



# COMPARATIVE MICROBIAL STUDIES FOR MULTIPLE BRANDS OF TRIPHALA CHURNA

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## Abstract

Ayurvedic Medicines are gaining popularity all over the world due to the phototherapeutics Market. Triphala Churna is used as an effective medicine to Aid in digestion and weight loss, regulating blood sugar levels. Triphala churna contains Amalaki (*Phyllanthus emblica*), Bibhitaki (*Terminalia bellerica*), and Haritaki (*Terminalia chebula*).as the constituents. Since most of the herbal formulations are sold as OTC products they are consumed by paediatrics, geriatrics, and adults so screening of such products by performing a Microbial Limit Test is necessary. Comparative study was conducted by performing MLT for various brands of Triphala which are Baidyanath Dabur, Santulan, Zandu, United Pharma. These brands were screened for antimicrobial activity against organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.Results of Triphala churna indicated that Amla (*Emblca Officinalis*), Harde (*Terminalia chebula*), and Baheda (*Terminalia bellerica*) as are claimed to have antiviral, antifungal, and antibacterial effects. From the brand's Zandu showed the best antimicrobial activity.

**Keywords:** Triphala, Amalaki, Bibhitaki, Haritaki, Microbial Limit Test

## Introduction:

Ayurveda is one of the oldest medical systems, which approaches health and personalized medicine holistically [1]. Triphala is polyherbal Ayurvedic medicine that contains the fruits of three plants (hence called “tri-phala”) [2,3].This herbal concoction consists of the plant species *Emblca officinalis* (*Amalaki*), *Terminalia bellerica* (*Bibhitaki*), and *Terminalia chebula* (*Haritaki*) [2].These constituents have antimicrobial activity, and are shown to affect

the gastrointestinal tract beneficially [4,5]. Additionally, the fruits present in triphala have antioxidant activity, along with radical scavenging abilities [6].

Amla, universally known as 'Indian gooseberry', has high amounts of vitamin-C, and consists of secondary metabolites like emblicanin-A and emblicanin-B, tannins, gallic acid, pyrogallol, and pectin. It is used to manage constipation and fever, as well as to relieve cough and asthma. Its fruit has immune-boosting activity, and is used in various nutraceuticals [7].

Bibhitaki contains glucoside, tannins, gallic acid, ellagic acid, ethylgalate, gallylglucose, chebulanic acid. It has wide therapeutic uses as an antibacterial, anticancer and analgesic agent. It also shows hepatoprotective and immune-modulatory action [8].

Haritaki, also called 'King of Medicines', has several therapeutic benefits. It is used to treat asthma, piles and cough; and is shown to have astringent, stomachic, laxative and purgative activity [9].

Since most of the Ayurvedic medicines are sold as over the counter (OTC) products, and are consumed by people of all ages, there is a need to study the presence of microbes in such products using methods like the microbial limit test [10]. Additionally, such evaluation is necessary due to a lack in regulatory requirements for manufacture and sale of Ayurvedic products [11].

The present study aims to quantitatively estimate the presence of microbes in Triphala churna, based on its individual guidelines. The microbial limit test quantitatively and qualitatively assesses the presence of certain viable microorganisms in non-sterile pharmaceutical, healthcare or cosmetic products, or in raw materials [12].

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 10<sup>5</sup> per gram, 10<sup>7</sup> per gram (for topical use) and the total yeast & mould count should be 10<sup>3</sup> per gram [13].

## Methods :

Five different brands of Sitopaladi were sampled for the study. These brands include Zandu, Baidyanath, Santulan, Dabur and United Pharma. 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].

### Preparation of Sample

10g Churna was weighed from various brands and added to 100ml flask of water individually.

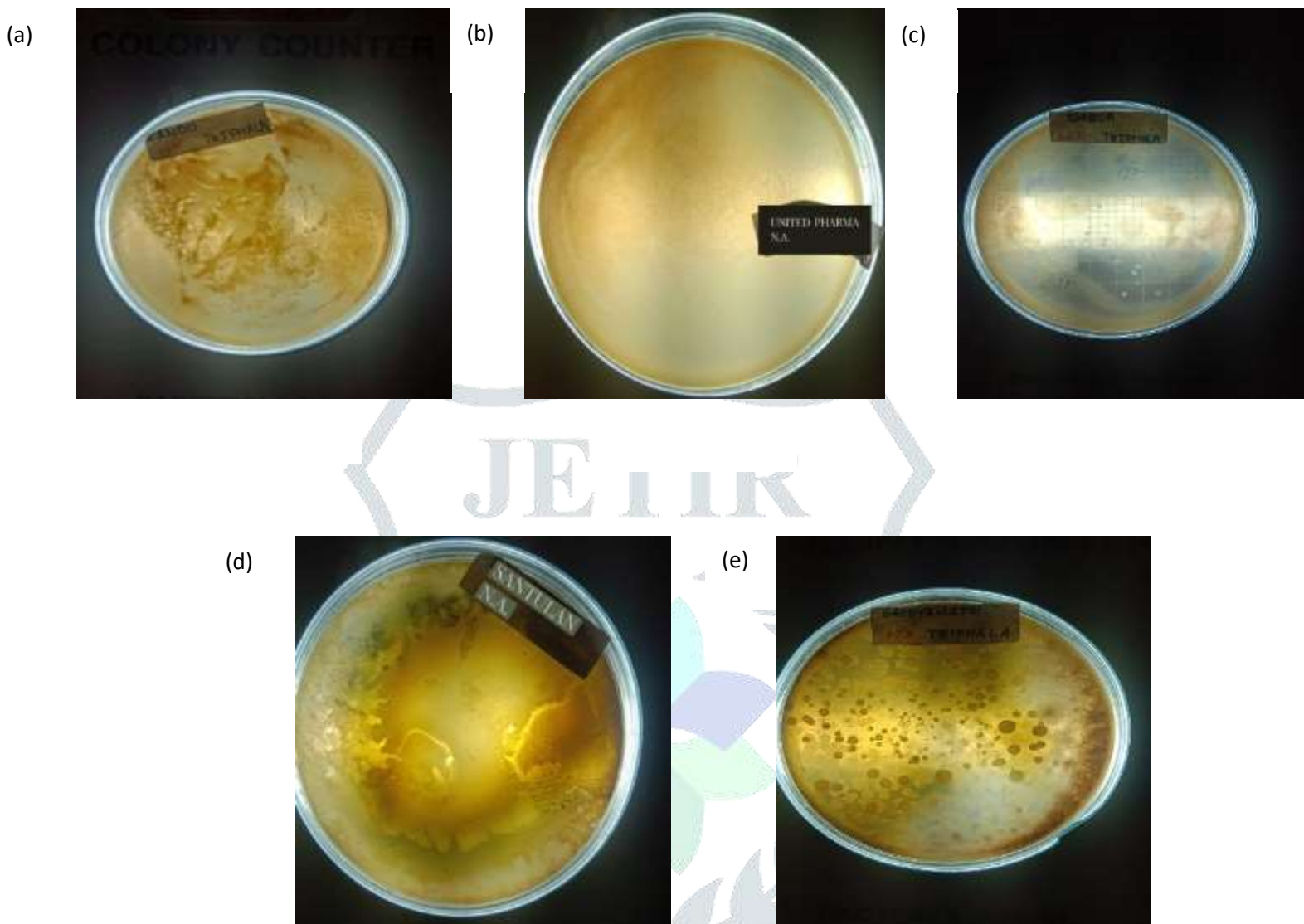
10 gm of Churna from different brands were added to each 100 ml Flask of Nutrient broth and were incubated at 35°C for 24hrs.

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 24hrs. 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.



### Growth of Organism In Nutrient Agar Petriplate



### Growth of Organism In Potato Dextrose Agar Petriplate

(a)



(b)



(c)



(d)



(e)



**Observation**

<b><u>Sr. No.</u></b>	<b><u>Brand name</u></b>	<b><u>Observations in Nutrient Agar (NA)</u></b> 24hrs at 35°C	<b><u>Observations in Potato Dextrose Agar (PDA)</u></b> 24hrs at 35°C
1.	Baidyanath	Matt growth	Matt growth
2.	Dabur	Matt growth	Matt growth
3.	Santulan	Matt growth	Matt growth
4.	Zandu	Matt growth	Matt growth
5.	United Pharma	Matt growth	Matt growth

**Detection of specific organism****Escherichia coli**

0.1ml of pretreated 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 color) after incubation indicated the presence of Escherichia coli.

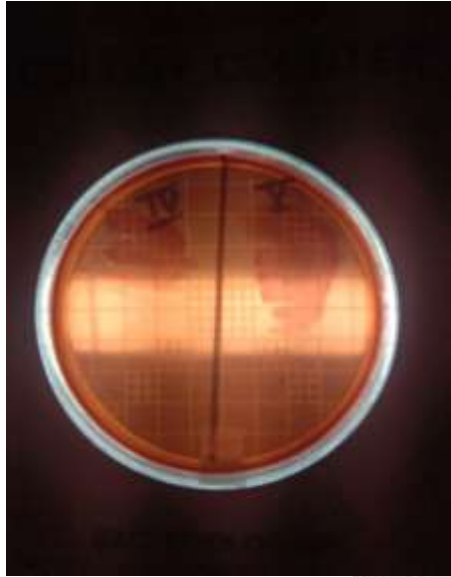
So samples from brands of Sitopaladi Churna like Zandu, Baidyanath, Santulan, Dabur and United Pharma were tested which were found to be Negative for Escherichia coli after Confirmatory Test.

**Confirmatory test:****Mac Conkeys Agar :**

Section 1: TRIPHALA “United Pharma” Section 2: TRIPHALA “Dabur”

Section 3: TRIPHALA “Santulan” Section 4: TRIPHALA “Baidyanath”

Section 5: TRIPHALA “Zandu”



### **Salmonella typhi :**

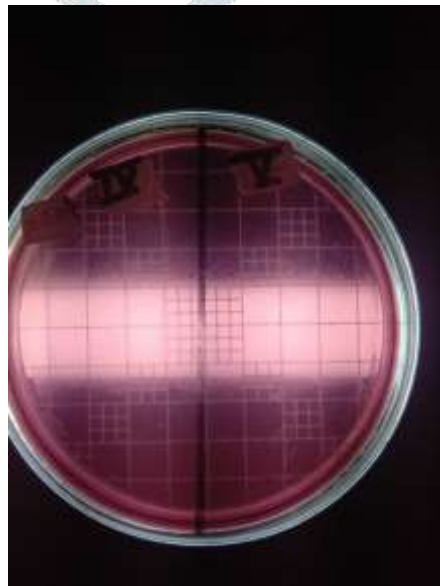
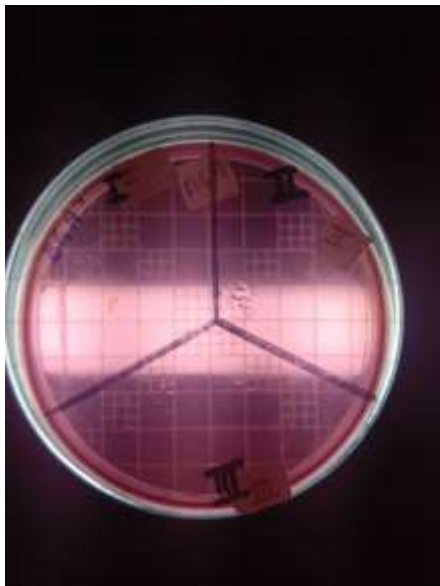
The 0.1 ml of pre-treated sample was added in sterile Triple Sugar Iron Broth using sterile inoculating needle in Test tube. If detected positive For Glucose And Production Of Hydrogen sulphide (H<sub>2</sub>S) indicates the presence of Salmonella typhi.

So samples from brands of Sitopaladi Churna like Zandu, Baidyanath, Santulan, Dabur and United Pharmawere tested which were found to be Negative for Salmonella typhi after Confirmatory Test .

Section 1: TRIPHALA “United Pharma”      Section 2: TRIPHALA “Dabur”

Section 3: TRIPHALA “Santulan”      Section 4: TRIPHALA “Baidyanath”

Section 5: TRIPHALA “Zandu”





Looking at the **Colonies** on the Petri plate of "**Triphala Churna**" belonging to the **Brand: Baidyanath** it was assumed that it might be "**Candida albicans**"



#### **OBSERVATION**

On "**Microscopic view**" it was Observed that it does not resemble shape & structure of "**Candida albicans**".

**RESULT : NEGATIVE**



## Result

All the samples passed the MLT as per IP standards.

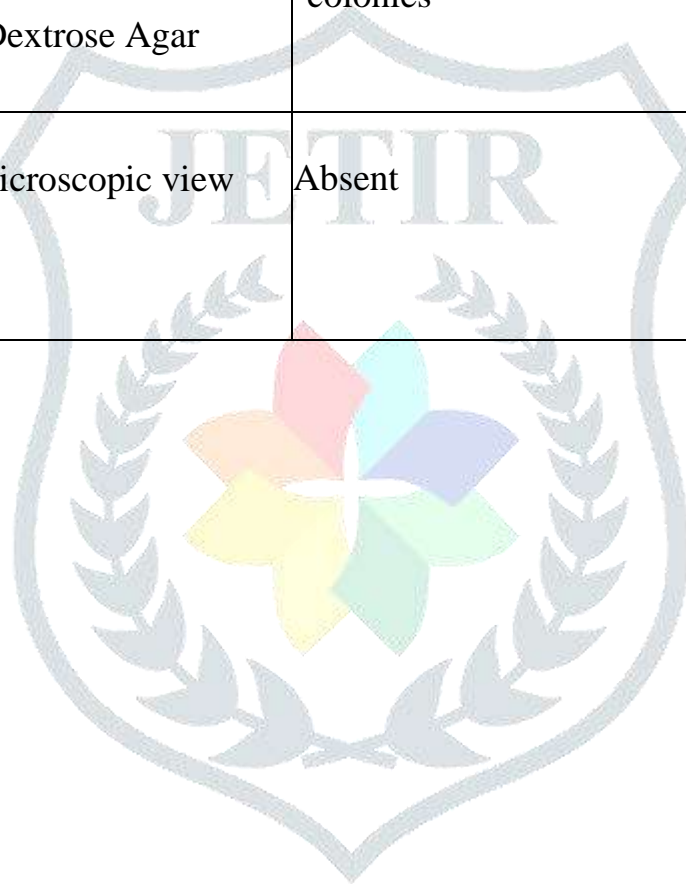
Matt growth was observed in all samples of Triphala in petri plates of NA and PDA, respectively indicating the presence of microbes above the prescribed limit. However, on testing for specific organisms, all tests were reported negative, hence all brands of Triphala samples passed the microbial limit test. As it didn't surpass the microbial limit test mentioned in ASU pharmacopoeias therefore it passes the test.

## Conclusion

Out of all the brands for Triphala, Zandu seemed the best one due to the absence of observation of any colonies after incubation of the sample in NA and PDA.

ORGANISM	TEST	OBSERVATION	RESULT
<i><u>Escherichia coli</u></i>	Mac Conkey Broth + Durham Tube	Air Bubbles in Durhams Tube	May be present
	Mac Conkey Agar	Colonies visible on Agar plate	May be present
	INDOLE TEST	No pink colour ring at the top	<i><u>Escherichiacol</u></i> <u>i</u> absent

<u>Salmonellatyp hi</u>	Brilliant Green Agar	No pink colour colonies	May be absent
	TRIPLE SUGAR IRON TEST	No colour change and no H <sub>2</sub> S produced	<u>Salmonella typhi</u> absent
Fungi	Potato  Dextrose Agar	No coloured colonies	Absent
<u>Candida albicans</u>	Microscopic view	Absent	Absent



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