

Comparative Microbial Studies for Multiple Brands of Sitopaladi Churna

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Submitted: 01-07-2022

Accepted: 10-07-2022

ABSTRACT: Ayurvedic medicines are gaining popularity all over the world due to phytotherapeutic market. Sitopaladi Churna is used as effective medicine for digestive, respiratory and allergic disorders. Sitopaladi churna contains *Piperum longum* (Piperaceae), *Elettaria cardamom* (Zingiberaceae), *Cinnamomum zeylanicum* (Lauraceae) as the constituents. Since most of the herbal formulations are sold as OTC products they are consumed by paediatrics, geriatrics as well as adults so screening of such products by performing Microbial Limit Test is of necessity. Comparative study was conducted by performing MLT for various brands of Sitopaladi which are Zandu, Baidyanath, Rasashala, Aushadhi and Santulan. These brands were screened for antimicrobial activity against organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Zandu showed the best antimicrobial activity when compared to the rest of the brands.

Keywords: Microbial limit test, Ayurveda, Sitopaladi, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*

I. INTRODUCTION

Disease causing microbes such as *Escherichia coli*, *Salmonella typhi* and *Candida albicans* pose severe health issues like bloody diarrhoea, fever, nausea and vomiting. Ayurvedic formulations have gained popularity to combat such common diseases[1]. Ayurveda is one of the richest and oldest systems of medicine which aims at maintaining health and curing diseases[2]. Herbal Ayurvedic medicines are gaining popularity all over the world due to the growth of the phytotherapeutic market[3].

However, clear cut guidelines for drug standardization of Ayurvedic formulations are not yet established. It hence poses a great challenge in the mainstreaming of Ayurvedic medicine to make it more widely acceptable and used by the masses[1,4]. The need of the hour is hence ensuring

the establishment of quality control parameters for Ayurvedic formulations; specially to tackle issues like adulteration and substitution[3,5]. Additionally, since most of the herbal formulations are sold as OTC products, they are consumed by children, adults as well as the elderly, hence analysis of these products by performing microbial limit test is necessary[6].

In the present study, we aimed to quantitatively estimate the presence of microbes in Sitopaladi Churna, an Ayurvedic product, using microbial limit tests. Sitopaladi Churna is a polyherbal Ayurvedic medicine that contains *Piperum longum* (Piperaceae), *Elettaria cardamom* (Zingiberaceae), *Cinnamomum zeylanicum* (Lauraceae) [1,7]. It is usually prescribed for diseases ranging from cold, cough associated with bronchitis, *topleurodynia* and intercostal neuralgia [7]. It has also been used for relieving gastric disorder [1].

Piperum longum (Piperaceae) contains the ingredient piperine, which possess strong antimicrobial, antioxidant, and antiparasitic activity[8]. A minimum concentration of 12.5 mg/mL of piperine is required for antibacterial activity, and 25.0 mg/ml for antifungal activity[9].

The microbial limit test is a parameter which quantitatively and qualitatively assesses presence of certain viable microorganisms in non-sterile pharmaceutical, healthcare or cosmetic products, or in raw materials[10].

According to AYUSH, the limits as prescribed in ASU Pharmacopoeias i.e., *Staphylococcus aureus* (per gram), *Salmonella* sp. (per gram), *Pseudomonas aeruginosa* (percent) and *Escherichia coli* should be absent. The total microbial plate count (TPC) should be 105 per gram, 107 per gram (for topical use) and the total yeast & mould count should be 103 per gram[11].

II. MATERIALS AND METHOD

Five different brands of Sitopaladi and Triphala Churna were sampled for the study. These brands include Zandu, Baidyanath, Rasashala, Aushadhi and Santulan. For each sample, we performed the microbial limit test (MLT), pertaining to the guidelines as stated in Indian Pharmacopoeia. MLT includes determination of total aerobic microbial count, followed by test for specific organism. Total aerobic microbial count helped us to determine if any mesophilic bacteria or fungi were present in the sample [12].

2.1. Preparation of samples

10gm Churna was weighed from various brands and added to 100ml flask of water individually. 10 gm of Churna from different brands were added to each 100 ml flask of Nutrient broth and were incubated at 35°C for 24 hours.

1 ml of sample from each flask was taken in petriplate followed by addition of 20 ml Nutrient Agar by Pour Plate Technique. These petriplates were incubated at 35°C for 24 hours. This method was performed to obtain viable aerobic bacterial count. The colonies of bacteria were counted as colony forming units per gram of sample by colony counter method.

To obtain the fungal count, 1 ml of sample from each flask was taken in petriplate followed by addition of 20 ml Potato Dextrose Agar by Pour Plate Technique. These plates were incubated at 35°C for 24 hours.

2.2. Detection of specific organism Escherichia coli

Primary test: 1ml of pre-treated sample was added to 5ml of sterile MacConkey Broth in test tube. Inverted Durham's tube was placed in the test tube. This was incubated at 30°C to 35°C for 18 to 24 hours.

If the contents of the tube showed acid and gas (air bubbles in inverted Durham's tube), secondary test following same procedure, but using 0.1ml of pre-treated sample was carried out. If secondary test showed positive result, we proceeded for confirmatory test.

Confirmatory test: 0.1ml of pre-treated sample was added into 5ml of sterile MacConkey Broth in test tube followed by 5ml of peptone water. To detect the presence of indole ring 0.5ml of Kovacs reagent was added later. The presence of acid, gas and indole (red colour) after incubation indicates the presence of Escherichia coli.

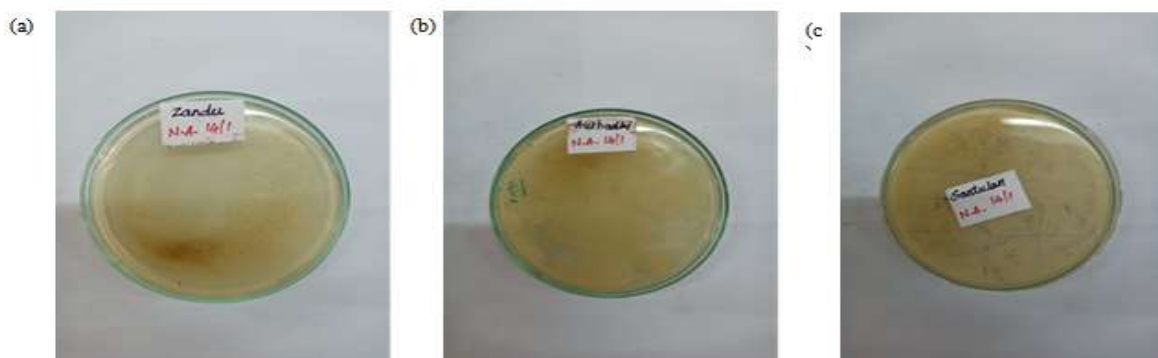
2.3. Detection of specific organism Salmonella typhi

Primary test: 1ml of sample was transferred to sterile Brilliant Green Agar and incubated at 30°C to 35°C for 18 to 24 hours. If small, transparent, pinkish or white colonies were observed, we proceeded for confirmatory test.

Confirmatory test: The 0.1 ml of pre-treated sample was added in Sterile triple Sugar Iron broth using sterile inoculating needle in test tube. If detected positive for glucose and production of hydrogen sulphide (H₂S) indicates the presence of Salmonella typhi.

III. RESULT AND DISCUSSION

All the samples passed the MLT as per IP standards. Matt growth was observed in two, and three samples of Sitopaladi in petri plates of nutrient agar and potato dextrose agar respectively: indicating the presence of microbes above the prescribed limit (Fig. 1, 2) (Table 1). However, on testing for specific organisms, all tests were reported negative, hence all Sitopaladi samples passed the microbial limit test (Fig. 3-6) (Table 2).



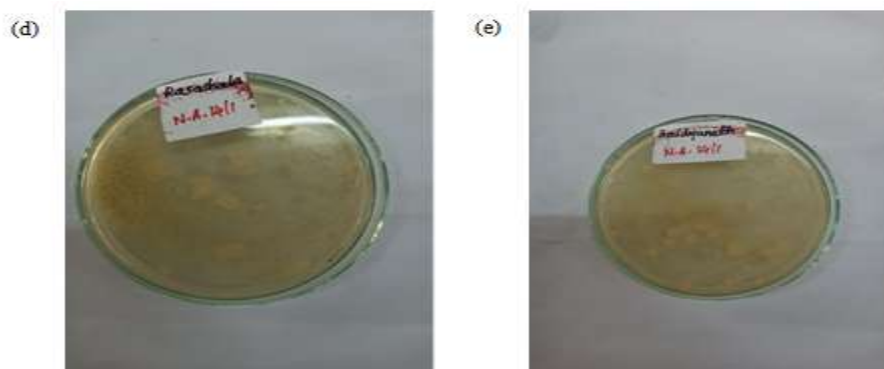


Fig. 1: Growth of organism in nutrient agar petri plate for each brand of Sitopaladi Churna – (a) Zandu, (b) Aushadhi, (c) Santulan, (d) Rasashala, and (e) Baidyanath.

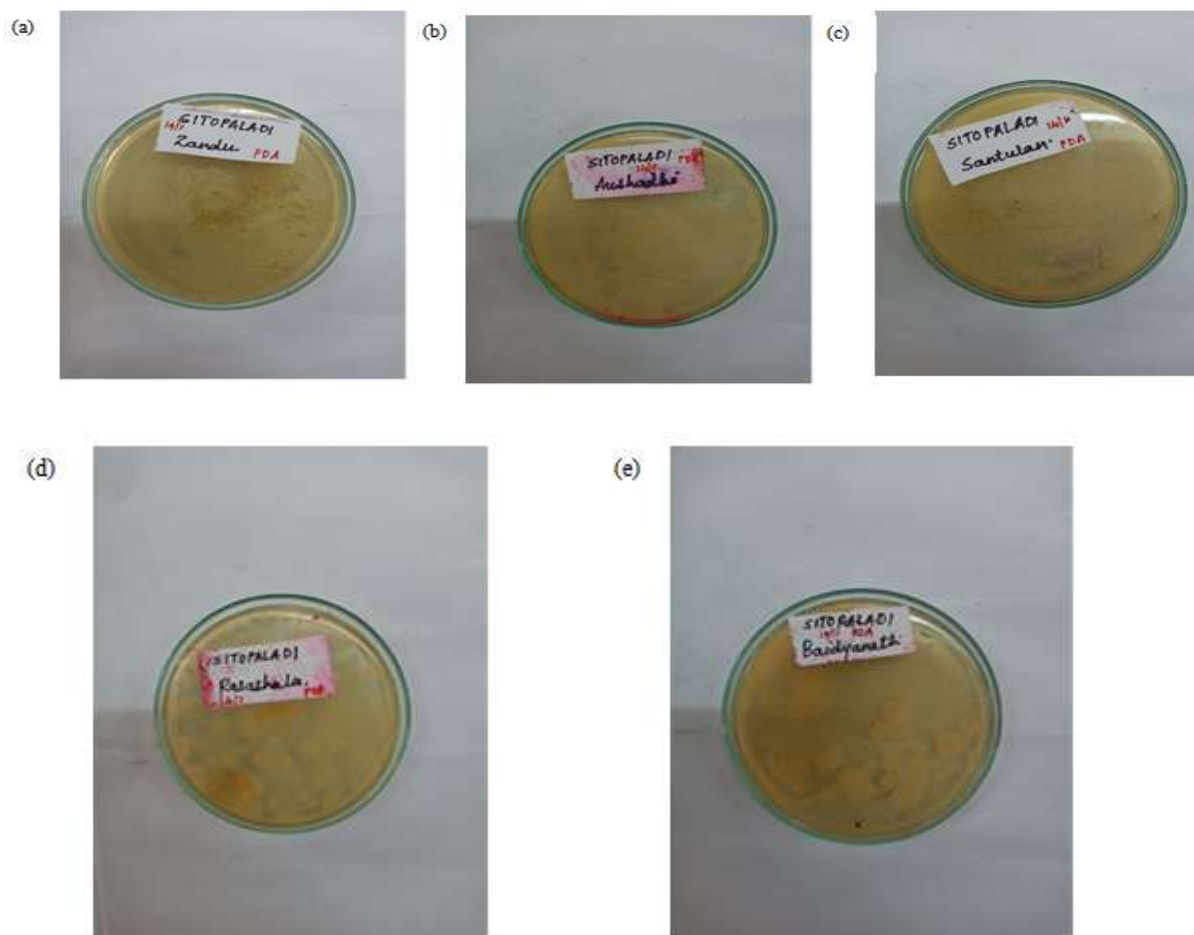


Fig. 2: Growth of organism in potato dextrose agar petri plate for each brand of Sitopaladi Churna – (a) Zandu, (b) Aushadhi, (c) Santulan, (d) Rasashala, and (e) Baidyanath.

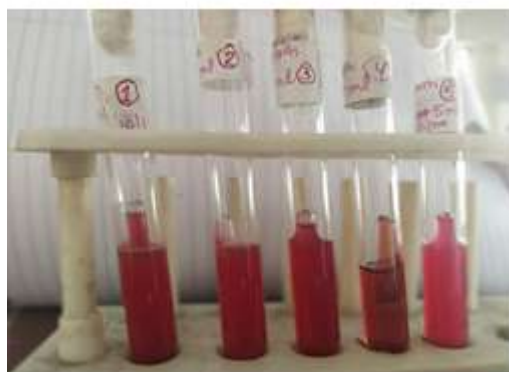


Fig. 3: Primary test for detection of *E. coli* for each brand of Sitopaladi Churna – (from left to right) (1) Zandu, (2) Aushadhi, (3) Santulan, (4) Rasashala, and (5) Baidyanath.

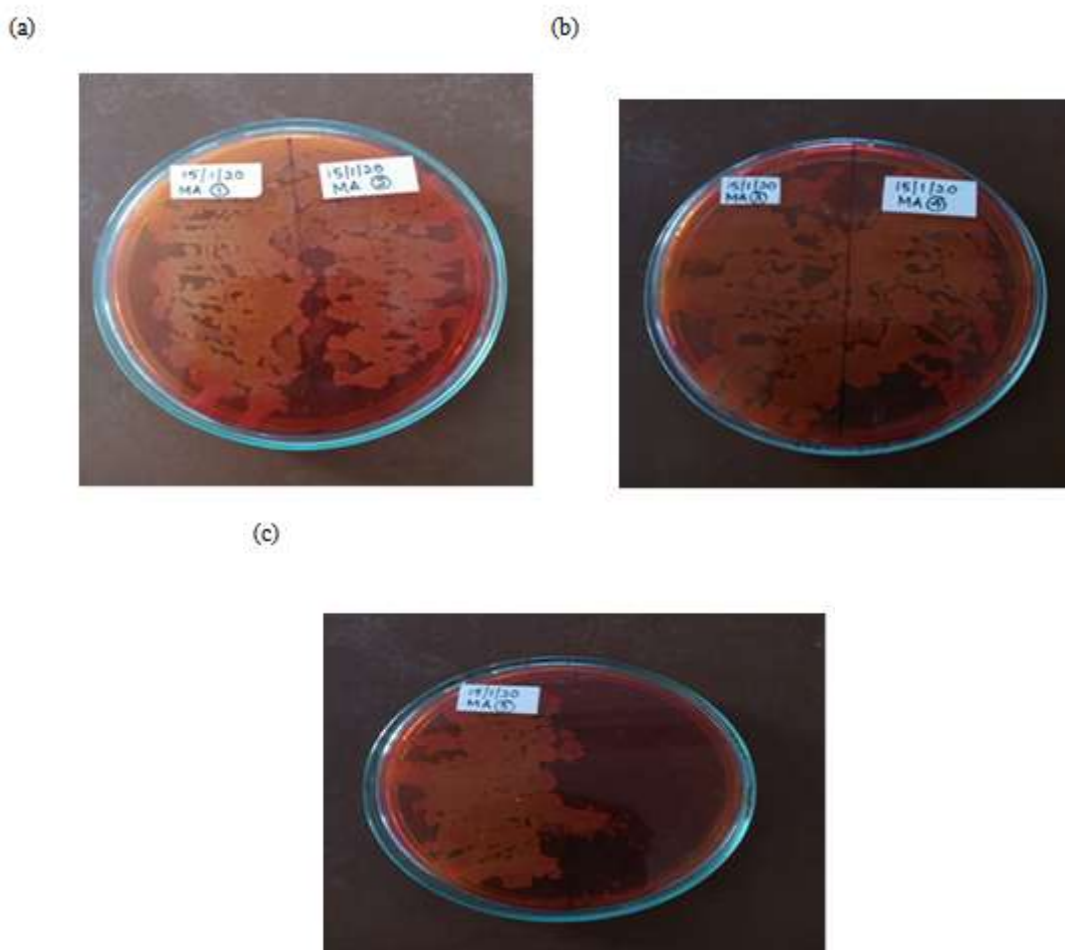


Fig. 4: Confirmatory test for detection of *E. coli* for each brand of Sitopaladi Churna– (a)Zandu(left) and Aushadhi(right), (b) Santulan(left) and Rasashala(right), and (c) Baidyanath.



Fig. 5: Primary test for detection of *Salmonella typhi* for each brand of Sitopaladi Churna – (from right to left) (1) Aushadhi, (2) Rasashala, (3) Santulan, (4) Baidyanath, and (5) Zandu.

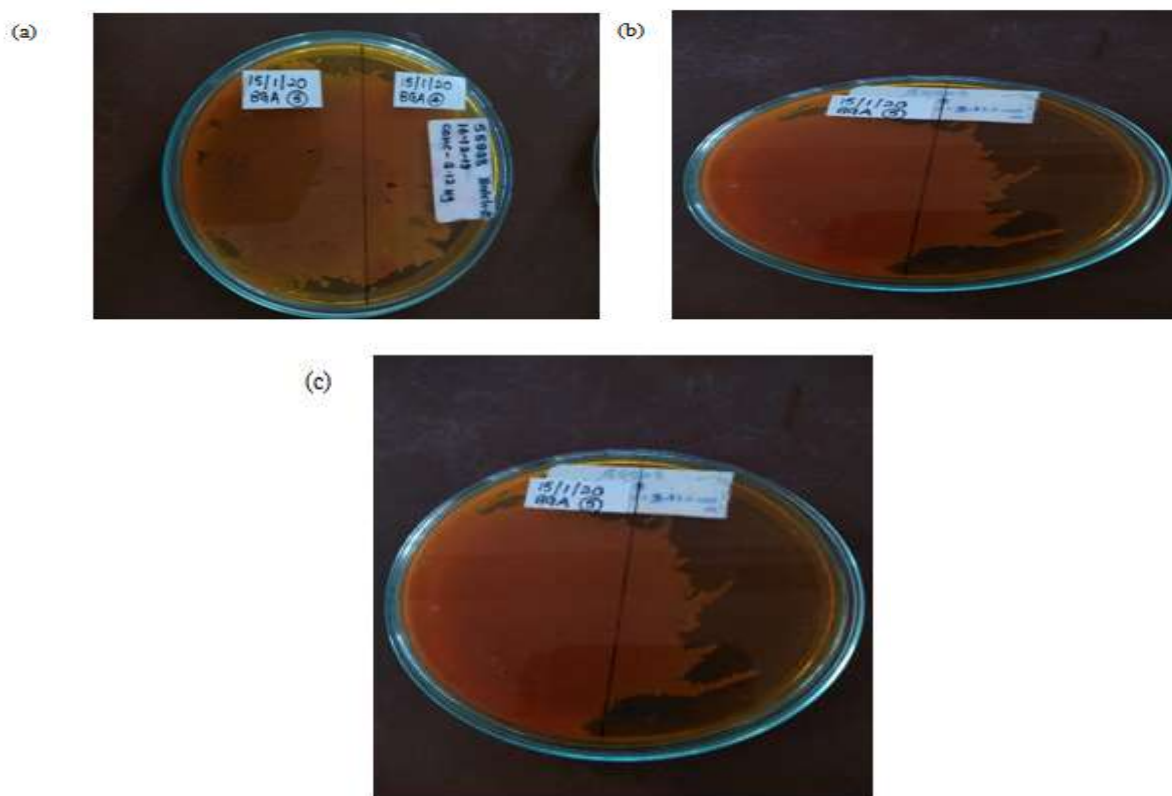


Fig. 6: Confirmatory test for detection of *Salmonella typhi* for each brand of Sitopaladi Churna – (a) Zandu(left) and Aushadhi(right), (b) Santulan(left) and Rasashala(right), and (c) Baidyanath.

Table 1: Observations of total aerobic microbial count of Triphala Churna based on plate count method using a colony counter.

Sr. No.	Brand name	Observations in Nutrient Agar	Observations in Potato
		(NA) 24 hours at 35°C	Dextrose Agar (PDA) 24 hours at 35°C
1.	Zandu	No colony	No colony
2.	Aushadhi	Matt growth	Matt growth
3.	Santulan	Matt growth	Matt growth
4.	Rasashala	16 colonies counted	Matt growth
5.	Baidyanath	46 colonies counted	12 colonies counted

Table 2: Detection of specific organisms.

Organism	Test	Observation	Inference
Escherichia coli	MacConkey Broth + Durham's Tube	Air bubbles in Durham's Tube	May be present
	MacConkey Agar	Colonies visible on Agar plate	May be present
	Indole test	No pink colour ring at the top	Escherichia coli absent
Salmonella typhi	Brilliant Green Agar	No pink colour colonies	May be absent
	Triple sugar iron test	No colour change and no H ₂ S produced	Salmonella typhi absent
Fungi	Potato Dextrose Agar	No coloured colonies	Fungi absent

IV. CONCLUSION:

Out of all the brands for Sitopaladi, Zandu seemed the best one due to the absence of observation of any colonies after incubation of the sample in nutrient agar and potato dextrose agar.

V. ACKNOWLEDGEMENT:

We wish to express our sincere and respectful thanks to our principal Dr Ashwini R. Madgulkar, AISSMS College of Pharmacy for her constant support and valuable suggestions. We are thankful to her for providing the necessary infrastructure and all the facilities required for carrying out the research. She has been very graceful to us from the commencement of the project till its completion. We take the opportunity

to express our gratitude for all teaching and non-teaching staff, and especially the librarian for teaching and also extending a hand of help when we required it the most of AISSMS College of Pharmacy, Pune.

Conflict of interests:

The authors declared no conflicts of interest.

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