

Evaluation of Microbial Contamination in Herbal Preparation Used in Respiratory Tract Infection

**Dr. Muzaffar Ahmed Farooqui¹, Mohammed Shakir Ghous², Arshiya Zulfen Mohd Fahim³,
Mir. Ashfaq Ali Mir Mushtaq Ali⁴, Shaikh Saniya Mohd Asif⁵, Quraishi Inshrah Fatema⁶**

Aurangabad Pharmacy College, Aurangabad, Maharashtra, India

Corresponding Author: farooqui.muzaffar@gmail.com¹

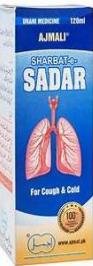
Abstract: *Herbal medicine is used in most part of India to prevent treat and cure for many diseases. In the light of COVID-19 pandemic most of Indians used herbal medicine for treatment of respiratory tract infection. A research method designed to evaluate the physicochemical properties and microbial contamination of herbal preparation used in respiratory tract infection. Several tests have been performed to evaluate physical and chemical properties such as total solids, alcohol and water-soluble extracts, loss on drying, pH and moisture content, and microbial contamination. Standard procedures are in place for the assessment of pathogens in herbal formulations. 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. Hence, herbal preparation shown all physicochemical properties in the limit values of Indian pharmacopoeias further these 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 of these finished medicines promises to meet the standards of the Pharmacopoeia.*

Keywords: Herbal Preparations, Respiratory Tract Infection, Microbial Contamination, Pathogens, Bacteria, Standards Etc

I. INTRODUCTION

The quality and safety of herbal preparations are of great concern because quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance and must therefore be investigated. [1] In developing countries, herbal extracts are recognized as pharmaceutical medicine and are used as complementary medicine and without adequate supervision they are released directly into the market. Hence, high microbial contamination may occur in these products [2,3]. In Nigeria, there appears to be an overwhelming increase in public awareness and usage of herbal medicinal products in the treatment and prevention of diseases. This may not be unconnected to the active mass media advertisements embarked upon by the producers and marketers of the herbal medicinal products who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of the people particularly in the rural areas and the reservations by the public due to prevalence of fake, substandard or counterfeit drugs in the market. These have placed the herbal medicinal products as a ready alternative to conventional dosage forms in the treatment of infections and diseases. With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals [4]

II. HERBAL PREPARATION USED IN RTI'S

Name	Figure	Uses	Reference
Zinda Tilismaat		t is easy to consume since few drops of it can be taken not only with water, but other fluids like tea and coffee as well. Apart from that, rubbing a few drops of the Zinda tilismath oil on affected areas like chest, nose and neck provides a great sigh of relief.	5
Adulsa Syrup		Adulsa Cough Syrup is an Ayurvedic cough syrup which helps in relieving dry and wet cough. It has a unique triple action formula which provides relief from sore throat, Bronchitis and helps in boosting body's natural immunity. It is formulated with ayurvedic ingredients and is non alcoholic and non drowsy in nature.	6
Sharbate sadar		<ul style="list-style-type: none"> • Anti-catarrhal (reduces excess mucous) • Anti-phlegmatic • Whooping Cough, dry Cough • Common cold, sore throat • Respiratory infections • Lung Infection 	7,8

III. MATERIALS AND METHODS

3.1 Materials

All marketed preparation were purchased from arshad unani medicals store, Aurangabad. All other ingredient were used analytical grade.

3.2 Methods

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. [9]

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. [10]

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. [11]

Evaluation of Loss on Drying

For evaluation of loss of drying, first of all weigh accurately about 1.5 g of the drug in 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. [12]

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 extractable value was calculated as % (w/w) with reference to air-dried drug.[13]

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. [14]

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 soluble 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. [15]

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. [16]

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. [17]

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.[18]

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 was assessed.[18]

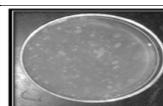
IV. RESULTS AND DISCUSSIONS

All preparation were tested for Total solid content, alcohol and water-soluble extractive values, pH assessment ,total yeast and mold count ,total aerobic viable count, assessment for *S. Typhi* ,assessment for *E. coli* , and assessment for *S. aureus*.[As shown in table 1 and table 2]

Table 1: Microbial analysis in preparation of herbal marketed product

Microbial Analysis	Standatd	Zinda Tilismaat	Adulsa Syrup	Sharbate sadar
Total solid content	As per I.P.	Found optimum	Found optimum	Found optimum
Alcohol- and water-soluble extractive values	As per I.P.	Found optimum	Found optimum	Found optimum
Loss on drying	As per I.P.	Found optimum	Found optimum	Found optimum
pH assessment	As per I.P.	Found optimum	Found optimum	Found optimum
Moisture level assessment	As per I.P.	Found optimum	Found optimum	Found optimum
Total yeast and mold count	103 CFU/g	103 CFU/g	103 CFU/g	103 CFU/g
Total aerobic viable count	105 CFU/g	105 CFU/g	105 CFU/g	105 CFU/g
Assessment for <i>E. coli</i>	Not present	Not present	Not present	Not present
Assessment for <i>S.Typhi</i>	Not present	Not present	Not present	Not present
Assessment for <i>S. aureus</i>	Not present	Not present	Not present	Not present

Table 2: Microbial growth was indicated in Petri dish in herbal marketed product

SR NO	Microbial Contamination in	Yeast and mold Count	<i>Escherichia coli</i>	<i>Salmonella Typhi</i>	<i>Streptococcus aureus</i>
1	Zinda Tilismaat				
2	Adulsa Syrup				
3	Sharbate sadar				

From the above conducted study it was found that the values for all the evaluated parameters were normal or were found significant compared with the standard values as per Indian Pharmacopoeia [Table 1]. Microbial growth was indicated in Petri dish [Table 2]

V. CONCLUSION

Some herbal preparation which used in respiratory tract infection were assessed for various parameters 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 permissible level. Further assessment for pathogenic bacteria such as *E. coli*, *S. Typhi*, and *S. aureus* is shown absence. Hence, herbal preparations 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*). This will help the once who interested in usage of these drugs and for scholar, students and readers.

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