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COMPREHENSIVE ASSESSMENT OF LONG TERM STABILITY AND MICROBIAL SAFETY STUDIES OF BRAHMARASAYAN

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Abstract:
This study presents a comprehensive assessment of the long-term stability and microbial safety of the Brahamrasayana formulation, a renowned Ayurvedic product with holistic health benefits. The formulation, prepared in 2022 to exacting standards, underwent rigorous stability testing to ensure its quality over time. In 2023, a revisit to the study facilitated a comparative analysis of the formulation's stability with the previous year's findings. Microbiological analyses, including Total Plate Count, Yeast and Mold Count, and assessment of specific microbial strains (Escherichia coli, Salmonella sp., Pseudomonas sp., Streptococcus sp., and Shigella sp.), were conducted. The results of this comparative study offer valuable insights into the formulation's ability to maintain long-term stability and microbial safety. The findings contribute to ongoing quality assurance efforts, guiding formulation improvements and affirming the product's adherence to stringent quality standards. This research is crucial for ensuring the continued efficacy and safety profile of Brahamrasayana, enhancing its reliability as a high-quality Ayurvedic formulation.

Introduction:

Brahmarasayana" is a term that seems to be a combination of two concepts: "Brahma" and "Rasayana." Brahma: In Hinduism, "Brahma" refers to the ultimate, supreme reality or the universal soul. It is often considered the creator deity in the Hindu trimurti, which also includes Vishnu (the preserver) and Shiva (the destroyer). Rasayana: Rasayana is a concept from Ayurveda, an ancient system of medicine that originated in India. Rasayana refers to a branch of Ayurveda that deals with rejuvenation, revitalization, and promoting longevity. It involves various therapies, dietary recommendations, and herbal preparations aimed at improving overall health and well-being.

Materials and methods:

Sample: Brahamrasayana Formulation
Media: The media Sabouraud Dextrose Agar (SDA), Tryptic Soy Agar (TSA), Cholramphenicol Yeast Glucose Agar (CYGA) media, Xylose Lysine Deoxycholate (XLD) Agar, Brilliant Green Agar (BGA) media, Asparagine proline medium, Buffer Peptone Water (BPW) and Aquadest, Rubbing alcohol.

Experimental:

Preparation of Brahamrasayana

The fruits of amalaki are taken in the number of 1000 and are steamed on the vapour of milk like flour-paste. When they are well steamed, they are taken out, dried in shade and powdered after removing the seeds. This is impregnated with the juice of one thousand fresh fruits of amalaki and added with the powder of salaparni, punarnava, jivanti, nagabala, brahmasuvacala, mandukaparni, satavari, sankhpuspi, pippali, vaca, vidanga, kapikacchu, guduci,candana, aguru, madhuka, flowers of madhuka, utpala, kamala, jati, taruni and yuthika in the quantity one eighth of the amalaka powder. This is again impregnated with the juice of nagabala in the quantity of 48gm and dried in shade. Then in double quantity ghee or ghee honey mixed are added to it and is made in the shape of small boluses. Thus, is kept in strong and clean vessel united with ghee and is stored underground within the heap of ashes for a fortnight after having performed the protective rise through the knowers of atherveda. After the fortnight is over, this should be taken out and added with the ash of gold, silver, copper, coral and iron in one eighth quantity [9,10].

Comprehensive stability study for prepared formulation

In 2022, we initiated a comprehensive stability study on the Brahamrasayana formulation, meticulously prepared to exacting standards. This Ayurvedic formulation, renowned for its holistic health benefits, underwent rigorous testing to assess its stability over time. In 2023, we revisited the study to compare the formulation's stability with the previous year's findings. Microbiological analyses were conducted, encompassing essential parameters such as Total Plate Count, Yeast and Mold Count, as well as evaluating the impact on specific microbial strains, including Escherichia coli, Salmonella sp., Pseudomonas sp., Streptococcus sp., and Shigella sp. The comparative study aimed to provide valuable insights into the formulation's long-term stability and microbial safety, ensuring that the Brahamrasayana product maintained its efficacy and safety profile over the elapsed period. The results contribute to ongoing quality assurance efforts, guiding formulation improvements and affirming the product's adherence to stringent quality standards.

Total plate number

Transfer 10 g homogenized blend samples (if it is solid) by using sterile spatula or sterile pipettes under aseptic conditions to a 90 mL sterile buffered water blank. Shake this dilution vigorously. It is 1: 10 Dilution. Take 1 mL from the initial suspension and transfer to 9 mL blank. Mix thoroughly; preferably by using a mechanical stirrer, repeat this procedure using further dilutions if necessary.

Inoculation and incubation

Take two sterile Petri dishes; transfer aseptically 1ml of the test sample if liquid or 1 mL of the initial suspension in the case of other products. Repeat the procedure described above using further dilutions. Pour about 15 mL of the plate count agar, previously melted and maintained at 45 °C $\pm$ 1°C in a water bath from a culture bottle into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed 15min. Carefully mix the inoculum with the medium and allow it to solidify. After solidification, the agar plates should be incubated for 3 days at 30 °C $\pm$ 1°C. It is advisable to examine the plates at the end of three days for bacterial colonies.

After that it is calculated using given formula, Use counts from plates containing fewer than 150 colonies. The number of bacteria per gram or per millilitre is equal to

$$Total\ Plate\ Number = \frac{\Sigma C}{(n1 + 0.1n2)\ d}$$

ΣC = the sum of the colonies counted on all the plates.
n_1 = the number of plates counted in the first dilution.
n_2 = the number of plates counted in the second dilution.
d = dilution from which the first count was obtained.

If no colonies obtained on plates from the initial suspension the number of bacteria grams of the product should be reported as less than 10.

Yeast and Mold Number

Take 10 g homogenized blend samples (if it is solid) in 90 ml buffer peptone water. Serially dilute about 10^{-6}. Take two sterile Petri dishes; transfer aseptically 1ml of the test sample if liquid or 1 mL of the initial suspension in the case of other products. Repeat the procedure described above using further dilutions. Pour about 15 mL of the chloramphenicol yeast glucose agar, previously melted and maintained at 45°C $\pm$ 1°C in a water bath from a culture bottle into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed 15min. Carefully mix the inoculum with the medium and allow it to solidify. After solidification, the agar plates should be incubated for 5-7 days at 25 °C $\pm$ 1°C. In case of meat and meat products plates are incubated at 30°C $\pm$ 1 °C It is advisable to examine the plates at the end of three days for yeast colonies as they are likely to be overgrown by mold growth, If only

yeast counts are required, add 0.25% of sterile sodium propionate solution to the plate at the time of pouring to inhibit the growth of molds.

***E Coli* contamination measurement**

Take 25 g of blend test sample in a sterile stomacher bag or in a screw cap bottle and add into 225 mL sterile diluting fluid (0.1% peptone) to yield 1:10 dilution. Make further dilutions series up to 10^{-6} if necessary. Spread out 0.1 ml from each dilution tube evenly on to MacConkey agar or Eosin methylene blue agar or Tergitol 7 agar and incubate at 37°C for 24 hrs.

***Salmonella sp.* contamination measurement**

Add 25 gm of sample in 225 ml Buffered peptone water (BPW). Also inoculate 10 ml BPW with standard culture of salmonella. Incubate at 37°C for not less than 18 hrs $\pm$ 2 hrs. 0.1 ml of above preenrichment inoculum is added to 10 ml of RVS medium and transfer 1ml pre-enriched inoculum into the sterile 10 ml of MKTTn broth. Incubate the RVS medium at 41.5°C for 24 hrs $\pm$ 3hrs and MKTTn broth at 37°C for 24hrs $\pm$ 3hrs. After total incubation period of RVS broth and MKTTn broth streak on Xylose Lysine Deoxycholate (XLD) agar and second isolation medium (BGA/BSA) and incubate at 37°C for 24hrs $\pm$ 3hrs. The presence of red colonies, with or without a black dot in the center, indicates the presence of characteristics.

***Stephylococcus sp.* contamination measurement**

For solid sample blend the sample in a sterile blender or in a stomacher then take 25g of blend test sample in a sterile stomacher bag or in a screw cap bottle and add into 225 mL sterile diluting fluid (0.1% peptone) or buffered peptone water to yield 1: 10 dilution. Make further dilutions series up to 10^{-6} if necessary. Spread out 0.1 mL or 1 mL from each dilution tube evenly on to prepared pre-dried Baired parker agar plate either on the surface of one large agar plate(140mm) or three small agar plates(90mm). In both cases, prepare duplicates by using two large plates or six small ones allow the plates to dry with their lids on for about 15 min at laboratory temperature. Invert the plates prepared and incubate for 24 hrs (±2 hrs) at 37°C. After incubation for 24 hrs ±2 hrs mark on the bottom of the plate the positions of any typical colonies present. Take for enumeration only those plates that contain at the maximum 150 colonies with typical and atypical colonies at two successive dilutions. One of the plates shall contain at least 15 colonies.

***P. aeruginosa* contamination measurement**

Transfer 10 g homogenized blend samples (if it is solid) or 10 mL thoroughly mixed samples (if it is liquid) by using sterile spatula or sterile pipettes under aseptic conditions to a 90 mL sterile diluent. Shake this dilution vigorously. It is 1: 10 Dilution. Take 1 mL from the initial suspension and transfer to 9 mL sterile diluent. Mix thoroughly; preferably by using a mechanical stirrer, repeat this procedure using further dilutions if necessary.

Inoculation and incubation

Take two sterile Petri dishes; transfer aseptically 1 mL or 0.1 mL of the test sample if liquid or 1 mL of the initial suspension in the case of other products. Repeat the procedure described above using further dilutions. Pour about 15 mL of the CFC agar, previously melted and maintained at 45- 50 °C in a water bath from a culture bottle int each Petri dishes. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed15 min. Carefully mix the inoculum with the medium and allow it to solidify. After solidification the agar plates should be incubated for 48 hrs. at 25°C $\pm$ 1°C. It is advisable to examine the plates at the end of 48 hrs. for bacterial colonies. Count the colonies on each plate and retain plates containing 15 to 300 colonies. Select at random five colonies from each retained plate.

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Results and Discussion:

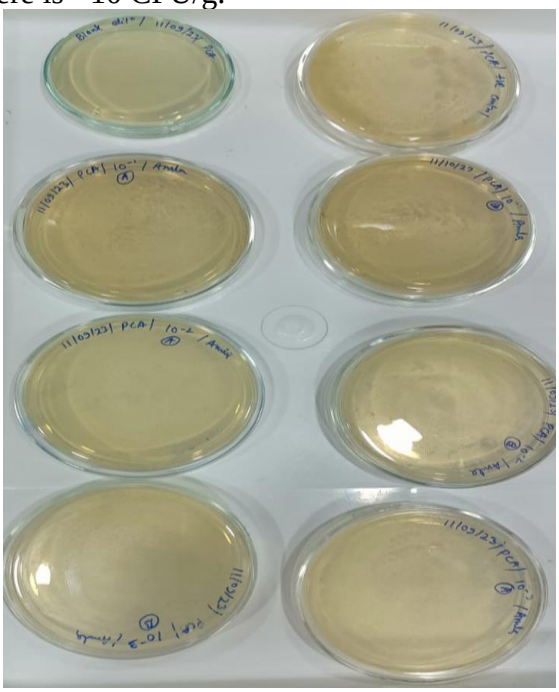
Total Plate Count

The result of TPC of powder sample is shown in table no.01

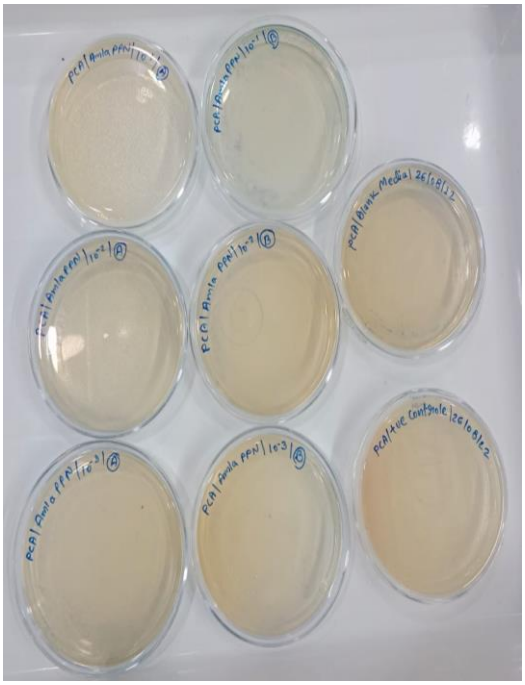
Table 1: TPC results on medicinal Brahamrasayana formulation

Sr no.	Group Identification	Dilutions	Results
1	TPC 2022	10^{-1}	<10 CFU/g
		10^{-2}	
		10^{-3}	
		10^{-4}	
		10^{-5}	
2	TPC 2023	10^{-1}	<10 CFU/g
		10^{-2}	
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the total plate count test in duplicate, in the first and second TPC on Brahamrasayana formulation from a dilution of 10-1 to 10-5 the number of colonies was obtained, the result of the first TPC was<10 CFU/g. This means that in TPC there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the TPC value to meet the requirements, there is <10 CFU/g.



Total Plate Count Year 2023



Total Plate Count Year 2022

Yeast and Mold number

The yeast and mold number test was carried out by the Duplo test. The result of yeast and mold number on powder sample of 2022 and 2023 are shown in table no.02

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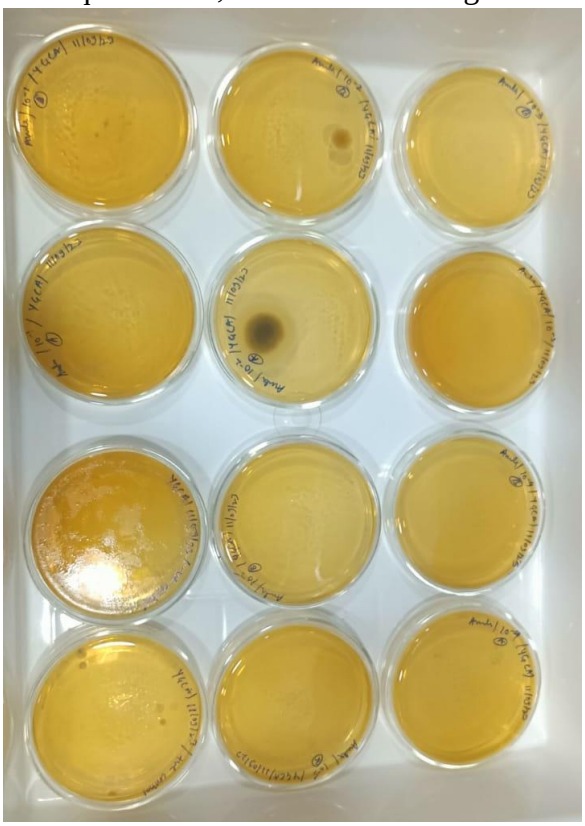
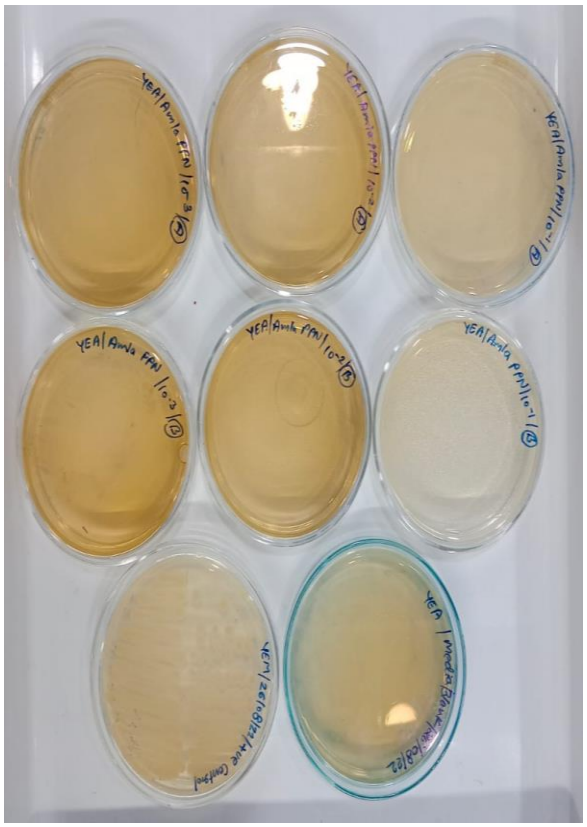
Table 2: Yeast and mold number results on Brahamrasayana formulation

Sr no.	Group Identification	Dilutions	Results
1	YMN 2022	10^{-1}	<10 CFU/g
		10^{-2}	
		10^{-3}	
		10^{-4}	
		10^{-5}	
2	YMN 2023	10^{-1}	<10 CFU/g
		10^{-2}	
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the Yeast and Mold count test in duplicate, in the first and second YMN on Brahamrasayana formulation from a dilution of 10^{-1} to 10^{-5} the number of colonies was obtained, the result of the first YMN was <10 CFU/g. This means that in YMN there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the YMN value to meet the requirements, there is <10 CFU/g.



Yeast and Mold Count Year 2023



Yeast and Mold Count Year 2022

Yeast and mold require 5 to 7 days to exhibit visible growth on solid media. These microorganisms may originate from drinking water or raw materials. The detection of yeast and mold in medicinal products poses a significant risk to consumers. Consequently, proactive measures must be

implemented to prevent the growth of yeast, mold, and bacteria in medicinal products, ensuring their safety for public health.

Escherichia coli

The *Escherichia coli* test is carried out by the Duplo test. The result of *E. coli* contamination in instant powder sample is shown in table 3.

E. coli test for powdered herbal medicine in sample 1 was tested with TSB and turbidity was produced, then the next test was carried out on MCB media and obtained a yellow color which means positive, then tested on MCA media and transparent colonies were produced. Then in the second sample carried out on the TSB test, the turbidity was marked positive, then the test was carried out on MCB media and the results were violet and there was turbidity, then continued for testing on MCA media and obtained no colony growth.

Table 3: *Escherichia coli* test results on Brahamrasayana formulation

Sr No.	Sample	TSB Media	MCB Media	MCA Media
1	*E Coli*	Cloudy	Cloudy violet	Colony not detected
2	*E Coli*	Cloudy	Cloudy violet	Colony not detected

Escherichia coli stands out as the predominant pathogen responsible for foodborne outbreaks. Strains of pathogenic E. coli have been linked to various health issues in humans, including diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and other manifestations.

E. Coli Year 2023

E. Coli Year 2022

Salmonella sp.

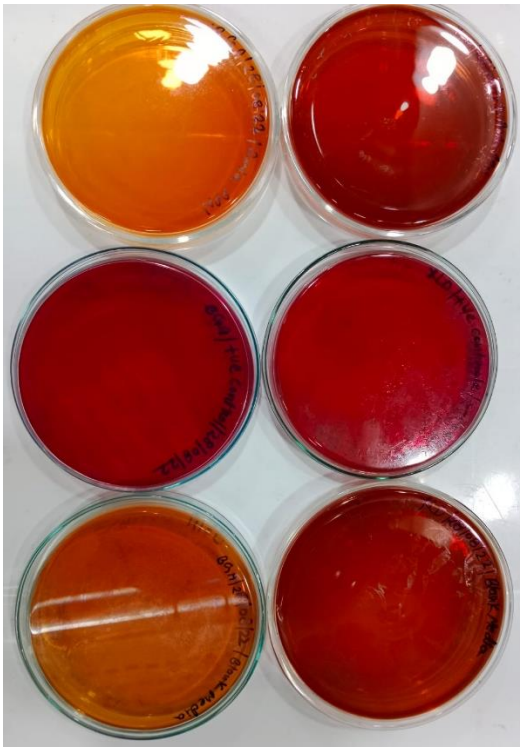
The *Salmonella sp.* test is carried out by the Duplo test. The result of *Salmonella sp.* contamination in powder sample is shown in table 4

Table 4: *Salmonella sp.* test results on Brahamrasayana formulation

Sr No.	*Salmonella*	Standard	Sample
1	*XLD*	Pink with black centre	Absent, No growth

2	BGA	Pink to red color change	No growth

Salmonella sp. Year 2023



Salmonella sp. Year 2022

Stephylococcus sp

The *Stephylococcus sp.* test is carried out by the Duplo test. The result of *Salmonella sp.* contamination in powder sample is shown in table 5.

Table 5: *Stephylococcus sp* test results on Brahamrasayana formulation

Sr No.	*Salmonella*	Standard	Sample
1	BPA	Sky black color colonies	No growth
2	Blood agar-Golden yellow colonies	No growth found	-

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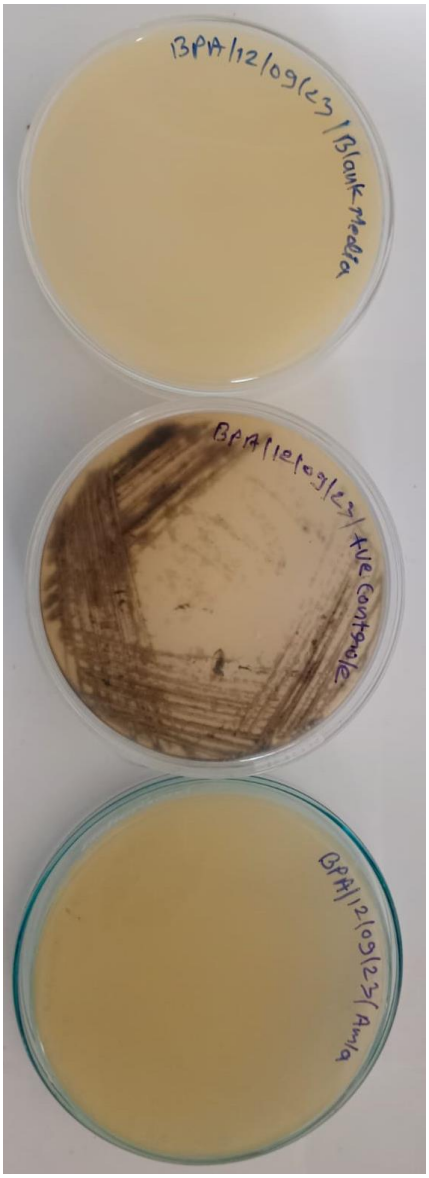
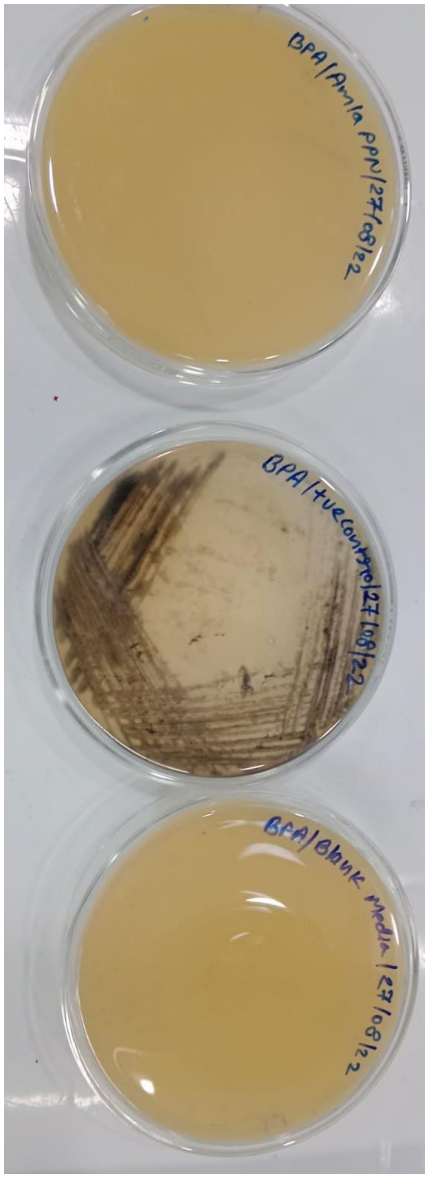


Stephylococcus sp* Year *2023



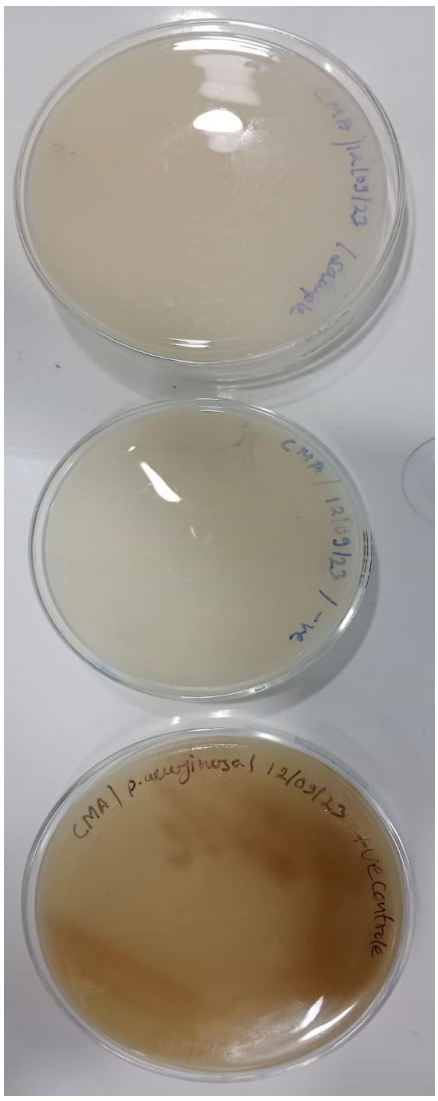
Stephylococcus sp* Year *2022

P. aeruginosa sp

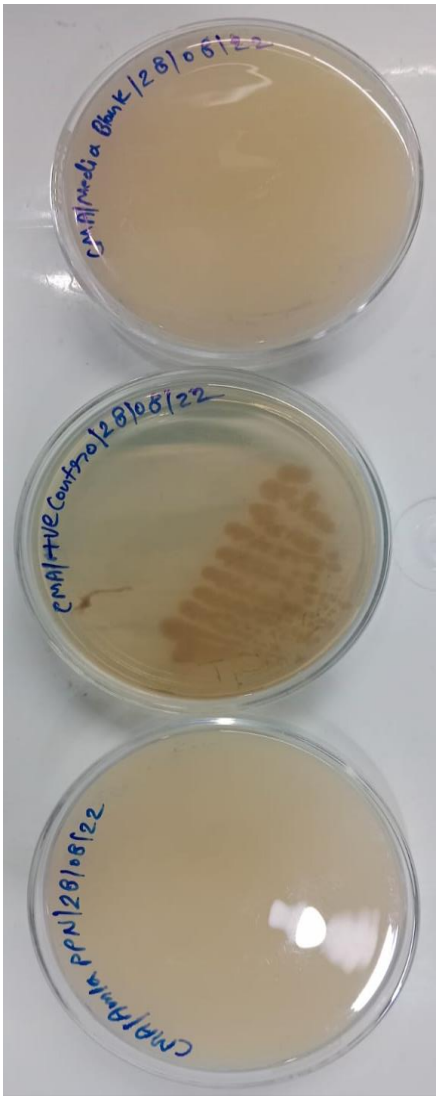
The *Salmonella sp.* test is carried out by the Duplo test. The result of *Salmonella sp.* contamination in powder sample is shown in table 6.

Table 6: *P. aeruginosa sp* test results on Brahamrasayana formulation

Sr No.	*P. aeruginosa*	Standard	Sample
1	Gram staining	No color change	No color change
2	Oxidase test	No blue color	No blue color
3	Catalase test	Negative	Negative
4	Hugh-Leif son's test	Negative	Negative



***Pseodomonas* sp. Year 2023**



***Pseodomonas* sp. Year 2022**

Conclusion:

The results of the evaluation of microbiological contamination parameters of traditional herbal medicinal preparation named as Brahamrasayana powder preparations for Total Plate Count , Yeast and mold Number values were <10 CFU/g, *Escherichia coli, Salmonella sp., Pseudomonas sp., Streptococcus sp.,* and *Shigella sp.* was negative/g. Brahamrasayana traditional medicinal preparations, namely instant powder medicine, can be consumed because they have met the safety and quality requirements according to PerKa BPOM No. 32 of 2019.

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