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Analytical study of some marketed herbal oral liquid preparations to rule out microbial limit tests

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Abstract
Ayurveda is a branch of life sciences which deals with study of total human existence. Ayurvedic medications are studied under the branch of Rasashastra and Bhaishajya Kalpana. In Rasashastra formulations are prepared by using minerals, animal products and toxic herbominerals. While in Bhishajya Kalpana preparations are done by using herbs and animal origin products like milk, urine, bones etc. On the basis of external form formulations can be divided in

1. Solid preparations like Tablets, powders etc.
2. Liquid preparations like Syrup, Decoction etc.
3. Semi solid like cream, Avaleha, Lepa etc.
4. Gaseous or fumes like Dhupana, medicated smokes

Keywords: Herbal oral liquid preparations, culture, plate method, microbial limit test

Introduction

Aims and Objectives

1) To check presence and number of aerobic microorganisms in the samples of collected herbal oral liquid preparations.
2) To verify microbial limits tests of given for herbal oral liquid preparations.
3) To check the safety of collected herbal oral liquid preparations.

Need of the study

Raw medicinal plant materials may have great number of bacteria and molds on their surface which may be originated from soil or naturally occurring micro flora of herbs. Current practices of harvesting, handling, and production may add load of contamination of *Escherichia coli* and molds. If such contamination not kept under control, the qualities of medicinal preparations get compromised. Many of Indian ayurveda physicians prepare their formulations in their own clinics or pharmacies. Many times these formulations are not subjected to aseptic conditions as required by modern pharmaceutical industries.

Authentic drugs having potent compositions and their efficient bioactivity are crucial for treatment of any disease. Herbal preparations should meet these standard criteria. There are lot of problems faced by ayurveda pharmacists like improper identification of authentic plants, genetic variability, variable growing conditions, and differences in harvesting procedures, malpractices in storage & processing and lack of knowledge about active pharmacological principles.

As and when all these domains are in proper collaboration potent, safe and authentic preparations can be made for use of mankind. Microbial load is one of the major causes to deteriorate herbal preparations. So present study is carried out.

Materials and Methods

A) Oral herbal liquid preparations: Five oral liquid herbal preparations were taken from market. For privacy policy names of those preparations are kept hidden. These samples are named as sample A, B, C, D and E. These samples were subjected to analytical procedures.

B) Instruments: Hot air oven, pH meter, incubator, refrigerator, laminar air flow, digital colony counter, digital balance.

C) Nutritional media used in microbial limit test:

1. Soyabean casein digest medium: It is used for the cultivation of wide variety of microorganisms. It is recommended for various microbial tests and sterility tests.
   Composition: Casein enzyme hydrolysate, Papaic digest of Soyabean, Sodium chloride, Dipotassium hydrogen phosphate and distilled water.
2. Mannition salt agar: It is used as selective medium for the isolation of pathogenic *Staphylococci.*
   Composition: Mannitiol, Peptone, Sodium chloride, Phenol red, Agar and distilled water.
3. Cetrimide agar: It is used as a selective medium for the isolated *Pseudomonas aeroginosa* from pus or sputum.
   Composition: Pancreatic digest of gelatin, Magnesium chloride, Potassium sulphate, Agar, Cetlytrimethylammonium bromide, Glycerin and distilled water.
4. Brilliant green agar medium: It is used as a medium for selective isolation and identification of *Salmonellae*
   Composition: Peptone, Yeast extract, Lactose, Sucrose, Sodium chloride, Phenol red, Brilliant green, agar and distilled water.
5. Macconkey agar medium: It is used for selection and recovery of the Enterobacteriaceae and related gram negative bacilli.
   Composition: Pancreatic digest of gelatin, Pancreatic digest of Casein, Peptic digest of animal tissue, Lactose, Sodium chloride, Neutral red, Crystal violet and distilled water.
6. Potato dextrose agar medium: It is a general purpose medium used for yeasts and molds.
   Composition: Potato infusion, Agar and Glucose.

Preparation of sample: Depending upon the nature of crude or processed medicinal plants materials, grinding, dissolution, dilution, suspension or emulsification processes are followed for the preparation of the drug sample. In this study five liquid samples were used directly. The samples were prepared by adding 10 ml of the drug sample in 100ml of distilled water, that means dilution factor 1:10. Further dilution was done as per need. Sampling was done in previously sterilized containers.

Method of experiment: Plate count method was used.
Experimental work: In this experiment five drug samples were tested for microbial limit tests to rule out presence and count of Staphylococcus, Streptococci, Salmonella, E.coli, Pseudomonas, Yeast and molds.

Plate method: Following plate method two types of counts were done, total aerobic microbial count and total combined *yeast* and *molds* count.

1. Total aerobic count

- Suitable medium was prepared after autoclaving at 121^0 C and 15 Ibs pressure for 30 mins.
- Petri dishes were used having 9 -10 cm diameter.
- 15 – 20 ml of medium was added for cultivation of bacteria and plates were allowed to solidify.
- Previously prepared and measured volume of drug sample of volume not less than 0.1 ml was spread over the surface of medium.
- Plates were allowed to incubation in incubator at 30 – 35^0C for 48-72 hours.
- Counting done. The number of colonies on medium was counted by using digital colony counter.

Total combined yeasts and molds count

- The procedure for total combined *yeasts* and *molds* was the same as for the aerobic count, except using Soyabean casein digest agar medium, potato dextrose agar medium was used.
- The plates were incubated at 20- 25 ^{0}C for 2 days, the fungi require the low temperature and take around 5 days to grow.

Experimental observations

Table 1: Number of organisms found after culture of testes samples

Sr. no.	Name of the sample	No. of organisms/ ml of the sample					
		Staphylococci	***Streptococci***	***Salmonella***	***E. coli***	***Pseudomonas***	***Yeasts* and *molds***
1	A	00	5600	4000	00	00	5600
2	B	00	3400	2100	1500	1000	4800
3	C	00	3000	2400	00	00	6000
4	D	1600	5400	1600	200	3000	4700
5	E	00	2600	3500	00	00	7000

Formula

No. of organisms/ml = No. of colonies × dilution factor À Volume of the sample

Microbial Limits for the herbal oral preparations given by the WHO

Table 2: Microbial limit for herbal oral preparations

Sr. no.	Name of the limit	Value given by WHO
1	*Streptococci* limit	10^{5} /ml
2	*Staphylococci* limit	None / ml
3	*Pseudomonas* limit	None / ml
4	*Salmonella* limit	None / ml
5	*E.Coli* limit	10 /ml
6	*Yeasts* and *Molds* limit	10^3 / ml

Results

1. Sample A has shown presence of *Salmonella* above prescribed limit.
2. Sample B has shown presence of *Salmonella* and *E.Coli* above prescribed limit.
3. Sample Chas shown presence of *Salmonella* above prescribed limit.
4. Sample D has shown presence of *Streptococci*, *Salmonella*, *E.Coli* and *Pseudomonas* above prescribed limit.
5. Sample E has shown presence of *Salmonella* above prescribed limit.

Conclusion

Herbal oral preparations tested in this study couldn't meet

the standards led down by WHO. Sample D showed maximum numbers of microbes in variety and quantity. Stringent measures for quality control of preparations should be followed. Standard operating procedures should be followed right from cultivation of herbs to storage of final products.

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