Original Research Article Microbiology

International Journal of Pharma and Bio Sciences

MICROBIAL QUALITY ASSESSMENT OF SELECTED MARKETED HERBAL MEDICINAL FORMULATIONS

SEEMADEVI S. KADAM*

Satara College of Pharmacy, Satara, Department of Pharmaceutics, Affiliated to Shivaji University, Kolhapur-415004, Maharashtra, India

ABSTRACT

The use of herbal medicine has constantly been an element of human culture, as some plants possess significant remedial properties which can be used to heal diseases in humans and other animals. The widespread utilization of these herbal medicinal products in the healing and management of diseases within communities of Maharashtra, India has made it essential to examine the microbial quality of these products by taking into consideration the values set by regulatory bodies. The aim of present study was to evaluate the microbiological quality of selected marketed herbal medicinal formulations that are collected from the local market at Satara, Maharashtra. This study was therefore designed to evaluate the microbial quality of selected marketed herbal medicinal formulations which were collected from the local market at Satara, Maharashtra (India). Fifteen samples of marketed herbal medicinal formulations were collected from the retail pharmacy of Satara city. The examination of microbial load from all selected samples was carried out according to Indian Pharmacopoeia. Out of fifteen samples; fourteen samples were found to be contaminated with aerobic bacteria. The total aerobic bacterial count was beyond the prescribed limit given in IP whereas, all fifteen samples exhibited fungal contamination beyond acceptable limit *Staphylococcus aureus* was the most frequently isolated bacterium from 12 samples of marketed herbal medicinal formulations. *Escherichia coli* was found to be present followed in 4 samples, *Salmonella typhi* was found to be present in 3 samples, whereas, *Pseudomonas aeruginosa* was found in one sample. The current analysis signified the contamination of select marketed herbal medicinal formulations by microorganisms. Hence, such products can adversely affect health status of consumers as well as stability of the products. Therefore the quality assurance of these products should be thoroughly monitored during production and distribution of herbal drugs as well as Good Manufacturing Practices at Pharmaceutical level are legally required for the manufacturing of herbal medicinal products.

Keywords: ***Microbial contamination Total aerobic bacterial count, marketed herbal medicinal formulations, microbial quality.***

SEEMADEVI S. KADAM*

Satara College of Pharmacy, Satara, Department of Pharmaceutics, Affiliated to Shivaji University, Kolhapur-415004, Maharashtra, India

Corresponding Author

Received on: 21-05-2019
Revised and Accepted on: 19-07-2019
DOI: http://dx.doi.org/10.22376/ijpbs.2019.10.3.b209-213

INTRODUCTION

The utilization of herbal medicines in human health care has increased to a large extent in both developed as well as in developing countries[1].The medicinal plants and their parts employed as such on industrial level for drug formulations are called raw materials[2].The raw materials used in the preparation of medicine have undeviating impact on the efficiency of the drug. The plant parts used in herbal formulations may provide different nutrients required for growth of microorganisms and thus enhance their multiplication. Many factors are involved in deciding quality of herbal drugs like environment, method of collection, cultivation, harvest, post harvest, transportation and storage conditions. This gives rise to the substandard quality of herbal products with little or no therapeutic efficiency. Some researchers have reported fungi from part of plants used in herbal formulations[3,4]. The objective of our study was to detect the microbial load on some selected marketed herbal medicinal formulations and to scrutinize the intensity of contamination as per Indian Pharmacopoeia.

MATERIALS AND METHODS

Collection of marketed herbal medicinal formulations

A total of fifteen (15) samples of marketed herbal medicinal formulations including five (5) powders/Churna, six (6) solutions and four (4) capsules were collected from the local market of Satara, Maharashtra State India for the evaluation of their contamination with total aerobic count and specific microbes.

Determination of bacterial and fungal load

A stock solution of the sample was prepared by adding one gram (1 gm) of the sample into 10 ml of 0.1% sterile peptone water and shaken thoroughly. A ten-fold serial dilution of the sample was made. This was done until 10^{-7} dilution was achieved. 0.1 ml was then pipetted out from the 10^{-3} dilution onto the surface of Petri plates containing 20 ml of a solidified and sterilized Nutrient Agar and Potato Dextrose Agar, and then spread evenly with sterile glass spreader. The Nutrient Agar plates were incubated at 37^{o}C for 48 hours for bacterial count, and Potato Dextrose Agar plates were incubated at 24^{o}C for 72 hours for fungal count. Counting of the colonies was done using the Stuart Digital colony counter [5].

Test for specific microbes using differential and selective media

Staphylococcus aureus

0.5 gm of the sample was added into Nutrient Broth and incubated at 37^{o}C for 24 hours. The sample was then patterned on Vogel-Johnson Agar and incubated at 37^{o}C for twenty-four hours. Colonies showing golden yellow colour or colourless colonies were considered to be of *Staphylococcus* and were subjected to biochemical tests such as catalase as well as slide and tube coagulase for the confirmation of *Staphylococcus aureus*[6].

Escherichia coli

1 gm/1 ml of the pre-treated sample was added to 15 ml of sterilized Nutrient Broth and incubated at 37^{o}C for 48 hours for the purpose of enrichment.

Primary test- 1 ml of enriched culture was added to 5 ml Mac-Conkey's Broth, shaken and incubated at 37^{o}C for 48 hours. If acid and gas production takes place, it indicates the presence of *Escherichia coli.*

Secondary test- 0.5 ml of enriched culture was added to 5 ml peptone water and incubated at 37^{o}C for 24 hours, and then to this tube, Xylene and Kovac's reagent were added. Formation of pink-coloured ring confirmed the presence of *Escherichia coli*[7].

Pseudomonas aeruginosa

1 gm sample was added into 10 ml Nutrient Broth and incubated at 37^{o}C for 24 hours. The diluted enriched sample was streaked onto Cetrimide Agar plate. After the incubation at 37^{o}C for 24 hours, the green colonies were tested for oxidase reaction and sub-cultured into Triple sugar iron medium. Growth of bacteria and the reaction results were observed [8].

Salmonella typhi

Enrichment was done by transferring 1 gm of the sample into 10 ml of Selenite F Broth and incubated at 37^{o}C for 24 hours. The enriched culture after incubation was streaked on duplicate plates of freshly prepared Deoxycholate Citrate Agar, and incubated along with the control plate of Deoxycholate Citrate Agar at 37^{o}C for 24 hours. After incubation, typical black and green colonies were regarded as positive for *Salmonella*. Green colonies were tested for Triple Sugar Iron (TSI) test[9].

RESULTS

Bacterial and fungal count obtained from the selected water-insoluble marketed herbal medicinal preparations are summarized in Table 1. According to Indian Pharmacopoeia (2010), the utmost tolerable count of total viable aerobic bacteria for water-insoluble herbal medicinal formulations is 10^3 CFU/gm[10]. In this respect, out of nine selected water-insoluble marketed herbal medicinal formulations, none of the formulation was found to be within Indian Pharmacopoeial standards. The utmost tolerable count of total viable aerobic bacteria for water-soluble marketed herbal medicinal formulations is 10^2 CFU/ml[10]. In this respect, out of six selected water-soluble marketed herbal medicinal formulations, none of the formulation was found to be within Indian Pharmacopoeial standards. Total aerobic bacterial count was found to be present beyond the maximum permissible limit as per IP in 14/15 (93.33%) samples ranging between 1×10^5-4×10^8.Bacterial and fungal count obtained from the selected water-soluble marketed herbal medicinal preparations are summarized in Table 2. According to Indian Pharmacopoeia (2010), the maximum acceptable count of fungi from water-insoluble herbal medicinal formulations is 10^2 CFU/gm [10]. In this respect, out of six selected water-insoluble marketed herbal medicinal formulations, no any formulation was found to be within Indian Pharmacopoeial standards. The maximum acceptable count of fungi from selected water-soluble marketed herbal medicinal formulations is 10^1 CFU/ml[10]. In this respect, out of six selected water-soluble marketed herbal medicinal formulations, none of the formulation was found to be within Indian Pharmacopoeial

standards. Total fungal count was found to be beyond the maximum permissible limits as per IP in 15/15 (100%) samples ranging between 1X105 and 4.5×10^8. Microbial contaminants isolated from the selected marketed herbal medicinal formulations are summarized in Table 3. *Staphylococcus aureus* was the most often isolated bacterium from 80% (12 samples) of the selected marketed herbal medicinal formulations, while *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* found in 26% (4 samples), 20% (3 samples) and 6.66% (1 sample), respectively. Samples D, H and J were found to be free from four specific microbes i.e. *S.aureus*, *E. coli*, *S.typhi and P.aeruginosa*.

Table I
Total aerobic bacterial and fungal count in selected water-insoluble herbal medicinal formulations

Sample code	Total Aerobic Bacterial Count (CFU/gm)		Total Fungal Count (CFU/gm)	
	Observed values	Maximum acceptable limit as per IP	Observed values	Maximum acceptable limit as per IP
A	1.2×10^6	10^3 CFU/gm	2.1×10^6	10^2 CFU/gm
B	3×10^6		6×10^6	
E	1×10^5		4.5×10^6	
G	1×10^5		4×10^5	
H	0.2×10^5		1×10^5	
I	0.8×10^5		1.3×10^5	
L	3×10^8		9×10^5	
N	2×10^5		6.2×10^6	
O	4×10^8		4.5×10^8	

Table II
Total aerobic bacterial and fungal count in selected water-soluble herbal medicinal formulations

Sample code	Total Aerobic Bacterial Count (CFU/ml)		Total Fungal Count (CFU/ml)	
	Observed values	Maximum acceptable limit as per IP	Observed values	Maximum acceptable limit as per IP
C	-	10^2 CFU/ml	6.1×10^6	10^1 CFU/ml
D	6×10^5		10×10^4	
F	1.1×10^5		2×10^5	
J	2.7×10^5		3×10^5	
K	1.1×10^6		1×10^5	
M	3×10^5		3×10^5	

Table III
Presence or absence of microbial contaminants in selected marketed herbal medicinal formulations

Sample code	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*
A	Present	Present	Present	Present
B	Present	Absent	Absent	Absent
C	Present	Absent	Absent	Absent
D	Absent	Absent	Absent	Absent
E	Present	Present	Absent	Absent
F	Present	Present	Absent	Absent
G	Present	Present	Absent	Absent
H	Absent	Absent	Absent	Absent
I	Present	Absent	Absent	Absent
J	Absent	Absent	Absent	Absent
K	Present	Absent	Absent	Absent
L	Present	Absent	Present	Absent
M	Present	Absent	Absent	Absent
N	Present	Absent	Present	Absent
O	Present	Absent	Absent	Absent

DISCUSSION

India has an immense diversity in medicinal herbal resources. According to WHO survey 70% to 80% of the population in developing countries uses herbal drugs[11]for the management of various diseases and the production of these medicines is escalating. Traditional herbal medicines and their preparations have been broadly used in India as well as overseas since several years[12]. However, there are a few industrial organizations in India that perform quality assessment on herbal drugs. Despite of standardization parameters and quality evaluation, the contribution of India in the International herbal market is not up to the mark[13]. In Traditional Systems of Medicine, the drugs are primarily dispensed as water decoctions of ethanolic extracts, fresh plant parts, juices, or crude powder. Thus, parts of medicinal plant should be genuine and free from microbial contamination. This is the motive for the World Health Organization to lay down specific guidelines for the assessment of the safety, efficacy, and quality of herbal medicines as a requirement for global harmonization[14]. Still, many Ayurvedic industries do not follow Good manufacturing Practices (GMP) and are ISO-certified. Microbial and fungal contamination not only influences the chemical composition, but also reduces the remedial potency of herbal drugs[15]. The principal obstacle that prevents India from becoming a herbal giant is microbial contamination of these herbal drugs. Fungal contamination of herbal raw materials and products is also a serious issue which needs a special attention during manufacturing of these formulations. Plant materials used for medicinal purposes should be stored appropriately and the development of bacteria and fungi should be inhibited[16]. India can be a chief competitor in the global herbal market if the herbal preparations are manufactured according to the regulatory guidelines. In the present investigation, the microbial quality of select marketed herbal medicinal preparations have been assessed. The present results have shown that, all the select marketed herbal medicinal formulations were found to be contaminated with bacteria, fungi, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Salmonella typhi* (*S. typhi*) above the acceptable limits as specified in Indian Pharmacopoeia. *E. coli*, *S.aureus*, *P.aeruginosa*, *S.typhi*, *Shigella* spp. and other Gram positive and Gram negative strains of bacteria have been accounted tocause solemn health hazards[17].The genus, *Escherichia,* was named after the German paediatrician, Theodor Escherich. It consists of facultative anaerobic Gram-negative bacilli, and belongs to the family Enterobacteriaceae. *E. coli* is broadly disseminated anaerobe, residing in the large intestine of humans and warm-blooded animals. Although, the majority of *E. coli* strains live in the colon and do not always cause disease in healthy individuals, a number of pathogenic strains can cause intestinal and extra intestinal diseases, both in healthy as well as immunologically-weak persons[18]. When *E. coli* strains attain certain genetic material, they can turn out to be pathogenic. Gastroenteritis, urinary tract infections and neonatal meningitis can be caused by potent strains of *E. coli*. In some cases, peritonitis, mastitis, Gram-negative pneumonia, septicaemia and haemolytic-uremic syndrome is also caused by virulent strains of *E. coli*. The first portrayal of “micrococci” isolated from furuncles and abscesses was provided by Sir Alexander Ogston and Louis Pasteur in 1880. *Staphylococcus aureus* is derived from the Greek words, ‘Staphyle’ means ‘bunch of grapes’, ‘coccus’ means ‘round-shaped’ and ‘aureus’ means ‘golden’, as most of the colonies on the agar plates show distinguishing orange-yellow colour, signifying the presence of *S. aureus*. *S. aureus* have the aptitude of growing in temperatures ranging between 7°-48.5°C with a possible growth temperature of 30°-37°C. Accordingly, variation in the temperatures during storage of the herbal medicinal products may result in the production of *S. aureus* in them. *S. aureus* can also build up in a broad range of pH ranging 4.2-9.3 with an optimum pH of 7-7.5. Hence, alteration in the pH during manufacturing of the herbal medicinal products may lead to the growth of *S. aureus* in them. *S. aureus* is a flexible pathogen, causing a large number of diseases from localised skin and soft-tissue infections to life-threatening septicaemia. *S. aureus* can also cause bloodstream infections[19].*Salmonella* infection may be a widespread microorganism malady that influences the enteric tract. *Salmonella* usually exists in the animal and human intestines. *Salmonella* infection is not life-threatening; however, if the *Salmonella* infection spreads beyond the intestines, the life-threatening complications may develop. The development of complications can be more hazardous, particularly in infants, older people, young children, pregnant women, transplant recipients and immunologically-weak persons[20].*Pseudomonas* is an enormously adaptable Gram-negative bacterium with a capability of budding in the broad spectrum of environments. *Pseudomonas aeruginosa* can cause urinary tract infections, respiratory tract infections, bacteraemia, dermatitis, bone and joint infections, soft tissue infections, GI infections etc.[21].

CONCLUSION

The marketed herbal medicinal formulations were vastly contaminated with specific micro-organisms and thus, the quality assurance should be systematically imposed and scrutinized in the manufacture and distribution of the herbal preparations from the commencement to the finished products. This suggests that even if the herbal medicines can be used as an effectual means for the treatment of various diseases, methods of preparation and handling should be effectively checked by undertaking the microbial examination to avoid contamination of these herbal medicinal preparations throughout the production process.

FUNDING ACKNOWLEDGEMENT

The author would like to acknowledge the financial support for the study provided by the Shivaji University, Kolhapur, INDIA (Grant Number - SU/C & UD section/UGK/6/453-6) and the Principal and Vice Principal, Satara College of Pharmacy, Satara, are also acknowledged.

CONFLICT OF INTEREST

Conflict of Interest declared none.

REFERENCES

1. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M. Trends in Alternative Medicine Use in the United States, 1990-1997. JAMA. 1998;280(18):1569. DOI: 10.1001/jama.280.18.1569
2. Chopra RN NS. Glossary of Indian medicinal plants, Publication and information directorate,. New Delhi: Council of Scientific And Industrial Research; 1956. 256-7 p.
3. Kedzia B HE. Occurrence of Bacillus spores in crude Drugs. Herb poll. 1983;29(34):287–300.
4. Kumari V, Chourasia HK RA. Aflatoxin contamination in seeds of medicinal value. Curr Sci. 1989;58(9):512–3.
5. Colle JG MR. Test for the identification of bacteria: Practical Medical Microbiology. Churchill Living Stone (Pub) New York. 1996. p 131-51
6. Shukla SK, Stemper ME, Ramaswamy S V, Conradt JM, Reich R, Graviss EA. Molecular Characteristics of Nosocomial and Native American Community-Associated Methicillin-Resistant Staphylococcus aureus Clones from Rural Wisconsin. J Clin Microbiol. 2004;42(8):3752–7. DOI: 10.1128/jcm.42.8.3752-3757.2004
7. Prescott LM, Harley JP K DA. Isolation of pure bacterial cultures from specimens. Microbiology International.. 8th ed. Boston: WCB, McGraw's Hill Company; 1999. 714-96 p.
8. Riewpaiboon A. Quality predictors of traditional drugs produced in Thailand. Mahidol Univ J Pharm Sci. 2001;28:17–27.
9. Lamikanra A. Essential Microbiology for students and practitioner of Pharmacy, Medicine and microbiology.. Lagos: Amkra books; 1999. 406 p.
10. Indian Pharmacopoeia.Controller of Publication, Govt. of India, Ministry of Health and Family Welfare; New Delhi .2010.
11. WHO. Regulatory situation of herbal medicine: A worldwide review. Geneva: World Health Organization; 1998.
12. Kamboj VP. Herbal medicine. Curr Sci. 2000;78(1):35–9.
13. Sharma A, Shanker C, Tyagi LK, Singh M RC. Herbal medicine for market potential in India: an overview. Acad J Plant Sci. 2008;1(2):26–36.
14. WH Organization. Guidelines for the assessment of herbal medicines. Geneva: World Health Organization; 1991.
15. Dubey NK, Kumar A, Singh P SR. Microbial contamination of raw materials: A major reason for the decline of India's share in the global herbal market. Curr Sci. 2008;95(6).
16. Anonymous, the National formulary of Unani India, Govt. of India, Ministry of health and family planning, Part I, 1981.
17. Noor R, Huda N, Rahman F, Bashar T, Munshi SK. Microbial contamination in herbal medicines available in Bangladesh. Bangladesh Med Res Counc Bull . 2014;39(3):124–9. DOI: 10.3329/bmrcb.v39i3.20313
18. Gomes TAT, Elias WP, Scaletsky ICA, Guth BEC, Rodrigues JF, Piazza RMF. Diarrheagenic Escherichia coli. Brazilian J Microbiol. 2016;47:3–30. DOI: 10.1016/j.bjm.2016.10.015
19. Raho GB AB. Escherichia coli and Staphylococcus aureus most common source of infection. Méndez-Vilas A, editor. The Battle against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs; 2015.
20. Salmonella infection. Patient Care & Health Info [Interent]. 2018[cited 2018 Sep 7]. https://www.mayoclinic.org/diseases-conditions/salmonella/symptoms-causes/syc-20355329.
21. Juhas M. Pseudomonas aeruginosa essentials: an update on investigation of essential genes. Microbiology. 2015;161(11):2053–60. DOI: 10.1099/mic.0.000161