Research Paper **Phytopharmacology Research Journal (PRJ) Open Access**

Received: 1 Feburary 2025 | Revised: 15 May 2025 | Accepted: 30 July 2025 | Published online : 31 July 2025 | ISSN : 2959-9687

Microbiological Evaluation of Traditional Herbal Medicines Commonly Practiced in Khyber Pakhtunkhwa, Pakistan

Muhammad Sohail Anwar, Abad Khan*, Lateef Ahmad, Saqib Jahan, Abdul Mateen

Department of Pharmacy, University of Swabi (23340), Khyber Pakhtunkhwa, Pakistan

SohailAnwar@uoswabi.edu.pk
lateef.ahmad@uoswabi.edu.pk
saqibjahan@uoswabi.edu.pk
abdulmateen@uoswabi.edu.pk

***Correspondence**
Abad Khan
Drabadkhan@uoswabi.edu.pk

Funding information
None

Abstract

Traditional medicine remains a widely practiced approach to managing various health conditions across both developed and developing nations. In fact, despite significant progress in modern medical science, nearly 70–80% of people in developing regions continue to rely on alternative or non-conventional therapies as their primary source of healthcare. In this study, traditional herbal remedies recommended for particular diseases and prepared in different dosage forms were obtained from local practitioners across various districts of Khyber Pakhtunkhwa, Pakistan. The samples were screened for the detection of any microbial burden and also evaluated for selected strains of bacteria and fungi using standard methods, involved the inoculation of samples on culture media including; Nutrient Agar, Mannitol Salt Agar, MacConkey Agar and Sabouraud Dextrose Agar). The plates were then observed after an incubation period of 3 days bacteria at 32.5°C and molds at 25°C. Morphological characters were observed from their physical appearance, while microscopic characterization was done by Gram staining and biochemical tests. Microbial contamination was identified in 70% of the analyzed samples, with Staphylococcus aureus being the predominant microorganism (52%), followed by Escherichia coli (29%), Salmonella spp. (14%), and Candida albicans (8%) among the contaminated samples. These findings highlight the critical need for regulatory authorities to implement effective measures to prevent microbial contamination in traditional medicines and to establish standardization protocols, thereby ensuring public health safety in Khyber Pakhtunkhwa, Pakistan.

KEYWORDS

Traditional Herbal Medicines, Microbial Burden, Bacterial & Fungal

1.0 INTRODUCTION

Traditional methods of treating illnesses have been practiced by humans for centuries and continue to be used alongside modern medicine. Despite significant progress in contemporary science, these remedies have persisted as an “invisible mainstream” within healthcare systems. [1]. Although biochemical medicine and allopathic formulations have become increasingly available, studies reveal a growing reliance on traditional medicine in developing countries. [2]. The utilization of traditional herbal medicine among various Asian populations has been reported to range between 22% and 65%. [3].

The traditional medicine sector has emerged as a significant component of the healthcare system in Pakistan, particularly within rural and tribal regions. [4]. People who trust traditional healers, hakims, homeopaths, and in some cases even unqualified practitioners, often turn to alternative medicine. A common belief among the general population is that traditional remedies are natural and therefore safe. The centuries-long use of various plants and their components is also viewed as evidence of their safety. However, serious concerns remain regarding the absence of proper regulation and the potential toxicity associated with such medicines. (Edzard Ernst, 2002b).

The widespread use and accessibility of traditional remedies have raised concerns about their safety, effectiveness, and the accountability of practitioners who prescribe them. [5]. Traditional medicines lack

stringent regulation and are readily accessible through health-food shops, supermarkets, and online platforms. In the absence of proper oversight, these products are highly susceptible to contamination, particularly with microbial loads. [6], (E Ernst, 2000). Most of the traditional medicines are self-prescribed and commonly used to treat, manage, or control both minor and chronic conditions; however, some of these preparations may contain harmful substances such as heavy metals and pathogenic microorganisms. [7].

Microbial contamination represents a major concern associated with herbal products. [8]. Non-conventional remedies carry the risk of contamination with various microorganisms. Several studies have documented the presence of potential microbial contaminants in such preparations used for different health conditions. [9]. The inappropriate cleaning of containers and drug materials, prolonged drying and storage in compromised environment, improper transportation, inadequate hygiene and congenital climatic conditions render the traditional medicines to infestations and exposed them to hazardous microbial contamination [10]. Unintentional contamination with microbial or chemical agents at any stage of production can compromise the quality, safety, and efficacy of traditional medicines [11]. Improper manufacturing techniques, along with unhygienic processing and packaging, constitute the main sources of microbial contamination in herbal preparations [12]. Contaminants that cause serious health hazards include bacterial strains like Salmonella, Escherichia coli, Staphylococcus aureus, Shigella species also some fungal spores. [13]. Mycotoxins are secondary metabolites produced by certain molds, comprising compounds that can be highly toxic, mutagenic, or teratogenic. [14].

Many traditional medicinal preparations, used in various forms, may harbor large numbers of microorganisms originating from the soil, typically adhering to the roots, seeds, stems, leaves and flowers of the plants. [15]. Raw materials collected through unscientific methods are frequently exposed to pathogenic contaminants and may undergo deterioration caused by microorganisms both before harvesting and during subsequent handling and storage. Consequently, the absence of strict regulations for herbal supplements poses a potential health risk, primarily due to their susceptibility to contamination with pathogenic microorganisms. [16]. Contamination of non-conventional medicines not only renders them toxic but also alters their physical, chemical, and organoleptic properties, which may lead to degradation of active ingredients or their transformation into toxic compounds. [17]. Both microorganisms and their metabolites are hazardous, as they can contribute to the development of various diseases even at low concentrations. [18].

Consequently, the presence of microbial contaminants in these products can negatively impact consumer health, particularly by increasing the risk of infections in immunocompromised individuals, thereby posing a global health concern. Ensuring the safety of herbal product consumers is therefore crucial, especially among elderly populations. [19]. According to WHO guidelines for assessing technically, the microbial quality of herbal preparations, the detection of microbial contaminants and the application of various limit tests for total viable aerobic bacteria and fungi detection serve as key indicators of quality. Similarly, the British Pharmacopoeia (2004) specifies that Shigella and Salmonella species must be completely absent from herbal medicines intended for internal use at all stages of production. [16]. Prolonged consumption of microorganism-contaminated herbal medicines may expose users to pathogenic microbes, thereby increasing the risk of infections and other serious health complications. [20]. To prevent and control microbial contamination in traditional medicines, it is essential to implement best practice guidelines such as Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP), alongside quality assurance measures like Good Agricultural and Collection Practices (GACP) for medicinal plants and herbal products. [21].

With these considerations in mind, the present study is aimed at evaluating traditional medicines for potential adulterants and contaminants, with the objective of raising awareness among the public and regulatory authorities about the risks linked to their widespread use.

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

All culture media (fluid soybean–casein digest medium, soybean–casein digest agar medium, , Vogel–Johnson agar medium, mannitol–salt agar medium, Sabouraud dextrose broth, MacConkey agar medium , fluid lactose medium, , cetrimide agar medium selenite–cystine medium, fluid tetrathionate medium, brilliant green agar medium, bismuth sulfite agar medium, triple sugar–iron agar medium, and Sabouraud dextrose agar) and chemicals (potassium tellurite, glycerin, potassium iodide, iodine, and brilliant green) were procured from Merck Chemical Co. (Darmstadt, Germany). The indicator microorganisms— Escherichia coli, Staphylococcus aureus , Salmonella typhi Pseudomonas aeruginosa and Candida albicans (USP 30, 2007)—were obtained from the Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Pakistan.

2.2 Apparatus and Equipment

The apparatus and equipment used in the study used were Incubator (Model J-DECO, JISICO, Korea), Autoclave (Model J-DECO, JISICO, Korea), Laminar Flow Hood (Tech, DAIHAN LAB TECH, Co. Ltd, Korea), Colony Counter (UTS- Digital colony counter), Dry Oven (Model J- DECO, JISICO, Korea), Compound Microscope (Viola MC-30, MICROS, Austria), Hot Plate (Model SI-301 (ES-3815), China) and Incinerator (JISICO, Korea).

2.3 Samples Collection

Samples were collected from previously selected districts of Khyber Pakhtunkhwa, Pakistan, through groups of volunteers who approached local practitioners (quacks/hakeems) while posing as patients with specific ailments. The diseases chosen for the study were selected based on common local practices and the specialties of the respective practitioners. The obtained samples included mixtures, compounded preparations, or locally formulated herbal products, often presented as pastes, powders, creams, tablets or manually filled capsules. These preparations were prescribed for a wide range of conditions, including stomach disorders, sexual dysfunctions, hypertension, piles, arthritis, diabetes mellitus, leukemia, and renal disorders. The details of samples are given in Table 1. The representative images are shown in Figure 1.

Table-1. Collected Herbal Medicines for Various Diseases

S. No	District of collection	Available Dosage Form	Sample identification code	Intended Disease
1	Swat	Syrup	M-01	Gastric disorder
2	Mardan	Paste	M-04	Piles
3	//	Capsule	M-06	Gastric disorder
4	//	Paste	M-10	Piles
5	Peshawar	Paste	M-13	Sexual tonic
6	Swabi	Pills	M-17	Gastric disorder
7	//	Ointment	M-19	Arthritis
8	//	Tablets	M-21	Piles
9	Bannu	Pills	M-22	Arthritis
10	//	Paste	M-27	Sexual tonic
11	//	Powder	M-33	Arthritis
12	//	Powder	M-34	Arthritis
13	//	Powder	M-35	Arthritis
14	Buner	Tablets	M-52	Piles
15	//	Paste	M-53	Piles
16	//	Powder	M-56	Gastric disorder
17	//	Capsule	M-57	Kidney stone
18	Mardan	Paste	M-64	Arthritis
19	//	Paste	M-66	Gastric disorder
20	//	Tablets	M-67	Arthritis
21	//	Paste	M-70	Hepatitis
22	Swabi	Syrup	M-72	Kidney stone
23	Nowshera	Powder	M-75	Sexual tonic
24	Swabi	Syrup	M-77	Kidney stone
25	Buner	Paste	M-80	Sexual tonic
26	Mardan	Capsule	M-83	Piles
27	Charsadda	Powder	M-85	Kidney stone
28	Charsadda	Tablets	M-89	Gastric disorder
29	Malakand	Tablets	M-92	Sexual tonic

30	Dir (U)	Tablets	M-95	Arthritis



Fig. 1: The collected herbal medicines from various districts of Khyber Pakhtunkhwa.

2.4 Samples preparation

The collected samples were prepared for analysis as per following protocols.

2.4.1 Preparation of Media for Culturing Microbes

The cultures used for the purpose of bacterial and fungal growth were;

i. Sodium thioglycolate medium (Oxoid, England) was prepared (28.0 g/1000 mL) distilled water.
ii. Sabouraud dextrose medium (Oxoid, England) was prepared (65.0 g/1000 mL) distilled water.
iii. Nutrient agar medium (Oxoid, England) was prepared (28.0 g/1000 mL) distilled water.
iv. MacConkey agar media was prepared (51.53 gm/1000 ml) distilled water.

The prepared culture media solutions were heated with continuous mixing to prevent clump formation, followed by autoclaving.

2.4.2 Preparation of Petri Dishes

Sterile plastic Petri plates were used for media preparation. Nutrient agar, Sabouraud dextrose agar, sodium thioglycolate medium, and MacConkey agar were poured into plates under aseptic conditions in a laminar flow hood. Prior to pouring, the media were cooled to an appropriate temperature preventing from contamination and also bubble formation and care was taken to prevent bubble formation. The plates were sealed with Parafilm, labeled accordingly, and then incubated at 37 °C for 24 hours.

2.4.3 Preparation of Normal Saline solution

An isotonic sodium chloride solution (0.9% w/v) was prepared and then filtered with sterile filter paper discs.

2.4.4 Preparation of Stock Solution and other relevant Dilutions

A stock solution for each sample was prepared by dispersing 1 g of the material in 10 mL of normal saline with gentle stirring. To reduce viable microbial cells, the dispersions were allowed to stand for five minutes. The serial dilutions from the stock solutions were then prepared accordingly, with each subsequent dilution made at a 1:10 ratio using sterile normal saline. All prepared dilutions were appropriately labeled.

2.2.5 Inoculation of Drug Samples

All solid dosage form samples were aseptically ground into fine particles using a sterile pestle and mortar. Serial dilutions of the samples were applied to agar plates using a micropipette delivering 0.1 mL of the sample, which was evenly spread across the surface and allowed to dry. The inoculated plates were then incubated at 37 °C for 24 hours and subsequently examined for microbial growth. Emerging colonies were sub-cultured onto selective sterile media in Petri dishes to obtain pure isolates. The purified microbial colonies were then counted and quantified using a colony counter.

The collected traditional medicine samples were then subjected to the following microbial examinations:

i. Detection of microbial presence or absence,
ii. Gram staining of positive samples and
iii. Presence or absence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella spp.* and *Candida albican*.

3.0 RESULTS

3.1 Detection of microbial presence or absence using the plate method

The prepared samples were analyzed and examined for microbial growth. Inoculation was carried out using sterile loops on Petri dishes containing nutrient agar as the culture medium. The inoculated plates were incubated at 37 °C for 24 hours. From the total samples

(thirty) tested, twenty-one showed positive microbial growth. The detection percentage in collected samples was 70%. The details of the screened drug samples are given in Table 2.

Table-2. Result of the screened samples

S. No	Sample Identification code	Marketed Dosage Form	Growth results
1	M-01	Syrup	Negative
2	M-04	Paste	//
3	M-06	Capsule	Positive
4	M-10	Paste	//
5	M-13	//	//
6	M-17	Pills	//
7	M-19	Ointment	//
8	M-21	Tablets	//
9	M-22	Pills	//
10	M-27	Paste	Negative
11	M-33	Powder	//
12	M-34	//	Positive
13	M-35	//	//
14	M-52	Tablets	//
15	M-53	Paste	Negative
16	M-56	Powder	Positive
17	M-57	Capsule	//
18	M-64	Paste	//
19	M-66	//	//
20	M-67	Tablets	Negative
21	M-70	Paste	Positive
22	M-72	Syrup	Negative
23	M-75	Powder	Positive
24	M-77	Syrup	Negative
25	M-80	Paste	Positive
26	M-83	Capsule	//
27	M-85	Powder	//
28	M-89	Tablets	//
29	M-92	//	//
30	M-95	//	Negative

3.2 Gram staining of positive samples

i. The microbial colonies observed were subjected to gram staining protocols in order to differentiate gram-positive and gram-negative bacteria. This procedure was performed on all purified microbial cultures obtained from the microbial analysis of collected samples. Gram staining differentiates bacterial species based on the structural make up in their cell walls. Bacteria with a thick peptidoglycan layer in their cell walls, retain the crystal violet dye and appear violet (Gram-positive), whereas those with a thinner peptidoglycan layer do not retain the dye after decolorization procedure, instead take up the counterstain and appear red (gram-negative). The Gram staining procedure consisted of the following three steps

ii. Heat fixation: A single colony was carefully picked, suspended in a droplet of water on a glass slide, smeared evenly, air-dried, and then heat-fixed.

iii. Primary stain: Crystal violet reagent was applied to the smear for 60 seconds, followed by gentle rinsing with water for 30 seconds.

iv. Moderant: A drop of Gram's iodine was added to the slide to form the crystal violet–iodine (CV–I) complex, which was allowed to stand for one minute.

v. Decolorization: The crystal violet–iodine (CV–I) complex was then decolorized with ethanol until all excess color was removed.

vi. Counter staining: Safranin dye was applied to the slide and allowed to stand for one minute, followed by rinsing under running tap water for 30 seconds. The Gram-positive and Gram-negative bacteria were then observed based on their staining characteristics in Figure 2.

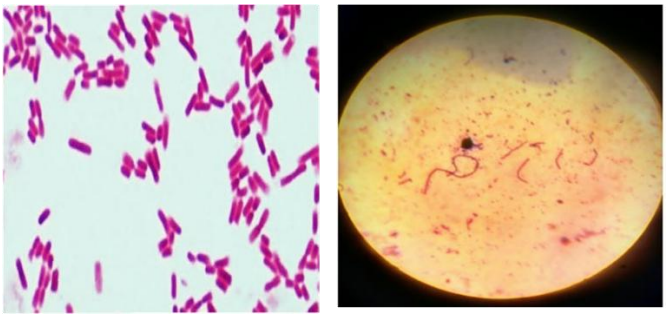


Fig. 2: Gram- negative and gram-positive bacteria under microscope

3.3 Isolation and Identification of Microorganisms

Serial dilution was performed for all samples. For each sample, three test tubes containing 9 mL of distilled water were prepared. One milliliter of the stock solution was added to the first tube, mixed thoroughly, and 1 mL from this tube was transferred to the second tube, followed by 1 mL to the third tube, achieving a threefold dilution. Using a sterile loop, aliquots from the last dilution were inoculated onto sterilized petri

dishes containing nutrient agar to produce countable bacterial colonies. The inoculated petri dishes were incubated at 37 °C for 24 hours to allow growth of bacteria. Bacterial isolates were further confirmed on selective media: Eosin Methylene Blue (EMB) and MacConkey agar for Escherichia coli, and Mannitol Salt Agar (MSA) for Staphylococcus aureus. Fungal growth observed on Sabouraud Dextrose Agar was stained using potassium hydroxide, a primary reagent for fungal identification, and the fungal elements were examined under a compound microscope.

Mannitol Salt Agar (MSA) plates were used to cultivate Staphylococcus aureus. Samples were inoculated onto MSA and incubated at 37 °C for 24 hours. Following incubation, petri dishes were examined for yellow-colored colonies indicative of S. aureus. On Eosin Methylene Blue (EMB) agar, Escherichia coli was identified by its characteristic metallic green sheen.



Fig.3: The images show growth of E. coli showing lactose fermentation on MacConkey agar, Colonies of E. coli on Eosin Methylene Blue, and growth of S. aureus showing Mannitol fermentation on Mannito Salt Agar

3.4 Selected Microorganisms

3.4.1 Staphylococcus aureus

Staphylococcus aureus was identified in six samples during the microbial burden screening, out of a total of twenty-one samples that tested positive for microorganisms, representing 28% of the contaminated samples.

S. aureus is a significant human pathogen causes a variety of infections, including boils, abscesses, pneumonia, and toxic shock syndrome. Although it commonly resides on human skin and mucous membranes without causing harm, it can lead to disease if it enters the body. S. aureus is also a frequent cause of foodborne illness, which can result in severe abdominal cramps and vomiting. Most strains remain susceptible to a wide range of antibiotics, allowing infections to be effectively treated.[22].

3.4.2 Escherichia coli

Escherichia coli was detected in seven samples, accounting for 33% of the contaminated samples.

E. coli is an infectious bacterium associated with gastroenteritis, urinary tract infections, and neonatal meningitis. It commonly causes symptoms such as severe abdominal cramps, diarrhea, and fever. In rare cases, highly virulent strains may lead to bowel necrosis.[23]. *E. coli* is the most common cause of urinary tract infections in humans and is responsible for a variety of gastrointestinal diseases. Its pathogenicity is generally attributed to the presence of one or more virulence factors, such as invasiveness factors or heat-labile toxins, which are often identified through specific virulence markers or serotypes associated with these factors. [24].

3.4.3 Salmonella Spp.

Salmonella spp. were detected in three samples, representing 14% of the contaminated samples.

Salmonella is a genus of rod-shaped, Gram-negative bacteria in the family Enterobacteriaceae, responsible for acute diarrheal diseases that contribute significantly to global morbidity and mortality each year, according to the World Health Organization (WHO)[25]. Salmonellosis is one of the most common food-borne infections. Infections caused by Salmonella are classified into minor and major forms. Minor salmonellosis, caused by non-typhoid Salmonella strains, typically results in self-limiting diarrhea and rarely progresses to bacteremia or meningitis. Major salmonellosis, on the other hand, is exemplified by typhoid fever, which presents with symptoms such as fever, headache, malaise, and occasionally cough.[26]. The primary reservoirs of Salmonella are the intestines of humans and animals. Transmission occurs through a variety of sources, with eggs, meat, dairy products, vegetables, and contaminated water being the most significant. [27].

3.4.4 Candida Albicans

The sample with ID M-85, a powder formulation prescribed for kidney stones, tested positive for *Candida albicans*.

Candida albicans is a fungal species capable of causing infections in humans and can be potentially toxic. Fungal contamination in traditional medicines may lead to chemical alterations of the product, resulting in spoilage. This not only diminishes the therapeutic efficacy of the drug but may also cause severe toxic and adverse effects.[28]. Candidiasis, caused by *Candida albicans*, can be life-threatening if left untreated. [29].

3.4.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in soil and water. It is an opportunistic pathogen responsible for approximately 10–20% of all hospital-acquired infections. This bacterium can cause infections in the respiratory, urinary, and gastrointestinal tracts, and is also associated with keratitis, otitis media, and bacteremia. [30]. Patients with weakened immune systems—such as those with HIV, burn injuries, or cystic fibrosis—are particularly susceptible to *P. aeruginosa* infections. These infections are often challenging to treat with conventional antibiotic therapies. [31].

The summary of the microbiological evaluation of collected samples is given in Table 4, Table 5 and Table 6. The percentage detection of microorganisms is given in Figure 4.

Table-3. Identified Microbes in analyzed samples

S. No	Sample Identification code	Bacterium identified
1	M-06	*Escherichia Coli*
2	M-10	*Salmonella sp., S. aureus*
3	M-13	*Escherichia Coli*
4	M-17	*S. aureus*
5	M-19	-
6	M-21	*Escherichia Coli*
7	M-22	-
8	M-34	*S. aureus*
9	M-35	*S. aureus*
10	M-52	*Escherichia Coli*
11	M-56	*S. aureus*
12	M-57	*S. aureus*
13	M-64	*Salmonella sp*
14	M-66	*Escherichia Coli*
15	M-70	*S. aureus*
16	M-75	*S. aureus, Escherichia Coli*
17	M-80	*Salmonella sp*
18	M-83	*S. aureus*
19	M-85	*Candida Albicans*
20	M-89	*S. aureus*
21	M-92	*S. aureus*

Table-4. Summary of Analyzed Samples for Microbial Burden

S. No.	Analysis details	Detection of microbes in total number of samples	Detection %age
1	Samples with microbial burden	21	70%
2	Samples without microbial burden	09	30%

Table-5. Summary of Analyzed Samples for Microbial Burden

S. No	Selected microbes	Detected No.	%age detection
1	*S. aureus*	11	52 %
2	*P. aeruginosa*	ND	NA
3	*Escherichia Coli*	06	29 %
4	*Salmonella spp.*	03	14 %
5	*C. Albicans*	01	08%

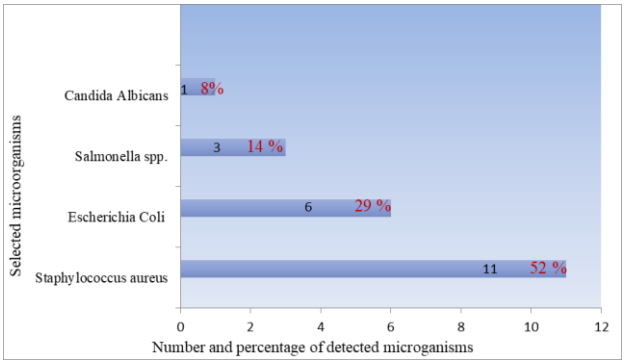


Fig.4: Number of Detected Microorganisms

4.0 DISCUSSION

The collected samples were subjected to microbiological evaluation, revealing that 70% of the screened samples were contaminated with microorganisms. *S. aureus*, *E. coli*, *Salmonella spp*., and *C. albicans* were detected in these formulations. Some of these microorganisms are pathogenic and capable of causing various infectious diseases. The inclusion of traditional herbal medicine across the globe has raised concerns about different negative effects on health, specifically in 3rd world countries like Brazil, Paraguay, Chile and other South American nations where the use of these traditional medicine is on ahigh rise due to easy availability and low cost. However, the contamination may be attributed to poor hygienic practices and inadequate handling of the herbal preparations, insufficient environmental controls such as temperature and moisture during processing, use of contaminated raw materials, repeated use of packaging containers, substandard storage and transportation conditions, absence of preservatives in the formulations, and a lack of regulatory oversight and quality control protocols for traditional medicines [32]. An important aspect of microbial growth is in practice in China. This aspect discusses and practices the fermentation of available traditional medicines in market. Research has demonstrated that microbial polysaccharides—particularly those derived from medicinal fungi—exhibit strong biological activity. Examples include polysaccharides from *Grifola frondosa*, *Ganoderma lucidum*, and *Phellinus igniarius* (PL), which show that in some instances the microbial growth can be of great benefit in order to have the active constituent of ones own desire and need of the product and patients. [33]. Spores are predominantly present on dried plant material, where they show high resistance to chemicals and various environmental conditions, making them a leading cause of food poisoning. The detection of *Enterobacteriaceae* and *E. coli* indicates fecal contamination, while the presence of coliforms serves as a marker of poor hygienic conditions [34]. A similar study was performed to check the growth of microbes in traditional medicine and was found that *Escherichia coli*, *Salmonella spp*., *Pseudomonas aeruginosa* and *Staphylococcus aureus* was some of major microbes found during the study. The consumption of homemade and commercial herbal medicines poses significant health risks for the elderly, primarily due to the absence of standardized microbial quality controls. The findings in this study revealed bacterial and fungal counts exceeding safety thresholds, and we also identified pathogenic bacteria within these herbal preparations. In terms of the sex distribution of the elderly participants, most were female. This finding aligns with gender-based comparative studies on medicinal plant knowledge, which highlight the social role of women—particularly wives and daughters—in healthcare practices, including recognizing illnesses, understanding prognoses, and administering initial treatments [32]. This study with the growth of bacteria and fungi in the samples used by the residents of rural areas of Khyber Pakhtunkhwa, Pakistan revealed an alarming situation, because of their extensive use. For having more reliable results, the colony forming units data would have a better impact on the interpretation of results and pathogenicity.

5.0 CONCULSION

Traditional herbal medicines often contain high levels of microorganisms, as proper decontamination protocols are frequently not followed. Such microbial contamination can cause serious health hazards, especially to most vulnerable sector of population, such as elders and children. Moreover, microbial presence can alter the physical, chemical, and organoleptic properties of these medicines, potentially converting active ingredients into toxic compounds. Immunocompromised patients are especially at risk, and exposure may lead to severe metabolic harm or even death. The findings of this study highlight the need for strict regulation of these products, including thorough testing and evaluation, to ensure their safe therapeutic use. To minimize contamination, control measures should be implemented, including standardized operating procedures, sourcing raw materials from registered suppliers, and adopting standard biological procedures during formulation to manage microbial bioburden effectively.

REFERENCES

[1] B.T. Shaikh, J. Hatcher, Complementary and alternative medicine in Pakistan: prospects and limitations, Evidence-Based Complementary and Alternative Medicine, 2 (2005) 139-142.

[2] E. Ernst, M. Pittler, B. Wider, K. Boddy, Complementary/alternative medicine for supportive cancer care: development of the evidence-base, Supportive Care in Cancer, 15 (2007) 565-568.

[3] Z. Aziz, N. Tey, Herbal medicines: prevalence and predictors of use among Malaysian adults, Complementary Therapies in Medicine, 17 (2009) 44-50.

[4] S. Hussain, A. Saeed, M. Ahmed, A. Qazi, Contemporary role and future prospects of medicinal plants in the health care system and pharmaceutical industries of Pakistan, URL http://www. telmedpak. com/doctorsarticles.(accessed on 10/18/2015), (2006).

[5] K. Chan, Progress in traditional Chinese medicine, Trends in pharmacological sciences, 16 (1995) 182-187.

[6] C.M. Judkins, P. Teale, D.J. Hall, The role of banned substance residue analysis in the control of dietary supplement contamination, Drug Testing and Analysis, 2 (2010) 417-420.

[7] J. Pfeffer, C.T. Fong, The end of business schools? Less success than meets the eye, Academy of management learning & education, 1 (2002) 78-95.

[8] Q. Shan, Y. Wang, K. Chen, Z. Liang, J. Li, Y. Zhang, K. Zhang, J. Liu, D.F. Voytas, X. Zheng, Rapid and efficient gene modification in rice and Brachypodium using TALENs, Molecular plant, 6 (2013) 1365.

[9] M.J. Bahr, K.J. Vincent, M.J. Arthur, A.V. Fowler, D.E. Smart, M.C. Wright, I.M. Clark, R.C. Benyon, J.P. Iredale, D.A. Mann, Control of the tissue inhibitor of metalloproteinases-1 promoter in culture-activated rat hepatic stellate cells: Regulation by activator protein-1 DNA binding proteins, Hepatology, 29 (1999) 839-848.

[10] R. Zamir, A. Hosen, M.O. Ullah, N. Nahar, Microbial and heavy metal contaminant of antidiabetic herbal preparations formulated in Bangladesh, Evidence-Based Complementary and Alternative Medicine, 2015 (2015).

[11] I. Kosalec, J. Cvek, S. Tomic, Contaminants of medicinal herbs and herbal products, Arhiv za higijenu rada i toksikologiju, 60 (2009) 485.

[12] A. Okem, C. Southway, A. Ndhlala, J. Van Staden, Determination of total and bioavailable heavy and trace metals in South African commercial herbal concoctions using ICP-OES, South African Journal of Botany, 82 (2012) 75-82.

[13] O. Okunola, A. Uzairu, G. Ndukwe, Levels of trace metals in soil and vegetation along major and minor roads in metropolitan city of Kaduna, Nigeria, African journal of Biotechnology, 6 (2007).

[14] E. Boydak, M. Alpaslan, M. Hayta, S. GERCek, M. Simsek, Seed composition of soybeans grown in the Harran region of Turkey as affected by row spacing and irrigation, Journal of agricultural and food chemistry, 50 (2002) 4718-4720.

[15] D. Adeyemo, A. Adeleye, Emotional intelligence, religiosity and self-efficacy as predictors of psychological well-being among secondary school adolescents in Ogbomoso, Nigeria, Europe's Journal of Psychology, 4 (2008) 22-31.

[16] R. Noor, N. Huda, F. Rahman, T. Bashar, S. Munshi, Microbial contamination in herbal medicines available in Bangladesh, Bangladesh Medical Research Council Bulletin, 39 (2013) 124-129.

[17] V. Mugoyela, K.D. Mwambete, Microbial contamination of nonsterile pharmaceuticals in public hospital settings, Therapeutics and Clinical Risk Management, 6 (2010) 443.

[18] F. Oluyemisi, O. Henry, O. Peter, Standardization of herbal medicines-A review, International Journal of Biodiversity and Conservation, 4 (2012) 101-112.

[19] E.B. Famewo, A.M. Clarke, A.J. Afolayan, Identification of bacterial contaminants in polyherbal medicines used for the treatment of tuberculosis in Amatole District of the Eastern Cape Province, South Africa, using rapid 16S rRNA technique, Journal of Health, Population and Nutrition, 35 (2016) 1-9.

[20] K.M. Hassan, P.M. Njogu, N.M. Njuguna, S.N. Ndwigah, Microbiological contamination of herbal medicinal products marketed in Kenya for chronic diseases: a case study of Nairobi metropolis, Journal

of Herbal Medicine, (2021) 100475.
[21] H.S. Husni, Pathogenic Microorganisms, Toxigenic Fungi, Heavy Metal accumulation, and Toxic Materials Contamination in Natural Products, Systematic Reviews in Pharmacy, 11 (2020) 1772-1776.
[22] J.-Y. Lee, Y. Nagano, J.P. Taylor, K.L. Lim, T.-P. Yao, Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy, Journal of Cell Biology, 189 (2010) 671-679.
[23] M. Besser, Richard E, M. Griffin, Patricia M, M. Slutsker, MPH, Laurence, Escherichia coli O157: H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease, Annual review of medicine, 50 (1999) 355-367.
[24] W.E. Stamm, R.A. Weinstein, R.E. Dixon, Comparison of endemic and epidemic nosocomial infections, The American journal of medicine, 70 (1981) 393-397.
[25] J.M. Besser, Salmonella epidemiology: A whirlwind of change, Food microbiology, 71 (2018) 55-59.
[26] P. Wattiau, C. Boland, S. Bertrand, Methodologies for Salmonella enterica subsp. enterica subtyping: gold standards and alternatives, Applied and environmental microbiology, 77 (2011) 7877-7885.
[27] F. Brenner, R. Villar, F. Angulo, R. Tauxe, B. Swaminathan, Salmonella nomenclature, Journal of clinical microbiology, 38 (2000) 2465-2467.
[28] R.N. Harris, R.M. Brucker, J.B. Walke, M.H. Becker, C.R. Schwantes, D.C. Flaherty, B.A. Lam, D.C. Woodhams, C.J. Briggs, V.T. Vredenburg, Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus, The ISME journal, 3 (2009) 818-824.
[29] D. Gupta, D.M. Thappa, Deep Fungal Infections, Comprehensive Approach to Infections in Dermatology, 158 (2016).
[30] T. Ikeno, K. Fukuda, M. Ogawa, M. Honda, T. Tanabe, H. Taniguchi, Small and rough colony Pseudomonas aeruginosa with elevated biofilm formation ability isolated in hospitalized patients, Microbiology and immunology, 51 (2007) 929-938.
[31] M. Whiteley, E. Greenberg, Promoter specificity elements in Pseudomonas aeruginosa quorum-sensing-controlled genes, Journal of Bacteriology, 183 (2001) 5529-5534.
[32] C.M. de Sousa Lima, M.A.T. Fujishima, B. de Paula Lima, P.C. Mastroianni, F.F.O. de Sousa, J.O.J.B.C.M. da Silva, Therapies, Microbial contamination in herbal medicines: a serious health hazard to elderly consumers, 20 (2020) 17.
[33] T. Wu, N. Wang, Y. Zhang, X.J.A.J.M.R. Xu, Advances in the study on microbial fermentation and transformation of traditional Chinese medicine, 7 (2013) 1644-1650.
[34] D. Saida, E.J.J.M.P.S. ouar Ibtissemb, Microbial contamination of medicinal plants, 2 (2023) 01.