

SCREENING OF MICROBIAL CONTAMINANTS IN HERBAL MEDICINES FROM AYURVEDIC SHOPS IN MYSURU, KARNATAKA, INDIA

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Abstract: Herbal drugs have increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs. These have the potential of contamination with different microorganisms which could change the physicochemical characteristics as well as toxicity to pharmaceutical preparations. Hence this study was undertaken to unearth the microbial contamination of herbal medicines marketed in some Ayurvedic shops in Mysuru, Karnataka, India. Out of our six samples studied, four (tablets) samples showed very high microbial load, where in two liquid samples the microbial load was low. In all these samples *Penicillium* spp. and *Aspergillus* spp. were common and all the six samples confirmed gram positive bacilli. The current analysis signifies the contamination of marketed herbal medicinal formulations by microorganisms. Consequently, such products can adversely affect health category of consumers as well as solidity of the products. Therefore the quality assurance of these products should be thoroughly monitored during production and allocation of herbal drugs as well as good manufacturing practices at pharmaceutical level are legally required for the manufacturing of herbal medicinal products.

Key Words: Microbial contaminants, *Aspergillus* spp., *Paecilomyces* spp., *Penicillium* spp., *Diplobacilli*, Herbal medicine.

1. INTRODUCTION:

In modern decades, the use of herbal products has increased in developed countries, due in part to the widespread assumption that “natural” implies “harmless”. However, with their popularity and global market expansion, the safety of herbal products has become a major concern in public health. Lack of regulation and loose distribution channels (including internet sales) may result in adverse reactions attributable to the poor quality of herbal products. The most common causes are adulteration of herbal products with undeclared potent pharmaceutical substances, substitution or misidentification with toxic plant species, incorrect dosing, interactions with conventional medicines, and use of products contaminated with potentially hazardous substances, such as microbial metabolites (e.g. mycotoxins), radioactive particles, heavy metals, and agrochemical residues [1] and [2]. The use of the traditional herbal medicinal products (THMPs) has been increasing worldwide due to the readily availability of raw materials and low cost compared to the synthetic industrial preparations. With this trend in mind the safety and quality of THMPs need to be addressed so as to protect the community [3]. Pharmaceuticals are used in a variety of ways in prevention, treatment and diagnosis of diseases. In recent year's manufacturers of pharmaceuticals have improved the non sterile quality of nonsterile pharmaceuticals such that today such products contain only minimal bioburden [4]. Contamination is the undesired introduction of impurities (chemical or microbiologic or foreign matter) onto a starting material intermediate product or finished herbal product during production, packaging, storage or transport of this product [5].

The exploitation of herbal medicine has constantly been an element of human culture, as some plants possess significant remedial properties which can be used to heal diseases in humans and other animals. The widespread utilization of these herbal medicinal products in the healing and management of diseases within communities, India has made it vital to examine the microbial quality of these products by taking into consideration the values set by regulatory bodies [6]. The presence of microbial contaminants in non sterile pharmaceutical preparations will not only cause spoilage of copious productions but is proved to be a latent health hazard to the consumer [7]. The present study evaluated the microbial contamination in herbal medicines from Ayurvedic shops in Mysuru, Karnataka, India so as to inform relevant stakeholders for appropriate safety interventions.

Being natural, herbal products are often perceives as safe but studies have shown that they may not be free of pathogenic microorganisms [8]. More than 70% of India's 1.1 billion populations still use herbal medicines. In 1970, the Indian Medical Central Council Act, which aimed to standardize qualifications for Ayurveda practitioners and

provide accredited institution for its study and research, was passed by the Parliament of India. State sponsored Central Council for Research in Ayurvedic Science is designed to do research in Ayurveda. Herbal medicine is on the rise worldwide, and safety issues allied with herbal medicines may also have an exacerbated impact in elderly because this population has an increased susceptibility and sensitivity to health complications due to ageing process [9]. As of 2013, India has over 200 training centres offer degree in traditional ayurvedic medicines. In November 09, 2014, India formed the ministry of AYUSH (Ayurveda, Yoga, Unani, Siddhi and Homeopathy). National Ayurveda day is celebrated in India on the birth of the Lord Dhanvantari and was celebrated on November 05 in 2018. Recently WHO announced that India is the second largest exporter of medicinal plants in the world. India stands second to China in this ranking, and produce 70% of the herbal medicines demand across the globe.

2. MATERIALS AND METHODS:

2.1. Sample collection:

A total of 6 different herbal medicinal samples were purchased from Ayurvedic shops in Mysuru, namely Anandabhairav Ras (S-1), Chandraprabhavati (S-2), Trishun (S-3), Fifatrol (S-4), Dashmularishta (S-5) and Ashokarishta (S-6). The herbal samples were collected in sterile bag and brought to laboratory.

Samples of herbal medicines



Figure 1. A-Anandabhairav Ras; B-Chandraprabhavati; C-Trishun; D-Fifatrol; E- Dashmularishta; F-Ashokarishta

Anandabhairav Ras

Anandabhairav Ras is an ayurvedic medicine in tablet form. It is used in the treatment of acute and chronic fever, diarrhoea, rheumatoid arthritis etc. This medicine should be strictly taken under medical supervision.

Chandraprabavati

Chandra refers to moon in Sanskrit. This formulation works similar to the Moon which removes darkness in night. It is useful in disturbed metabolism of kapha dosha as well as vata dosha. It is also good medicine for diabetes, tumor, cysts, obesity and general weakness, reproductive related problems.

Fifatrol

Fifatrol is a natural effective remedy for infection, flu and cold. It is enriched with immune enhancing herbs to fight against bacteria, viruses and allergens and provides fast relief from nasal congestion, sore throat, body ache and head ache. Fifatrol is rationale combination of vital phytoconstituents, immunomodulators and antioxidants.

Trishun

Herbal Trishun strengthens immunity and fights against cold. Trishun should be taken with warm water, tea or coffee, to treat against cold and influenza effectively. Zandu Trishun tablet has combined benefits of Sudarshan Churna and Tribhuvan Kirti Rasa. It has the capacity to give relief to pain and fever.

Dashmularishta

Dashmularishta is a liquid ayurveda medicine, used in the treatment of Anemia, after delivery care of mother, cold, cough, digestive disorders etc. It contains about 5-7 % of self generated natural alcohol in it. This self generated alcohol and the water present in the product acts as a media to deliver water and alcohol soluble as the active herbal components to the body which is also called as Dashmularishtam.

Ashokarishta

Ashokarishta is an ayurvedic medicine and extensively used in ayurvedic treatment of heavy menstrual bleeding and other gynaecological complaints. It contains 5 - 10 % of self generated alcohol, which acts as medium for herbal active principles. This is also called as ashokarishtam. It is used in ayurveda treatments of many diseases like menstruation pain, heavy periods, fever, bleeding disorders such as nasal bleeding, bleeding hemorrhoids and inflammation.

2.2. Processing of samples:

Serial dilution was carried out by adding 1 ml stock solution containing 9 ml of physiological saline. The sample was dispensed in 9 ml of saline water using sterile pipette and were mixed to obtain 10 ml of dilute solution. 1ml of samples were transferred to ten test tubes from 10⁻¹ - 10⁻⁹ to obtain distinct microbial colonies.

2.2.1. Media preparation:

NA media

Nutrient Agar medium is very commonly used for isolation and growth of bacterial colonies. 5gm of peptone, 3gm of beef extract, 5.0 gm of NaCl, 15gm of agar was added to 1000 ml distilled water, homogenised and autoclave the content at 121 °C at 15 psi. Sterile plates were poured with NA and used for isolation of bacteria.

PDA media

Potato dextrose agar medium is very commonly used for isolation and growth of fungal colonies. 200 gm of sliced potatoes were boiled for 20 min to get potato infusion which was filtered through cheese cloth. 15 gm of agar as solidifying agent and 20 gm of dextrose was mixed homogenised in one litre distilled water, autoclaved for 15 min at 121°C and 15 psi. Sterile plates were poured with PDA and used for isolation of fungi.

2.2.2. Inoculation of Herbal samples:

Inoculation of samples was done by using spread plate method. About 10-15 ml of media was poured to sterile Petri plates and allowed to solidify. 0.5 ml of each diluted samples was transferred on media and spread evenly using L-shaped spreader, incubated at 37 °C for 24 hours for bacteria and 27 °C for 6 -7 days for fungi.

2.2.3. Isolation and identification of Bacteria and Fungi:

Bacteria - Gram staining: The test was carried out to differentiate between gram positive and gram negative bacteria. A loopful of bacterial culture was transferred to clean slide contained drop of water. The culture was heat fixed by slightly moving the slide over Bunsen burner flame. A few drops of crystal violet stain as primary stain were dropped on to the fixed culture, for a minute and was rinsed off using distilled water, later drops of Grams iodine (mordant) was added on the smear and allowed to stand for a minute and washed with distilled water. Few drops of decolourising agent ethanol was added onto the smear for 30 seconds and rinsed off. Safranin a counter stain was added and was left 2-3min and the slide was gently was under running tap water. The air dried slides were observed under 100x and 40x magnification.

Fungi: Small amount of fungal culture was transferred to clean slide using sterile needle. A drop of cotton blue stain was used for fungal slide preparation and observed under 100x and 40x magnification.

3. RESULTS:

3.1. Bacterial contamination in herbal medicines:

Sample 1 to 4 was high in bacterial contamination, whereas sample 5 and 6 showed least bacterial contamination. Gram-positive diplobacilli was more and evident in all the samples compared to Gram-negative bacilli and cocci.

Table 1: Bacterial contamination in the herbal medicines

Sl. No.	Samples	Cocci		Bacilli	
		Gram Positive	Gram Negative	Gram Positive	Gram Negative
1	Anandabhairav Ras	✓	-	✓	-

2	Chandraprabhavati	-	-	✓	✓
3	Trishun	✓	-	✓	-
4	Fifatrol	✓	-	✓	✓
5	Dashmularishta	-	-	✓	-
6	Ashokarishta	-	-	✓	-

Bacterial and Fungal colonies of Herbal Medicines

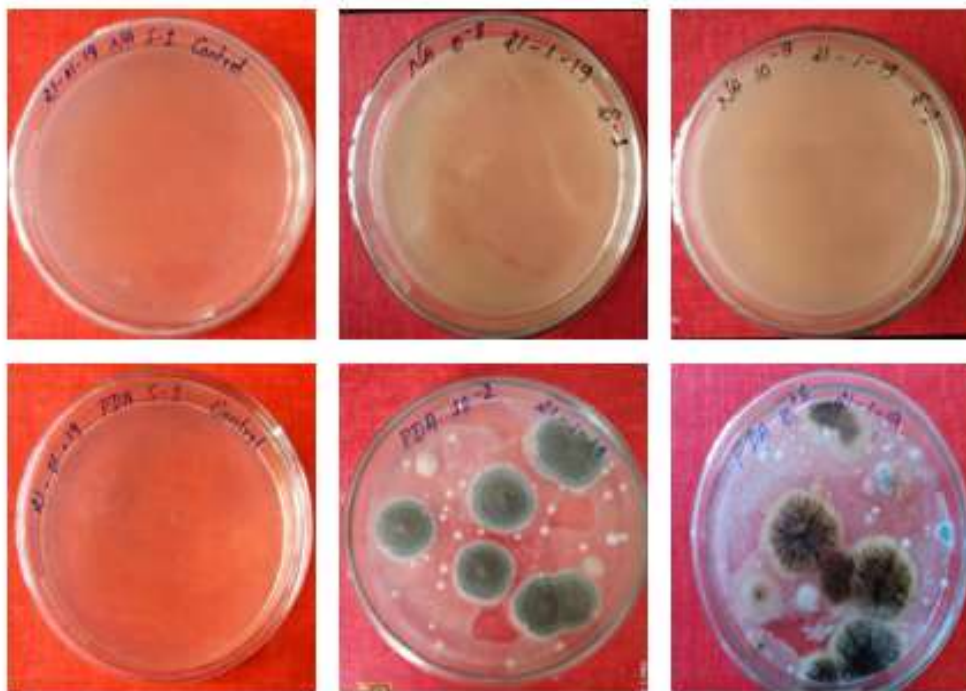


Figure 2. Bacterial and Fungal plates of Anandabhairav Ras

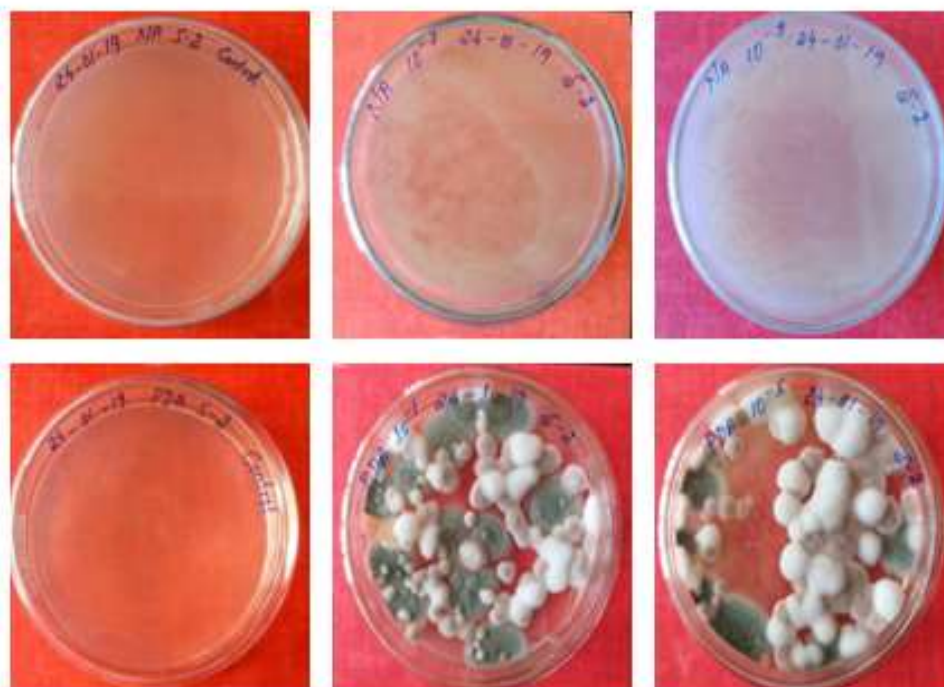


Figure 3. Bacterial and Fungal plates of Chandraprabhavati

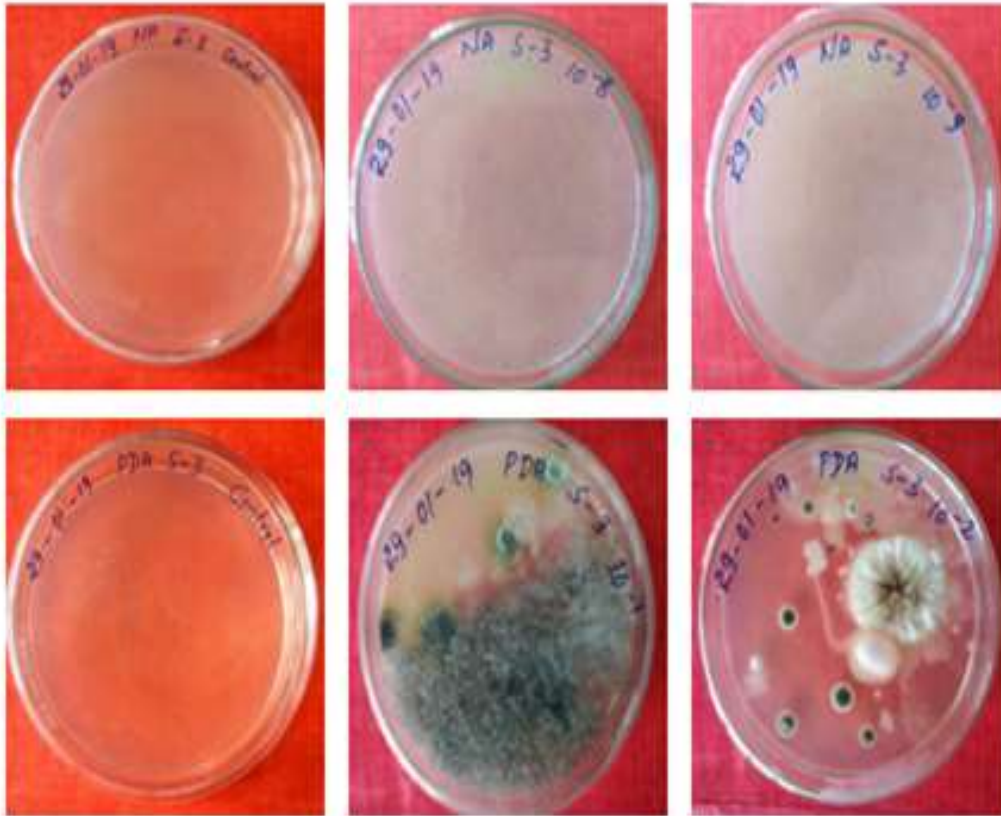


Figure 4. Bacterial and Fungal plates of Trishun

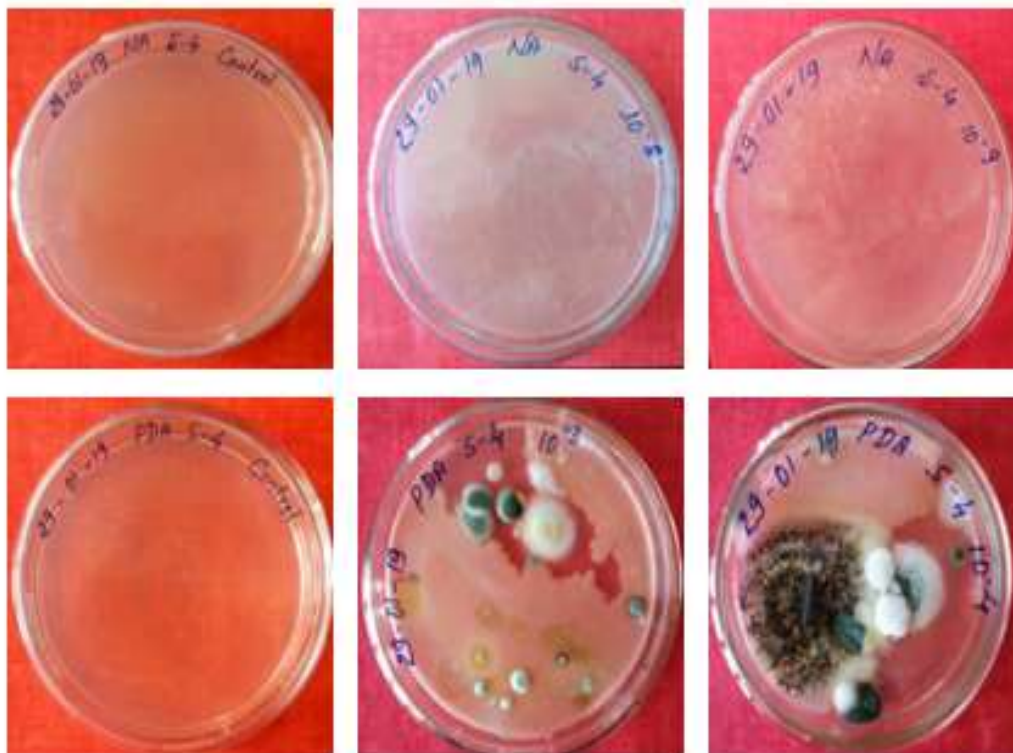


Figure 5. Bacterial and Fungal plates of Fifatrol

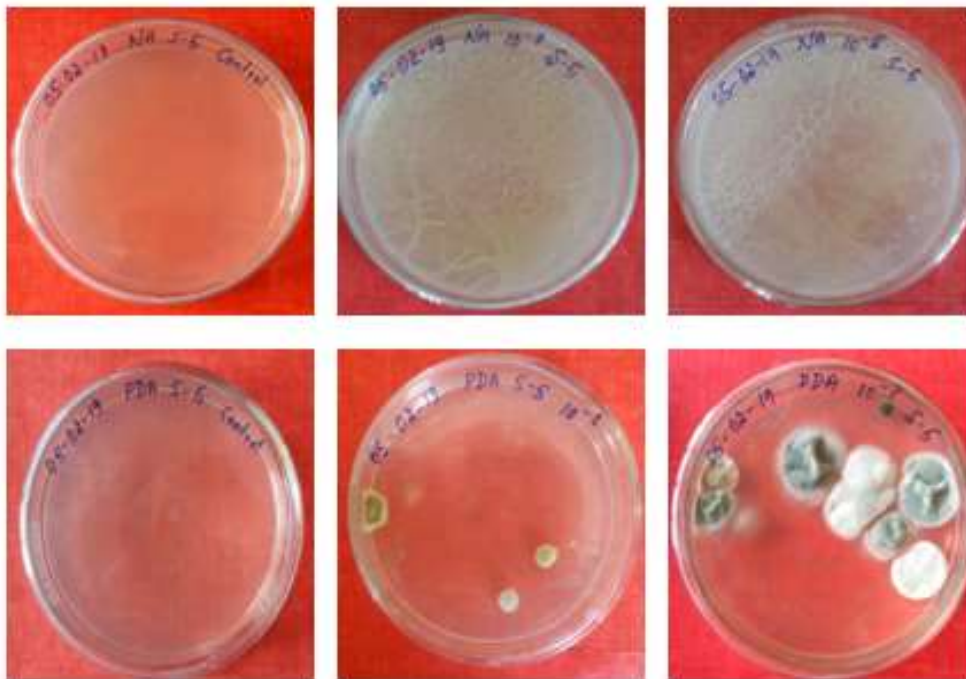


Figure 6. Bacterial and Fungal plates of Dashmularishta

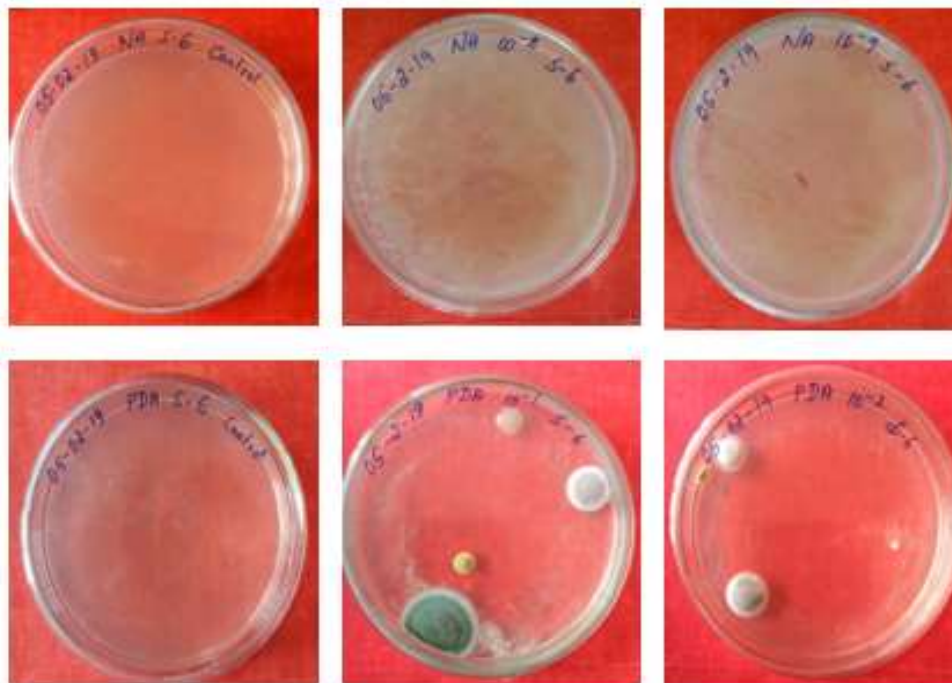


Figure 7. Bacterial and Fungal plates of Ashokarishta

Fungal contamination in herbal medicines

Among the six samples, sample-2 showed high fungal incidence. Sample 1 to 6 *Penicillium* spp. and *Aspergillus* spp. were common. *Rizopus* and *Cladosporium* spp. were reported from sample 3 and 4. *Paecilomyces* spp. was reported from sample 2 and 5. Sample 5 and 6 showed very a small number of colonies. The fungi identified from sample 5 and 6 were *Aspergillus* spp, *Paecilomyces* spp. and *Penicillium* spp.

Table 2: Fungal contamination in herbal medicines

Sl. No.	Sample Name	Fungal Species
1	Anandabhairav Ras	<i>A. flavus</i> and <i>Penicillium</i> spp.
2	Chandraprabhavati	<i>Paecilomyces</i> spp. and <i>Penicillium</i> spp.
3	Trishun	<i>A. flavus</i> , <i>Cladosporium</i> spp. and <i>Rizopus</i> spp
4	Fifatrol	<i>A. flavus</i> and <i>Cladosporium</i> spp.
5	Dashmularishta	<i>Penicillium</i> spp. <i>Aspergillus</i> spp. and <i>Paecilomyces</i>
6	Ashokarishta	<i>Aspergillus</i> spp. <i>Paecilomyces</i> and <i>Penicillium</i> spp.

Microscopic Views of Bacterial and Fungal contaminants in Herbal Medicines

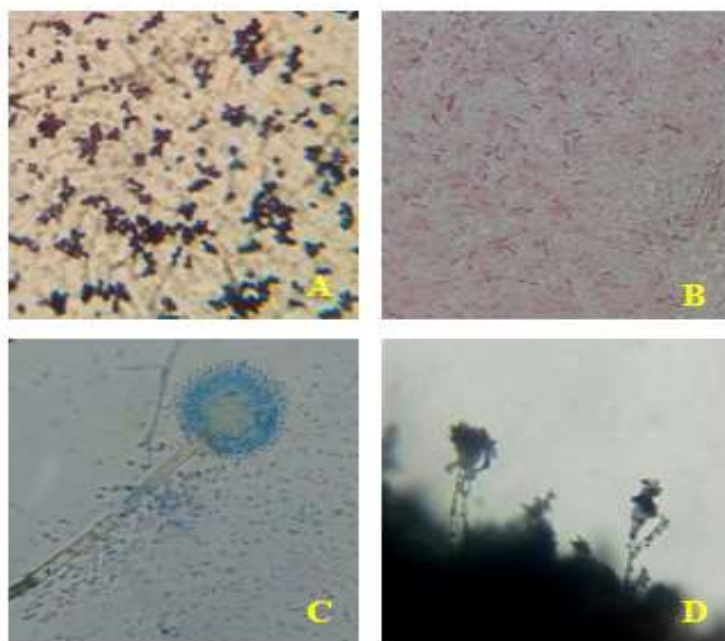


Figure 8. Bacterial and Fungal species in Anandabhairav Ras under 100x and 40x magnification
 A: gram +ve cocci; B: gram +ve bacilli; C: *A. flavus*; D: *Penicillium* spp.

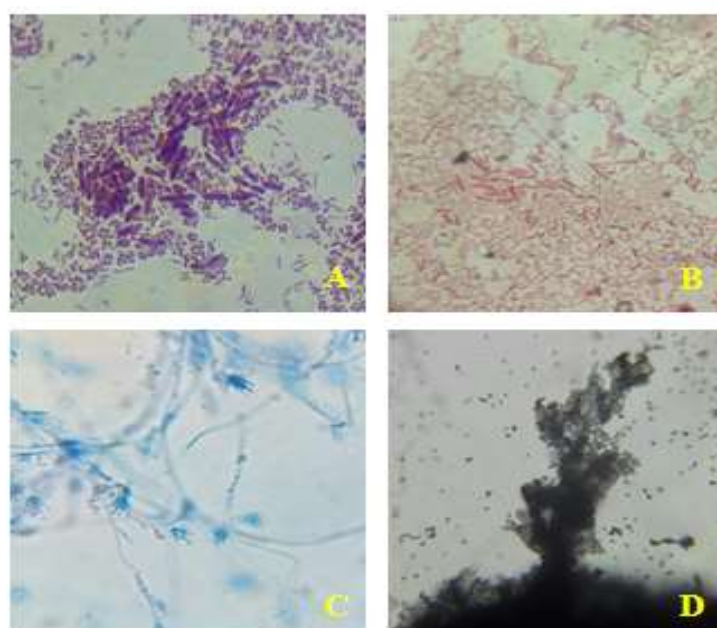


Figure 9. Bacterial and Fungal species in Chandraprabhavati under 100x and 40x magnification

A: gram +ve bacilli; B: gram -ve bacilli; C: *Paecilomyces* spp.; D: *Penicillium* spp.

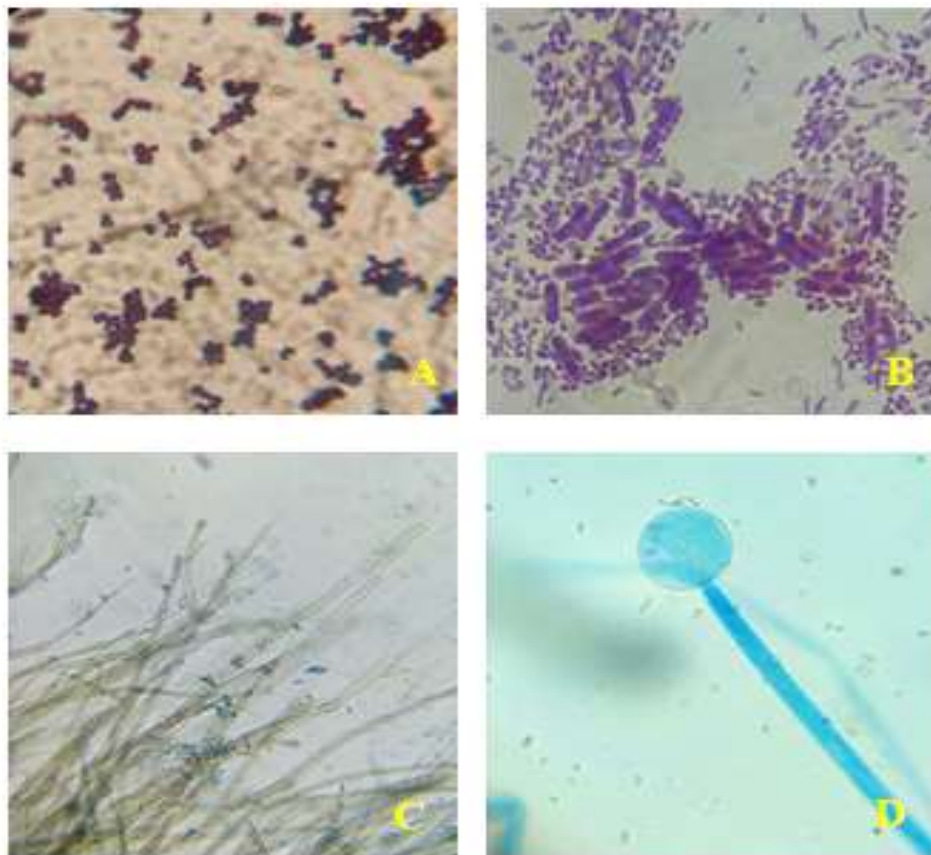


Figure.10. Bacterial and Fungal species in Trishun under 100x and 40x magnification
A: gram +ve cocci; B: gram +ve bacilli; C: *Cladosporium* spp.; D: *Rhizopus* spp.

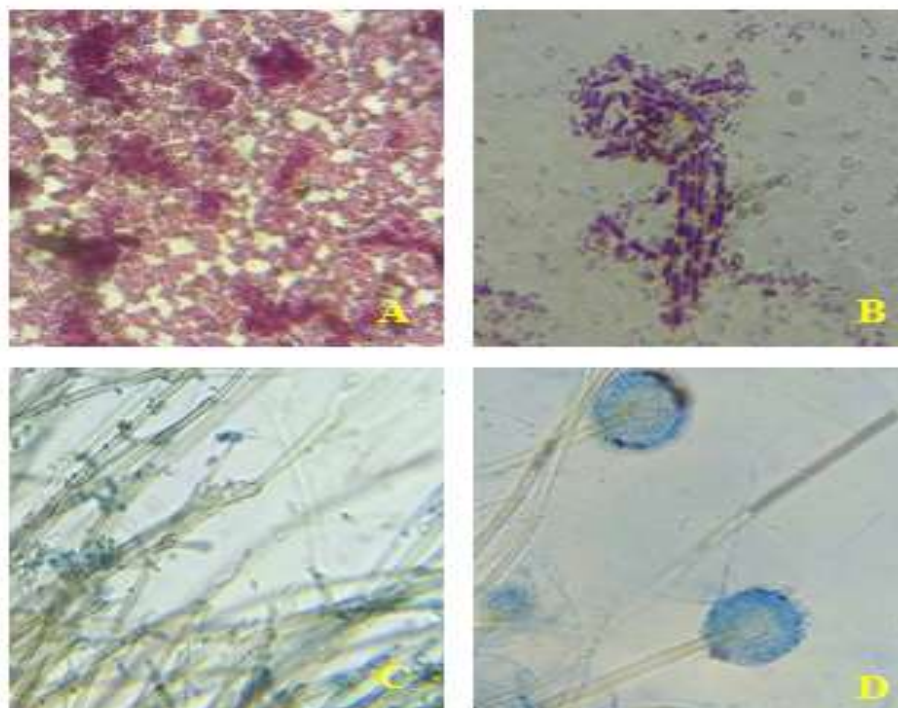


Figure 11. Bacterial and Fungal species in Fifatrol under 100x and 40x magnification
A: gram +ve cocci; B: gram +ve bacilli; C: *Cladosporium* spp.; D: *A. flavus*.

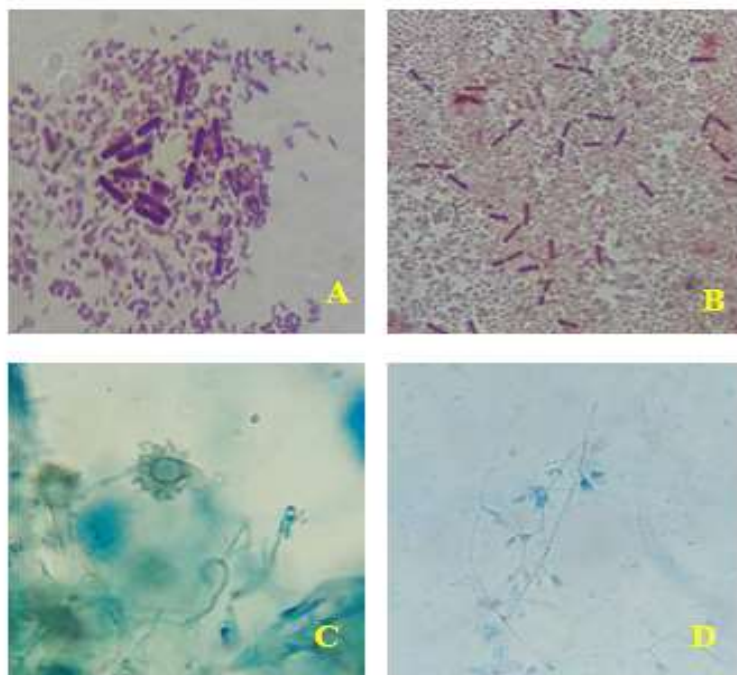


Figure. 12. Bacterial and Fungal species in Dashmularishta under 100x and 40x magnification
A: gram +ve bacilli B: gram +ve bacilli; C: *Aspergillus* spp: D: *Paecilomyces* spp.

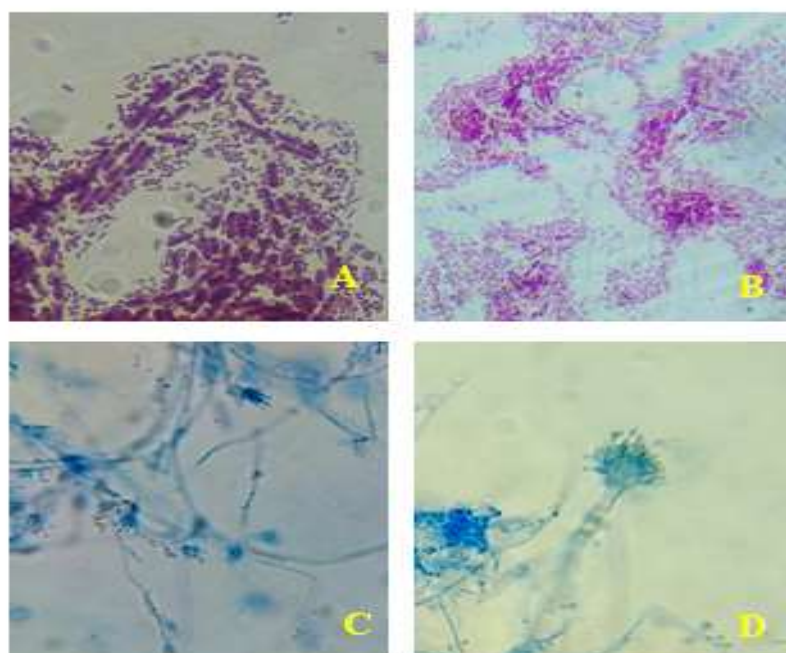


Figure. 13. Bacterial and Fungal species in Ashokarishta under 100x and 40x magnification
A: gram +ve bacilli B: gram +ve bacilli; C: *Paecilomyces* spp.; D: *Aspergillus* spp.

4. DISCUSSION:

Contamination of pharmaceuticals with microorganisms irrespective whether they are harmful or non pathogenic can bring about changes in physicochemical characteristics of the medicines. Although sterility is not a requirement in official compendia for nonsterile, bioburdens need to be within acceptable limits [10]. According to WHO estimates, the present demand for medicinal plant is about US \$14billion a year and by the year 2050 of would be about US \$5 trillion. Now people rely more on herbal drugs because of the high price and harmful side effects of synthetic drugs and this trend is growing not only in developing countries but in developed countries and many

developing countries continued to get benefit from the rich acquaintance of Herbalism. The aim of our study was to examine the microbial contamination in herbal medicines. In India where many people rely upon herbal medicines for medication. Recently the Drug Administration in India has set a standard guideline for manufacturing herbal medicines and the regulators also have finalized the testing criteria to boost herbal sector but the process was slow earlier in the absence of definite testing criteria.

Microbial contaminants of pharmaceutical preparations are a widespread hitch which has been reported for several non-sterile medicaments [11]. The presence of bacteria [12] microbial contaminants in herbal medicines depends on several environmental factors which have an impact on overall quality of herbal products. The microbes isolated and identified in this study such as gram positive bacilli which are ubiquitous but undesirable because of their spoilage potential, and their presence in a product suggests poor environmental hygiene during processing or heavily contaminated or adulterated raw materials [13] and [14]. Fungi like *Aspergillus* and *Rizopus* may be as a result of environmental contamination and bacterial contaminants were predominantly gram-positive bacilli which are in accordance with the results obtained by [15]. Similar findings were recorded by [16] where the microbial contamination might have been caused by poor handling of products during use. *Aspergillus* spp., *Trichoderma* spp., *Penicillium* spp. and *Candida* spp. were responsible for several diseases was also observed [15]. The presence of microbial contaminants in these medicines can reduce or even inactive the therapeutic activity of the product agreeing with our results. Several fatal infectious outbreaks may be associated with the use of heavily contaminated raw materials of natural origin with pathogens leading to secondary infections. During the preparation, standardisation of some physical characteristics such as moisture content, p^H and the assessment of microbial contamination level are mandatory.

Our results disclose that the microbial load in samples used varied considerably where tablets showed high rate of contamination compared to ashokarishta, this is due to self generating alcohols in it. However, the study emphasis on the fact that manufacturers should ensure the lowest possible level of microorganisms in the raw materials, finished dosage forms and the packaging components to maintain appropriate quality, safety and potency of medicines. Evaluation of pharmaceutical and microbial qualities of some herbal medicines in south western Nigeria where 21 different herbal medicines were analyzed [17] and the microbial load of the product varied considerably namely *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. and some fungi.

Our findings correlates with the results obtained [18] with microbial contaminations in herbal medicines collected in Saudi Arabia where the samples showed the presence of predominant *Aspergillus* spp., *Bacillus* spp., *Shigella* spp. and *Enterobacter* spp. Our results are in lines with the works of [19] on fungal contamination of raw materials of herbal drugs marketed in Kaduna metropolis detected from several samples including *Aspergillus* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Escherichia coli* [20]. Supportive studies of [21] on microbial contamination on herbal medicines available in Bangladesh and the presence of microbes in samples thus suggest the fact that aseptic handling is necessary during processing of herbal medicines. Contamination of locally produced herbal medicines sold in Gombe main market and assessment of contamination of herbal products carried was evident that the samples were contaminated with *Staphylococcus aureus* and *Escherichia coli* [22]. The problems may be due to lack of sanitation and the inadequate management of human and animal dejections which are the most important factors of water used to prepare herbal drugs [23]. These findings demonstrate important risks for individuals associated with herbal medicine and the need for shadowing and establishment of stern control procedures in the production/ preparations and marketing of these samples to guarantee quality. Campaigns linked to primary health care units and family health programs with the aim of guiding the proper preparations of herbal medicines should be initiated.

5. SUMMARY:

The medicinal benefits of herbs have been known for centuries and the World Health Organization survey indicated that about 70-80 % of the world population particularly in developing countries rely on non-conventional medicines mainly of herbal origin for their primary health care as these are accessible and cheap. Therefore, the quality and safety of herbal medicinal preparation are of big concern. The menace of herbal contamination has become a growing concern worldwide, particularly Asia and Africa. Herbal preparation methodology and handling may be one of the important factor for contamination and resulting in diseases.

In our study herbal medicinal samples were likely to be contaminated with wide variety of Bacteria and Fungi. Tested samples showed bacterial and fungal contaminants like *Aspergillus* spp. *Paecilomyces* spp. and *Penicillium* spp. and gram positive diplobacilli, gram negative bacilli and gram positive cocci. The presence of large number of bacteria and fungi in herbal medicines may be due to the methods of their preparation or equipment and materials used in preparing the herbal medicines. It is recommended that, though herbal medicines are used as effective means for treatment of various diseases, yet the methods of preparation and handling should be seriously checked to avoid

contamination of these herbal medicinal plants during preparation and package. The quality assurance of these products should be thoroughly enforced and monitored in the production and of herbal preparations.

6. CONCLUSIONS:

The findings reiterate the need for constant quality assessment of herbal materials in the market to ensure that they are safe and suitable for human consumption and do not impose risk on consumer's health. The importance and potential for microbial contamination of pharmaceuticals is now widely recognized in the pharmaceutical industry and attempts to safeguard from contamination include, strict manufacturing practices such as raw material testing, equipment refinement and automation, microbiological testing and validation of water systems, monitoring of the environment, training of personnel, wearing of gloves, masks, hats and laboratory uniforms and packing of products in individual waterproof, tamperproof wrapping employing effective preservatives. The letdown of firm observation of good manufacturing practice at any stage of production may wholly affect the quality of products. Awareness programs should be implemented by national regulatory agencies to prevent or reduce the consumption of products outside the minimum standards of quality. Strategies for health education, campaigns and workshops for public on the safe use of these herbal medicines are imperative for health and patient safety.

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COMPETING INTERESTS: The authors declare no competing interests.

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