PROJECT RESEARCH WORK

ON

QUANTITATIVE AND QUALITATIVE ANALYSIS OF MICROBIAL LOADS IN SOME HERBAL MEDICINES SOLD IN ILORIN

PRESENTED BY: GROUP 3

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CERTIFICATION

This is to certify that the project titled QUALITATIVE AND QUANTITATIVE ANALYSIS OF MICROBIAL LOADS FOUND IN SOME HERBAL MEDICINES SOLD IN ILORIN was successfully written and submitted by EJIGA DAVID UGBEDEOJO with Matric Number HND/23/SLT/FT/0005 to the department of science laboratory technology, Microbiology unit, institute of applied science (IAS), Kwara state polytechnics, ilorin and has been read and approved in partial fulfillment of the requirements for the award of Higher national diploma in Science Laboratory Technology (Microbiology unit), Under the supervision of MRS. OTUYO MUJIBAT. This project has been examined and approved as meeting the required standard for the above academic award.

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(Head of Department)

DEDICATION

This project report is dedicated to the Almighty God, The king of kings and the Lord of Lords for his Grace, mercy and enablement throughout this project research. May his name be lifted high.

ACKNOWLEDGEMENT

My first and foremost appreciation Goes to the Almighty God, Creator of the universe, the giver of life for the Grace and enablement given unto me on this project report, I give him all glory for the strength, wisdom, and grace to complete this project successfully.

Also my sincere gratitude goes to my project supervisor **MRS. OTUYO MUJIBAT** for her valuable guidance, support, and encouragement throughout the course of this work. I also appreciate the Head of Department, lecturers, and staff of the Microbiology unit for their contributions toward my academic growth. Special thanks to my ever loving parents **MR. EJIGA AUSTINE AND MRS. EJIGA JOY** for their help and support both financially and through constant prayers. And to my Honourably friends and loved ones and everyone who supported me directly or indirectly, I say a big thank you for your constant prayers, support and motivation. May God bless you all.**EJIGA DAVID UGBEDEOJO.**

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ABSTRACT

This study evaluated the microbial load and isolated microbial species from herbal concoctions sold in Ilorin, Kwara State, Nigeria. Four sealed herbal samples labeled J, K, O, and F were randomly collected from vendors and subjected to bacteriological and mycological analyses. The total bacterial count ranged from 1.4×10^{7} to 2.0×10^{7} CFU/ml, while the fungal count ranged from 5.3×10^{5} to 8.2×10^{5} CFU/ml. Biochemical tests and gram staining identified several bacterial isolates including Staphylococcus aureus, Pseudomonas spp., and E. colispp. Fungal isolates included Aspergillus niger and Aspergillus flavus. The results revealed microbial loads exceeding acceptable limits for oral herbal preparations, raising public health concerns. The study underscores the need for better regulation and hygienic preparation of herbal products in local markets.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Herbal medicine, a form of complementary and alternative medicine, is becoming increasingly popular in both developing and developed countries. A World Health Organization (WHO) survey indicates that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (Okunlola et al., 2007). WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicine (WHO, 2000).

In Nigeria, there appears to be an overwhelming increase in the public

awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases. This may not be unconnected to the active mass media advertisement embarked upon by the producers and marketers of the herbal medicinal products (HMPs) who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of people particularly in the rural areas and the reservations by the public due to the prevalence of fake, substandard or counterfeit drugs in the market. These have placed the HMPs as a ready alternative to conventional dosage forms in the treatment of diseases. With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. Although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects which may be due to factors such as adulteration, substitution, contamination, misidentification, lack of standardization, incorrect preparation and/or dosage, inappropriate labeling and/or advertisement (Okunlola et al., 2007). In contrast to

chemically defined medicinal products, the biopharmaceutical quality and behavior of HMPs are often not well documented. The WHO Good Manufacturing Practice Guidelines have provided technical guidelines to national regulatory authorities, scientific organizations, and manufacturers to undertake an assessment of the documentation/submission/dossiers in respect of herbal medicinal products. In Nigeria, the National Agency for Food Drug Administration and Control (NAFDAC) is responsible for drug administration and control of the quality of medicinal products including HMPs generally available in the market.

Factors that influence the contamination of medicinal herbs are several. These include certain environmental factors, mainly humidity, rainfall, storage conditions of crude and processed medicinal-plant materials, and handling and hygiene of people handling the herbs. Also, microorganisms derived from soil, air and water may contaminate the herbal raw materials leading to pathogenic effects to humans (de Freitas Araújo and Bauab 2012). The presence of such pathogens limits the use of medicinal plants and also exerts an

important impact on the overall therapeutic quality of herbal drugs and preparations. Also, since the presence of such pathogenic microorganisms constitutes a potential hazard to human health, reducing their concentration from their source is critical.

Escherichia coli and *Pseudomonas* spp. are the typical pathogens seen in herbal extract powders. Fungal species such as *Rhizhopus, Penicillium*, *Aspergillus* are also reported. Although bacterial endospores and fungal spores are considered as the two dominating groups of contaminants seen on medicinal plants, the presence of pathogenic bacteria like *B. cereus*, *Aeromonas hydro-phila*, *Shigella* spp., *Enterobacter agglomerans*, *E. cloacae*, *Vibrio fluvialis*, *Pasteurella multocida*, *S. epider-midis*, *Acinetobacter iwoffii*, *Klebsiell*a spp., and *B. subtilis* have been reported in plant samples analyzed recently (Yewale et al. 2020).

1.2 Problem Statement

Herbal medicines are widely consumed in Ilorin, Nigeria, due to their perceived efficacy, affordability, and cultural acceptance. However,

the safety and quality of these herbal products are often compromised due to inadequate regulation, poor handling, and lack of standardized production processes. One critical concern is the presence of microbial contaminants, including bacteria, fungi, and pathogenic microorganisms, which pose serious health risks to consumers.

1.3 Aim and Objectives

To assess the microbial loads in selected herbal medicines sold in Ilorin through quantitative and qualitative analysis, with the goal of evaluating their safety and compliance with acceptable microbial standards.

Objectives:

- To quantify microbial loads in herbal medicines using standard microbiological techniques.
- To identify and characterize microbial contaminants (bacteria and

fungi) present in the herbal medicines.

- To evaluate the potential health risks associated with the identified microbial contaminants.

1.4 Significance of the Study

This study will help identify potential health risks associated with microbial contamination in herbal medicines, ensuring consumer safety by raising awareness of the need for quality control. The findings will highlight the importance of implementing stricter quality assurance measures during the production, storage, and distribution of herbal medicines in Ilorin. By addressing microbial contamination, the study can contribute to reducing the incidence of infections or illnesses caused by contaminated herbal products. Ensuring the microbiological safety of herbal medicines will enhance their credibility and acceptance, both locally and internationally, while promoting the safe use of traditional remedies. The study will serve as a reference for future research in microbiological analysis of herbal medicines and provide insights for academic and professional

discussions on improving herbal medicine practices. By identifying gaps in the production and handling processes, the study can help herbal medicine producers improve their products, potentially boosting consumer trust and market value.

1.5 TYPES OF HERBAL MEDICINE:

- **Decoctions:** Decoctions involve boiling tough plant materials such as bark, roots, or seeds to extract active phytochemicals that are not easily released through simple infusion. This method allows for the extraction of alkaloids, glycosides, and tannins, making it suitable for medicinal herbs used to treat chronic diseases, gastrointestinal issues, or fever (Okwu et al., 2021). Decoctions are widely used in African traditional medicine and have been documented as effective in treating malaria, respiratory illnesses, and pain-related disorders.
- **Tinctures:** Tinctures are alcohol-based or vinegar-based extractions of herbs that preserve both water-soluble and alcohol-soluble compounds. They are potent and long-lasting,

often used in low doses due to their concentrated nature. According to Akinmoladun et al. (2020), tinctures are particularly effective in managing infections and chronic inflammatory conditions and are commonly used in Western herbalism and naturopathic medicine. The alcohol base also acts as a preservative, allowing these products to maintain their efficacy over extended periods.

- **Infusions:** Infusions are made by steeping soft parts of a plant such as leaves or flowers in hot water, often referred to as herbal teas. They are ideal for extracting vitamins, volatile oils, and antioxidants, making them effective for managing mild symptoms like colds, digestive issues, and stress (Moghadam et al., 2020). Infusions are safe, easy to prepare, and commonly used in both traditional and modern herbal practices. Popular examples include chamomile for sleep and lemongrass for digestion.
- **Capsules and Tablets:** Capsules and tablets represent the pharmaceutical modernization of herbal medicine. They contain

either powdered herbs or standardized extracts, ensuring accurate dosage and extended shelf life. According to Abubakar and Haque (2020), the use of encapsulated herbal products is increasing due to consumer demand for more standardized, safe, and palatable formulations. Capsules are often used for chronic conditions like arthritis, liver disorders, and hormonal imbalances.

- **Ointments and Creams:** Herbal ointments and creams are external preparations used for skin conditions like burns, wounds, eczema, or fungal infections. They combine plant extracts with carriers such as beeswax or petroleum jelly. These preparations allow active ingredients to be applied directly to the site of infection or irritation. As noted by Chikezie et al. (2022), ointments containing aloe vera, neem, or turmeric show strong antimicrobial and anti-inflammatory activity, making them ideal for dermal healing and skin care.
- **Essential Oils:** Essential oils are concentrated, volatile plant extracts obtained via steam distillation or cold pressing. They

are primarily used in aromatherapy, inhalation, massage, and topical applications. Moghadam et al. (2020) emphasize that essential oils like lavender and peppermint possess antimicrobial, anxiolytic, and analgesic effects. However, due to their high potency, they must be diluted before use to prevent skin irritation or systemic toxicity.

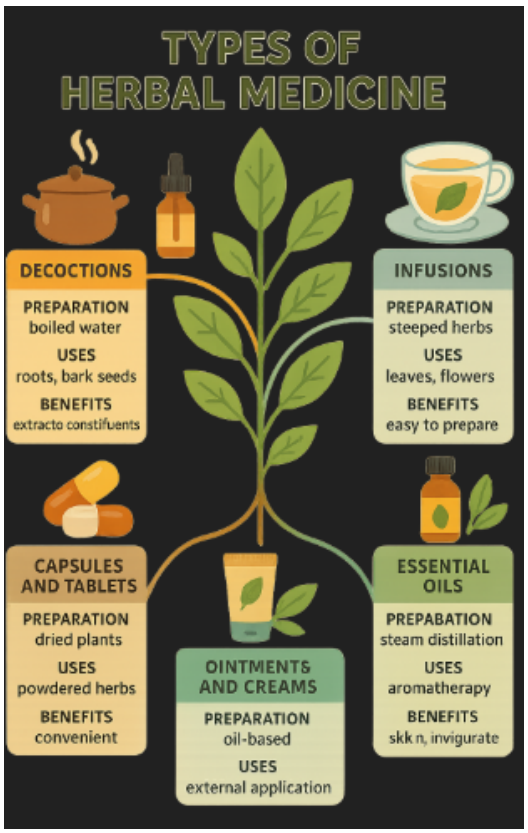

Fig.1

A visual representation connecting the six types (Decoctions, Tinctures, Infusions, Capsules and Tablets, Ointments and Creams, Essential Oils) of herbal medicine with their preparation, uses, and benefits

Source: (Okwu et al., 2021).

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Herbal Medicines

Herbal medicines also referred to as phytomedicines, involve the use of plant-derived materials for therapeutic purposes. These remedies have been a cornerstone of healthcare systems globally, particularly in traditional medicine practices. The growing interest in herbal medicines stems from their perceived safety, affordability, cultural acceptance, and efficacy in treating various ailments. Herbal medicine has ancient origins, dating back to prehistoric times. Early human civilizations relied on plants for food, shelter, and healing. The first documented use of herbal remedies can be traced to the Sumerians (circa 3000 BCE), who recorded their medicinal practices on clay tablets. Similarly, the Ebers Papyrus of ancient Egypt (circa 1550 BCE) listed over 800 herbal formulations used for various ailments (Petrovska, 2012).In Asia, Traditional Chinese Medicine (TCM) and Ayurveda have long histories of utilizing herbal

preparations. The Chinese text, *Shennong Bencao Jing* (circa 200 CE), cataloged hundreds of medicinal plants. Ayurveda, originating in India over 3,000 years ago, emphasizes the use of herbs like turmeric, ashwagandha, and neem for holistic health (Patwardhan et al., 2005). Indigenous practices in Africa and the Americas also showcase extensive knowledge of medicinal plants passed down through generations.

Herbal medicines play a vital role in global healthcare systems, especially in developing countries where access to modern pharmaceuticals may be limited. The World Health Organization (WHO) estimates that approximately 80% of the population in some regions relies on traditional medicine, including herbal remedies, as their primary healthcare source (WHO, 2002). In industrialized nations, the demand for natural and alternative therapies has surged due to growing concerns about the side effects of synthetic drugs. Herbal medicines are often more affordable and accessible than synthetic drugs, making them a vital resource in low-income settings. Many

medicinal plants can be cultivated locally, reducing dependency on imported pharmaceuticals. Traditional medicine systems align with cultural beliefs and practices, enhancing their acceptance and use. For instance, Ayurveda in India and TCM in China are deeply rooted in their respective cultures. Herbal remedies are often viewed as safer alternatives to synthetic drugs, particularly for chronic conditions. However, this perception requires careful evaluation, as natural does not always equate to safe (Noor et al., 2013). Many modern pharmaceuticals have origins in herbal medicine. For example, aspirin was derived from willow bark, and the anti-malarial drug artemisinin was isolated from *Artemisia annua* (Klayman, 1985). Research is crucial for validating the safety and efficacy of herbal medicines. Advances in pharmacology, molecular biology, and analytical techniques have enabled the identification of active compounds in medicinal plants. For instance, the isolation of taxol from the Pacific yew tree (*Taxus brevifolia*) revolutionized cancer treatment (Wani et al., 1971). Collaborative efforts between traditional healers and scientists can bridge knowledge gaps, ensuring that valuable

traditional remedies are preserved and scientifically validated.

Herbal medicine is generally considered as an integral part of dietary supplement. There is a growing interest in herbal medicine due to its long history of application and general belief that herbs are natural and intrinsically safe. According to WHO, approximately 4 billion of people, 80% of the world population, rely on traditional herbal medicine for their primary health care (Akerele, 1993). In recent years, utilisation of herbal remedies as a dietary supplement for disease prevention or as alternative/complementary medicine (CAM) for disease treatment has become increasingly popular. A wide variety of herbal medicine/products are readily available in the market all over the world. Herbal medicine is used in ways that differ from the ways conventional pharmacologic drugs are used. Because herbs have nutritional elements, and because pharmaceutical elements interact with one another polyvalently, the clinical effects may have greater depth and breadth than those seen in drug therapy. Patient prescriptions are based on both the pharmacology AND the traditional indications for the herbs.

Herbal medicine is conceived from different points of view depending on the place where it is used, but its definitions have the same conception, that is, it is the use of plants or parts of the plant to treat a disease and achieve health wellness. The herbal medicines include

herbs, herbal materials, herbal preparations, and finished herbal products that contain as active ingredient parts of plants, or other plant materials, or combinations (WHO, 2019).

Humankind in its early years used nature to implement suitable conditions for its survival, improving its quality of life over the years. Since those ancient years the knowledge of herbal medicine based on tradition has served to cure the ailments of the humankind (González Rodríguez & Cardentey García, 2016). Herbal medicine includes numerous substances extracted from plants, homemade infusions, plants harvested for medicinal purposes, and products that have to be approved by government regulatory bodies (Carrillo Esper, Lara Caldera, & Ruiz Morales, 2010). Much of the herbal remedies are obtained by alcoholic, acetonic, or aqueous extraction and sometimes without manipulation of seeds, leaves, stem, bark, or roots of these plants (Carrillo Esper et al., 2010).

The use of herbal medicine has increased around the world due to its presumptive efficiency, availability, and general acceptance. Approximately 80% of the general population, especially in developing countries, use medicinal herbs for primary health care (Taddei Bringas, Santillana Macedo, Romero Cancio, & Romero Tellez, 1999; WHO, 1993).

Archaeological evidence indicates that the use of medicinal plants

dates back to the Paleolithic age, approximately 60,000 years ago (Solecki, 1975). Written evidence of herbal remedies dates back over 5,000 years to the Sumerians, who compiled lists of plants (Samuelsson, 2004). Some ancient cultures wrote about plants and their medical uses in books called herbals. In ancient Egypt, herbs were mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs (Nunn, 2002). In ancient Egypt, the Ebers papyrus dates from about 1550 BCE and covers more than 700 compounds, mainly of plant origin (Loewen, 2005). The earliest known Greek herbals came from Theophrastus of Eresos who, in the 4th century BCE, wrote Historia Plantarum, from Diocles of Carystus in the 3rd century BCE, and from Krateuas in the 1st century BCE. Only a few fragments of these works have survived intact, but from what remains, scholars have noted an overlap with the Egyptian herbals (Scarborough, 1978).

Herbal medicine (also called herbalism, phytomedicine or phytotherapy) is the study of pharmacognosy and the use of medicinal plants, which are a basis of traditional medicine (Fabricant & Farnsworth, 2001). Scientific evidence for the effectiveness of many herbal treatments remains limited, prompting ongoing regulatory evaluation and research into their safety and efficacy (WHO, 2004). Standards for purity or dosage are generally not

provided. The scope of herbal medicine sometimes includes fungal and bee products, as well as minerals, shells and certain animal parts.

Since ancient times, herbal medicines have been used by many different countries throughout the world to treat illnesses and to assist bodily functions. The use of herbal medicines in human health care has developed substantially in both developed and developing countries and it is continually expanding (Tilburt & Kaptchuk, 2008). About 60 to 80% of the population of every country of the developing world relies on herbal or indigenous forms of medicine (WHO, 2013). The reasons for the patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries. The World Health Organization survey indicated that about 70–80% of the world population, particularly in developing countries, rely on non-conventional medicines mainly of herbal origins for their primary health care (WHO, 2013).

The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties, which can be used to cure human and other animal diseases. The use of these herbal remedies has increased significantly in the last two to three decades in Nigeria (Olowokudejo et al., 2008). This has led to the production of herbal products with bogus claims that it can cure all forms of ailments. With the ever-increasing use of herbal medicines

and the global expansion of the herbal medicines market, safety has become a concern for both health authorities and the public at large (Ekor, 2014). This is because it is difficult to attest to the hygienic nature of the sanitary conditions from the point of collection of the herbs to processing and finally to the point of preparation. Therefore, the quality and safety of herbal preparations are of great concern because quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance and must therefore be investigated (Kunle et al., 2012).

In developing countries, herbal extracts are recognized as pharmaceutical medicine and are used as complementary medicine and without adequate supervision they are released directly into the market. Hence, high microbial contamination may occur in these products (Okunlola et al., 2007). In Nigeria, there appears to be an overwhelming increase in public awareness and usage of herbal medicinal products in the treatment and prevention of diseases. This may not be unconnected to the active mass media advertisements embarked upon by the producers and marketers of the herbal medicinal products who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of

the people particularly in the rural areas and the reservations by the public due to prevalence of fake, substandard or counterfeit drugs in the market (Oreagba et al., 2011). These have placed the herbal medicinal products as a ready alternative to conventional dosage forms in the treatment of infections and diseases. With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals.

Although herbal remedies are often perceived as being natural and therefore safe, microbial contamination of medicinal herbal preparations could be influenced by environmental factors such as temperature, humidity, rainfall during pre-harvesting and post-harvesting periods, handling practices, the production of these herbal medicines by unlicensed vendors with no or poor educational levels, and also poor knowledge of food hygiene (Martins et al., 2001).

The storage conditions of crude and processed medicinal-plant materials are also not left out in the contamination of this herbal medicine. The presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicines (Rios & Recio, 2005). It is desirable that the microbiological status of medicinal plants which are susceptible to microbial attack be ascertained before their usage.

The widespread disregard for possible association of microorganisms to the production of some herbal drugs has tarnished the reputation of many herbal drugs. Without proper microbial analysis, there is no assurance that the herb contained in the bottle is the same as what is stated on the outside label and that it is free from contaminations (Ekor, 2014).

Paraherbalism is the pseudoscientific use of plant or animal extracts as medicine, relying on unproven beliefs about the safety and effectiveness of minimally processed natural substances (Ernst, 2002).

Herbal medicine has been used since at least the Paleolithic era, with written records from ancient Sumer, Egypt, Greece, China, and India documenting its development and application over millennia (Balick & Cox, 1996). Modern herbal medicine is widely used globally, especially in Asia and Africa. Traditional medicine systems involve long-standing, culturally-embedded practices using local herbs, animal products, and spiritual elements. These systems have influenced and contributed to modern pharmacology (Rates, 2001). Herbalists believe that plants, having evolved defenses against environmental stressors, produce beneficial phytochemicals, often extracted from roots or leaves, that can be used in medicine.

Sick animals often seek out and eat plants containing compounds like tannins and alkaloids to help purge parasites—a behavior observed by scientists and sometimes cited by indigenous healers as the source of their knowledge (Huffman, 2003).

Herbal plants are a natural source of compounds that can be used against many diseases today. Informal street merchants and traditional health practitioners primarily offer consumers semi-processed herbal preparations that are commonly prepared in small batches. In the preparation of the herbal concoctions, fresh or dry plant material can be used. The plant material can either be macerated in water for several days or generally boiled in hot water. Moreover, the increased cost of new and more effective antimicrobial remedies together with their side effects and lack of health care facilities in some rural areas, makes the search for safer, more effective, and affordable alternative remedies imperatives. In South Africa, herbal products that are sold by informal traders are usually claimed to be immune and energy boosters, blood cleansers, detoxifiers, and aphrodisiacs. There also seemed to be a proportional high demand for plant-based medicines, in that the estimated annual market value of phytomedicines stood at 75 to 150 million USD.

The broad use of traditional herbal remedies has encouraged manufacturers, private traders, and street merchants to capitalize on this upsurge by increasing the availability of herbal remedies to those

who desire them. The signs of urbanization are witnessed by the increase in herbal shops, informal street traders, and the wide distribution of herbal remedies in pharmacies and supermarkets. (Osuntokun OT, et al., Duan Q, Zhou M, etal.,)

Herbal medicine encompasses a wide range of plant-based remedies used for various health purposes. These can range from traditional teas and tinctures to more modern forms like capsules and ointments. Common examples include Echinacea for immune support, Ginger for nausea, and Ginkgo biloba for circulation.

Many plants are used in traditional medications as herbal preparations for human health-care and they are being promoted as natural and safe without any side effects. As the use of herbal preparations by patients is increasing day by day, there is a need for pharmacists and physicians to have knowledge about the safety of these preparations. The transportation and congenial climatic conditions can render the raw materials for herbal drugs prone to fungal infestations. The raw materials collected using unscientific methods are commonly exposed to many pathogenic contaminants

and are often deteriorated by pathogenic microorganisms before harvesting, and also during handling and storage. Therefore, lack of regulation for herbal supplements presents potential health risk, largely their contamination chances with pathogenic microorganisms. However, only a few surveillance studies have been conducted to assess this threat. Present study figured out the biological threats in herbal medicines and added the knowledge of proliferating bacteria, yeasts and moulds in such medicines. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. Such medicinal preparations have been used since ancient times to treat a wide range of diseases(Noor et al., 2013). However, the medicinal use of herbs went into a rapid decline in the Western countries when more predictable synthetic drugs were made commonly available. In contrast, many developing countries continued to get benefit from the rich knowledge of medical herbalist.

2.2 Microbial Contamination in Herbal Products

Herbal products, often marketed as natural remedies, are susceptible

to microbial contamination during cultivation, processing, storage, and distribution. Contaminants may include bacteria, fungi, or their metabolites, posing risks to consumer health.

S/N	ORGANISMS	EXAMPLES	RISK POSTING
1	Bacteria	*Escherichia coli*	Indicates fecal contamination and poor hygiene during production.
		Salmonella spp.	Can cause severe gastrointestinal infections
		Clostridium spp.	Known for toxin production leading to botulism in extreme cases.
2	Fungi	*Aspergillus spp.*	Produces aflatoxins, which are carcinogenic and hepatotoxic (Martins et al. 2012).
		Penicillium	Can produce harmful

		spp.	mycotoxins under improper storage conditions.
3	**Yeasts and Molds**		These can grow during storage, especially in high-moisture environments, leading to spoilage and potential allergic reactions (Prakash et al. 2014).

Methods prescribed in the British Pharmacopoeia (2004) were used to test microbial quality of the herbal medicines**10**. In this general method, certain selective media were used. A feature common to all selective media was that the sub-lethally injured organisms could not be detected. Soybean-Casein Digest agar media were used to enumerate the total bacterial population. Sabouraud Dextrose agar (SDA) was used for the identification and enumeration of total fungi. Xylose-Lysine Deoxycholate (XLD) agar for the isolation of

Salmonella spp. and *Shigella* spp., and MacConkey (MAC) agar media for isolation of coliforms were used. The condition of the test for microbial contamination was designed to minimize accidental contamination of the material being examined. The precautions taken during the study did not affect any microorganism. The risk of microbial contamination may have an exacerbated impact in the elderly population because this population has increased susceptibility to the consumption of herbal medicines and sensitivity to health complications due to the aging process. The aging process, according to some studies (de Sousa et al, 2020), is characterized by morphological changes and physiological, biochemical and psychological factors that lead to a decrease in an individual's ability to adapt to the environment.

2.3 Regulatory Standards for Microbial Loads in Herbal Medicines

The purpose of this document is to propose to Member States a framework for facilitating the registration of traditional medicines.

The proposed framework, which has a regional perspective, is intended to accelerate the registration and circulation of standardized African traditional medicines within the WHO African Region, on the basis of criteria of pharmaceutical quality, safety of use, and therapeutic efficacy (World Health Organization, 2023).

The guidelines are aimed at national drug regulatory authorities, manufacturers, traditional health practitioners, and institutions engaged in drug research and development, who should ensure that their products satisfy the requirements laid down in this document. The guidelines can also be used for training and teaching purposes, in particular, for teaching undergraduate and postgraduate pharmacy, and for training pharmacy technicians, nurses, and medical students, related health professions, and agencies involved in the development of traditional medicine in the WHO African Region (World Health Organization, 2023).

Many countries in the WHO African Region are encountering

problems in providing their people with equitable access to health care; at present, only about one-half of the population in the region has access to conventional health care (World Health Organization, 2022). Such considerations aside, traditional medicines continue to h care needs (World Health Organization, 2022).

In countries for which more detailed data are available, the percentage of the population that uses traditional medicine ranges from 90% in Burundi and Ethiopia, to 80% in Burkina Faso, the Democratic Republic of Congo, and South Africa; 70% in Benin, Côte d'Ivoire, Ghana, Mali, and Rwanda; and 60% in Tanzania and Uganda (Odhiambo, 2023).

2.4 Health Implication of Herbal Medicines

Herbal medicines have long been celebrated for their therapeutic properties, especially in managing chronic conditions such as diabetes, arthritis, and hypertension. Derived from natural sources, these plant-based remedies are often perceived as safer alternatives to synthetic drugs. Studies have confirmed the antioxidant, anti-

inflammatory, antimicrobial, and immunomodulatory activities of various herbs, which can contribute positively to health when used appropriately (Abubakar & Haque, 2020). For instance, compounds like flavonoids, alkaloids, and terpenoids found in many medicinal herbs help neutralize free radicals, support immune responses, and reduce oxidative stress.

Despite their benefits, the unregulated use of herbal medicines poses significant health risks. Many herbal products sold in markets especially in low-resource settings—lack proper quality control and may contain contaminants such as heavy metals, pathogenic microorganisms, or pesticides (Okwu et al., 2021). This contamination may result in acute or chronic health issues, including liver or kidney toxicity, gastrointestinal disturbances, or even carcinogenic effects. In Nigeria and other African countries, where traditional herbal remedies are often prepared and sold informally, the risks of microbial load or adulteration are especially high.

Another major concern is the potential for herb-drug interactions.

Herbal compounds can affect the absorption, metabolism, or elimination of conventional drugs, thereby reducing drug efficacy or increasing toxicity. For example, herbs like St. John's wort and ginseng are known to interfere with drugs metabolized by liver enzymes, leading to therapeutic failure or adverse effects (Moghadam et al., 2020). Unfortunately, many patients do not disclose their use of herbal remedies to healthcare providers, increasing the likelihood of preventable complications during treatment.

Additionally, some herbal products have been linked to organ-specific toxicities. Prolonged use of herbs like kava, comfrey, or aristolochia has been associated with hepatotoxicity and nephrotoxicity due to the accumulation of toxic alkaloids (Chikezie et al., 2022). In pregnant women, certain herbal preparations can induce uterine contractions, leading to miscarriage or premature labor. Children and the elderly, with their more sensitive physiological systems, are also at greater risk of adverse outcomes from improperly dosed or impure herbal formulations.

To ensure safety, there is a growing need for standardization, regulation, and public education on herbal medicines. Governments and health agencies must work to regulate herbal product production and encourage research to validate their safety and efficacy. Meanwhile, public health practitioners should inform communities about the risks of self-medication and emphasize the importance of consulting qualified professionals before using herbal remedies (Akinmoladun et al., 2020). Integrating traditional knowledge with modern clinical practices may help maximize the benefits of herbal medicines while minimizing health risks.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out on a sealed herbal mixture sold in Ilorin, Kwara State.

3.2 Sample Collection

Four (4) herbal concoction samples were randomly collected from four different herbal vendors in Ilorin, Kwara State. The herbal products includes were J, K,O and F. The samples were carefully and aseptically transported to the laboratory in their packages for analysis.

3.3 Preparation of Culture Media

All media used were prepared according to manufacturer's

instruction. The media were weighed and dissolved in the appropriate volume of water and sterilized in the autoclave at 121°C at 15psi for 15minutes. Media used include Nutrient Agar, MacConkey Agar and Potato Dextrose Agar.

3.4 Materials

Glass wares used for this study include; petri dish, measuring cylinder, microscope glass slides, glass rod, beakers, Micropipettes and test tubes, equipment use for this study include; autoclave, refrigerator, microscope, incubator, oven, electronic beam balance, Bunsen burner and water bath, distilled water, Lugol' iodine, crystal violet, alcohol and decolorizer, nutrient agar, , Potato Dextrose agar, foil paper, needle and syringes, aluminum foil paper, paper tape, detergent, wire loops, cotton wool.

3.5 Bacteriological and Mycological Analysis

3.5.1 Total Bacterial and Fungal Count

Fourfold serial dilutions were carried out in (four) 4 test tubes. Nine milliliters (9 ml) of distilled water were pipette into the tubes and plugged with stopper and sterilized by autoclaving at 121^{o}C at 15psi

for 15 minutes and allowed to cool after it has been brought out from the autoclave. One milliliter (1 ml) of the sample was diluted serially using a sterile pipette. Test tubes 10^3 were plated on the Potato Dextrsoe Agar (PDA) agar, test tube 10^3 were plated on the Nutrient Agar (NA) agar all the volume of culture plated were 1ml. The plates were then incubated at 37^{o}C for 24 hours for bacteria and fungi incubated at 27^{o}C for 3-5 days respectively. At the end of 24 hours of incubation for bacteria and 3-5 days for fungi, standard bacterial count and fungal count were recorded. The count was expressed as colony forming units (CFU) as described by Okafo *et al.* (2019) and Anie (2018).

3.6 Isolation of Microorganisms

After the 24hours of incubation, the plates were removed from the incubator, the colony were observed on the plates and counted using colony counter, and the pure cultures were obtained by transferring the distinct colonies into sterile solid nutrient agar plates using sterile inoculating loop and then streaked. The plates were then incubated at 37^{o}C for 24-48hours and sub-cultured until satisfactorily

pure cultures were obtained. The agar slant were then prepared dispensing molten nutrient agar into sterilized McCartney bottle and set into slant position. The distinct pure isolates obtained were then aseptically inoculated further onto nutrient agar slants in McCartney bottles and incubated 37^{o}C and stored in the refrigerator at 4^{o}C. The stock cultures were to serve as a source of reference whenever tests would be carried out on the isolates.

3.7 Characteristics of Microbial Isolates

3.7.1 Bacterial Characterization of Isolates

Morphological characterization of each distinct colony was observed and recorded by noting the color, shape, pigmentation, elevation, size of the colonies and microscopic view was done using a compound microscope with X100 oil immersion lens. Bacteria isolates were then further characterized by their physiological characteristic though the biochemical reactions of isolates to some reagents and media.

3.7.2 Gram Staining

Gram stain is one of the differential stains that are used to

characterize bacteria into: either Gram positive bacteria or Gram-negative bacteria. A thin smear of each of the pure 24 hours old culture was prepared on clean grease-free slide, fixed by passing over gentle flame. The smear was flooded by crystal violet solution for 1 minute and rinsed with water. The smear was again flooded with Lugol's iodine for 30- 60 seconds and rinsed with water, decolourized with 70% alcohol for 15 seconds and was rinsed with water. The slide was counter stained with safranin for 60 seconds and rinsed with water. The smear was mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while Gram positive organisms appeared purple.

3.8 Biochemical Test

Different bacterial isolates were carried out on the basis of the result of four tests; Indole, Methyl red, Voges Proskauer, Catalse, Oxidase, Citrate utilization, with their standard methods.

3.8.1 Indole Test

Indole test procedures was done as described by Islam (2018). This determined the ability of bacteria to split amino acid tryptophan

to form compound indole. One percent tryptophan 10ml broth was taken in test tubes and inoculated by fresh pure culture obtained from pure colonies. After 48 hours of incubation period at 37^{o}C, the test tubes were shaken gently. Five drops of Kovács reagent was added directly to the tubes. These were also shaken gently and allowed to stand for twenty (20) minutes. Two test tubes were used per isolate with one being a control. Control test tube contained one percent tryptophan broth and inoculated by fresh pure culture obtained from pure colonies. Formations of red colouration at the top layer indicated positive while yellow colouration indicated negative results, respectively.

3.8.2 Methylred Test

This test is used to check acid production in the medium usually for coliform organisms which ferment dextrose rapidly causing a fall in the pH. MR-VP broth was prepared and 10 ml of the broth was dispensed into test tubes and sterilized. Inoculation was subsequently done and incubated at 37^{o}C for 2 days. After incubating for 48 hours, the broth was aseptically divided into 2 portions. To the

first portion, 2-3 drop of methyl red indicator is added and observed for colour change. A red colour change indicates a positive reaction, that is, there is gas production while a yellow colour change indicates a negative reaction (Ellis and Goodacre, 2016).

3.8.3 Catalase Test

Catalase test was done according to the procedure described by Ahmed *et al*. (2017) to determine aerobic and anaerobic bacteria and it was important in differentiating morphologically similar Enterococcus *Staphylococcus (catalase positive)* and *Streptococcus* spp (catalase negative). Three ml of catalase reagent (3% H2O2) was put on a glass slide. Single colony from the pure culture of bacteria from each sampled site was scooped with a glass rod and submerged in the reagent and observed for bubble formation which indicated positive test while absence of bubbles formation indicated negative results.

3.8.4 Citrate Test

This test was performed according to procedure described by Aligwekwe (2018) by inoculating the bacteria into Simmon‟s citrate

medium obtained from pure colonies as explained in section 3.3.2. This was employed in determining the ability of bacteria to utilize sodium citrate as its only carbon and energy source. The inoculated medium was incubated for 48 to 72 hours to allow complete utilization of Simmon‟s citrate medium by microorganisms. The colour of the medium indicated the result. If the colour of media changed from green to blue then the bacteria was citrate positive while if the media retained the green colour after incubation period it indicated citrate negative bacteria.

CHAPTER FOUR

RESULTS

4.1 Total Bacteria Counts and Total Coliform Counts (CFU/ml)

The results obtained from the total bacteria count of herbs samples are reported in Table 4.1 which has the ranged from $5.0x10^4$cfu/ml to $15.0x10^5$ cfu/ml with sample K having the highest value while sample J had the lowest value respectively.

4.2 Total Fungal Counts (CFU/ml)

The results obtained from the total fungal count of herbs samples are reported in Table 4.2 which has the ranged from $2.0x10^3$cfu/ml to 0 $x10^3$ cfu/ml with sample O and F having the highest value of $2.0x10^3$cfu/ml while sample K had no growth.

4.3 Biochemical Test

Table 4.3 shows the various biochemical test carried out on different isolates ranging from indole to citrate and others as shown in the table.

4.4 Colonial Morphology Characterization

Table 4.4a presented the colonial morphology of the bacteria

isolates gotten from the herbal samples. Table 4.4b show the colonial morphology of the fungi isolates respectively.

Table 4.1: Total Bacterial Counts (CFU/ml)

Samples	TBC ($x10^4$)
J	5
K	15
O	13
F	7

KEYS: TBC -Total Bacteria Counts

Table 4.2: Total Fungal Counts (CFU/ml)

Samples	TFC (x103)
J	1
K	0
O	2
F	2

KEYS: TFC -Total Fungal Counts

Table 4.3: Biochemical and Microscopic Characterization of

Bacteria Isolated from the Herbs Sample

Isolates	Ind	MR	VP	Cit	Cat	Gram reaction	Shape	Probable Organism
1	+	+	-	-	+	-	Cocci	*E. coli*
2	-	+	-	+	+	-	Rod	*Staphylococcus sp.*
3	+	-	-	+	+	-	Rod	*Pseudomonas sp.*
4	-	+	-	+	+	-	Rod	*Staphylococcus sp.*
5	+	-	-	+	+	-	Rod	*Pseudomonas sp.*

Keys: - = negative + = positive

Table 4.4a: Colonial Morphology Characterization of Bacterial Isolates

Isolate	Colour	Elevation	Form	Surface	Size
1	Off-white	Convex	Circular	Mucoid	Small
2	Milkish	Raised	Circular	Smooth	Large
3	Whitish	Raised	Circular	Moist	Large
4	Yellow-whitish	Raised	Circular	Smooth	Large
5	Whitish	Raised	Circular	Moist	Large

Table 4.4b: Colonial Morphology Characterization of Fungal Isolates

ISOLATES	COLONIAL DESCRIPTION	LIKELY ORGANISMS
1	Colony exhibited white mycelium growth initially with black centre which spread or increases with time. The margins of the fungi were whitish. The colony had The reverse face of the plate was yellowish.	*Aspergillus niger*
2	Yellow-brown and powdery with whitish mycelium at the edges	*Aspergillus flavus*

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The findings of this study revealed significant bacterial and fungal loads in the herbal preparations examined. Sample K recorded the highest bacterial count (2.0×10^7 CFU/ml), which is far above the acceptable limit of 10^4–10^5 CFU/ml for oral liquid herbal products as recommended by the World Health Organization (WHO, 2007).

Among the bacterial isolates identified were *Staphylococcus aureus*, a known pathogen responsible for foodborne illness and skin infections, *E. coli*, often associated with urinary tract infections, and *Pseudomonas spp.*, an opportunistic pathogen. These findings align with previous studies. For instance, Adeyemi *et al.* (2020) isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa* from herbal

mixtures sold in Lagos, Nigeria. Similarly, Yakubu and Ighodaro (2021) found *Enterobacter spp.*

The predominant fungi identified were *Aspergillus niger* and *Aspergillus flavus*, both of which are capable of producing mycotoxins. *A. flavus*, in particular, produces aflatoxins which are potent hepatocarcinogens. These findings agree with those of Chinedu *et al.* (2022), who also found *Aspergillus spp.* In 70% of herbal remedies sampled from urban markets in Abuja. Okafo et al. (2019) reported bacterial loads in local herbal remedies ranging between 10^5–10^7 CFU/ml, similar to our findings. Musa et al. (2021) emphasized the contamination risk posed by poor handling and storage practices of herbal drugs in Ilorin, which echoes the current study. In a previous study, Ajayi and Alade (2023) reported that over 60% of tested herbal mixtures in Ibadan had detectable levels of *Aspergillus flavus*.

These findings highlight the consistent trend of microbial contamination in locally prepared herbal medicines across Nigeria, suggesting systemic issues in production hygiene.

5.2 Conclusion

The microbial evaluation of herbal concoctions sold in Ilorin revealed unacceptable levels of bacterial and fungal contamination. The presence of pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas spp.,* and *Aspergillus spp*. Raises significant health concerns. These findings affirm that many locally sold herbal mixtures do not meet microbiological safety standards and could be harmful, especially to immunocompromised individuals.

5.3 Recommendations

- Relevant government agencies like NAFDAC should intensify inspections and ensure that herbal products meet microbial safety standards before being sold to the public.
- Consumers should be educated on the potential risks associated with the use of unregulated herbal medicines.
- Herbal vendors should be trained on proper hygiene, preparation, and storage of herbal concoctions.
- Future studies should include molecular identification of

isolates and screening for antibiotic resistance and mycotoxin production.

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