Sushil Sharma et al /J. Pharm. Sci. & Res. Vol. 8(5), 2016, 279-288

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Biodeterioration of Therapeutically Important Phytoconstituents and Nutrients of Stored Triphala Powder by Associated Fungal Species

Sushil Sharma[1, 2]*, Madhu Gupta[1], Rekha Bhadauria[1] and S P Bajpai [2]

[1]*Mycology and Plant Pathology laboratory, School of Studies in Botany, Jiwaji University Gwalior (M.P.) India-474011*

[2]*Department of Environmental Studies, Amity University Madhya Pradesh, Gwalior (M.P.) India-474005*

**Abstract**

The study was aimed to investigate the associated mycobiota and their effect on the quality of Triphala powder. Results showed the presence of various fungal species in commercially available Triphala powder with varying degree of CFU (colony forming unit) and frequency of occurrence. *Aspergillus* was recorded as the most dominant genus with five species. A large variation in quantity of phytoconstituents was observed among commercially available Triphala powder samples. Artificially infested samples showed the variation in the level of therapeutically important phytoconstituents after different storage periods. The study proved the role of fungal species in depletion of phytoconstiuents during storage although the capability to degrade the phytoconstituents varied from species to species.

**Keywords** Biodeterioration, mycobiota, phytoconstituents, triphala

## INTRODUCTION

Triphala powder is one of the most widely used herbal laxative and rejuvenating tonic of Indian system of medicine [1]. It is easily available in the global herbal market and can also be prepared at home [2]. It is a powdered mixture of dried fruit pulp of three important myrobalans i.e. *Emblica officinalis* Gaertn., *Terminalia belerica* Roxb. and *Terminalia chebula* Retz. in equal proportion [3]. This secret formula for the maintenance of a healthy digestive system is described in the ancient books on Ayurveda, the *Charak Samhita* and *Susruta Samhita* which date back to 1500 B.C. [4]. The power behind Triphala's benefits comes from the ingredients that make up the formula [5]. According to Ayurveda, Triphala powder is a safe and non habit forming formulation hence can be recommended for all age groups [6]. It corrects constipation, cleanses and tonifies the gastrointestinal tract, detoxifies the whole body and improves digestion and assimilation [7]. Triphala further aids in digestion by eliminating toxins from the intestinal tract [8]. Triphala is also effective in weight loss, for those who suffer from obesity [9]. Being a *rasayana* (a herb associated with rejuvenation), it arrests aging, enhances intelligence, memory, strength, youth, luster, sweetness of voice and vigor [4] According to Ayurvedic practitioners, daily use of Triphala promotes appetite and ensures good digestion. It is also proven highly effective as anti-inflammatory [10], anticancer [ 1, 11-14] antimutagenic [15], radioprotective [16], antidiabetic [17], antioxidant [18-20], hepatoprotective [21], immunomodulatory [22], wound healer [23,24] and anti-aging agent [4]. The major chemical compounds reported from this formulation are phenolics (25-38%) comprising mainly of tannin (~35%), gallic acid (3%-7%), elagic acid (~2 %), chebulagic acid (~ 5%), chebulinic acid (~5%) and ascorbic acid (0.050-0.33%) [8, 6, 25]. Polyphenols have already been proven as a major therapeutic constituent of Triphala [6, 11, 15]. The purgative action of Triphala is also believed due to polyphenolic constituent, chebulagic acid [4]. Ascorbic acid is another important therapeutic constituent of Triphala. Beside its well known antioxidant potential, it is reported to act as a cofactor for the synthesis of collagen during healing of dermal wound [23]. Triphala is believed to be responsible for low incidence of pancreatic cancer among the Indian population [14]. The role of ascorbic acid to act as an antitumor agent against ovarian, pancreatic and glioblastoma xenografts in mice is well studied [26]. But being a combination of three different fruits, Triphala is rich in nutrients that make it highly vulnerable to fungal invasion and contamination. Various kinds of microorganisms normally adhere to raw materials [27] which may act as supplementary contaminants because no sincere attention is paid during handling of these raw materials except washing. The direct presence of inoculums of fungi in or on raw or processed plant materials may not be that much harmful. But under favorable conditions i.e. a relative humidity of at least 65 %, moisture content of >13% [28], the inoculums of storage fungi may germinate on products and start consuming such products for growth and development, thereby causing undesirable changes of varied nature in the raw material or processed products including quality of nutrients, loss of active therapeutic constituents, poisoning of the products by production of mycotoxins, reduction in shelf life and finally loss of potency and market value [29, 30]. Therefore an attempt was made to study the role of these fungi on the quality of Triphala powder. A special emphasis was given upon their role in the depletion of therapeutically active phytoconstituents and nutrients.

## MATERIALS AND METHODS

### Experimental material

On the basis of a survey carried out in five major cities of Gwalior District. Top ten best selling and most popular brands among common people with latest manufacturing dates were collected from the market of Gwalior district,

Madhya Pradesh, India during September–November, 2010 and subsequently analyzed for various parameters during January-April, 2011. Collected samples were coded as 'Mult.D', 'Mult.B', 'Mult.Z', 'Mult.DI', 'Reg.B', 'Reg.U', 'Reg.S', 'Loc.1', 'Loc.2' and 'HP'. After noting product related details (date of Mfg), pH, moisture content and physical appearance of Triphala powder, all the samples were analyzed for the associated mycobiota. This was followed by quantitative estimation of therapeutically active constituents (total phenolics, tannin, and ascorbic acid) and some important nutrients (total soluble sugar and protein).

To study the effects of these fungal contaminants on the concentrations of therapeuticay important phytonstituents, fresh samples of Triphala powder with latest manufacturing dates (October, 2011) were collected during December, 2011 and study was performed during January-June, 2012.

**Methods**

**Measurement of pH.** 5% suspension of Triphala powder was prepared in distilled water, shaken constantly for one hour. pH of the suspension was measured using an electronic pH meter ( MK-V). Each sample was analyzed in triplicate.

**Measurement of moisture content.** 2 g powder of Triphala churn was taken in previously dried and tarred flat weighing bottle in triplicate and kept in the oven at 105 $^{o}$C. Dried until two consecutive weighing do not differ by more than 5 mg [31]. Total moisture content was calculated by the formula:

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

**Morphological analysis of Triphala powder.** Triphala powder sample was examined morphologically under magnifying glass for their physical appearance like; color, powder fineness and the presence of any visual clumps

**Isolation and identification of mycobiota.** Fungi were isolated from the Triphala powder samples by pour plate technique, using Czapek Dox agar media (pH 7.3) supplemented with chloramphenicol. Under aseptic condition 1gm sample was suspended in 9 ml of sterilized distilled water and serial dilutions were made up to $10^{-5}$. One ml aliquot of each sample from appropriate dilution was poured in sterilized petri plates (in triplicate) and appropriate amount of media was added in petri plates and mixed well. After solidification, plates were incubated at 25±2$^{o}$ C and growth of fungal colonies was recorded at various time intervals. After seven days of incubation period, pure culture of each fungal isolate was prepared by using PDA media for identification purpose.

Colony forming units per gram (CFU/g) were calculated using following formula:

$CFU = N \times 10^{-n}$

where N= Total number of colonies, n= dilution

Whereas frequency of occurrence (FO) was calculated using the formula:

FO = number of sample containing a fungal species/total number of samples evaluated×100

Isolated fungal species (each fungal isolates) were identified on the basis of morphology (shape, size, growth rate and color of the colonies) and microscopic characteristics (characteristics of mycelium, size, shape, color and arrangement of conidia, spore, conidiophores, sporangiophores, vesicle, sterigmata etc.) as described by Thom and Raper [32] Gilman [33] Barnett [34] Jamaluddin et al. [35] Samson et al.[36, 37]

**Estimation of phytoconstituents.** Therapeutically important phytoconstituents (total phenolics, tannin, and ascorbic acid) along with some nutritional components (total sugar and protein) were analyzed during the study. The active constituents were selected on the basis of their therapeutic values whereas the nutrients were selected due to their potential to act as an indicator of fungal growth and metabolism.

Analysis of total phenolics and tannin was carried out in accordance with the Makkar et al. [38] whereas ascorbic acid was estimated by a titrimetric method as suggested by Roe [39] Total sugar and protein was estimated by the standard method as proposed by Dubois et al. [40] and Lowery et al. [41] respectively. All the experiments were performed in triplicates and the results were expressed in percentage on dry matter basis.

**Study of effect of storage fungi on Triphala powder.** To study the effect of storage fungi on herbal formulation, fresh samples of Triphala powder were infested with spores of frequently occurring fungal species. As Triphala is retailed and consumed in powder form, therefore to imitate the exact market and retailing condition 'dry inoculation technique' as described by Coutinho et al. [42] with some modification was used during this experiment.

After preliminary studies of various brands of Triphala powder available in the market, the least contaminated (total CFU<$10^{2}$), most rich in term of phytoconstituents and overall superior among all tested brands in term of various other quality parameters was selected (manuscript communicated). Samples of the selected brand of the same batch with latest manufacturing dates were procured in bulk. Triphala powder of each pack was emptied into a sterilized container in aseptic conditions and mixed well for uniformity. Prior to infestation with test fungi, pH, moisture content, powder appearance, Total CFU and the initial concentrations of therapeutically important phytoconstituents and nutrients of Triphala powder were estimated (in triplicate) using standard methods. To avoid the loss of heat sensitive phytoconstiuents and to maintain the integrity and consistency of powder, samples were refrained from sterilization.

**Selection of test organisms and amount of inoculums.** On the basis of frequency of occurrence, three most commonly occurring fungal species viz. *Aspergillus niger*, *A. fumigatus* and *A. flavus* were used as test organisms

during the investigation. While amount of inoculum ($10^5$ spores /g) was set on the basis of highest total CFU reported from the commercially available Triphala powder samples available in the market.

**Preparation of inoculums.** 6 mm disks from seven day old cultures were taken with the help of cork borer and shaken for 2 minutes in 10 ml of distilled water. After removal of disks, suspension was filtered through Whatman filter paper followed by drying. Spores were collected using brush and mixed with Triphala powder in the ratio of $10^5$ spores /g. Spore count was calculated by counting the approximate number of spores in one 6 mm disk of each fungal species by suspending the disk in 10 ml of distilled water followed by shaking and subsequently counting the spores/ ml with the help of haemocytometer.

**Inoculation of test fungi.** Four groups containing packs of 50 g of Triphala powder in triplicate were prepared. The first group was inoculated with spores of *Aspergillus niger*; second with *A. flavus* and third with *A. fumigatus*. The fourth group without any fungal inoculum was kept as control.

Samples of Triphala powder were inoculated with a dried spore mass of the test fungi ($1\times 10^5$ spores/g) and mixed well for uniform distribution of spores that was ensured by visualizing the Triphala powder (mounted on glass slides in glycerin drop) under binocular microscope at 400X . Each group (in triplicate) of infested Triphala powder samples were sealed with paraffin wax to make containers air tight to reproduce the actual retailing conditions of Triphala powder. Control without any test fungi was also maintained. All the infested samples along with control were stored at room temperature (30-32 $^oC$). Phytochemical analysis of all the infested samples and control was performed in triplicates at various intervals i.e. at the interval of one, three and six months (30, 60 and 180 days) of storage period. Final results of each time interval/ storage period were compared with initial readings.

**Statistical analysis.** One way ANOVA at $p < 0.05$ followed by Tukey HSD test was used to determine significance level and Pearson's correlation coefficient was performed to test the correlation between various factors using the Minitab-16 software.

## RESULTS

### pH, moisture content and appearance of Triphala powder

As Table 1 shows, pH of the samples was in the range of 3.67 -6.24 whereas percent moisture content was recorded in between 4.16-7.49%. Except one sample with exceptionally high pH, not much variation was observed in pH of the rest of the samples. While a good variation was observed in percent moisture content of Triphala powder samples. Color of collected samples was found to range between khaki to dark brown.

### Associated mycobiota of Triphala

During present investigation, samples of various brands of commercially available Triphala powder were found contaminated with varied number of fungal species with total CFU ranging from $1.66\times10^3$ to $7.33\times10^5$. As depicted in Table 2 & 3, a total seven fungal species viz. *Aspergillus niger, A. flavus, A. fumigatus, A. nidulans, A. wentii, Alternaria alternata and Curvularia lunata* were isolated from the samples of Triphala powder during the study. Among these, *Aspergilli* were the most dominant with 5 species. *A. niger* was recorded as the most prevalent fungal species with frequency of occurrence of 80% and CFU range of $3.3\times10^2$ to $2.66\times10^4$. This was followed by *A. fumigatus* (50%; $3.3\times10^2$-$1.33\times10^5$), *A. flavus* (30%; $1\times10^3$-$1.33\times10^5$), *A. nidulans* (20 %; $6.6\times10^2$-$6.66\times10^5$), *A. alternata* (20%; $3.3\times10^4$-$1.33\times10^5$), *Curvularia lunata* (10%; $3.3\times10^4$) and *A. wentii* ( 10%; $1.33\times10^3$). The majority of the samples were with fungal load of $> 10^3$ CFU/g .

### Phytoconstituents of Triphala

A significant variation ($p<0.05$) in a concentration of therapeutically important phytoconstituents was observed among the samples of various brands (Table 4). The percent concentration of total phenolics (tannic acid equivalent) was found in between 21.96±0.45 to 40.99± 1.70 %. The highest concentration of total phenolics was recorded from Triphala powder samples 'Mult.Z' (40.99±1.70%) and least concentration was recorded in the samples 'HP' (21.96±0.45%). Similarly in case of tannin, concentration was recorded in the range of 18.83±0.61 (HP) to 36.86±1.49% (Mult.Z). The concentration of the ascorbic acid was recorded in between 0.041±0.002 ('Loc.1') to 0.159±0.005 % ('Reg.B'). While the highest concentration (13.32±0.14) of total sugar was recorded from the sample 'Loc.2' and minimum (3.22±0.88) from sample Mult.B. Protein concentration (in term of bovine serum albumin) of Triphala powder samples was recorded in the range of 6.86±0.25 (Mult.B) to 10.90±0.60 % ('HP').

### Effect of fungal contaminants on Triphala powder

Three dominant fungal species viz., *Aspergillus niger*, *A. flavus* and *A. fumigatus*, isolated earlier from the Triphala powder were selected during the investigation to study the effect of these storage fungi on phytochemical properties of Triphala powder.

A generalized results revealed that besides the natural loss of phytoconstituents during storage, fungi have a capability to metabolize certain phytoconstituents for their growth and development. Although capability varied from species to species. During present investigation significant difference ($p< 0.05$) in a concentration of certain phytoconstituents was observed during storage of 30, 60 and 180 days, in the infested samples as compared to control and initial readings.

**Table 1** The pH, moisture content and color of collected Triphala powder samples

| | Triphala Powder Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mult. D** | **Mult. B** | **Mult. Z** | **Mult. DI** | **Reg. B** | **Reg. U** | **Reg. S** | **Loc. 1** | **Loc.2** | **HP** |
| **pH** | 3.75 ±0.04 | 3.72 ±0.02 | 3.68 ±0.06 | 6.24 ±0.02 | 3.68 ±0.03 | 3.65 ±0.03 | 3.67 ±0.02 | 4.08 ±0.07 | 3.73 ±0.04 | 4.31 ±0.03 |
| **Moisture Content (%)** | 5.44 ±0.68 | 6.33 ±0.72 | 5.89 ±0.45 | 4.16 ±0.65 | 6.2 ±0.73 | 7.49 ±0.52 | 7.3 ±036 | 5.77 ±085 | 7.04 ±0.67 | 5.96 ±0.74 |
| **Color** | LB[a] | B[b] | K[c] | LB | K | K | LB | B | B | DB[d] |

[a] Light brown; [b] Brown; [c] Khaki; [d] Dark Brown

**Table 2** Fungi isolated from Triphala powder with their respective CFU (colony forming units) ranges and frequency of occurrence.

| Sr. No. | Isolated fungal species | CFU Range | Frequency of Occurrence (%) |
|---|---|---|---|
| **1** | *Aspergillus niger* | $3.33\times10^2$-$2.66\times10^4$ | 80 |
| **2** | *Aspergillus flavus* | $1\times10^3$-$1.33\times10^5$ | 30 |
| **3** | *Aspergillus fumigatus* | $3.3\times10^2$-$1.33\times10^5$ | 50 |
| **4** | *Aspergillus nidulans* | $6.6\times10^2$-$6.66\times10^5$ | 20 |
| **5** | *Aspergillus wentii* | $1.33\times10^3$ | 10 |
| **6** | *Alternaria alternata* | $3.3\times10^4$-$1.33\times10^5$ | 20 |
| **7** | *Curvularia lunata* | $3.3\times10^4$ | 10 |

**Table 3** Distribution of fungal species in Triphala powder samples

| | Triphala Powder Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Isolated fungal species** | **Mult. D** | **Mult. B** | **Mult. Z** | **Mult. DI** | **Reg. B** | **Reg. U** | **Reg. S** | **Loc. 1** | **Loc. 2** | **HP** |
| ***Aspergillus niger*** | + | + | + | + | + | – | – | + | + | + |
| ***A. flavus*** | – | – | – | + | – | – | – | + | – | + |
| ***A. fumigatus*** | – | – | + | – | – | + | – | + | + | + |
| ***A. nidulans*** | – | + | – | – | – | – | + | – | – | – |
| ***A. wentii*** | – | – | – | – | – | – | – | + | – | – |
| ***Alternaria alternata*** | – | – | – | – | – | + | + | – | – | – |
| ***Curvularia lunata*** | – | – | – | – | – | – | + | – | – | – |

**Table 4** Product related details and phytochemical profile of collected Triphala powder samples

| Brand Code | Date of Mfg.[y] | Date of Collection | Total CFU | Total Phenolics | Tannin | Ascorbic Acid (%) | Total sol. Sugar | Protein |
|---|---|---|---|---|---|---|---|---|
| **Mult.D** | July/10 | October/10 | $2.66\times10^4$ | *36.68±0.55$^{abc}$** | 32.16±0.77$^{abc}$ | 0.084 ±0.006$^{b}$ | 9.41±0.87$^{bc}$ | 8.12±0.16$^{cde}$ |
| **Mult.B** | Jan/09 | September/10 | $1.88\times10^4$ | 40.38±1.85$^{ab}$ | 36.38±1.91$^{ab}$ | 0.042±0.004$^{d}$ | 3.22±0.88$^{d}$ | 6.86±0.25$^{f}$ |
| **Mult.Z** | Sep/10 | October/10 | $1.66\times10^3$ | 40.99±1.70$^{a}$ | 36.86±1.49$^{a}$ | 0.085±0.002$^{b}$ | 9.10±0.47$^{c}$ | 7.35±0.26$^{ef}$ |
| **Mult.DI** | Oct/10 | November/10 | $2.66\times10^3$ | 39.11±0.84$^{ab}$ | 35.29±0.99$^{ab}$ | 0.053±0.003$^{c}$ | 4.83±0.17$^{d}$ | 7.86±0.25$^{de}$ |
| **Reg. B** | July/10 | October/10 | $6.33\times10^3$ | 36.94±2.32$^{abc}$ | 33.09±2.64$^{abc}$ | 0.159±0.005$^{a}$ | 9.65±2.81$^{bc}$ | 8.37±0.26$^{bcd}$ |
| **Reg.U** | March/10 | October/10 | $2.66\times10^5$ | 37.33±2.63$^{abc}$ | 33.44±2.70$^{abc}$ | 0.076±0.005$^{b}$ | 8.53±0.31$^{c}$ | 8.88±0.98$^{bc}$ |
| **Reg.S** | April/10 | November/10 | $7.33\times10^5$ | 33.6.±0.50$^{c}$ | 29.39±0.38$^{c}$ | 0.058±0.002$^{c}$ | 3.53±0.53$^{d}$ | 9.18±0.24$^{b}$ |
| **Loc. 1** | NM[x] | November/10 | $3.0\times10^4$ | 24.7±1.77$^{d}$ | 21±1.75$^{d}$ | 0.041±0.002$^{d}$ | 10.75±0.83$^{abc}$ | 10.07±0.19$^{a}$ |
| **Loc.2** | NM | November/10 | $2\times10^3$ | 35.52±2.65$^{bc}$ | 31.45±1.03$^{bc}$ | 0.050±0.001$^{cd}$ | 13.32±0.14$^{a}$ | 8.36±0.37$^{bcd}$ |
| **HP** | NM | October/10 | $3.5\times10^4$ | 21.96±0.45$^{d}$ | 18.83±0.61$^{d}$ | 0.058±0.002$^{c}$ | 12.45±1.04$^{a}$ | 10.90±0.60$^{a}$ |

***Mean values of three replicates ± Standard Deviation. [x]: Not mentioned; [y]: Date of manufacturing**

****Means in columns that do not share a superscript letter are significantly different (One way ANOVA at P <0.05 followed by Tukey HSD test).**

**Table 5** Evaluation of phytoconstituents of infested Triphala powder samples at various intervals

| | | After 1 Month | | | | After 3 Months | | | | After 6 Months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Initial con.** | **Control** | ***A. n*[x]** | ***A. f*[y]** | ***A. fu*[z]** | **Control** | ***A. n*** | ***A. f*** | ***A. fu*** | **Control** | ***A. n*** | ***A. f*** | ***A. fu*** |
| **Total Phenolics** | **44.77 ± 2.38** | 43.38 ±1.07[a] | 43.27 ±2.08[a] | 42.81 ±1.45[a] | 42.51 ±3.46[a] | 41.15 ±0.71[a] | 34.48 ±1.17[bc] | 36.18 ±0.90[b] | 32.72 ±0.59[c] | 37.27 ±1.56[a] | 29.59 ±0.91[b] | 29.62 ±2.64[b] | 28.79 ±1.05[b] |
| **Tannin** | **41.86 ± 2.37** | 40.31 ±1.37[a] | 40.46 ±1.94[a] | 40.02 ±1.62[a] | 39.73 ±3.67[a] | 38.15 ±0.70[a] | 31.63 ±1.03[bc] | 33.56 ±0.71[b] | 29.76 ±0.67[c] | 34.38 ±1.54[a] | 26.59 ±0.90[b] | 26.85 ±2.73[b] | 26.20 ±1.0[b] |
| **Ascorbic Acid** | **0.095 ± 0.002** | 0.094 ±0.004[a] | 0.092 ±0.002[a] | 0.091 ±0.002[a] | 0.094 ±0.004[a] | 0.090 ±0.002[b] | 0.088 ±0.01[b] | 0.081 ±0.004[a] | 0.080 ±0.02[a] | 0.088 ±0.003[a] | 0.085 ±0.003[a] | 0.069 ±0.004[b] | 0.069 ±0.001[b] |
| **Total sol. Sugar** | **8.79 ± 0.08** | 8.74 ±0.11[a] | 8.31 ±0.10[a] | 8.66 ±0.07[a] | 8.46 ±0.47[a] | 8.71 ±0.06[b] | 8.81 ±0.13[ab] | 8.98 ±0.08[a] | 9.02 ±0.06[a] | 8.95 ±0.11[a] | 9.15 ±0.43[a] | 9.23 ±0.59[a] | 9.14 ±0.15[a] |
| **Protein** | **9.61 ± 1.18** | 9.36 ±0.40[b] | 11.15 ±0.24[a] | 10.50 ±0.90[ab] | 10.49 ±0.78[ab] | 9.87 ±0.24[a] | 10.36 ±0.54[a] | 10.74 ±0.36[a] | 10.55 ±0.27[a] | 9.81 ±0.33[b] | 10.55[a] ±0.12 | 10.71 ±0.29[a] | 10.81 ±0. 27[a] |

***Mean values of three replicates ± standard deviation; ([x]): *Aspergillus niger*; ([y]): *Aspergillus flavus*; ([z]): *Aspergillus fumigatus***
****Means in rows ( group 1: after 1 months; group 2; after 3 months; group 3: after 6 months) that do not share a superscript letter are significantly different (One way ANOVA at P <0.05 followed by Tukey HSD test).**

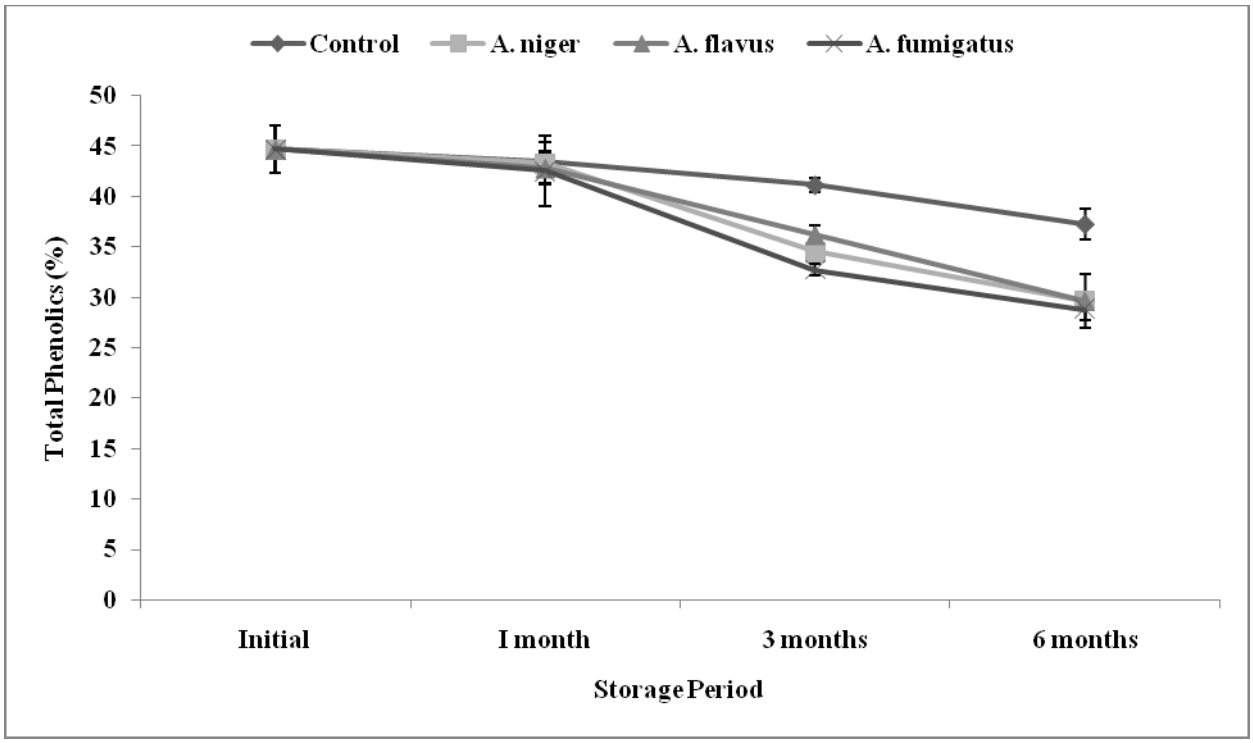


**Fig. 1** Effect of *A. niger*, *A. flavus* and *A. fumigatus* on total phenolics concentration of Triphala powder

**pH, moisture content and appearance of Triphala powder**

Prior to infestation with test fungi and subsequently after infestation, pH, moisture content and morphological appearance of Triphala powder was noted. The initial pH of the samples was 3.91±0.02 while moisture content was recorded as 10.68±0.57%. Initially powder of Triphala was khaki in color but after six months of storage it appeared light brown in color in treated as well as control samples. No significant changes were recorded in moisture content and pH of the *in vitro* infested samples after one, three and six months of storage period (data not shown).

**Effect on total phenolics concentration**

As depicted in Table 5 and Figure 1, in comparison to the initial concentration of total phenolics (44.77 ± 2.38 %), a gradual decrease was observed in concentration of total phenolics of Triphala powder samples inoculated with test fungi as compared to the control sample. In case of infested samples total phenolics concentration was recorded as 43.27±2.08, 34.48±1.17 and 29.59±0.91 % ( in *Aspergillus niger*), 42.81±1.45, 36.18±0.90 and 29.62±2.64 % (in *A. flavus*) , 42.51±3.46, 32.72±0.59 and 28.79±1.05 % ( in *A. fumigatus*) after one, three and six months of storage periods respectively. While in control it was 43.38±1.07, 41.15±0.71 and 37.27±1.56 % after similar time intervals.

A significant difference in total phenolic concentration was observed after three and six months of storage period. Findings revealed that the decrease in total phenolics concentration was comparatively high in *A. fumigatus* treated samples (5.04, 26.91 and 35.69 % ) followed by *A. niger* (3.35, 22.98 and 33.90 %) and *A. flavus* (4.37, 19.18 and 33.83 % ) infested samples as compared to control (3.10, 8.08 and 16.75 %), after 1, 3 and 6 months of storage

periods respectively. In all three groups of infested samples, a sharp decrease in total phenolic concentration was recorded between 90 to 180 days of storage as compared to over 30 and 90 days of storage period (Table 6 and Fig. 1).

**Effect on tannin concentration**

Tannin is an important and major part of total phenolics concentration of Triphala powder. During present investigation, initial concentration of tannin was recorded as 41.86±2.37 %. While in treated samples it was 40.46±1.94, 31.63±1.03 and 26.59±0.90 % (in *A. niger* ), 40.02±1.62, 33.56±0.71 and 26.85±2.73 % (in *A. flavus*), 39.73±3.67, 29.76±0.67 and 26.20±1.0 % (in *A. fumigatus*) after one, three and six month's of storage periods respectively. At a similar time intervals tannin concentration of control was 40.31±1.37, 38.15±0.70 and 34.38±1.54 % (Table 5). In control a decrease of 3.70, 8.86 and 17.86 % in tannin concentration was observed during one, three and six months of storage periods respectively whereas in treated samples the decrease was 3.34%, 24.43 % and 36.47% ( in *A. niger* ), 4.39, 19.82 and 35.85% (in *A. flavus*), 5.08, 28.90 and 37.41 % (in *A. fumigatus*) after one , three and six months of storage periods respectively. During investigation it was found that the tannin concentration was more or less constant in the control and treated samples during the initial to 30 days of storage. But a sharp decrease in tannin concentration was recorded after 90 and 180 days of storage especially in *A. fumigatus* followed by *A. niger* and *A. flavus* treated samples. (Table 6 and Fig. 2).

**Effect on ascorbic acid concentration**

*Emblica officinalis*, an important ingredient of Triphala powder is a richest source of ascorbic acid. During present investigation initial concentration of ascorbic acid in Triphala powder was recorded as 0.095±0.002 %. A minor difference was observed in the values of ascorbic acid concentration of *A. niger* treated (0.092±0.002, 0.088±0.01 and 0.085±0.003 %) samples as compared to control ( 0.094± 0.004, 0.090±0.002 and 0.088 ±0.003 %) after one, three and six months of storage periods respectively (Table 5). In comparison to control (1.05, 5.26 and 7.36 %) a decrease of 3.15, 7.36 and 10.5 % in ascorbic acid concentration was observed in *A. niger* treated samples after one, three and six months of storage periods respectively. While after similar time intervals the ascorbic acid concentration was 0.091±0.002, 0.081±0.42 and 0.069±0.004 % in the case of *A. flavus* and 0.094±0.004, 0.080±0.02 and 0.069±0.001% in *A. fumigatus* treated samples. A decrease of 4.21, 14.73 and 27.36 % in *A. flavus* and 1.05, 15.78 and 27.36 % in *A. fumigatus* treated samples was observed after one three and six months of storage periods respectively. Results indicate that among all the treatments only *A. flavus* and *A. fumigatus* were found more effective to affect the concentration of ascorbic acid as compare to *A. niger* treated samples. After six months of storage a maximum decrease of 27.36% was recorded from *A. flavus* and *A. fumigatus* treated sample, which was quite high in comparison to control (7.36 %) clearly indicating the role of these fungal species in degradation of ascorbic acid (Table 6 and Fig. 3).

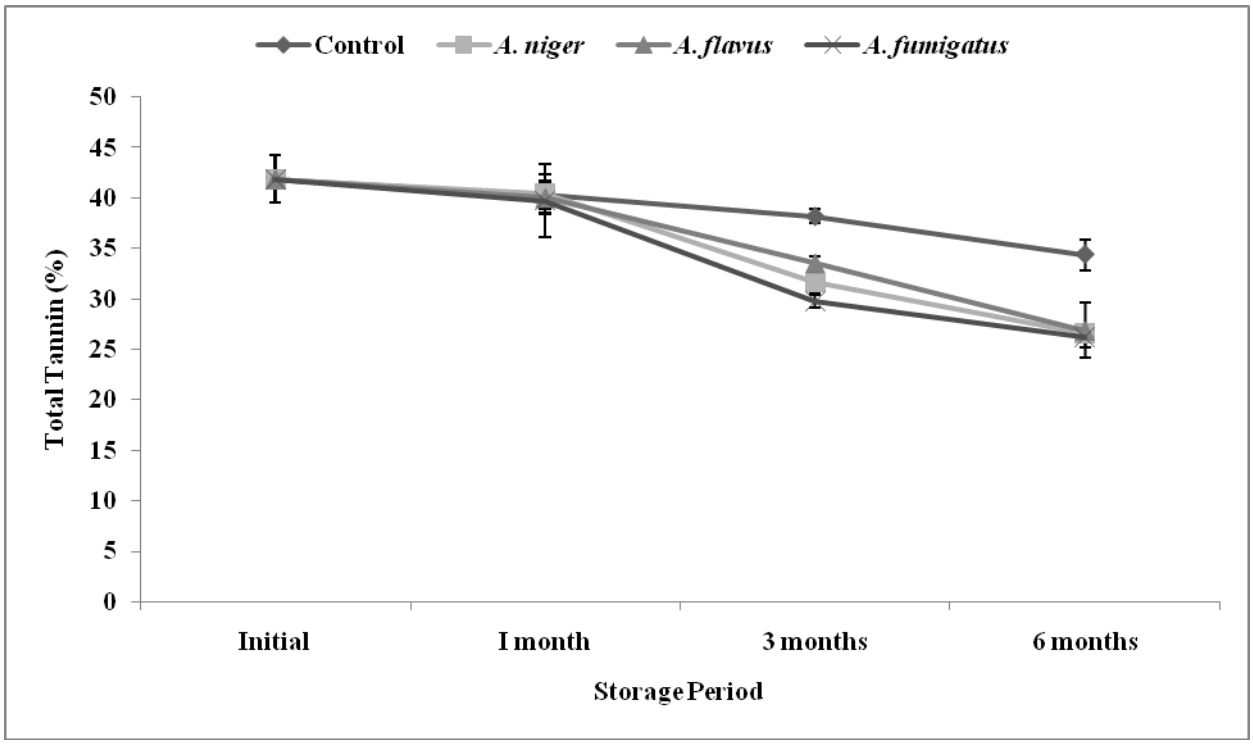


**Fig. 2** Effect of *A. niger*, *A. flavus* and *A. fumigatus* on tannin concentration of Triphala powder

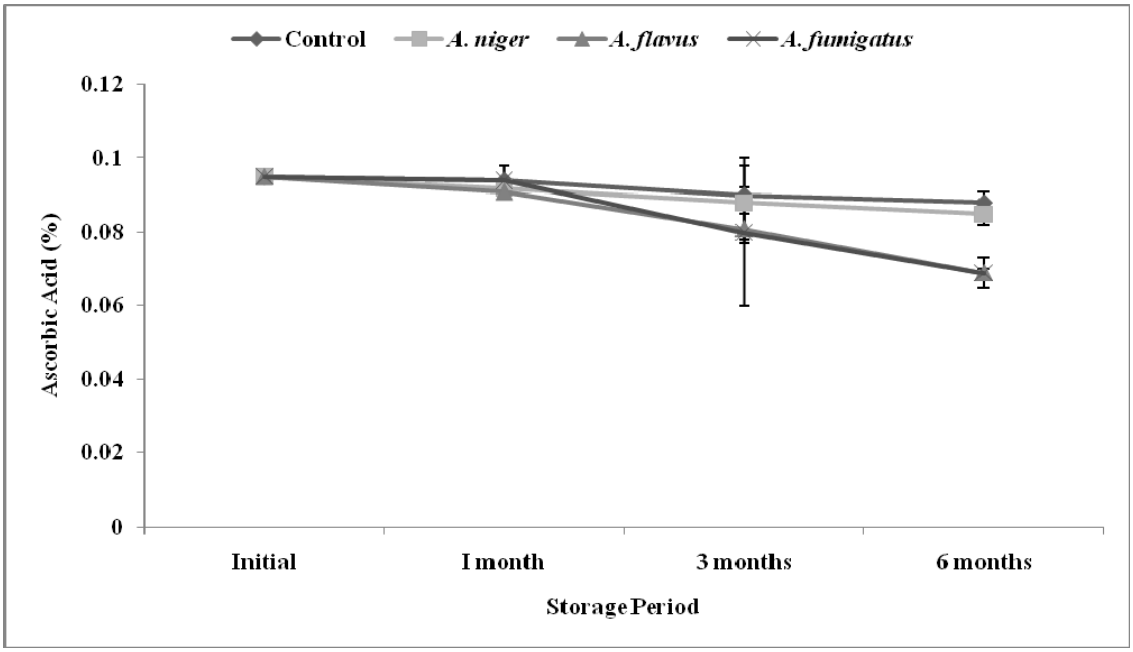


**Fig. 3** Effect of *A. niger*, *A. flavus* and *A. fumigatus* on ascorbic acid concentration of Triphala powder

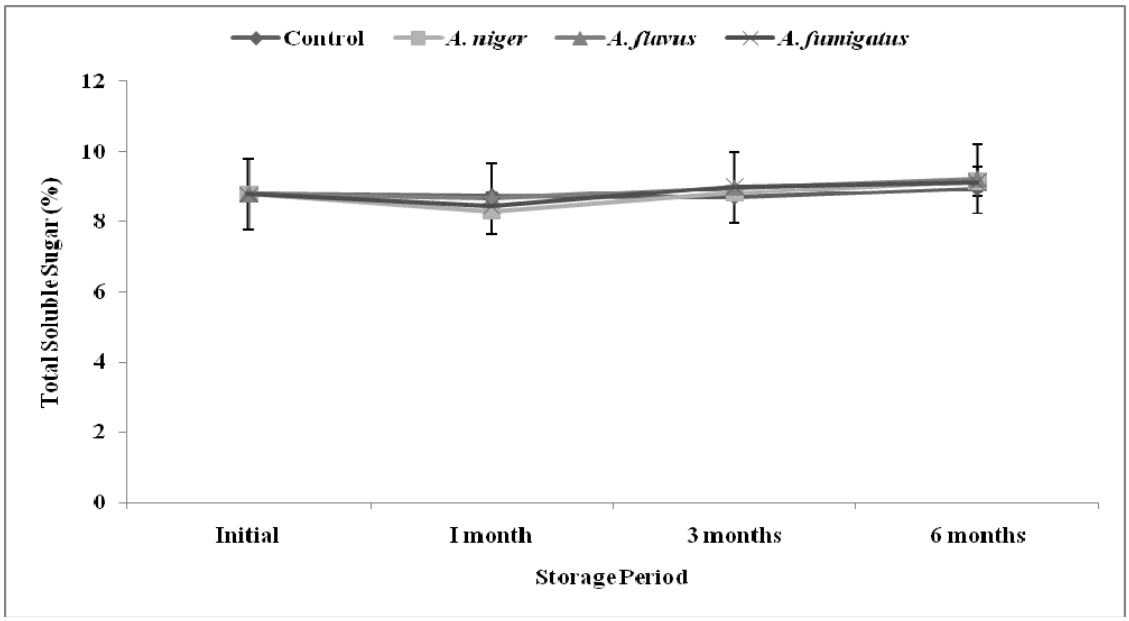


**Fig. 4** Effect of *A. niger*, *A. flavus* and *A. fumigatus* on total soluble sugar concentration of Triphala powder

**Table 6** Total percent changes in concentration of phytoconstituents of Triphala powder after different time intervals

| | **Control** | | | ***A. niger*** | | | ***A. flavus*** | | | ***A. fumigatus*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time interval (months)** | **1** | **3** | **6** | **1** | **3** | **6** | **1** | **3** | **6** | **1** | **3** | **6** |
| **Total phenolics** | –3.10 | –8.08 | –16.75 | –3.35 | –22.98 | –33.90 | –4.37 | –19.18 | –33.83 | –5.04 | –26.91 | –35.69 |
| **Tannin** | –3.70 | –8.86 | –17.86 | –3.34 | –24.43 | –36.47 | –4.39 | –19.82 | –35.85 | –5.08 | –28.90 | –37.41 |
| **Ascorbic acid** | –1.05 | –5.26 | –7.36 | –3.15 | –7.36 | –10.5 | –4.21 | –14.73 | –27.36 | –1.05 | –15.78 | –27.36 |
| **Total sugar** | –0.56 | –0.91 | +1.82 | –5.46 | +0.22 | +4 | –1.47 | +2.16 | +5 | –3.75 | +3.98 | +2.61 |
| **Protein** | –2.60 | +2.70 | +2.08 | +16.02 | +7.80 | +9.78 | +9.26 | +11.75 | +11.4 | +9.15 | +9.78 | +12.48 |

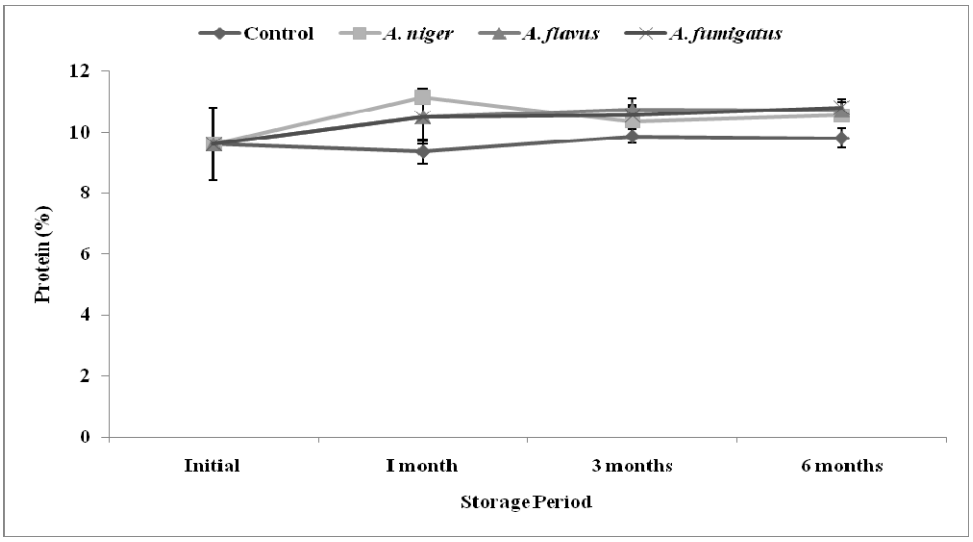


**Fig. 5** Effect of *A. niger*, *A. flavus* and *A. fumigatus* on protein concentration of Triphala powder

**Effect on total soluble sugar concentration**

During study, a small rise in total soluble sugar concentration was observed during storage. Increase in concentration was comparatively high in treated samples as compared to control.

The concentration in *A. niger* treated samples and control were 8.31±0.10, 8.81±0.13, 9.15±0.43 % and 8.74±0.11, 8.71±0.06 and 8.95±0.11% after one, three and six months of storage time respectively. An increase of 0.22 and 4% in total soluble sugar concentration was observed in *A. niger* treated samples after three and six months of storage period whereas a decreased value (5.46%) was noted after one month of storage. A rise of 1.82 % in total soluble sugar was recorded in the control (8.95±0.11%) as compared to the initial value (8.79±0.08 %) after six months, but a decrease of 0.56 and 0.91% was recorded after one and three months storage periods respectively. Triphala powder infested with *A. flavus* and *A. fumigatus* also showed a rise in total soluble sugar concentration during storage. After one, three and six months of storage period, the concentration of total sugar was recorded as 8.66±0.07, 8.98±0.08 and 9.23±0.59% in *A. flavus* and 8.46±0.47, 9.02±0.06 and 9.14±0.15% in *A. fumigatus* treated samples. In case of *A. flavus* trated samples, during initial one month of storage, sugar concentration was found to decrease (1.47%) after that a slight increase of 2.16 and 5% in total soluble sugar concentration was recorded after three and six months of storage respectively. While in case of *A. fumigatus* a decrease of 3.75% after one month and an increase of 3.98% and 2.61% was recorded after six and three months of storage periods respectively. Results showed decreased concentration of total soluble sugar after one month of storage after that an increase in concentration was observed in treated samples. The highest increase in sugar concentration was observed in *A. flavus* treated samples as compare to *A. niger* and *A. fumigatus* (Table 5, 6 and Fig. 4).

**Effect on protein concentration**

Results of present investigation showed a slight increase in protein concentration during storage. Initial protein concentration was 9.61±1.18 %, later on it was recorded as 9.36±0.40, 9.87±0.24 and 9.81±0.33 % in control after one, three and six months respectively. Only an increase of 2.08% in protein concentration of control samples was recorded after six months of storage. While in case of infested samples, protein concentration was 11.15±0.24, 10.36±0.54 and 10.55±0.12% in *A. niger* treated group, 10.50±0.90, 10.74±0.36 and 10.71±0.36 % in *A. flavus* and 10.49±0.78, 10.55±0.27 and 10.81±0.27 % in *A. fumigatus* treated samples after one, three and six months of storage periods respectively.

In comparison to control, infested samples showed increased protein concentration. Although there was no significant difference in protein concentration after one, three and six months of storage period in all the three treated groups (Table 5, 6 and Fig. 5).

## DISCUSSION

Microbial contamination of herbal drugs is an important factor which is generally responsible for maintaining the quality and efficacy of the formulations [29, 43]. Being a plant based formulation; Triphala powder is highly vulnerable to fungal contamination. During present investigation, in majority of the samples, total CFU was more than $10^3$spores/g. As per the guidelines of the World Health Organization [31], total CFU (colony forming units) of moulds should not exceed more than $10^3$ fungal spores/ g in medicinal plant formulations for internal use. There are plenty of reports available on the fungal contamination of herbal drugs but very few reports are available fungal contamination of Triphala powder. In our study, the occurrence of *Aspergillus* was observed in almost all of the samples. These results are in accordance with the observations of Gautam and Bhadauria [44] and Sharma et al. [45] Several authors also reported the *Aspergillus* as most dominant genera and *A. niger* as most prevalent species in herbal drug samples [27, 30, 43, 46, 47]. This is in agreement with our study, where *A. niger* was detected as predominant fungal species of Triphala powder. According to Pitt and Hocking [48] high incidence of black aspergilli can be justified on the basis of the black color of the spores that apparently provide protection from sunlight

and UV rays during sun drying. Secondly, this high occurrence of aspergilli and low frequency or absence of other fungi may be due to high phenolic contents of Triphala powder (21 to 44% of the dry weight) as reports of various researchers suggests that phenolics are ineffective against the storage fungi especially *Aspergillus* group as compare to pathogenic and field fungi. [49-51].

Triphala also possess a good amount of therapeutically important phytoconstituents. The major phytoconstituents reported from Triphala powder are phenolics including tannin, and ascorbic acid [6, 8, 25]. These phytoconstituents are believed to be responsible for various therapeutic uses of Triphala powder. In the present study, a sharp decrease in the concentration of total phenolics and tannin was observed in Triphala powder samples infested with storage fungi. This can be attributed to the capability of *Aspergillus* species to utilize the phenolics and tannin for their growth and development. This fact is also supported by the findings of Diepeningen et al. [52] Santos and Linardi [53] Hernandez et al [54], Stoilova et al. [55] Leitao et al. [56] Ventura et al. [57] Belmares et al. [58] Silva et al. [59], who have reported species of *Aspergillus* including *A. niger*, *A. flavus*, *A. fumigatus*, *A. awamori,* and *Penicillium chrysogenum* with phenol and tannin degrading capability and highly efficient in utilizing phenolics as sole carbon source. Tannin and phenolics degrading capability is associated with tannase enzymes produced by these fungi which efficiently break tannin and phenolics in simpler compound for further utilization [59, 60]. Stoilova et al. [55] reported that even conidia of *Aspergillus awamori* can degrade the phenolics. These reports confirm our findings where a good amount of phenolics including tannin were found to decrease in infested samples of Triphala powder after a storage period of six months. Other important constituents i.e. ascorbic acid was also found to decrease in infested Triphala powder samples as compared to control. Degradation of ascorbic acid has also been reported in sugarcane [61] and in the fruits of apricot [62]. Present investigation also revealed an increase in concentration of sugar and protein in Triphala powder infested with fungal species. An increase in sugar and protein concentration in agro industrial wastes [63]: dried leaves of Creosote bush [58] and in red chillies [64] have also been reported after fungal infestation. According to Belmares et al. [58] increase in sugar content can be attributed to capability of *Aspergillus* species to degrade plant polymers like cellulose and starch into simpler compounds whereas an increase in protein concentration may be due to increase in fungal biomass.

## CONCLUSION

Results of present investigation showed that fungi associated with Triphala powder have capability to degrade the phytochemicals. As the polyphenols and ascorbic acid are reported as major therapeutic components of the Triphala powder. A variation in the level of active constituents among marketed samples and their subsequent reduction by associated mycobiota may result into the poor efficacy of the formulation and reduction in shelf life. Increased level of sugars and protein can further make Triphala vulnerable for microbial contamination during consumption and subsequent storage. Therefore, it is the need of time to formulate the guidelines related to quality control of Ayurvedic formulations. So that fungal contamination can be brought under control. Specific precautions should be taken at the time of harvest, collection and storage of raw materials as they are the primary source of the fungal contamination.

## ACKNOWLEDGEMENT

The authors (SS and MG) are highly thankful to School of Studies in Botany Jiwaji University Gwalior (M.P.) India for providing necessary facilities to carry out this research work. The first author (SS) also extends thanks to retailers of herbal medicines, local manufacturers of Triphala and herbal practitioners for providing Triphala powder samples for research purpose.

## REFERENCES

1. Deep G. Dhiman M. Rao AR. et al. Chemopreventive potential of Triphala (a composite Indian drug) on benzo (a) pyrene induced forestomach tumorigenesis in murine tumor model system. J Exp Cl Cancer Res. 2005; 24: 555-563.
2. Pawar V. Lahorkar P. Narayana DBA. Development of a RP-HPLC method for analysis of Triphala curna and its applicability to test variations in Triphala curna preparations. Indian J Pharma Sci. 2009; 71: 386-386.
3. The Ayurvedic Pharmacopeia of India (formulations) 2007. Government of India, Ministry of Health and Family Welfare, Department of Ayush, New Delhi. Part II, Vol. I, 13
4. Gupta M. Therapeutic use of the polyherbal drug triphala in geriatric diseases. Int J Pharma Biosci. 2009; 1: 1-13.
5. Baliga MS. Meera S. Mathai B. et al. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: A review. Chinese J Integ Med. 2012; 18: 946-954.
6. Naik GH. Priyadarsini I. Mohan H. Free radical scavenging reactions and phytochemical analysis of Triphala, an Ayurvedic formulation. Curr Sci. 2006; 90: 1100-1105.
7. Juss SS. Triphala- the wonder drug. Indian Med Gaz. 1997; 131: 194-196.
8. Mukherjee PK. Rai S. Bhattacharyya S. et al. Clinical Study of Triphala -A well known phytomedicine from India. Iranian J Pharmacol Therap. 2006; 5: 51-54.
9. Hashimoto M. Nakajima Y. Antiobesity agents, alphaamylase inhibitors, lipase inhibitors, foods and beverages containing plant extracts. Japanese Kokai Tokkyo Koho. 1997; 9: 227, 398.
10. Rasool M. Sabina EP. Anti-inflamatery effect of Indian Ayurvedic formulation Triphala on adjuvant induced arthritic mice. Phyt Res. 2007; 21: 889-894.
11. Kaur S. Michael H. Arora S. et al. The *in vitro* cytotoxic and apoptotic activity of Triphala—an Indian herbal drug. J Ethnopharmacol. 2005; 97: 15-20.
12. Sandhya T. Lathika KM. Pandey BN. et al. Potential of traditional Ayurvedic formulation. Triphala as a novel anticancer drug. Can Lett. 2006; 231: 206-214.
13. Sandhya T. Mishra KP. Cytotoxic response of breast cancer cell lines. MCF 7 and T 47 D to triphala and its modification by antioxidants. Can Lett. 2006; 238: 304-313
14. Shi Y. Sahu RP. Srivastva SK. Triphala inhibits both *in vitro* and *in vivo* xenograft growth of pancreatic tumor cells by inducing apoptosis. BMC Cancer. 2008; doi:10.1186/1471-2407-8-294.
15. Kaur S. Arora S. Kaur K. et al. The in vitro antimutagenic activity of Triphala – an Indian herbal drug. Food Chem Toxicol. 2002; 40: 527-534.
16. Jagetia GC. Baliga MS. Malagi KJ. et al. The evaluation of the radioprotective effect of Triphala (an Ayurvedic rejuvenating drug) in the mice exposed to γ-radiation. Phytomed. 2002; 9: 99-108.
17. Sabu MC. Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol. 2002; 81: 155-160.

18. Naik GH. Priyadarsini KI. Bhagirathi RG. et al. In vitro antioxidant studies and free radical reactions of Triphala. an Ayurvedic formulation and its constituents. Phyt Res. 2005; 19: 582-586.
19. Dhanalakshmi S. Srikumar R. Manikandan S. et al. Antioxidant property of Triphala on cold stress induced oxidative stress in experimental rats. J Health Sci. 2006; 52: 843-847.
20. Singh R. Singh B. Kumar N. et al. Antioxidant activity of Triphala a combination of *Terminalia chebula. Terminalia bellerica* and *Emblica officinalis.* J Food Biochem. 2010; 34: 222-232.
21. Rasoll MK. Sabina EP. Lavanya K. et al. Therapeutic effect of Indian Ayurvedic formulation Triphala on acetaminophen induce hepatotoxicity in mice. J Pharmacol Toxicol. 2007; 2: 725-731.
22. Srikumar R. Parthasarathy NJ. Devi RS et al. Immunomodulatory activity of Triphala on neutrophil functions. Bio Pharma Bull. 2005; 28: 1398-1403.
23. Kumar MS. Kirubanandan S. Sripriya R. et al. Triphala promotes healing of infected full- thikness dermal wound. The J Surg Res. 2008; 144: 94-101.
24. Kumar MS. Kirubanandan S. Sripriya R. et al. Triphala incorporated collagen sponge—A smart biomaterial for infected dermal wound healing. J Surg Res. 2010; 158: 162-170.
25. Sharma DK. Varshneya C. Mehta M. Total phenolic content and antioxidant activity of Triphala (an Ayurvedic formulation) and its constituents. American J PharmTech Res. 2012; 2: 458-465.
26. Chen Q. Espey MG. Sun AY. et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Nat Acad Sci. 2008; 105: 11105-11109.
27. Gautam AK. Sharma S. Bhadauria R. Detection of toxigenic fungi and mycotoxins in medicinally important powdered herbal drugs. The Int J Microbiol. 2009; 7.
28. Atanda SA. Pessu PO. Agoda S. et al. Fungi and mycotoxins in stored foods. African J Microbiol Res. 2011; 5: 4373-4382
29. Dutta GR. Roy AK. Mycoflora associated with Strychnos seeds and deterioration of their active principles under storage. Indian Phytopath. 1987; 40: 520-524.
30. Singh P. Srivastava B. Kumar A. et al. Fungal contamination of raw materials of some herbal drugs and recommendation of *Cinnamomum camphora* oil as herbal fungitoxicant. Microbial Ecol. 2008; 56: 555-560.
31. World Health Organization. "Quality control methods for medicinal plant materials revised draft update." Working document. 2005; QAS/05.131/Rev.1.
32. Thom C. Raper KB. A manual of Aspergilli. Willima and Willikins company. USA; 1945.
33. Gilman JC. A manual of soil fungi. 2nd Ed. Ames. Iowa: Iowa state college press; 1971.
34. Barnett HL. Illustrated genera of imperfect fungi. Burgess publishing company. Minnesota; 1969.
35. Jamaluddin. Goswami MG. Ojha BM. Fungi of India 1989-2001. Scientific publisher (India). Jodhpur; 2004.
36. Samson RA. Hong S. Peterson SW. et al. Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya.* Stud Mycol. 2007a; 59: 147-203.
37. Samson RA. Noonim P. Meijer M. et al. Diagnostic tools to identify black aspergilli. Stud Mycol. 2007b; 59: 129-145.
38. Makkar HPS. Bluemmel M. Borowy NK. et al. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agri. 1993; 61: 161–165.
39. Roe JH. Chemical determination of ascorbic. dehydroascorbic and diketogluconic acids. Methods Biochem Anal. 1954; 1: 115-139.
40. Dubois MK. Gilles JK. Hamilton PA. et al. A colorimetric method for the determination of sugars. Nature. 1951; 168: 167.
41. Lowry OH. Rosebrough NJ. Fan AL. et al. Protein measurement with the Folin-Phenol Reagent. The J Biol Chem. 1951; 193: 265-275.
42. Coutinho WM. Andrade DD. Almeida PBA. et al. Development of a powder formulation of *Aspergillus parasiticus* for dry inoculation of peanut kernels. Tropical Pl Path. 2011; 36: 414-418.
43. Kumar A. Shukla R. Singh P. et al. Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *Cymbopogon flexuosus* essential oil and its components as antifungal. Int Biodet Biodeg. 2009; 63: 712-716.
44. Gautam AK. Bhadauria R. Diversity of fungi and mycotoxins associated with stored Triphala churn and its ingredients. J Biol Sci. 2011; 11: 226-235.
45. Sharma S. Gupta M. Bhadauria R. Mycobiota of Commercially Available Triphala Powder: A well known dietary supplement of Indian system of medicine. J Mycol. 2013; DOI http://dx.doi.org/10.1155/2014/836036
46. Tahir A. Aftab M. Microbial assessment of herbal drugs available in local market of Lahore. Can J App Sci. 2011; 1: 97-103.
47. Mishra PK. Shukla R. Singh P. et al. Antifungal and antiaflatoxigenic efficacy of *Caesulia axillaris* Roxb. essential oil against fungi deteriorating some herbal raw materials. and its antioxidant activity. Ind Crops Prod. 2012; 36: 74-80.
48. Pitt JI. Hocking AD. Fungi and Food spoilage. Blackie Academics & Professional. London; 1997.
49. Cowan MM. Plant Products as Antimicrobial Agents. Cl Microbiol Rev. 1999; 12: 564–582.
50. Miller JD. Fielder DA. Dowd PF. et al. Isolation of 4-acetyl-benzoxazolin-2-one (4-ABOA) and diferuloyputrescine from an extract of Gibberella Ear rot-resistant corn that blocks mycotoxin biosynthesis. and insect toxicity of 4-ABOA and related compounds. Biochem Syst Ecol. 1996; 24: 647–658.
51. Samapundo S. Meulenaer BDe. Nimoh DO. et al. Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? Food Microbiol. 2007; 24: 465-473.
52. Diepeningen AD. Debets AJM. Varga J. et al. Efficient degradation of tannic acid by black *Aspergillus* species. Mycol Res. 2004; 108: 919-925.
53. Santos VL. Linardi VR. Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential. Proc Biochem. 2004; 39: 1001-1006.
54. Hernandez MC. Esquivel JCC. Lara F. et al. Isolation and evaluation of tannin degrading fungal strains from the Mexican desert. Zeitschrift Naturforsch. 2005; 60: 844-848.
55. Stoilova I. Krastanov A. Stanchev V. et al. Biodegradation of high amounts of phenol. catechol. 2. 4-dichlorophenol and 2. 6-dimethoxyphenol by *Aspergillus awamori* cells. Enz Microbial Tech. 2006; 39: 1036-1041.
56. Leitao AL. Duarte MP. Oliveria JS. Degradation of phenol by a halotolerant strain of *Penicillium chrysogenum*. Int Biod Biodeg. 2007; 59: 220-225.
57. Ventura J. Belmares R. Carbo AA. et al. Fungal biodegradation of tannin frim Creosote bush (*Larrea tridentate*) and Tar bush (*Fluorensia cernua*) for gallic and ellagic acid production. Food Tech Biotech. 2008; 46: 213-217.
58. Belmares R. Garza Y. Rodríguez R. et al. Composition and fungal degradation of tannins present in semiarid plants. Elect J Environ Agri Food Chem. 2009; 8: 500-511.
59. Silva IS. Menezes CR. Franciscon E. et al. Degradation of lignosulfonic and tannic acids by lignolytic soil fungi cultivated under icroaerobic conditions. Braz Arch Biol Tech. 2010; 53: 693-699.
60. Enemuor SC. Odibo FJ. Culture conditions for the production of a tannase of *Aspergillus tamari* IMI 388810 (B). African J Biotech. 2009; 8: 2554-2557.
61. Singh AP. Lal R. Solomon S. Change in ascorbic acid content in sugarcane affected with smut fungus (*Ustilago scitaminea* Syd.). Sugar Tech. 2002; 4: 72-73.
62. Bhadwal J. Sharma YP. Changes in ascorbic acid content of fresh Apricot fruits under fungal pathogenesis. Environ Conser J. 2010; 11: 33-36.
63. Iyayi EA. Changes in cellulose. sugar and crude protein content of agro-industrial by-products fermented with *Aspergillus niger*. *Aspergillus flavus* and *Penicillium* sp. African J Biotech. 2004; 3: 186-188.
64. Tripathi S. Mishra HN. Nutritional changes in powdered Red Pepper upon *in vitro* infection of *Aspergillus flavus*. Braz J Microbiol. 2009; 40: 139-144.