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MICROBIOLOGICAL QUALITY ASSESMENT OF SHATAVARI CHURNA

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ABSTRACT

Traditional medicines derived from medicinal plants are used by more than 60% of the world's population. Even though several differences exist between herbal and conventional pharmacological treatments, herbal medicines can be tested for efficacy using conventional trial of methodology. Shatavari churna is obtained from roots of *Asparagus racemosus Will*, family Liliaceae, is available in the market in various dosage forms like churna, kalpa, tablets, capsules and jams. Shatavari is consumed by pregnant and lactating women as it helps to maintain healthy levels of breast milk production, it is consumed by pediatrics as it helps boost immunity.[1] Microbial test is important aspect to maintain quality of herbal drugs. This includes analytical and microbiological testing as per pharmacopeia. To maintain potency of natural sources and excipients used in pharmaceutical preparation microbial limit test is performed. The microbial quality assessment of Shatavari churna is performed to check whether it's safety, efficacy and purity is maintained on long term storage. The present study focuses on microbiological evaluation of Shatavari churna.

KEYWORDS: Shatamuli, microbial limit test, Asparagus

INTRODUCTION

Shatavari has an important role in maintaining healthy female reproductive system, healthy levels of breast milk production, supports already balanced hormones in male and female reproductive system, soothing effect on digestive tract, healthy peristalsis of bowels, moisturizing support of respiratory tract, promotes healthy energy levels and strength, supports immune system and possesses natural antioxidant properties.[3] Used internally for

infertility, loss of libido, threatened miscarriage, menopausal problems.[9] It nourishes and cleanses the blood and the female reproductive organs.[6] It is a good food for menopause or for those who have had hysterectomies, as it stimulates many female hormones. It nourishes the ovum and increases fertility.[7] It is used to treat sexual debility, impotence, spermatorrhea and inflammation of sexual organs. Shatavari is also useful for hyperacidity, stomach ulcers, dysentery and bronchial infections.[4] [8]

Shatavari is an herbal drug, is obtained from roots of *Asparagus racemosus Will*, belonging to family Liliaceae. It is widely distributed throughout the tropical region of Africa, Australia and Asia.[5] It is also found in Himalayan ranges upto an altitude of 4000-4500 feet. It occurs as a wildly grown plant in the dry and deciduous forests of Maharashtra state of India.[2]

The present study of microbial assessment was carried out on shatavari churna with 3 formulations 2 of which were marketed formulations and 2 was prepared in -house. One contained the preservatives methyl paraben and propyl paraben in the ratio of 9:1 while the other was not added with it.[1]

Microbial contamination and high concentrations of microbes is the major drawback of herbal formulations. Microbial contamination is caused by organisms like *E.coli, Salmonella species,* yeast and moulds and other enterobacteria. Microbial growth results due to improper processing or drying and storage conditions of drug prior to the formulation and in the process of formulation into the dosage form. Though popular, there is lack of modern quality standards for ayurvedic formulation like shatavari churna. So it is difficult to maintain/ reproduce desired quality standards batch after batch consistently. It is essential to limit the number of such microbes within the values mentioned by the WHO. WHO has defined certain guidelines for the efficacy, purity and safety of the HERBAL DRUGS FOR INTERNAL USE.

The limits are.

a) Yeast and mould- maximum 10^3/gm.
b) *E.coli*- maximum 10/gm.
c) *Salmonella* species- absent.
d) Other enterobacteria- maximum 10^3/gm.

Preservatives methyl paraben and propyl paraben were used since, these are safe and widely used since many years.[15] [16] [17] Natural preservatives cannot be used as they have a typical taste which decreases their patient compliance.

MATERIAL AND METHOD

MATERIAL[13]

a) Marketed preparations (brands):- Dhanvantari and Sharangdhar.
b) Media:- Nutrient broth, Nutrient agar & Sabraud dextrose agar (Primary testing).
c) Selective media: Mc Conkey's Agar, Cetrimide Agar, Vogel Jhonson Agar, Brilliant green Agar (Secondary testing).
d) Preservative (In house preparation of churna) Methyl paraben : Propyl paraben -9:1[3]
e) Sterile petriplate, sterile test tubes, sterile pipettes, cotton.
f) Hot air oven.
g) Autoclave.
h) Desiccator.
i) Incubator.

2.2] METHOD[10] [12]

Shatavari churna of various marketed brands was procured. Namely, Dhanvantari and Sharangdhar. For primary testing, nutrient broth, nutrient agar and sabraud dextrose agar was prepared. These Medias were autoclaved. 1g churna each brand was inoculated in 10 ml nutrient broth. This is called inoculum. Incubate this for 24 hrs. By pour plate technique, using asceptic conditions, add 1 ml inoculum to nutrient agar and 1 ml inoculum to sabraud dextrose agar. Perform this for each of the 2 brands. Incubate the nutrient agar at 37ºC for 24 hrs and sabraud dextrose agar at 25ºC for 24 hrs.

To the study the action of preservative, In house churna was prepared. For the preparation, collection and authentication of shatavari roots from the botanical garden of our college. These roots are then washed and peeled. They were dried at 60ºC-70ºC for 8 hrs for 5 consecutive days. These were then grinded into fine powder. This was preserved in a desicator. The churna was added with preservative methyl paraben and propyl paraben in ratio of 9:1.[1]

WITHOUT PRESERVATIVE IN HOUSE CHURNA

1g churna was added to 10 ml Nutrient Broth. This is called an Inoculum. Incubate the inoculum for 24 hrs. By pour plate technique, using aseptic conditions, add 1 ml inoculum to 10 ml Nutrient Agar and 10 ml Sabraud Dextrose Agar. Incubate Nutrient Agar for 24 hrs at 37^0C and Sabraud Dextrose Agar for 24 hrs at 25ºC. Growth was observed in both the Medias. The growth was inoculated in the specific agar for secondary testing, using streak plate technique. The growth was observed beyond limits in all the Medias.

WITH PRESERVATIVE METHYL PARABEN: PROPYL PARABEN = 9:1.

1g churna was added to 10 ml Nutrient Broth. This is called an Inoculum. Incubate the inoculum for 24 hrs. By pour plate technique, using aseptic conditions, add 1 ml inoculum to 10 ml Nutrient Agar and 10 ml Sabraud Dextrose Agar. Incubate Nutrient Agar for 24 hrs at 37ºC and Sabraud Dextrose Agar for 24 hrs at 25ºC. Growth was observed in both the medias. The growth was inoculated in the specific agar for secondary testing, using streak plate technique. Only the Mc Conkeys Agar showed the presence of colonies. Hence, to confirm, Kovac Indole Reagent, Eosin Methylene blue agar test was performed. No red ring and shiny colonies indicating the presence of *E. coli* was seen. So, absence of *E. coli* was confirmed.

WITH PRESERVATIVE AND NEUTRALIZER (POLYSORBATE 80).

1g churna was added to 10 ml Nutrient Broth. This is called an Inoculum. Incubate the inoculum for 24 hrs. By pour plate technique, using aseptic conditions, add 1 ml inoculum to 10 ml Nutrient Agar and 10 ml Sabraud Dextrose Agar. Incubate Nutrient Agar for 24 hrs at 37^0C and Sabraud Dextrose Agar for 24 hrs at 25ºC. Growth was observed in both the Medias. The growth was inoculated in the specific agar for secondary testing, using streak plate technique. No growth was seen in the specific Medias.

RESULT AND DISCUSSION

PRIMARY TESTING: In primary testing, where pour plate technique was used and inoculum was inoculated on to the nutrient agar and sabraud dextrose agar, the plates showed both bacterial and fungal growth. Hence, secondary testing was performed to confirm the specific organism.

TABLE 1.1 Total viable count in cfu/ml in marketed and in-house formulations.

Growth	Marketed Formulation 1	Marketed Formulation 2	Marketed Formulation 3	Inhouse Formulation 1	Inhouse Formulation 2
Bacterial	1000	1420	1270	1100	00
Fungal	60	50	70	40	00

SECONDARY TESTING: Secondary testing was performed only for the marketed formulations and in-house formulation which was not added with preservatives. All of them showed presence of organisms on enrichment Medias. The one with preservative added did not show the presence of organisms in primary tests so no secondary test was performed.

Standards for the microbial testing in ayurvedic formulations according to The Ayurvedic Pharmacopoeia of India.[18]

Table 2: Standards According To The Ayurvedic Pharmacopoeia Of India.

Sr. No.	Parameters	Permissible limits for herbal extracts and powders
1	*Escherichia coli*	Absent
2	*Staphylococcus aureus*	Absent
3	*Pseudomonas aeroginosa*	Absent
4	*Salmonella shiegella*	Absent
5	Total microbial plate count (TPC)	10^5/g
6	Total Yeast and Mould	10^3/g

Table 3: Observation of Specific Micro Organism in Marketed and In-House Formulation.

Growth	Marketed Formulation 1	Marketed Formulation 2	Marketed Formulation 3	Inhouse Formulation 1
Escherichia coli	P	p	P	P
Staphylococcus aureus	P	P	P	P
Pseudomonas aeroginosa	P	P	P	P
Salmonella shiegella	P	P	P	P

P- Presence of Microbial Growth was seen; A- No growth of micro-organisms was seen.

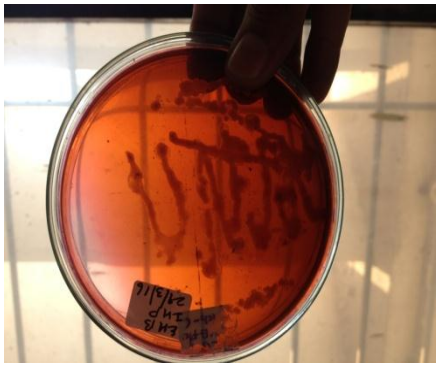

Fig 1:- Growth of organism on EHB.

Fig 2:- Growth of organisms in BGA.

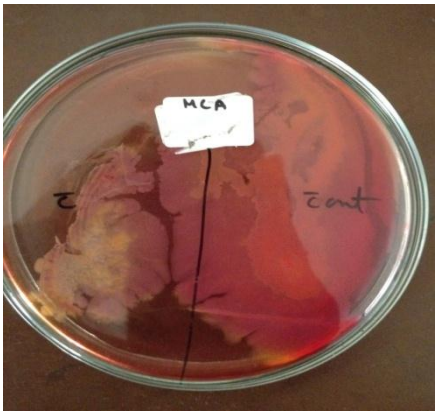

Fig 3:- Growth of organism in MCA.

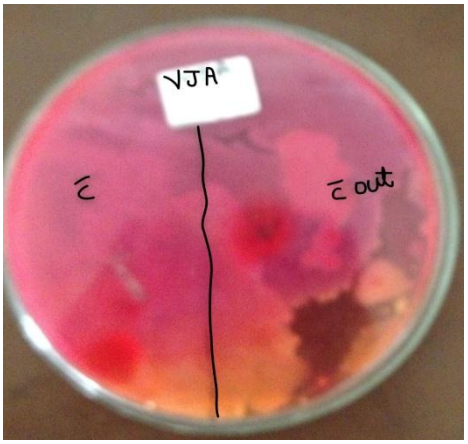

Fig 4:- Growth of organisms in VJA.

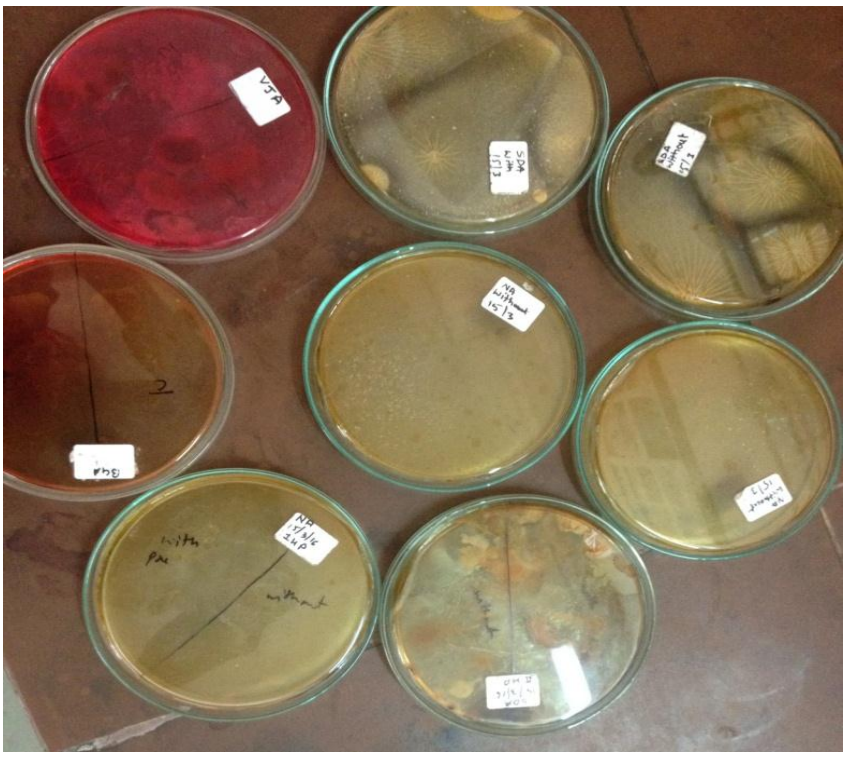

Fig 5: All agar plates for bacterial and fungal growth.

CONCLUSION

According to experiment performed, we see that the marketed churna as well as the churna prepared without preservative showed growth of micro-organisms. This implies that further ahead even if churna is prepared without the preservative will show growth. Hence to store the preparation, addition of preservative is necessary. Ayurvedic formulation are not tested for their viable count, their long term storage goal cannot be achieved. Growth of micro-organism led to deterioration of safety, efficacy and purity of the formulation. Addition of preservative prevents any microbial growth facilitating their long term storage without decreasing the efficacy.

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