochemical tests, including catalase, motility, citrate utilization, urease, indole, oxidase, methyl red, Voges–Proskauer, and sugar fermentation tests, following standard procedures described by Holt *et al.* (1994) and Cheesbrough (2006).

Lactophenol Cotton Blue (LPCB) Staining

A portion of each fungal colony was transferred onto a clean glass slide, stained with lactophenol cotton blue, covered with a coverslip, and examined microscopically at ×10 and ×40 objectives. Fungal structures such as hyphae, conidiophores, conidia, pseudohyphae, and chlamydospores, where present, were used for identification.

Congo Red Agar Test for Biofilm Production

Congo Red Agar was used for qualitative screening of biofilm production by the bacterial isolates. Brain Heart Infusion agar supplemented with 5% (w/v) sucrose was prepared, and 0.08% (w/v) Congo red solution was added after cooling to approximately 55°C. Pure bacterial isolates were streaked onto the prepared medium and incubated aerobically at 37°C for 24 hours. Black colonies with a dry or crystalline appearance were recorded as positive for biofilm production, while red or pink colonies were recorded as negative. The procedure followed Harika *et al.* (2020), based on Freeman *et al.* (1989).

Detection and Characterization of Suspected Microplastics and Associated Biofilms

The herbal medicine samples were subjected to oxidative digestion with 30% hydrogen peroxide (H_2O_2) at 60°C for four days (96 hours) to reduce the organic herbal material. After digestion and cooling, 0.75 L (750 mL) of each undiluted sample was filtered through a sterile 0.45 µm membrane using a membrane filtration apparatus. The filters were removed with sterile forceps and air-dried at room temperature. The dried filters were examined under a light microscope at ×10 and ×40 magnifications. Suspected microplastic particles were identified based on observable morphological characteristics, counted manually, and classified as fibres, fragments, or beads. Microorganisms observed in association with the particles were recorded. Isolates that showed positive biofilm production on Congo Red Agar and had microorganisms observed in association with suspected microplastic particles were classified as microplastic-associated biofilms.

Table 1: Microbial loads (CFU/mL) of the herbal medicine samples on different media.

Sample	EMB Agar (CFU/mL)	MRS Agar (CFU/mL)	Nutrient Agar (CFU/mL)
A	2.02×10^5	1.88×10^5	2.34×10^5
B	3.20×10^4	9.70×10^4	6.30×10^4
C	1.27×10^5	1.47×10^5	1.82×10^5
D	1.60×10^5	8.20×10^4	1.60×10^4
E	1.00×10^5	1.55×10^5	1.77×10^5
F	5.20×10^4	1.87×10^5	3.20×10^4
G	1.09×10^5	4.90×10^4	1.77×10^5

Key: A = Eke Nibo; B = Oyenwochichi Nibo; C = Red Cross, Army Barracks, Amawbia; D = Eke Amawbia; E = First Gate, Eke Awka; F = Gbarimgba, Eke Awka; G = Ifite School Gate, Ifite Awka.

Biosafety and Quality Control

Standard microbiological safety procedures were followed throughout the study. Personal protective equipment was used, aseptic techniques were maintained, and work surfaces were disinfected before and after laboratory procedures. Media, glassware, and instruments were sterilized appropriately, while microbial cultures and contaminated materials were properly sterilized and disposed of. For quality control, culture media were prepared according to manufacturers' instructions and their sterility was checked using uninoculated plates. Reagents were properly prepared and stored, laboratory equipment was cleaned and used according to standard procedures, and samples and filters were kept covered as much as possible to reduce contamination from airborne particles. Colony counts and observations were carefully recorded to minimize errors.

RESULTS PRESENTATION

Table 1 shows the microbial loads of the herbal medicine samples. Sample A recorded the highest count of 2.02×10^5 CFU/mL on EMB Agar and 2.34×10^5 CFU/mL on Nutrient Agar, while Sample F recorded the highest count of 1.87×10^5 CFU/mL on MRS Agar.

Table 2 shows the cultural characteristics of bacterial isolates. The isolates showed varying cultural characteristics, with

Table 2: Cultural characteristics of bacterial isolates.

Isolate	Media	Colour	Opacity	Margin	Elevation	Texture	Surface
A	EMB	Green metallic sheen	Opaque	Entire	Convex	Moist	Smooth
B	EMB	Colourless	Translucent	Irregular	Flat	Moist	Smooth
C	MRS	Creamy-white	Opaque	Undulate	Umbonate	Dry	Wrinkled
D	EMB	Dark purple	Opaque	Entire	Convex	Mucoid	Smooth
E	EMB	Green metallic sheen	Opaque	Entire	Convex	Moist	Smooth

Table 3: Cultural characteristics of mold isolates.

Isolate	Media	Colour (top)	Colour (reverse)	Opacity	Margin	Elevation	Texture	Surface
F	SDA	Yellow-Green	Creamy	Opaque	Entire	Flat	Velvety	Dry

Table 4: Cultural characteristics of yeast isolates.

Isolate	Media	Colour	Opacity	Margin	Elevation	Texture	Surface
G	SDA	Creamy-white	Opaque	Entire	Convex	Butyrous	Smooth

isolates A and E appearing green metallic in colour, while isolate C appeared creamy-white.

Table 3 shows the cultural characteristics of the mold isolate. Isolate F appeared yellow-green on the top and creamy on the reverse, opaque, with an entire margin, flat elevation, velvety texture and a dry surface.

Table 4 shows the cultural characteristics of the yeast isolate. Isolate G appeared creamy-white in colour, opaque, with an entire margin, convex elevation, butyrous texture and smooth surface.

Table 5 shows the biochemical characteristics of the bacterial isolates. The probable organisms identified were *Escherichia coli*, *Proteus mirabilis*, *Bacillus spp.* and *Klebsiella pneumoniae*.

Table 6 shows the microscopic characteristics of the mold isolate. Isolate F showed septate and branched hyphae with conidia, while budding cells were absent. The probable organism was identified as *Aspergillus spp*.

Table 7 shows the microscopic characteristics of the yeast isolate. It appeared Gram-positive with oval budding yeast cells and blastoconidia, occurring singly and in pairs. The probable organism was identified as *Saccharomyces spp*.

Table 6: Microscopic characteristics of mold isolate.

Isolate	Hyphae	Asexual Spore	Budding cells	Probable Organism
F	Septate / Branched	Conidia	Absent	*Aspergillus spp.*

Table 7: Microscopic characteristics of yeast isolate.

Isolate	Gram Reaction	Morphology	Blastoconidia	Arrangement	Pseudohyphae	Probable Organism
G	Gram-positive	Oval budding yeast cells	Present	Cells occur singly and in pairs	Absent	*Saccharomyces spp.*

Isolate	Gram Reaction	Motility	Urease	Citrate	Catalase	MR	VP	Indole	Oxidase	Lact.	Suc.	Fruc.	Gluc.	Probable Organism
A	Gram-negative rod	+	−	−	+	+	−	+	−	AG	−	AG	AG	*Escherichia coli*
B	Gram-negative rod	+	+	+	+	+	−	−	−	−	−	A+	AG	*Proteus mirabilis*
C	Gram-positive rod	+	−	+	+	−	+	−	−	−	A+	A+	A+	*Bacillus spp.*
D	Gram-negative rod	−	+	+	+	−	+	−	−	AG	AG	AG	AG	*Klebsiella pneumoniae*
E	Gram-negative rod	+	−	−	+	+	−	+	−	AG	−	AG	AG	*Escherichia coli*

Key:

Abbreviation	Meaning	Abbreviation	Meaning	Abbreviation	Meaning
+ / −	Positive / Negative	MR	Methyl Red test	Suc.	Sucrose
AG	Acid and gas production	VP	Voges–Proskauer test	Fruc.	Fructose
A+	Acid production only	Lact.	Lactose	Gluc.	Glucose

Table 8: Distribution and enumeration of suspected microplastic particles in liquid herbal medicine samples.

Sample	Micro-fragments	Micro-beads	Micro -fibre	Total No. of Microplastics
A	501	45	216	762
B	301	0	0	301
C	481	0	0	481
D	80	6	22	108
E	152	0	48	200
F	188	0	7	195
G	207	4	26	237

Table 8 shows the distribution and enumeration of microplastic particles in the liquid herbal medicine samples. Microfragments were the most abundant, while Sample A had the highest total number of suspected microplastics at 762 and Sample D had the lowest at 108.

Table 9 shows the biofilm production by microbial isolates on Congo Red Agar. Biofilm/slime production was observed in *Proteus mirabilis* and *Klebsiella pneumoniae*, while the other isolates showed no biofilm production.

Table 10 shows the microplastic-associated biofilm formation observed through microscopy. Biofilm-like material was observed on microfragments associated with *Proteus mirabilis* and *Klebsiella pneumoniae*, while no biofilm was observed in the other isolates.

DISCUSSION

The results demonstrated microbial contamination, suspected microplastic contamination, and biofilm formation by some bacterial isolates in the locally prepared and sold liquid herbal medicines examined. The bacterial isolates recovered were *Escherichia coli*, *Proteus mirabilis*, *Bacillus spp.*, and *Klebsiella pneumoniae*, while *Aspergillus spp.* and *Saccharomyces spp.* were recovered among the fungi. Suspected microplastics occurred in all seven samples, with microfragments, microfibres, and microbeads detected at varying levels. Biofilm production was demonstrated by *P. mirabilis* and *K. pneumoniae*, which were also associated with biofilm-like material on microplastic surfaces. The bacterial counts varied among samples and culture media, reflecting differences in the nutritional and selective properties of the media. Ahiabor *et al.* (2024) similarly reported considerable variation in microbial loads among herbal medicine products. The recovery of *E. coli*, *P. mirabilis*, *Bacillus spp.*, and *K. pneumoniae* is consistent with previous reports of bacterial contamination in herbal medicines (Archibong *et al.*, 2017; Ilozue and Okoye, 2024). Their presence may be associated with contaminated plant materials, water, dust, handling, utensils, processing surfaces, and storage conditions. The detection of *E. coli* is particularly relevant as it can indicate faecal or sanitary contamination, although its presence does not imply that every strain is pathogenic. The occurrence of *Bacillus spp.* may also reflect their widespread distribution in soil, dust, water, and plant materials, as well as the ability of their spores to survive adverse conditions. The fungal isolates comprised *Aspergillus spp.* and *Saccharomyces spp*. The mold exhibited septate, branched hyphae and conidiospores, while the yeast showed oval budding cells. The recovery of *Aspergillus spp.* agrees with Ahiabor *et al.* (2024), who reported fungal contamination in 70% of the African studies reviewed and identified *Aspergillus* as the most frequently reported fungal genus. Fungal contamination may result from contaminated plant materials, air, dust, handling, and storage. Suspected microplastics were detected in all seven samples, with microfragments being the most abundant particle type, followed by microfibres and microbeads. Sample A recorded the highest number of suspected particles, whereas Sample D recorded the lowest, indicating variation in contamination between products. Packaging, handling, transportation, and storage conditions may contribute to this variation. Pirsaheb

Table 9: Biofilm production by microbial isolates on Congo Red Agar.

Isolate	Probable Organism	Congo Red Reaction	Results/Interpretation
A	*Escherichia coli*	Pink, smooth colonies	No biofilm production
B	*Proteus mirabilis*	Black, crystalline colonies	Biofilm/slime production
C	*Bacillus spp.*	Red, smooth colonies	No biofilm production
D	*Klebsiella pneumoniae*	Black, crystalline colonies	Biofilm/slime production
E	*Escherichia coli*	Pink, smooth colonies	No biofilm production
F	*Aspergillus spp.*	Red, smooth colonies	No biofilm production
G	*Saccharomyces spp.*	Red, smooth colonies	No biofilm production

Table 10: Microplastic-associated biofilm formation observation through microscopy.

Isolates	Probable Organism	Microplastic Particle	Biofilm Observation	Result
A	*Escherichia coli*	Microfragments	No biofilm observed	Negative
B	*Proteus mirabilis*	Microfragments	Biofilm-like material observed	Positive
C	*Bacillus spp.*	Microfragments	No biofilm observed	Negative
D	*Klebsiella pneumoniae*	Microfragments	Biofilm-like material observed	Positive
E	*Escherichia coli*	Microfragments	No biofilm observed	Negative
F	*Aspergillus spp.*	Microfragments	No biofilm observed	Negative
G	*Saccharomyces spp.*	Microfragments	No biofilm observed	Negative

et al. (2025) detected microplastics in 92% of bottled herbal distillates examined, while Yang *et al.* (2025) reported microplastic release from packaging used for liquid herbal medicines. These findings support the possibility that plastic packaging contributed to the contamination observed in the present study. Microbial and microplastic contamination showed some overlap but were not directly proportional across all samples. Sample A had both the highest microplastic count and high bacterial counts, whereas Sample D had the lowest microplastic count but a comparatively high EMB count. This suggests that microbial and microplastic contamination were influenced by multiple factors rather than a single common source. Biofilm production was demonstrated by *P. mirabilis* and *K. pneumoniae* using Congo Red Agar, with both producing black, crystalline colonies. Microscopic examination also showed biofilm-like material associated with microplastic microfragments for these two isolates, whereas such material was not observed for the other isolates. This agreement between the culture-based test and microscopic observation suggests an association between these bacterial isolates and the suspected microplastic particles. Microplastic surfaces can support microbial attachment and biofilm development, with factors such as surface roughness, hydrophobicity, polymer characteristics, and environmental conditions influencing colonization (Huang *et al.*, 2024; Ventura *et al.*, 2024; Ormsby and Quilliam, 2026). The presence of microbial loads alongside suspected microplastics is relevant to the quality of locally marketed herbal medicines. Similarly, the observed material on the particles represents biofilm-like material and does not by itself establish the complete composition or viability of the attached microorganisms. Overall, the findings indicate that the herbal medicines examined contained microbial contaminants and suspected microplastics, with some bacterial isolates demonstrating biofilm-forming activity and association with microplastic surfaces. The results highlight the need for improved hygienic practices, appropriate packaging and storage, microbiological monitoring, and further investigation of microplastic contamination and microbial colonization in herbal medicinal products.

CONCLUSION

This study demonstrated the occurrence of microbial contamination and suspected microplastic particles in locally prepared and sold liquid herbal medicines in Awka, Anambra State. Considerable bacterial and fungal loads were recorded, with *Escherichia coli*, *Proteus mirabilis*, *Bacillus spp.*, *Klebsiella pneumoniae*, *Aspergillus spp.* and *Saccharomyces spp.* recovered from the samples. Suspected microplastic particles were observed in all seven samples, with microfragments being the most abundant type. Biofilm production was demonstrated in *P. mirabilis* and *K. pneumoniae*, which were also the only isolates associated with observed biofilm-like material on particle surfaces. The study therefore highlights the occurrence of microbial and suspected plastic particle contamination in the examined herbal medicines and the possible association of some bacterial isolates with these particles.

RECOMMENDATIONS

1. Improved hygiene should be maintained during the preparation, handling, packaging, transportation and storage of locally sold herbal medicines to reduce microbial contamination.
2. Producers and vendors should use clean raw materials, water, utensils and suitable packaging materials, while proper storage conditions should be maintained (NAFDAC, 2021a).
3. Regular microbiological quality assessment of herbal medicines should be encouraged by relevant regulatory authorities (NAFDAC, 2021b), with attention to emerging contaminants such as microplastic particles and their association with microbial biofilms.
4. Further studies should examine a larger number of herbal medicine samples, determine the polymer composition of the suspected particles and further investigate the relationship between particle contamination and biofilm formation.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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