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RESEARCH ARTICLE

QUALITY AND SAFETY ASSESSMENT OF HERBAL ANALGESIC PREPARATIONS IN ADEN, YEMEN: HEAVY METAL AND MICROBIAL CONTAMINATION PERSPECTIVE

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Abstract

Herbal medicine products (HMPs) are widely used in Yemen to treat pain and inflammation; however, concerns regarding their safety persist due to the absence of official regulations governing the quality control and evaluation of herbal products. This study aimed to detect the presence of heavy metals and microbial contamination in herbal analgesic products (HAPs) commonly sold in Aden, Yemen. Five widely used herbal analgesic preparations were randomly collected from local herbal markets. Heavy metals, including lead (Pb), cadmium (Cd), cobalt (Co), zinc (Zn), iron (Fe), silver (Ag), and nickel (Ni), were quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Microbial contamination was assessed using standard microbiological techniques, with specific screening for *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas spp*. Both bacterial and fungal contamination levels were compared against international pharmaceutical safety standards. Results indicated that heavy metal concentrations in all samples were within permissible limits recommended by the WHO, USFDA, and the European Pharmacopoeia. Pb was not detected in any sample. However, microbial analysis revealed bacterial and fungal contamination in all samples, with total counts exceeding the safety thresholds prescribed by the WHO. *Escherichia coli* was detected in two products. These findings highlight potential public health risks associated with unregulated HAPs and underline the need for stringent quality control and regulatory oversight in Yemen.

Keywords: Herbal analgesic products; Heavy metals; Microbial contamination.

1. Introduction

Herbal medicinal products (HMPs) are widely used in Yemen and low- and middle-income countries as first-line remedies for common ailments, including pain relief, owing to their cultural acceptance, accessibility, and perceived safety. However, their safety is questionable due to the absence of official regulations for the quality control and evaluation of herbal products.

Contamination of HMPs with toxic heavy metals and pathogenic microorganisms poses a substantial public-health concern, particularly where regulatory oversight and good manufacturing practice (GMP) are weak or fragmented. Plants may accumulate metals from contaminated soils, irrigation water, agrochemicals, or environmental deposition, and traditional processing or informal production practices can introduce microbial contamination or fail to remove contaminants. These combined risks increase the potential for acute infections and chronic toxicities following regular consumption of contaminated herbal analgesics, underscoring the need for systematic quality control and risk assessment in resource-limited settings such as Yemen. A global review concluded that heavy-metal contamination in HMPs is a persistent safety problem, with several studies reporting levels above recommended safety thresholds in many regions [1]. Toxic metals such as Pb, Cd, and As can accumulate in body tissues and cause severe health effects, while microbial contamination may result from poor hygienic practices during processing and storage [2].

Although the World Health Organization (WHO) promotes the integration of herbal medicines into healthcare systems, it also emphasizes the need for

stringent quality control due to common issues of contamination and efficacy [3,4]. In Yemen, the regulatory framework for herbal products remains weak, and data regarding their contamination levels is limited.

Chronic exposure to heavy metals is associated with neurological, cardiovascular, developmental, and immunological disorders [5]. On the microbial front, contamination by bacteria and fungi—especially pathogenic species like *E. coli*, *Salmonella*, and *Staphylococcus aureus*—poses significant health risks [6,7].

Regionally focused systematic analyses confirm these findings for low- and middle-income countries (LMICs): a 2023 systematic review of contamination in HMPs from LMICs found frequent detection of bacterial contamination and toxic metals at concentrations posing potential health risks, and highlighted the challenge of weak regulatory systems and inconsistent quality assurance practices [8].

Microbial contamination is a recurrent finding in marketed HMPs, with studies reporting both high total viable counts and isolation of potentially pathogenic species (e.g., *Escherichia coli*, *Salmonella*, *Staphylococcus aureus, and various Aspergillus spp*.). Contamination may occur during harvesting, drying, storage, or preparation under unhygienic conditions. Beyond immediate gastroenteritis risk, contaminated herbal products can serve as reservoirs for antimicrobial-resistant organisms, complicating treatment in resource-limited health systems [9].

Chronic ingestion of heavy metals via contaminated herbal remedies is associated with multisystem toxicities: lead exposure is linked to neurodevelopmental impairment and hematologic disorders; cadmium to renal dysfunction and bone disease; arsenic to dermatologic changes and cancers; and mercury to neurotoxicity. The cumulative exposure risk is especially relevant for populations with frequent herbal product consumption and vulnerable subgroups (children, pregnant women). Microbial contamination compounds acute risks (gastrointestinal disease, sepsis) and may contribute to long-term public-health burdens [1].

Several global studies have reported contamination of herbal medicines with toxic metals and microorganisms. In China, a study found that over 30% of traditional herbal preparations contained at least one heavy metal above permissible limits, notably Pb, Cd, As, and Hg [1]. Elevated metal concentrations were also reported in herbal products from Qatar [10], Iran [11], Bangladesh [12], and Mexico [13]. Similarly, microbial contamination remains widespread, as revealed by studies in Brazil and Malawi [14,15], which detected bacterial and fungal growth in over half of the tested herbal samples. A study in the Ghanaian market [17] reported contamination in 76.7% of African preparations. A systematic review confirmed that nearly half of global herbal samples exceeded microbial safety limits [8]. These findings collectively underscore the urgent need for standardized quality control and regulatory monitoring of HMPs worldwide.

Despite global recognition of contamination risks, data from many regions remain sparse, particularly for specific product categories such as herbal analgesics sold in Yemeni markets. Given the high reliance on such remedies, the fragile regulatory setting, and limited laboratory infrastructure, localized studies that combine sensitive metal analysis with microbiological profiling are essential to characterize exposure, inform risk communication, and prioritize interventions. This study addresses this gap by applying validated ICP-OES/AAS methodologies and standard microbiological assays to a representative sample of herbal analgesic preparations in Aden, Yemen.

2. Materials and Methods

2.1 Sample Collection

Five HAPs were randomly obtained from herbal markets in different districts of Aden. Samples were labeled, coded, and stored appropriately before analysis. Information concerning the herbal products can be found in Table 1.

Table 1: Information related to the selected HAPs.

No.	Sample Code	Labeled Ingredients	Batch No.	Manufacturing Date	Expiry Date	Country of Manufacture
1	**Sample A**	Anise, Black seed, Clove, Thyme, Bay Laurel, Ginger, Rosemary, Mint	No: 23080	January 2023	January 2026	Yemen – Sana'a
2	**Sample B**	None	No: 15571	January 2023	January 2026	Yemen – Taiz
3	**Sample C**	None	None	January 2022	January 2025	Yemen – Aden
4	**Sample D**	None	None	September 2023	September 2026	Yemen – Aden
5	**Sample E**	None	None	January 2023	January 2026	Yemen – Aden

2.2 Heavy Metal Analysis

Sample Digestion: Microwave-assisted acid digestion was performed using 10 mL concentrated nitric acid (HNO_3) per 1 g of homogenized sample**.** The specific conditions and requirements for the digestion procedure are outlined in Table 2. After cooling, digests were diluted to 50 mL with deionized water.

Table 2: Parameters for the digestion of the samples.

Phase	Duration	Energy (W)	Temperature (°C)
1	10 min	800 W	180°C
2	10 min	1200 W	220°C
3	10 min	1200 W	240°C

2.3 ICP-OES analysis

The PerkinElmer ICP-OES (USA) instrument was used with a Counts per Second (CPS) detector. The calibration curve spanned the concentration range of 1.0-7.5 ppm. The operational parameters for the ICP-OES instrument are listed in Table 3.

Table 3: Parameters of ICP-OES.

Parameter	Value	Parameter	Value
RF Power	1550 W	Nebulizer Gas Flow	1.03 L/min
Plasma Gas Flow	15.0 L/min	Pump Speed	0.10 RPS
Auxiliary Gas Flow	0.90 L/min	Quantification Unit	CPS

2.4 Microbial Analysis

Culture Media Preparation

Standard agar media were prepared, autoclaved, cooled, and poured into Petri dishes. The initial dilution was carried out by diluting 5 g of the sample in 45 mL of Buffered Peptone Water (BPW). Further dilutions (10^{-2} to 10^{-7}) were prepared by transferring 1 mL of the previous dilution into 9 mL of BPW. Then 1 mL of each dilution was added to Plate Count Agar or Soybean Casein Digest Agar, mixed gently, and poured into a sterile Petri dish. The plates were incubated at 37°C for 48 hours for bacterial contamination. Sabouraud Dextrose Agar or Sabouraud Chloramphenicol Agar was mixed and poured into Petri dishes, and the plates were incubated at 33°C for 5–7 days. The pathogen detection media and colony appearances are illustrated in Table 4, according to USP and WHO [18,19]:

Table 4: Media for pathogenic investigation.

Pathogen	Media	Colony Appearance
S. aureus	Mannitol Salt Agar	Yellow colonies
P. aeruginosa	Cetrimide Agar	Green/blue
Salmonella spp.	XLD Agar	Black colonies
E. coli	MacConkey / EMB	Pink (MacConkey), metallic green sheen (EMB)
Candida spp.	HiCrome Agar	Green colonies

Gram Staining

Confirmed identity of isolates: Gram-positive cocci (Sample B) & Gram-positive bacilli (Sample E).

3. Results and Discussion

3.1 Heavy Metal Contamination

Heavy metal exposure represents a major health hazard to human well-being, constituting a significant threat as environmental pollutants (approximately one-fourth of human diseases). Being non-biodegradable, they accumulate in the environment and enter the food chain, posing multiple health risks to animals and humans [20]. All detected heavy metal levels were within the acceptable limits by the WHO, USFDA, and the European Pharmacopoeia. Lead was undetected in all samples. Iron (Fe) was highest in Sample E (9.2625 ppm) and Sample B (2.848 ppm), Zinc (Zn) was highest in Sample B (1.711 ppm) and Sample A (1.645 ppm), Cadmium (Cd) and Nickel (Ni) were detected at trace levels [18,19].

Table 5: Heavy Metal Concentrations in the Five Samples.

Sample	Ni (ppm) ± SD	Co (ppm)± SD	Fe (ppm)± SD	Zn (ppm)± SD	Cd (ppm)± SD	Ag (ppm)± SD	Pb (ppm)± SD
Sample A	0.0535 ± 0.013	0.014 ± 0.009	8.034 ± 0.190	1.6455 ± 0.037	0.3055 ± 0.021	0.0125 ± 0.013	0.000
Sample B	0.0265 ± 0.0007	0.006 ± 0.0	2.848± 0.038	1.7115± 0.0007	0.001 ± 0.0	0.0055 ± 0.0007	0.000
Sample C	0.017 ± 0.004	0.001 ± 0.001	2.5005 ± 0.603	0.307± 0.386	0.0025 ± 0.007	0.0035 ± 0.0007	0.000
Sample D	0.026 ± 0.007	0.003 ± 0.004	6.099 ± 0.034	0.841± 1.141	0.0035 ± 0.002	0.007 ± 0.0056	0.000
Sample E	0.0215 ± 0.0007	0.0055 ± 0.006	9.2625 ± 0.265	0.0305 ± 0.0035	1.3785 ± 0.526	0.014 ± 0.0155	0.000

The Permissible Limits of Heavy Metals in Herbal Drug Products (WHO Guidelines) are as follows: Cadmium (Cd) ≤ 0.3 ppm, Lead (Pb) ≤ 10 ppm, Lead (Pb) ≤ 10 ppm, Cobalt (Co) ≤ 0.1 ppm, Zinc (Zn) ≤ 50 ppm, Iron (Fe) ≤ 20 ppm, Silver (Ag) ≤ 1 ppm, Nickel (Ni)≤ 1 ppm [19].

These results demonstrate a disparity with international literature, which often documents extensive contamination, including heightened Cr and Pb concentrations (Bangladesh), diverse metal contaminants (Mexico), and health-hazardous heavy metal levels (Saudi herbal products) [12, 13, 9]. Also, these results contrast with reports from other regions showing elevated contamination levels. For instance, a study found that 30.5% of 1,773 traditional herbal samples globally contained at least one metal exceeding regulatory thresholds, with Pb, Cd, As, and Hg presenting unacceptable health risks [1]. Similarly, a study in Oman identified elevated concentrations of Mn, Al, and As in some commercial herbal products marketed for weight loss [10], while research in Persian reported excessive Cd levels in several Persian herbal preparations [11]. The lower levels observed in the present study may reflect cleaner raw materials, environmental factors, or improved handling practices. Nonetheless, continuous monitoring and enforcement of quality-control measures remain crucial, especially under Yemen's limited regulatory capacity.

3.2 Microbial Contamination

Microbial contamination poses a major threat to consumer safety, particularly within herbal medicines, where it is the most common form of contamination. The detection of pathogenic microbial contaminants in herbal products represents a significant safety concern, as these agents pose a direct risk of inducing severe user infections [21]. All samples exceeded acceptable microbial limits for total viable aerobic count (TVC) range 1.2×10^7 to 5.0×10^8 CFU/g. The fungal count was within the acceptable range in Samples A, C, and E; Sample D slightly exceeded; Sample B showed a high fungal load. *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were not detected in any sample. The results were shown in Table 6. The results of microbial contamination are shown in Figures 1 &2.

Table 6: The result of microbial contamination of samples.

Sample	Normal Range (CFU/g ≤ 10^5)	Result (CFU/g)	Interpretation	Pathogens Detected
Sample A	≤ 1.0×10^5	1.8×10^7	Exceeds normal limit	None
Sample B	≤ 1.0×10^5	5.0×10^8	Exceeds normal limit	*E. coli* (Gram+ cocci)
Sample C	≤ 1.0×10^5	1.2×10^7	Exceeds normal limit	None
Sample D	≤ 1.0×10^5	1.5×10^7	Exceeds normal limit	None
Sample E	≤ 1.0×10^5	9.4×10^7	Exceeds normal limit	*E. coli* (Gram+ bacilli)

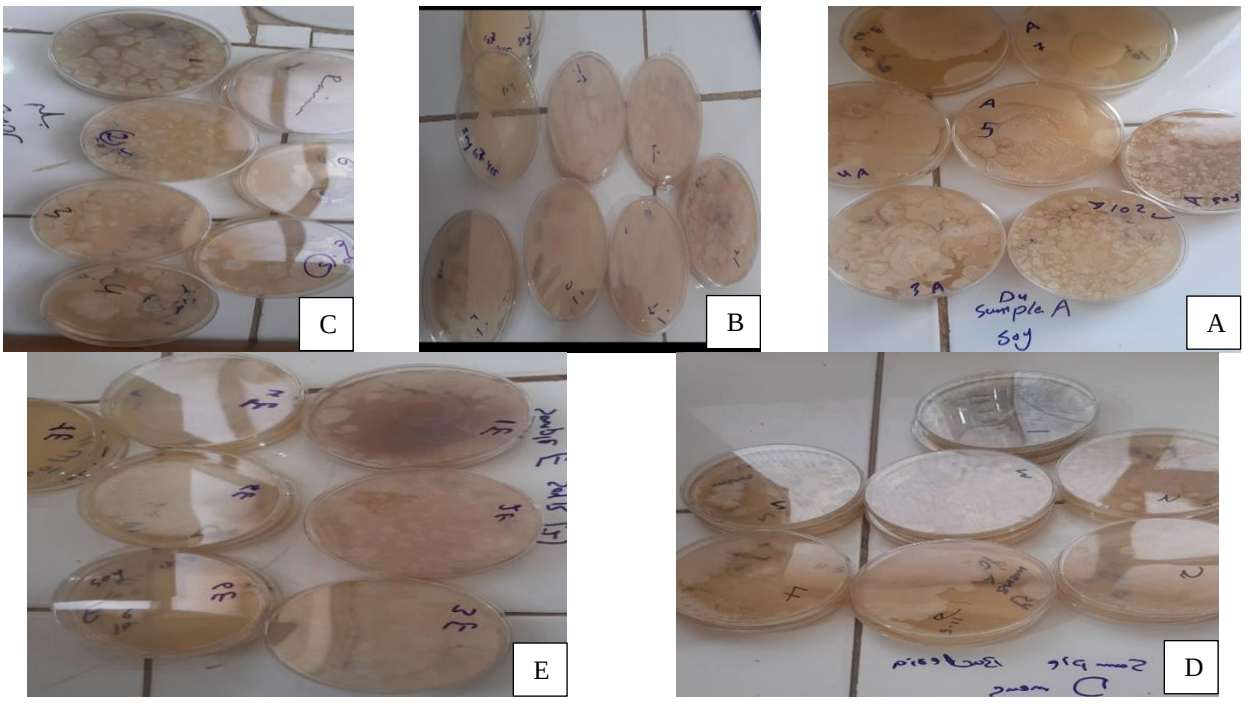

Fig.1: (A) Microbial Load and Contaminant Profile of Soybean Samples A, B,C,D,E

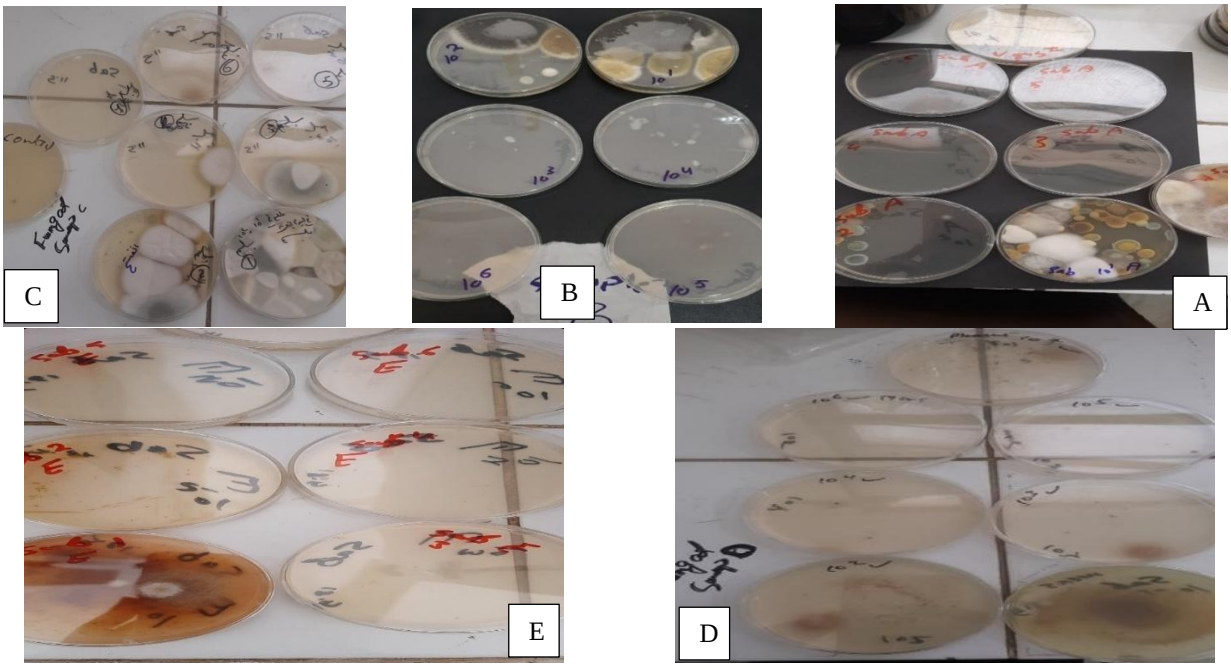

Fig. 2: Microbial Load and Contaminant Profile of Soybean Samples A, B, C, D, E

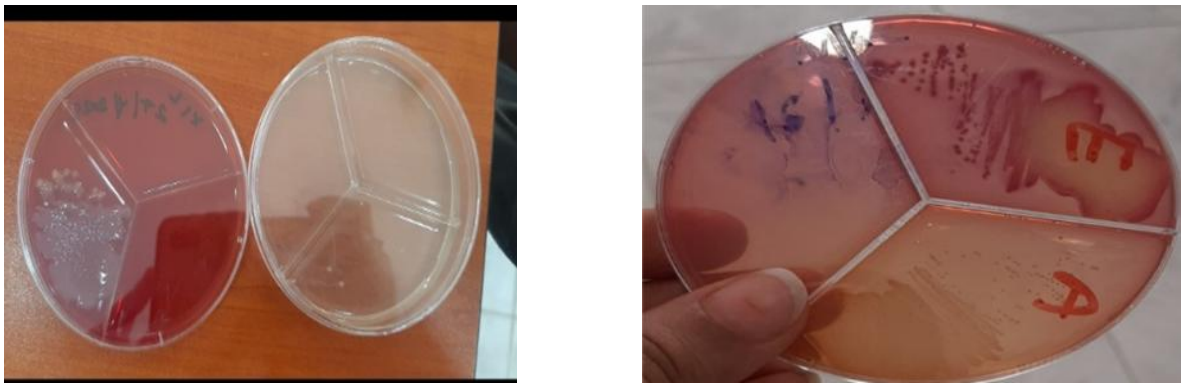

Fig. 3: Escherichia coli shown in pink on MacConkey agar for Samples B & E.

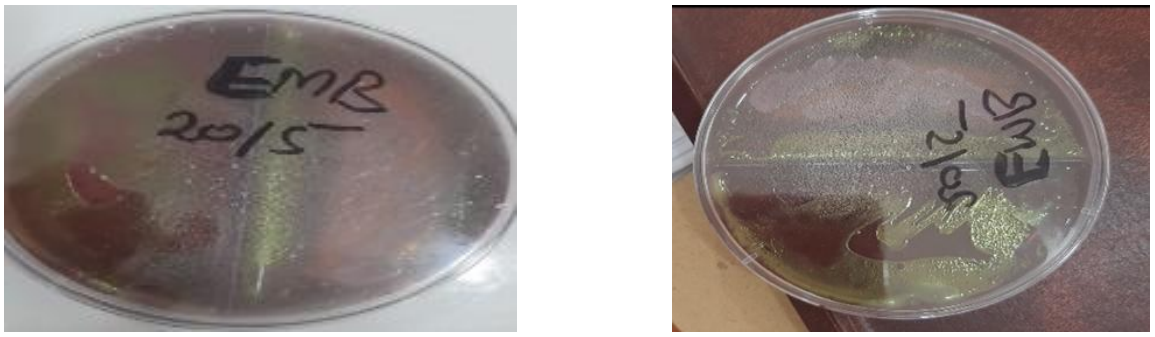

Fig. 4: Escherichia coli shown as a bright green metallic sheen on Eosin Methylene Blue (EMB) agar for Samples B & E.

Analysis of the pathogenic contamination type revealed that *Escherichia coli* was detected in samples B and E, as evidenced by the characteristic pink coloration observed on MacConkey agar in Figure 3. Confirmation of Escherichia coli presence was achieved using Eosin Methylene Blue (EMB) agar, on which samples B and E yielded colonies displaying the characteristic bright green metallic sheen (Figure 4). In contrast, pathogenic bacteria such as *Salmonella spp*., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were not detected in the tested samples, as evidenced by the absence of growth on Xylose Lysine Deoxycholate (XLD) agar, Cetrimide agar, and Mannitol Salt agar, respectively.

Microbiological analysis of the tested HAPs revealed varying degrees of contamination, with both bacterial and fungal growth exceeding the permissible pharmacopoeial limits. *E.coli* was detected in two samples, indicating possible fecal contamination and poor hygienic practices during preparation or storage. These findings corroborate prior international reports that recognize microbial contamination as a significant safety concern associated with herbal medicines. In Brazil, a study reported bacterial contamination in more than 50% of analyzed herbal samples and fungal growth in 35.6%, with *S. aureus*, *E. coli*, and *Salmonella spp*. being the predominant isolates [14]. Similarly, in Malawi reported that 68.9% of herbal medicines contained coliforms [15]. In Nigeria, researchers observed high microbial loads, with *Proteus spp.* and *Candida spp*. Dominating [16], while contamination was observed in 76.7% of African herbal preparations, mainly with *Bacillus* and *Pseudomonas aeruginosa* [17]. In Saudi Arabia, a study detected bacterial growth in 94% of herbal products, including Staphylococcus spp. and other Gram-negative bacteria [9] .

Although the degree of contamination identified in this study was relatively low compared to certain international reports, the confirmed presence of E. coli and fungal contaminants nonetheless constitutes a serious potential public health concern. The detection of fecal indicator bacteria reflects inadequate quality control, non-compliance with Good Manufacturing Practices (GMP), and the absence of microbial testing in the informal herbal market in Yemen. These findings reinforce the need for strict microbial safety standards, routine monitoring, and public awareness programs to ensure the microbiological quality of HMPs.

4. Conclusion

This study underscores the coexistence of acceptable heavy metal concentrations with notable microbial contamination in HAPs marketed in Aden, Yemen. The detection of *E. coli* and total viable aerobic counts exceeding pharmacopeial limits indicates lapses in hygienic practices during production or storage and represents potential public health hazards. These findings emphasize the urgent need for stringent microbiological quality control, regular market surveillance, and the implementation of GMP to ensure the safety and efficacy of herbal products in regions with limited regulatory oversight. It is important to note that the sample size used in this study (N = 5) is relatively small, which represents a limitation of the work. Therefore, the findings should be considered preliminary and warrant confirmation through broader, large-scale survey studies to validate and generalize the results.

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مقالة بحثية

تقييم الجودة والسلامة للمستحضرات العشبية المسكّنة في عدن، اليمن: منظور التلوث بالمعادن الثقيلة والملوثات الميكروبية

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المُلخّص

تُستخدم المستحضرات العشبية الطبية على نطاق واسع في اليمن لعلاج الألم والالتهابات، إلا أن المخاوف المتعلقة بسلامتها لا تزال قائمة بسبب غياب اللوائح الرسمية الخاصة بمراقبة الجودة وتقييم المنتجات العشبية. هدفت هذه الدراسة إلى الكشف عن وجود المعادن الثقيلة والتلوث الميكروبي في المستحضرات العشبية المسكنة الشائعة في مدينة عدن، اليمن. تم جمع خمس عينات من المستحضرات العشبية المسكنة المنتشرة في الأسواق المحلية بشكل عشوائي. جرى تحديد تراكيز المعادن الثقيلة بما في ذلك الرصاص(Pb) ، الكادميوم(Cd) ، الكوبالت(Co) ، الزنك (Zn)، الحديد(Fe) ، الفضة(Ag) ، والنيكل (Ni) باستخدام مطيافية الانبعاث البصري بالبلازما المقترنة حثيًا .(ICP-OES) كما تم تقييم التلوث الميكروبي باستخدام الطرق الميكروبيولوجية القياسية، مع فحص خاص للكشف عن الإشريكية القولونية (*Escherichia coli*) والسالمونيلا (*Salmonella spp.*) والمكورات العنقودية الذهبية (*Staphylococcus aureus*) والزائفة (*Pseudomonas spp.*). تمت مقارنة مستويات التلوث البكتيري والفطري بالمعايير الدولية لسلامة المستحضرات الدوائية. أظهرت النتائج أن تراكيز المعادن الثقيلة في جميع العينات كانت ضمن الحدود المسموح بها من قبل كل من منظمة الصحة العالمية (WHO) وإدارة الغذاء والدواء الأمريكية (USFDA) والدستور الدوائي الأوروبي، ولم يُكتشف الرصاص في أي من العينات. ومع ذلك، أظهرت التحاليل الميكروبية وجود تلوث بكتيري وفطري في جميع العينات، حيث تجاوزت الأعداد الكلية الحدود الموصى بها من قبل منظمة الصحة العالمية، كما تم الكشف عن الإشريكية القولونية في منتجين اثنين. تشير هذه النتائج إلى وجود مخاطر صحية محتملة مرتبطة بتداول المستحضرات العشبية المسكنة غير الخاضعة للرقابة، وتؤكد على الحاجة الملحّة إلى تطبيق إجراءات صارمة لمراقبة الجودة ووضع لوائح تنظيمية فعّالة في اليمن.

الكلمات المفتاحية: المستحضرات المسكنة؛ المعادن الثقيلة؛ التلوث الميكروبي.

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