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Evaluation of Microbial Contamination in Herbal Drugs

Aparajita Gupta[1], Himanshu Upadhyay[2], Sachin Pandey[3]

[1]Department of Microbiology, Dolphin P. G College, Fatehgarh Sahib, Punjab, India
Email: *aparajitag88[at]gmail.com*

[2]Department of Microbiology, Dolphin P. G College, Fatehgarh Sahib, Punjab, India
Email: *uhimanshu22[at]gmail.com*

[3]Department of Microbiology, Dolphin P. G College, Fatehgarh Sahib, Punjab, India
Email: *apandeysp342[at]gmail.com*

Abstract: ***Herbal remedies are becoming more and more well - liked as alternatives to conventional pharmaceuticals because of their alleged natural origins and potential health advantages. However, worries about microbial contamination in herbal medications have surfaced, raising concerns about their safety and effectiveness. In this study, the microbiological load of herbal medicines—specifically Triphala, Shatavari, and Ashwagandha—was assessed, along with the effects of various time periods on microbial contamination. Samples of the herbal medicines were cultivated on Nutrient Agar Medium (NAM) plates for bacterial evaluation and Sabouraud Dextrose Agar (SDA) plates for fungal assessment in order to look into microbial contamination. To observe the changes in microbial load over time, the 30th and 60th days were chosen as the two time windows. The study's findings showed that the microbial burden significantly increased across the designated time periods. On NAM plates, Triphala and Shatavari had the highest bacterial loads of all the herbal medications tested, pointing to a higher potential of bacterial contamination. Triphala had an average bacterial count of $18x10^{-3}$ colony - forming units per gram (cfu/g), compared to Shatavari's $9x10^{-3}$cfu/g. These data imply a significant bacterial burden in these herbal medications, which may jeopardise their efficacy and safety. On SDA plates, Ashwagandha displayed the highest fungal load in terms of contamination by fungi. The presence of fungus and the potential dangers of fungal contamination in this herbal medication are highlighted by the fungal count for ashwagandha, which was around $7x10^{-3}$cfu/g. The observed rise in microbial load with time suggests the possibility of microbial development and contamination during various intervals in time. These results highlight the requirement for strict quality control procedures and monitoring guidelines in the manufacture and storage of herbal medicines.***

Keywords: Herbal medications, Conventional Pharmaceuticals, Microbial contamination, Jeopardise

1. Introduction

Herbal drugs have gained significant popularity in traditional medicine systems for their therapeutic properties and potential health benefits. However, ensuring the safety and quality of these herbal products is of utmost importance. Microbial contamination poses a considerable risk to the efficacy and safety of herbal drugs, as it can introduce harmful bacteria and fungi. Thus, assessing the microbial load in herbal drugs is crucial to safeguard public health and maintain product quality.

Despite the fact that the use of herbal medicines has been around for almost 60, 000 years, in the last ten years it has gained more attention and interest due to the observed drug resistance of infectious pathogens to commonly used antimicrobial agents as well as the significant side effects and high cost of treatment associated with commonly used synthetic drugs [1].

Herbal medicine is a traditional method frequently used to cure illnesses. Alternative and complementary medicine (CAM) is another name for it. It is gaining popularity as developments in clinical research, analysis, and quality control demonstrate the effectiveness of herbal medicine in the treatment and prevention of disease. According to the World Health Organization (WHO), over 80% of the world's population (four billion people) use herbal medicine for some part of primary healthcare [2].

Although the efficacy of a significant number of herbal products has been established, the use of herbal extracts as drugs and health supplements has shown promising potential to treat human diseases. However, many of these products remain untested and are not adequately monitored due to a lack of suitable quality controls, etc [3].

Asthma, eczema, irritable bowel syndrome, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, and cancer are just a few of the ailments that have been reported to be treated with herbal medication [9].

The bioefficacy of an herbal formulation containing extracts of Panax ginseng and Ginkgo biloba in enhancing cognitive function and memory in elderly individuals. The findings of this study revealed that the herbal formulation significantly improved cognitive performance and memory recall compared to a control group [4].

These studies, along with many others, highlight the bioefficacy of herbal drugs and their potential therapeutic applications. The diverse range of bioactive compounds present in herbal drugs offers a promising avenue for the development of novel therapeutic.

In the world, there are 20, 000 plant species that are utilised to make various medications, according to the WHO. Over 6000 plants are used in India for traditional, folk, and herbal

medicine, which satisfies approximately 75% of the medical needs of Third World nations [5].

Significant economic advantages are being realised on the national and international markets for medical plants, with anticipated global sales of herbal goods reaching US$60 million in 2000 [6].

Herbal drugs provided valuable insights into conventional and controlled microbial cultivation techniques for biomass production. Their work emphasized the need for effective bioprocess parameters to minimize microbial contamination during cultivation and processing [7].

Additionally, herbal drugs stressed the importance of evaluating medicinal plant products as antimicrobial agents using various techniques, highlighting the potential health risks associated with microbial contamination [8].

2. Materials and Methods

Herbal drug sample collection
The test herbal drug material selected for project work was Triphala powder (*Emblicaofficinalis, Terminaliabellerica* and *Terminaliachebula*), Shatavari powder (*Asparagus racemosus*) and Ashwagandha powder (*Withaniasomnifera*). The herbal drug material was collected from the local market in Chandigarh. Samples were packed in cleaned and sterile beakers and stored at room temperature until analysis.

Testing for Microbial Load in Herbal Drugs
The spread plate method is used for the estimation of microbial load in herbal drugs.

Preparation of agar plates:
The autoclaved nutrient agar media and sabouraud dextrose agar was poured into sterile petri plates in Laminar air flow and allowed to solidify completely.

Isolation of Bacteria and Fungi from Herbal Drugs Samples
1 gm of the processed herbal drug was mixed in 9 ml sterile distilled water in a test tube. Herbal drug suspension was made by vortexing the tubes and thereafter the larger herbal drug particles were allowed to settle down by keeping the suspension undisturbed for 10 mins. The resultant suspension was called master sample. Master sample was further serially diluted 6 folds 10^{-1} to 10^{-6} concentration. The dilution were carefully poured in aseptic conditions on Nutrient agar medium plates used for bacterial colonies and sabouraud dextrose agar medium plates for fungal colonies in the laminar air flow. Plates were incubated at 37°C for 24 hrsof NAM cultured plates and 25 °C for 6 - 7 days of SDA cultured plates and observed for appearance of distinct individual colonies. Procedure was repeated for all other herbal drug sample and leveled. This step is revised after 30^{th} and 60^{th}days interval for measuring the microbial load.

Observation of colonies After the incubation the petri plates were observed in Digital Colony Counter for counting the bacterial and fungal colonies from Nutrient agar plates and Sabouraud dextrose agar plates.

Calculation of Bacteria and Fungi colonies observed
After the observation of colonies mean of each is calculated from all petri plates at different dilutions.

3. Result and Discussion

In our study, results indicates that the microbial load is been increased when cultured at different time intervals gap i. e 30^{th} and 60^{th} days. The triphala and shatavari showed the highest bacterial load in NAM plates and Ashwagandha shows the highest fungal load in SDA plates which tell about the microbial contamination during different time interval gaps. In triphala and shatavarithe highest bacterial count detected was $18x10^3$cfu/g and $9x10^3$cfu/g. On the other hand, ashwgandha shows the highest fungal counts about $7x10^3$cfu/g. Table no.1, 2, 3, 4, 5 & 6.

1) Triphala Powder
The microbial load of triphala powder during months storage period at the room temperature has been presented in the table 1 for bacteria and table 2 for fungi.

Table 1: Total bacterial count of triphala powder

Test herbal drug	Storage duration	Dilution	Total Bacterial Count (TBC)	
			Average value	TBC (CFU/g)
TRIPHALA	1^{ST}	10^4	3	$3x10^4$
		10^5	2	$2 x10^5$
		10^6	2	$2 x10^6$
	30^{th}	10^4	10	$10 x10^4$
		10^5	6	$6 x10^5$
		10^6	2	$2 x10^6$
	60^{th}	10^4	18	$18 x10^4$
		10^5	6	$6 x10^5$
		10^6	4	$4 x10^6$

Table 2: Total fungal count of triphala powder

Test herbal drug	Storage duration	Dilution	Total Fungal Count (TFC)	
			Average value	TBC (CFU/g)
TRIPHALA	1^{ST}	10^3	2	$2x10^4$
		10^4	1	$1x10^5$
		10^5	1	$1x10^6$
	30^{th}	10^3	2	$3 x10^4$
		10^4	1	$2 x10^5$
		10^5	1	$1 x10^6$
	60^{th}	10^3	6	$4 x10^4$
		10^4	3	$3 x10^5$
		10^5	2	$2 x10^6$

2) Ashwgandha Powder
The microbial load of ashwgandha powder during 3 months storage period at the room temperature has been presented in the table 3 for bacteria and table 4 for fungi.

Table 3: Total bacterial count of ashwgandha powder.

Test herbal drug	Storage duration	Dilution	Total Bacterial Count (TBC)	
			Average value	TBC (CFU/g)
ASHWGANDHA	1^{ST}	10^4	3	$3x10^4$
		10^5	2	$2 x10^5$
		10^6	1	$1x10^6$
	30^{th}	10^4	5	$5 x10^4$
		10^5	2	$2 x10^5$
		10^6	1	$1 x10^6$
	60^{th}	10^4	8	$8 x10^4$
		10^5	4	$4 x10^5$
		10^6	2	$2 x10^6$

Table 4: Total fungal count of ashwgandha powder

Test herbal drug	Storage duration	Dilution	Total Fungal Count (TFC)	
			Average value	TBC (CFU/g)
ASHWGANDHA	1ST	10^3	3	$3x10^4$
		10^4	2	$2x10^5$
		10^5	1	$1x10^6$
	30th	10^3	4	$4 x10^4$
		10^4	3	$3 x10^5$
		10^5	2	$2 x10^6$
	60th	10^3	7	$7 x10^4$
		10^4	3	$3 x10^5$
		10^5	2	$2x10^6$

3) Shatavari Powder

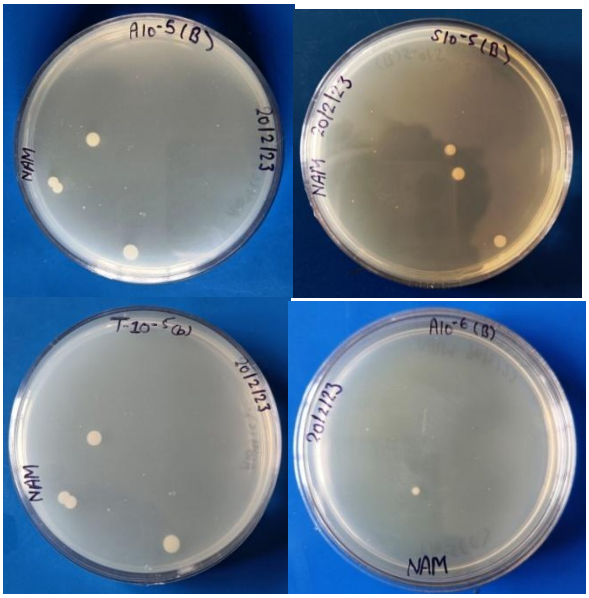
The microbial load of shatavari powder during 3 months storage period at the room temperature has been presented in the table 5 for bacteria and table 6 for fungi.

Table 5: Total bacterial count of shatavari powder

Test herbal drug	Storage duration	Dilution	Total Bacterial Count (TBC)	
			Average value	TBC (CFU/g)
SHATAVARI	1ST	10^4	2	$2x10^4$
		10^5	2	$2 x10^5$
		10^6	1	$1x10^6$
	30th	10^4	7	$7 x10^4$
		10^5	5	$5 x10^5$
		10^6	3	$3 x10^6$
	60th	10^4	9	$9 x10^4$
		10^5	6	$6 x10^5$
		10^6	3	$3 x10^6$

Table 6: Total fungal count of shatavari powder

Test herbal drug	Storage duration	Dilution	Total Fungal Count (TFC)	
			Average value	TBC (CFU/g)
SHATAVARI	1ST	10^3	2	$2x10^4$
		10^4	1	$1x10^5$
		10^5	1	$1x10^6$
	30th	10^3	3	$3 x10^4$
		10^4	2	$2 x10^5$
		10^5	1	$1 x10^6$
	60th	10^3	4	$4 x10^4$
		10^4	3	$3 x10^5$
		10^5	2	$2 x10^6$



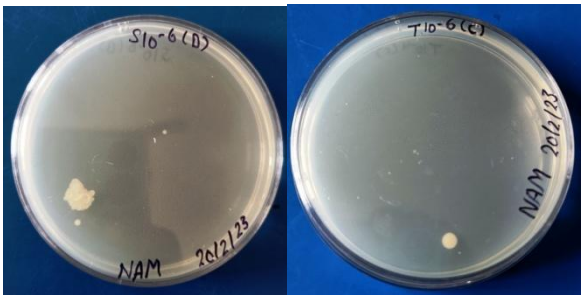


Figure 1: Showing bacterial colony in herbal drugs on 30th Days

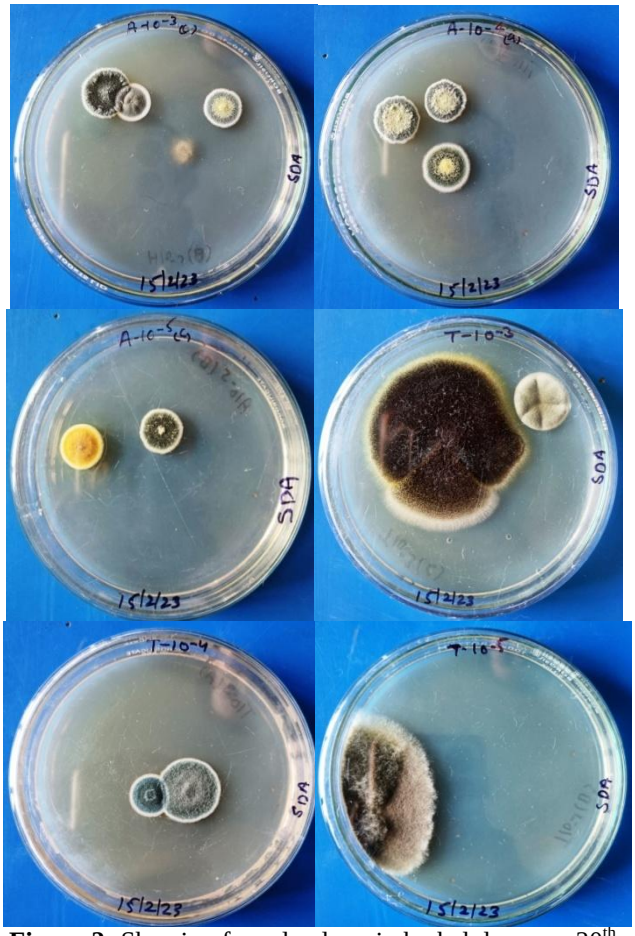
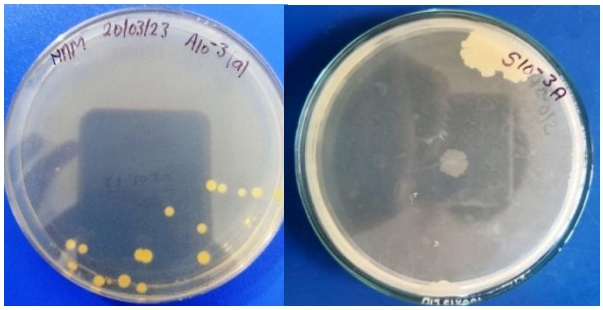


Figure 2: Showing fungal colony in herbal drugs on 30th Days



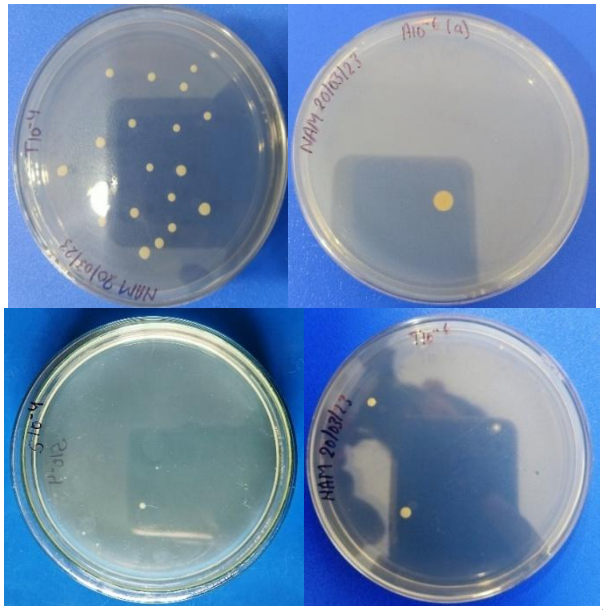


Figure 3: Showing bacterial colony in herbal drugs on 60th Days

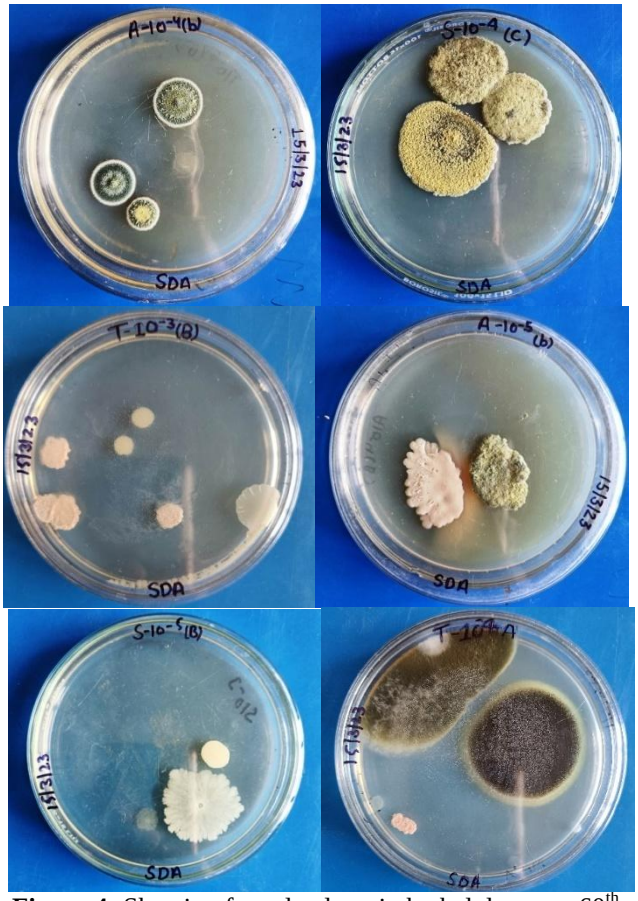


Figure 4: Showing fungal colony in herbal drugs on 60th Days

3 Conclusion

In conclusion, the evaluation of microbial contamination in herbal drugs revealed concerning results. The microbial load increased significantly over time, indicating the presence of contamination. Triphala and shatavari exhibited the highest bacterial load, with counts of 18x10^3 cfu/g and 9x10^3 cfu/g, respectively, while ashwagandha had the highest fungal load, with a count of 7x10^3 cfu/g.

Our findings, therefore emphasize the importance of implementing stringent quality control measures in the production and storage of herbal drugs. The presence of high bacterial and fungal counts raises concerns about the safety and efficacy of these herbal products. Microbial contamination can pose serious health risks to consumers, especially those with weakened immune systems or pre - existing medical conditions.

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