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Microbial Contamination of the Traditional Medicine Used by the *Pnar* Tribe of Meghalaya, India

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Abstract: This study examined microbial contamination in herbal medicinal products in Jaintia Hills Districts, Meghalaya, India. Analysis of 40 samples revealed widespread bacterial and fungal growth, exceeding safety limits in 30% of samples. Also, Aerobic bacteria counts ranged from 64 × 10^5 to 130 × 10^6 CFU/g, surpassing WHO and Ayurveda Pharmacopeia of India guidelines. Similarly, 87.5% of samples showed significant fungal growth, with 12.5% exceeding safety limits. Bacterial analysis revealed *E. coli* (35%) which indicates fecal contamination and poor hygiene. *S. aureus* (53%) suggests poor handling and preparation practices. *P. aeruginosa* (20%) is another indicator of poor hygiene and potential contamination. The presence of these pathogens in herbal products is a serious concern globally, and it's crucial to address this issue to ensure consumer safety. The three pathogens *E. coli*, *S. aureus* and *P. aeruginosa* can cause a range of health problems, from mild to severe, including gastrointestinal issues (diarrhea, vomiting, abdominal pain), skin and wound infections, respiratory problems (pneumonia, bronchitis), life-threatening conditions (sepsis, meningitis, urinary tract infections).

Keywords: Herbal Medicinal Products (HMPs), World Health Organization (WHO), *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Total viable count (TVC), Colony forming units (CFU), Jaintia Hills Districts, Meghalaya, India

I. INTRODUCTION

The World Health Organization's (WHO) definition of Herbal Medicinal Products (HMPs). HMPs are medicinal products that contain only herbal drugs or herbal drug preparations as active ingredients. Herbal drugs refer to raw materials, such as plants, used for medicinal purposes. Herbal drug preparations include processed herbal materials, like extracts, tinctures, or powders. Mixed seasoning products are blends of multiple herbs. Excipients, like binders or fillers, may be added to HMPs to enhance their absorption or stability. Traditional herbal remedies may include non-plant materials like animal or mineral substances in certain regions. However, products containing chemically defined active substances, artificial compounds, and isolated constituents from herbs (like isolated alkaloids or glycosides) are not classified as herbal products according to the WHO definition [48].

Despite the clear definition and framework for herbal medicinal products, the industry still faces significant challenges, particularly when it comes to microbial contamination. This contamination can lead to compromised product quality and reduced competitiveness in international markets [24]. The definition of contamination provided highlights the various ways in which herbal products can become contaminated, including chemical impurities, microbiological contaminants (such as bacteria, fungi, or viruses), and foreign matter (such as heavy metals or other substances) [26].

This contamination can occur at any stage of production, including starting materials (e.g., raw herbs), intermediate products (e.g., extracts or powders), finished products (e.g., capsules or tablets), sampling, packaging, or repackaging or storage or transport. The introduction of microorganisms in herbal medicinal products can alter the physicochemical characteristics of the product, which may lead to harmful effects on the quality of the herbal medicinal products [10].

The sources of contamination in the herbal medicinal products are as follows
i) Environmental conditions in which the medicinal plants are grown or collected.
ii) Drying and processing conditions of the herbal medicinal products.
iii) The conditions under which the herbal medicinal products are stored and transported.
iv) Insanitary utilization of herbal medicinal products by the patients.
v) The manufacturing procedures, when the ready-made herbal medicinal products are prepared [11, 20].

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Herbal products can be contaminated with various substances, including pesticides, toxic metals, and microorganisms. The main microorganisms found in herbal products are, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Studies have shown that bacterial contamination is a significant problem in traditional medicine samples, highlighting the need for strict quality control measures. Fungal contamination, particularly with *Aspergillu*s and *Penicillium* species, is also a significant concern due to the potential production of mycotoxins. Consuming contaminated traditional medicines can lead to chronic health issues, such as liver damage, kidney damage, immune system suppression and cancer. [46,49]. Consuming herbal products contaminated with microorganisms like *E. coli* can lead to serious health issues. *E. coli* is a type of bacteria that is commonly found in the large intestine of humans and warm-blooded animals. While most *E. coli* strains are harmless and do not cause disease in healthy individuals, some pathogenic strains can cause a range of diseases, including intestinal diseases such as diarrhea, urinary tract infections, inflammatory bowel disease, extra-intestinal diseases such as pneumonia, meningitis, and sepsis. These diseases can affect both healthy individuals and those with compromised immune systems, such as the elderly, young children, and people with chronic illnesses [15 and 39]. The genus *Escherichia* was named after Theodor Escherich, *E. coli* is a facultative anaerobic Gram-negative bacillus, belonging to the family Enterobacteriaceae. *E. coli* strains can become pathogenic when they acquire certain genetic material, such as virulence factors, which enable them to cause disease. Virulent strains of *E. coli* can cause a range of serious infections, including gastroenteritis (diarrheal disease), urinary tract infections (UTIs), neonatal meningitis (infection of the lining of the brain and spinal cord in newborns), peritonitis (inflammation of the lining of the abdominal cavity), mastitis (infection of the breast tissue), gram-negative pneumonia (lung infection), septicemia (bloodstream infection), hemolytic-uremic syndrome (a potentially life-threatening condition that can cause kidney failure and anemia) [11].

Alexander Ogston and Louis Pasteur first described "micrococci" isolated from furuncles and abscesses in 1880. "Staphyle" (Greek: σταφυλή) meaning "bunch of grapes" (likely due to the cluster-like appearance of the bacteria), "Coccus" (Greek: κόκκος) meaning "round-shaped" (referring to the bacteria's spherical shape) and "Aureus" (Latin: aureus) meaning "golden" (due to the characteristic orange-yellow color of the colonies on agar plates). The temperature growth range of *S. aureus* is 7°C - 48.5°C, optimum growth temperature is 30 °C - 37° C. This information helps us understand the growth conditions and characteristics of *S. aureus*, which is an important pathogen in human medicine. Alterations in temperature during the storage of herbal medicinal products can create an environment conducive to the growth of *S. aureus*. Additionally, *S. aureus* can thrive in a wide range of pH conditions, from 4.2 to 9.3, with an optimum pH range of 7-7.5. This adaptability makes *S. aureus* a resilient and potentially problematic contaminant in herbal products. Alterations in pH during the manufacturing process can create an environment conducive to the growth of *S. aureus* in herbal medicinal products. *S. aureus* is a highly adaptable and opportunistic pathogen that can cause a wide range of diseases, including localized skin and soft-tissue infections (e.g., abscesses, furuncles, cellulitis), life-threatening septicemia (bloodstream infection), bloodstream infections (bacteremia), pneumonia, meningitis, osteomyelitis (bone infection), endocarditis (heart valve infection) [15].

Pseudomonas is a tremendously versatile Gram-negative bacterium with the ability to flourish in a broad spectrum of environments. Pseudomonas aeruginosa can cause urinary tract infections, respiratory tract infections, bacteremia, dermatitis, bone and joint infections, soft tissue infections, GI infections etc [21]. Several studies have been done around the world to analyze the microbial content of herbal medicines in Thailand [23], Nigeria [4, 7, 25 and 9], Kenya [22, 19], Bangladesh [28, 2], Ghana [36, 13], Iran [27] India [43, 8, 17 and 34, 33, 35]

II. METHODOLOGY

A. *Study area*.

The present study was conducted in thirty-one villages spread throughout the Jaintia Hills District of Meghalaya, India (Fig 1). Selection of the study area was based on the availability of traditional healers in these villages and their willingness to share their medicines for analysis. The Jaintia Hills (located at latitude-25.4, longitude-92.1) are composed of two districts viz., East Jaintia Hills District and West Jaintia Hills District East Jaintia Hills Districts located at the easternmost part of Meghalaya and cover an area of 2040 sq. km.

It is bounded by Bangladesh in the south, North Cachar Hills district in the east and West Jaintia Hills district in the north and west. West Jaintia Hills District is bounded by Assam in the North, Bangladesh and East Jaintia Hills District in the South, Assam in the East and East Khasi Hills District in the West. The total population of Jaintia Hills is 3, 95,124 [6] .

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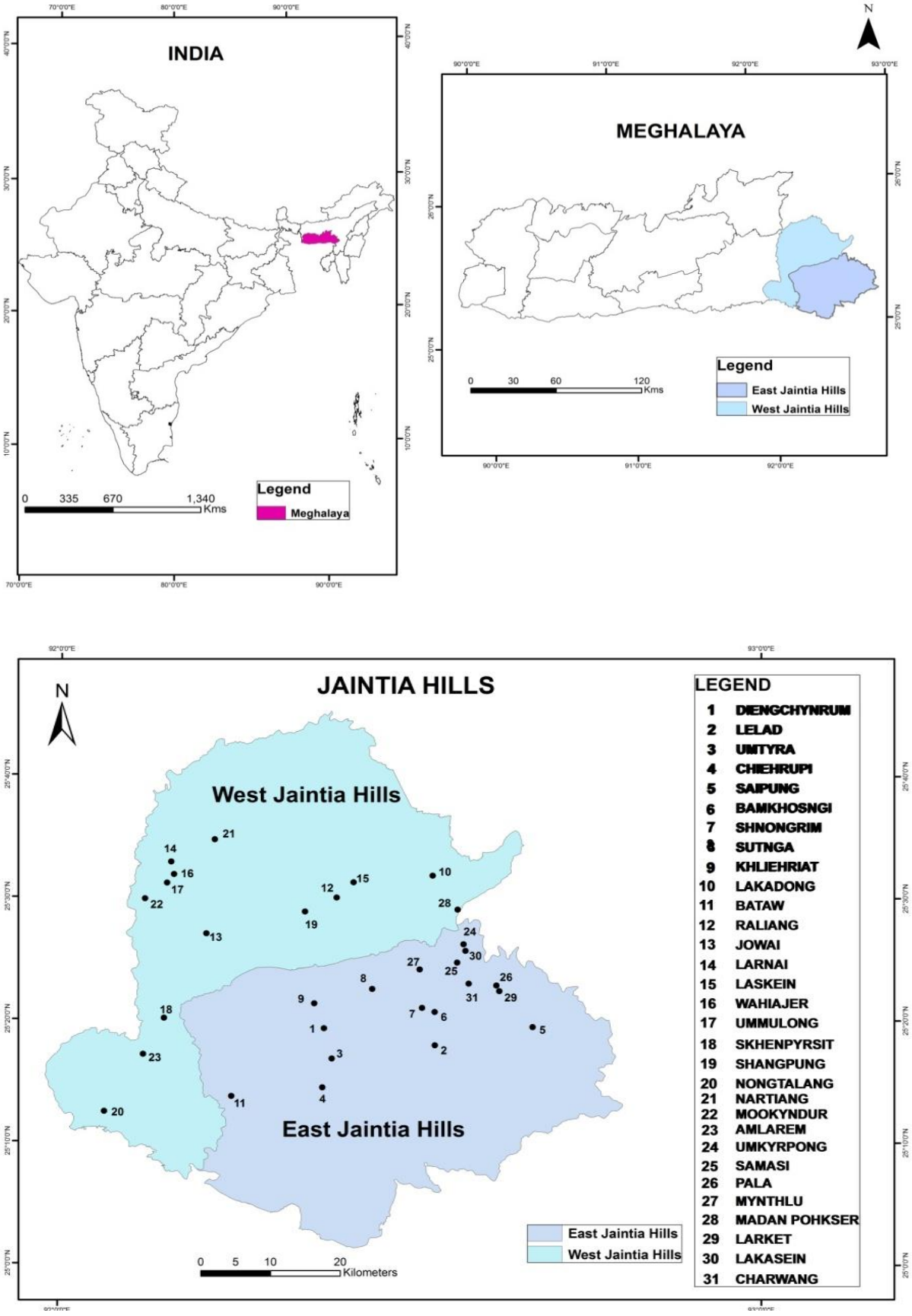

Fig. 1-Location map of Jaintia hills showing the study villages

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B. *Sample Collection and Preparation for Laboratory Analysis*.
Lots sizes of approximately 15 - 20 g for each product purchased were taken and packed aseptically in sterile zipped polythene bags. In the laboratory, sample containers were opened under aseptic conditions and 1 g of product was placed into a sterile container containing sterile Buffered Sodium Chloride-Peptone Solution pH 7.0, and the volume was adjusted to 10 ml. Then, 1 ml of the prepared homogenate inoculum obtained by vortex was transferred into a test tube containing 9 ml of sterile physiological saline. The procedure was repeated up to 6 serial dilutions and in the last dilution 1 ml of inoculum was discarded [47].

B. *Determination of total viable counts*.
Plate count for bacteria: The samples for total viable colony (TVC) count determination were the serially diluted specimens in sterile physiological normal saline in the 10^{-1} to 10^{-10} dilutions. Using a sterile pipette, 1 ml of each dilution was inoculated into Nutrient Agar (NA) plate in triplicate. The plates were incubated at 37°C for 24 hours (±3 hours). Colony counts were done by taking 300 colonies per plate as the maximum consistent with good evaluation [47]. The colony forming units (cfu)/g or (cfu)/ ml were calculated as follows,

CFU/ml – (Number of colonies ×dilution factor) / volume of culture plate [30]
Plate count for fungi: For fungi, the same serial dilution method as for bacteria was followed but Potato Dextrose Agar with antibiotics was used in place of Nutrient agar and the plates were incubated at 20^0 to 25^0 C for 5 days unless a more reliable count was obtained in a shorter time. The Colony Forming Units were calculated using plates with not more than 100 colonies [47].

C. *Isolation and identification of Escherichia coli*.
Having dissolved 1 g of the preparation being examined using lactose broth the volume was adjusted to 10 ml with the same medium. 90 ml of nutrient medium was inoculated with a quantity of the solution, thus obtained containing 1 g or 1 ml of the preparation being examined. The mixture was incubated at 35^0C to 37^0C for 24 to 48 hours. Isolation of *Escherichia coli* was achieved by streaking the pre-enriched culture from nutrient medium onto MacConkey agar plates to obtain pure colonies The plates were inverted and incubated at 44.5ºC for 24 to 48 hours. *E*. coli on MacConkey agar appeared bright pinky-red in color. Presumptive *E*. *coli* colonies were identified at the species level by Gram staining and IMViC tests [47].

D. *Isolation and identification of Staphylococcus aureus*:
1 g of the sample being examined was dissolved using lactose broth in place of buffered sodium chloride-peptone solution, and the volume was adjusted to 10 ml with the same medium.90 ml of soybean-casein digest medium was inoculated with a quantity of the solution, thus obtained containing 1 g or 1 ml of the preparation being examined. The mixture was incubated at 35^0C to 37^0C for 24 to 48 hours. Isolation of the *S. aureus* was achieved by streaking the pre-enriched culture from soybean-casein digest onto selective differential agar plates of freshly prepared Mannitol Salt Agar (MSA); a selective and differential medium used for the isolation of pathogenic staphylococci. The plates were incubated at 37°C for 18 to 24 hours under aerobic conditions. Colonies showing golden yellow color or colorless were presumed to be Staphylococcus spp. On MSA, pathogenic *S. aureus* produces small colonies surrounded by yellow zones as a result of mannitol sugar fermentation. If, upon examination of the incubated plates, none of them contained colonies having the characteristics for the media used, the sample met the requirements for the absence of *Staphylococcus aureus*. The presumtive colonies were subcultured onto nutrient agar for purification and masking the effect of acid produced during the fermentation of MSA. The colonies selected from nutrient agar were subjected to Gram stain, to check the morphology and staining characteristics. The gram-positive cocci organisms were subjected to a catalase test to differentiate between *Staphylococcus* spp. and *Streptococcus* spp. Catalase-positive colonies were further subjected to coagulase and deoxyribonuclease tests. If bubbles appear (due to the production of oxygen gas) the bacteria are catalase-positive. If no bubbles appear, the bacteria are catalase-negative. For deoxyribonuclease tests, if a clear zone is observed around the colony on the Dnase Agar plate, the organism is positive. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative [47].

E. *Isolation and identification of Pseudomonas aeruginosa*.
Having dissolved 1 g of the sample being examined using lactose broth the volume was adjusted to 10 ml with the same medium. 90 ml of soybean-casein digest medium was inoculated with a quantity of the solution, thus obtained containing 1 g or 1 ml of the preparation being examined. The mixture was incubated at 35^0 C to 37^0 C for 24 to 48 hours. The medium was examined for growth, if growth was present, a portion of the medium was streaked on the surface of the cetrimide agar medium, covered, and incubated at 35^0 C to 37^0 C for 18 to 24 hours. Colonies showing greenish coloration under UV light and on Gram staining appeared to be Negative rod-shaped bacilli were presumed to be *P*. *aeruginosa*.

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The representative suspect colonies from cetrimide agar were streaked onto Pseudomonas Agar medium for the detection of fluorescein and the Pseudomonas agar medium for the detection of pyocyanin. The inoculated media were incubated at 33^0C to 37^0C for not less than 3 days. The streaked surfaces were examined under ultra-violet light to determine colonies which is blue for Pseudomonas agar medium for the detection of fluorescein and yellow for Pseudomonas agar medium for the detection of pyocyanin. If growth of suspect colonies occurs, 2 or 3 drops of a freshly prepared 1 per cent w/v solution of N,N,N1,N1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper were smear with the colony; if there is development of a pink color, changing to purple, the sample is positive for P. *aeruginosa*. If there is no development of a pink color, changing to purple, the sample meets the requirements of the test for the absence of *Pseudomonas aeruginosa* [47].

III. RESULTS AND DISCUSSIONS

A. *Total viable count*.

Among the 40 samples examined, all displayed observable bacterial growth, regardless of contamination thresholds. Of these, 30% exceeded the safety limits established by WHO guidelines and the Ayurveda Pharmacopeia of India, with aerobic bacteria counts ranging from 64 × 10^5 to 130 × 10^6 CFU/g. 87.5% of the samples displayed noticeable fungal growth, regardless of acceptable contamination levels. Among them, 12.5% exceeded the safety thresholds for fungal growth (CFU/g ≤ 103), with counts ranging from 32 × 103 to 98 × 104 CFU/g. Similar results have been reported around the world where the herbal medicines exceeded the established by WHO and or different agencies like Nigeria [4 and 7], Thailand [23], Iran [27], Ghana [36], Bangladesh [28] Kenya [22,19]. In India, several studies have been reported on the contamination of herbal medicines whose results for bacterial and fungal viable count were similar to this study's findings [33- 35, 17, 50, 16]. Microscopic analysis suggested fungal contamination that was similar to another study that demonstrated the presence of fungal species known to be able to produce mycotoxins, such as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus parasiticus* [1]. Many studies have also demonstrated the presence of mycotoxins in preparations derived from medicinal plants [3]. Characterization of fungal colonies, although not required by the WHO standards [47], is important in assessing the risk of analyzing products.

B. *Determination of specific microorganisms*.

A prevalence rate of 35%,53 %, and 20% for *E. coli*, *S. aureus*, and *P. aeruginosa* respectively was recorded. Out of the 40 samples examined, *E. coli* was detected in 14 samples, *S. aureus* was found to be present in 21 samples and *P. aeruginosa* was detected in 8 samples. Several studies have been conducted around the world where the evaluation of *E. coli*, *S. aureus* and *P. aeruginosa* is done for example in Thailand [23], in Nigeria [7] and Iran (27) reported similar results to these study findings. In India, Bais et al., showed the presence of *E. coli*, *S. aureus*, and *P. aeruginosa* [34]. Evaluation of the microbiological quality of hepatoprotective herbal formulations of various brands marketed in Yavatmal, India showed the presence of *E. coli* in 4 samples, *S. aureus* in 3 samples and *P. aeruginosa* in 4 samples [33] Another study done to determine the presence of microbial content in the Ashwagandha indicates the presence of *E. coli* in 3 samples, *S. aureus* in 4 samples and *P. aeruginosa* [35]. In a study conducted in Trychi City, Tamil Nadu, the samples were found to be contaminated with more than one bacterial pathogen such as *S. aureus*, *E. coli*, *P. aeuroginosa*, *Shigella* sp and *Salmonella* sp. [17]. However, contradicting the findings of this study, a study conducted in Meerut, India reported that the herbal medicines were not contaminated with *S. aureus*, *E. coli*, or *P. aeruginosa* [8]. Factors contributing to microbial contamination in TMPs: exposure to a polluted environment, inadequate handling of raw materials, substandard packaging, use of contaminated containers, and harvesting from polluted surroundings (14). *Escherichia coli* is a bacterium commonly inhabiting the lower intestine of warm-blooded animals. While many *E. coli* strains are harmless, certain types can induce severe food poisoning. Shiga toxin-producing *E. coli* (STEC) is a strain capable of causing significant foodborne illness. Although most cases resolve on their own, some instances can progress to life-threatening conditions like hemolytic uremic syndrome (HUS), particularly affecting young children and the elderly (WHO 2018). The presence of *E. coli*, an intestinal bacterium, indicates contamination by feces in the production line, revealing poor hygiene during the preparation and storage of these herbal medicines [5, 29]. Staphylococcus aureus the most predominant bacteria isolated from contaminated samples is a normal flora of the nasal passage and skin, however, their presence in herbal preparations to be ingested orally may result in food poisoning and gastro-intestinal infection (51). Staphylococcus aureus ranks among the most prevalent bacterial infections in humans, responsible for a plethora of ailments including bacteremia, infective endocarditis, various skin and soft tissue infections (such as impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, infections related to prosthetic devices, pulmonary infections (like pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections [37, 40]. The severity and nature of the infection hinge on the strain of the bacterium and the site of invasion, potentially leading to invasive infections and/or diseases mediated by toxins [37, 12]. The pathophysiology exhibits considerable diversity contingent upon the specific type of *S. aureus* infection [37].

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Tactics employed by these bacteria to evade the host immune response encompass the production of an antiphagocytic capsule, sequestration of host antibodies, antigen masking facilitated by Protein A, biofilm formation, intracellular survival, and inhibition of leukocyte chemotaxis [41 and 40]. Pseudomonas aeruginosa is a soil-derived bacterium implicated in urinary tract and respiratory infections. Its presence suggests improper washing and handling of herbs [29]. It notably affects patients with conditions such as burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous drug addiction. This bacterium commonly contaminates healthcare settings, leading to outbreaks traced back to various hospital items. Extended hospital stays often result in colonization by *P. aeruginosa*, heightening the risk of infection. Severe cases can manifest as malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, or septicemia. Recovery prospects correlate with the severity of the patient's underlying condition. The introduction of antipseudomonal aminoglycosides and penicillins has significantly improved prognosis, particularly ticarcillin and carbenicillin for neutropenic patients. Prompt initiation of therapy is crucial for optimal outcomes. Recent years have witnessed the introduction of new drugs with antipseudomonal activity, including penicillins, cephalosporins, and other β-lactams, presenting promising avenues for therapy in these infections [14]

IV. CONCLUSION

In conclusion, the assessment of microbial contamination in herbal medicinal products across various regions underscores the pervasive issue of compromised product quality and potential health risks associated with their consumption. Studies conducted in diverse geographic locations, including Nigeria, Thailand, Iran, Ghana, Bangladesh, Kenya, and India, consistently reveal alarming levels of bacterial and fungal contamination exceeding permissible limits. *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are among the frequently identified pathogens, indicating fecal contamination, poor hygiene during preparation, and inadequate storage conditions as contributing factors.

The presence of these microbial contaminants poses significant health hazards, ranging from mild gastrointestinal discomfort to life-threatening conditions like food poisoning, septicemia, and systemic infections, especially in vulnerable populations such as children and the elderly. Additionally, the diversity of bacterial and fungal species detected underscores the complexity of the contamination issue and the need for comprehensive mitigation strategies. Efforts to improve herbal product quality must address multiple facets of the production and supply chain, including sourcing of raw materials, manufacturing processes, packaging, and storage conditions. Regulatory authorities need to enforce stringent quality control measures, including adherence to international pharmacopoeial standards, to ensure the safety and efficacy of herbal medicines.

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DECLARATIONS

List of abbreviations: Not applicable.

Ethical approval and consent to participate: This study was undertaken with the approval of the Local Traditional Healer of Jaintia Hills of Meghalaya, northeast India.

Consent for publication: Not applicable

Conflict of interest: The authors have no conflicts of interest to disclose.

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Author contributions: DS and DP conceived the idea and designed the collection of samples. DS conducted a collection of samples and experiments. DS and DP wrote the manuscript. All authors have read and approved the final version of the manuscript.

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TABLE I TOTAL VIABLE COUNT (TVC) OF THE TRADITIONAL MEDICINE PRODUCTS (TMPS) USED BY THE HEALERS OF JAINTIA HILLS DISTRICTS.

Sample no.	**Bacteria**							**Fungi**				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	CFU/g	10^{-1}	10^{-2}	10^{-3}	10^{-4}	CFU/g
1	TNTC	172.33	36.00	8.33	NIL	NIL	172.33×102	3	NIL	NIL	NIL	3×101
2	TNTC	TNTC	138.67	83.00	16.67	NIL	138.67×103	15.5	2.5	NIL	NIL	15.5×101
3	TNTC	TNTC	TNTC	TNTC	76.33	10.00	76.33×105	TNTC	TNTC	54	5.5	54×103
4	TNTC	TNTC	TNTC	TNTC	138.33	68.33	138.33×105	TNTC	TNTC	32	2.5	32×103
5	TNTC	TNTC	191.67	106.67	44.67	12.00	191.67×103	24	1	NIL	NIL	24×101
6	TNTC	TNTC	TNTC	TNTC	170.67	106.67	170.67×105	TNTC	TNTC	TNTC	26	26×104
7	TNTC	TNTC	TNTC	TNTC	TNTC	130.00	130.00×106	TNTC	TNTC	TNTC	80	80×104
8	TNTC	TNTC	161.67	55.00	13.00	NIL	161.67×103	125	26	2.5	1	125×101
9	TNTC	TNTC	198.67	161.67	132.00	94.00	198.67×103	TNTC	TNTC	TNTC	93	93×104
10	TNTC	TNTC	TNTC	53.00	28.00	6.33	53.00×104	TNTC	34	7	NIL	34×102
11	TNTC	TNTC	TNTC	177.00	32.33	NIL	177.00×104	49	10	1	NIL	49×101
12	TNTC	TNTC	166.33	64.33	9.00	NIL	166.33×103	1	1	1	NIL	1×101
13	TNTC	TNTC	TNTC	TNTC	117.33	89.67	117.33×105	TNTC	77	15	NIL	77×102
14	TNTC	TNTC	TNTC	TNTC	127.33	47.00	127.33×105	TNTC	31.5	11	NIL	31.5×102
15	TNTC	TNTC	105.00	28.33	NIL	NIL	105.00×103	TNTC	37.5	5	4	37.5×102
16	TNTC	TNTC	195.33	92.00	25.67	NIL	195.33×103	TNTC	94	9	NIL	94×102
17	TNTC	TNTC	143.00	52.67	9.33	NIL	143.00×103	58.5	7	1.5	NIL	58.5×101
18	TNTC	TNTC	TNTC	TNTC	69.33	26.00	69.33×105	59	12.5	1	NIL	59×101
19	TNTC	147.33	41.00	3.00	NIL	NIL	147.33×102	2.5	NIL	NIL	NIL	2.5×101
20	TNTC	TNTC	170.33	51.00	11.33	5.00	170.33×103	34.5	NIL	NIL	NIL	34.5×101
21	TNT	TNT	TNT	TNT	TNT	67.67	67.67×106	20.5	3	1	NI	20.5×10

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	C	C	C	C	C						L	1
22	TNTC	TNTC	TNTC	TNTC	TNTC	58.33	58.33×106	15.5	10	1.5	NIL	15.5×101
23	TNTC	TNTC	132.00	73.67	15.67	NIL	132.00×103	23	2	NIL	NIL	23×101
24	TNTC	TNTC	167.00	73.33	27.67	5.00	167.00×103	24	17	7	NIL	24×101
25	TNTC	TNTC	TNTC	TNTC	70.33	30.33	70.33×105	5	NIL	NIL	NIL	5×101
26	TNTC	TNTC	192.33	163.67	45.00	NIL	192.33×103	68	11	NIL	NIL	68×101
27	TNTC	TNTC	82.00	41.67	8.00	NIL	82.00×103	79.5	11.5	3	NIL	79.5×101
28	TNTC	TNTC	TNTC	TNTC	64.00	30.33	64.00×105	TNTC	56	11	2	56×102
29	TNTC	TNTC	154.67	99.00	16.67	NIL	154.67×103	63.5	2	1	NIL	63.5×101
30	TNTC	TNTC	159.67	77.00	NIL	NIL	159.67×103	19	8.5	2.5	10	19×101
31	TNTC	TNTC	110.33	75.00	28.67	NIL	110.33×103	TNTC	36.5	12.5	1	36.5×102
32	TNTC	TNTC	TNTC	TNTC	TNTC	63.67	63.67×106	25	1	NIL	NIL	25×101
33	TNTC	TNTC	147.00	94.33	28.00	NIL	147.00×103	17.5	5	NIL	NIL	17.5×101
34	TNTC	TNTC	TNTC	163.33	63.33	10.00	163.33×104	5.5	NIL	NIL	NIL	5.5×101
35	TNTC	TNTC	TNTC	109.67	73.67	8.67	109.67×104	TNTC	31	4	NIL	31×102
36	TNTC	TNTC	TNTC	TNTC	TNTC	63.00	63.00×106	14.5	16	NIL	NIL	16×102
37	TNTC	TNTC	167.33	84.33	33.67	NIL	167.33×103	17.5	4.5	2	NIL	17.5×101
38	TNTC	TNTC	140.00	47.00	6.67	NIL	140.00×103	47	3.5	NIL	NIL	47×101
39	TNTC	TNTC	141.33	52.33	15.00	NIL	141.33×103	14	1	NIL	NIL	14×102
40	TNTC	112.00	49.00	14.67	NIL	NIL	112.00×102	72	3.5	5	NIL	72×101
1	TNTC	172.33	36.00	8.33	NIL	NIL	172.33×102	3	NIL	NIL	NIL	3×101

(Abbreviation:TNTC=Too Numerous to Count andCFU= Colony Forming Unit)