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Assessment of Biological Contaminants in Energy Stimulating Herbal Medicines Collected from Dhaka City, Bangladesh

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Ubiquitous nature of erectile dysfunction (ED) has placed it as one of the most rampant health care problem and therefore consumption of energy stimulating herbal medicines (ESHMs) has increased in Bangladesh. However, these herbal medicines reaching consumers without maintaining proper screening procedure, which bring a threat to public health safety. An analysis of biological contaminants (microbial load) of these herbal medicines available in Bangladesh was investigated. In most of samples, the total bacterial counts (TBC) $6 \times 10^7 - 32 \times 10^{11}$ cfu/mL and lactobacillus count $6 \times 10^8 - 12 \times 10^{11}$ cfu/mL exceed the maximum value as percribed by WHO.

Keywords: Erectile dysfunction, Herbal medicines, Drug safety, Microbial diversity, WHO Guidelines.

INTRODUCTION

Repetitive inability to get on or keep on stable erection, which is sufficient enough for sexual intercourse is called erectile dysfunction (ED) [1,2]. Erectile dysfunction is one of the most proliferating health care problems. Other sexual problems like lack of sexual desire (libido), ejaculation complications, orgasm hitches lead to an inconsistent ability to reach orgasm and a trend to sustain only brief erections (premature ejaculation) [3,4] are closely associated with ED. An assessment depicts a number of of nearly 15 million to 30 million men worldwide suffering from ED [1]. Daily chores and exhaustion in nerve-wracking life style of modern days has been correlated with ED, which has surfaced the number of male living in misery of shame owing to sexual dysfunction.

Synthetic drugs are offered and/or used to treat these types of problems by physicians. Oral testosterone is effective in minimizing ED in some men with low levels of natural testosterone, but it is often ineffective and may effect liver damage [2]. Other drugs such as yohimbine, papaverine, phentolamine, and alprostadil are also used with caution [5]. However, these type of conventional drugs have been associated with serious hostile health effects. Side effects of drugs like blood pressure drugs, antihistamines, antidepressants, tranquilizers, appetite suppressants, and cimetidine (an ulcer drug) can accelerate ED [6]. These instances have led patients to search for natural treatments which are plants based medicines also called herbal medicines. Use of energy stimulating herbal medicines (ESHMs) for sexual impotence and ED have been reported widely in Asian countries [6]. As a result, there is a rampant usage of non-certified or unlicensed herbal products [7]. However, drug safety of herbal medicines is often face questions as lack of proper Quality Control (QC) and insufficient labeling leads to sacrifice of herbal drug safety [8-11]. These herbal medicines are the commonest route, which does not have to meet specific standards of safety and quality neither is required to be accompanied by safety information for the consumer [8].

A medicinal plants host a wide spectrum of microorganisms, which may be due to a series of influences from animal and inanimate sources. Presence of bacterial endospores and fungal spores in herbal plants have been reported. From source there is possibility of transformation of microbial load to herbal medicines due to wrong handling during production. Findings have shown the presence of potential contaminants (*Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp. and other Gram positive and Gram negative strains of bacteria) in herbal medicines [12-15]. Certain fungal genera produce mycotoxins, which is a potential health hazard chemical. Inges-

tion of adherent fungal flora with herbal drugs is associated with human disorders. Not only the microbes but also the low molecular weight metabolites from molds are known as chemical contaminants. Understanding the risk an inquiry into evaluation of biological contaminants (microbial type) in ESHMs available in Bangladesh was undertaken in this study.

EXPERIMENTAL

Sample collection: Out of total 9 samples, three samples (no. 1-3) were collected from Mirpur city, four samples (no. 4-6) from Mohammadpur and the remaining (no. 7-9) were collected from Kalabagan area. The rationale for selection of the study area were justified as herbal drugs selling hotspots. Few samples were provided in finished commercial pack by retailers and rest of those were not packaged. Non-packaged drugs were collected in sterile glass cans. All samples collected from the sites were labeled from ESHM-1 to ESHM-9 to conceal the identity of the manufacturer. Labeled samples were taken to Microbiological Research Laboratory, Department of Nutrition and Food Engineering, Daffodil International University, Dhaka, Bangladesh for microbial quality assessment.

All the glasswares used for microbial diversity assessment were sterilized by autoclaving (at 121 ºC for 20 min). The petri-dishes, test tube and pipet were prepared in duplicates. UV-C Germicidal lamps interior was sterilized for 15 min and UV-C germicidal lamp was also used to sterilize the interior and contents before usage.

Preparation of media: Culture media were made according to the specific instruction manual booklet provided by the manufacturer followed by sterilization using autoclave (model: LTE J7090, LTE Scientific Ltd., England) at 121 ºC for 15 to 20 min. The sterile media were then dispensed or poured into sterilized petri-dishes and allowed to cool and checked for sterility of blindly selected incubated media (37 ºC for 24 h).

Cultivation of microbes: It is done using different media *viz*. nutrient agar, plate count agar, Lactobacillus M.R.S. agar, potato dextrose agar and MacConkey agar. In brief, suspension was made by taking an appropriate amount in 100 mL distilled water. The pH of the suspension was checked and neutralized accordingly. The suspension was heated till all the contents dissolved followed by autoclave sterilization at 121 ºC and 15 1bs pressure for 15 min. Cool the solution to 40 to 42 ºC and poured into sterile petri plates after mixing.

Salmonella Shigella agar: About 6.3 g of agar was suspended in 100 mL distilled water and then heated the solution till boiling at 100 ºC for 25 min to dissolve the suspended mass. On cooling, the dissolved mass was fused well and poured into sterile petri plates.

Total bacterial count (TBC): With the help of a sterile loop of 0.1 mL water brand was spread on the surface of a sterile nutrient agar. Ketoconazole (0.05 mg/mL) was added into the nutrient agar plates to inhibit fungal growth, followed by incubation in an inverted position at 35 ºC for 16- 24 h. As a result, bacterial colonies were formed. Each colony was sub cultured and later stored for characterization and identification.

Total fungal count (TFC): The spread plate method was conducted for total fungal count. Water sample (0.1 mL) was dispensed into a sterile potato dextrose agar (PDA) plate and spread evenly over the agar surface. Chloramphenicol (0.05 mg/mL) was employed onto the agar plate to inhibit bacterial growth followed by incubation in an inverted position at 35 ºC for 72 h. Each colony was sub-cultured on sterile PDA plates and later stored in sterile PDA slants.

Total coliform count (TCC): Each water sample (100 mL) was passed through a membrane filter and the filter transferred onto the surface of plates containing MacConkey agar (MCA) aseptically followed by incubation in an inverted position at 35 ºC for 48 h.

Total Lactobacillus count: Each water sample (100 mL) was passed through a membrane filter followed by aseptic transferring onto the surface of Lactobacillus M.R.S. agar kept in plates. Incubation was carried out in an inverted position at 35 ºC for 48 h.

RESULTS AND DISCUSSION

The average microbial counts of the herbal medicines are summarized in Table-1. During this study, the average temperature varied between varied from 23 to 28 ºC at Bangladesh Meteorological Department, Climate Division, with a pH range 4.85-8.06.

Absence of total fungal count (TFC) and other pathogenic microorganisms like total coliform count (TCC) and total Salmonella Segilla count (TSSC) were affirmed in all nine herbal medicines. Although, medicinal herbs are susceptible to indigenous fungi in the soil where they were grown in pre-harvest stage and the dried part of medicinal herbs may be exposed to

TABLE-1
AVERAGE MICROBIAL COUNTS OF THE ENERGY STIMULATING HERBAL MEDICINES (ESHMs)

Sample ID	Total bacteria count (TBC) (cfu/mL)	Total fungal count (TFC) (cfu/mL)	Total coliform count (TCC) (cfu/mL)	Total Lactobacillus count (cfu/mL)	Total Salmonella Segilla count (TSSC) (cfu/mL)
ESHM-1	12×10^9	Absent	Absent	9×10^{11}	Absent
ESHM-2	16×10^8	Absent	Absent	6×10^8	Absent
ESHM-3	32×10^{11}	Absent	Absent	Absent	Absent
ESHM-4	42×10^8	Absent	Absent	Absent	Absent
ESHM-5	14×10^{11}	Absent	Absent	7×10^8	Absent
ESHM-6	6×10^7	Absent	Absent	Absent	Absent
ESHM-7	10×10^{10}	Absent	Absent	20×10^9	Absent
ESHM-8	3×10^8	Absent	Absent	Absent	Absent
ESHM-9	8×10^{11}	Absent	Absent	12×10^{11}	Absent

fungal contamination during post-harvest [16]. Absence of fungal count may be due to natural barriers and antimicrobial substances of different chemicals present in plant species. These organic chemicals (oils, peptides, liquid and extracts contained by certain plants) employ inhibitory effects on microbial growth and stability [17].

Null computation of total coliform were also seen which is an indication that the raw materials from which the herbal drugs have been prepared like leaves or extracts and water were free from human and animal wastes (feedlots, pets, septic systems). Contamination from seepage or discharge from septic tanks, sewage treatment facilities and natural soil and plant bacteria were also unlikely which has been resulted in absence in TCC. Moreover, presence of fecal coliform indicates possible presence of other harmful microorganisms like *Sammonella* [18]. In agreement with this study, present investigation lead us to absence of *Salmonella* and *Shigella* population (Table-1). Transmission of *Shigella* follows the fecal-oral route followed by spreading from person-to-person and through consumption of water or food contaminated with feces from infected individuals.

The ESHMs were found contaminated with varying degrees of bacteria which is in agreement with previous study on microbial quality of herbal drugs [19]. Out of the nine ESHM analyzed, the total bacterial counts (TBCs) were between 6×10^7 cfu/mL and 32×10^{11} cfu/mL. ESHM-3 ensured the highest TBC count (32×10^{11} cfu/mL) followed by ESHM-5 (14×10^{11} cfu/mL), ESHM-9 (8×10^{11} cfu/mL) and ESHM-7 (10×10^{10} cfu/ mL. Sample ESHM-6 was found containing restricted TBC count (6×10^7 cfu/mL), which is the lowest among all the nine samples. Aerobic bacterial population was found in all of the ESHMs beyond safe standard range set by WHO for TABC (10^5 cfu/mL) [13]. Contact of air borne microbiological conta-minants with the drug samples during harvesting period could be the reason for the contamination of herbal drug samples with TABC. Moreover handling of herbs by farmers to manu-facturer may pave entry for aerobic bacteria [20]. Some aerobic bacteria like *Salmonella*, *Staphylococcus* and *Escherichia coli* can cause serious illness and serious infections damages to organs [21].

Among the nine samples analyzed, five samples (ESHM-1, ESHM-2, ESHM-5, ESHM-7 and ESHM-9) were found contaminated with Lactobacillus. The Lactobacillus counts ranged between 6×10^8 cfu/ mL and 12×10^{11} cfu/mL. ESHM-9 had the highest Lactobacillus counts (12×10^{11} cfu/mL), followed by ESHM-1 (9×10^{11} cfu/mL), ESHM-7 (20×10^9 cfu/mL) and ESHM-5 (7×10^8 cfu/mL). ESHM-2 had minimum originate Lactobacillus (6×10^8 cfu/mL). All the five samples exceed the safe limit set as by WHO [14] for Lactobacillus.

Conclusion

Assessment of microbial diversity in terms of the TBC, TFC, TCC, Lactobacillus counts and TSSC in nine ESHMs available in Bangladesh was performed. Absence of TFC and other pathogenic microorganisms like TCC and TSSC was observed in all nine energy stimulating herbal medicines (ESHMs). However, presence of TBC in all ESHMs were found which exceed the maximum limit as percribed by WHO. Moreover, estimation of Lactobacillus counts in five ESHMs (ESHM-1, ESHM-2, ESHM-5, ESHM-7 and ESHM-9) were found unsafe according to WHO guidelines. The types of microbial contamination found reveals that the ESHMs were not subjected to aseptic conditions during various stages of preparation, packaging, storage and transportation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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