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Natural Occurrence of Diverse Fungal Species and their Toxins in Dried Fruits of E*mblica officinalis, Terminalia chebula* and *T. bellerica*- Constituents of Triphala, an Important Ayurvedic Preparation of India

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Abstract: *This investigation was designed to throw light on the microbial status of some herbal materials used in 'triphala preparation'. Dried fruit samples of Phyllanthus emblica (Amla), Terminalia belerica (Baheda) and Terminalia chebula (Harada) were collected from random market sources of Jammu & Kashmir state (India). Mycological analysis was carried out for the detection and enumeration of fungi using standard media. Fungal contamination was detected from all the samples. Nearly half of the spoilage fungi belonged to Aspergillus, Penicillium and their perfect states. Four measures of diversity were considered viz., species richness (S), Shannon-Wiener's diversity index (H'), Simpson Dominance index (Cd) and Berger Parker's Dominance index (d') to study the diversity of recovered fungi. Samples were also investigated from mycotoxilogical point of view to identify mycotoxins associated with triphala constituents. In view of the natural fungal contamination that was detected, analyzed samples were also found to be positive for various mycotoxins including aflatoxins (B_1, B_2), ochratoxin A, patulin and citrinin. The high levels of these toxins in dried fruits of E. officinalis, T. bellerica and T. chebula besides reducing the effectiveness of their medicinal value, may also pose serious threat to human beings due to its hepatotoxic, nephrotoxic and cytotoxic effects. On the basis of this study, it is concluded that before making triphala preparations, it is very essential to assess the quality of these three fruits for mycobial and mycotoxin contamination. Further, emphasis has to be laid on various economical ways of decontaminating the herbal drug preparations.*

Keywords: *Triphala, Phyllanthus emblica, Terminalia bellirica , Terminalia chebula , Aflatoxins, Ochratoxin A , Patulin , Citrinin, Medicinal plants.*

I. Introduction

The use of Ayurveda is one of the oldest, richest and most diverse tradition associated with the use of medicinal plants in India (Tandon *et al.*, 2004). In this system of medicine, different parts of the medicinal plants are used in crude as well as powdered form. Over 8000 plant species have been reported for the preparation of some 25,000 formulations that are used to treat various ailments (Dubey *et al*., 2004). According to Ayurveda, perfect digestion is the basis of all health and 'triphala churn' is a wonderful ayurvedic drug that acts as a perfect tonic for proper digestion (Pandey *et al.*, 2008). Triphala literally means "three fruits, in an equiproportional (1:1:1) mixture and this consists of dried fruit powder of three important medicinal plants, namely *Emblica officinalis* Garetn. (Amla), *Terminalia bellerica* Gaertn. Roxb. (Baheda) and *Terminalia chebula* Retz. (Harada). This formulation is based on a formula that can be used to bring balance to all body's constitutions. Amla, one of the three ingredients in triphala, is the richest known natural source of vitamin C, which helps in the production of collagen that is necessary to keep skin supple and thick. Baheda contains tannins, sitosterol and gallic acid used mainly as tonic, laxative, in cough, in piles and dyspepsia. Harada contains 24-32% tannins used to cure acidity and dysentery. The fruit decoction is also used in bleeding and ulceration of the gums (Wealth of India, 1952; 1976). Triphala has many specific effects but it is particularly rejuvenating for the digestive tract and also cures dyspepsia, anaemia, impurity of blood, hyperlipidaemia, skin diseases, excessive heat and irritation of eyes (Juss, 1997).

The constituents of triphala,that is, dried fruit powder of *Emblica officinalis*, *Terminalia bellirica* and *Terminalia chebula* are subjected to fungal contamination at various steps of its preparation. The fruits get contaminated during growth (while the fruits are on tree), after harvesting (when fruits are dried), processing and during storage. Post-harvest spoilage by filamentous fungi is one of the most common threats associated with processed and stored herbal products. The unscientific methods of harvesting, collection, storage of raw materials, processing and poor storage of herbal drugs, retailed in the markets often openly in the unhygienic conditions are the main causes of fungal infections (Essono *et al.*, 2007). The fungal contaminants

have been reported to affect the chemical composition of the raw materials and thereby, decrease the medicinal potency of the herbal drugs (Roy, 2003). In addition, mycotoxins produced by them in these herbal drugs can also cause several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs, etc. (Rai and Mehrotra 2005; Trucksess and Scott, 2008).

Occurrence of mycotoxin contamination in dried fruits of *E. officinalis*, *T. belerica* and *T. chebula* seem to be quite inevitable. The warm and humid climatic conditions of major areas of J&K, along with the poor conditions of extended storage could also promote the germination and growth of storage fungi. Therefore, the present study was conducted to investigate the status of mould and mycotoxin contamination associated with these three important components of triphala in order to assess the possible exposure of local users to the contaminated herbal drugs.

II. Materials And Methods

Realizing the importance of triphala in healing some minor and major ailments and visualizing the existing primitive methods of storing this botanical, selection of three medicinal plants viz., *E. officinalis*, *T. belerica* and *T. chebula* was made for the present study. During the investigation period, dried market samples of the fruits of selected medicinal plants were collected in pre-sterilized polythene bags from various wholesale and retail shops.

Isolation of mycoflora associated with the market samples

Surface mycoflora associated with the market samples of dried fruits of *E. officinalis*, *T. belerica* and *T. chebula* was determined by using surface washing technique (Singh and Kainsa, 1983). In this method, 5g of sample broken into small pieces was taken in an Erlenmeyer flask of 250ml capacity containing 25ml sterilized distilled water and subjected to horizontal shaking for 30 minutes on a rotary shaker. The liquid was then decanted and centrifuged at 3000 r.p.m., for 15 minutes. The residue thus obtained was mixed with 15ml sterilized distilled water and shaken vigorously to obtain a homogenous suspension. This suspension was poured in sterilized Petri-plates at the rate of 1ml / plate with the help of sterilized pipette. For the recovery of maximum number of fungal propagules from the surface of each sample, three different media – Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Dichloran 18% Glycerol Agar (DG 18) and Malt Salt Agar (MSA) were used. The medium was poured by making gentle rotational movements of the Petri-plates so as to ensure uniform spreading of the sample. Petri-plates thus prepared were incubated at 28±2ºC and after 7 days of incubation, observations for the development of different fungal species were made. Percent frequency of each fungal species was calculated by using the formula given below:

$$\text{Frequency (\%)} = \frac{\text{Number of samples from which an organism was isolated}}{\text{Total number of samples tested}} \times 100$$

The data was used in making comparison of different diversity indices. Shannon–Wiener's index (*H'*) (Shannon and Wiener, 1963) and Simpson's Dominance index (*Cd*) (Simpson, 1949) were calculated to determine the heterogeneity of samples (Table 1). Berger- Parker Dominance index (Berger and Parker, 1970) was also calculated to augment the interpretation of Shannon values.

Table 1. Diversity indices.

Diversity indices	Formula used
Shannon–Wiener's index (*H'*)	$-\sum_{i=1}^{s} p_i \ln p_i$
Simpson's Dominance index (*Cd*)	$\sum_{i=1}^{s} (p_i)^2$
Berger- Parker Dominance index (*d'*)	$\frac{n}{N}$

Where, p_i is the relative importance value of species i; n = number of individuals in a species and N = total number of individuals of all species.

Detection of mycotoxins:

Preparation of TLC plates

Glass plates of 20×10cm size were thoroughly cleaned with detergent, air dried and then wiped with cotton moistened in acetone to remove all grease. Silica gel-GF 254 (TLC grade) and distilled water (1:2 w/v) was taken in a stoppered round bottom flask and shaken thoroughly for 2 minutes to make uniform slurry. This

slurry was applied on glass plates with applicator so as to get uniform thickness of 0.25cm. The coated plates were allowed to air dry for 2 hours and then activated in a hot air oven at 110°C for 1 hour. These plates were then cooled to room temperature and stored in a desiccator till further use.

Extraction of mycotoxins from dried medicinal plant samples

Samples of dried fruits of *Emblica officinalis, Terminalia bellerica* and *T. chebula* obtained from different market were analysed for mycotoxin contamination by multi-mycotoxin detection method developed by Stoloff *et al*. (1971). In this method, 25g of finely ground sample was taken in 250ml Erlenmeyer flask containing 100ml mixture of acetonitrile and 4% potassium chloride (90:10v/v). This solution was put on a mechanical shaker for 30 minutes and then filtered through Whatman no. 41 filter paper. 50ml of this filtrate was taken in 250ml separating funnel, defatted and extracted twice with 50ml of iso-octane. The upper iso-octane layer was discarded. To the lower acetonitrile layer, 12.5ml of water was added, shaken and extracted thrice with 20ml of chloroform each time. The chloroform acetonitrile layer was filtered through Whatman no. 41 filter paper having a bed of anhydrous sodium sulphate. The extract was collected in a beaker and then evaporated to dryness on a water bath. The residue was dissolved in 1ml of benzene : acetonitrile (98:2 v/v) solution and stored in a clean screw cap vial for quantitative and qualitative analysis.

Known amount of sample extracts and standards of investigated mycotoxins obtained from Sigma Aldrich Co. were spotted on activated TLC (Thin Layer Chromatographic) plates and developed with different solvent systems.

Estimation of aflatoxins

For qualitative estimation of aflatoxins, known amount of sample extracts (50µl) were applied on activated TLC plates with the help of a micropipette and then developed with a solvent system consisting of toluene: iso-amyl alcohol : methanol (90: 32 :2 v/v). Various spots of aflatoxins were located under long wave UV light (365nm) and marked with a sharp needle. The standard aflatoxins (B_1, B_2, G_1 and G_2) were also spotted on the TLC plates as reference spots. After comparing the fluorescence colour and Rf value, chemical confirmation of aflatoxins was done by spraying 0.25% H_2SO_4 which changed the blue fluorescent spots to yellow (Stack and Pohland, 1975).

Quantitative estimation of aflatoxins was done spectrophotometrically by following the method of Nabney and Nesbitt (1965). Silica gel of each spot was scraped and collected individually in clean dry centrifuge tubes. For extraction, 5ml of cold methanol was added to each tube and centrifuged at 3000 r.p.m. for 3 minutes. Aflatoxin content was determined by recording the ultraviolet absorption spectrum of the methanolic solution in ELICO (SL 164 Double Beam) UV / VIS spectrophotometer set at 360nm for aflatoxin B_1 and 362nm for aflatoxin B_2.

Estimation of ochratoxin A

Known amount of sample extract (50 µl) was spotted on the activated TLC plate along with ochratoxin A (OTA) standard solution and developed in a solvent system consisting of toluene : ethyl acetate : 90% formic acid (50:40:10 v/v). Under long wave UV light, OTA is observed as a bluish green spot. Confirmation of OTA was done with ammonia fumes, which changes the blue-green fluorescent spot to deep blue colour.

For quantitative estimation, detected spots of ochratoxin A were marked and scraped from the TLC plate. Extraction of ochratoxin A was done with 5ml cold methanol by centrifuging it at 3000 r.p.m. for 3 minutes and its concentration was determined spectrophotometrically at 333nm (Bacha *et al*., 1988).

Estimation of citrinin and patulin

Qualitative estimation of citrinin and patulin was done by applying known amount (50µl) of sample extract on the activated TLC plate with the help of a micropipette. Standards of patulin and citrinin were also spotted on the TLC plates as reference spots. The loaded plates were developed in a solvent system consisting of toluene : ethyl acetate : chloroform (80:70:50 v/v) and 1 ml of 90% formic acid.

For the detection of citrinin, developed plates were directly visualized under 365nm UV light and they showed yellow fluorescent spots. Further, chemical confirmation of citrinin was done by spraying aluminium chloride (dissolved 2g reagent grade $AlCl_3 6H_2O$ in 100ml of ethanol) on the plate. Heated it for 5 minutes at 110°C, cooled it and observed under 365nm UV light. This chemical test changed the yellow fluorescence of citrinin to sky blue fluorescence (Gimeno and Martins, 1983). Quantitative estimation of citrinin was done by marking yellow fluorescent spots under long wave UV light (365nm), scraped them individually in clean, dry centrifuge tubes, dissolved them in 5ml of cold methanol and centrifuged at 3000 r.p.m. for 3 minutes. Colour intensity of supernatant was immediately determined in a spectrophotometer (Bacha *et al*., 1988).

For the detection of patulin, developed TLC plates were sprayed with freshly prepared phenylhydrazine hydrochloride (by dissolving 2g in 100ml of water) and then heated the plate for 5 minutes at 110°C

(Subramanian, 1982). Under visible light, patulin showed yellow spots. Quantitative estimation of patulin was done by spectrophotometric method as given by Bacha *et al*. (1988). In this method, silica gel of each yellow spot was scraped in a clean dry centrifuge tube, dissolved in 5ml of n-butanol and centrifuged at 3000 r.p.m. for 3 minutes. Colour intensity of supernatant was immediately determined in a spectrophotometer at 540nm (Subramanian, 1982).

III. Results and Discussion

Analysis of associated mycoflora

During the period of investigation, a total of 90 market samples of dried fruits of *Emblica officinalis, Terminalia bellerica* and *Terminalia chebula* were collected in pre-sterilized polythene bags from eight districts of J&K State (viz., Kathua, Jammu, Udhampur, Rajouri, Poonch, Doda, Srinagar and Leh). These samples were screened for the mycobial load by using surface washing technique and three media (dichloran rose bengal chloramphenicol agar, dichloran 18 percent glycerol agar and malt salt agar) having different chemical composition. While recovering surface mycoflora, dichloran rose bengal chloramphenicol agar medium could trap maximum number of fungal species, dichloran 18% glycerol agar medium helped to recover a wide range of non-fastidious xerophilic fungi including most of the *Penicillium* and *Aspergillus* species, whereas, malt salt agar was more useful in recovering the members of *Aspergillus glaucus* group. This indicates that nutritional requirements of various fungi differ and there is no single medium, which can help in the recovery of all the fungi.

Analysis of mycobial load of dried market samples of *Emblica officinalis* (amla) collected from eight districts of Jammu and Kashmir State shows that a very large number of fungal species (55) were associated with them, which belonged to 23 fungal genera (table 2). Nearly half of the spoilage fungi belonged to *Aspergillus, Penicillium* and their perfect states. Two fungal species, *Ascotricha guamensis* and *Acremonium sp.* were recovered only from the dried fruit samples of *E. officinalis* indicating thereby, the substrate specificity of these fungal species. Fluctuation in the number of fungal species recorded in the present investigation may be due to the fluctuating storage conditions and the length of storage period.

Dried fruits of *Terminalia bellerica* (bahera) which form the second important component of 'triphala' were also analysed for mycoflora. Perusal of data presented in table 2 shows that their fruit samples collected from different districts of Jammu and Kashmir State elaborated a total of 42 fungal species belonging to 20 genera. As observed in other medicinal dried fruits, *Aspergillus niger* was recovered from all the samples collected from eight districts. Association of some fungal species like *Chrysosporium luteum, Fusarium pallidoroseum, Penicillium vinaceum, Scopulariopsis candida* and *Phoma sorghina* was detected only with these samples.

Dried fruits of *Terminalia chebula* commonly known as 'harad' form the third important component of 'triphala'. In addition, they are also used in a number of other herbal formulations. Dried fruit samples of *T. chebula* collected from eight districts of J&K State revealed an association of 45 fungal species belonging to 19 genera. Of these, 4 fungal species were dominant, 4 fairly dominant, 7 sub-dominant and 30 were observed to be of rare occurrence. *Aspergillus niger* was recovered from all the dried fruit samples of *T. chebula*, indicating the ubiquitous nature of this fungal species. In addition, *Sartorya fumigata, Beauveria* sp. and three species of *Drechslera* viz., *D. sorokiana, D. revenelli* and *D. teres* were recovered only from this host.

During the market survey conducted in eight districts of Jammu and Kashmir State, dried fruits of *E. officinalis, T. bellerica* and *T. chebula* were found stored in open metal/plastic containers/ wooden boxes/gunny bags or on the bare ground. This may expose the dried herbals directly to biotic environmental factors**,** air pollutants and other contaminants including microbial contamination (Essono *et al*., 2007). Variation in morphological appearance, that is, change in colour texture and appearance was also found in triphala powder and its three components, which may be due to prolonged storage under unfavourable environmental factors**.** Fungal infection in some fruits was visually noticed, which was possibly due to injuries during harvesting, transportation and drying. Presence of 73 fungal species belonging to 28 genera revealed the broad mycobiota association with stored Triphala ingredients. Species of *Aspergillus* and *Penicillium* were reported as dominating mycoflora in stored herbal drugs (Mandeel, 2005) which was also seen in present study.

Assessment of Diversity indices

Diversity indices computed for the fungal species recovered from market samples of dried fruits of *Emblica officinalis, Terminalia bellerica* and *Terminalia chebula* are given in table 3. These diversity indices show differences in values of species richness (*S*), Shannon-Wiener's diversity index (*H'*), Simpson dominance index (*Cd*) and Berger-Parker's dominance index (d). However, the values of diversity indices for *Terminalia bellerica* and *Terminalia chebula* are more or less similar, which may be due to similar kind of nutrients and similar substrate composition in the fruits of *Terminalia* species that elaborate similar kind of fungal diversity. Higher species richness (*S*) and Shannon-Wiener's diversity (*H'*) was recorded in *E. officinalis* (*S* = 55 species

and *H'* = 1.634) as compared to those for *Terminalia bellerica* and *Terminalia chebula* (*S* = 42 and 45 species and *H'* = 1.534 and 1.524 respectively). The detection of these high values from the surface of dried fruits of *E. officinalis* reveals that it is a very good source of nutrients for the surface growing moulds. Moreover, dried fruits of *E. officinalis* absorb moisture readily from the air, which induces germination and thus increases the fungal inoculum responsible for biodeterioration, thus leading to higher diversity of the fungal species. Low fungal load in *Terminalia* can also be attributed to its greater antifungal activity (Chattopadhyay and Bhattacharyya, 2007). Contrary to this, low values for Simpson dominance index (*Cd* = 0.028) and Berger Parker dominance index (*d'* = 0.053) were obtained for *E. officinalis* in comparison to *Terminalia bellerica* and *Terminalia chebula* (*Cd* =0.033 and 0.035 and *d'* =0.061 and 0.065 respectively) which is probably due to the fact that *Aspergillus niger* was recovered from all the *T. bellerica* and *T. chebula* samples collected from eight districts of Jammu and Kashmir. Thus more representation of *Aspergillus niger* than species of other fungal genera lead to high Simpson dominance values of these plants.

Analysis of associated mycotoxins

Among the investigated dried fruit samples of *Emblica officinalis*, 13.0 percent were positive for aflatoxin contamination but the amount of toxin detected in the positive samples was quite high with afla B_1 ranging from 0.74 to 2.12mg/kg and afla B_2 ranging from 0.30 to 1.07mg/kg (Table 4). This suggests that dried fruits of *E. officinalis* are very suitable for aflatoxin production, provided the storage conditions are favourable and there is presence of toxigenic *A. flavus* spores. Perusal of data presented in table 3 also shows that about 20.0 percent dried fruit samples of *Terminalia bellerica*, were found to be positive for aflatoxin contamination. Range of afla B_1 varied from 0.32 to 1.47 mg/kg, whereas, that of afla B_2 was comparatively less, varying between 0.11 to 0.74 mg/kg. Dried fruit samples of *Terminalia chebula* also revealed the presence of both aflatoxin B_1 and B_2 . The concentration of afla B_1 ranged between 0.50 to 1.27mg/kg whereas, afla B_2 was detected (0.48mg/kg) from only one market sample. Presence of high concentration of aflatoxins in the three constituents of 'triphala' viz., *Terminalia chebula, T. bellerica* and *E. officinalis* is a matter of great concern as these are commonly used in Ayurvedic medicines. Earlier, aflatoxin contamination from constituents of 'triphala' have been recorded by Singh (2003) and Gautam and Bhadauria (2011).

Detection of aflatoxin B_1 and B_2; from triphala ingredients justifies the presence of *Aspergillus flavus* and *A. parasiticus* from the samples. Earlier, some other researchers have also reported aflatoxin contamination from drugs of plant origin (Aziz *et al*., 1998; Gautam and Bhadauria, 2008). Detection of aflatoxins from herbal drugs is of concern as they are hepatotoxic, immunosuppressive, carcinogenic and mutagenic for consumers. Aflatoxin B_1 is the most potent mycotoxin evaluated as a Group 1 carcinogen (Shephard, 2004). In the present investigation, the dried fruits of the tested medicinal plants were contaminated with very high concentration of aflatoxins in comparison to the permissible tolerance limits of 30 ppb (van Egmond and Jonker, 2004; European Commission, 2006). However, the magnitude of aflatoxin contamination varied with the type of dehydrated medicinal plant, storage practices, geographical factors, seasonal changes and varying aflatoxigenic potential of the associated *A. flavus* strains. Similar observations were recorded by Bilgrami *et al.* (1983) while investigating some dehydrated commodities from Bihar.

Market samples of dried medicinal plants were also investigated for the contamination of ochratoxin A (OTA). Among the investigated dried fruits, at least 20 percent samples of *E. officinalis* were contaminated with OTA and the range of contamination in positive samples varied from 0.23 to 0.83mg/kg. In case of dried fruits of *Terminalia bellerica* and *T. chebula*, only 10 percent samples were contaminated with OTA. The range of OTA contamination in *T. bellirica* varied from 0.63 to 1.05mg/kg, whereas in *T. chebula* it was slightly less, varying from 0.42 to 0.78mg/kg. Reports of OTA contamination from dried medicinal plants and their crude products have been provided by other ethanomycologists also (Roy and Chourasia 2001; Singh, 2003).

Data presented in table 4 also indicates that among the investigated mycotoxins, patulin contamination was detected to be maximum in the investigated samples. Dried fruits of *Terminalia chebula* and *T. bellerica* proved to be excellent substrates for patulin production as in both the cases, 76 and 63 percent of the collected samples respectively were positive for this toxin. In addition, the positive samples of *T. bellerica* and *T. chebula* showed a very high level of patulin concentration (upto31.0mg/kg and 36.0mg/kg respectively). Similarly, atleast 66 percent of dried fruits of *E. officinalis* were found to be positive for patulin contamination and a concentration as high as 33.75mg/kg was detected. So far, very high patulin contamination has been reported only from rotted rosaceous fruits and their products (Beretta *et al*., 2000; WHO, 2000; Singh, 2002). But in the present investigation, patulin appeared as the second major mould contaminant and the concentration detected was more than the specified action level of 10µg/kg and 50µg/kg (EC, 2006; Lawley, 2013). As most of the herbal formulations are used in the powdered form without processing or with minimal processing, detection of patulin in dried medicinal plants may pose a health hazard as patulin is a heat stable mycotoxin and can survive pasteurization process like other mycotoxins (IARC, 1986; Harrison, 1989).

Citrinin contamination was also detected from the samples, the maximum being 30 percent in dried fruits of *E.officinalis* , which amounted to 2.07mg/kg. Dried fruit samples of *Terminalia bellirica* showed 13.33 percent contamination and the range of citrinin concentration varied from 0.22 to 1.16mg/kg. Only 6.7 percent samples of *Terminalia chebula* were found to be positive for citrinin contamination. Quantitative analysis of citrinin from these positive samples showed a contamination range upto 0.40mg/kg. It was further observed that in many samples of *Emblica officinalis, Terminalia bellerica* and *Terminalia chebula*, citrinin co-occurred with patulin .

IV. Conclusion

Prolonged storage of medicinally important herbal raw material and drugs in unfavourable climatic conditions increases the association of mycotoxic fungi and their mycotoxins, which may pose a potential risk to consumers health. Looking into the importance of 'triphala churn' and its ingredients in curing various human ailments and an increasing number of consumers relying on them, it is a necessity to undertake safety measures against fungal and mycotoxin contamination of raw materials. In view of this, it is essential to scrutinize these herbal raw materials for mycotoxic contaminants before processing them. Moreover, even after processing these herbal drugs, contamination by mycotoxins need to be checked prior to packing and launching for public use.

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.**Table 2** : Percentage frequency of mycoflora associated with dried fruits samples

Fungal species	***Emblica officinalis***	***Terminalia bellirica***	***Terminalia chebula***
Acremonium sp.	37.5	-	-
Alternaria alternata	62.5	37.5	50.0
Ascotricha guamensis	12.5	-	-
Aspergillus flavus	87.5	75.0	87.5
A. flavus var. columnaris	12.5	-	-
A. fumigatus	50.0	75.0	87.5
A. japonicus	12.5	-	12.5
A. niger	87.5	100.0	100.0
A. niveus	12.5	-	12.5
A. ochraceus	12.5	12.5	25.0
A. sydowii	62.5	75.0	87.5
A. tamari	25.0	25.0	-
A. terreus	37.5	37.5	25.0
A. ustus	12.5	-	12.5
A. versicolor	-	25.0	-
A. viridae-nutans	12.5	-	-
Beauveria sp.	-	-	12.5
Botryophialophora sp.	37.5	-	-
Chaetomium brasiliense	12.5	-	-
C. cochliodes	12.5	-	-
C. globosum	12.5	37.5	25.0
C. olivaceum	12.5	12.5	-
Chrysosporium luteum	-	12.5	-
Cladosporium cladosporioides	25.0	25.0	37.5
C. oxysporum	-	25.0	12.5
C. sphaerospermum	75.0	87.5	62.5
Curvularia brachyspora	50.0	37.5	12.5
C. lunata	75.0	50.0	50.0
Drechslera australiensis	-	-	12.5
D. hawaiiensis	25.0	25.0	-
D. ravenelli	-	-	12.5
D. teres	-	-	12.5
D. sorokiana	-	-	12.5
Emericella nidulans	37.5	50.0	62.5
E. echinulata	12.5	-	12.5
E. rugulosa	-	12.5	-
E. quadrilineata	12.5	-	-
E. variecolor	25.0	-	-
Eurotium amstelodami	87.5	62.5	75.0
E. chevalieri	37.5	62.5	12.5
E. rubrum	12.5	62.5	25.0
Fusarium pallidoroseum	-	12.5	-
F. solani	75.0	-	12.5
F. subglutinans	37.5	-	-
Lasiodiplodia theobromae	12.5	-	-
Microascus cinereus	12.5	12.5	-

Monascus ruber	12.5	-	-
Mucor geophilus	-	25.0	-
M. mucedo	25.0	37.5	12.5
Nodulisporium didymosporum	12.5	12.5	-
Paecilomyces herbarum	12.5	-	12.5
P. variotii	25.0	-	12.5
Penicillium brevicompactum	25.0	25.0	50.0
P. chrysogenum	25.0	62.5	50.0
P. citreonigrum	-	-	12.5
P. citrinum	50.0	50.0	50.0
P. corylophilum	12.5	-	-
P. implicatum	12.5	-	-
P. islandicum	37.5	25.0	12.5
P. oxalicum	12.5	25.0	25.0
P. paxilli	-	-	12.5
P. purpurogenum	12.5	-	12.5
P. restrictum	-	25.0	12.5
P. vinaceum	-	12.5	-
Phoma sorghina	-	12.5	-
Rhizopus oryzae	12.5	-	-
R. stolonifer	37.5	37.5	50.0
Scopulariopsis brevicaulis	37.5	50.0	25.0
S. candida	-	12.5	-
Syncephalastrum racemosum	37.5	12.5	75.0
Sartorya fumigate	-	12.5	12.5
Thielavia sepedonium	-	12.5	-
T. terricola	12.5	-	12.5
Trichoderma harzianum	12.5	-	12.5
No. of fungal species recovered	**55**	**42**	**45**

-, not detected

Table 3: Diversity indices computed for the recovered fungal species.

Diversity indices	*Emblica officinalis*	*Terminalia bellirica*	*Terminalia chebula*
Species richness (*S*)	55	42	45
Shannon-Wiener's diversity index (*H'*)	1.634	1.534	1.524
Simpson dominance index (*Cd*)	0.028	0.034	0.037
Berger-Parker's dominance index (d')	0.053	0.067	0.07

Table 4: Mycotoxins detected from dried fruit samples of *Emblica officinalis, Terminalia bellerica, Terminalia chebula.*

Medicinal plants	Samples found positive for mycotoxin contamination (mg/kg)				
	AFB_1	AFB_2	OTA	PAT	CIT
Emblica officinalis	2 (0.74-2.12)	2 (0.30-1.07)	6 (0.23-0.83)	20 (33.75)	9 (2.07)
Terminalia bellirica	3 (0.32-1.47)	3 (0.11-0.74)	3 (0.63-1.05)	19 (31.0)	4 (0.22-1.10)
Terminalia chebula	3 (0.50-1.27)	3 (0.48)	3 (0.42-0.78)	23 (36.0)	2 (0.40)

-, not detected

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