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ASSESSMENT OF MYCOFLORA OF RAW MATERIAL USED IN ASAVA AND ARISHTA PREPARATION IN AYURVEDA

Prachi A. Dua[1]*, Dr. Sanjeev D. Patankar[2] and Dr. Vijay S. Wadhai[3]

[1*]Ph.D. Scholar, Dept. of Microbiology, S P. College of Arts, Commerce and Science, Chandrapur.

[2]Principal, S.S. Jaiswal College of Arts, Commerce and Science, Arjuni Mor.

[3]Head of Dept. of Microbiology, S P.College of Arts, Commerce and Science, Chandrapur.

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***Correspondence for Author**
Prachi A. Dua
Ph.D. Scholar, Dept. of Microbiology, S P. College of Arts, Commerce and Science, Chandrapur.

ABSTRACT

The study was designed to investigate and identify the common fungi present on the surface of the raw material used for asava and arishta preparation. The raw materials were collected from the local vendor in the nearby market. Mycological analysis of the samples was carried out for the detection of fungi on the standard media. Some of these isolates obtained are reported to produce certain secondary metabolites. Five different raw materials viz. nagarmotha (*Cyperus rotundus*), sugandhbala (*Pavonia odorata*), dhyatiphool (*Woodfordia fructicosa*), gudwel (*Tinospora cardifolia*) and sunthi (*Zingiber officinale*) were collected. The common fungal isolates obtained belonged to the species Aspergillus, Mucor, Rhizopus, Penicillium and some filamentous yeast were also obtained.

KEYWORDS: Asavas, Arishtas, Mycology, mycotoxins.

INTRODUCTION

The use of Ayurveda is one of the oldest, richest and the most diverse traditions associated with the use of medicinal plants in India.[1] Until synthetic drugs were developed in the nineteenth century, herbs were the basis for nearly all medicinal therapy. Today, herbs are still found in 40% of the prescriptions, and the interest for the use of herbal remedies instead of chemical drugs is increasing because of lesser side effects. Since ancient times, medicinal plants have been considered as main ingredients in ayurvedic formulations for the treatment

of various diseases and they have never lost their importance with emergence of modern science.[2-3]

Woodfordia fructicosa is being used as a source of medicinal agent for antihelminthic, astringent, emetic, febrifuge, sedative and stimulant. The decoction of the flower is used for biliousness, burns, diabetes, hemorrhage, leprosy and skin diseases.[4] *Zingiber officinale* is used for treating diabetes, high blood pressure, cancer and many other illnesses.[5] It contains a number of antioxidants such as β-carotene, ascorbic acid, terpenoids, alkaloids and polyphenols such as flavonoids, flavones, glycosides, rutin etc.[6] Ginger has been used as a spice and as natural additives for more than 2000 years.[7] Also ginger has many medicinal properties. Studies have shown that long term intake of ginger has hypoglycemic and hypolipidaemic effect.[8] In traditional Indian and Chinese medicine, ginger has been used to treat a wide range of ailments including stomaches, diarrhea, nausea, asthama, respiratory disorders,[9] *Cyperus rotundus* is a multivalent plant widely used in traditional medicine around the world for the treatment of various diseases. This herb has been given special recognition in ayurveda due to its multifaceted therapeutic benefits to cure various diseases. Studies have shown that this medicinal plant has several pharmacological activities including anti inflammatory, antidiabetic, antidiarrhoeal, antipyretic, analgesic activities etc.[10-12] *Tinospora cardifolia* is also widely used in Ayurvedic medicines for treatment of various ailments. It is reported that extract of *Tinospora cardifolia* has good immunomodulating effect.[14] It also has the ability to scavenge free radicals and to inhibit radical induced membrane damage.[15] It also has hypoglycemic and hypolipidaemic activity.[16] It has the ability to protect the liver from various diseases,[17] It also has been found to be non- toxic in acute toxicity studies.[18] *Pavonia odorata* is another herb commonly used in Ayurvedic medicines. This plant has anti-inflammatory and spasmolytic effect. The roots are generally used in stomaches, demulscent, astringent. It is used in dysentery, ulcers and bleeding disorders.[19] These raw materials are individually as well used in combination with others for the preparation of different Ayurvedic formulations. As per the regulatory norms, these raw materials should be properly handled so that the product is free from risk. The plant material carry a huge number of bacteria and fungi mainly originating in soil. Aerobic sporulating bacteria frequently predominate in this to which additional contamination and microbial growth occur during harvesting, handling and production.[20-21] Research has also shown a steady and marked presence of fungi in various herbal formulations and their raw materials,[22– 27] High incidences of fungal contaminants may deteriorate the quality of the

products by degradation of phytoconstituents, thereby reducing the potency and shelf life of the drug and rendering it unfit for consumption.[24,28] Besides the deteriorative potential, the presence of fungi in herbal formulations is also a public health issue due to the possibility of mycotoxin production,[25,26,29] As the use of Ayurvedic medicines and these herbs is increasing, there is an urgent need to have knowledge about the safe handling of these preparations.

MATERIALS AND METHODS

a) Source of Samples

A total of 20 samples of *Cyperus rotundus*, *Pavenia odorata*, *Woodfordia fructicosa* and *Zingiber officinale* were randomly collected from the local vendors of the nearby market. The samples were transported to the laboratory immediately and stored in air – tight containers at room temperature till further analysis.

b) Measurement of Moisture Content

Moisture content was measured prior to rinsing of raw materials in distilled water. For moisture content, weighed amount of individual samples were dried at 100^{o} C for 24 hrs. and the difference in weight was calculated according to Essono et al.[13]

$$\text{Moisture Content} = \frac{\text{Initial Wt.} - \text{Final Wt.}}{\text{Initial Wt.}} \times 100$$

c) Mycoflora Isolation

Ten grams of each sample was mixed aseptically in 90ml of sterile distilled water and shaken vigorously. Appropriate serial dilutions were made and 1ml of the dilution was transferred aseptically to sterilized petri-plates containing growth media. For mycobiota analysis, freshly prepared potato dextrose agar medium was used,[24] Triplicate of each sample was incubated at 25 ± 2^{0} C for three days. After incubation, plates were examined visually. Identification of fungal species was done by cultural and morphological characteristics.[30]

RESULTS AND DISCUSSION

A high level of fungal contamination was observed in the herbal samples. The samples were contaminated with fungal and yeast species. Different fungal species belonging to genera *Aspergillus*, *Penicillium* and *Trichoderma* were isolated during the study. Some filamentous yeast were also isolated. *Aspergillus* was the most dominant genus with species *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus terreus*. Species of *Penicillum*,

Rhizopus and *Trichoderma* were present in very few samples. These results are in accordance with the observations of Gautam and Bhaduria[29]. Similar results were observed by Sharma etal,[27] *Aspergillus niger* is the most prevalent species in herbal drug samples,[22,24,26,27,31-39]

These herbal samples high phenolic content. This may be the probable reason of the high occurrence of Aspergilli and low frequency or absence of other fungi. Reports of various researchers also approved the inhibitory effect of phenolics against pathogenic, field fungi and ineffectiveness in controlling the growth of storage fungi, esp. *Aspergillus* group.[40-43]

Being plant material, the herbs are more prone to fungal contamination. The contamination occurs at different stages. Drying and storage are the two important steps that determine the rate of contamination. Any availability of sufficient moisture in the stored material results in microbial contamination. Water availability is the major factor in controlling the microbial contamination of medicinal plant material. Higher moisture content may also be one of the reasons for high fungal contamination. Moisture content along with substrate type, nutrient availability and the presence of secondary metabolite also affect the extent of fungal contamination.[24,35,38]

Duration of storage also has been reported to influence the level of fungal contamination of stored plant commodities,[44] But storage period is not the sole factor in determining the fungal load and diversity; it is coupled with moisture content of the samples. Samples which are properly stored and have low moisture content are less contaminated as compared to the samples with high moisture content. This is in accordance with Oyebangi etal,[45] and Quezada.[46] who reported an increase in fungal load with increasing storage time along with increased moisture content.

The species of *Aspergillus* and *Penicillium* may be a matter of great concern due to the mycotoxic effect of their secondary metabolites. Hence, the presence of wide range of fungi in these medicinally important herbal drugs showed that there is a potential risk for mycotoxins contamination especially during prolonged storage in poor conditions without temperature and moisture control.[47,48]

Taking into consideration the above facts and the increased use of herbal drugs in the society along with poor quality control measures taken by manufacturers and vendors leave a great question mark on the safety on consumers health.[49]

CONCLUSION

In the present investigation, considerable yeast and mould contamination was detected in the herbal drugs selected for study. The presence of these microbes is strongly influenced by the extent of storage and the moisture content of the sample. The fungal contaminants produce mycotoxins which may have adverse effects on the consumer which will be a setback for the Indian herbal industry.

India can come up as the major nation and play the lead role in the production and proliferation of standardized and therapeutically effective herbal formulations. This is possible only if the herbal raw materials are assessed using standard norms and techniques.[49,50] Therefore, the Indian herbal industries need to strengthen their quality control measures for the herbal medicines coming to the local Indian market, so that the contamination may be reduced to the globally expectable limit.

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