

ASSESSMENT OF MICROBIAL QUALITY IN MARKETED HERBAL DRUGS SOLD IN TRICHY CITY

K. Rajapandiyan*, S. Shanthi and S. Vidya

Post Graduate and Research Department of Microbiology,
Srimad Andavan Arts and Science college, Trichy, Tamil Nadu, India.

ABSTRACT

The increase in the consumption of natural drugs has made their use a Public Health problem due to the possibility of access to products without adequate conditions of use. These natural drugs have the potential of contamination with different microorganisms. This is due to raw materials contamination and unhygienic production conditions. In this study, microbiological quality of some marketed herbal drugs from public markets, in the city of Trichy, Tamil Nadu, India was examined. 12 different herbal medicines were evaluated for microbial contamination by USP (United States Pharmacopoeia) microbial limit test for enumeration and identification. Total aerobic count showed that most of the marketed herbal drugs had above WHO bacteriological limit. Isolation and identification of microbes showed that the samples were contaminated with more than one bacterial pathogens such as *S.aureus*, *E.coli*, *P.aeruginosa*, *Shigella* sp and *Salmonella* sp. This study is clinically significant because most of the herbal medicines which are sold in Trichy market for herbal medications that are highly contaminated with potentially pathogenic bacterial flora due to poor quality control and preparation standards.

Keywords: Herbal Medicines, Total Viable count, Bacterial pathogens Enterobacteriaceae.

INTRODUCTION

India is reputed for the extraordinary richness of its flora, which totals more than tens thousands of species of herbal medicines. They are gaining in popularity because of typically low side-effect profiles (Wilt *et al.*, 2000), low cost (Vanderhoof, 2001) and a high level of acceptance by patients and the majority of the population. The plant material used as herbal drugs are organic in nature, it provides nutrition to microorganisms and facilitates the multiplication of microorganism which lead to contamination, deterioration and variation in composition. This give rise to inferior quality of herbal product with no therapeutic efficacy. The quality of herbal drug is also depend on many factors like environment, collection method, cultivation, harvest, post harvest processing, transport and storage practices. If herbal medicines are contamination by microbial agent during any of the production stage it can

lead to deterioration in safety and quality and can also cause health hazards to the consumer's in spite of curing the disease. Majority of information about herbs is usually based on the antimicrobial activity of medicinal plants in India, but there is very less literature regarding microbial contamination of herbal drugs. The main objectives of this research study is to determine the microbial quality of the herbal medicines packaged for human consumption particularly for adults, infants and children which is sold in herbal market, Trichy city, Tamil Nadu, India.

MATERIALS AND METHODS

Samples

The Herbal drug samples were collected from Herbal drug stores in Market at Trichy City, Tamil Nadu, and India. A total of 12 different (10 no in each drugs from different shops) raw herbals drug samples were selected based on the raw drugs

which to be sold more than 50 customers per day. All drugs samples were examined for their bacteriological profiles. Commercial and medicinal names are as follows:

1. Needredam (*Syzygium cumini* linn) 2. Thanner vitan kilanzhu (*Asparagus racemosus*) 3. Jadikai 4. Jadi pathiri (*Myristica fragrans*) 5. Orilai (*Hybanthus enneaspermus*) 6. Omum (*Apium leptophyllum*) 7. Kadukai (*Terminalia chebula*) 8. Arisithipili (*Piper longum* linn) 9. Ammanpacharisi (*Euphorbia hirta*). 10. Thathapoo (*Tirdex procumbens*) 11. Ficus glomerata (Athi). 12. Sivadai (*Operculina turpithumsilva manso*)

All samples had been powdered by the sterile processors in aseptic condition. Before processing

for bacteriological examinations. For Bacteriological determinations, 1g quantities of samples were dissolved in 9 mL of sterile distilled water. Serial dilutions were made and viability assessed using the pour plate method. The plates were incubated at 37°C for 24h. The plate was placed on a colony counter and the number of colony forming units was taken. The microbial content was taken as the mean of duplicate determinations. The media utilized were Nutrient agar (HiMedia, Mumbai), Baird-Parker agar (HiMedia, Mumbai), xylose -lysine desoxycholate agar (HiMedia, Mumbai), MacConkey agar (HiMedia, Mumbai). Then all isolates were identified by using standard biochemical tests (J.G Holt 1994).

RESULTS

Table 1: Bacterial and fungal Total Viable count of marketed herbal drugs

S. No.	Common name	scientfi name	TVC (Range)
1	Needredam	<i>Syzygium cumini</i> linn	7.4X10 ³ - TNTC
2	Thanner vitan kilanzhu	<i>Asparagus racemosus</i>	50X10 ³ - TNTC
3	Jadikai	<i>Myristica fragrans</i>	3X10 ³ - TNTC
4	Jadi pathiri	<i>Cinnamomum tamala</i>	40X10 ³ - 32X10 ⁵
5	Orilai	<i>Hybanthus enneaspermus</i> (Linn.)	3X10 ³ - 56X10 ³
6	Omum	<i>Apium leptophyllum</i>	7X10 ³ - 56X10 ³
7	Kadukai	<i>Terminalia chebula</i>	7X10 ³ - 200X10 ³
8	Arisithipili	<i>Piper longum</i> linn	6X10 ³ - 40X10 ³
9	Amman pacharisi	<i>Euphorbia hirta</i>	4X10 ³ - 280X10 ³
10	Thathapoo	<i>Tirdex procumbens</i>	4X10 ³ - TNTC
11	Athi	<i>Ficus glomerata</i>	TNTC - TNTC
12	Sivadai	<i>Operculina turpithumsilva manso</i>	TNTC - TNTC

Table 2: Profile Indicator microorganisms in marketed herbal drugs

Bacterial pathogens	No of contaminated samples
<i>S.aureus</i>	71
<i>E.coli</i>	48
<i>Salmonella</i>	6
<i>Shigella</i>	16
<i>Pseudomonas sp</i>	31

The microbial level of marketed herbal medicines which are used in this study as depicted in the table1. Microbial counts by plate count method ranged between 3X10³ to TNTC (Too numerous to count). Bacterial viable count was highly observed in Thanner vitan kilanzhu followed by Jadi pathiri, Needredam, Omum, Kadukai, Arisithipili and Amman pacharisi. The presences of indicator organisms in the samples were reported in table.2. On the basis of standard biochemical test five

different bacterial pathogens were identified. *S.aureus* was the predominant bacterial pathogens isolated from marketed herbal drugs followed by *E.coli*, *P.aeuroginosa*, *Shigella* sp and *Salmonella* sp respectively.

DISCUSSION

All the herbal medications used in this study were orally consumed remedies in form of alcoholic spirits, lime, oil, and water-extracted remedies. In

addition, none of the analysed marketed herbal samples had any form of food-based tests carried out by the manufacturers, which probably accounted for the high recovery rates of coliforms. 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. Based on US pharmacopoeia (USP 30), the total aerobic microbial count of herbal medicine was not more than 10^5 cfu/g. Thus, the microbial load of marketed herbal medicine which is analysed in this study were not acceptable limit. These findings demonstrated that raw herbal medicine of natural origin had some initial microbial levels of contaminants which related to the growing and culture conditions of medicinal plants. Similar findings have also been obtained in an earlier study on the microbiological quality of some pharmaceutical raw materials (Westwood, 1971). Coliforms are members of the family Enterobacteriaceae and are the most reliable indicators of faecal contamination, thus the present study revealed their presence is an index of the degree of contamination, which may indicate a possible presence of harmful, disease-causing organisms (APHA 1992; Pelczar et al. 1996; Jay 1997). These bacteria make up approximately 10% of the intestinal microorganisms of humans and other animals. The significance of faecal coliforms is that if these specific bacteria are present, then other harmful microorganisms may also be present, such as *Salmonella*, *Shigella*, *E.coli*, *Pseudomonas* sp and *S.aureus* (Forest 2004; Hester 2004) and many more others.

The identification of the isolated pathogens in this study is in agreement with previous study by Bailey and Scott (1974), Buchanan and Gibbons (1974), Harrigan and McCance (1976), Andrews (1985), Kloos and Schleifer (1975), Varnam and Evans (1991), Mossel *et al.* (1995), Rowe-Taitt *et al.* (2004) and Prescott *et al.* (2005). All the pathogens which was isolated from the marketed herbal drugs samples in this study have been implicated in previous studies on gastroenteritis and other transmissible diseases (CDC 2002, Okeke and Nataro 2001, Ogunshe 2004). *Bacillus cereus* is a bacterium known to cause gastrointestinal infection, which is characterized by diarrhoea (McKillip 2000), while *Staphylococcus aureus* was implicated in gastrointestinal illness by earlier findings of workers such as Sears and Kaper (1996) and Brooks *et al.* (1998). According to Anon (1986),

diarrhoeal episodes of infective aetiology represent around 27% of those reported from *Shigella* species which are among the five most frequently identified pathogens in children with acute diarrhoea or dysentery, leading to a number of serious complications and high mortality rates (Thoerner *et al.*, 2003). 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, particularly with *Salmonella* and *Shigella* species which are the causative agents of harmful enteric diseases. These organisms may survive for extended periods of time in soil and thus, increase the risk of plant contamination. Moreover, in the absence of viable cells, microbial metabolites may be toxic (Beveridge, 1992). Similar results were obtained with the herbal powder samples contaminated by *E. coli* and *Salmonella* species and herbal tablets contaminated by *E. coli* (Bahri Najafi et al., 2001). According to WHO report (2002), *Salmonella* food poisoning is a major problem globally and has increased in incidence in many continents in the last 25 years. *Salmonella* can infect plants cells and successfully evade all the defense mechanisms of plants. It is already known that *Salmonella* can survive for up to 900 days in contaminated soils, which creates a rich source of infection for plant material.

However with this study, the hazard of microbial contamination of marketed herbal which is sold in market to human health has been demonstrated. Taking into consideration the above facts and increased use of herbal drugs in the society along-with poor quality control measures taken by the manufactures and vendors leave a great question mark on the safety of consumers' health. It may be concluded from this study that most herbal medicines sold in herbal market in Trichy are likely to be contaminated with a wide variety of potentially pathogenic bacteria that there is insufficient quality control in their production and distribution. India can come up as the major nation and play the lead role in the production and proliferation of standardized and therapeutically effective herbal medicines. This is possible only if the herbal products are assessed using standard norms and techniques. Thus there exists a strong need of continuous monitoring and quality control of herbal medicines coming to the local Indian market. Moreover the issues raised should be considered by medical and paramedical

practitioners, the government and the entire citizen of the nation.

REFERENCES

1. Andrews WH. A review of culture methods and their relation to rapid methods for the detection of Salmonella in foods. *Food Technology*. 1985. 39:77-82.
2. Anon. Consensus development conference statement: Traveller's diarrhoea. Review of Infectious diseases. 1986. S223-S227.
3. APHA. Standard methods for the examination of water and wastewater, 18th Ed. 1992. Table 9225: I, p. 9-66. American Public Health Association, Washington D.C.
4. Bahri Najafi R, Ghanadi A, Rahimpour E. Microbial control of some Iranian herbal drugs. *Iranian J. Basic Med. Sci.* 2001. 4(Pt 1): 1-6.
5. Bailey WR, Scott EG. Diagnostic microbiology. 1974. The C.V. Mosby Company, Saint Louis, USA.
6. Beveridge EG. Microbial spoilage and preservation of pharmaceutical products. In: Hugo WB, Russel AD (eds.) *Pharmaceutical Microbiology*. 1992. 5th ed. Blackwell Scientific London. pp. 369-390.
7. Brooks GF, Butel JS, Moore SA. *Medical Microbiology*. 1998. 21st edn. Appleton and Lange, Norwalk, CT.
8. Buchanan RE, Gibbons NE. *Bergey's Manual of Determinative Bacteriology*. 1974. 8th edition. Williams and Wilkins Co. Baltimore, USA.
9. CDC. Coliform bacteria and drinking water. 2002. Centers for Disease Control and Prevention, Atlanta. <http://www.bfhd.wa.gov/2002>.
10. Forest J. Fecal Coliforms. 2004. University of Iowa Hygienic Laboratory Manual, Vol. 36, No. 2, p. 4.
11. Harrigan WF, McCance ME. *Laboratory Methods in Food Dairy and Microbiology*. 1976. p. 342. Academic Press, London, New York, San Francisco, USA.
12. Hester K. Fecal Coliforms. 2004. University of Iowa Hygienic Laboratory Manual, Vol. 36, No. 2, pp. 5-6.
13. Jay JM. *Modern food microbiology*. 1997. 3rd Ed. , pp.409-435. CBA Publishers Delhi, India.
14. John Holt G., Krieg N. R., Sneath P. H. A., Staley J. T, and S. T. Williams. *Bergey's Manual of Determinative Microbiology*. 1994. 9th ed. Baltimore: Williams & Wilkins.
15. Kloos WE, Schleifer KH. Isolation and characterization of staphylococci from human skin, II. 1975. *International Journal of Systemic Bacteriology* 25:62-79.
16. McKillip JL. Prevalence and expression of enterotoxins in *Bacillus cereus* and other *Bacillus* spp., a literature review. 2000. *Antonie Leeuwenhoek* 77:393-399.
17. Mossel DA, Jansen JT, Struijk CB. Taking the professional liability for the assurance of the microbiological safety of food and catered meals seriously: preparing for the next millennium by adoption and elaboration of the autonomous total quality strategy, pp 9-29. In: 1995. Proceedings of the 4th International Symposium on Microbiology of Food and Cosmetics in Europe. Ispra, Italy, European Common Market Research Center.
18. Ogunshe AAO. Characterization and selection of *Lactobacillus* species as probiotics for the control of infantile bacterial gastroenteritis. 2004. PhD Thesis, University of Ibadan, Nigeria.
19. Okeke I, Nataro JP. Enteroaggregative *E. coli*. 2001. *Lancet: Infectious Diseases* 1:304-307.
20. Pelczar MJ, Chan ECS, Krieg NR. *Microbiology*. International edition. 1996. Tata McGraw Hill Publishing Company Ltd., New Delhi.
21. Prescott LM, Harley JP, Klein DA. *Microbiology*. 2005. 6th Ed., pp. 501-502. WCB/McGraw-Hill, USA.
22. Rowe-Taitt CR, Shubin YS, Angel R, Ligler FS. Detection of *Salmonella enterica* serovar Typhmuri by using a rapid, array-based immunosensor. 2004. *Applied and Environmental Microbiology* 69:152-158.
23. Sears CL, Kaper JB. Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. 1996. *Microbiological Reviews* 60:167-215
24. Thoerner P, Kingombe CIB, Bogli-Stuber K, Bissig-Choi B, Wassenar TM, Frey J, Jemmi T. 2003. PCR detection of virulence genes in *Yersinia enterocolitica* and

- Yersinia pseudotuberculosis* and investigation of virulence gene distribution. *Applied and Environmental Microbiology* 69:1810-1816.
25. Vanderhoof JA. Probiotics: future directions. 2001. *Annals of Journal of Clinical Nutrition*. 73:1152S-1155S.
 26. Varnam AH, Evans MG. Food borne pathogens an illustrated text. 1991. Wolfe Publishing Ltd. London.
 27. Westwood N. Microbial contamination of some pharmaceutical raw materials. 1971. *Pharm. J.* 207: 99-102.
 28. Wilt TJ, Ishani A, Rutks I, MacDonald R. Phytotherapy for benign prostatic hyperplasia. 2000. *Public Health Nutrition* 3:459-472.