

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Antifungal Potential of Triphala Churna Ingredients against *Aspergillus* Species Associated with Them During Storage

¹Ajay K. Gautam, ²Shubhi Avasthi, ²Anu Sharma and ²Rekha Bhadauria

¹Department of Botany, Abhilashi Institute of Life Sciences,
Mandi-175008, Himachal Pradesh, India

²Mycology and Plant Pathology Laboratory, School of Studies in Botany,
Jiwaji University Gwalior-474011, Madhya Pradesh, India

Abstracts: The present study describes the antifungal potential of fruit and powdered ingredients of triphala churna, i.e. *Emblica officinalis* (Gaertn.) (Amla), *Terminalia bellirica* (Gaertn.) Roxb. (Baheda) and *Terminalia chebula* (Retz.) (Harada), collected from the market of Gwalior (M.P.), India. Water extracts of all the fruits and powdered samples were tested (*in vitro*) for their antifungal activities by poisoned food technique against different *Aspergillus* species (*A. flavus*, *A. fumigatus*, *A. versicolor*, *A. terreus* and *A. niger*) associated with them during storage. All extracts displayed varied levels i.e. very low to very high antifungal activities on four *Aspergillus* species. The aqueous extracts of fresh fruits (37.96±7.59%) was observed to be most effective than dry fruits (34.95±7.59%) and powder (25.07±6.05%). *Terminalia chebula* (fresh and dry) extracts were found most active against the four *Aspergillus* species with 49.15 and 40.8% inhibition, respectively. None of the extracts were found effective against the growth of *A. niger*. All fruits and powdered aqueous extracts were observed to be ineffective against the *A. niger*. The variability in antifungal activity of aqueous extracts in the present study may be useful to study the relationship between antifungal potential of herbal drugs and prevalence of fungal contaminant during their storage.

Key words: Triphala churna ingredients, fungal contamination, antifungal activity and *Aspergillus* species

INTRODUCTION

Plants have been used to treat or prevent illness since before recorded history. “Vrikshayurveda”, compiled before the beginning of Christian era, “Rig Veda” in 2000 B. C. have mentioned the use of a number of plants as medicines (Tandon *et al.*, 2004). Nearly 80% population of the globe in developing countries still rely on traditional medicine for their primary healthcare (Shrikumar and Ravi, 2007) and about 25% of all modern medicine are directly or indirectly derived from plants (Calixto, 2000). This medicinal potential of herbal drugs is due to the presence of numerous biologically active compounds, which have been also reported to exhibit antimicrobial and insecticidal properties (Suliaman *et al.*, 2007; Mann *et al.*, 2008; Mahesh and Satish, 2008; Gayathri and Kannabiran, 2009). Triphala churna a wonderful ancient drug of Ayurveda, considered as a perfect tonic for proper digestion (Pandey *et al.*, 2008). Triphala, literally means “three fruits, is a combination of *Emblica officinalis* (Gaertn.) (Amla), *Terminalia bellirica* (Gaertn.) Roxb. (Baheda) and *Terminalia chebula* (Retz.) (Harada), which are medicinally important in raw and

powdered form. This wonderful ayurvedic drug aids recovery from bowel complaints, lowers blood and cholesterol levels primarily, which are all afflictions that many of us fall victim to at some point in our lives (Juss, 1997). But as the case with other herbal drugs, raw and powdered ingredients of triphala churna i.e. *E. officinalis*, *T. bellirica* and *T. chebula* are also subject to operations of contamination by various microorganisms 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 also one of the most common threats associated with stored raw and processed herbal products. Fungal contamination of stored herbal drugs not only linked to discoloration, quality deterioration, and reduction in commercial values as well as in therapeutic potential but may produces secondary metabolites like mycotoxins (Roy *et al.*, 1988). Presence of mycotoxins may degrade the product, leading to decrease in the phytochemical and medicinal properties of herbal drugs and their raw materials which may reduce the antimicrobial potential also (Truckesses and Scott, 2008). Contamination of triphala churna, its raw and powdered ingredients by different *Aspergillus* species

has already been reported (Gautam and Bhadauria, 2008; 2009, 2011). Therefore, the main objective of the present study was to examine the antifungal efficacy of raw and powdered ingredients of triphala churna against different *Aspergillus* species, which are associated with them during their storage.

MATERIALS AND METHODS

Fresh fruits of all the three medicinal plants were collected at the time of experiment. Sun dried fruits and powder form of these fruits were collected from the market of Gwalior (M.P.), India, in clean labeled packets and stored in the laboratory at the room temperature.

Isolation of mycoflora: Isolation of mycoflora from stored fruit samples of *E. officinalis*, *T. bellirica* and *T. chebula* was done by using Potato Dextrose Agar (PDA) and Czapek Dox Agar culture media. Small pieces of fruits samples were inoculated in each plate in triplicates along with a control (without samples). After inoculation plates were incubated for seven days at 25±1°C. The plates were examined after 3 to 7 days of incubation period. Morphological and cultural characteristics of different fungal isolates were studied and were identified on the basis of color, colony shape and change in their shape during growth (Gilman, 2001). Frequently occurring *Aspergillus* isolates were further confirmed at IARI, New Delhi. Pure culture of different *Aspergillus* isolates isolated from stored fruit samples were prepared on Czapek Dox agar media for five days at the optimum temperature.

Test organisms: Isolates of frequently isolated *Aspergillus* spp. (*A. flavus* #7413.09; *A. fumigatus* #7408.09; *A. versicolor*, *A. terreus* #7406.09 and *A. niger*

#7414.09) were selected to test the effect of different fruit and powder extracts on their mycelial growth.

Preparation of aqueous extracts of fruit and powdered samples:

The fresh, sun dried fruits and powders of *E. officinalis*, *T. bellirica*, and *T. chebula* were analysed in the present study. The detailed descriptions about the samples (NISCAIR, 2010) are listed in Table 1. The fresh and sun dried fruit samples were first surface sterilized with 0.01% HgCl₂, washed with sterilized distilled water and then air dried. Hundred gram of grinded powder of each fruit sample were taken in a beaker containing water 100 mL sterilized distilled water. Similarly, 100 g of powdered samples of *E. officinalis*, *T. bellirica* and *T. chebula* (sold in the market) were taken in a beaker containing 100 mL sterilized distilled water and boiled at 80°C for 10 min water bath (Awuah, 1989). The material was homogenized for 5 min, filtered through muslin cloth and filtrate was centrifuged at 5000 rpm for 15 min. The clear supernatant was collected. This was considered as 100% basic stock (Kiran and Adiver, 2006).

Screening of antifungal activity: Antifungal screening was carried out using poisoned food technique. Different concentrations of aqueous extracts (5, 10, and 20%) of fruit and powdered samples were incorporated. Small disk of the fungus culture was cut with a sterile cork borer from seven days old culture of test fungus (five isolates of *Aspergillus flavus*, *A. fumigatus*, *A. versicolor*, *A. terreus* and *A. niger*) and transferred aseptically in the centre of a petri dish containing the medium with different concentrations of extract. Three plates of each sample were used for each concentration as replicates. Control was kept where the culture disk were grown under same conditions on Czapek agar media without any extract. Inoculated plates were incubated at 27°C until mycelial growth of fungus covered the whole surface of medium in

Table 1: Ethnobotanical and phytochemical data of triphala ingredients

| Botanical Name/Family | Common name | Part used | Known phytochemicals of part used | Traditional use |
|---|-------------|-----------|--|---|
| <i>Emblica officinalis</i> / Euphorbiaceae | Amla | Fruit | Moisture (81.2%), protein (0.5 mg), fat (0.1 mg), minerals (0.7 mg), fibers (3.4 mg), carbohydrates (14.11 mg), calcium (0.05 mg), phosphorus (0.02 mg), iron (1.2 mg), nicotinic acid (0.2 mg) and vitamin C (600 mg) | Jaundice, dyspepsia and coughs, indigestion, anemia and cardiac problems, etc. |
| <i>Terminalia bellirica</i> / Combretaceae | Baheda | Fruit | Moisture (6.1%), tannin (21.4%), and water extractables (44%), lovibond color (0.5%) | Bitter, acrid, astringent, laxative, germicidal and antipyretic and is applied in diverse range of conditions including cough, tuberculosis, eye disease, dyspepsia, diarrhea, and dysentery, inflammation of the small intestine, biliousness, flatulence, liver disease and leprosy |
| <i>Terminalia chebula</i> / Combretaceae | Harada | Fruit | Chebulinic acid, tannic acid, anthraquinone, Chebulagic acid, Corilagin | Digestive, antiseptic, alterative, laxative, diuretic and carminative, eye diseases, diabetes, chronic and recurrent fever, anemia, hypertension |

control treatment. The fungus colony diameter was measured every 24 h. The colony diameter was compared with control (New, 1971). The efficacy of fruit and powder extracts in terms of percentage inhibition of mycelial growth was calculated by using the following formula (Singh and Tripathi, 1999):

$$\frac{dc-dt}{dc} \times 100$$

Where:

dc = Average increase in mycelial growth in control

dt = Average increase in mycelial growth in treatment

RESULTS

A total of six *Aspergillus* isolates namely, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A. versicolor* and *A. parasiticus* were isolated from fruit samples along with species of *Penicillium*, *Alternaria*, *Curvularia*, *Syncephalastrum* and *Rhizopus*. Frequently occurring *Aspergillus* species were confirmed further and used as test organism. The efficacy of fresh and shade dried fruits and powder extracts of *E. officinalis*, *T. bellirica* and *T. chebula* were evaluated in the present study. All the fruit and powdered extracts had shown more or less inhibitory effect on mycelial growth of the test organisms. The antifungal potential of the aqueous extracts was assessed by zone diameter (mm) measurement. The effectiveness of the extracts increased with an increase in concentration and it was recorded maximum at 20% concentration. Strong antifungal activity was shown by fresh fruits as compared to their dry fruits and powder extracts. All the aqueous extracts showed antimicrobial activity against at least four of the types of *Aspergillus* isolates tested, i.e. *A. terreus*, *A. flavus*, *A. versicolor*, *A. fumigatus*. None of the aqueous extracts were found effective against *A. niger* (Table 2).

The percent inhibition of aqueous extracts of fruit and powdered samples on mycelial growth of *Aspergillus* isolates were summarized in Table 3. The results revealed that fresh fruits were found more inhibitory (37.96±11.52) than dry (34.95±7.59) and powdered (25.07±6.05) samples. Among fruits extracts maximum inhibition of mycelial growth of fungi was obtained with fresh fruit aqueous extract of *T. chebula* (49.15±9.73) followed by dry fruits of *T. chebula* (40.8±9.13), fresh fruits of *T. bellirica* (38.61±12.07), dry fruits of *E. officinalis* (37.7±8.40). Minimum percent inhibition in mycelial growth was observed in dry and fresh fruits of *T. bellirica* (26.37±4.80) and *E. officinalis* (26.12±15.44), respectively.

Among powdered samples of all the three fruits extracts maximum inhibition of mycelial growth of fungi was obtained with aqueous extract of *T. chebula* (31.69±5.69) followed by *E. officinalis* powder (23.95±2.58). Minimum percent inhibition in mycelial growth was observed in *T. bellirica* powder (19.66±2.41). The gradation of antifungal potential i.e. low (+), medium (++) , high (+++), very high (++++), and no inhibition (-) of fruit and powdered samples against *Aspergillus* spp. is summarized in Table 4.

The detailed results about inhibition of mycelial growth *Aspergillus* isolates by different concentrations of aqueous extracts of fruits and powdered samples are depicted in Table 3. Our data revealed that the percent inhibition of mycelial growth was observed in the range of 60 to 17.31%. Among all the *Aspergillus* isolates, highest inhibition was recorded in *A. terreus* (60.5%) and *A. flavus* (53.3%) from fresh fruits of *T. chebula* at 20% conc. The next best growth inhibition form aqueous extracts was observed in fresh fruit of *T. bellirica* on *A. terreus* (70%) at 20% conc.; fresh fruit of *T. chebula* on *A. terreus* (63.6%) at 10% conc; triphala churna on *A. terreus* (62.5%) and *A. flavus* (62.5%) at 20% ; dry fruit of *T. chebula* on *A. flavus* (62.5%) at 20% and at 10% on *A. terreus* (62.5%) fresh and dry fruit of *E. officinalis* on *A. versicolor* (60%) and *A. terreus* (60%) at 20% ; fresh fruit of *T. bellirica* on *A. terreus* (60%) at 10% conc. Percent inhibition ranging from 58 to 50% was recorded at 20% conc. in fresh and dry fruit of *T. bellirica* and *T. chebula* on *A. terreus* (58.3%) and *A. versicolor* (58.3%); dry fruit of *E. officinalis* on *A. versicolor* (55.5%); fresh fruit of *T. chebula* on *A. fumigatus* (53.8%); dry fruit of *E. officinalis* on *A. flavus* (50%); fresh fruit of *T. bellirica* on *A. flavus* (50%); while at 10 % conc. of dry fruit of *T. chebula* it was 50% in *A. flavus*. It was interesting to note that, effect of aqueous extract of dry fruits of *E. officinalis* was found more effective in reducing the mycelial growth as compared to fresh fruits and powder extracts. One another investigation observed during present study was that as concentration of extracts increased, increase in diameter of mycelial growth of *A. niger* was found, which means aqueous extracts of all the samples were not effective against *A. niger*.

Table 2: Antifungal effect (%) of fruit and powdered samples on mycelial growth of *Aspergillus* isolates

| Samples | Fresh fruit | Dry fruit | Powder |
|-----------------------|-------------|------------|------------|
| <i>E. officinalis</i> | 26.12±15.44 | 37.70±8.40 | 23.95±2.58 |
| <i>T. bellirica</i> | 38.61±12.07 | 26.37±4.80 | 19.66±2.41 |
| <i>T. chebula</i> | 49.15±9.73 | 40.80±9.13 | 31.67±5.69 |
| Mean value* | 37.96±7.59 | 34.95±7.59 | 25.07±6.05 |

*Data given are means of three replicates±SD

Table 3: Percent inhibition of mycelial growth of *Aspergillus* isolates

| Type of extract | Conc. of extract (%) | <i>Aspergillus flavus</i> | <i>Aspergillus fumigatus</i> | <i>Aspergillus versicolor</i> | <i>Aspergillus terreus</i> | <i>Aspergillus niger</i> |
|---|----------------------|---------------------------|------------------------------|-------------------------------|----------------------------|--------------------------|
| <i>Emblica officinalis</i> (Amla) | | | | | | |
| Fresh fruits | 5 | 14.2 | 5.8 | 33.3 | 6.66 | NE |
| | 10 | 28.5 | 11.7 | 50 | 20 | NE |
| | 20 | 35.7 | 21.4 | 60 | 26.6 | NE |
| Mean±SD | | 26.1±10.94 | 12.9±7.87 | 47.8±13.5 | 17.7± 10.1 | NE |
| Dry fruits | 5 | 20 | 10 | 22.2 | 30 | NE |
| | 10 | 40 | 30 | 44.4 | 50 | NE |
| | 20 | 50 | 40 | 55.5 | 60 | NE |
| Mean±SD | | 36.7±15.3 | 26.7± 15.3 | 40.7± 11 | 46.7± 15.3 | NE |
| Powder | 5 | 10 | 7.6 | 16.6 | 10 | NE |
| | 10 | 22.2 | 23 | 25 | 20 | NE |
| | 20 | 33.3 | 38.4 | 41.6 | 40 | NE |
| Mean±SD | | 21.8±11.7 | 23±15.4 | 27.7±12.7 | 23.3±15.3 | NE |
| <i>Terminalia bellirica</i> (Baheda) | | | | | | |
| Fresh fruits | 5 | 16.6 | 20 | 12.5 | 40 | NE |
| | 10 | 33.3 | 33.3 | 37.5 | 60 | NE |
| | 20 | 50 | 46.6 | 43.7 | 70 | NE |
| Mean±SD | | 33.3±16.7 | 33.3±13.3 | 31.2±16.5 | 56.6±15.27 | NE |
| Dry fruits | 5 | 12.5 | 10 | 9.5 | 16.6 | NE |
| | 10 | 25 | 25 | 23.8 | 25 | NE |
| | 20 | 37.5 | 40 | 33.3 | 58.3 | NE |
| Mean±SD | | 25±12.5 | 25±15 | 22.2±11.9 | 33.3±22.05 | NE |
| Powder | 5 | 8 | 7.14 | 5.33 | 15.4 | NE |
| | 10 | 20 | 21.4 | 20 | 23 | NE |
| | 20 | 30 | 28.5 | 26.6 | 30.7 | NE |
| Mean±SD | | 19.3±11.01 | 19.01±10.9 | 17.31±10.9 | 23.03±7.65 | NE |
| <i>Terminalia chebula</i> (Harada) | | | | | | |
| Fresh fruits | 5 | 30 | 23 | 33.3 | 45.4 | NE |
| | 10 | 60 | 38.4 | 41.6 | 63.6 | NE |
| | 20 | 70 | 53.8 | 58.3 | 72.7 | NE |
| Mean±SD | | 53.3±20.8 | 38.4±15.4 | 44.4±12.7 | 60.5±13.9 | NE |
| Dry fruits | 5 | 25 | 13.3 | 25 | 37.5 | NE |
| | 10 | 50 | 33.3 | 37.5 | 52.8 | NE |
| | 20 | 62.5 | 46.6 | 43.7 | 62.5 | NE |
| Mean±SD | | 45.8±19.09 | 31.06±16.7 | 35.4±9.5 | 50.9±12.6 | NE |
| Powder | 5 | 14.2 | 11.11 | 23.8 | 25 | NE |
| | 10 | 28.5 | 27.7 | 33.33 | 41.6 | NE |
| | 20 | 42.8 | 38.8 | 42.8 | 50 | NE |
| Mean±SD | | 28.5±14.3 | 25.87±13.9 | 33.3±9.5 | 38.8±12.7 | NE |

Mean of three replicates±SD, NE: Not effective

Table 4: Antifungal activity of various concentrations of fruit and powder extracts on different fungal species

| Fruit/powder sample (Type of extract) | Conc. of fruit extract/powder (%) | <i>Aspergillus flavus</i> | <i>Aspergillus fumigatus</i> | <i>Aspergillus versicolor</i> | <i>Aspergillus terreus</i> | <i>Aspergillus niger</i> |
|---------------------------------------|-----------------------------------|---------------------------|------------------------------|-------------------------------|----------------------------|--------------------------|
| <i>Terminalia chebula</i> | | | | | | |
| Fresh fruits | 5 | ++ | + | +++ | + | - |
| | 10 | ++ | ++ | +++ | ++ | - |
| | 20 | +++ | ++ | ++++ | ++ | - |
| Dry fruits | 5 | ++ | + | ++ | ++ | - |
| | 10 | +++ | ++ | +++ | +++ | - |
| | 20 | +++ | +++ | ++++ | ++++ | - |
| Powder | 5 | + | + | + | + | - |
| | 10 | ++ | ++ | ++ | + | - |
| | 20 | +++ | +++ | +++ | +++ | - |
| <i>Terminalia bellirica</i> | | | | | | |
| Fresh fruits | 5 | ++ | ++ | ++ | +++ | - |
| | 10 | +++ | +++ | +++ | ++++ | - |
| | 20 | +++ | +++ | +++ | ++++ | - |
| Dry fruits | 5 | ++ | + | + | ++ | - |
| | 10 | ++ | ++ | ++ | ++ | - |
| | 20 | +++ | +++ | +++ | ++++ | - |
| Powder | 5 | + | + | + | ++ | - |
| | 10 | ++ | ++ | ++ | ++ | - |
| | 20 | ++ | ++ | ++ | +++ | - |
| <i>Emblica officinalis</i> | | | | | | |
| Fresh fruits | 5 | ++ | ++ | +++ | +++ | - |
| | 10 | ++++ | +++ | +++ | ++++ | - |
| | 20 | ++++ | ++++ | ++++ | ++++ | - |

Table 4: Continue

| Fruit/powder sample (Type of extract) | Conc. of fruit extract/powder (%) | <i>Aspergillus flavus</i> | <i>Aspergillus fumigatus</i> | <i>Aspergillus versicolor</i> | <i>Aspergillus terreus</i> | <i>Aspergillus niger</i> |
|--|--------------------------------------|---------------------------|------------------------------|-------------------------------|----------------------------|--------------------------|
| Dry fruits | 5 | ++ | + | ++ | +++ | - |
| | 10 | +++ | +++ | +++ | ++++ | - |
| | 20 | ++++ | +++ | +++ | ++++ | - |
| Powder | 5 | + | + | ++ | ++ | - |
| | 10 | ++ | ++ | +++ | +++ | - |
| | 20 | +++ | +++ | +++ | +++ | - |

+, Positive antifungal activity low inhibition. ++, Positive antifungal activity medium inhibition. +++, Positive antifungal activity High inhibition. +++++, Positive antifungal activity very high inhibition. -, No inhibition

DISCUSSION

The isolation of *Aspergillus* spp. (*A. flavus*, *A. terreus*, *A. versicolor*, *A. fumigatus* and *A. niger*) as dominating fungi from fruit samples of *E. officinalis*, *T. bellirica* and *T. chebula* leading us to select them as test organism in the present study. The efficacy of fruit and powdered ingredients of Triphala churna i.e. *E. officinalis*, *T. bellirica* and *T. chebula* was investigated against various *Aspergillus* species. *Aspergillus* was reported earlier as one of the dominating fungal genera isolated from stored herbal drugs (Dubey *et al.*, 2004; Roy and Chourasia, 1990; Aziz *et al.*, 1998; Bugno *et al.*, 2006; Gautam and Bhadauria, 2008; Gautam and Bhadauria, 2011). This fungal contaminant associated with stored herbal drugs decreases their medicinal potential as well as antimicrobial potential (Bugno *et al.*, 2006; Truckesses and Scott, 2008).

During this study, an increase in antifungal activity of the extracts was observed by increase in their concentrations. This finding agrees with the earlier reports (Banso *et al.*, 1999) that higher concentration of antimicrobial substance showed appreciation in growth inhibition. The fact that results of this study showed that fruits and powder extracts of *E. officinalis*, *T. bellirica*, *T. chebula* have antifungal property that justify their use as traditional medicinal plants (Mann *et al.*, 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Ogundipe *et al.*, 1998; Ibrahim, 1997). All the plants, plant parts differ significantly in their antimicrobial properties due to presence of varied concentrations of active compounds which may be responsible for their antimicrobial activities. Strong antifungal activity was shown by fresh fruits as compared to their dry fruits and powder except in case of *E. officinalis*. In *E. officinalis* dry fruits shown more antifungal activity as compared to their dry fruits, as in the fresh fruits the main ingredient is moisture (about 80%) which is completely eliminated upon drying. The difference observed in fungicidal activity of these fresh, dry and powdered aqueous extract is likely due to in the presence of active principles and their solubility in water which is also agrees with the previous reports (Qasem and Aau-Blan, 1996; Amadioha 2001). The active principles

present in plants are influenced by many factors which includes, age of plants, extracting solvents, method of extraction and time of harvesting (Calixto, 2000). It has been observed that active principles are destroyed by enzymatic processes that continue for long periods of time after plant collection (Okigbo and Nmeke, 2005). *Aspergillus niger* was reported earlier as a major *Aspergillus* isolate from stored herbal drugs (Gautam and Bhadauria, 2008; 2009; 2011), which may justifies the ineffectiveness of all fruit and powdered extracts against this fungi.

CONCLUSION

On the basis of antifungal activity of fruits and powder ingredients of triphala churna in present study, we can reveal that shelf life decrease during storage. Decreased shelf life along with prolonged storage can reduce the efficacy of stored herbal drugs. Therefore, the study is an important step towards quality control practices and management of storage fungi. Moreover, this parameter can be helpful to study the relationship between antifungal activity and frequently occurring fungal contaminants in stored herbal drugs.

ACKNOWLEDGMENTS

This work was financed by Madhya Pradesh council of Science and Technology (MPCST), Bhopal, India. The Head, School of Studies in Botany, Jiwaji University, Gwalior is gratefully acknowledged for providing necessary laboratory facilities.

REFERENCES

- Amadioha, A.C., 2001. Fungitoxic effect of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch. Phytopathol. Plant Protect., 33: 499-507.
- Awuah, R.T., 1989. Fungitoxic effects of extracts from some West African plants. Ann. Applied Biol., 115: 451-453.
- Aziz, N.H., Y.A. Youssef, M.Z. El-Fouly and L.A. Moussa, 1998. Contamination of some medicinal plant samples and spices by fungi and their mycotoxins. Bot. Bull. Acad. Sci., 39: 279-285.

- Banso, A., S.O. Adeyemo and P. Jeremiah, 1999. Antimicrobial properties of *Vernonia amygdalina* extract. J. Applied Sci. Manag., 3: 9-11.
- Bugno, A., A.A.B. Almodovar, T.C. Pereira, T.D.J.A. Pinto and M. Sabino, 2006. Occurrence of toxigenic fungi in herbal drugs. Braz. J. Microbiol., 37: 47-51.
- Calixto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (Phytotherapeutic agents). Braz. J. Med. Biol. Res., 33: 179-189.
- Dubey, N.K., R. Kumar and P. Tripathi, 2004. Global promotion of herbal medicine: India's opportunity. Curr. Sci., 86: 37-41.
- Gautam, A.K. and R. Bhadauria, 2008. Occurrence of toxigenic moulds and mycotoxins in ayurvedic medicine trifla churn. J. Myco. Plant Path., 38: 664-666.
- Gautam, A.K. and R. Bhadauria, 2009. Mycoflora and mycotoxins in some important stored crude and powdered herbal drugs. Biol. Forum Int. J., 1: 1-7.
- Gautam, A.K. and R. Bhadauria, 2011. Diversity of fungi and mycotoxins associated with stored triphala churn and its ingredients. J. Biol. Sci., 11: 226-235.
- Gayathri, M. and K. Kannabiran, 2009. Antimicrobial activity of *Hemidesmus indicus*, *Ficus bengalensis* and *Pterocarpus marsupium* Roxb. Indian J. Pharm. Sci., 71: 578-581.
- Gilman, J.C., 2001. A Manual of Soil Fungi. Oxford and IBH Publishing Corporation, New Delhi, India.
- Ibrahim, M.B., 1997. Antimicrobial effects of extract leaf stem and root bark of *Anogeissus leiocarpus* on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. J. Pharm. Dev., 2: 20-30.
- Juss, S.S., 1997. Triphala D: The wonder drug. Ind. Med. Gazett., 131: 194-196.
- Kiran, K.S. and S.S. Adiver, 2006. Effect of plant extracts on *Sclerotium rolfsii*, the incitant of stem rot of groundnut. J. Mycol. Plant Pathol., 36: 77-79.
- Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci., 4: 839-843.
- Mam, A., A. Banso and L.C. Clifford, 2008. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania J. Health Res., 10: 34-38.
- NISCAIR, 2010. The Wealth of India: An Encyclopedia of India's Raw Material Resources. CSIR, New Delhi, India, ISBN: 81-85038-00-7.
- New, Y.L., 1971. Fungicides in Plant Disease Control. IBH Publishing Co., Oxford, UK., pp: 281- 291.
- Ogundipe, O., O. Akinbiyi and J.O. Moody, 1998. Antibacterial activities of essential ornamental plants. Niger. J. Nat. Prod. Med., 2: 46-47.
- Okigbo, R.N. and I.A. Nmeka, 2005. Control of yam tuber with leaf extracts of *Xylopiya aethiopica* and *Zingiber officinale*. Afr. J. Biotech., 4: 804-807.
- Pandey, M.M., S. Rastogi and A.K.S. Rawat, 2008. Indian herbal drug for general healthcare: An overview. Int. J. Alter. Med., Vol. 6.
- Qasem, J.R. and H.A. Aau-Blan, 1996. Fungicidal activity of some common weed extracts against different plant pathogenic fungi. J. Phytopathol., 144: 157-161.
- Roy, A.K. and H.K. Chourasia, 1990. Mycoflora, mycotoxins producibility and mycotoxins in traditional herbal drugs from India. J. Gen. Applie Microbiol., 36: 295-302.
- Roy, A.K., K.K. Sinha and H.K. Chourasia, 1988. Aflatoxin contamination of some common drug plants. Applied Environ. Microbiol., 54: 842-843.
- Shrikumar, S. and T.K. Ravi, 2007. Approaches towards development and promotion of herbal drugs. Phcog. Rev., 1: 180-184.
- Singh, J. and N.N. Tripathi, 1999. Inhibition of storage fungi of black gram (*Vigna mungo* L.) by some essential oils. Flavour Fragrance J., 14: 42-44.
- Suliman, A.M.E., I.M.O. El-Boshra and E.A.A. El-Khalifa, 2007. Nutritive value of clove (*Syzygium aromaticum*) and detection of antimicrobial effect of its bud oil. Res. J. Microbiol., 2: 266-271.
- Tandon, V., B. Kapoor and B.M. Gupta, 2004. Herbal drug research in India: A trend analysis using I J P as a marker (1995-August 2003). Ind. J. Pharmacol., 36: 99-100.
- Truckesses, M.W. and P.M. Scott, 2008. Mycotoxins in botanicals and dried fruits: A review. Food Addit. Contam., 25: 181-192.