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Microbial evaluation of commercial samples of dried *Amalaki* (*Phyllanthus emblica* Linn.) fruit rind procured from Herbal drug market in Kerala, India

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Abstract
Amalaki (*Phyllanthus emblica* Linn.) is one of the most popular and highly reputed drug used in Ayurveda. In Kerala, the dried fruit rind is used either as single or in combinations. In many ailments, it is directly administered for the internal use. As the fresh fruit contains more than 80% of water content, the chances of microbial contamination cannot be neglected in the dried drug and its formulations. The present study was undertaken to assess the microbial contamination in dried *Amalaki* fruit rind available in herbal raw drug markets in Kerala. 28 commercial samples (1 urban and 1 rural) of dried *Amalaki* fruit rind were collected from each 14 districts of Kerala. The microbial growth in the market samples were compared with the dried fruit rind of genuine sample and also with the standards mentioned in API. On microbial evaluation, *Aspergillus flavus* (6/28), *Aspegillus niger* (11/28) and *Escherichia coli*. (2/28) were mainly detected in market samples.

Keywords: *Amalaki*, *Phyllanthus emblica* Linn., Microbial contamination, Herbal drug market, Ayurveda.

1. Introduction
Ayurveda is popularised due the guaranteed safety and efficacy. The fruit of *Amalaki* (*Phyllanthus emblica* Linn., Family-*Euphorbiaceae*) is one of the most popular and highly reputed drug used in Ayurveda and is described as a *vayahsthapana* (anti-aging) drug. It is the main ingredient of many Ayurvedic formulations including the *Triphala curna*, *dhatrilouha*, and *Amruthotharam kashaya* to name a few. It is commonly known as Indian gooseberry. *Amalaki* fruit has multifaceted utilities in pharmaceutics, health suppliments and herbal cosmetics in both fresh and dried form. Its fruits are rich source of Vitamin C and is said to have the properties like *tridoshahara, rochana, deepana, medhya, chaksusya* and *keshya* [1].
Dried *Amalaki* fruit rind is a major ingredient of many Ayurvedic preparations. It was reported that Ayurvedic medicine manufacturer's in Kerala required 4, 00000 Kg *Nellikathodu* (dried *Amalaki* fruit rind) at the rate of 70 Rs per kg [2].
The fresh fruit of *Amalaki* contains more than 80% water content. Moisture is one of the major factors responsible for the deterioration of drugs and products. Low moisture content is always desirable for higher stability of drugs [3] .Presence of high amount of moisture in any drug may facilitate enzyme hydrolysis or enhance the growth of microbes which leads to deterioration[4]. So any improper management during the processing and storage may cause retention of moisture in the dried form also. It affects the stability and finally the quality and safety of the raw drug and thus the medicinal preparations. So the occurrence of harmful microbial growth can't be neglected in the dried fruit rind of *Amalaki* available in the herbal drug market. In many formulations like *triphala choorna*, along with other drugs, dried *Amalaki* fruit rind is used as a major ingredient and is directly administered internally without undergoing any processing. In this situation we cannot ensure the dried *Amalaki* fruit rind available in the market that we use for the medicinal purpose fulfil the safety and quality standards. So it is necessary to evaluate the microbial contamination in the dried *Amalaki* fruit rind available in the herbal drug market in Kerala.

2 Materials and methods
2.1 Sample collection:
The genuine sample of fresh *Amalaki* fruits were procured from Trivandrum district, Kerala and authenticated at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode. Voucher specimens were deposited at JNTBGRI Herbarium (TBGT 84561 dated 7/7/2016).

The sample was thoroughly cleaned, cut into small pieces, dried well and stored in labelled air tight container. Two market samples (One from urban area and one from rural area) were purchased from each of the 14 districts of Kerala. All the 28 market samples were grouped into Sample A (Urban samples) and Sample B (Rural Samples) and each sample of group A and group B was separately kept in labelled air tight containers.

2.2 Sample Evaluation

The microbial contamination in the samples was evaluated using pour plate method and colony counting method. Sabouraud dextrose agar, Nutrient agar, Macconkeys agar and peptone water were used as the culture media. The microbes were identified on the basis of morphological and cultural characteristics. The microbial count detected was compared with the limit values laid down in the API as well as in the WHO guidelines.

3 Results

The microbial contamination was evaluated in each sample and the results are shown in table 1 and figure 1

Table 1: Total microbial count present in the genuine and market samples

Sl. No	Samples	Total bacterial count	Total fungal count
1	Genuine	No growth	No growth
2	TVM A	No growth	20cfu/ml (*Aspergillus niger*)
3	TVM B	11cfu/ml (*Escherichia coli*)	12cfu/ml (*A. niger*)
4	KLM A	No growth	25 cfu/ml (*A. niger*)
5	KLM B	No growth	30 cfu/ml (*A. flavus*)
6	PTA A	No growth	15 cfu/ml (*A. niger*)
7	PTA B	No growth	No growth
8	ALP A	No growth	No growth
9	ALP B	No growth	No growth
10	KTYM A	No growth	35 cfu/ml (*A. flavus*)
11	KTYM B	No growth	No growth
12	IDUKKI A	No growth	No growth
13	IDUKKI B	No growth	No growth
14	EKM A	No growth	14 cfu/ml (*Mucor*)
15	EKM B	No growth	37 cfu/ml (*A. flavus*)
16	THSR A	No growth	34 cfu/ml (*A. niger*)
17	THSR B	No growth	40 cfu/ml (*A. flavus*)
18	PKD A	No growth	18 cfu/ml (*A. niger*)
19	PKD B	No growth	33 cfu/ml (*A. flavus*)
20	MLP A	4cfu/mg (*E. coli*)	No growth
21	MLP B	No growth	No growth
22	KZKD A	No growth	Nil
23	KZKD B	No growth	16 cfu/ml (*A. niger*)
24	WYND A	No growth	Nil
25	WYND B	No growth	34 cfu/ml (*A. niger*)
26	KANR A	No growth	36 cfu/ml (*A. niger*)
27	KANR B	No growth	14 cfu/ml (*A. niger*)
28	KSGD A	No growth	33 cfu/ml (*A. niger*)
29	KSGD B	No growth	27 cfu/ml (*A. flavus*)

- TVM- Trivandrum, KLM- Kollam, PTA- Pattanamthitta, ALP-Alappuzha, KTYM Kottayam, EKM-Ernakulam, THSR- Thrissur, PKD- Palakkad, MLP- Malapppuram, KZKD- Kozhikode, WYND - Wayanad, KANR - Kannur, KSGD-Kasarkode

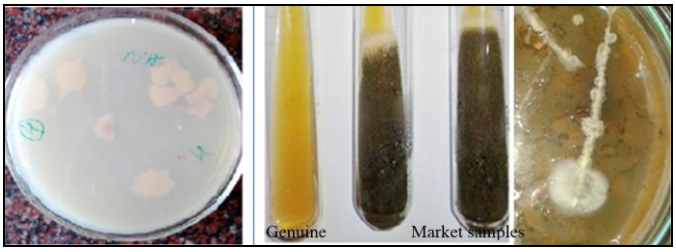

Fig 1: Microbial growth present in market samples

4 Discussion

On microbial evaluation of *Amalaki* samples, most of the market samples (18 /28) contained varying amount of fungal growth when compared to the genuine sample. No bacterial count was detected in genuine as well as market samples except Thiruvananthapuram B and Malappuram A samples. Out of 28 samples, 17 samples had fungal growth. But the total count was far below the limit mentioned in API.

Both raw and powdered herbal drugs harbour wide variety of contaminants including heavy metals, pesticides and microbial pathogens which seems to be the cause of deterioration of the quality of drug. The toxic contaminants may come from environment under which the medicinal plants are cultivated and collected. The conditions under which they are processed, stored and transported also contribute to the problem of toxic contamination.

The microbial contaminants are easily transferred by air and soil borne vectors as well as by irrigating water. The bacterial endospores and fungal spores are the two dominant groups of contaminants associated with medicinal plants. However, a broad diversity of bacterial and fungal cells can be found in the plant material. Among these microorganisms, pathogens may also occur and this fact particularly limits the utilization of these plants, besides quality reduction caused by microbe induced spoilage. Factors that determine the microbiological quality of medicinal plants can be categorized into intrinsic and extrinsic. The intrinsic factors are nature of plant and natural barriers, structure of plant, plant composition (antimicrobial compounds and agents) and intracellular microbial contamination. While extrinsic factors include

humidity, harvest method, post harvesting treatment, packaging and storage conditions and exogenous microbial contaminants [5].

In the present study, the fungi present in the contaminated samples were identified as *A. niger* (11/28), *A. flavus.* (6/28) and *Mucor* (1/28). Maximum fungal count was detected in Thrissur B sample. It was identified as *A. flavus*. Minimum fungal count was detected in Trivandrum B sample and it was identified as *A. niger*. Both the bacteria detected samples had only *E coli*. According to WHO, in plant materials for internal use, the bacterial count should not exceed 10 per gram. In Trivandrum B sample 11 cfu/gm of *E coli* and in Malappuram A sample 4 cfu/gms of *E coli* were detected. As per API, it should be nil. So the contamination in both the samples seems to be harmful.

It was reported that, the presence of wide range of fungi in medicinally important herbal fruits showed potential risk for mycotoxins contamination, especially during prolonged storage in poor conditions of temperature and moisture[6].The moisture content in the market samples may be one of the important causative factor responsible for the incidence of fungal contamination.

A study reported that, among *E. officinalis, T. bellerica* and *T. chebula*, maximum numbers of contaminated samples (7/25) as well as mycotoxins (4/6) were found in *Emblica* powder samples. The broadest spectra of fungal genera and species were recorded in *Amla* fruits (4 genera and 13 species). The genus *Aspergillus* was found to be the most dominant genus encountered with five species viz. *A. flavus, A. niger, A. parasiticus. A. fumigates* and *A. versicolor* while only three species viz. P. *Rubrum, P. Citrinum* and *P. viridicatum* were found in all the samples. The study also showed the potential risk of the use of these herbal fruits and their products for the consumers. *Aspergillus*, *Penicillium* and *Alternaria* are reported to have ability to produce mycotoxins like aflatoxins, ochratoxins and citrinin. Mycotoxins are the cause for the toxicological and immunologic problems in animals and human beings through the contamination of cereals, food and other commodities [6].

Studies have shown that occasional isolates of *A. niger* can produce Ochratoxin A. The presence of *A. flavus* in stored fruits is also alarming since this fungus initially colonizes the substrate and predisposes the infected substrate to mycotoxin contamination. The presence of different mycotoxins is of the matter of concern because aflatoxins are highly toxic, mutagenic, carcinogenic and teratogenic metabolites produced mainly by *A. flavus* and have been implicated as causative agents in human hepatic and extrahepatic carcinomas [6].

In the present study, the genus *Aspergillus* was found to be the most dominant genus with 2 species. Among them *A. niger* (11/28) was seen more frequently than the *flavus* species (6/28). Even though the samples contain only permissible limits of total fungal count it does not rule out its toxic potential.

Unscientific and improper drying, prolonged storage along with climatic conditions in Kerala, where the average temperature and relative humidity are comparatively high may be the factors which favour association of microbes with stored product. Plant materials which are especially rich in carbohydrates and getting less attention in storage are more prone to the attack of vulnerable moulds [7].

It was also reported that the fruits including *Amalaki* are mostly transported from the field to market without taking any care regarding the quality of fruit. In addition, these fruits are stored within open and unclean tin containers in the market, thereby exposing them to microbial infection [6]. Also, as the fresh fruit of *Amalaki* contain 81-85 % of water content, unscientific drying and storage of the drug will results in microbial contamination affecting the quality, safety and efficacy of the drug.

5 Conclusions

Section 9 A of the Drugs and Cosmetic Act, 1940 defines an adulterated drug as the one containing any harmful or toxic substance which may be injurious to health; or if any substance has been mixed with it so as to reduce its quality or strength [8]. The results of microbial evaluation of the market samples to that of the genuine sample and also the standards mentioned in API evidenced that the majority of market samples were contaminated and deteriorated with microbial growth. Hence the study reveals, the medicines prepared with such unsafe and contaminated commercial samples of dried *Amalaki* fruit which does not follow the quality standards can never bring the desired pharmacological actions. So adequate care should be taken in the collection, processing and storage of herbal drugs to avoid microbial contamination and also to get maximum concentration of active principles. The care should be taken during the harvesting also.

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