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Assessment of microbial quality of some marketed herbal medicinal formulations

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Abstract

This study was intended to assess the microbial quality of some marketed herbal medicinal formulations collected from Satara, Maharashtra (India). Ten samples i.e. five water-soluble and five water-insoluble marketed herbal medicinal formulations were collected from retail pharmacy of Satara city. The investigation of microbial load was performed according to Indian Pharmacopoeia. Total aerobic bacterial count was found in 9/10 samples. Total fungi count was found in 10/10 samples. *Staphylococcus aureus* was the most frequently isolated bacteria from 6 samples of marketed herbal medicinal formulations, while *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* followed in 5 samples, 4 samples, 3 samples, respectively. The present investigation indicated the contamination of marketed herbal medicinal formulations by microbes. Thus, evaluation techniques for contamination should be pointed out at every manufacturing step to assess the microbial contamination level on crude drug.

Keywords: Contamination, herbal medicines, marketed herbal medicinal formulations, microbial quality

1. Introduction

Nature has sanctified us with an incredibly wealthy botanical asset and a vast number of diverse plants are budding in different parts of the world. These medicinal plants are further processed for a range of formulations of cosmetics, food supplements etc. They are also used as spices and herbs in routine life. These plants are employed for their all-embracing applications, due to their antimicrobial, nutritional, antioxidant and other medicinal properties. Being an origin of herbal medicines (HM), in India HM is recognized as a central element of health care system, chiefly amid rural residents^[1]. In several countries like India, traditional ways of collection, storage and marketing are still used for processing of herbal raw materials. The defective post-harvest knowledge together with moist climatic circumstances makes the raw materials of herbal drugs prone to fungal infections. The fungal contamination has been reported to influence the chemical composition of the raw materials and, thus, reduces the therapeutic potency of the herbal drugs^[2]. The microbial contamination of the raw materials of herbal drugs is a foremost reason of decline of their demand in international herbal market. Medicinal herbs become toxic when they are contaminated with microbes, heavy metals, pesticides, and other harmful chemicals^[3]. The escalating utilization of medicinal herbs has led to public health problems where effectual surveillance has not scrutinized the use, efficacy, toxicity and quality of these products. Additionally, investigations tend to take place only when cases of toxic results are reported^[4]. There is a prevalent misapprehension that consuming herbs and plants is naturally and intrinsically safe. Though recently, news of adverse, even lethal effects linked to the utilization of herbs and herbal formulations has drawn greater attention^[5]. The present work has been carried out to check the microbial load on some selected marketed herbal medicinal formulations and was inspected to test the intensity of contamination as per Indian Pharmacopoeia.

2. Materials and Methods

2.1 Collection of marketed herbal medicinal formulations

A total of ten (10) samples of marketed herbal medicinal formulations, i.e. five (5) water-soluble samples and five (5) water-insoluble samples were collected from the local market of Satara, Maharashtra State, (India) for the assessment of their contamination with specific micro-organisms.

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2.2 Determination of bacterial and fungal load ^[6].

0.1 ml of the pretreated test sample was spread onto the surface of each of two Petri dishes containing 20 ml of solidified and sterilized Soybean Casein Digest Agar plate and Sabouraud Dextrose Agar plate, and then spread evenly with sterile glass spreader. The Soybean Casein Digest Agar plates were incubated at 30°C-35°C for 24-48 hours for bacterial count, and Sabouraud Dextrose Agar plates were incubated at 20°C-25°C for 4-5 days for fungal count and the number of colonies was counted on each plate.

2.3 Test for specific microbes- ^[6].

2.3.1 Test for *Staphylococcus aureus* (*S. aureus*)

10 ml of fluid Soybean Casein Digest broth medium was inoculated containing 1 gm/1 ml of the pretreated sample. The broth medium was mixed and incubated at 35°C-37°C for 24-48 hours. A portion of medium was streaked on the surface of Mannitol Salt Agar and Baird-Parker Agar and incubated at 35°C-37°C for 18-24 hours. After incubation, the plates were examined for colonies having characteristics yellow color colonies with yellow zones and black, shiny colonies surrounded by clear zones of 2-5mm for the media used- Mannitol Salt Agar and Baird-Parker Agar, respectively. After occurrence of growth, the coagulase test was carried out. The representative suspected colonies were transferred from agar surface of Mannitol Salt Agar to individual tubes, each containing 0.5ml of human plasma and incubated in water bath at 37°C, examined tubes at 3hours and subsequently at suitable intervals up to 24hours. The presence of coagulation in any degree indicated the presence of *S. aureus*.

2.3.2 Test for *Pseudomonas aeruginosa* (*P. aeruginosa*)

10 ml of fluid Soybean Casein Digest broth medium was inoculated containing 1 gm/1 ml of the pretreated sample. The broth medium was mixed and incubated at 35°C-37°C for 24-48 hours. A portion of medium was streaked on the surface of Cetrimide Agar medium and incubated at 35°C-37°C for 18-24 hours. After incubation, the plates were examined for colonies having characteristics generally colorless or yellowish colonies. After the development of suspected colonies confirming above characteristics, oxidase test was carried out. The representative suspected colonies from the surface of Cetrimide agar were streaked on the surface of Pseudomonas agar medium and incubated at 33°C-37°C for not less than 3 days. The plates were examined for colonies having characteristics generally greenish. After the growth of suspected colonies, 2-3 drops of a freshly prepared 1% w/v solution of N, N, N', N'-tetramethyl-4-phenylene diamine dihydrochloride was placed on filter paper and smeared with the colony; development of pink color, changing to purple, indicated the presence of *P. aeruginosa*.

2.3.3 Test for *Salmonella typhi* (*S. typhi*)

1 ml of pretreated sample was added to 10 ml of sterile Nutrient broth, shaken, and allowed to stand for 4 hours and shaken again. The broth was incubated at 35°C-37°C for 24

hours. The primary test was performed, by adding 1 ml of enriched culture to each of two tubes containing a) 10 ml of Selenite F broth and b) 10 ml of Tetra Thionate Bile Brilliant Green broth and incubated at 36°C-38°C for 48 hours. Sub-culturing from each of these two cultures was carried out on two of the following agar media: Deoxycholate-Citrate Agar and Xylose-Lysine-Deoxycholate Agar. The plates were incubated at 36°C-38°C for 18-24 hours and observed for typical colonies colorless and opaque colonies with or without black centers, red colonies with or without black centers, respectively. The secondary test was carried out after confirming the colonies on the basis of description. The secondary test was performed, by sub-culturing any one of typical colonies showing the positive characteristics in Triple Sugar Iron Agar, by first inoculating the surface of slope and then making a stab culture with same inoculating needle and at the same time inoculating a tube of Urea broth. The tubes were incubated at 36°C-38°C for 18-24 hours. The formation of acid and gas within the stab culture (with or while not concomitant blackening) and absence of acidity from surface growth in Triple Sugar Iron Agar, along side the absence of a red color in Urea broth, indicated the presence of *Salmonella*.

2.3.4 Test for *Escherichia coli* (*E. coli*)

1 ml of the pretreated sample was added to 15 ml of sterilized Nutrient broth, shaken and incubated at 37°C for 24 hours. The primary test was carried out, by adding 1 ml of enriched culture to a tube containing 5 ml of Mac-Conkey's broth, shaken and incubated at 36°C-38°C for 48 hours. The secondary test was carried out on the tubes showing presence of acid and gas. The secondary test was performed, by adding 0.1 ml of contents of tube containing a) 5 ml of Mac-Conkey's broth and b) 5 ml of peptone water and incubated at 43.5°C-44.5°C for 24 hours and examined tube a) for acid and gas and b) for indole. To test for indole, 0.5 ml of Kovac's reagent was added, shaken well and allowed to stand for 1 min., development of red color in a reagent layer, indicated the presence of indole. The presence of acid and gas and of indole in the secondary test confirmed the presence of *E. coli*.

3. Results

Bacteria and fungi isolated 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 10³ CFU/gm. ^[6] in this respect, out of five selected water-insoluble marketed herbal medicinal formulations, no any 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 10² CFU/ml. ^[6] in this respect, out of five selected water-soluble marketed herbal medicinal formulations, only one formulation was found to be free from aerobic bacteria. The aerobic bacteria was found to be absent in ASKR. Total aerobic bacterial count was found to be present in 9/10 samples ranging between 1×10³-2.08×10⁵.

Table 1: 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	IP standards	Observed values	IP standards
TTF	6.9×10 ⁴	10 ³ CFU/gm	1.2×10 ⁴	10 ² CFU/gm
UST	6.1×10 ⁴		1.2×10 ⁴	
NYM	4.1×10 ⁴		8×10 ³	
KM	7×10 ³		1×10 ⁴	
SPN	8.7×10 ⁴		9×10 ³	

Bacteria and fungi isolated 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 10² CFU/gm. [6] in this respect, out of five 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 10¹ CFU/ml. [6] in this respect, out of six selected water-soluble marketed herbal Medicinal formulations, no any formulation was found to be

within Indian Pharmacopoeial standards. Total fungal count was found to be in 10/10 samples ranging between 2×10³-4.08×10⁵.

Microbial contaminants isolated from the selected marketed herbal medicinal formulations are summarized in Table 3 and Table 4. *Staphylococcus aureus* was the most frequently isolated bacteria from 60% (6 samples) of the selected marketed herbal formulations, while *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* followed in 50% (5 samples), 40% (4 samples) and 30% (3 samples), respectively.

Table 2: Total aerobic bacterial and fungal count in selected water-soluble herbal medicinal formulations

Sample code	Total Aerobic Bacterial Count (CFU/gm)		Total Fungal Count (CFU/gm)	
	Observed values	IP standards	Observed values	IP standards
GRIW	2.08×10 ⁵	10 ² CFU/gm	8×10 ³	10 ¹ CFU/gm
KFL	1×10 ³		2×10 ³	
ASKR	--		4.08×10 ⁵	
ASGR	1×10 ³		6×10 ³	
RNK	5×10 ³		8.9×10 ⁴	

Table 3: Microbial contaminants isolated from the selected water-insoluble marketed herbal formulations

Sample code	Micro-organisms identified
TTF	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>
UST	<i>Staphylococcus aureus</i>
NYM	----
KM	<i>Staphylococcus aureus</i> , <i>Salmonella typhi</i>
SPN	<i>Staphylococcus aureus</i> , <i>Salmonella typhi</i>

Table 4: Microbial contaminants isolated from the selected water-soluble marketed herbal formulations

Sample code	Micro-organisms identified
GRIW	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i>
KFL	<i>Pseudomonas aeruginosa</i>
ASKR	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhi</i>
ASGR	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhi</i>
RNK	<i>Pseudomonas aeruginosa</i>

4. Discussion

In the perspective of India, where many people depend upon the herbal medicines for medication, the evaluation of pathogens in these medicines is immediately required. Presence of pathogens in herbal medicines can lessen or even inactivate the remedial activity of the products. This could be a solemn public health hazard causing mortality. Several lethal infectious outbreaks have been allied with the use of heavily contaminated raw materials of natural origin with pathogens. Based on this support, we attempted to spot and enumerate the pathogenic microorganisms in the samples of herbal formulations randomly collected from local markets. The results of this study illustrated that the microbial load of

the herbal products varied noticeably. The samples were contaminated to varying degrees with bacteria and fungi and pathogenic micro-organisms.

The microbiological quality of finished herbal product directly depends upon the microbial contamination in raw materials. Contamination occurring during manufacturing i.e. through contaminants, insects and animal vectors, products residue left in operators/or personnel, air, packaging materials and lastly during use by consumer or patients.

Every healthful product throughout production incorporates a distinctive set of physical and chemical conditions. Microbial contamination during manufacturing can be controlled by assessing or recognising the condition, which encourage the establishment of multiplication of microorganisms in the product which is being manufactured and to manufacturing environment.

The diverse supply of raw material shows different kinds of contamination. For example, the roots and rhizomes are cultivated beneath the soil and therefore they are usually contaminated with the soil micro-organisms, which may include fungal spores and bacterial spores. The surface/grooves/ furrows on dried fruits or the presence of sugars in fruits may be the source of environmental contamination. Microbial contamination in contemporary herbal raw material may occur simply throughout harvest. This contamination increases from contact with workers and from physical environment like soil, water, air, hands, containers and storage conditions etc. Some herbal raw material throughout washing might retain water or mechanical injury may occur, that contributes to microbial load.

The materials get contaminated with the microorganisms, during various stages of manufacture which are carried in the final formulations. If the manufacturing processes are harsh,

the micro-organisms present in the raw material get destroyed or injured. If injured, they might recover and can proliferate within the product [7,8].

The bulk of human pathogen noticed in the selected marketed herbal formulations was *Staphylococcus aureus*. *Staphylococcus aureus* is a major human pathogen that causes a wide range of clinical infections. It is top most reason for bacteraemia and infective carditis as well as osteoarticular, skin and soft tissue, Pleuro pulmonary, and device-related infections [9]. *Staphylococci* are Gram-positive bacteria, with diameters of 0.5-1.5µm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially colonise the human body. The *staphylococci* are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. The ability of *cocci aureus* to stick to the animate thing matrix and plasma proteins deposited on biomaterials could be a vital think about the pathological process of orthopaedic-device connected infections. *S. aureus* is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immuno-competent people in the community. This micro-organism is found naturally on the skin and within the nasopharynx of the human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening [10]. An estimated 20% to 30% of the human population are long-term carriers of *S. aureus* which may be found as a part of the conventional skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women. *S. aureus* is capable to cause a variety of diseases from minor skin infections like pimples, impetigo, boils, cellulitis, rubor, carbuncles, scalded skin syndrome and abscesses to serious diseases like pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia and sepsis [11].

The second most noticeable organism in the selected marketed herbal formulations was *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* could be a common bacteria, gram-negative expedient infectious agent capable of infecting humans with compromised natural defences inflicting severe pulmonic illness. It is one of the leading pathogen associated with nosocomial infections. The majority of mortalities in immuno-upset patients; fibrocystic disease of pancreas, may be attributed to the progressive decline of respiratory organ operate ensuing from chronic infection by pathogens like *P. aeruginosa* [12].

The next noticeable organism in the selected marketed herbal formulations was *Salmonella typhi*. *Salmonella* are gram-negative, rod-shaped bacilli capable of causing food poisoning, an unconstipated malady in humans. The bacteria live in the gut of infected humans and animals. There are thousands of subtypes of bacterium, but only about 12 that make people ill, usually with gastroenteritis. Signs and symptoms of *Salmonella*-induced intestinal flu include: abdomen cramps, bloody stools, chills, diarrhea, fever, headache, muscle pains, nausea, vomiting, and dizziness. Some people experience joint pain, known as reactive arthritis. It can last for months or years, and it can become chronic arthritis. During pregnancy, complications include dehydration and bacteremia, or bacteria in the blood. This can lead to meningitis. *Salmonella* can also pass to the fetus. The baby might have looseness of the bowels and fever once birth

and a risk of developing infectious disease [13].

The other most organisms found in the selected marketed herbal formulations was *Escherichia coli*. *Escherichia coli* are usual inhabitants of the human intestine. Most strains are harmless, but some strains acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasion factors and become pathogenic. These virulent strains are responsible for diarrheal infections worldwide, as well as neonatal meningitis, septicemia, and urinary tract infections (UTIs) [9].

This study highlighted on the fact that manufacturers should ensure the lowest possible level of micro-organisms in the raw materials, finished dosage forms and the packaging components to maintain appropriate quality, safety and potency of the herbal medicines. Quality has to be built throughout the process, beginning from the selection of propagating materials to the final products reaching to the consumers. Finally, based on the suggestive data previously reported and considering the contamination status as revealed from our study, we recommend that there is an urgent need for constant monitoring and control of the microbiological standards of herbal medicines available in the local markets.

5. Conclusion

The selected marketed herbal medicinal formulations were vastly contaminated with specific micro-organisms (90%), whereas, only 10% abide with the microbial limits as specified in Indian Pharmacopoeia Such medicinal products may assist transmission of infectious diseases in the population and thus present a public health problem. Therefore, it is necessary to widen the government rules to herbal medicinal formulations to make sure that their processing, preparation or manufacture obey the Good Manufacturing Practices, and consequently reduce the jeopardies to consumers and patients.

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