Research Article

Physicochemical property and microbial contamination assessment in marketed herbal preparations

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ABSTRACT

Aim: The study designed to assess the physicochemical properties and bacterial contamination of various herbal medicinal preparations sourced from Indian local market. **Methodology:** Standard procedures were carried out for the evaluation of physicochemical properties and contamination in finished herbal preparations. Various tests were performed to assess physical and chemicals properties such as total solid content, alcohol- and water-soluble extractive values, loss of drying, pH assessment, and moisture level along with microbial contamination. Standard procedures were carried out to assess the pathogenic bacteria in the herbal preparations. **Results:** Different test results such as total solid content, alcohol- and water-soluble extractive values, loss of drying, pH assessment, and moisture level are found to within the limits as per pharmacopeia standards. Further, total yeast count and total aerobic viable count are also found to be near the standard values. No any finished herbal preparation containing pathogenic bacteria. **Conclusion:** Hence, herbal preparation shown all physicochemical properties in the limit values of Indian pharmacopoeias further theses herbal medications in Indian local market area are not likely to be contaminated with potentially pathogenic bacteria (*Escherichia coli*, *Salmonella* Typhi, and *Staphylococcus aureus*). The quality promises of these finished herbal preparations which are according to pharmacopeia standards.

Keywords: Alcohol extractive values, herbal preparations, microbial contamination, pathogenic bacteria

Introduction

The medicinal benefits of herbs have been known for centuries. The application of herbal drugs for the cure of a good number of ailments is now a common occurrence in many communities, most especially among the rural populace.[1] The occurrence of infectious and non-infectious diseases among human population across the world is increasing day by day since many decades. Malaria, measles, and respiratory infections are the examples of infectious diseases.[2] According to the WHO, approximately more than 50% of the populations using herbal medicine for some aspect of primary health care in mainly Asia and Africa.[3]

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Ayurvedic, siddha, unani, and amchi systems of medicine

This system of medicine is indigenous to India. The word "Ayurveda" encompasses of Sanskrit words, like "Ayu" means "Life" and "Veda" means "Knowledge."[4] This system is a preventive and curative measure for the well-being of the humankind. As a medical system, it states that one can obtain knowledge about positive and negative ways of life, blissful, and depressed types of life, as well as, the very nature of life.[5] Siddha System of Medicine is one of the oldest systems of medicine in India. Siddha medicine represents the fundamental nature of Ayurveda (plant extracts), Unani, Acupressure (sensitive points), Reiki (energy field), etc., in the theories of Siddha medicine.[6] Unani System of Medicine is the mixture of current traditional medicinal system in Egypt, Syria, Iran, Iraq, China, India, and various other East countries. According to Unani System of Medicine, management of any disease depends on diagnosis of disease.[7] Amchi or Sowa-Rigpa System of Medicine is an earliest well-renowned traditional medicinal system,

which was accepted in Tibet, Mongolia, Nepal, Bhutan, and Himalayan region of India.[8] Folk medicines may well be framed because the whole development and performance adopted by the common community so as to spot and restore to health. These medicines play an important part in the protection of health and therapeutic diseases in rural or tribal population. Herbs and other remedies were used to prepare the folk medicines based on traditional beliefs. Thousands of herbal plants are used to prepare such medicines in India. There are several bases due to which Indian preferred treatments such as Allopathy, Siddha, Unani, Amchi, and folk medicine. Theses bases are least possibility of adverse effects, cheap (cost effective), easy availability, good acceptability, and curable. In clinical survey, knowing adverse effects of seasoning herbal drugs are not routine and their news is even less frequent.[9,10] Finished product or mixed product to that with chemicals outlined active substances is further, as well as artificial compounds and/or isolated constituents from seasoning materials, does not seem to be thought-about to be herbal.[11]

Herbal preparation and its standardization

Although, flavoring commodities became gradually standard all over the world, one in every of the obstructions in its acceptance is that the lack of normal internal control profile. The quality of flavoring medication, that is, the profile of the constituents within the finished preparation has implications in effectiveness and safety.[12] Many herbal preparations are deteriorating to compete in the international market due to high microbial load, as plant materials are extremely prone to microbial contamination.[13]

Indian herbal preparations scenario

Ethical and conventional preparations and home remedies of Ayurveda, Siddha, and Unani Systems of Medicine are about $1 billion with an inadequate export of $80 million.[14] The turnover of Indian herbal products industry is about Rs. 2300 crores as against the turnover of pharmaceutical industry of Rs. 14,500 crores with a growth rate of 15% per annum.[15]

Aneesh *et al.*, in the year 2009, studied that India ranks third in the class of herbal finished products in worldwide market share.[16] Contamination of herbal preparations is defined as, "the undesired introduction of adulteration of a chemical or microbiological nature, or of foreign substance, into or onto a starting material, intermediate product or finished flavored product throughout production, sampling, packaging or repackaging, storage, or transport."[17] The sources of contamination in the herbal medicinal products are like environmental conditions in which the medicinal plants are grown or collected, drying and processing conditions of the herbal medicinal products. The conditions under which the herbal finished products are stored and transported, unhygienic use of herbal medicinal products by the patients.[18] Different contaminants such as pesticides, toxic metals, and microorganisms may be associated with the herbal preparations. The microorganisms mainly present in the herbal preparations are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhi, *Pseudomonas aeruginosa,* etc.[19] Hence, variation in the pH during manufacturing of the herbal finished products may lead to the augmentation of *S. aureus* in them. *S. aureus* is an adaptable pathogen, causing a great numeral of diseases from localized skin and soft-tissue infections to life-threatening septicemia. *S. aureus* can also cause blood stream infections.[20] Salmonella infection may be a common microorganism problem that affects the gastrointestinal tract. The development of complications can be dodgy, especially in infants, older people, young children, pregnant women, and transplant recipients and immunologically weak people.[21] Pseudomonas is an extremely versatile Gram-negative bacterium with an ability of flourishing in the broad spectrum of environments. *Pseudomonas aeruginosa* can cause urinary tract infections, respiratory tract infections, bacteremia, dermatitis, bone and joint infections, soft-tissue infections, GI infections, etc.[22]

Control of microbial contamination in Herbal preparations

Most of the herbal preparations in the market today had not gone through the drug approval process. Most of the commercially available herbal products do not even comply with the preliminary regulations. The World Health Assembly, the world's highest health policy setting agency, comprised health ministers from 194 member nations – in its resolutions, WHA 31.33 (1978), WHA 40.33 (1987), and WHA 42.43 (1989) have stressed the necessity to confirm the standard using fashionable management techniques and applying appropriate international standards.[23] In India, herbal medicines are ruled by Drugs and Cosmetics Act, 1940 and Drugs and Cosmetics Act rules of 1945. They govern the import, manufacturing, distribution, and sale of drugs and cosmetics.[24] Herbal remedies and medicinal plants to be incorporated in the modern system (allopathic) must follow the regulations of Drug Controller General of India (DCGI).[25] Hence, due to all these reasons, regularity of herbal preparations is crucial to evaluate the superiority of the drug.

Methodology

The study of quantitative and qualitative evaluations of microbes is intended to carry out the in the experimental samples. It consists of various tests for fungi and pathogenic bacteria. During experiment, care must be taken to avoid contamination of samples from outside. When the samples of test include an activity of antimicrobials or if it possesses substances with antimicrobial characteristic, such antimicrobial characteristics have to be removed through filtering, deactivation, dilution, or neutralization or other suitable mediums. The investigations have to be performed for samples made by combining different portion roughly selected from the separate products or ingredients. When the sample is said to be diluted with fluid means, the test has to be carried out rapidly. Focus has to be on the biohazard prevention and efficient quality control.

Sampling of herbal preparations

Various herbal medicine outlets and retail shops are chosen to purchase different herbal preparation as samples for the current study. Different herbal preparations include for the evaluation of microorganism quality such as Bonnisan Drops (Himalaya Herbal Healthcare Limited) [Figure 1], Naunehal Herbal Gripe Water (Hamdard Laboratories,

Figure 1: Bonnisan drop

Figure 2: Naunehal Herbal Gripe Water

Figure 3: Sharbat-e-Faulad

India) [Figure 2], Sharbat-e-Faulad (Hamdard Laboratories, India) [Figure 3], Majun Chobchini (Hakeems Natural Lab) [Figure 4], and Majun Dabidul Ward (Hamdard Laboratories, India) [Figure 5] which were taken to assess the microbial contamination.

Figure 4: Majun Chobchini

Figure 5: Majun Dabidul Ward

Evaluation of physical as well as physiochemical properties of herbal preparations

Physical evaluations were performed and this includes total solid content, alcohol- and water-soluble extractive values, and alcohol content which have been analyzed and estimated based on the Indian Pharmacopoeia method.[26]

Evaluation of pH of herbal preparations

According to the Norris and Ribbons, various herbal preparations were analyzed for pH evaluation, and for this method, Hanna microprocessor machine was used to find out the pH of the herbal products. The sample is diluted into 100 ml of sterilized distilled water in a beaker and thus the sample solution of 10% was produced as a homogenous solution. The pH meter as mentioned above was used to check it.[27]

Evaluation of moisture level

For the evaluation of moisture content in herbal preparation, the halogen moisture analyzer was used. First of all, 1 g of herbal

preparation was taken in a pan and the machine itself automated the entire process. It will analyze the moisture content in sample and after analyzing it gives out reading.[28]

Evaluation of loss on drying

For evaluation of loss of drying, first of all weigh accurately about 1.5 g of the drug in a tared porcelain dish and further dried at 105°C in oven to get constant weight and then weighed again after drying. From the difference in weight, the percentage loss on drying with reference to the air-dried substance was calculated.[29]

Evaluation of extractive value

For evaluation of extractive value in herbal preparations, first of all accurately weighed herbal formulation and macerated with hundred ml of alcohol (95%) for 24 h in an airtight container. The contents were regularly shaken during the first 6 h and then allowed to remain for 18 h. After 24 h, the extracts was filtered and filtrate was evaporated; finally, the extract was dried at 105°C to a constant weight and extractible value was calculated as % (w/w) with reference to air-dried drug.[30]

Evaluation of total solid content

For the evaluation of solid contents, 4 g of herbal preparations were placed in a previously clean, dry, and weighed evaporating dish. After placing the sample in evaporating dish, it was weighed again to confirm the exact weight of the herbal preparation. After proper weighting of the samples, herbal preparations were evaporated by placing the evaporating dish on the hot plate. After evaporation of liquid portion of the preparations, the weight and thus % of the solid contents of herbal formulation left after complete drying was calculated.[31]

Evaluation of number of total aerobic microbes

For the evaluation of total aerobics, first of all, 10 g of the sample were suspended in 100 ml of buffered sodium chloride-peptone solution with pH 7. After mixing with buffer, Polysorbate 80 of 0.1% w/v was added to support the suspension of poorly wettable substances. After mixing with Polysorbate 80, about 15 ml of the liquefied casein soybean digest agar and 1 ml of the preparation were added to two Petri dishes incubated at 30–35° C for 4 days and kept at not more than 45°C. Following this, the Petri dishes were monitored, and colonies count was taken.[32]

Evaluation of yeast and mold (fungus)

For the evaluation of yeast and mold, first take 10 g of the sample which is further suspended in 100 ml of phosphate buffer with pH 7.2. After suspension formation, 1 ml of the prepared mixture was then added to the 15 ml of liquefied potato dextrose agar medium as two partitions in Petri dishes. This was later incubated for 7 days at 25°C. Following this, the dishes were then monitored, and count for total colonies was taken.[33]

Evaluation of *E. coli*

For the evaluation of *E. coli*, 10 g of herbal preparation was taken in sterile capped jar and which is further suspended it in a total of 100 ml of buffered lactose broth through vigorous shaking along with addition of Polysorbate 80 in 0.1% w/v. After this, the solution was transferred into a sterile container that can be capped with a screw and added 50 ml of nutrient broth. After shaking, the mixture was incubation for a total of 1 day at 37°C. Then, the dishes were evaluated for the availability of acid as well as gas according to established protocol.[34]

Evaluation of Salmonella assessment

For the evaluation of Salmonella, 1 g of herbal preparation as sample was suspended in 100 ml of nutrient broth in a sterile screw capped container which is further allowed it untouched for a total of 240 min and after shaking it was incubation at a temperature of 35°C not more than 37°C for a time period of 1 day. From the improved culture, took 1 ml and added it to two cylinders that were already loaded with 10 ml of selenite F stock as well as Tetrathionate Bile-Brilliant Green broth. These were kept under incubation at 36°C to a maximum temperature of 38°C for 2 days. Further sample was cultured in brilliant green agar as well as in bismuth sulfite agar. After this process, these plates were kept under incubation at 37°C for 1 day. These plates were kept under observation for the appearance of pink or black-green colonies.[35]

Evaluation of *S. aureus* assessment

For the evaluation of *S. aureus,* first of all, 10 g of the herbal preparation as sample was suspended in 100 ml of nutrient broth and kept untouched for a period of 240 h and then shaken which is further incubation at 37°C for 1 day. From this, 1 ml was taken to Soybean-Casein Digest Media and was assessed for the growth availability. Part of the medium was then streaked in the plates containing Vogel-Johnson Agar and Mannitol Salt Agar Medium. These Petri plates were also kept under incubation at 37°C for 18 h. Appearances of yellow as well as black colonies identified as *Staphylococcus* and were assessed.[36]

Results

Bonnisan drops

Total solid content in Bonnisan drop was observed it passes as per Indian Pharmacopeia, 2018 (when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by unaided eye), alcohol- and water-soluble extractive values were found to be 0.386, pH assessment was found as 6.78 ± 0.02, total yeast and mold count, the standard value is 103 CFU/gm and observed value is 102 CFU/gm, total aerobic viable count, the standard value is 105 CFU/gm and the observed value is 105 CFU/gm, assessment for *E. coli*, assessment for *S. Typhi*, and assessment for *S. aureus*, from the above conducted study, it was found that the values for all the evaluated parameters were normal or wear found significant compared with the standard values as per Indian Pharmacopoeia [Table 1]. Microbial growth was indicated in Petri dish [Figure 6].

Naunehal herbal gripe water

Naunehal herbal gripe water passes according to Indian Pharmacopeia (2018) for total solid content. Assessment of total solid content was done by visual inspection which further found to be clear and

practically free from particles, alcohol- and water-soluble extractive values were found to be 0.287, pH assessment was found as 6.89 ± 0.02, total yeast and mold count, the standard value is 103 CFU/gm and observed value is 102 CFU/gm, total aerobic viable count, the standard value is 105 CFU/gm and the observed value is 102 CFU/gm, assessment for *E. coli*, assessment for *S. Typhi,* and assessment for *S. aureus*, from the above conducted study, it was found that the values for all the evaluated parameters were normal or wear found significant compared with the standard values as per Indian Pharmacopoeia [Table 2]. Microbial growth was indicated in Petri dish [Figure 7].

Sharbat-e-faulad

Sharbat-e-Faulad passes according to Indian Pharmacopeia (2018) for total solid content. Assessment of total solid content was done by visual inspection which further found to be clear and practically free from particles, alcohol- and water-soluble extractive values were found to be 0.325, pH assessment was found as 6.45 ± 0.03, total yeast and mold count, the standard value is 103 CFU/gm and observed value is 103 CFU/gm, total aerobic viable count, the standard value is 105 CFU/gm and the observed value is 104 CFU/gm, assessment for *E. coli*, assessment for *S. Typhi*, and assessment for *S. aureus*, from the above conducted study, it was found that the values for all the evaluated parameters were normal or wear found significant compared with the standard values as per Indian Pharmacopoeia [Table 3]. Microbial growth was indicated in Petri dish [Figure 8].

Table 1: Microbial analysis of (Bonnisan drop)

Microbial analysis	Standard value	Observed value
Total solid content	As per I.P	Found optimum
Alcohol- and water-soluble extractive values	As per I.P	Found optimum
Loss on drying	As per I.P	Found optimum
pH assessment	As per I.P	Found optimum
Moisture level assessment	As per I.P	Found optimum
Total yeast and mold count	103 CFU/gm	102 CFU/gm
Total aerobic viable count	105 CFU/gm	105 CFU/gm
Assessment for *Escherichia coli*	Not present	Not present
Assessment for *Salmonella* Typhi	Not present	Not present
Test for *Staphylococcus aureus*	Not present	Not present

Table 2: Microbial analysis of (Naunehal Herbal Gripe water)

Microbial analysis	Standard value	Observed value
Total solid content	As per I.P	Found optimum
Alcohol- and water-soluble extractive values	As per I.P	Found optimum
Loss on drying	As per I.P	Found optimum
pH assessment	As per I.P	Found optimum
Moisture level assessment	As per I.P	Found optimum
Total yeast and mold count	103 CFU/gm	102 CFU/gm
Total aerobic viable count	105 CFU/gm	102 CFU/gm
Assessment for *Escherichia coli*	Not present	Not present
Assessment for *Salmonella* Typhi	Not present	Not present
Assessment for *Staphylococcus aureus*	Not present	Not present

Majun chobchini

Alcohol- and water-soluble extractive values for Majun Chobchini was found to be 8.1% w/w, loss on drying observed as 5.95%, pH assessment was found as 5.40 ± 0.03, moisture level assessment was found as 7.54%, total yeast and mold count, the standard value is 103 CFU/gm and observed value is 102 CFU/gm, total aerobic viable count, the standard value is 105 CFU/gm and the observed value is 105 CFU/gm, assessment for *E. coli*, assessment for *S. Typhi*, and assessment for *S. aureus*, from the above conducted study, it was found that the values for all the evaluated parameters were normal or wear found significant compared with the standard values as per Indian Pharmacopoeia [Table 4]. Microbial growth was indicated in Petri dish [Figure 9].

Majun dabidul ward

Alcohol- and water-soluble extractive values for Majun Dabidul Ward was found to be 8.6% w/w, loss on drying observed as 6.34%, pH assessment was found as 6.23 ± 0.02, moisture level assessment was found as 7.23%, total yeast and mold count, the standard value is 103 CFU/gm and observed value is 102 CFU/gm, total aerobic viable count, the standard value is 105 CFU/gm and the observed value is 103 CFU/

Figure 6: Microbial growth analysis of Bonnisan Drop

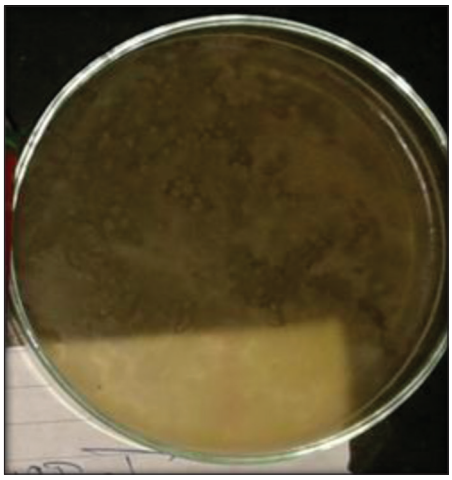

Figure 7: Microbial growth analysis of Naunehal Herbal Gripe Water

gm, assessment for *E. coli*, assessment for *S. Typhi,* and assessment for *S. aureus*, from the above conducted study, it was found that the values for all the evaluated parameters were normal or wear found significant compared with the standard values as per Indian Pharmacopoeia [Table 5]. Microbial growth was indicated in Petri dish [Figure 10].

Discussion

In the powdered herbal preparations, moisture content showed significant difference with other preparations. As per the IP and European agency for the assessment of gerbil finished products, recommended moisture content should be incorporated in the list of comprehensive specifications. Below 8%/gm moisture content is recommended in the herbal finished products according to Food and

Table 3: Microbial analysis of (Sharbat-e-Faulad)

Microbial analysis	Standard value	Observed value
Total solid content	As per I.P	Found optimum
Alcohol- and water-soluble extractive values	As per I.P	Found optimum
Loss on drying	As per I.P	Found optimum
pH assessment	As per I.P	Found optimum
Moisture level assessment	As per I.P	Found optimum
Total yeast and mold count	103 CFU/gm	103 CFU/gm
Total aerobic viable count	105 CFU/gm	104 CFU/gm
Assessment for *Escherichia coli*	Not present	Not present
Assessment for *Salmonella* Typhi	Not present	Not present
Assessment for *Staphylococcus aureus*	Not present	Not present

Table 4: Microbial analysis of (Majun Chobchini)

Microbial analysis	Standard value	Observed value
Total solid content	As per I.P	Found optimum
Alcohol- and water-soluble extractive values	As per I.P	Found optimum
Loss on drying	As per I.P	Found optimum
pH assessment	As per I.P	Found optimum
Moisture level assessment	As per I.P	Found optimum
Total yeast and mold count	103 CFU/gm	102 CFU/gm
Total aerobic viable count	105 CFU/gm	105 CFU/gm
Assessment for *Escherichia coli*	Not present	Not present
Assessment for *Salmonella* Typhi	Not present	Not present
Assessment for *Staphylococcus aureus*	Not present	Not present

Table 5: Microbial analysis of (Majun Dabidul Ward)

Microbial analysis	Standard value	Observed value
Total solid content	As per I.P	Found optimum
Alcohol- and water-soluble extractive values	As per I.P	Found optimum
Loss on drying	As per I.P	Found optimum
pH assessment	As per I.P	Found optimum
Moisture level assessment	As per I.P	Found optimum
Total yeast and mold count	103 CFU/gm	102 CFU/gm
Total aerobic viable count	105 CFU/gm	103 CFU/gm
Assessment for *Escherichia coli*	Not present	Not present
Assessment for *Salmonella* Typhi	Not present	Not present
Assessment for *Staphylococcus aureus*	Not present	Not present

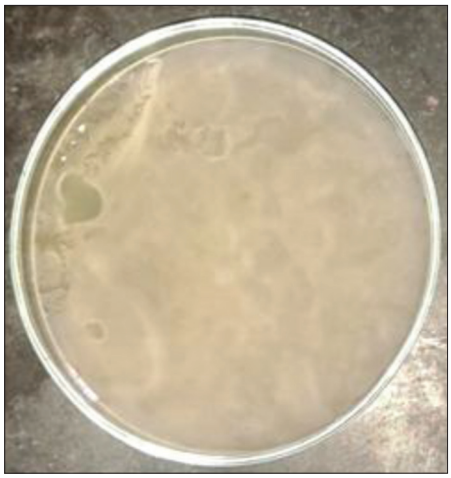

Figure 8: Microbial growth analysis of Sharbat-e-Faulad

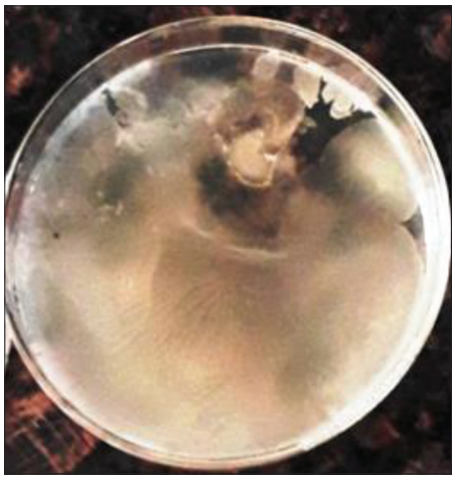

Figure 9: Microbial growth analysis of Majun Chobchini

Figure 10: Microbial growth analysis of Majun Dabidul Ward

Drug Administration.[37] With same line of research, current study samples of herbal preparation products shown moisture content within the limits of standards.

Current study shows that herbal preparations are slightly acidic which is similar to previous studies. It was already shown in the previous studies that the stability and less bacterial contamination were shown in acidic environments whereas neutral and alkaline pH was more liable to instability of product and higher contamination.[38]

According to the previous studies, the limits of bacterial contamination are like total aerobic bacteria (10^5 cfu/g) and Gram-negative organisms (10^3 cfu/g),[39] and with further in our current study, total aerobic and Gram-negative organism were found within the limits of pharmacopoeias. Pathogenic bacteria (*E. coli, S. Typhi*, and *S. aureus*) are absent in the current study.

Conclusion

In the current study, sampled herbal preparation is assessed for a variety of parameter and shown total solid content, alcohol- and water-soluble extractive values, pH assessment, total yeast and mold count, and total aerobic viable count in a permitted level. Further, assessment for pathogenic bacteria such as *E. coli, S. Typhi*, and *S. aureus* shown absence. Finally, all herbal preparations were found high quality when compared with the pharmacopoeia standards.

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