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Determination of microbial load in reference to stability study of Churnas (powders)

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ABSTRACT

The aim of broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents[1]. For the present study the trial drugs taken i.e. *Haritaki Churna, Vibhitaki Churna, Amalaki Churna* and *Triphala Churna* retain their potency i.e. no deterioration is observed in terms of physical, chemical and microbiological parameters after one month, two months, four months and six months duration in the Stability chambers - Temperature 45^0C ± 2 & Relative Humidity 75% ± 5 and in ordinary conditions of temperature and humidity. This shelf life period may be applicable to other Churnas having similar method of preparation and constituents having similar range of phyto-chemicals, carbohydrates, cellulose etc.

Key words: Hc. (Haritaki churna) Vc. (Vibhitaki churna) Ac. (Amalaki Churna) Tc. (Triphala churna) Dilution method, Diffusion methods, Mueller Hinton agar (pH 7.2 to 7.4)

INTRODUCTION

It is well known that infectious diseases account for high proportion of health problem, especially in developing countries. Microorganisms have developed resistance to many antibiotics and this have created immense clinical problem in the treatment of infectious disease (Davis-'1994). This resistance has increased due to indiscriminate use of commercial anti microbial drugs commonly used in the treatment of infectious disease. This situation has forced scientists to search for new antimicrobial molecules from various sources, including plants (Karaman et al., 2003). Infection is considered as one of the main factors responsible for diarrhea in children. Secondary metabolites produced by plants constitute source bioactive substances and nowadays the scientific interest has increased for new drugs of plant origin.

Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into a nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate. For broth dilution, often determined in 36-well microtiter plate format, bacteria are inoculated into a liquid growth medium in the presence of different concentrations of an antimicrobial agent. Growth is assessed after incubation for a defined period of time (16–20 hrs.) and the MIC value is read. This protocol applies only to aerobic bacteria and can be completed in 3 days.

The following tests i.e. Dilution method Diffusion method (Stoke's method Kirby – Bauer method) were designed for the estimation of the number of viable aerobic micro-organism present and for detecting the presence of designated

microbial species in pharmaceutical substance. The term "growth" is used to designate the presence and presumed proliferation of viable micro-organism. In the present paper the antimicrobial properties of all single drugs i.e., *Haritaki, Amalaki, Vibhitaki churna* and all these three plant mixed as a *Triphala churna* were carried out on different pathogens which may help in ascertaining the mode of action as well as further specific use of the present combination in future studies.

MATERIALS AND METHODS

The drugs specified in different groups according to the need of analysis for authentication of the data generated.

The trial drugs are divided into two batches, one batch-A samples are kept in ordinary conditions of temperature, and humidity and second batch-B is kept in stability chamber under controlled relative humidity (75% ± RH and temperature (45°C± 2°C).and Batch-C are control group. Each batch consisting four groups and each group contains four samples. Other than this one group containing four samples of freshly prepared Haritaki Vibhitaki, Amalaki and Triphala churna are analyzed in all above stated parameters as control group for both the batches, a total of 36 samples are subjected for analysis.

Anti microbial susceptibility testing can be carried out by-

1. Dilution method
2. Diffusion method

Diffusion method is done by two methods

- Stoke's method
- Kirby – Bauer method

In routine laboratory modified Kirby-Bauer method is used as suggested by NCCLS (national committee for clinical laboratory services), USA (2000).

Material required for antimicrobial study and MIC determination are:[2]

1. Mueller Hinton agar (pH 7.2 to 7.4)[3]
2. Luria Bertani broth (pH 7.2)
3. Sterile distilled water
4. Bacterial stain
   - *Escherichia coli ATCC 25992*
   - *Staphylococcus aurens*
   - Salmonella Typhi
   - *Salmonella* Paratyphi A.
   - *Salmonella* Paratyphi B
   - *Salmonella* Typhimurium

Procedures

Taken a Mullar Hinton (M.H) plate and growth on the plate with particular organism

- Pure bacterial isolates were grown on

Table 1: Permissible Limits of Microbial load & Pathogens[5]

Microbial load	Permissible Limit As per WHO		
	For in the crude plant materials (cfu per gram)	Contamination For plant materials that have been pretreated (for topical use) (cfu per gram)	For other plant material for internal use (cfu per gram)
Total viable Aerobic Count	-	$<10^7$	$<10^5$
Escherichia., coli	10^4	10^2	10g
Total Yeast & mould count	10^5	10^4	10^3
Total Enterobacteriaceae	-	10^4	10^3
Salmonella spp.	-	None	None
Staphylococcus aureus	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Coliforms	Absent	Absent	Absent

Table 2: Showing Analytical Specifications of Samples of Control Group Batch- C and Batch - A (Samples in ordinary conditions of Temperature and humidity)

S. No.	Name of Taste	Name of Batch																			
		Batch-C				Batch-B															
						Group I				Group II				Group III				Group IV			
		Hc	Vc	Ac	Tc	H1	V1	A1	T1	H2	V2	A2	T2	H3	V3	A 3	T3	H4	V4	A4	T4
A.	Microbial load																				
	Total viable	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	aerobic countTotal	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
B.	*Enterobacteriaceae*	Nil	Nil	Nil	Nil	Nil	Ni	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Total fungal count																				
	Test for specific pathogens																				
	Escherichia coli		Absent				Absent				Absent				Absent				Absent		
	Salmonella sp.		Absent				Absent				Absent				Absent				Absent		
	Staphylococcus aureus		Absent				Absent				Absent				Absent				Absent		

Table 3: Showing, Analytical Specifications of Samples of Control Group Batch - C and Batch - B (Stability chambers - Temperature 450C ± 2 & Relative Humidity 75% ± 5).

S. No.	Name of Taste	Name of Batch																			
		Batch-C				Batch-B															
						Group I				Group II				Group III				Group IV			
		Hc	Vc	Ac	Tc	H1	V1	A1	T1	H2	V2	A2	T2	H3	V3	A 3	T3	H4	V4	A4	T4
A.	Microbial load																				
	Total viable	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	aerobic countTotal	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
B.	*Enterobacteriaceae*	Nil	Nil	Nil	Nil	Nil	Ni	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Total fungal count																				
	Test for specific pathogens																				
	Escherichia coli		Absent				Absent				Absent				Absent				Absent		
	Salmonella sp.		Absent				Absent				Absent				Absent				Absent		
	Staphylococcus aureus		Absent				Absent				Absent				Absent				Absent		

nutrient agar plate.

´ Bacterial suspension of 106 cfu / µl was plated spread on the dry plate of M.H.agar by cotton swab

´ Sample preparations in the volume of 10 ml were placed on the plate.

´ Then incubated for 18hrs 37^{0}C.

Observation

´ On the day of manufacturing of *Haritaki*, *Vibhitaki*, *Amalaki* and *Triphala churna* the bacterial load was examined and it was found that all the four preparation are free from bacterial growth.

´ All the preparations were divided into control group Batch-C, trial batch-A, and batch-B, and then stored at room temperature and stability chamber respectively.

´ Then all the samples were further tested at the end 1 month, after 2 months, after 4 months, and 6 months.

No bacterial growth is seen in any of the samples examined on the above time duration.

RESULTS AND DISCUSSION

Medicinal plant materials normally have a great number of bacteria and moulds. Often of soil origin while a large number of bacteria and fungi from the naturally occurring micro flora of herbs, aerobic spore forming bacteria frequently predominate. Current practice of harvesting, handling and production often causes additional contamination and microbial growth. The determination of *E. coli* and mould may indicate good production and harvesting practices. Specific pathogen (*E . coli, Salmonella spp., S. aureus*), total viable aerobic count, total *Enterobacteriaceae* and total fungal count were not seen in all batches of churna up to six months.

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