International Journal of Frontiers in
Chemistry and Pharmacy Research
Journal homepage: https://frontiersrj.com/journals/ijfcpr/
ISSN: 2783-0462 (Online)

(RESEARCH ARTICLE)

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Microbiological analysis in different brands of Hepato-protective liquid herbal medicines available in the Dhaka city

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International Journal of Frontiers in Chemistry and Pharmacy Research, 2023, 03(01), 053–057

Publication history: Received on 26 July 2023; revised on 09 September 2023; accepted on 11 September 2023

Article DOI: https://doi.org/10.53294/ijfcpr.2023.3.1.0053

Abstract

Introduction: The use of herbal medicine is on the rise worldwide, and safety issues associated with herbal medicines may have an impact on the population who are using it. Herbal medicines are generally considered as safe based on their long-standing use in various cultures. However, there are concerns regarding the safety especially microbial growth of many of the liquid herbal medicines.

Objective: To isolate and identify the organisms from the Herbal Preparations used by the mass people.

Methods: A total of 27 different Hepato-protective herbal medicines (HPHMs) were purchased randomly from identified herbal shops and retail outlets in different parts of Dhaka city. All collected samples were analyzed by the Pour Plate method for microorganisms in the Department of Microbiology, Bangladesh University of Health Sciences (BUHS), Mirpur, Dhaka.

Results: The results of the culture of the studied Hepato-protective drugs showed “No growth” of any Bacteria among 14 samples (51.9%). Growth was found in the remaining 13 samples (48.1%). There was “No growth” of any fungi in all Hepato-protective liquid herbal medicines.

Conclusions: The levels of viable bacteria in the studied samples were found to be above safety limits. Pathogenic bacteria were also isolated from these herbal medicines. So, the use of homemade and commercial herbal medicines is a major risk to the health of the people who use these therapies due to the lack of microbial quality standards. Therefore, this warrants immediate need of management of herbal medicines to scale up its quality & safety issue.

Keywords: Hepatoprotective herbal medicines; Bacteria; Fungus

1. Introduction

The use of herbal treatment is an old practice usually employed in disease treatments. The term "herbal drugs" denotes plants and plant parts that have been converted into phytopharmaceuticals using some simple processes involving harvesting, drying, and storage. It is sometimes referred to as complementary and alternative medicine (CAM). Many

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synthetic drugs listed as conventional medication were originally derived from plants, for example, the antimalarial drug quinine from Cinchona species[1]. The World Health Organization (WHO) estimates that four billion people (about 80 percent of the world population) use herbal medicine for some aspect of primary health care[2].

However, the medicinal use of herbs went into a rapid decline in 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 herbalism. For example, Ayurvedic medicine in India, Kampo medicine in Japan, traditional Chinese Medicine (TCM), and Unani medicine in the Middle East and South Asia are still being used by a large majority of people[3]. The World Health Organization (WHO) survey indicated that about 70-80% of the world's population particularly in developing countries rely on non-conventional medicines mainly of herbal origins for their primary health care. This is because herbal medicines are relatively accessible and cheaper than synthetic drugs[4].

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[5]. Lack of regulation for herbal supplements presents potential health risks, largely their contamination chances with pathogenic microorganisms. However, only a few surveillance studies have been conducted to assess this threat[6]. The present study figured out the biological threats in herbal medicines and added the knowledge of proliferating bacteria, yeasts, and molds in such medicines.

1.1. Rationale of the Study

In Ayurveda, plant materials have been used to protect against liver injury by various chemicals and dietary agents. Herbal drugs are safe and have the potential to cure such diseases, so they have gained popularity in recent years. Long-term uses of these medicines are very cost-effective too. Microbial contamination of these drugs may cause deterioration of the quality of drugs making them ineffective. Organisms present in the drugs may also cause various diseases. So, the study on microbial contamination of Hepato-protective drugs is very much justified[7].

2. Methodology

This study was a cross-sectional (experimental) study conducted at the Department of Microbiology, Bangladesh University of Health Sciences (BUHS) from January 2019 to December 2019 for one year.

2.1. Materials and methods

A total of 27 different Hepato-protective herbal medicines (HPHMs) were purchased randomly from identified herbal shops and retail outlets in different parts of Dhaka city. All collected samples were analyzed for microorganisms in the Department of Microbiology, BUHS, Mirpur, Dhaka. DNA sequencing of isolated bacteria was performed in the Microbiology Department of Jessore University of Science and Technology.

2.2. Bacteriological analyses

MacConkey agar, Blood agar, and Saboraud's dextrose agar were used (Oxoid) for culture and isolation of bacteria and fungus. Identification of organisms was done in standard ways.

2.2.1. Total aerobic bacterial plate count

After 24-48 hours, all the colonies were counted. A magnifying colony counter was used in counting small embedded colonies. Bacterial load was calculated by using the formula, CFU/ml= CFU* dilution factor* 1/ aliquot[7]

2.2.2. Morphological and Biochemical Identification

Microscopic examination and gram staining of the isolates was performed. The morphological characteristics (shape, size, edge, elevation, and opacity) of colonies on the Blood agar plate were recorded. After overnight incubation, the colony count was made from each plate and an average was made for each drug. In case of no growth, the culture plate was incubated for up to 48 hours. If there was no growth after 48 hours the culture was taken as "No growth"[8].

Biochemical tests were performed for gram-positive bacteria and gram-negative bacteria[9]. Depending on the similarity of morphological characteristics, biochemical tests, and microscopic characteristics, bacteria were grouped into four categories, Group A, Group B Group C, and Group D respectively. One bacteria from each of 1st three groups, in total 3 bacteria, was selected for genome sequencing.

2.3. Quality Control

For quality control - *Escherichia coli* (ATCC25922) used as control organisms

3. Results

The colonies on each plate were counted and the values were averaged after the overnight incubation period. The culture plate was incubated for up to 48 hours in the absence of growth. After 48 hours, a culture is said to have "No growth" if no growth has occurred. The results are explained in the following tables.

Table 1 Result of culture of Hepato-protective drugs (n=27)

Sl.#	Status of bacterial culture in Hepatotoxic drugs	No.(%)	Status of fungal culture in Hepatotoxic drugs	No.(%)
1.	No growth	14(51.9%)	No growth	27(100%)
2	Growth present	13(48.1%)	Growth present	0(0%)

Table 1**.** shows the result of the culture of Hepato-protective drugs. There was "No growth" of any Bacteria in 14(51.9%) Hepato-protective drugs and growth was found in 13(48.1%) drugs. There was "No growth" of any fungi in all samples.

Table 2 Species of bacteria grown in Hepato-protective drugs(n=13)

Sl#	Species of bacteria	No.(%)
1.	*Bacillus species*	12(92.3%)
2.	*Enterobacter sp.*	01(7.7%)

Table-2 shows the growth of bacteria in Hepatoprotective drugs. Out of 13 samples having growth of bacteria, *Bacillus sp*. was found in 12 (92.3%) and Enterobacter species in one(7.7%).

Table 3 A load of bacteria was isolated from 13 Hepatoprotective Herbal Medicines (HPHM) and assigned in groups.

Name of Drugs	Name of Bacteria Identified by Biochemical Characteristics	Colony count	Group of Organisms having the same Biochemical report
HPHM6 HPHM7 HPHM13 HPHM14 HPHM12	Gram-positive *Bacillus* Species	3.7 x 10^4/g	Group A Isolates
HPHM10 HPHM11 HPHM15 HPHM5	Gram-positive *Bacillus* Species	3.2 x 10^4/g	Group B Isolates
HPHM17 HPHM21 HPHM15	Gram-positive *Bacillus* Species	3.7 x 10^4/g	Group C Isolates
HPHM19	Enterobacter sp.	3.0 x 10^2/g	Group D Isolates

The table shows three strains of *Bacillus species* were identified from 12 drugs and one *Enterobacter sp*. from one drug. These strains are assigned as Group A, Group B, Group C, and Group D respectively. Bacterial load in group A was 3.7x10^4/g, in group B, 3.2x10^4/g, in group C, 3.7x 10^4/g and in group D, 3x 10^2/g.

4. Discussion

Hepato-protective herbal drugs are used for treating various forms of hepatitis. This use of Hepatoprotective drugs is increasing day by day. These Hepato-protective drugs should be free from microbial infection which may damage the quality of the herbal drugs. This study was designed to assess the microbial quality of the drugs.

There was “No growth” of any Bacteria in 14(51.9%) Hepato-protective drugs and growth was found in 13(48.1%) drugs. *Enterobacter* was found in one (3.7%) sample (HPHM19) and *Bacillus species* was isolated in 12(44.4%) samples. There was "No growth" of any fungi in all samples. In a similar study by Lima et al(2020)[10], out of 31 samples, *Escherichia coli* was found in 12.9% of samples, *Pseudomonas aeruginosa* in 8.3%, *Staphylococcus aureus* in 20.4% of samples. In the study of Szajewske et al[11], out of 5 products, Lactobacillus sp. and Bifidobacterium sp were found in 3 products (60%). In the study of Hasan et al[12], out of 86 herbal products, 26(30.2 %) showed contamination with Enterobacteriaceae, where salmonella(24.4%), E.coli(24.4%) and 41.5% were Enterobacteriaceae were isolated.

Three strains of bacillus species were identified from 12 drugs. These strains are assigned as Group A, Group B, and Group C. Bacterial load in Group A was $3.7x10^4$/g, in Group B $3.2x10^4$/g, In group C $3.7x\ 10^4$/g, and Group D $3x\ 10^2$/g. However, in all the herbal drugs in our study, the bacterial count varied from $3x10^2$ to $3.7x10^4$ cfu/ml. which is within the acceptable limit(CFU/ml $\leq$ $1x\ 10^5$ for aerobic bacteria and 10^3/ml for Enterobacteria other than *Escherichia coli*) (WHO,2007)[7]. No fungus was detected in any drug. In the study of Lima et al, (2020)[10], The bacterial load was within the permissible range($\leq 10^5$cfu/ml) in 10.6% of cases and outside the permissible range ($> 10^5$ cfu/ml) was 10.6% of cases. They also found fungal growth in 3.0% of cases within the acceptable limit and 3.0% more than the acceptable limit. In the study of Hassan (2021)[12], out of 86 herbal products 41(47.7%) failed to comply with the pharmacological specification for microbiological quality.

5. Conclusion

The use of homemade and commercial herbal medicines is a major risk, to the health of the people who use these therapies, due to the lack of microbial quality standards. *Bacillus sublists, Bacillus altitudinis*, and *Bacillus pumilus* were found in the herbal medicines studied. A load of bacteria was also studied and found within normal limits. No growth of any fungus was found in this study. Therefore, this warrants immediate need of management of herbal medicines to scale up its quality & safety issue.

Compliance with ethical standards

Acknowledgments

The authors would like to acknowledge and express gratitude to the Bangabandhu Science and Technology Fellowship Trust, Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing the Post-Doctoral Fellowship program. International Science Programme (ISP), Asian Network of Research on Antidiabetic Plants (ANRAP) and Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing partial funds for this study. The authors are also grateful to the Department of Microbiology and Department of Chemistry, BUHS, for giving the opportunity to conduct this study.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Reference

[1] Iwu, M.W., Duncan, A.R. and Okunji, C.O., New antimicrobials of plant origin. *Perspectives on new crops and new uses. ASHS Press, Alexandria,* 1999; 457 :462.

[2] Ekor, M., The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology* 2014; 4:177.

[3] Pan, S.Y., Zhou, S.F., Gao, S.H., et al. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine*, *2013*.Volume 2013. Article ID 627375. https://doi.org/10.1155/2013/627375.

[4] Chan, K., Some aspects of toxic contaminants in herbal medicines. *Chemosphere* 2003; 52(9):1361-1371.

[5] Noor, R., Huda, N., Rahman, F. et al. 2013. Microbial contamination in herbal medicines available in Bangladesh. *Bangladesh Medical Research Council Bulletin* 2013;39(3):124-129.

[6] Newell, D.G., Koopmans, M., Verhoef, L.et al. Food-borne diseases—the challenges of 20 years ago persist while new ones continue to emerge. *International journal of food microbiology*, 2010;139: S3-S15.

[7] World Health Organization. WHO guidelines for assessing the quality of herbal medicines concerning contaminants and residues Press, Geneva, Switzerland. 2007.

[8] Ahmed MR, Asna SMZH, Khan MSH, Rokeya B,Mosihuzzaman M and Sayed MA. Microbial Contamination of Some Antidiabetic Herbal Preparations Available in Bangladesh. European Journal of Medicinal Plants 13(1): 1-5, 2016, Article no.EJMP.23556

[9] Dimri, A.G., Prasad, R., Chauhan, A., et al. 2018. Characterization of soil actinomycete isolates against gram-positive and gram-negative food-borne bacteria. *IJEP* 2018; 38:1004-1015.

[10] De Sousa Lima, C.M., Fujishima, M.A.T., de Paula Lima, B. *et al.* Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. *BMC Complement Med Ther* 2020; 20: 17. https://doi.org/10.1186/s12906-019-2723-1

[11] Szajewske H, Fordymacka A, Bardowsky J et al. Microbial and genetic analysis of probiotic products licensed for medicine purposes. Med Sci Manit 2004;10(9):346-350.

[12] Hassan KM, Njogu PM, Njuguana NM et al. Microbiological Contamination of Herbal Medicinal Products Marketed in Kenya for a Chronic Disease: A case study of Nairobi Metropolis. Journal of Herbal Medicine, Elsiveir 2021;29: 100475.