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Microbial Contamination in *Musta* (*Cyperus rotundus* Linn.) Sourced From Herbal Drug Markets in Kerala

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Abstract

The plant *Musta* (*Cyperus rotundus* Linn.) holds significant importance in Ayurveda, were the dried rhizome or tuber is widely utilised either singly or in combination with other herbs. Owing to its subterranean nature, there is a potential risk of microbial contamination in the raw drug and subsequent formulations prepared from it. This study is to assess the level of microbiological contamination in the dried tuber or rhizome of *Musta* that is sold in herbal raw medicine market places in the state of Kerala. Nine samples from the commercial sector, representing urban, rural and manufacturing unit sources were collected from North, Central and South zones in Kerala. The microbial proliferation in these samples was analysed with the standards specified in the Ayurvedic Pharmacopoeia of India (API). Upon microbial assessment predominant findings in the market samples included the presence of mixed colonies of Escherichia coli, Bacillus species, Klebsiella species, Staphylococcus and the fungal species *Aspergillus niger*. These results points to the importance of monitoring and addressing microbial contamination of *Musta* to ensure adherence to safety and quality standards.

Introduction

Ayurveda is a science dedicated to maintaining optimal health, alleviating diseases and promoting overall well-being and life balance. Plants play a significant role in achieving objectives of Ayurveda with approximately 90% of Ayurvedic preparations consisting of plant-based ingredients.(1) The efficacy of these medicinal preparations is closely associated with the authenticity, quality and purity of the herbal medications used.

Cyperus rotundus Linn., commonly known as '*Musta*' in Ayurveda, emerges as a versatile herb with rich utility in traditional Indian medicine. Despite being classified as one among the top ten harmful weeds in the world for agricultural fields *Musta* possesses valuable medicinal properties. It serves as a natural reservoir for a diverse range of bioactive phytochemicals, offering multifaceted medicinal and therapeutic benefits.(2) *Musta* is a key ingredient in various Ayurvedic formulations including *Mustharishta*, *Shadangapaniya*, *Balachathurbadra Curna* and *Chyavanaprasha*, to name a few.(3) *Musta* is attributed with properties like *Grahi, Deepana*, *Pachana, Lekhana* and alleviating *Pitha* and *Kapha.*(4)

The microbial burden in plants is the outcome of various factors.

Herbal drugs owing to their origin are susceptible to microbial contamination from soil, air and water eventually paving the way for the introduction of pathogenic microorganisms to humans.[5] Microbial contamination can be influenced by various environmental elements including rainfall, temperature and humidity during pre-and post-harvesting periods. Additionally, handling procedures and place of storage of medicinal plant materials both raw and processed can also have an impact.[5] The microbial presence in pharmaceutical products can lower their therapeutic activity.

The two main types of harmful substances linked to medicinal plants are endospores from bacteria and spores of fungi.[6] It is well known that certain fungi can produce mycotoxins which are harmful compounds when the right conditions are met. Aflatoxin is the most toxic mycotoxin that is currently recognized.[7] The impact of a mycotoxin is determined by its toxicity, the degree of exposure, the age of the individual, nutritional status and any potential synergistic effects with other substances they may be exposed to.[8] Any medicine that has a high moisture content may deteriorate more quickly due to enzyme hydrolysis or microbial development.[9] Therefore, any incorrect processing or storage methods could result in moisture retention in the dried form as well. Thus, an assessment of the contamination by microbes of the dried medicinal plants under marketing is necessary. *Musta* seems to be very susceptible to microbial contamination and hence, the present study is taken up.

MATERIALS AND METHODS

Sample collection:

Kerala is geographically segmented into three zones: North (N), Central (C) and South (S). Three market samples from each of these zones were collected and systematically labelled as NU (North Urban) NR (North Rural) NM (North Manufacturing Unit) CR (Central Rural) CU (Central Urban) CM (Central Manufacturing Unit) SR (South Rural) SU (South Urban) SM (South Manufacturing Unit).

Media Preparation and Sample Evaluation

Microbial contamination in the samples was assessed through the broth method and colony counting method. The Nutrient Broth (HiMedia) was made by dissolving 13g in 100ml of water that was distilled, autoclaving the mixture at 121°C, fifteen lbs for 15 minutes. The process of creating nutrient agar media involved dissolving 37g of nutrient agar in 1000ml of distilled water, then autoclaving at 121°C, fifteen lbs for 15 minutes. After being cooled to 50°C, the media was put on to petri plates that had already been sanitized and labelled. It was then allowed to harden in a Laminar airflow chamber. Each sample (1g) was mixed with 10ml of sterile distilled water, centrifuged at 6000 rpm and the resulting supernatant was collected.

The microbial species were recognized based on their morphology and cultural traits. The overall amount of living bacterial and fungal colony-forming units per gram (CFU/g) was calculated for the assessment of microbial contamination. The discovered microbial load was compared to the permissible values specified in the API.

Results

The results of bacterial evaluation of market sample of *Musta* are depicted in Figure 1 along with corresponding observations detailed in Table 1. Figure 2 illustrates the results of the fungal assessment and the corresponding observations are documented in Table 2.

Table 1: Total amount of Aerobic bacteria found in the *Musta* samples.

Sample	Number of colonies	CFU/g	Identified variant
NR	594	$0.297*10^5$	Mixed colony of Klebsiella species and E. coli
NU	543	$0.2715*10^5$	Mixed colony of Bacillus species and E. coli
NM	201	$0.1005*10^5$	Mixed colony of E.coli and Bacillus species
CR	414	$0.207*10^5$	Mixed colony of E. coli and Klebsiella species
CU	466	$0.233*10^5$	Mixed colony of E.coli and Bacillus species
CM	311	$0.1555*10^5$	Mixed colony of E. coli and Bacillus species
SR	5	$0.0025*10^5$	Colony of Bacillus species.
SU	3	$0.0015*10^5$	Mixed colonies of E.coli and Bacillus species
SM	7	$0.0035*10^5$	Mixed colonies of Klebsiella and staphylococcus species

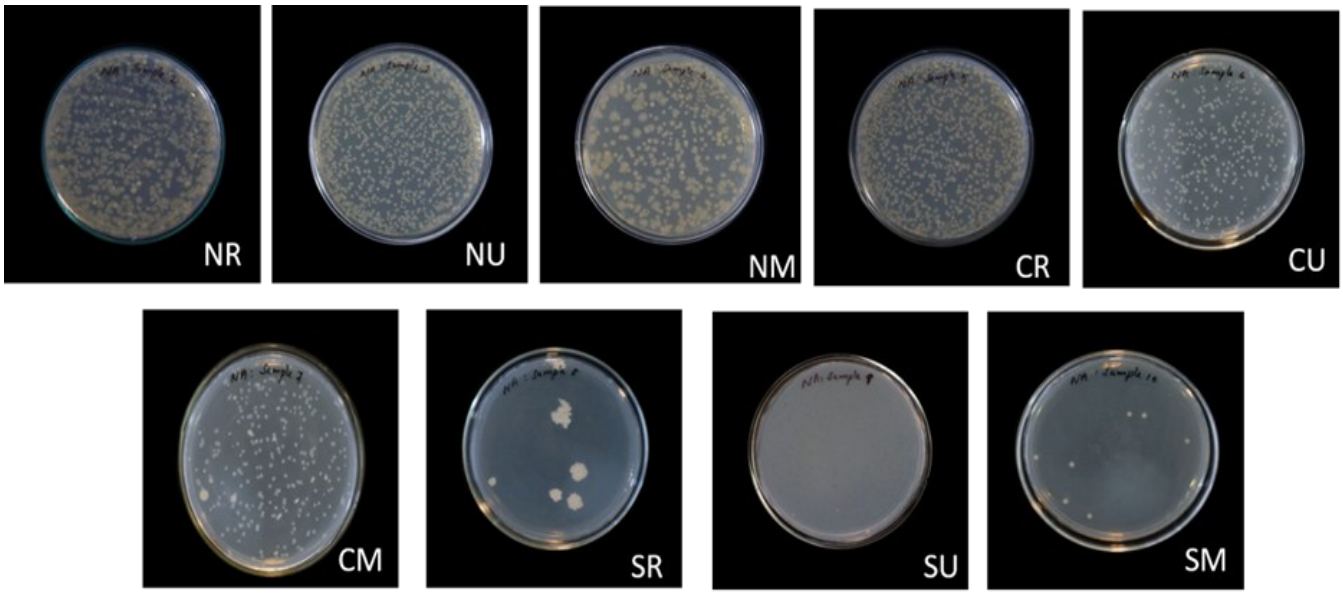

Fig 1 : Aerobic bacterial growth in Market samples of *Musta*

NR Sample **CR Sample**

Fig 2 : Fungal growth in market samples of *Musta*

Discussion

The efficacy of powdered herbal drug is liable to be compromised by the presence of pollutants such as heavy metals, pesticides and microorganisms. This contamination stems from various sources including the growth environment, processing, storage and transportation phases. Intrinsic factors such as plant constitution and extrinsic factors such as harvesting techniques play pivotal roles in determining microbial quality.[6]

In the evaluation of Musta samples from various markets, varied levels of microbial growth were observed. Mixed colonies of E. coli, Bacillus, Klebsiella and Staphylococcus species were noted. Although most samples met API regulations for total bacterial plate count, presence of E. coli violated the API norms. Contamination with E. coli is a matter of concern. Heavy growth of *Aspergillus niger* in the market samples probably caused by prolonged storage under unfavourable condition has the potential to produce Ochratoxin A. which can lead to severe health problems upon consumption.[7] [8]

Microbial presence in stored samples could have stemmed from improper drying techniques heightening moisture levels, prolonged storage periods and the prevailing atmospheric conditions in Kerala marked by high

Fig 2 : Fungal growth in market samples of *Musta*

Sample	Identified variant
NR	*Aspergillus niger*
CR	*Aspergillus niger*

average temperatures and increased relative humidity levels.[9] The efficacy of drug safety and overall standard could all suffer as a result of this relationship.

Conclusion

Despite the microbial count remaining below the allowable threshold set by API, the presence of E. coli, which ideally should be absent, raises concerns regarding the purity of market samples of Musta. This underscores the importance of implementing adequate measures to prevent microbial contamination during the collection, processing and storage of herbal products.

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