

Original Article

COMPARATIVE STUDY OF MYCOFLORA, ANTIBACTERIAL ACTIVITY AND PHYTOCHEMISTRY OF SELECTED FRESH AND STORED MEDICINAL FRUITS

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ABSTRACT

Objective: To understand the mycoflora, phytochemical constituents and antibacterial potential of fresh and stored herbal fruits of selected plants, extensively used in herbal medicines.

Methods: Mycoflora analysis of *Terminalia bellerica*, *Phyllanthus emblica* and *Myristica fragrans* fruits was done employing serial dilution method, colony forming unit (CFU) and relative density of each fungi was recorded. The diversity of fungi associated with test fruits was calculated using various diversity indices. Methanol extracts of test fruits was subjected to preliminary phytochemical analysis, presence or absence of alkaloids, flavonoids, tannins, saponins, terpenoids, quinones and cardiac glycosides was detected. Antibacterial potential of test fruits was studied by well diffusion method.

Results: Fresh fruits were free of fungal contamination, whereas stored fruits were found in association with various fungi. A total of 64 isolates of 29 species belonging to 13 genera were identified from stored fruits. *Aspergillus* was predominant followed by *Penicillium*. *T. bellerica* was highly contaminated (1×10^5 CFU/g). Stored fruits of *M. fragrans* recorded high fungal diversity with highest Simpson's diversity index ($D-1=0.840$) and Shannon-Wiener index ($H=2.888$). *P. emblica* exhibited high species richness with high Margalef Richness ($R=2.925$), Menhinick index ($E=1.830$) and Berger Parker Dominance ($d'=0.418$). Phytochemical analysis of fresh and stored fruits did not show a significant difference in the presence or absence of tested phyto-constituents. Stored fruit extract recorded up to 41% increase in antibacterial activity.

Conclusion: Results suggests the need of proper training to the herbal material handler's right from the harvest to retail selling, and also points out the need to assess the fungal contamination of herbal materials before using them for herbal drug manufacture.

Keywords: Medicinal fruits, Storage condition, Mycoflora, *Aspergillus niger*, Bioactivity

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INTRODUCTION

Plants have been used in the prevention, treatment and cure of disorders and diseases since ancient times [1]. Medicinal plants are widely used as raw material for pharmaceutical preparations and as a supplement for dietetic products, specifically for self-medication [2]. In recent decades, the use of herbal preparations has increased in developing and developed countries, due to the belief that being natural, they are harmless [3]. With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these herbal materials and preparations, the safety and quality of raw materials have become a major concern for health authorities, pharmaceutical industries and the public [4]. The quality of raw material used has a direct bearing on the safety and efficacy of the herbal drug.

Unfortunately, reports of people experiencing negative effects, caused by the use of herbal drugs, has been increasing. One of the reasons for such problem could be the poor quality of herbal drug raw material and insufficient attention being paid to the quality assurance and control of these herbal medicines. Although world health organisation (WHO) has developed guidelines for the quality control of herbal drugs which provide a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants, there is still a lacuna between the available knowledge and implementation, because farmers and other relevant persons like producers, handlers and processors of herbal drugs are not much aware of WHO's guidelines. As a result, the quality control measures are ignored by the practitioners, resulting in an inferior quality of herbal drugs with lots of contaminants like heavy metals, pesticides and microbes [5].

Many of the herbal drugs are plant products, and they can be infested by bacteria and fungi, especially moulds. Unscientific method of cultivation and collection, inappropriate harvesting and cleaning, unsuitable transportation, inadequate drying and storage,

poor hygiene of producer and congenial climatic conditions render the raw plant materials prone to microbial contamination in general and fungal infestation in particular [6]. Fungi are the predominant contaminants that survive during drying and storage. Mould contamination has been reported to alter the phytoconstituent of herbal drug raw materials [7] which in turn alters the therapeutic value of the herbs.

Considering these, three fruits widely used in herbal medicine viz., *Terminalia bellerica* (Gaertn.) Roxb. *Phyllanthus emblica* Linn. and *Myristica fragrans* Hoult. were selected for the study to understand the changes if any in the mycoflora, phytochemistry and antibacterial activity of fresh and stored fruits.

MATERIALS AND METHODS

Collection of samples

Dried and stored fruit samples of *Terminalia bellerica* (Gaertn.) Roxb. *Phyllanthus emblica* Linn. and *Myristica fragrans* Hoult. were randomly collected from different retail shops of the Mysuru city (Geographical coordinates in decimal degrees: Latitude 12.2979100° and Longitude: 76.6392500°). Fresh fruits of *P. emblica*, *T. bellerica* and *M. fragrans* were collected from Biligiri Ranga hills (Geographical coordinates in decimal degree: Latitude 11.9° and Longitude: 77.233333°) and Chandravana herbal farm of Mysuru (Geographical coordinates in decimal degree: Latitude 12.2979100° and Longitude: 76.6392500°). The samples were collected in sterilized lock covers to avoid further contamination and stored in airtight containers at 4°C until further analysis. All the specimens were deposited at Centre for Innovative Studies in Herbal Drug technology, DOS in Botany, University of Mysore, Mysuru and voucher number was obtained as MGB-CISHDT-RR-TB-SF-M-0008a, MGB-CISHDT-RR-PE-SF-M-0006a and MGB-CISHDT-RR-MF-SF-M-0005a for stored fruits of *T. bellerica*, *P. emblica* and *M. fragrans*

respectively, and MGB-CISHDT-RR-TB-FF-M-0008b, MGB-CISHDT-RR-PE-FF-M-0006b and MGB-CISHDT-RR-MF-FF-M-0005b for fresh fruits of *T. bellerica*, *P. emblica* and *M. fragrans* respectively.

Chemicals and reagents

Chemicals/Reagents: Source

Nutrient Agar, Nutrient Broth and Czapek Dox Agar: HiMedia Laboratories Pvt. Ltd., Mumbai.

Mercuric chloride: Chemicals division, Glaxo Laboratories (India) Ltd., Mumbai

Ferric chloride, Potassium iodide and Sodium iodide: Fisher Scientific, Thermos electron LLS India Pvt. Ltd, Navi Mumbai

Hcl: Qualigens fine chemicals glaxosmithkline pharmaceutical limited, Mumbai

Bismuth carbonate, Lead acetate, Zinc, H₂SO₄ and Acetic anhydride: Fisher Scientific India Pvt. Ltd. Sion (East) Mumbai

Sodium bicarbonate, Glacial acetic acid: Sisco Research Laboratory Pvt. Ltd. Bombay

Methanol, Chloroform and Ethyl acetate: SDFCL sd fine chemicals limited, Mumbai

Mycoflora analysis

Serial dilution method [8] was employed to determine the colony forming unit (CFU) of fungi. One gram of each sample transferred into 20 ml screw capped bottles containing 9 ml of sterile distilled water and was mechanically homogenized at a constant speed for 15 min on an electronic shaker. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate ten-fold serial dilutions (1:10) were prepared and 1 ml aliquot of each dilution was aseptically surface plated and distributed uniformly on culture medium with the help of sterilized L-shaped glass spreader. Freshly prepared czapek dox agar (CDA) medium served as the culture medium. Plates were incubated at 28±2 °C for 7 d and observed daily. Subsequently, the CFU, diversity and density of the fungi were recorded. Morphologically different mould colonies were individually sub-cultured by hypha tip method on CDA medium and their pure cultures were maintained.

Colony forming units of fungi per gram of fruit (CFU/g) was calculated using the formula

CFU = $N \times 10^{-n}$ where N = total no of colonies, n = dilution [9]

All the isolated fungal species were identified on the basis of their cultural and morphological characteristics with the help of standard manuals.

The relative density of each fungi was calculated using the formula:

$$\text{Relative density of fungi} = \frac{\text{No. of colony of individual fungus}}{\text{Total no. of colonies of all fungal species}} \times 100$$

Calculation of diversity indices

Shannon Weiner index (H) [$H' = -3.3219 \sum n_i/N \log n_i/N$], Margalef's richness index (R) [$d = S-1/\log N$] and Menhinick index [$D_{mm} = S/\sqrt{N}$] was calculated to determine the Species richness of fungi in the herbal fruits. Evenness of fungal distribution was calculated through Berger Parkers dominance index (d') [$d' = n_{max}/N$]. The diversity of fungi was determined by Simpson's diversity index (1-D) [$1-D = 1 - \sum n_i(n_i-1)/N(N-1)$]. Online webpage calculator (http://www.alyoung.com/labs/biodiversity_calculator.html) was used for the calculation of diversity indices.

Extract preparation

The fresh and stored fruits of *P. emblica*, *T. bellerica*, and *M. fragrans* were powdered with the help of a waring blender. 100g of each of the powder was extracted with methanol by cold extraction method. The extract was concentrated by evaporation and preserved at 4 °C

until subjected to antibacterial activity assay [10] and phytochemical analysis.

Preliminary phytochemical analysis

The methanol extracts of the fruits were subjected to the detection of alkaloids, flavonoids, tannins, saponins, terpenoids, quinones and cardiac glycosides following the methods of Trease *et al.*, and Sofowara [11, 12].

Test bacteria

Authentic pure cultures of phytopathogenic bacteria *Xanthomonas campestris* pv. *vesicatoria* (MTCC 2286) and *Xanthomonas campestris* pv. *campestris* (NCIM 5028) were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh and National Centre of Industrial Microorganism, NCL, Pune respectively. *Xanthomonas oryzae* pv. *oryzae*, isolated from the diseased plant was obtained from the culture collections of Centre for Innovative Studies in Herbal Drug Technology, Department of Studies in Botany, University of Mysore, Mysuru. All the test bacteria were sub-cultural on nutrient agar. Two-day old nutrient broth cultures of the test bacteria were used for assay.

Antibacterial activity assay

Antibacterial activity of methanol extract was determined by well diffusion method on nutrient agar medium. Wells (6 mm) were made in nutrient agar plates using sterile cork borer and 50µl of inoculums of test bacteria was spread on the solid plates with a sterile swab. Then 100µl of the extract was poured into the wells. The treatment also included 100µl of methanol separately which served as control. The plates were incubated for 24 h at 37 °C and zone of inhibition if any around the wells were measured in mm and recorded [13].

RESULTS

Mycoflora analysis

Following serial dilution method, a total of 64 isolates of fungi were recorded from the stored herbal fruits while fresh fruit samples were free from fungal contamination. A maximum number of 24 isolates belonging to 12 species viz., *Aspergillus niger*, *A. tamarii*, *Penicillium citrinum*, *Penicillium oxalicum*, *P. commune*, *Penicillium* sp., *Cladosporium* sp., *Heteroccephalum*, *Chaetomium* sp., *Pestalotiopsis* sp., *Mycocleptodiscus* sp. and one sterile fungi were recorded from *Phyllanthus emblica* (table 1). Eighteen isolates belonging to 10 species viz., *Aspergillus niger*, *Aspergillus flavus*, *A. minutus*, *A. candidus*, *A. terricola*, *Curvularia trifolii*, *C. lunata*, *Penicillium pinophilum*, *Alternaria helanathi* and one sterile fungus were isolated from *Terminalia bellerica* (table 2). Stored fruits of *M. fragrans* recorded 22 isolates belonging to 11 species viz., *Aspergillus niger*, *Aspergillus flavus*, *A. parasiticus*, *A. ochraceous*, *A. terreus*, *A. versicolor*, *Penicillium purpurogenum*, *P. commune*, *Helminthosporium sorghicola* and *Rhizopus* sp. (table 3).

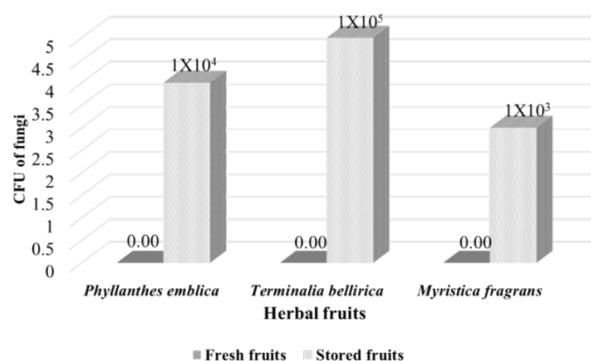


Fig. 1: Fungal contamination of fresh and stored herbal fruits

Stored fruits of *P. emblica* was found in association with 12 different fungal species belonging to 8 genera. The genus *Penicillium* was found to be predominant recording 4 species followed by *Aspergillus*

with two species. Among 12 species, *A. niger* recorded the highest relative density (82.608) followed by *P. commune* with a density of 31.63% (table 1). In case of *T. bellerica* 10 species of 5 different genera were observed among these the genus *Aspergillus* was found to be predominant comprising 5 species, this was followed by *Curvularia* (2 species). *A. niger* and *A. flavus* recorded highest relative density of 61.90 and 52.38 respectively in the stored fruits of *T. bellerica* (table 2). Eleven different species belonging to 4 genera were isolated from stored fruits of *M. fragrans*, with 7 species

Aspergillus being the predominant one, followed by *Penicillium* (2 species). *A. versicolor* was found to be predominant in stored fruits of *M. fragrans* with the highest relative density of 60 (table 3). Fig. 1 presents the CFU of fungi per gram of test fruits.

Highest CFU of 1×10^5 was recorded in *T. bellerica* followed by 1×10^4 in *P. emblica* and 1×10^3 in *M. fragrans*. CFU of each fungal isolate is presented in the tables 1-3. *A. niger* was found to be most predominant fungi in all the test fruits.

Table 1: Diversity, CFU and density of fungal contaminants isolated from fresh and stored fruits of *Phyllanthus emblica*

S. No.	Fungal contaminants	Stored fruits		Fresh fruits	
		CFU/ml	Density (%)	CFU/ml	Density (%)
1	<i>Aspergillus niger</i>	6×10^2	82.608	00	00
2	<i>A. tamarii</i>	7×10^2	4.545	00	00
3	<i>Penicillium citrinum</i>	3×10^2	13.636	00	00
4	<i>P. oxalicum</i>	4×10^4	18.181	00	00
5	<i>P. commune</i>	7×10^2	31.818	00	00
6	<i>Cladosporium</i> sp.	1×10^2	4.545	00	00
7	<i>Heterociphalum</i>	1×10^2	4.545	00	00
8	<i>Mycloptodiscus</i> sp.	1×10^2	4.545	00	00
9	<i>Pestalotiopsis</i> sp.	1×10^1	7.142	00	00
10	<i>Penicillium</i> sp.	2×10^2	14.286	00	00
11	<i>Chaetomium</i> sp.	2×10^2	14.286	00	00
12	Sterile fungi-1	1×10^1	14.286	00	00

Table 2: Diversity, CFU and density of fungal contaminants isolated from fresh and stored fruits *Terminalia bellerica*

S. No.	Fungal contaminants	Stored fruits		Fresh fruits	
		CFU/ml	Density (%)	CFU/ml	Density (%)
1	<i>Aspergillus niger</i>	6×10^1	61.90	00	00
2	<i>A. flavus</i>	5×10^2	52.38	00	00
3	<i>A. minutus</i>	1×10^4	4.76	00	00
4	<i>A. candidus</i>	2×10^2	9.52	00	00
5	<i>A. terricola</i>	2×10^2	9.52	00	00
6	<i>Curvularia trifolii</i>	3×10^3	14.28	00	00
7	<i>Curvularia lunata</i>	2×10^2	7.142	00	00
8	<i>Penicillium pinophilum</i>	3×10^3	7.142	00	00
9	<i>Alternaria helanathi</i>	6×10^1	21.42	00	00
10	Sterile fungi-2	2×10^2	7.142	00	00

Table 3: Diversity, CFU and density of fungal contaminants isolated from *Myristica fragrans*

S. No.	Fungal contaminants	Stored fruits		Fresh fruits	
		CFU/ml	Density (%)	CFU/ml	Density (%)
1	<i>Aspergillus niger</i>	13×10^1	43.75	00	00
2	<i>A. flavus</i>	12×10^2	51.42	00	00
3	<i>A. ochraceous</i>	4×10^1	5.714	00	00
4	<i>A. parasiticus</i>	2×10^2	34.7	00	00
5	<i>A. terreus</i>	5×10^1	11.42	00	00
6	<i>A. versicolor</i>	20×10^1	60.00	00	00
7	<i>Aspergillus</i> sp.	8×10^1	37.5	00	00
8	<i>Penicillium commune</i>	2×10^1	6.25	00	00
9	<i>P. purpurogenum</i>	2×10^2	6.25	00	00
10	<i>Rhisopus</i> sp.	6×10^2	8.571	00	00
11	<i>Helminthosporium sorghicola</i>	6×10^3	8.571	00	00

Table 4: Diversity indices of fungi associated with stored herbal fruits

Diversity indices	<i>P. emblica</i>	<i>T. bellerica</i>	<i>M. fragrans</i>
Species richness	12	10	11
Simpson's diversity index (1-D)	0.794	0.799	0.840
Shannon-Wiener index (H)	2.788	2.625	2.888
Margalef Richness index (R)	2.925	2.474	2.295
Menhinick index of evenness (E)	1.83	1.622	1.246
Berger Parker Dominance index (d')	0.418	0.352	0.269

Diversity indices

The diversity indices of fungi associated with stored herbal fruits is presented in table 4. Highest diversity of fungi was observed in fruits of *M. fragrans* with Simpson's diversity index of D-1=0.840 and Shannon-Wiener index of H=2.888 followed by *P. emblica* (D-

1=0.799; H=2.788) and *T. bellerica* (D-1=0.794; H=2.625). High species richness (12) of fungi was recorded in *P. emblica* fruits showing high Margalef Richness index (R=2.925), Menhinick index of evenness (E=1.830) and Berger Parker Dominance index (d'=0.418) values followed by *T. bellerica* (R=2.474; E=1.622; d'=0.352) and *M. fragrans* (R=2.295; E=1.246; d'=0.269).

Preliminary phytochemical analysis

The methanol extract of both stored and fresh fruits of *P. emblica* showed the presence of alkaloids, flavonoids, tannins, saponins,

steroids, phenols and quinones. Fresh and stored sample of *T. bellerica* was found to possess flavonoids, tannins, steroids, terpenoids, phenols and quinones. Flavonoids, steroids and terpenoids were detected in *M. fragrans* extract (table 5).

Table 5: Phytochemical analysis of solvent extracts of herbal fruits

Phytochemical tests	ME of stored <i>T. bellerica</i>	ME of fresh <i>T. bellerica</i>	ME of stored <i>P. emblica</i>	ME of fresh <i>P. emblica</i>	ME of stored <i>M. fragrans</i>	ME of fresh <i>M. fragrans</i>
Alkaloids	-	-	+	+	-	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	-	-
Saponins	-	-	+	+	-	-
Steroids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Phenols	+	+	+	+	-	-
Quinones	+	+	+	+	-	-
Cardiac glycosides	+	+	+	+	+	+

+indicate the presence and- indicate the absence

Antibacterial activity assay

Methanol extracts of all test fruits recorded variable activity against all the tested bacteria with a zone of inhibition ranging between 18 mm to 30 mm (table 6). Stored fruits exhibited comparatively higher antibacterial activity than the fresh fruits. An increase of 7 mm zone

of inhibition was observed against *Xanthomonas campestris* pv. *vesicatoria* in the contaminated fruits of *M. fragrans* and an increase of 6 mm was observed against *Xanthomonas oryzae* pv. *oryzae* in the contaminated *T. bellerica* fruit. Similarly, an increase of 3 mm inhibition was recorded in stored fruits of *P. emblica* against *Xanthomonas campestris* pv. *vesicatoria* (table 6).

Table 6: Antibacterial activity of methanol extracts of Terminalia bellerica, P. emblica and M. fragrans against test bacteria (Zone of inhibition in mm)

Test bacteria	Zone of inhibition in mm					
	ME of stored fruits of <i>T. bellerica</i>	ME of fresh fruits of <i>T. bellerica</i>	ME of stored fruits of <i>P. emblica</i>	ME of fresh fruits of <i>P. emblica</i>	ME of stored fruits of <i>M. fragrans</i>	ME of fresh fruits of <i>M. fragrans</i>
<i>X. c. pv. c.</i>	27.02±0.01	27.00±0.00	28.01±0.01	25.02±0.01	24.00±0.00	17.00±0.00
<i>X. c. pv. v.</i>	30.01±0.01	30.02±0.01	27.03±0.01	26.05±0.00	26.05±0.00	24.01±0.01
<i>X. o. pv. o.</i>	27.01±0.01	21.01±0.01	28.01±0.00	28.01±0.00	19.01±0.00	18.01±0.00

Values are the mean of three replicates±standard Error, P value=0.00 ≤ 0.05, Note: ME-Methanol extract; *X. c. pv. v.*-*Xanthomonas campestris* pv. *vesicatoria*, *X. c. pv. c.*-*Xanthomonas campestris* pv. *campestris* and *X. o. pv. o.*-*Xanthomonas oryzae* pv. *Oryzae*

DISCUSSION

The traditional system of herbal medicine has become a topic of global importance both as medicinal and economical [14] since they are considered as rich sources of lead compounds and quietly safe for both human use and environment-friendly [15]. Considering this in the present study, assessment of fungal contamination of selected fresh and stored herbal fruits was done by employing serial dilution method. Results revealed that the stored fruits were contaminated with various fungi whereas fresh fruits were found to be free of fungal contaminants.

The occurrence of the high diversity of fungi of 64 isolates of 29 species belonging to 13 genera indicates the association of broad spectrum of mycobiota in stored fruits. Among these *P. emblica* was found to be highly contaminated with 12 species of fungi belonging to 8 genera followed by *M. fragrans* with 11 species, belonging to 4 genera and *T. bellerica* with 10 species, belonging to 5 genera.

Fungal contamination of *M. fragrans* [14, 16] *P. emblica* [18, 19] and *T. bellerica* [20] has been reported. Results of the previous study correlates with the results obtained in the present investigation, but the diversity and density of fungal contaminants recovered varies. Akhund et al., [19] have recorded *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium* and *Curvularia* from *P. emblica* stored fruits and reported that *A. niger* and *A. flavus* are the predominant fungi. Gautam and Bhadauria [18] have isolated *Aspergillus*, *Penicillium*, *Helminthosporium*, *Rhizopus*, *Syncephalastrum*, *Alternaria*, and *Curvularia* from *P. emblica* and *T. bellerica*. Rajeshwari and Raveesha [21] have reported fungal contamination of *A. calamus* roots, *M. fragrans* mace, *C. angustifolia* and *C. asiatica* leaves, *T.*

cordifolia and *W. somnifera* stem collected from the retail herbal shops of Mysuru. Mycoflora analysis of the herbal fruits is done in various parts of the world including India, but no reports are available on fungal contamination of *M. fragrans*, *P. emblica* and *T. bellerica* fruits of Mysuru, which is an important hub of the herbal drug raw materials markets. Thus the present work is the first to report on the mycoflora of the stored herbal drug fruits from this region.

Diversity indices calculation revealed the occurrence of highest fungal diversity on *M. fragrans* recording high Simpson's diversity index (D-1=0.840) and Shannon-Wiener index (H=2.888). Although *M. fragrans* did not possess a high number of fungal species, the highest diversity was recorded in *M. fragrans*, as the term diversity considers not only the species richness but also the even distribution of species. On the other hand, *P. emblica* recorded high species richness (12), high dominance and evenness (R=2.925; E=1.83; d'=0.418) but not the diversity. This pattern of results may be due to the fact that, *A. niger* being fast growing and dominant may inhibit the growth of other fungal species. This is justified by the results observed in this investigation, wherein *A. niger* was recorded with high density (82.608) on *P. emblica* than on the *T. bellerica* (61.90) and *M. fragrans* (43.75). Sharma et al. [22] have studied species richness (S), Shannon-Wiener's diversity index (H'), Simpson Dominance index (Cd) and Berger Parker's Dominance index (d') diversity indices of fungi recorded from dried fruit samples of *Phyllanthus emblica*, *Terminalia bellerica* and *Terminalia chebula* from Jammu and Kashmir. Unlike the results of present study, they have recorded high species richness (55) and diversity (Shannon-Wiener's diversity index=1.634) and least Simpson dominance index (0.028) and Berger-Parker's dominance index (0.053) on *P. emblica* fruits.

During the collection of stored fruits from the market, it was observed that the samples were displayed on open metal/plastic containers/wooden boxes/gunny bags or on the bare ground in local general stores, causing direct exposure to airborne bio-pollutants which may have resulted in fungal contamination. Apparently, healthy fresh fruit samples were collected directly from the plant and brought to the laboratory in sterilized lock covers and analyzed and did not show any fungal contamination.

The genus *Aspergillus* was found to be predominant genus recording 12 species. Apart from this 6 species of *Penicillium*, 2 species of *Curvularia* and one species each of *Alternaria*, *Helminthosporium*, *Cladosporium*, *Heteroccephalum*, *Chaetomium*, *Pestalotiopsis*, *Mycodypteria* and 2 sterile fungi were recorded. Species of *Aspergillus* and *Penicillium* has been reported as the dominant mycoflora in some herbal drug raw materials collected from Tokyo (Japan); Hunan, Hubei and Guangxi province (China); Gwalior (North India) [23-25] the results of the present study also revealed the same. The association of *A. niger* in high density from all fruit samples, which are used by consumers, should be taken seriously as some strains can produce mycotoxins like ochratoxin A [26].

Fungal contaminants have been reported to affect the chemical compositions of the herbal drug raw materials on which they grow and thereby alter the medicinal property [7]. In order to understand the effect of fungal contamination on phytochemical constituents of both fresh (free of fungal contamination) and stored fruits (contaminated with fungi), methanol extracts of the same were screened for the phytochemical constituents. The extracts of test samples were tested for the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phenols, quinones and cardiac glycosides. Methanol was the preferred solvent for extraction since it is known to dissolve most of the secondary metabolites due to its polar nature.

Previous reports state the presence of alkaloids, tannins [27], flavonoids, saponins, phenols, anthraquinones, cardiac glycosides, coumarins, anthocyanin, chalcones, emodins, and triterpenoids [28] in *M. fragrans*. Dhale and Mogle [29] have found the presence of alkaloids, glyceroids, phenols, tannins, lignin, saponins, flavonoids and terpenoids in *P. emblica*. Devi et al. [30] and Abraham et al. [31] have detected alkaloids, flavonoids, tannins, glycosides, phenols and saponin in *T. bellerica*.

In the present investigation methanol extracts of fresh and stored fruits of *P. emblica* showed positive results for all the tested phytochemicals. Fresh and stored fruit samples of *T. bellerica* was found to possess flavanoid, tannins, steroid, terpenoids, phenols and cardiac glycosides. *M. fragrans* fresh and stored samples showed the presence of flavonoids, steroids, terpenoids and cardiac glycosides. Results did not show any significant difference in the presence or absence of test phytochemical constituents between fresh and stored herbal fruits. However, there could be adding some new compounds which have not been tested. Further quantitative and qualitative analysis need to be done to draw clear inferences on the role of fungal contamination on phytochemical constituents of tested fruits.

To understand the antibacterial potential of methanol extracts of selected fresh and stored herbal fruits, antibacterial activity assay was carried out against three plant pathogenic bacteria. Methanol extracts of all the tested samples showed antibacterial activity against all the test bacteria with varying zones of inhibition ranging between 24 mm and 30 mm. The presence of more phytochemicals in an extract correlates with more potential activity exhibited by that extract as the *M. fragrans* extract which showed presence of 4 phytochemicals (flavonoids, steroids, terpenoids and cardio glycosides) out of 9 tested, exhibited least antibacterial activity zone between 17 and 26 mm whereas extracts of *P. emblica* and *T. bellerica* recording presence of all and 7 phytochemicals out of 9 tested exhibited zone of inhibition range between 25-28 mm and 21-30 mm respectively. Antimicrobial activity of *P. emblica* [29, 32], *M. fragrans* [27, 33] and *T. bellerica* [34-36] is reported by researchers against some human pathogenic bacteria but not against plant pathogenic bacteria. Results of the present investigation demonstrate the antimicrobial potential of test fruits on phytopathogenic bacteria.

Although stored fruits were found in association various fungi they exhibited a comparatively larger zone of inhibition i.e. upto 41 % more than the fresh fruit extracts which were free of contamination. Stored fruit of *M. fragrans* showed an increase of 41% inhibition against *Xanthomonas campestris* pv. *campestris*, 8.33% against *Xanthomonas campestris* pv. *vesicatoria* and 5.55% against *Xanthomonas oryzae* pv. *oryzae*. An increase of 28% of zone of inhibition was observed in stored fruits of *T. bellerica* against *Xanthomonas oryzae* pv. *oryzae*. An increase of 12% and 3.8% zone of inhibition was recorded in stored fruits of *P. emblica* against *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *vesicatoria* respectively. This may be due to the predisposition of stored fruits to stress conditions during drying and fungal infestation that might have resulted in the production of more or new secondary metabolites, responsible for the antibacterial property. However, this needs further investigation on quantification and characterization of active principle present in fresh and stored fruits for the better understanding.

CONCLUSION

Analysis of fresh fruit samples showed that fruits were free of fungal contaminants while the stored fruits were contaminated with fungi, but it was within the maximum permitted limit prescribed by WHO. However the occurrence of a high diversity of fungi that are capable of causing bio-deterioration and producing fungi mycotoxins viz., *Aspergillus*, *Penicillium* and *Alternaria* is a cause of concern. Results clearly indicate the need for adaptation of appropriate methods for harvesting, collection, transportation, handling and storage of herbal drug raw materials. Maintenance of hygienic condition may be helpful in the prevention of fungal contamination. Though there was no significant difference in the phytochemical constituents as well as the antibacterial potential of both fresh and stored samples. Long term storage may affect the quality of the herbal fruits, due to increased growth of mycobiota, which in turn may affect the phytochemical composition and antibacterial potential. Hence proper handling and storage methodology is a prerequisite for the maintenance of herbal drug raw materials. The results suggest proper training need to be provided herbal material handlers right from harvesting to retail selling.

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AUTHORS CONTRIBUTION

First (Sushma K. S.): Acquisition of data

Second Author (Rajeshwari P.): Data interpretation and Drafting the manuscript

Third author (K. A. Raveesha): Conception and design of study

CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors

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