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Microbiological Contamination and Anti-bacterial Traits of Common Oral Herbal Medicinal Products within Dhaka Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SQ and NIT performed the experiments Authors MS and LP wrote the protocol. Author MSM managed the literature searches. Authors KKD and MA managed the analyses of the study. Author RN designed the study, wrote the first draft of the manuscript and critically revised the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Present study endeavored to examine the growth and survival of microorganisms within 6 categories of oral herbal medicines commonly used by the community within Dhaka metropolis.
Methodology: Samples were analyzed for the presence of bacteria and fungi up to 14 days. The microbial analysis was conducted by conventional cultural and biochemical methods. The *in vitro* anti-bacterial activity of the medicines was also detected employing agar well diffusion method.
Results: Initially all samples were found to be contaminated with total viable bacteria (10^2-10^4cfu/ml); however, the fungal and pathogenic growth was not observed. In course of time, the bacterial and fungal load increased up to 10^6cfu/ml and 10^3cfu/ml, respectively in most of the samples up to 14 days. The staphylococcal growth commenced after 48 hours in all samples and vigorously increased in two samples up to 10^5cfu/ml. Two categories of samples were found to be populated with *Klebsiella* spp.

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(10^2cfu/ ml); while other pathogenic bacteria were completely absent. Out of 6 categories of samples tested, 4 were found to exhibit the anti-bacterial trait against a few bacteria examined. Significant activity was found for sample 1 against *E. coli*, and sample 3 against *E. coli* and *Staphylococcus* spp. Sample 2 exhibited moderate activity against 4 test bacteria; while sample 4 was also noticed to be moderately active against 2 test bacteria.

Conclusion: Overall, together with the trivial anti-bacterial features, the appearance of massive bacteria and fungi after 14 days in most of the samples with an excessive staphylococcal load may pose the probable health risks to the medicine users.

Keywords: Oral herbal medicines; pathogenic bacteria; fungi; microbiological stability; consumer safety; public health.

1. INTRODUCTION

Herbal medicines play an important role in healthcare throughout the world and are known to possess antiulcer, antipyretic, anti-diabetic, and even the anti-cancerous activity including the combating potential against an array of clinical complications [1-9]. Besides the advancement in antibiotics, medicinal herbs have long been in use with an objective of managing public health especially in Asia [10]. Together with adverse side effects and toxicity, the emergence of drug-resistant bacteria appears to be a major limitation of using antibiotics [11-24]. In this context, herbal medicines appeared as alternative source of healthcare management throughout the world, with 21,000 plants currently being used for medication purposes [4].

Indeed, the anti-bacterial features of natural medicines made them as a suitable swap of the synthetic drugs [25-29]. However, even being advantageous over antibiotics in the aspect of combating against drug-resistant pathogens, conversely herbs may harbor an array of microorganisms, aerobic spores, and the fungal population, which are likely to originate from the soil or may gain access from the manure used in the plantation fields [30,31]. Also, the method of harvesting herbs, processing and handling, transportation and improper storage and distribution may also impart microbial flaw [32-35].

In Bangladesh more than 500 of medicinal plants have so far been enlisted of which a huge pharmacological evaluation and ethno-medicinal survey have been conducted; however, the microbiological aspects still have not been chalked out [36,37]. Moreover, while a number of study of microbial contamination of the pharmaceutical products have been conducted, the microbiological study on the medicinal herbal products is still in its infancy [36,38-40]. Besides, the knowledge on anti-microbial potential of the herbal medicines is needed to correctly interpret the efficacy of the herbal products. Another important aspect of using the herbal medicines underlies on the strategies for the safe application of these products [1]. The evidence-based knowledge on microbiological and pharmacological aspects on herbal medicine may help the operative management of herbs [1]. Being a developing country, availability, cost effectiveness and accuracy of medication are largely required for the overall public health management in Bangladesh. Based on these facts, current investigation attempted to measure the prevalence of pathogenic bacteria and fungi in the commonly used products with a time course motif, and further to determine the anti-bacterial traits of the tested herbal medicines.

2. MATERIALS AND METHODS

2.1 Study Area, Sampling and Sample Processing

Five samples from each of the six (6) categories were randomly collected from different registered private drug stores with appropriate dates of manufacturing and expiry within the city of Dhaka during the time frame of January 2013 to February 2014 according to the standard sampling method [41]. All the samples were aseptically processed followed by homogenizing 10ml of each samples with 90 ml normal saline and diluted up to 10^{-6} for microbiological assessment at 0, 48 and 72 hours. For control, un-inoculated 100 ml of sterile saline was used kept both at 37°C and 25°C.

2.2 Total Microbial Count

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1ml of each sample from dilutions 10^{-2}, 10^{-4} and 10^{-6} was introduced onto the nutrient agar and Sabouraud's dextrose agar plates (Hi-Media Laboratories Pvt. Ltd., India), respectively, by means of spread plate technique [42]. Aliquots from the control saline were also introduced onto these plates. Plates were incubated at 37°C for 24 hours and at 25°C for 48 hours for total viable bacteria and fungi, respectively. All the experiments were done in triplicate to confirm the reproducibility of the results.

2.3 Estimation of Specific Pathogenic Microorganisms

From the dilutions 10^{-3} and 10^{-5}, 0.1ml of each sample was spread onto the membrane fecal coliform agar and MacConkey agar (Hi-Media Laboratories Pvt. Ltd., India) for the triplicate enumeration of total fecal coliform (TFC), and coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hours at 44.5°C and 37°C for fecal coliform and coliforms, correspondingly. Likewise, *Staphylococcus* spp. and actinomycetes were isolated onto Mannitol Salt Agar and Actinomycetes agar (Hi-Media Laboratories Pvt. Ltd., India), respectively by adding 0.1ml of diluted sample each, and all the plates were then incubated at 37°C for 24 hours.

Ten (10) ml of sample was transferred into 90ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella*, and *Vibrio* spp., respectively and incubated at 37°C for 6 hours. After incubation, the samples were diluted up to 10^{-6} and then 0.1ml of samples from 10^{-3} and 10^{-5} dilutions were spread onto *Salmonella-Shigella* agar (Hi-Media Laboratories Pvt. Ltd., India) and thiosulfate citrate bile salt sucrose agar (Hi-Media Laboratories Pvt. Ltd., India) for the isolation of *Salmonella* spp. and *Shigella* spp., and *Vibrio* spp., respectively. The plates were incubated at 37°C for 48 hours for the detection of typical colonies. Finally, all the isolates were biochemically examined following standard procedures [42,43]. All the experiments were done in triplicate.

2.4 Assessment of Antibacterial Activity of Herbal Medicine

The antibacterial activity of the samples was detected employing agar well diffusion method [44]. Suspensions (with standard turbidity compared to that of the McFarland standard of 0.5) of each of the test bacteria; i.e., the laboratory strains of *Pseudomonas* spp., *Listeria* spp., *Bacillus* spp., *Vibrio* spp., *Salmonella* spp., *Klebsiella* spp., *Staphylococcus* spp., *E. coli* was spread evenly over the Muller Hinton Agar (Hi-Media Laboratories Pvt. Ltd., India) which

in turn resulted in the uniform lawns. Wells were made in the Muller Hinton Agar, and 100 µl of each of the samples was then introduced (with a concentration of around 11 mg/ml) separately in the specified well along with a positive control (Gentamicin, 10µg) and a negative control (normal saline). Presence of clear zone around the sample solution (if any) was indicative of the presence of antibacterial activity of the samples tested and the diameter of inhibition zone was recorded.

3. RESULTS AND DISCUSSION

The use of herbal medication in Bangladesh is quite abundant including homeopathy, Ayurveda, Unani medicines, etc. [36,37,45]. However, contamination of herbal medicines by bacteria and fungi, and even with the specific pathogens may commence due to improper handling during harvesting, processing, manufacturing, distribution or storage of the medicinal plant samples [30-36,46]. Thus a periodic examination of the microbiological status of such medicines along with their anti-microbial potency is required to ensure a sustainable health management system.

3.1 Increase of Microorganisms in the Herbal Medicine Samples in Course of Time

3.1.1 Growth and survival of total viable bacteria (TVB) and fungi

Of the sealed samples studied, all were found to be contaminated with total viable bacteria (TVB) within a range of 10^2-10^4cfu/ml; however, no pathogen was detected along with the complete absence of fungal population Table 1. The study continued afterward once the cap was opened in order to demonstrate further microbial growth, which in turn might focus on the contamination from the environment or due to improper handling. In course of time up to 14 days, the TVB bio-burden was noticed to be increased by 3-4 logs (~10^6cfu/ml in most of the samples), which was beyond the acceptable microbial limits [46].

Initially no fungal growth was observed Table 1. Up to 14 days, sample 1 was observed to be totally free from fungal growth. However, in samples 2-6, fungal proliferation was noticed to be initiated within 48-72 hours, and after 14 days, these samples were found to harbour fungal burden with an average load of 10^3cfu/ml.

3.1.2 Growth initiation and proliferation of pathogenic bacteria in course of time

Like the pharmaceutical oral products, pathogenic spoilage of oral herbal medicines is not unlikely due to handling malpractice during harvesting of herbs, processing, manufacturing, storage or usage. Indeed herbs are more likely to harbor a huge number of pathogenic bacteria including *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and others, usually originating in the plantation soils or may be disseminated from organic fertilizer [30,31,46].

In our study, initially all the samples were found free from any pathogenic bacterium. However, with a concomitant increase in TVB, the staphylococcal load was observed to be elevated within a range of 10^2-10^5cfu/ml after 14 days, especially in samples 1 and 2 Table 1. Notably the growth commenced after 48 hours of unsealing the medicinal samples which is assumptive of the possible contamination from the surrounding environment or users.

Table 1. Microbiological growth by time in the samples

Samples	Duration of test	TVB (cfu/ml)	Fungi (cfu/ml)	*Kebsiella* spp. (cfu/ml)	*Staphylococcus* spp. (cfu/ml)
Sample 1	0 hour	3.0×10^3	0	0	0
(N=5)	48 hours	1.5×10^3	0	0	0
	72 hours	4.5×10^4	0	0	2.6×10^3
	14 days	2.8×10^6	0	0	3.6×10^4
Sample 2	0 hour	3.6×10^4	0	0	0
(N=5)	48 hours	4.2×10^4	0	2.9×10^2	0
	72 hours	5.2×10^5	2.8×10^2	3.1×10^2	1.0×10^2
	14 days	1.2×10^6	1.1×10^3	3.3×10^2	4.3×10^5
Sample 3	0 hour	5.0×10^4	0	0	0
(N=5)	48 hours	3.6×10^4	2.2×10^2	2.2×10^2	0
	72 hours	4.6×10^6	3.1×10^2	3.6×10^2	3.9×10^2
	14 days	2.6×10^6	7.6×10^3	3.4×10^2	6.7×10^3
Sample 4	0 hour	3.6×10^2	0	0	0
(N=5)	48 hours	4.3×10^4	0	0	0
	72 hours	5.1×10^5	3.1×10^3	0	4.0×10^2
	14 days	2.0×10^6	2.1×10^3	0	2.7×10^3
Sample 5	0 hour	5.2×10^2	0	0	0
(N=5)	48 hours	6.2×10^4	0	0	0
	72 hours	3.1×10^5	1.5×10^2	0	4.1×10^2
	14 days	2.2×10^6	1.4×10^3	0	2.2×10^3
Sample 6	0 hour	4.9×10^2	0	0	0
(N=5)	48 hours	4.5×10^4	0	0	0
	72 hours	4.8×10^5	3.1×10^3	0	1.0×10^2
	14 days	4.3×10^5	1.7×10^3	0	1.4×10^2

The average load has been shown. Escherichia coli, fecal coliforms, Vibrio spp., Salmonella spp., Shigella spp., Bacillus spp., Listeria spp. and actinomycetes are totally absent in all the times tested. TVB=Total viable bacteria; Microbial limits [46]=Total aerobic bacteria: 10^5cfu/ ml

Two categories of samples (samples 2 and 3) were found to be populated with *Klebsiella* spp. (10^2 cfu/ ml) after 48 hours which remained at its level till 14 days. All samples were found to be completely free from other pathogenic bacteria including *Escherichia coli*, fecal coliforms, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp., *Listeria* spp. and actinomycetes as tested up to 14 days. Even a minor presence of *Klebsiella* spp. was noticed together with the observation of the complete absence of most of the pathogenic bacteria, the huge number of total viable bacteria (TVB) and *Staphylococcus* spp. might lead to possible health risks to the consumers. Both the TVB and staphylococcal contamination is assumed to take place during plant harvesting or from the manure; and certainly due to the unhygienic handling both by the manufacturers and by the users [30,31,46].

3.2 *In vitro* Anti-bacterial Activity of the Herbal Medicine Samples

A number of reports revealed the antagonistic feature of natural herbs against a wide range of microorganisms and hence suggesting them as an alternative of the antibiotics [25-27,47-50]. However, such study is still very much limited in Bangladesh. In our study, 4 samples were found to exhibit the anti-bacterial activity against a few test bacteria used Table 2. The activity of sample 1 was scored against *E. coli*, *Vibrio* spp. and *Pseudomonas* spp.; sample 2 was found to exhibit the anti-bacterial activity against *E. coli*, *Salmonella* spp., *Vibrio* spp. and *Staphylococcus* spp.; sample 3 against *E. coli*, *Salmonella* spp., and *Staphylococcus* spp.; and sample 4 showed the activity against *Salmonella* spp. and *Vibrio* spp. only Table 2. Samples 5 and 6 were completely devoid of such anti-bacterial trait.

Table 2. Antimicrobial activity of the herbal medicine samples tested

Herbal medicine samples	Zone of inhibition (mm) against test bacteria							
	E. coli	***Klebsiella* spp.**	***Salmonella* spp.**	***Vibrio* spp.**	***Pseudomonas* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***Listeria* spp.**
Sample 1	17.12	0	0	13.42	9	0	0	0
Sample 2	14.45	0	15	12.95	0	0	15	0
Sample 3	17.65	0	15	0	0	0	17.23	0
Sample 4	0	0	6	13.05	0	0	0	0
Positive control	16.5	17	20.5	13.55	23.8	17.75	25.35	18.5
Negative control	0	0	0	0	0	0	0	0

The experiments were conducted three times independently at 0 hour, and the results were found to reproducible. One representative data has been shown. Samples 1-4 were found to be inactive against Klebsiella spp. and Listeria spp.; while samples 5 and 6 were not found to exhibit any anti-bacterial activity against all the test bacteria. As the positive control, gentamicin 10 □g was used, and as the negative control, saline was used

Relatively elevated anti-bacterial activity was scored for sample 1 against *E. coli*, sample 3 against *E. coli* and *Staphylococcus* spp. Sample 2 was found to be moderately active against *E. coli*, *Salmonella* spp., *Staphylococcus* spp. and *Vibrio* spp. Sample 4 was also found to be moderately active against *Vibrio* spp. with a lower anti-bacterial activity against *Salmonella* spp. In case of samples 1-4, including the remaining samples 5 and 6, no anti-bacterial activity was demonstrated against *Klebsiella* spp., *Bacillus* spp. and *Listeria* spp.

As stated earlier, in Bangladesh, while the pharmaceutical finished product quality control and the ethno-medicinal survey of medicinal herbs have been conducted; the microbiological study specifically of the herbal medicines is still insufficient [36,37]. The sustainability of bacteria and fungi in the herbal medicines tested in our study reveals the inappropriate practice of hygiene maintenance during manufacturing and processing systems. The growth and proliferation of pathogenic bacteria in course of time in the herbal products refer to (i) the maintenance of good manufacturing practice (GMP) along with total quality management (TQM) during manufacturing and processing of medicines; and (ii) the emergence of aseptic caution during the usage of the medicines. Besides, the complete absence of anti-bacterial traits in the majority of the samples studied unveiled the probable ineffectiveness of the herbal medicines against pathogenic infection.

4. CONCLUSION

According to our study, most of the pathogenic bacteria were found to be absent in all samples; however, the increasing trend of total viable bacteria and fungi together with the staphylococcal load indicated the level of microbial contamination which may lead to the deterioration of the product shelf life as well as may pose threat to the overall public heath safety. In context of all samples, the relatively minor anti-bacterial activity demands better formulation to be specific towards the elimination of pathogenic bacteria.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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