



Microbial Contamination of Some Antidiabetic Herbal Preparations Available in Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MRA and SMZHA wrote the protocol, managed the literature searches, wrote the first draft of the manuscript, designed the study, managed the experimental process of the study and statistical analysis of data of the study performed. Authors MRA and MSHK responded to the reviewers comments. Authors BR, MM and MAS corrected the manuscript and gave the valuable suggestions to write and perform the research. All authors read and approved the final manuscript.

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ABSTRACT

Herbal Medicines (HM) are being used in our country for a long time but the type and load of the microbial agents has not been isolated in locally produced finished products of HM. The present study was designed to assess the microbial load, genus & species of the microbes contaminating with HM. Seven different Antidiabetic Herbal Preparations (ADHPs) were purchased randomly and analyzed for microbial contaminants. Blood agar, Mac Conkey agar, Chocolate agar and Sabouraud's dextrose agar were used (Oxoid) for culturing and isolation of bacteria and fungus.

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Identification of organisms were done as standard ways. Total aerobic bacterial plate count was done as per the method of Brown, Poxton and Wilkinson. Out of 07 antidiabetic solid and liquid samples, except ADHP-3, *Bacillus subtilis* ($3.5 - 4.0 \times 10^4$ cfu/g) was isolated from solid ADHPs and *Enterococcus* spp. (1.0×10^4 cfu/ml) was isolated from liquid ADHP, but all samples were free from fungi (yeasts and moulds). However presence of bacteria in these samples indicates the possibility of increased number of bacteria. So, the sample should be handled in any step maintaining standard sterility of the environment, instrument and involved personnel. The result of present study showed the contamination rate within tolerable level but the presence of bacteria in these samples was not desirable.

Keywords: Herbal formulations; antidiabetic herbal preparations; microbial contamination; antibacterial assessment.

1. INTRODUCTION

Herbal medicines embrace herbal materials, herbs, herbal formulations and finished herbal drugs. These types of herbal formulations have been used since ancient times to treat a extensive range of diseases [1-3]. Many developing countries continued to get benefit from the rich knowledge of medical herbalism. A Good numbers of people are still being used Ayurvedic medicine in India, Kampo medicine in Japan, traditional Chinese medicine (TCM), and Unani medicine in the Middle East and South Asia [4]. About 70-80% of the world population relies on non-conventional medicines mainly of herbal origins for their primary health care particularly in the developing countries, because herbal medicines are relatively accessible and cheaper than the synthetic drugs [5].

The quality and safety of herbal preparations are also of great concern. The efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of supreme importance [6].

The quality criteria for herbal formulations are based on a clear scientific explanation of the unprocessed materials. Comprehensive quality criteria for herbal drugs due to 'professional secrecy' of herbalists is difficult to establish, but in order to improve the purity and safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristic such as moisture content, pH and microbiological contamination levels are desirable [7]. Earlier studies have established the presence of potential contaminants in herbal preparations [8]. The presence contaminants that serious health hazard are pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp. and other Gram positive and Gram negative strains of

bacteria [9-13]. Primarily a good number of inhabitants in Bangladesh are believed to depend on herbal formulations for their medical needs. Unfortunately, no researches have been carried out to determine the microbiological safety of these herbal products in Dhaka metropolis, Bangladesh to the best of our knowledge. In this present study, the level of contamination of powdered herbal formulations marketed locally in Dhaka city with selected pathogenic bacteria and the susceptibility of these contaminants.

2. MATERIALS AND METHODS

2.1 Sample Collection and Study Area

A total of 07 different antidiabetic herbal preparations (ADHPs) were purchased randomly from identified herbal shops and retail outlets in different parts of Dhaka city. All collected samples were analyzed for microbial contaminations at the Department of Microbiology, Bangladesh University of Health Sciences (BUHS), Mirpur, Dhaka.

2.2 Chemical and Reagent

All chemicals and Reagents were of analytical grade and procured from Oxoid Ltd, UK. Experiments were done carefully with appropriate control. Chemicals and reagents used were:

For culture: Blood agar, Mac Conkey agar, Chocolate agar and Saboraud's dextrose agar.

For Gram's stain: 1% Crystal violet, Lugol's iodine, Acetone, Dilute carbol fuchsin.

Others: Hydrogen peroxide, Bile esculin, 6.5% Sodium chloride in trypticase soy broth and Normal saline.

2.3 Bacteriological Analyses

Blood agar, MacConkey agar, Chocolate agar and Saboraud's dextrose agar were used (Oxoid) for culturing and isolation of bacteria and fungus [14]. Identification of organisms were done as standard ways [15,16].

2.4 Preparation of Media

All dehydrated media were prepared according to manufacturer's instructions. The sterile media were dispensed or poured into sterilized Petri dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37°C for 24 hrs.

2.5 Total Aerobic Bacterial Plate Count

The method as mentioned by Brown, Poxton and wilkinson was used. For liquid drugs 1:10

dilutions were made. For tablets and capsules 1 g was dissolved in 10 ml Normal saline and then 1:10 dilutions were made. 100 µl of the dilutions were placed on the surface of plates and spread widely with sterile inoculation wire. The count was calculated from average colony count/plate [17].

3. RESULTS

After overnight incubation, the colonies in each plate were counted and the values were averaged. In case of no growth the culture plate was incubated for up to 48 hours. If there is no growth after 48 hours the culture is taken as "No growth". The result is given in Table 1. It was noted that "No growth" of any Bacteria was found in one drug and No growth of any fungi was found in all drugs. In one drug, Enterococcus (*Streptococcus faecalis*) was found and in others *bacillus subtilis* was isolated.

Table 1. Selected bacteria isolated from the antidiabetic herbal preparations (ADHPs)

Name of drug	Name of bacteria isolated	Colony count	Name of fungus isolated
ADHP-1	<i>Bacillus Subtillis</i>	3.5 x 10 ⁴ /g	N/G
ADHP-2	<i>Enterococcus (Streptococcus faecalis)</i>	1.0 x 10 ⁴ /ml	N/G
ADHP-3	N/G	00	N/G
ADHP-4	<i>Bacillus Subtillis</i>	3.7 x 10 ⁴ /g	N/G
ADHP-5	<i>Bacillus Subtillis</i>	3.2 x 10 ⁴ /g	N/G
ADHP-6	<i>Bacillus Subtillis</i>	3.8 x 10 ⁴ /g	N/G
ADHP-7	<i>Bacillus Subtillis</i>	4.0 x 10 ⁴ /g	N/G

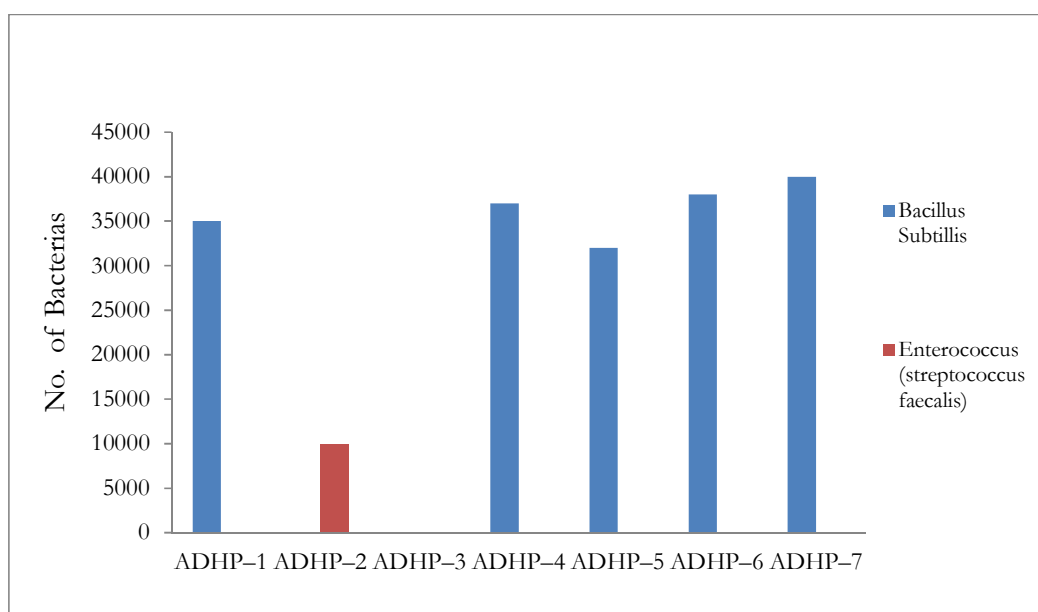


Fig. 1. Average bacterial count of 07 ADHPs

4. DISCUSSION

Many people of Bangladesh rely upon the herbal medicine for medication; the assessment of pathogens in these medicines is urgently required. The present study was attempted to identify and quantify the pathogenic microorganisms in samples randomly collected from local markets of Dhaka city.

More than 650 medicinal plant species have been identified to be in use with around 25 plants having high medicinal value in Bangladesh. A standard guideline for manufacturing herbal medicines has been set by the "Drug Administration" in Bangladesh in recent times. The regulators have also finalized the testing criteria to boost the herbal sectors but the progression was slow earlier in the lack of specific testing criteria [18]. The present study was performed to identify and enumerate the microbial contaminations in commercial herbal medicines considering nearby situation.

In this study 7 ADHPs were studied of which one showed no growth of any bacteria or fungi. The organisms found in our study were *Bacillus subtilis* in 5 (71.43%) ADHPs and *Enterococcus* in one (14.29%) and were free from *Salmonella*, *Shigella*, *Escherichia coli*, other coliforms and fungi (yeast and mould). In the study of Abba, 2009, organisms were isolated from all herbal preparations [5]. Out 150 herbal preparations, *Salmonella typhi* was found in 70 (46.67%), *Shigella* spp. in 29 (19.33%), *Escherichia coli* in 88 (58.67%), and *Staphylococcus aureus* in 98 (65.33%). However, Noor R, 2013 in their study found *coliform* in one (1.18%), fungus in 10 (11.76%), no *salmonella* and *shigella* spp. in any sample [18]. Like present study, Shah B and Pokhrel N, 2012 found predominantly *Bacillus subtilis* in samples [19].

Bacterial load of our study was *Bacillus subtilis* $3.5-4.0 \times 10^4$ cfu/g of solid ADHPs and *Enterococcus* spp. 1.0×10^4 cfu/ml of liquid ADHP. However, this very much within the standard limit of microbial contamination according to British Pharmacopeia (2004). The limits of microbial contamination are: 10^5 cfu/g or ml for total aerobic bacteria, 10^4 cfu/g or ml for yeasts and moulds, 10^3 cfu/g for *Enterobacteriaceae* and other gram negative organism and *E. coli* and *Salmonella* should be absent [20].

5. CONCLUSION

Microbial contaminations of the experimental drugs were within the acceptable limit. Samples should be randomly collected from market to maintain the quality of drugs for testing the microbial contaminations. In general, microbial contamination can approach from raw materials, during processing of raw materials and manufacturing of finished products. The results if goes outside the acceptable limit, it should be informed to the concerned manufactures. So that, they can take appropriate measures to maintain the quality. ADHPs should attain to consumers without any kind of contaminations; qualities have to be continued throughout the beginning of process from the selection of raw material up to the finished product. Appealing these particulars into our consideration, regulatory agencies should keep regular monitoring to ensure the safety of herbal medicines.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization (WHO). WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems; 2004. Available:<http://www.who.int/medicinedocs/index/assoc/s7148e/s7148e.pdf> (Accessed November 20, 2011)
2. Khanyile ZC, Singh N, Smith M, Shode FO, Mngomezulz S, Dewir YH. Comparative assessment of bacterial

- contamination in commercial herbal products of *Lessertia futescens*. American-Eurasian J Agric. & Environ Sci. 2009;5(4): 494–499.
3. Kulkarni C, Deshpande A, More S. Assessment of microbial contamination in commercial herbal oral medicinal liquids. International Journal of Pharma Research and Development. 1999;2(9):191-194.
 4. Mosihuzzaman M, Choudhury MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. 2008 IUPAC, Pure and Applied Chemistry. 2008;80:2195–2230.
 5. Abba D, Inabo HI, Yakubu SE, Olonitola OS. Contamination of herbal medicinal products marketed in Kaduna metropolis with selected pathogenic bacteria. Afr J Tradit Complement Altern Med. 2009;6(1): 70–77.
 6. World Health Organization (WHO), author Research guidelines for evaluating the safety and efficacy of herbal medicines. Manila: World Health Organization regional office for the western pacific; 1993.
 7. Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drugs standard be achieved. Drugs Information J. 1998;32:101–110.
 8. De Smet PAGM. Overview of herbal quality control. Drugs Information J. 1999; 33:717–724.
 9. Arias ML, Chaves C, Alfaro D. Microbiological analysis of some herbal infusions used as medicines. Rev Biomed. 1999;10(1):1–6.
Available:<http://www.imbiomed.com.max/uay.English/ZYu91-01.html>
 10. Erich C, Wolfgang K, Brigitte K. Microbiological status of commercially available medicinal herbal drugs- A screenings study. Planta Med. 2001;67: 263–269. [PubMed]
 11. Wolfgang K, Erich C, Brigitte K. Microbial contamination of medicinal plants- A review. Planta Medica. 2002;68:5–15. [PubMed]
 12. Adeleye IA, Okogi G, Ojo EO. Microbial contamination of herbal preparations in Lagos, Nigeria. J Health, Population and Nutrition. 2005;23(3):296–297. [PubMed]
 13. Okunlola A, Adewoyin AB, Odeku AO. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in south western Nigeria. Trop J Pharmaceut Res. 2007;6(1):661–670.
 14. Prescott LM, Harley JP, Klein DA. Isolation of pure bacterial cultures from specimens: Microbiology International. 4th edition. Boston: WCB McGraw's Hill Companies. 1999;714–796.
 15. Lucia Martin Texeira, Maria DA Gloria, Siqueira Carvalho, Patricia Lynn Shewmaker, Richard R. Facklam, Karen C Carroll, Guido Funke, James H Jorgensen, Marie Louise Landry, David W Warnock, (eds.). Manual of Clinical Microbiology, 10th edition, Washington DC, 2011;1:350-364.
 16. Niall A Logan, Alex R Hoffmaster, Sean V Shadomy, Kendra E Stauffer. *Bacillus* and other Aerobic Endospore-Forming Bacteria. In: James Versalovic, Karen C Carroll, Guido Funke, James H Jorgensen, Marie Louise Landry, David W Warnock, (eds.). Manual of Clinical Microbiology, 10th edition, Washington DC. 2011;1:381-402.
 17. Brown R, Poxton IR, Wilkinson JF. Centrifuges, colorimeters and bacterial counts. In: Collee JG, Dgiud JP, Frase AG, Marmion BP, (eds). Mackie and McCartney Practical Medical Microbiology. Churchil Livingstone. 13th edition. 1989;2:246.
 18. Noor R, Huda N, Rahman F, Bashar T, Munshi SK, Microbial contamination in herbal medicines available in Bangladesh. Bangladesh Med Res Counc Bull. 2013;39: 124-129.
 19. Bibha Shah, Nabaraj Pokhrel. Microbial quality and antibacterial activity of herbal medicines. Nepal Journal of Science and Technology. 2012;13(2):191-196.
 20. British Pharmacopoeia Comission. Appendix XVI (A–D). In: British Pharmacopoeia, Volume IV. Wielka. Brytania, Medicines Commission. 2004;331-351.

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