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Microbiological Analysis Of Herbal Medicines Collected From Different Areas Of Dhaka, Bangladesh

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Abstract: *The present study was attempted to determine the microbiological analysis of commonly available herbal medicines. In this regard, ten marketed herbal drugs from some herbal selling stores in the city of Dhaka, Bangladesh were collected and then examined microbiologically. Total aerobic count showed that most of these drugs were above the acceptable bacteriological limit. The samples contained the microbial contamination with viable bacteria and fungi upto 10^7 cfu/ml. Among the specific bacterial isolates, Staphylococcus spp. and Pseudomonas spp. were prevalent in all samples. Presence of Klebsiella spp. was also documented. The study of antibiogram profile revealed that Pseudomonas spp. was highly resistant against azithromycin, streptomycin and ampicillin whereas Klebsiella spp. was only resistant against ampicillin. This study was significant from the public health point of view as most of the herbal medicines were highly contaminated with potentially pathogenic bacterial flora having the drug resistant properties.*

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I. Introduction

Herbal medicine is a major form of treatment for more than 70% of the world's population. The use of medicinal products containing as active ingredients exclusively plant material and/or vegetable drug preparations used to treat various health conditions. Practice with herbal medicines has a long tradition of use outside conventional medicine. It is becoming more mainstream as improvements in analysis and quality control, along with advances in clinical research, show the value of herbal medicine in treating and preventing disease. Throughout history, people have used plants for both food and medicine. Medicinal herbs are defined as plants or parts of plants such as the leaves, stems, roots, flowers, and seeds that contain organic chemicals with effective healing properties [1]. The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [2]. Traditional knowledge is the indigenous knowledge developed over hundreds of years through direct contact with the environment. Individuals with traditional knowledge have a vast understanding of how plants and animals affect people. Because of this knowledge, much of the world's population uses herbal medicine globally as their primary health care choice [3].

In herbal medicines microbes might come along with the raw materials or due to contaminated processing unit. Microbes might contaminate herbal medicines during storage and improper handling as well. Although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effect. And this might be due to factors such as adulteration, substitution, contamination, misidentification, and lack of standardization, incorrect preparation, dosage, inappropriate labeling and advertisement [4]. Most environmental factors leading to contamination can be controlled by implementing the standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for manufacturing safe and effective herbal remedies [5]. Thus, it is far safer to go to a trained professional herbalist to receive a more regulated herbal product than to purchase an over-the-counter one.

However, at present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and in homeopathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes. The commercial value of various innumerable drugs and pharmaceuticals derived from tropical forest systems on worldwide basis is projected at 20 billion dollars a year [2]. Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics [6,7].

Considering all these fact, the study has been designed to investigate the microbiological contamination of herbal medicines, currently used by Bangladeshi people to treat their illness. Besides the antibiogram profile of the selected isolates was also carried out.

II. Material And Methods

Study area, sampling and sample processing

10 herbal medicine samples were collected from different areas of Bangladesh during February 2017-April 2017 following standard protocol [8, 9]. The samples were prepared for the microbiological assay by homogenization of 10 ml sample into 90 ml sterile normal saline and serial dilution was done up to 10^{-5}, according to the standard methods as described by Cappuccino & Sherman in 1996 [10].

Microbiological Analysis

Estimation of Total Viable Bacteria and Fungi

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 ml of each sample from the dilutions 10^{-3} and 10^{-4} was introduced onto the nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates, respectively, by means of spread plate technique. Plates were incubated at 37 ^{o}C for 24 hours and at 25 ^{o}C for 48 hours for the isolation and enumeration of total viable bacteria and fungi, respectively [8, 10, 11, 12].

Estimation of *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp. and *Pseudomonas* spp.

From the dilutions 10^{-3} and 10^{-4}, 0.1 ml of each sample was spread onto the MacConkey agar for the enumeration of coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hours at 37 ^{o}C for the detection of coliforms,. Likewise, *Staphylococcus* spp. and *Pseudomonas* spp. were isolated onto Mannitol Salt Agar (MSA) and *Pseudomonas* agar, respectively by adding 0.1 ml of the each diluted sample, and all the plates were then incubated at 37 ^{o}C for 24 hours [8, 10, 11, 12].

Confirmative biochemical identification:

Finally, a series of biochemical tests were conducted to confirm the identity of all the isolates as described previously. Some biochemical tests such as Methyl Red-Voges- Proskauer (MR-VP) test, Citrate test, MIU test and Triple sugar iron (TSI) etc were performed for the identification of bacterial isolates [10].

Study of antibiogram profile:

Isolates were tested against four common antibacterial drugs by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) following standard protocols [13]. Briefly, a single colony of each isolate was introduced into 2 ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were used to spread the suspension evenly over the entire MHA agar surface. Antibiotic discs (azithromycin-15µg, gentamycin-10 µg, streptomycin-10 µg, ampicillin-10 µg) were then placed onto the surface of the inoculated plates. After incubation, diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant.

III. Result

The microbial level of marketed herbal medicines which were used in this study is depicted in the table 1. Microbial counts by plate count method ranged between 10^{4} -10^{7}. The presences of indicator organisms in the samples were reported in table 1. On the basis of standard biochemical test three different bacterial pathogens were identified as shown in table no.2.

Quantification of the bacterial isolates:

Out of 10 liquid samples, 3 samples showed very high bacterial load. Almost all the samples also showed high fungal load. Nine samples were found to be contaminated by coliforms which are intestinal bacteria and are indicator for contamination by feces. None of the samples showed contamination by *Salmonella* spp and *Shigella* spp. All the samples showed high growth for *Pseudomonas* spp. and *Klebsiella* spp. These results are depicted in Table no:1.

Table 1: Microbiological load in the tested herbal medicine samples (cfu/ml)

Sample	Total bacterial count (cfu/ml)	*Klebsiella* spp. (cfu/ml)	*Staphylococcus* spp. (cfu/ml)	*Pseudomonas* spp. (cfu/ml)	Total fungal count (cfu/ml)
1.Jambadyarista	2.5×10^7	2.5×10^6	7.0×10^5	1.0×10^4	3.9×10^6
2.Arjunarista	2.9×10^7	1.9×10^5	4.4×10^5	2.3×10^5	3.1×10^6
3.Mehabajra Rasayan	1.6×10^6	2.8×10^6	5.6×10^5	8.8×10^4	1.4×10^6
4.Kutjarista	2.8×10^7	1.6×10^6	4.8×10^5	2.3×10^5	1.1×10^6
5.Livolima	1.2×10^7	-	5.6×10^5	2.9×10^5	3.6×10^5
6.Amalaki Rasayan	1.5×10^7	2.3×10^5	6.8×10^5	8.0×10^4	1.8×10^6
7.Ashokarista	2.2×10^7	2.9×10^4	6.0×10^5	1.9×10^6	2.7×10^6
8.Patrangasav	2.7×10^7	1.1×10^6	3.6×10^5	1.9×10^4	5.8×10^5
9.Jirakadyarista	2.7×10^6	4.2×10^5	1.5×10^5	1.4×10^5	9.2×10^5
10.Mahadraksharista	2.4×10^6	9.2×10^5	1.4×10^5	2.3×10^5	9.2×10^5

The average load is shown here.
Microbial limits according to World Health Organization, 2007 [14]
Total aerobic bacteria 10^5 cfu/ml, *Escherichia coli* 10^1 cfu/ml, *Salmonella* spp. absent, Enterobacteria 10^3 cfu/ml

Biochemical identification of the bacterial isolates among the samples studied:
Among the bacterial isolates *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. were presumptively identified through the standard biochemical tests from the herbal medicines as revealed in Table no. 2.

Table no 2: Biochemical identification of the bacterial isolates

Isolated Strain	TSI			H_2S reaction	MR Test	VP Test	Citrate Test	MIU Test
	Slant	Butt	Gas					
Pseudomonas spp.	Y	Y	-	-	-	-	+	-
Staphylococcus spp.	Y	R	+	+	+	-	+	+
Klebsiella spp.	Y	Y	+	-	+	+	-	+

TSI-Triple Sugar Iron Test, Y-Yellow (Acid), R-Red (Alkaline), MR-Methyl red, VP-Voges-Proskauer

Antibiogram profile of the bacterial isolates found from the herbal medicines:
The antibiogram profile of the pathogenic isolates found in these herbal medicine samples was tested. *Pseudomonas* spp. was highly resistant against azithromycin, streptomycin and ampicillin whereas *Klebsiella* spp. was only resistant against ampicillin as shown in Table no: 3. Resistance gene might be evolved due to point mutation, genetic disorders, and mechanistic factors or by epidemiological factors [11]. Such drug resistance properties may render these pathogens causing serious health hazards because of ineffective treatment of the sufferers by the commonly prescribed antibiotics.

Table no 3: Study of antibiogram profile of the bacterial isolates

Name of Antibiotic disc	***Pseudomonas* spp.**		***Klebsiella* spp.**	
	Resistant %	***Sensitive %***	***Resistant %***	***Sensitive %***
Azithromycin	100%	0%	0%	100%
Streptomycin	100%	0%	0%	100%
Ampicillin	100%	0%	100%	0%
Gentamycin	0%	100%	0%	100%

IV. Discussion

The risk of the presence of microorganisms in a pharmaceutical product depends on finality of the use, its nature and its potential damage that may be caused to the consumers. The total aerobic microbial count of herbal medicine was not more than 10^7 cfu/ml. Thus, the microbial load of marketed herbal medicine which was analyzed in this study was not in acceptable limit according to WHO guideline, 2007 [14]. Harmful microorganisms may be present in herbal medicines as reported by some previous authors such as *Salmonella, Shigella, E. coli, Pseudomonas* spp. and *S. auereus* [15]. The identification of the isolated pathogens in this study is in agreement with previous study by Sharmin et al., 2014 [8]. All the pathogens which were isolated from the marketed herbal drugs in this study have been implicated in previous studies on gastroenteritis and other transmissible diseases [16, 17]. *Staphylococcus aureus* was a cause in gastrointestinal illness by earlier findings of workers such as Rajapandiyan et al, 2013 [18]. The microbial levels associated with these marketed

herbal drugs could be attributed to their source of origin and their nutritive values and low standard of processing. The presence of bacterial load in herbal drugs constitutes a health hazard. Moreover, in the absence of viable cells, microbial metabolites may be toxic. Similar results were obtained with the herbal powder samples contaminated by *E. coli* and *Salmonella* species and herbal tablets contaminated by *E. coli* [19]. There are also some results obtained with the herbal medicines available in Dhaka Bangladesh by Sharmain et al., 2014 [8]. The results in that study showed that the microbial load of the herbal products varied considerably. The samples were contaminated to varying degrees with bacteria and fungi. In case of individual product, most of them met with the given microbiological standard but few of them could not pass the entire test.

V. Conclusion

It may be concluded from this study that most herbal medicines sold in herbal market in Dhaka city are likely to be contaminated with a wide range of potentially pathogenic bacteria and there is inadequate quality control in their production and distribution. Bangladesh can come up as the chief nation and play the lead role in the production and proliferation of standardized and therapeutically helpful herbal medicines. This can happen only if the herbal products are assessed using standard guidelines and techniques. Thus there is a strong need of incessant monitoring and quality control of herbal medicines coming to the local Bangladeshi market. Quality has to be built throughout the process beginning from the selection of propagating materials to the final products reaching to the consumers. Moreover the issues raised should be measured by medical and paramedical practitioners, the government and the whole citizen of the nation.

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