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Evaluation of Microbial Quality of Selected Herbal Raw Materials Marketed in Sri Lanka

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ABSTRACT

Ayurveda medical system is one of the earliest documented health care systems in Asia and it is the second major health care providing service in Sri Lanka. Commercial manufacturing of Ayurveda pharmaceuticals has been legislated for the last six decades. *Well-documented standards for commercially available herbal raw materials are not regulated in manufacturing process. Therefore, identifying standard parameters for herbal raw materials are highly significant. Microbial quality of commercially available* dried fruits of *Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus emblica* that *are widely used in various types of Ayurveda pharmaceuticals were evaluated and compared with recommended microbial count with reference to establishing microbial standards of herbal raw materials. Randomly collected dried crushed fruit samples were subjected to evaluate the microbial quality in terms of cfug^{-1} using pour plate method and spread plate method. Bacterial count of Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus emblica* were 1.85 x 10^4, 2.91 x $10^5$and 2.66 x 10^5 *cfug^{-1}* respectively. Yeast and mold count *of Terminalia chebula* were 2.83 x 10^4 *cfug^{-1}*, *Terminalia bellerica* were 2.9 x 10^4 *cfug^{-1}* and *Phyllanthus emblica* were 3.03 x 10^4 *cfug^{-1}*. Bacterial count of tested samples of *Terminalia bellerica* and *Phyllanthus emblica* were higher than the value recommended by WHO for dried powdered herbal materials (1x 10^5cfu/g). Yeast and mold count of tested samples were higher than the recommended value (1x 10^3cfu/g). Improper collection, processing, transportation and storing methods of raw materials could be the reasons for higher microbial count of the samples. Therefore, improving and regulating standards for processing method and supply chain in raw materials is required for enhancing quality, safety and efficacy of herbal pharmaceuticals.

KEYWORDS: *Microbial Quality; Terminalia chebula; Terminalia bellerica; Phyllanthus emblica*

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INTRODUCTION

Ayurveda is one of the oldest medical systems in the world which have been developed since thousands of years ago. It helps to improve physical, mental and spiritual health of the people, but not just the treatment of a disease but overall wellbeing of an individual as well (Jayasundar, 2010). Ayurveda system is having a potential to improve public health with low cost compare to modern medicines. Due to long term acceptability of system, people are interesting to get remedies for improving their health and prevent the diseases (Kumari and Kotecha, 2016). Etiology, signs and symptoms and treatment protocols has been mentioned in authentic Ayurveda texts such as *Charak Samhitha, Sushruta Samhitha* and *Vagbhata*. Ayurveda pharmaceuticals that are manufactured using various parts of plants, animal products and minerals are acting as valuable sources for curing and preventing diseases (Shinde *et al*., 2009). Medicinal plants constitute a source of raw material for both Ayurveda and modern medicine (Surekha *et al.,* 2016). At present, herbal medicines are getting more popular with the global trend of people returning to natural or herbal therapies (Pohtam *et al*., 2019) and demand has been increased for plant derived products in developed and developing countries (Yadav *et al*., 2008). Roots, barks, whole plant, fruits, leaves, flowers, rhizomes and plant seeds are the ingredients of the pharmaceuticals (Kankanamalage *et al.,* 2013). Lack of usage of standardization techniques and parameters in poly-herbal formulations has been created difficulties in validating the quality, efficacy and safety of the herbal product (Pal and Shukla, 2003). Good Manufacturing Practices (GMP), process standardization and product standardization are the key events of manufacturing good quality herbal products (Masand *et al.,* 2014). Nowadays classical and modern instrumental method of analysis using reliable, specific and sensitive techniques are contributing to improve quality and standards of herbal pharmaceuticals worldwide (Yadav *et al*., 2011). Qualitative and quantitative values in standardization process is carrying an assurance of quality, efficacy, safety and reproducibility in Ayurveda pharmaceutical manufacturing process. The traditional process in identification of plant materials, variation of properties of botanicals due to time and environmental factors, bad practices in commercialization and improper supply chain of raw materials leads to highlight the need of establishing quality control and standardization protocols for herbal pharmaceuticals (Shinde *et al*., 2009). Proper identification of raw materials using morphological and

microscopic characteristics, adequate analytical and microbiological methods are essential to ensure the quality and standards of herbal pharmaceuticals. Microbiological contaminants with spoilages of herbal products George *et al*., 2014), excessive heavy metal contamination and presence of pesticide residues have been reported due to mal practices of commercial herbal pharmaceutical manufacturing industry. Environment factors such as temperature, humidity and rainfall during pre- harvesting, harvesting and post- harvesting periods are the common factors of microbial contamination of raw materials and finished products (Marcelo *et al*., 2012: Rajapandyan *et al*., 2013: Masand *et al.,* 2014). Due to the organic nature of herbal materials, it could be act as a nutritional media for overgrowth of microorganisms that resulting deterioration of raw materials or finished products and also create many of legislative problems in the norm of export and import process in global market (Masand *et al.,* 2014). Deteriorated raw materials with large count of microorganisms lead to manufacturing substandard finished products. With this background, microbiological standards as well as the application of Good Manufacturing Practice (GMP) have been considered as standardization parameters in developing and developed countries to assure the hygienic quality of non-sterile pharmaceutical preparations (Esimone *et al.,* 2001).

Though the legislations are available for monitoring the herbal medicines in developed countries, it is not yet established in developing countries. With this background, determine microbial quality of selected raw materials which are commonly used in Ayurveda pharmaceutical preparations are utmost significant. Findings of these studies will be helpful to identify current quality status of raw materials and initiating legislative activities and standardization process in Sri Lanka. Considering these background, this research project was designed to evaluate the microbial quality of selected raw materials in terms of $cfug^{-1}$ and to compare the results with previously recorded data.

MATERIALS AND METHOD

Fruits of *Terminalia chebula* (*Harithaki*), *Terminalia bellerica* (*Vibhithaki*) and *Phyllanthus emblica* (*Amalaki*) that are widely used as main ingredients in different type of Ayurveda pharmaceuticals were selected for the evaluation. 100g of dried crushed fruits without seeds of each plant materials that are marketed in herbal outlets were randomly collected from Colombo, Gampaha and Kurunegala district in Sri Lanka. Standard glassware and microbiological media were used to evaluate microbial quality of the tested samples. Glassware was soaked in a bath with a mild detergent for 24 hours. Then they were rinsed well and dried in the room temperature on racks in the laboratory. They were kept in stainless steel container, glass pipettes 1.00ml and 10.00ml, with cotton plugs at the mouthpiece, kept in another stainless steel container. Both were sterilized under 160^0C for three hours in hot air oven and kept for auto cooling. Culture media such as Plate Count Agar and Sabouraud Dextrose Agar for the enumeration of bacteria and yeast were prepared according to the instructions prescribed in the Oxoid manual. Then all collected samples were aseptically powdered separately by using Grinder and 1g of each powder was dissolved in 9ml sterile distilled water were labeled as 10^{-1} and mixed well. Then 1ml of mixture was aseptically transferred to the test tube containing 9ml sterile distilled water and mixed well using vortex mixture were labeled as 10^{-2}. Following the same the procedure 10^{-3} and 10^{-4} were prepared.

Enumeration of bacteria and fungi in the samples

Each prepared sample was subjected to evaluate the microbial quality in terms of colony forming unit ml^{-1}. Pour Plate method was used for enumeration of bacteria and spread plate method was used for fungi.1ml of each dilution samples were pippeted out and poured in to labeled Petri dishes. Then approximately 15-20ml of Sterile Plate count agar was poured. Mixed well and kept for solidification. After solidification plates were incubated at 37^0C for 24 hours in Incubator. After incubation period, plates that were containing less than 30 colonies and more than 300 colonies were exempted and remaining plates were subjected to enumeration of bacterial count.

For counting of yeast and mold, 1ml of each dilution series of samples were aseptically poured in to previously prepared sterilized Petri plates and approximately 15-20ml sterilized Sabouraud Dextrose Agar were filled to the plates and mixed well and kept under room temperature for three days. After incubation period, plates that were containing less than 30 colonies and more than 300 colonies were exempted and remaining plates were subjected to enumeration of yeast and mold count.

RESULTS AND DISCUSSION

Viable bacteria count of *Terminalia chebula* samples collected from Gampaha district was $1.8\times10^4 cfug^{-1}$, Colombo district was 6.5×10^4 $cfug^{-1}$ and it was 1.9×10^4 $cfug^{-1}$ in samples collected from Kurunegala district. Value of bacterial count of *Terminalia bellerica* was 2.95×10^5 $cfug^{-1}$, 3.22×10^5 $cfug^{-1}$ and 2.57×10^5 $cfug^{-1}$ respectively. Bacterial count of *Phyllanthus emblica* samples collected from Gamapaha , Colombo and Kurunegala districts were having 2.11×10^5 $cfug^{-1}$, 2.18×10^5 $cfug^{-1}$ and 3.71×10^5 $cfug^{-1}$

The results of this study revealed that bacterial count of the *Terminalia chebula* (Aralu) was lower than $1x10^5$cfu/g and bacterial count of *Terminalia bellerica* (Bulu) and *Phyllanthus emblica* (Nelli) were higher than the standards which was given in WHO.

Yeast and mold count of *Terminalia chebula* collected from Gampaha district was $4\times10^4 cfug^{-1}$, Colombo district was $2.5\times10^4 cfug^{-1}$ and it was $2\times10^4 cfug^{-1}$ in samples collected from Kurunegala district. Value of yeast and mold count of *Terminalia bellerica* was 4.1×10^4 $cfug^{-1}$, 3.0×10^4 $cfug^{-1}$ and 1.6×10^4 $cfug^{-1}$ respectively. Yeast and mold count of *Phyllanthus emblica* samples collected from Gamapaha , Colombo and Kurunegala districts were having 3.2×10^4 $cfug^{-1}$, 2.8×10^4 $cfug^{-1}$ and 3.1×10^4 $cfug^{-1}$. The results of this study revealed that yeast and mold count of all samples was higher than $1x10^3$cfu/g, which was given in WHO standards (WHO, 2007).

Improper practices of cultivation and collection, inappropriate techniques of harvesting and cleaning, unsuitable transportation, prolonged drying and storage, inadequate hygiene and natural climatic conditions render the raw plant materials prone to infestations and exposed

them to many microbial contaminants (Tatjana *et al*., 2012).High microbial count could be impact on herbal raw materials by deterioration and variation of compositions, appearance, texture, taste, smell and medicinal properties. In 2012, Tatjana *et al*.and Kunle have been emphasized that pathogenic and toxic bacteria and fungi such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species, Escherichia coli, Aspergillus*, *Alternaria* and *Rhizopus* could be present in raw materials that are having high number of microbial count. Therefore, further studies should be carried out to isolate and identify bacteria and fungi that are presence in the raw materials to testify quality, safety and efficacy of the raw materials.

According to the results of study carried out by Rajapandyan *et al* 2013, having large number of microbial count in tested raw materials indicate the need of adaptation to the proper storing management system of herbal raw materials for better standard herbal pharmaceutical industry. Regulating, monitoring and directives for Good Manufacturing Practice (GMP), Good Agricultural Practice (GAP) are essential in cultivation, harvesting and storage of herbal raw materials in terms of upgrading quality, safety and efficacy of herbal products. By adapting to the WHO guide lines and recommendations (WHO, 2003) of storing conditions such as appropriate low temperature, (2-8ºC) for fresh medicinal plant materials, -20ºC for frozen herbal products, the microbial quality could be maintain (Ramady *et al*., 2015). Applying proper drying techniques, selection of suitable material for packaging of raw herbs also helpful to minimize the microbial count (Masand *et al*., 2004).

CONCLUSION

Legislation for application of good cultivation practices, good harvesting practices, safe handling, transportation and proper storage of herbal raw materials have been identified as the major requirements for standardization of herbal raw materials.

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