Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2017, 7(5):64-73

ISSN : 2249-7412
CODEN (USA): AJPSKY

Detection of Aflatoxins and Use of Scanning Electron Microscope for the Identification of Fungal species in Some Commonly Used Spices

Zafar Alam Mahmood*, Najma Shaheen, Farhana Tasleem, Shahlla Imam, Safia Abidi and Iqbal Azhar

Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan

ABSTRACT

There is a possibility for the presence of fungi as well as mycotoxins in spices due to the improper methods used before and after harvesting, without controlling temperature and moisture during prolong storage period or may be during transportation. The purpose of this study was to detect and identify fungal contamination in some commonly used spices in Pakistan. Spices are available in Pakistan markets in loose packing and they are easily contaminated with dust, waste water and human/animal excreta. In present study five different spices such as black pepper (Piper nigrum), cardamom large (Amomum subulatum), cinnamon (Cinamomum zeylanicum), coriander (Coriandrum sativum) and red chili (Capsicum annuum) were used for detection of fungus as well as aflatoxins. During fungal contamination test out of five spices, with the exception cinnamon all four spices were found contaminated with various fungal species. The most common organisms identified were Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Aspergillus foetidus, Aspergillus fumigatus and Aspergillus ellipticus. In total, twelve different fungal species have been detected and also identified through scanning electron microscope (SEM). Thin layer chromatography (TLC) assay was performed for aflatoxins detection and the results revealed that red chili was heavily contaminated with aflatoxins than other selected spices.

Keywords: Spices, Fungi, Aflatoxins, SEM, TLC, Contamination

INTRODUCTION

Spices represent different parts of plants and herbs, mostly in dried form and consumed for aroma (such as black pepper, cardamom small, cinnamon bark and coriander), color (such as red chili and turmeric) and as a preservative (such as cinnamon, clove, red chili and turmeric) in food and around 50 are being used in cuisine globally. Spices can be obtained from different parts of the plant, such as bark (cinnamon) buds (clove), flowers (lavender), fruits (red chili and cardamom small), leaves (coriander) rhizomes (turmeric), roots (heeng), seeds (cumin seed), etc. [1-3]. Most of the spices are highly valuable in medication due to their associated health benefits such as anti-allergens, anti-microbial, anti-diabetics, anti-cancers and anti-oxidants effects, etc. [4].

Black pepper (*Piper nigrum* L., family Piperaceae) is a smooth woody flowering plant [5,6]. Cultivated in Bengal, Malaysia, Assam, Vietnam, China, Thailand, Srilanka and India [6-8]. Piperine is a major constituent of black pepper have anti-pyretic, anti-inflammatory, analgesic, anti-fungal, anti-depressant, anti-spasmodic, diaphoretic, anti-septic, anti-toxic, aphrodisiac, diuretic, febrifuge and rubefacient activities [7,9]. Oil of black pepper also used for relief in rheumatism pain, flu, cold, exhaustion, fever, emotional and physical coldness, muscular aches, nerve tonic, stimulate appetite, increase the flow of saliva and encourages peristalsis tones [6,10]. *Amomum subulatum* Roxb. L. is the second largest genus of the family Zingiberaceae.

It is a perennial spice cultivated in moist and shady areas. This well-known ancient spice also known as cardamom large and cultivated in sub Himalayan and North Easter Himalayan of India, Sikkim, Darjeeling hills, hills of Nepal

and Bhutan. The seed of *Amomum subulatum* Roxb. L. contain essential oil with 1-8 cineol as a main constituent. Other important constituents are limonene Sabine, β-pine, α-terpineol, spathulenol, α-pinene, β-selinene, etc. The seed is used as an analgesic, alexipharmic, anti-diabetic, anti-malarial, anti-emetic, anti-microbial, in teeth and gum infections, snake venom and scorpion venom, remedy for throat and respiratory troubles. Seeds are also used as a flavoring agent and as a spice produces pleasant odor in foods [11-16].

The ever green small plant cinnamon (*Cinnamomum zeylanicum* L.) belongs to the family Lauraceae Cinnamon spice achieved by drying the center portion of bark and it is available in market as quills and powder form. This plant commonly cultivated in Indonesia, South Asia, Sri Lanka, South India and Madagascar. The major constituents of cinnamon bark oil are cinnamaldehyde and eugenol and other important constituents are cinnamic acid, coumarin cinnamyl acetate, borneol, β-caryophyllene etc. The nutritional phytochemical constituents are carbohydrates, proteins, flavonoids, saponins, tannins, glycosides and tri terpenoids, etc. [17-19]. Bark is used as an anti-allergic. anti-microbial, anti-inflammatory, anti-septic, anti-diabetic, anti-obesity, anti-oxidant, also effective in Alzheimer's disease, liver disease and in tooth ache treatment [15,17,18].

Coriander (*Coriander sativum* L.) is an annual spice belongs to the family Apiaceae. This ancient spice also known as Chinese parsley and Dhania. Coriander is extensively cultivated in Europe, South America, Pakistan, Turkey, China, India, Malaysia, France, Spain, North Africa, Thailand, Italy, Morocco [20,21]. The major phytochemical constituents of coriander seeds are linalool, α-pinene, graniol, camphor, geranyl acetate, oleic acid, linoleic acid and palmitic acid [22]. The major and nutritional components are flavonoids, amino acid, saponins, tannins, steroids, sterols, carbohydrates and cardiac glycosides [23,24]. It has many pharmacological activities such as anti-microbial, anti-cancer, anti-convulsant, anti-diarrheal, anti-hypertensive, anti-oxidant, anti-pyretic and anti-ulcer.

It also useful in heavy metal detoxification, rheumatism, insomnia, nausea and headache [20,22,25-28]. *Capsicum annuum* L. is an annual spice and belongs to the family Solanaceae. Commonly known as bell pepper, green pepper, sweet pepper, cherry pepper and chili pepper. It is widely grown in America, Mexico, India, Indonesia, China, Ethopia, Spain, Portugal, South Africa and Central Europe. The active component of red chili are capsaicin ($C_{18}H_{27}NO_3$) (trans-8 methyl-N-vanillyl-6-nonenamide) and di hydro capsaicin (8 methyl-N vanillyl nonan amide [29-31]. Nutritional compounds are also investigated such as carbohydrates, protein, alkaloids, phenol compounds, saponins, tannins and flavonoids, etc. [32]. Red chili have analgesic, anti-carcinogenic, anti-coagulant, anti-microbial, anti-oxidant, anti-tumor, arteriosclerosis, bronchitis and carminative activities. It is also effective in cough, otalgia and increased blood circulation as well as in rheumatism [15,30-35].

Fungi produce toxic substances such as mycotoxin that can be distinguished according to their chemical structure, fungal origin and biological activity. More than 200 mycotoxins have been reported in which aflatoxins considered to the most dangerous and widely studied mycotoxin. The aflatoxins were firstly isolated and recognized when more than 100,000 turkeys died in the United Kingdom and high incidence in Kenya and hatchery trout in the United States. Aflatoxins are potent carcinogen mainly produced by secondary metabolism of *Aspergillus flavus, Aspergillus nomius* or *Aspergillus parasiticus*. *Aspergillus flavus* is common and prevalent in nature. There are four major aflatoxins B_1, B_2, G_1 and G_2. Metabolic derivative of AFB_1 and ABF_2 are AFM_1 and AFM_2 respectively and both originated from metabolism of few animals and normally found in milk and urine. Aflatoxins have a wide presence in various kinds of sample matrices, such as vegetables, meat, fruits, spices, milk, cereals, oils, etc.

Spices are commonly used as natural preservative for food. Mustard and ginger may accelerate the growth of aflatoxigenic fungi whereas few other spices such as pepper, cinnamon act as anti-aflatoxigenic or antifungal [36-40]. The spices mostly grown in tropical and sub-tropical regions, which have extreme ranges of humidity, rain fall and temperature, provide favorable condition for fungal growth [41]. The high temperature and steam treatment used to reduce the microbial contamination is the major cause of loss of volatile oils from spice which effects level of the flavor, aroma and color of spices as well. Further, steam is also responsible for increase the moisture levels of spices which affects the quality of spice or long term storage and may allow the fungi to grow and produces aflatoxins toxic substances such as mycotoxin that can be distinguished according to their chemical structure, fungal origin and biological activity.

More than 200 mycotoxins have been reported in which aflatoxins considered to the most dangerous and widely studied mycotoxin [1,36]. Spices are available in markets in loose packing and they are easily contaminated with dust,

waste water and human/animal excreta. Many countries like Spain, UK, Australia, Turkey, Morocco and Pakistan have been reported that spices and spices products are commonly contaminated with aflatoxins are the most toxic compounds causing cancer in liver and other body organs in human. Foods after affected by aflatoxins can cause serious human health issues. Malnutrition, impaired immune function and stunted growth in children and a number of disabilities and death have also been reported due to extensive use of aflatoxins. The critical impact of aflatoxins in human ranges from acute hepatic toxicity to chronic disease such as haemorrhages, oedema, hepatocellular carcinoma, hepatitis, rye's syndrome, depressed immune response, liver cancer, pulmonary interstitial fibrosis, teratogenesis, tumor induction [42-45]. DNA mutation, disorder of cardio vascular and central nervous system [46].

The climate of Karachi (Pakistan) is hot and humid as compared to other city of Pakistan which invites the growth of fungi in food and the production of aflatoxins. In Karachi liver cancer is increasing day by day study shows that 34.6% liver cancer caused by alpha fetoprotein and 60% by hepatitis B and remaining through spices containing aflatoxins often sold in open market without any environmental control. Different countries like Kenya, Uganda and Thailand also indicated the relation between consumption of contaminated food with aflatoxins and the formation of liver cancer [47,48]. The present study therefore attracts the attention to potential risk may be caused as a result of using these contaminated spices.

MATERIALS AND METHODS

Collection of spices

Sample of spices black pepper (*Piper nigrum*), cardamom large (*Amomum subulatum*), cinnamon (*Cinamomum zeylanicum),* coriander (*Coriandrum sativum*) and red chili (*Capsicum annuum*) were purchased from local market Karachi, Pakistan. All ten samples were identified and their voucher specimen numbers are deposited in the department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Science.

Preparation of powder

The dried samples were cleaned and ground by grinder separately to get powder samples and stored in dry and air tight containers separately. These powder samples were used in preparation of extracts and in serial dilutions.

Preparation of extracts

Test samples (5.0 g each) were transferred as powdered to a conical flask and 50 ml of hexane, ethanol, chloroform, and distilled water were added as single solvent in each flask and stoppered. The mixtures were allowed to stand at room temperature for 24 h. Finally samples were filtered and used for aflatoxins detection test [49].

Preparation of sample for fungal contamination test

Each spice sample (1 g) was taken in a test tube separately and 9 ml distilled water was added and shaken well. Serial dilutions were prepared by transferring 1 ml of first dilution (10^{-1}) from each test tube into other 9 ml distill water containing test tube separately then the mixture of each test tube was shaken and to made 10^{-2} dilution. Procedure was repeated to obtain 10^{-3} through 10^{-6} dilution. For contamination test 10^{-4} to 10^{-6} dilutions were used [50].

Isolation and identification of fungal

With the help of sterilized pipette serially diluted each sample, i.e., 10^{-4}, 10^{-5}, 10^{-6} was taken and dropped 1 ml aliquots on to the surface in each Sabouraud Dextrose Agar (SDA) plate separately and the drops spread with the help of wire loop by using streaking method. Then the plates were moved left upright for dry purpose and were incubated at 37°C for 10 days. After 10 days fungal growth on plates were observed by visually and were identified by light microscope and scanning electron microscope [51].

Scanning electron microscopy test

Each dehydrated sample was placed separately on specimen stub with two sided adhesive tape and coated with thin layer of gold using quick auto coater model number JFC 1500. Many images of fungal species were observed and identified [52].

Aflatoxins detection test by TLC

TLC (20 × 20 cm × 0.2 mm coated with $60F_{254}$, Merck Germany) were spotted with sample extracts and placed in a TLC tank contained a solution of chloroform and acetone in the ratio of 88:12 (v/v) for 30 min at room temperature and the TLC plates were observed for the presence of aflatoxins by their characteristics fluorescence properties with the help of ultra violet (UV) light. The blue fluorescence corresponding to authentic aflatoxins B_1 and B_2 indicated the presence of AFB_1 and AFB_2 in samples. The green fluorescence corresponds to the authentic aflatoxins G_1 and G_2 [39].

RESULTS

Fungal contamination

The results revealed that four samples out of five were positive for different fungus species (Table 1). In total, twelve different fungal species have been detected and also identified through SEM (Figure 1). All fungal species were identified on the basis of colony color by light microscope and SEM's obtained data. The identification was based on the various studies reported by various research workers [52-58].

Aflatoxins contamination

The results of aflatoxins revealed (Table 2) that in test spices ethanol and chloroform extract of black pepper showed aflatoxins $G_{2,}$ ethanol extract of cardamom large showed aflatoxins B_2 and aflatoxins B_2 also showed in ethanol and

Table 1: Spices showing fungal contamination on SDA plates and characteristic features of colonies recorded through light and SEM

Name of spices	Fungal species	Colony color	Vesicle serration	Shape	Texture
Black pepper	*Aspergillus niger*	Dark brown to black	Biseriate	Globose	Smooth
	Aspergillus ellipticus	Grayish brown	Biseriate	Globose/Radiate	Rough
	Aspergillus fumigatus	Smoky gray	Uniseriate	Globose to ellipsoidal	Smooth to finely rough
	Aspergillus carbonaricus	Brownish black	Biseriate	Globose to radiate	Smooth to rough
	Aspergillus foetidus	Reddish brown	Biseriate	Globose to subglobose	Smooth
	Aspergillus flavus	Green	Biseriate	Elliptical	Smooth to finely rough
	Aspergillus parasiticus	White	Uniseriate	Globose to subglobose	Rough
Cardamom large	*Aspergillus niger*	Brown	Biseriate	Globose	Rough
	Aspergillus aculeatus	Brown to black	Uniseriate	Ellipsoidal	Rough
	Aspergillus tubingensis	Grayish black	Biseriate	Elliptical	Rough
Cinnamon	Not observed				
Coriander	*Aspergillus niger*	Brown	Biseriate	Globose	Rough
	Aspergillus spp. UFLADC 01	Light brown	Biseriate	Globose to subglobose	Spiny
	Aspergillus sclerotion niger	Black pigments on yellow	Biseriate	Globose	Smooth
	Aspergillus ibericus	Greenish black	Biseriate	Granular	Spine
Red chili	*Aspergillus uvarum*	Black	Uniseriate	Globose to sub globose	Spine
	Aspergillus flavus	Yellowish green	Biseriate	Elliptical	Rough

Table 2: Aflatoxins detection in spices by TLC

S. No.	Name of spices	Extracts used	Observations	Rf values of test samples observed as green and blue fluorescesing spots	Presence/Absence of aflatoxins type based on confirmatory test	Rf values of standard aflatoxins
1	Black pepper	I	+	Nil		
		II	+	0.32	G2	
		III	+	0.32	G2	
		IV	-	Nil		
2	Cardamom large	I	+	Nil		
		II	+	0.31	B2	
		III	-	Nil		
		IV	-	Nil		
3	Cinnamon	I	-	Nil		
		II	-	Nil		
		III	-	Nil		
		IV	-	Nil		
4	Coriander	I	-	Nil		
		II	-	0.31	B2	
		III	-	0.29	B2	
		IV	-	Nil		
5	Red chili	I	-	0.31	G2	
		II	+	0.32	G2	
		III	+	0.32	G2	
		IV	-	Nil		

Key:

I	*Hexane extract*	*IV*	*Distilled water extract*
II	*Ethanol extract*	+	*Positive*
III	*Chloroform extract*	-	*Negative*

chloroform extract of coriander, while aflatoxins G_2 showed in hexane, ethanol and chloroform extract of red chili, while all cinnamon extracts showed negative results of aflatoxins.

DISCUSSION

In recent years, consumption of spices has considerably increased in daily food preparation to enhance aroma and flavor. Spices like black pepper, cardamom large, coriander and red chili may be contaminated with fungus and aflatoxins due to the improper methods used before and after harvesting, without controlling temperature and moisture during prolong storage period or may be during transportation.

The morphological contamination of spices has great influence upon its quality and its application for any specific purpose. A number of investigators have performed microbiological contamination tests and reported various fungal species in various spices collected from domestic market [59,60]. The most commonly identified fungus organisms were *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus,* etc. [60]. Presence of these organisms and including some other fastidious organisms can greatly affect the quality of spices and may produce aflatoxins causing harmful effects upon human body. The European pharmacopoeia specifies that the total viable aerobic count should not be more than 10^2 fungi per gram of any sample taken for fungal contamination tests. During fungal contamination test out of five spices with the exception cinnamon, all four spices were found contaminated with various fungal species.

The most common organisms identified were *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Aspergillus foetidus, Aspergillus fumigatus* and *Aspergillus ellipticus.* The shape and texture of identified fungal species have mentioned along with the colony color (Table 1). In total, twelve different fungal species have been detected and also identified through SEM (Figure 1).

All fungus was identified on the basis of colony color and by light microscope and SEM's obtained data. On the basis of conidia ornamentation we distinguished the different species for example *Aspergillus foetidus*, *Aspergillus niger* and *Aspergillus tubingensis* species are difficult to distinguish on morphological basis but on the basis of conidial ornamentation *Aspergillus foetidus* could be distinguished, it has delicately spine in immature stage, when mature its conidia become smooth. *Aspergillus tubingensis* could be differentiating to *Aspergillums niger* to its white to pink

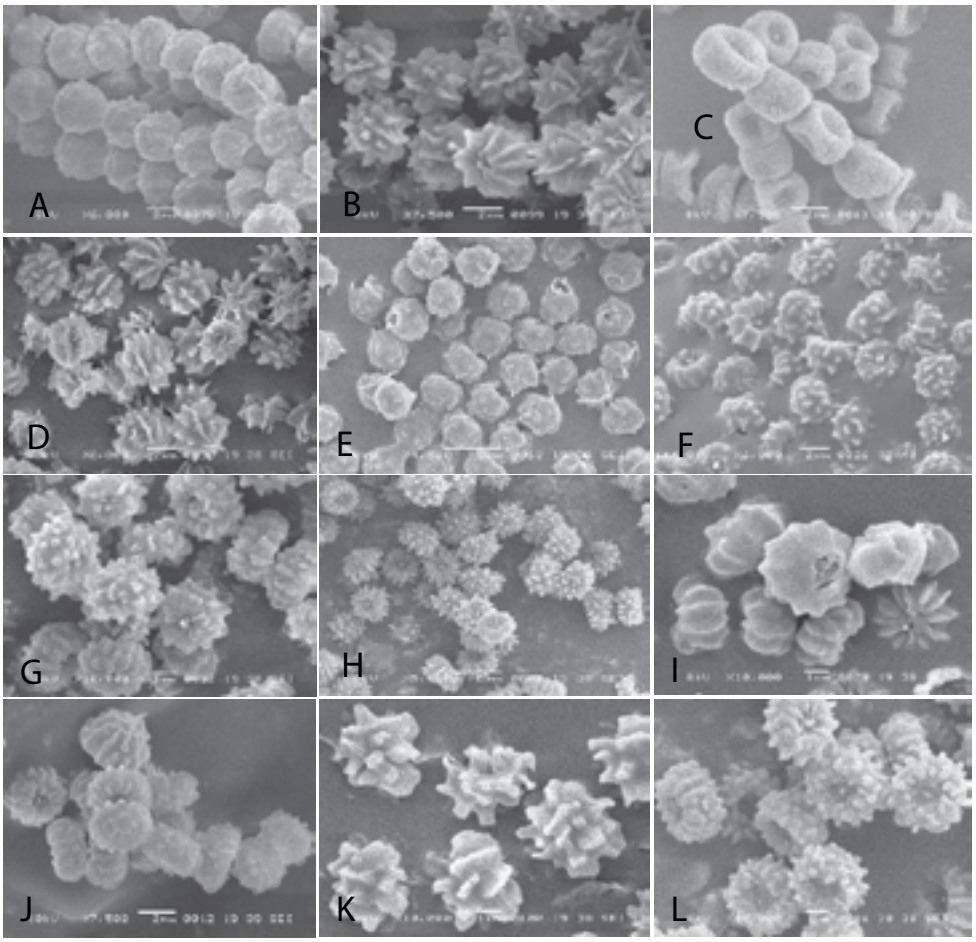

Figure 1: SEM images of identified fungal species

A-L: Identified fungal species, A: *Aspergillus niger,* B*: Aspergillus ellipticus,* C: *Aspergillus fumigatus,* D: *Aspergillus Carbonaricus,* E: *Aspergillus foetidus,* F: *Aspergillus flavus,* G: *Aspergillus parasiticus,* H: *Aspergillus aculeatus,* I: *Aspergillus tubingensis,* J: *Aspergillus* spp. UFLA DCA01, K: *Aspergillus ibericus,* L: *Aspergillus uvarum*

color sclerotia production.

Aspergillus ibericus and *Aspergillus carbonaricus* and *aspergillus ellipticus* are similar but can be differentiate on the basis of conidia ornamentation *Aspergillus carbonaricus* having bigger conidia than *Aspergillus ibericus* while *Aspergillus ellipticus conidias* were found in elliptical shape. *Aspergillus* spp. UFLA DCA 01 and *Aspergillus costaricansis* morphologically are similar but on the bases of conidial ornamentation they are distinguished each other *Aspergillus* spp. UFLA DCA 01 showed spiny to finely wrinkled conidia while *Aspergillus costaricansis* has smooth conidia to distinctly wrinkle and has larger vesicle size than *Aspergillus* spp. UFLA DCA 01 vesicle.

Aspergillus nidulans identified by its colony color with smooth wall and has short conidiophores. Ascospore of *A. nidulans* surrounded by Hulle cells. *Aspergillus japinocus* and *Aspergillus aculeatus* were found similar both have spiny conidia but *Aspergillus aculeatus* had large vesicle with ellipsoidal conidia while *Aspergillus japinocus* had globular conidia with small vesicle. *Aspergillus niger* and *Aspergillus lacticoffeatus* were found morphologically similar but distinguished morphologically by difference in conidia color and ornamentation. The identification of fungus species was confirmed of the various studies reported by various research workers [52-58].

Aflatoxins are potent carcinogen mainly produced as secondary metabolite of *Aspergillus flavus, Aspergillus parasiticusnomius or Aspergillus parasiticus*. The most common reported aflatoxins producing organism is *Aspergillus flavus*. In the present study (Table 2) red chili was found more contaminated with aflatoxins than other selected spices. Many researcher like Yernani et al. [39], Reddy et al. [30], Rajarajan et al. [61], Fufa and Urga [62] have also investigated the high level of aflatoxin in red chili and since it is an essential ingredient of food specially in sub-continent, therefore people who are consuming red chili are more prone to be affected by liver fungus due to the improper methods used before and after harvesting, without controlling temperature and moisture during prolong storage period or may be during transportation.

In present studies the absence of fungus species in cinnamon may be due to the unfavorable condition for the development of microbial species during production, storage and transportation and may be applicable to good manufacturing practice after harvesting like cleaning, drying and packing which may have minimized the growth of mould and production of aflatoxins in spice. Synder [63] investigated that cinnamon has strong anti-microbial effect; coriander seed has moderate anti-microbial effect while black pepper and red chili have weak anti-microbial effects [63]. Among test spices cinnamon was showed negative result of fungus because it contain eugenol, cinnamyldehyde, cinnamic acid, methoxy cinnamaldehyde and cinnamyl alcohol they have inhibitory effects to mycotoxigenic *Aspergillus* and *Aspergillus parasiticus* [64,65].

CONCLUSION

Spices are natural products and cultivated in tropical and non-tropical countries. Pre and post harvesting spices implicates a number of hygienic problems and lead to high microbial counts which affect quality of the spices and thus on the basis of microbial contamination results, it is believed that a requirement of a control system to maintain hygienic quality and good sanitary practice during different process of production of spices to improve the quality of spices is highly essential. The fungal contamination test must be performed before these can be used in food products.

REFERENCES

[1] Douglas M, Heyes J, Small field B. Herbs, spices and essential oil. Postharvest operations in developing countries. Food and agriculture organization of the United Nations. United Nations industrial development organization. **2005**, 1-60.

[2] Singh A, Sharma OK, Garg G. Natural products as preservatives. *Int J Pharm Biol Sci*, **2010**, 1: 601-612.

[3] Sahu TR. Economic botany. Spices. India: S L Kochhar Macmillian Publisher India Ltd., **2007.**

[4] Gladness E.T. Fungal contamination of selected commonly used spices in Tanzania. *J Adv Biol Biotechnol*, **2016**, 8: 1-8.

[5] Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, et al. Biological role of *Piper nigrum* L. (Black pepper). *Asian Pac J Trop Biomed.* **2012**, S: 1945-1953.

[6] Aziz S, Naher S, Abukawsar MD, Roy SK. Comparative studies on physico-chemical properties and GC-MS analysis of essential oil of the two varieties of the black pepper (*Piper nigrum* Linn.). *Int J Pharm Phytopharm Res*. **2012**, 2: 67-70.

[7] Trivedi MN, Khemani A, Vachhani UD, Shah CP, Santani DD. Pharmacognostic phytochemical analysis and antimicrobial activity of two Piper species. *Int J Comp Pharm*, **2011**, 7: 1-4.

[8] Reshmi SK, Sathya E, Devi PS. Isolation of piperdine from *Piper nigrum* and its anti -proliferative activity. *J Med Plants Res*, **2010**, 4: 153-1546.

[9] Nahak G, Sahu RK. Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. *J Appl Pharm Sci.* **2011,** 1: 153-157.

[10] Rai N, Yadav S, Verma AK, Tiwari L, Sharma RK. Quality specifications on *Piper nigrum* L. - A spice and herbal drug of Indian commerce. *Int J Food Sci Tech*. **2012**, 1: 1-11.

[11] Bisht VK, Negi JS, Bhandari AK, Sundriyal RC. Traditional phytochemical and biological activities. *Afr J Agric Res*. **201**1, 6: 5386 - 5390.

[12] Shukla SH, Mistry HA, Patel VG, Jogi BV. Pharmacognostical, preliminary phytochemical studies and analgesic activity of *Amomum subulatum* Roxb. *Pharma Science Monitor: An International Journal of Pharmaceutical Sciences,* **2010,** 1: 90-102.

[13] Gopal K, Baby C, Mohammed A. *Amomum subulatum* Roxb: An overview in all aspects. *Int Res J Pharm,* **2012,** 3: 96-99.

[14] Sharma G, Sharma R, Sharma E. Traditional knowledge systems in large cardamom farming: Biophysical and management diversity in Indian mountains regions. *Indian J Tradit Knowledge*, **2009**, 8: 17-22.

[15] Peter KV. Handbook of herbs and spices. Volume 1. Wood head publishing limited and CRC press LLC, **2001**.

[16] Kapoor IPS, Bandana S, Gurdip S. Essential oil and oleoresins of Cardamom (A.S.R) are as natural food preservatives for sweet orange juice. *J Food Process Eng,* **2011**, 34: 1101-1113.

[17] Vangalapati M, Satya SN, Prakash SDV, Avanigadda S. A review on pharmacological activities and clinical effects of Cinnamon species. *Res J Pharm Biol Chem Sci,* **2012,** 3: 653 - 663.

[18] Jakhetia V, Patel R, Khatri P, Pahuja N, Garg S, et al. Cinnamon: A pharmacological review. *J Adv Sci Res,* **2010,** 1: 19-23.

[19] Manurung SI, Parhusip A, Wibawa FK. Studies of antibacterial activity from cinnamon extract towards the damage of pathogenic bacteria. *Journal of Applied and Industrial Biotechnology*, **2008**, 1: 1-6.

[20] Pathak NL, Kasturi SB, Bhat NM. Phytochemical screening of *Coriander sativum* Linn. *Int J Pharm Sci*, **2011**, 9: 159-163.

[21] Maroufi K, Farahani HA, Darvishi HH. Importance of coriander (*Coriandrum sativum* L.) between the medicinal and aromatic plants. *Adv Environ Biol*, **2010**, 4: 433-436.

[22] Nimish PL, Sanjay KB, Nayna BM, Jaimik RD. Phytopharmacological properties of *Coriandrum sativum* as a potential medicinal tree. *J Appl Pharm Sci*, **2011,** 1: 20-25.

[23] Uma B, Prabhakar K, Rajendran S, Sarayu LY. Studies on GC/MS spectro -scopic analysis of some bioactive antimicrobial compounds from *Cinnamomum zeylanicum*. *J Med Plants Res,* **2009,** 8: 125-131.

[24] Nair SS, Nithyakala CM, Rozario RV, Jennifer J, Somashekraiah BV. Biochemical characterization of selected plant species and investigation of phytochemicals for *in vitro* antioxidant activity. *Int J Pharmacogn Phytochem Res,* **2012**, 4: 127-133.

[25] Momin AH, Acharya SS, Gajjar AV. *Coriandrum sativum* - Review of advances in phytopharmacology. *Int J Pharm Sci Res,* **2012,** 3: 1233-1239.

[26] Asgarpanah J, Kazemivash N. Phytochemistry, pharmacology and medicinal properties of *Coriandrum sativum* L. *Afr J Pharm Pharmacol*, **2012**, 6: 2340-2345.

[27] Wangensten H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. *Food Chem*, **2004**, 88: 293-297.

[28] Shivanand P. *Coriandrum sativum*: A biological description and its uses in the treatment of various diseases. *Int J Pharm Life Sci,* **2010,** 1: 119-126.

[29] Nadeem M, Riaz A. Cumin (*Cuminum cyminum*) as a potential source of antioxidants. *Pak J Food Sci*. **2012**, 22: 101-107.

[30] Reddy MVB, Sasikala P. Capsaicin and color extraction from different verities of green and red chili peppers of Andhrapradesh. *Int J Adv Sci Tech Res*, **2013**, 2: 554-572.

[31] Sunil P, Sanjay Y, Vinod S. Pharmacognostical investigation and standardization of *Capsicum annuum* L. roots. *Int J Pharmacogn Phytochem Res*, **2012**, 4: 21-24.

[32] Kouassi CK, Koffi NR, Guillaume LY, Yesse ZN, Koussemon M, et al. Profiles of bioactive compounds of some pepper fruit (*Capsicum* L.) varieties frown in cote D' ivoire. *Innov Rom Food Biotechnol,* **2012,** 11: 23-31.

[33] Omolo MA, Wong Z, Mergen AK, Hastings JC, Le NC, et al. Antimicrobial properties of chili peppers. *J Infect Dis Ther*, **2014**, 2: 2332-0877.

[34] Pawar SS, Bharude NV, Sonone SS, Desmukh RS, Raut AK, et al. Chilies as food, spice and medicine: A perspective. *Int J Pharm Biol Sci*, **2011**, 1: 311-31.

[35] Al-Snafi AE. 2015. The pharmacological importance of capsicum species (*Capsicum annuum* and *Capsicum frutescens*) grown in Iraq. *J Pharm Biol*, **2015,** 5: 124-142.

[36] Okello DK, Kaaya AN, Bisikwa J, Were M, Oloka HK. Management of aflatoxins in ground nuts. A manual for farmers, processors, traders and consumers in Uganda: National agricultural research organization, **2010**, 1-7.

[37] Otsuki T, Wilson JS, Sewadeh M. saving two in a billion: quantify the trade effect of European food safety standards on African exports. *Food Pol*, **2001**, 26**:** 495-514.

[38] Rajasinghe M, Abeywickrama K, Jayasekera R. Aflatoxigenic *Aspergillus flavus* and Aflatoxin formation in selected spices during storage. *Trop Agric Res Ext,* **2009,** 12: 1-6.

[39] Yerneni SG, Hari SS, Jaganthan R, Senthamarai M, Vasanthi NS, et al. Determination of the level of aflatoxin present in the marketed spices. *World J Sci Technol*, **2012**, 2: 31-34.

[40] Lizarraga-Paulin EG, Moreno-Martinez E, Miranda-Castro SP. Aflatoxins and their impact on human and animal health: An emerging problem. *Aflatoxins Biochem Mol Biol*, **2011**, 255-282.

[41] Halil T, Arslan R. Determination of aflatoxins B1 levels in organic spices and herbs. *ScientificWorldJournal*, **2013**, 2013: 874093.

[42] Khan MA, Asghar MA, Ahmed A, Iqbal J, Shamsuddin ZA. Reduction of aflatoxins in dundi-cut whole red chilies (*Capsicum indicum*) by manual sorting technique. Science, Technology and Development. **2013**, 32: 16-23.

[43] Bbosa GS, Kitya D, Lubega A, Ogwal-Okeng J, Anokbonggo WW, et al. Review of the biological health effects of aflatoxins on body organs and body systems. Aflatoxin-Recent advances and future prospects (chap 12). Intech Publisher, **2013,** 239-265.

[44] Munir MA, Saleem M, Mafik ZR, Ahmed M, Ali A. Incidence of Aflatoxins contamination in non-perishable food commodities. *J Pak Med Assoc,* **1989**, 39: 154.

[45] Sharma A, Sharma K. Protection of maize by storage fungi and aflatoxin production using botanicals. *Indian J Nat Prod Resour*, **2012**, 3: 215-221.

[46] Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, et al. Fungal and aflatoxin contamination of some human food commodities in Nigeria. *Afr J Food Sci*, **2010**, 4: 127-135.

[47] Abrar M, Anjum FM, Zahoor T, Nawaz H. Effect of storage period and irradiation doses on red chilies. *Pak J Nutri*, **2009**, 8: 1287-1291.

[48] Nizami F, Nizami HM, Ahinad M. Aflatoxin in uncooked commodities: Spices. *J Pak Med Assoc*, **1986**, 36: 109-111.

[49] Ugochukwu, SC, Arukwe UI, Ifeanyi O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Asian J Plant Sci Res*, **2013**, 3: 10-13.

[50] Thoha TB, Lzuka EH, Sikirat, Toyin AM, Omobowale AK, et al. Enumeration of microorganism in dried cassava powder (garri): A comparative study of four methods. *New York Sci J*, **2012**, 5: 63-66.

[51] Mandeel QA. Fungal contamination of some imported spices. *Mycopathologia*, **2005**, 159: 291-298.

[52] Silva DM, Batista LR, Rezende EF, Fungaro MHP, Sartori D, et al. Identification of fungi of the genus aspergillus section nigri using polyphasic taxonomy. *Braz J Microbiol*, **2011**, 42: 761-773.

[53] Simoes MF, Santos C, Lima N. Structural diversity of aspergillus (section Nigri) spores. *Microscopy and Microanalysis*, **2013**, 19: 1151-1158.

[54] Refai M, El-Yazid A, Hassan A. Monograph on *aspergillus* and aspergillosis in man, animals and birds. A guide for classification and identification of aspergilli, diseases caused by them, diagnosis and treatment, **2014**, 1-169.

[55] Serra R, Cabaries FJ, Perrone G, Castella G, Venancio A, et al. *Aspergillus ibericus*: A new species of section nigri isolated from grapes. *Micologia*, **2006**, 98: 295-306.

[56] Rodrigues P, Soares C, Kozakiewicz Z, Paterson RRM, Lima N, et al. Identification and characterization *of Aspergillus flavus* and aflatoxins. Communicating current research and educational topics and trends in applied microbiology. A Mendez-Vilas, **2007**, 527-334.

[57] Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M. Identification of aspergillus species using morphological characteristics. *Pak J Med Sci*, **2007**, 23: 867-872.

[58] Guan H, Yang L, Guo J, Ma X, Wang H, et al. Morphological and molecular identification of *Aspergillus versicolor* D−1 with selective reduction ability. *Asian J Tradit Med,* **2007**, 2: 39-44.

[59] Parveen S, Das S, Begum A, Sultana N, Hoque M, et al. Microbiological quality of three selected spices in Bangladesh. *Int Food Res J*, **2014**, 21: 1327-1330.

[60] Ahene RE, Odamtten GT, Owusu E. Fungal and bacterial contaminants of six spices and spice products in Ghana. *Afr J Environ Sci Technol*, **2011**, 5: 633-640.

[61] Rajarajan PN, Rajasekaran KM, Devi NKA. Aflatoxin contamination in agriculture commodities. *Indian J Pharm Biol Res*, **2013**, 1: 148-151.

[62] Fufa H, Urga K. Screening of aflatoxins in shiro and ground red pepper in Addis Ababa. *Ethiop Med J*, **1996**, 34: 243-249.

[63] Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. *J Food Saf,* **1988**, 9: 97-118.

[64] Elshafie AE, Al-Rashidduli TA, Al-Bahry SN, Bakheit CS. Fungi and aflatoxins associated with spices in the sultanate of Oman. *Mycopathologia,* **2002**, 00: 1-6.

[65] Al-Juraifani AA. Natural occurrence of fungi and aflatoxins of cinnamon in the Saudi Arabia. *Afr J Food Sci*, **2011**, 5: 460-465.