INTRODUCTION

Spices are characterized as any dried, aromatic vegetables or plant materials in the entire, crushed, or ground forms. They incorporate all the parts of herbaceous plants aside from the leaf, which is viewed as an herb (Billing & Sherman, 1998). Spices are utilized in little concentrations for their preservative and medicinal properties (Sethi, Dutta, Gupta, & Gupta, 2013) and also to enhance or change the taste of food (Al-Mofleh, 2010). In particular, spices have been employed since antiquated circumstances with a view to prevent food spoilage and deterioration and to increase the validity of food (Shan, Cai, Brooks, & Corke, 2007). Investigations have presumed that spices might be extremely helpful since bacteria can develop resistance to conventional anti-infection agents (Gull et al., 2012). Thus, spices have been utilized as elective drugs particularly among disease patients (Boon, Olatunde, & Zick, 2007). In addition, the herbal medicine has been authenticated to hinder the engendering of tumor cells (Dwivedi, Shrivastava, Hussain, Ganguly, & Bharadwaj, 2011).

Zingiber officinale (ginger) is one of the Zingiberaceae family (Bhargava, Dhabhai, Batra, Sharma, & Malhotra, 2012). Curcuma longa or “turmeric” as known commonly is vastly utilized as a spice and coloring agent, and it also exhibits stimulating properties (Luthra, Singh, & Chandra, 2001). Cumin seeds (Cuminum cyminum L.) belong to the Umbelliferae family and generally implanted in Egypt (Romeilah, Fayed, & Mahmoud, 2010). Nigella sativa L. belonging to...
the Ranunculaceae family is known as dark cumin (Ali & Blunden, 2003). *Salvia officinalis* or sage is a flavoring spice of the Lamiaceae family (Smidling, Mitic-Culafic, Vukovic-Gacic, Simic, & Knezevic-Vukcevic, 2008).

Spices are getting polluted by molds before the drying process during the treatment (Stankovic, Comic, & Kocic, 2006). In addition, the awful states of capacity, ventilation, and high moisture rate are prompt reasons for spoilage of spices by pathogenic microorganisms (Omafuvbe & Kolawole, 2004).

Aflatoxins and ochratoxins A are mycotoxins delivered by few contagious types of the *Aspergillus* and *Penicillium* genera (Frisvad, Thrane, & Samson, 2007). Mycotoxins induce dangerous consequences in living organisms since they exhibit carcinogenic, nephrotoxic, hepatotoxic, and immunotoxic effects (Cao et al., 2013). Aflatoxin and ochratoxin are examined in a variety of dietary products such as spices (Zinedine et al., 2007). Temperature and humidity are considered as the principal factors that control the production of mycotoxin (Giessler, Rodrigues, Handl, & Hofstetter, 2010). The International Agency for Research on Cancer (IARC, 2002) has ordered the naturally occurring blends of aflatoxins as human carcinogens (group 1) and ochratoxin A as potential carcinogen to human (group 2B).

Molecular techniques have been employed as robust methods for detection and identification of fungi (Hassan, Gaber, & El-Hallous, 2014). Consequently, rapid strategies such as polymerase chain reaction (PCR) have been utilized directly for testing food-borne pathogens and for confirmation and genotyping of food microbes. The basic feature of PCR is that organisms are not in need to be cultured, at least not for lengthy periods prior to their detection. Moreover, PCR-based methods that target DNA offer a good way for rapid diagnosis because of their outstanding specificity and sensitivity (Bandyopadhyay et al., 2016), particularly in developing species-specific primers using multicycopy sequences (Awoor et al., 2016).

In the present study, we have evaluated the mycobiota of five types of spices, determination of total AFs and OTs in the most common isolates of *A. flavus* and *A. niger* and also in some samples which contaminated by them, detection of AFs and OTs biosynthesis genes in the same isolates also comparison the genetic diversity by using ISSR-PCR.

## 2 | MATERIALS AND METHODS

### 2.1 | Collection of spice samples and mycological isolation

Fifty specimens of spices (10 samples for each) were gathered from different markets in two governorates: Sohag and Qena in Upper Egypt. Each sample was reserved in a sterile polyethylene pack and transferred to the mycological laboratory for fungal examination.

The dilution-plate technique was applied for the quantitative enumeration of contaminated spices, as mentioned by Christensen (1963). A known weight of tested spices (2 g of each of cumin and black cumin, and 0.3 g of turmeric, sage, and ginger samples) was mixed aseptically in 50 ml of sterile distilled water and shaken vigorously. One ml of the proper dilution was poured in sterilized Petri dishes. Fifteen ml of melted DRBC agar media g/L (peptone, 5; dextrose, 10; KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; dichloran, 0.002; agar, 15), rose bengal (0.025) and chloramphenicol (0.1) were added as bacteriostatic agents (Baylis, 2003), cooled to 45°C, and poured on the sample suspension in Petri plates which were turned to convey the suspension. Triplicates were read, and the cultures were incubated at 28°C for 7 days. The creating colonies of fungi were isolated, identified, and counted.

### 2.2 | Determination of total AFs and OTs producing potential of *Aspergillus flavus* and *A. niger* isolates and also in the samples

The total AFs and OTs producing ability of the isolates were examined by cultivating fungal strains in Czapek yeast extract agar (Ben Fredj, Chebil, & Mlik, 2009) medium for 5 days at 25°C. Total AFs and OTs were extracted by shaking 50 ml of fungal filtrate with methanol (100 ml).

The natural occurrence of total aflatoxins and ochratoxins in five samples (one of each type that highly contaminated) was determined by using a slightly modified method based on the Association of Official Analytic Chemists (AOAC) method (Trucksess et al., 1991). Methanol:water (80:20) solvent (100 ml) and 5 g NaCl were added to 100 g of ground each sample, and the mixture was shocked in a blender at maximum speed for 3 min. Filtration process occurred through fluted filter paper (Whatman 2V; Whatman plc), and the filtrate was diluted (1:4) with water and refiltered through glass-fiber filter paper. Two milliliters of the glass-fiber filtrate was placed on an AFs or OTs Test RWB SR Column (VICAM) and allowed to elute at 1–2 drops/s. The columns were washed two times with 5 ml water, and aflatoxin or ochratoxins were eluted from the column with 1 ml high-performance liquid chromatography (HPLC)-grade methanol. A bromine developer (1 ml) to the methanol extract was added, and the total AFs or OTs concentrations were read in a recalibrated VICAMSeries-4 fluorometer set at 360 nm excitation and 450 nm emissions (Lewis et al., 2005).

### 2.3 | Molecular detection of AFs and OTs biosynthesis genes

All the molecular steps were employed by Gene analyzer 3121 in Scientific Research Center, Biotechnology and Genetic Engineering Unit, Taif University, KSA.

DNA extraction and purification were performed using DNA Promega Kit DNeasy Blood & Tissue. Two published primer sets were employed for the specific detection of *omt*-A and *Aopks* genes (Criseo, Racco, & Romeo, 2008). The sequences of primers were as follows: *Aopks*-F 5′-CAGACCATCGAACTGCATGC-3′, *Aopks*-R 5′-CTGGCGTTCCAGTACCATGAG-3′, *omt*-A-F 5′-GAC CAATAAGCCACAGAC-3′, and *omt*-AR 5′-CTTGTGTAAGCTCTCTCGC-3′, respectively. The 549 and 320 bp fragments were
amplified, respectively. The used reaction volume in PCR was 25 μl (Hassan et al., 2014). The reactions achieved in a C1000TM Thermo Cycler Bio-Rad, Germany, involving with denaturation at 94°C for 5 min, followed by 36 cycles at 94°C for 45 s, annealing at 46°C for 45 s, and extension at 72°C for 1 min; then, final step was extended for 7 min at the same temperature (Criseo et al., 2008). The separation of PCR products was accomplished by agarose gel (1.3%) electrophoresis followed by staining with ethidium bromide.

2.4 | ISSR analysis

Primers utilized in ISSR analysis were obtained from Amersham Pharmacia Biotech. Following the experiments for optimization of component concentrations, PCR amplification of random primers was carried out in 25 μl volume containing 1 μl (20 ng) of genomic DNA, 12.5 μl of Go Taq® Green Master Mix, Promega, USA, 1 μl of primer (20 p.mol), and deionized distilled water (up to a total volume of 25 μl) as described by Hassan et al. (2014). For DNA amplification, the C1000TM Thermo Cycler Bio-Rad, Germany, was programmed under the conditions involving denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 30 s, primer annealing at 35°C for 1.5 min, primer extension at 72°C for 2.5 min, and final extension step at 72°C for 7 min. Amplified DNA products were analyzed by electrophoresis in 1.5% agarose gel run in TBE. The gels were stained with ethidium bromide (5 μg/ml). 100 bp DNA ladder (Solis Bio-Dyne®) was applied as a standard. DNA was visualized by UV illumination and then photographed by a Bio-Rad Gel Doc 2000 device.

2.4.1 | Data analysis

The amplification results of ISSR-PCR were recorded for the presence as "1" or absence as "0" and missing data as "9." The genetic associations between isolates were evaluated by calculating Jaccard’s similarity coefficient for pairwise comparisons depending on the proportion of shared bands produced by the primers. The dissimilarity matrix was generated using of neighbor joined technique, and the dendrogram was reconstructed. The computations were achieved utilizing the NTSYS-computer program version 2.01 (Rohlf, 2000). For principal component analysis, Jaccard’s similarity matrix was amplified.

2.5 | Cytotoxic effect on human cell lines

All the cytotoxic effect was carried out in Department of Pharmacognosy, National Research Centre El-Tahrir Str., Dokki, Giza 12622, Egypt.

2.5.1 | Preparation of extracts

Ten g of each powdered spice (ginger, cumin, turmeric, sage, and black cumin) was macerated with 50 ml hexane 80% at room temperature for 3 days. The stock solutions were prepared by adding 1ml dimethylsulfoxide (DMSO) to the residue after evaporation. All extracts were stored at 4°C until the cytotoxic test (Esmaeili, Hamzelo-Moghadam, Ghaffari, & Mosaddegh, 2014).

2.5.2 | Cell lines

The spice extracts were tested against the following cell lines: HCT116 [colon cell line], HePG 2 [human hepatocellular carcinoma cell line], MCF7 [human Caucasian breast adenocarcinoma], and PC3 [prostate cell line].

2.5.3 | MTT assay

Cell viability was assessed by the established mitochondrial reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. All the succeeding steps were completed under sterilized conditions using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium (for HCT116, HePG 2, MCF7, and PC3, 1% antibiotic-antimycotic combination (containing potassium penicillin 10,000 U/ml, streptomycin sulfate 10,000 µg/ml, and amphotericin B 25 µg/ml), and 1% L-glutamine at 37°C with 5% CO₂). Cells were batch-cultured for 10 days and then seeded at a concentration of 10 × 10³ cells/well in a fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 hr below 5% CO₂ using a water jacketed carbon dioxide incubator (Sheldon, TC2323). The media were aspirated, a fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the sample to give a final concentration of 100–50–25–12.5–6.25–3.125–0.78 and 1.56 µg/ml. After incubation for 48 hr, the medium was re-aspirated, and 40 ul MTT salt (2.5 µg/ml) was supplemented to each cavity followed by incubation for another four hours at 37°C below 5% CO₂. For the purpose of dissolving the formed crystals and stopping the reaction, 200 µl of 10% sodium dodecyl sulfate (SDS) in deionized water was supplemented to each cavity and subsequent incubation at 37°C for 24 hr. 100 µg/ml of Annona cherimolia extract was used as a positive control that known as natural cytotoxic agent and gives 100% lethality under the same circumstances (El-Menshawi et al., 2010).

The measurement at the absorbance of 595 nm and at a reference wavelength of 620 nm was performed using the microplate multiwell reader (Bio-Rad Laboratories Inc., model 3350). The statistical significance between the samples and the negative control (cells with vehicle) was estimated using independent t test by SPSS 11 program. DMSO is used for dissolving the plant extracts in final concentrations that do not exceed 0.2% (v/v) in cell assays. The percentage of the change in viability was calculated according to the equation: ((Reading of extract/Reading of negative control) −1) × 100. IC50 and IC90 were calculated by employing a probit analysis using SPSS 11 program.
3 | RESULTS

3.1 | Mycobiota contamination of spices

The mycobiota of five kinds of spices marketed in Upper Egypt was investigated in this study. Sage samples were found to be more contaminated than other samples (34,562.46 CFU/g of Sage) as illustrated in Table 1. Studies on the fungal contamination of sage are almost completely absent in the previous literature.

Eighteen genera comprised of 41 species, and 5 species varieties were obtained from 50 samples of spices that were bought from the markets in Upper Egypt, 2015. The highly isolated fungi in terms of abundance and frequency, which were recovered from the samples of spices, were as follows: *Aspergillus* sp. (71.32% and 100%), *Eurotium chevalieri* (11.76% and 40%), *Emericella nidulans* var. *lata* (11% and 22%), *Penicillium* sp. (4.45% and 66%), and sterile mycelia (1.55% and 34%). The remaining genera were obtained in low or moderate frequencies that formed 9.41% collectively.

3.2 | Total AFs and OTs producing potentials of *A. flavus* and *A. niger* isolates

Eight isolates of *A. flavus* and *A. niger* isolated from the spice samples were subjected to estimate their AFs and OTs producing potentials. The results are summarized in Table 2. The tested isolates had the abilities to produce total AFs and OTs with the exception of two isolates of *A. niger* from black cumin and sage samples which exhibited no detectable ochratoxin production. The range of AFs produced by *A. flavus* was 2.2–7.5 ppb. The levels of OTs produced by *A. niger* were 3.4 and 3.6 ppb.

3.3 | Natural incidence of total AFs and OTs in spice samples

Five samples out of 50 (which had the highest count of *A. flavus* and *A. niger*) were naturally contaminated with AFs and OTs. The concentration of the AFs in the spice samples ranged from 1.9 to 8.2 ppb with the highest amount found in cumin sample. Likewise, the reading of the OTs ranged from 4.1 to 6.7 ppb, and the highest reading was recorded in ginger sample, as shown in Table 3.

### Table 1

| Spices types         | Total fungal counts (CFU/g) | *Aspergillus* spp. occurrence (%) | AFs (PPB) | OTs (PPB) |
|----------------------|----------------------------|-----------------------------------|-----------|-----------|
| *Cuminum cyminum*    | 1,933.32                   | 58.19                             | 8.2       | 4.5       |
| *Curcuma longa*      | 15,836.52                  | 88.41                             | 2.6       | 4.1       |
| *Nigella sativa*     | 4,349.99                   | 85.05                             | 1.9       | 4.9       |
| *Salvia officinalis* | 34,562.46                  | 56.91                             | 6.5       | 5.4       |
| *Zingiber officinale*| 27,838.9                   | 78.24                             | 2.0       | 6.7       |
| Total                | 84,521.19                  | 366.8                             |           |           |

Note: Out of 10 samples from each type of spices, AFs and OTs were determined in the highest contaminant samples.

3.4 | Molecular characterization of AFs and OTs biosynthesis genes

The eight *Aspergillus* isolates identified genes clustered within a 70 kb DNA region in the chromosome that are comprised in the production of AFs and whose DNA sequences have been previously published (Criseo et al., 2008). The polymerase chain reaction (PCR) was applied using two sets of primer for different genes involved in aflatoxin and ochratoxin biosynthetic pathways. The bands of the fragments of *omt-A and Aopks* genes can be visualized at 320 and 549 bp, respectively (Figure 1). The DNA banding patterns of the examined aflatoxigenic and ochratoxigenic isolates were different. All aflatoxigenic *A. flavus* (i.e., isolates, no. 31, 32, 33, and 34) showed DNA fragments that corresponded to the presence of genes. In addition, two of the four *A. niger* isolates (no. 35 and 36) showed the investigated ochratoxin gene.

3.5 | ISSR-PCR analysis, genetic distances, and the cluster dendrogram

The genomic diversity of *Aspergillus* spp. was investigated by ISSR markers. Results of ISSR-PCR are illustrated in Figure 3. ISSR-PCR reactions were completed with eight *Aspergillus* isolates. Six different ISSR-PCR markers yielded 84 distinct bands of which 57 bands were polymorphic (68%) and only 27 bands were monomorphic (32%). The number of bands for individual ISSR primers varied from 10 bands for ISSR-16 to 19 bands for ISSR-28. The highest polymorphism was recorded for ISSR-19 and the lowest for ISSR-28. Moreover, the band size of ISSR-8 ranged from 220 to 2,000 bp (Figure 2).

The genetic similarity and intraspecies differentiation as well as the dendrogram constructed using neighbor-joint method based on Jaccard’s similarity coefficients ranging from 0.00 to 1.14 (Figure 3) were used here. According to the corresponding data, the dendrogram analysis showed that there is genetic distance among native *Aspergillus* sp. They were grouped into two large clusters: The first cluster consisted of *A. flavus*-31, *A. flavus*-32, *A. flavus*-33, and *A. flavus*-34, and the second main cluster included the four *A. niger* only. The result found that genetic distance among native *Aspergillus* sp. was comparatively low between each species and high within each species in general. The smallest genetic distance (0.00) was estimated between *A. niger*-35 and *A. niger*-38 (Figure 3).
| Genera and species | Spice types | ATC | Abundance | NCI | F% |
|-------------------|-------------|-----|-----------|-----|----|
| Acremonium       | C + S       | 80.56 | 0.1 | 2 | 4 |
| A. kiliense      | S           | 55.56 | 0.07 | 1 | 2 |
| A. hyalinulum    | C           | 25 | 0.03 | 1 | 2 |
| Alternaria       | C + S       | 525.1 | 0.62 | 6 | 12|
| A. alternata     | C + S       | 516.77 | 0.61 | 5 | 10|
| A. fugax         | C           | 8.33 | 0.01 | 1 | 2 |
| Aspergillus      | C + Cu + N + S + Z | 60,280.53 | 71.32 | 50 | 100 |
| A. egypticus     | C           | 50 | 0.05 | 2 | 4 |
| A. candidus      | S           | 166.7 | 0.28 | 2 | 4 |
| A. clavatus      | N           | 16.67 | 0.02 | 1 | 2 |
| A. flavipes      | C + N + Z   | 169.46 | 0.20 | 4 | 8 |
| A. flavus        | C + Cu + N + S + Z | 19,384.06 | 22.93 | 39 | 78 |
| A. flavus var. columnaris | C | 41.67 | 0.05 | 1 | 2 |
| A. fumigatus     | Cu + N + Z  | 461.21 | 0.55 | 6 | 12|
| A. niger         | C + Cu+ N + S + Z | 23,890.4 | 28.27 | 50 | 100 |
| A. ochraceus     | C + Cu + N + S + Z | 4,525.9 | 5.36 | 12 | 24|
| A. parasiticus  | S           | 55.57 | 0.07 | 1 | 2 |
| A. sydowii      | C + Cu + N + S + Z | 5,389.93 | 6.38 | 20 | 40|
| A. tamarii      | Z           | 111.13 | 0.13 | 1 | 2 |
| A. terreus var. africanus | C + Cu + N + S + Z | 875.17 | 1.04 | 8 | 16|
| A. terreus var. auratus | C + N + S + Z | 2,064.3 | 2.44 | 15 | 30 |
| A. versicolor    | C + S       | 2,064.3 | 2.44 | 3 | 6 |
| Bhushakala oliveconitra | S | 55.57 | 0.07 | 1 | 2 |
| Cladosporium oxysporum | C | 8.33 | 0.01 | 1 | 2 |
| Cochliobolus spicifer | C | 8.33 | 0.01 | 1 | 2 |
| Emericella nidulans var. lata | C + Cu + S + Z | 4,606.47 | 5.45 | 11 | 22 |
| Epicoccum purpurascens | C | 8.33 | 0.01 | 1 | 2 |
| Eurotium chevalieri | C + Cu+ S + Z | 9,940.84 | 11.76 | 20 | 40 |
| Fusarium         | C           | 33.3 | 0.04 | 3 | 6 |
| F. oxysporum     | C           | 8.33 | 0.01 | 1 | 2 |
| F. solani       | C           | 25 | 0.03 | 3 | 6 |
| Mucor hiemalis  | Cu + N+ S + Z | 700.13 | 0.83 | 7 | 14|
| Paecilomyces carneus | C | 16.67 | 0.02 | 1 | 2 |
| Papillospora     | Cu + Z     | 1,333.6 | 1.58 | 3 | 6 |
| P. immersia      | Z           | 777.93 | 0.92 | 1 | 2 |
| P. irregulara    | Cu + Z     | 555.67 | 0.66 | 2 | 4 |
| Penicillium      | C + Cu + N + S+ Z | 3,761.7 | 4.45 | 33 | 66|
| P. aurantiogriseum | N + Z | 119.46 | 0.14 | 3 | 6 |
| P. cambertii     | C           | 8.33 | 0.01 | 1 | 2 |
| P. chrysogenum   | C + Cu + N + S + Z | 2,289.2 | 2.71 | 27 | 54|
| P. corylophilum  | S           | 55.57 | 0.07 | 1 | 2 |
| P. duclauxii     | C + Cu + S + Z | 1,080.77 | 1.28 | 8 | 16|
| P. funiculosum   | S           | 55.57 | 0.07 | 1 | 2 |
| P. purpurogenum  | C + Cu + N | 72.23 | 0.09 | 3 | 6 |

(Continues)
In this experiment, the cytotoxic capability of five extracts of spices against four human tumor cell lines (HCT116, HePG2, MCF7, and PC3) was probed using the MTT assay (Table 4). The highest cytotoxic action was observed in ginger and sage. The crude extract of ginger showed cytotoxic activity against the tested cell lines (IC50: 43.0, 19.8, 43.0, and 18.9 μg/ml, respectively) and (IC90: 77.7, 36.4, 77.7 and 28.8 μg/ml, respectively). The extract of sage had also cytotoxic effects on the four cell lines (IC50: 59.2, 19.07, 59.2, and 22.5 μg/ml, respectively) and (IC90: 95, 36.1, 95, and 34.2 μg/ml, respectively). In contrast, the cytotoxicity of black cumin was found to be considerably low on the four cell lines.

**TABLE 2** (Continued)

| Genera and species | Spice types | ATC | Abundance | NCI | F% |
|--------------------|-------------|-----|-----------|-----|----|
| P. verruculosum    | C           | 16.67 | 0.02       | 2   | 4  |
| P. waksmanii       | C + Cu      | 63.9 | 0.08       | 2   | 4  |
| Phoma              | C + N + S   | 127.79 | 0.15       | 3   | 6  |
| P. exigua          | C + S       | 119.46 | 0.14       | 2   | 4  |
| P. eupryena        | N           | 8.33 | 0.01       | 1   | 2  |
| Rhizopus stolonifer| N + Z       | 72.24 | 0.09       | 3   | 6  |
| Stachybotrys atra var. microsporum | C + S | 350.07 | 0.41 | 4 | 8 |
| Sterile Mycelia    | C + Cu + N + S + Z | 1,308.57 | 1.55 | 17 | 34 |
| Ulocladium         | Cu + N+ S   | 1,303.03 | 1.54 | 10 | 20 |
| U. botrytis        | S           | 777.93 | 0.92       | 4   | 8  |
| U. tuberculatum    | Cu + N+ S   | 525.1 | 0.62       | 6   | 12 |
| Total count        |             | 84,521.199 |

Abbreviations: C, Cuminum cyminum; Cu, Curcuma longa; N, Nigella sativa; S, Salvia officinalis; Z, Zingiber officinale.

**TABLE 3** Frequency of single genes in Aspergillus flavus, A. niger isolates collected from spice samples

| Strain code number | Mycotoxigenic isolate | Source of isolation | Total aflatoxins (ppb) | Aflatoxin gene | Total ochratoxins (ppb) | Ochratoxin gene |
|--------------------|-----------------------|---------------------|------------------------|----------------|-------------------------|----------------|
| 31                 | A. flavus             | Curcuma longa       | 2.2                    | +             | 0                       | –              |
| 32                 | A. flavus             | Cuminum cyminum     | 5.2                    | +             | 0                       | –              |
| 33                 | A. flavus             | Zingiber officinale | 7.5                    | +             | 0                       | –              |
| 34                 | A. flavus             | Nigella sativa      | 2.4                    | +             | 0                       | –              |
| 35                 | A. niger              | Curcuma longa       | 0                      | –             | 3.6                     | +              |
| 36                 | A. niger              | Cuminum cyminum     | 0                      | –             | 3.4                     | +              |
| 37                 | A. niger              | Nigella sativa      | 0                      | –             | 0                       | –              |
| 38                 | A. niger              | Salvia officinialis | 0                      | –             | 0                       | –              |

Note: +, PCR amplification signal present; –, PCR amplification signal absent.

**3.6 | Cytotoxic potential of spice extracts**

In this experiment, the cytotoxic capability of five extracts of spices against four human tumor cell lines (HCT116, HePG2, MCF7, and PC3) was probed using the MTT assay (Table 4). The highest cytotoxic action was observed in ginger and sage. The crude extract of ginger showed cytotoxic activity against the tested cell lines (IC50: 43.0, 19.8, 43.0, and 18.9 μg/ml, respectively) and (IC90: 77.7, 36.4, 77.7 and 28.8 μg/ml, respectively). The extract of sage had also cytotoxic effects on the four cell lines (IC50: 59.2, 19.07, 59.2, and 22.5 μg/ml, respectively) and (IC90: 95, 36.1, 95, and 34.2 μg/ml, respectively). In contrast, the cytotoxicity of black cumin was found to be considerably low on the four cell lines.

**4 | DISCUSSION**

The species A. flavus and A. niger obtained in this study are described as mycobiota contaminated of spices due to the presence of fungi at preharvest or postharvest depend on storage temperature, seed moisture content, relative humidity, and fungal species. Gherbawy, Shebany, Hussein, and Maghraby (2015) found that crushed chili samples were the preferred mediums for fungal growth
Among the examined chili products, the highest level of fungal contamination was detected in one sample (11,335 × 10^3 CFU/g of crushed chili). The same results are in line with the findings of Saleem, El-Said, Mohamed, and Abdelnasser (2013), who reported that Alternaria, Aspergillus, Emericella, Mucor, Penicillium, Stachybotrys, and sterile mycelia were common genera contaminating anise and cumin seeds. El-Gali (2014) further showed that the most prevalent fungal genera isolated from 14 species of spices, including turmeric, cumin, and ginger, were Aspergillus spp., Penicillium spp., Alternaria spp., and Fusarium spp. Jeswal and Kumar (2015) found that Aspergillus flavus and A. niger were the commonest species isolated from 9 kinds of spices also comprising turmeric, cumin, and ginger.

In this study, 100% of tested A. flavus isolates were aflatoxigenic and 50% of the tested A. niger were ochratoxigenic, and similar results were obtained by Jeswal and Kumar (2015) who...
reported that all Aspergillus flavus isolates from tested spices had the ability to produce aflatoxins. Aspergillus species belonging to Circumdati, Nigri, and Flavi sections are the major producers of ochratoxins, whereas aflatoxins are mainly obtained from Aspergillus: section Flavi (Rank et al., 2011). Chourasia (1995) found the natural occurrence of aflatoxin B1, citrinin, ochratoxin A, and zearalenone in some herbs such as Cuminum cyminum and Zingiber officinale. Moreover, Jeswal and Kumar (2015) manifested that AFs amounts were higher than OTA since the ginger, turmeric, and cumin samples had a high percentage of aflatoxins (77.7%, 68.5%, and 64.3%, respectively). The OTA were reported in 55.5% and 57.1% of ginger and turmeric samples, respectively, and the cumin samples were free of OTA. AFB1 was detected in 23 samples out of 36 spices (63.9%) in a concentrations range of 0.10 to 26.50 μg/kg (Azzoune et al., 2016).

All aflatoxigenic A. flavus showed DNA fragments due to the presence of genes, and two of the four A. niger isolates showed the ochratoxin gene. Aflatoxigenic aspergilli were detected using PCR based on the intermediated enzymes including the sterigmatocystin O-methyltransfrase encoding gene omt‐1 and the regulatory gene aflR (Erami et al., 2007). El-Hamaky, Atef, Yazeed, and Refai (2016) found that a single fragment of about 549 was produced with two positive ochratoxigenic A. niger isolates.

The highest cytotoxic activity was observed in ginger and sage extracts. Spices had cytotoxic potential are useful in the development of cancer therapeutics which had increasing importance in the last decade. Nematollahi-Mahani, Rezaadreh-Kermani, Mehrabani, and Nakhaee (2007) reported that the ethanol extract of Teucrium polium exhibited cytotoxic effects on four cell lines. The IC50 value for each cell line was reported as 90 μg/ml for A-549, 106 μg/ml for BT-20, 140 μg/ml for MCF-7, and 120 μg/ml for PC-12 cells. Different concentrations of plant extracts (50, 100, and 150 μg/ml) were supplemented to HepG2 cells and were incubated for 48 hr. The experiments revealed that amla, green tea, liquorice, sarpagandha, and periwinkle were cytotoxic to the liver cancer cell line at all varied concentrations. The IC50 values for these were recorded to be less than 150 μg/ml (Rao, Timsina, & Nadumane, 2014). Furthermore, extracts of Salvia species especially S. miltiorrhiza and S. officinalis have been recently documented to possess high antitumor activity in vitro and in vivo (Jiang, Zhang, & Rupasinghe, 2016). Likewise, the ginger extracts have been recorded to manifest antiproliferative actions on many kinds of cancer cells, especially pancreatic cancer cells (Akimoto, Lizuka, Kanemastu, Yoshida, & Takenaga, 2015).

### 5 | CONCLUSION

The mycological analysis revealed that sage samples were highly contaminated and that the most prevalent fungi were Aspergillus sp. Cumin had the highest reading of AFs, and ginger was the highest for OTs. All the tested isolates of Aspergillus flavus and A. niger were positive for AFs and OTs production except two isolates of A. niger that exhibited no detectable OTs, omt-A gene was found in all A. flavus isolates, and Aopks genes were detected in 2 out of four isolates of A. niger. The most cytotoxic activity was determined in ginger and sage. Therefore, our findings reveal that the dryness of the spices after harvesting is the main factor to avoid the development of fungi during storage and open up the possibility that natural compound found in these spices may be used to develop new treatment modality for cancer. However, further investigation is needed to determine the mechanism for its action in carcinoma.

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### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

### ETHICAL REVIEW

All experimental protocols and procedures were reviewed and approved by the Department of Pharmacognosy, National Research Centre El-Tahrir Str., Dokki, Giza 12622, Egypt.

### INFORMED CONSENT

Written informed consent was obtained from all study participants.

### TABLE 4 Cytotoxic activity of spices against HCT116, HePG 2, MCF7, and PC3 human tumor cell lines

| Scientific Name        | IC50 (μg/ml) | IC90 (μg/ml) |
|------------------------|-------------|-------------|
|                        | HCT116 | HePG 2 | MCF7 | PC3 | HCT116 | HePG 2 | MCF7 | PC3 |
| Cuminum cyminum        | N      | N      | N    | 24.5| N      | N      | N    | 37.6|
| Curcuma longa          | 45.9   | N      | 45.9 | 21.7| 78.3   | N      | 78.3 | 34.4|
| Nigella sativa         | N      | N      | N    | N   | N      | N      | N    | N   |
| Salvia officinalis     | 59.2   | 19.07  | 59.2 | 22.5| 95.0   | 36.1   | 95.0  | 34.2|
| Zingiber officinale    | 43.0   | 19.8   | 43.0 | 18.9| 77.7   | 36.4   | 77.7  | 28.8|

Abbreviation: N, not effective.
REFERENCES

Akimoto, M., Lizuka, M., Kanemastu, R., Yoshida, M., & Takenaga, K. (2015). Anticancer effect of ginger extract against pancreatic cancer cells mainly through reactive oxygen species mediated autotic cell death. PLoS ONE, 10, 1–22. https://doi.org/10.1371/journal.pone.0126605

Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research, 17, 299–305. https://doi.org/10.1002/ptr.1309

Al-Mofleh, I. A. (2010). Spices, herbal xenobiotics and the stomach: Friends or foes? World Journal of Gastroenterology, 1622, 2710–2719. https://doi.org/10.3748/wjg.v16.i22.2710

Awour, A. O., Montgomery, J., Yard, E., Martin, C., Daniel, J., Zitomer, N., ... Elmore, S. (2016). Evaluation of efficacy, acceptability and palatability of calcium montmorillonite clay used to reduce aflatoxin B1 dietary exposure in a Crossover study in Kenya. Food Additives & Contaminants: Part A, 34, https://doi.org/10.1080/1944049.2016.1224933.

Azzoune, N., Mokrane, S., Ribai, A., Verheechke, C., Sabauou, N., & Mathieu, F. (2016). Contamination of common spices by aflatoxigenic fungi and aflatoxin B1 in Algeria. Quality Assurance and Safety of Crops & Foods, 8, 137–144.

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., ... Cotty, P. J. (2016). Biological control of aflatoxigenic and aflatoxin B1 in Algeria. BMC Women’s Health, 16, 1–4. https://doi.org/10.1186/s12905-016-0130-9

Baylis, C. L. (2003). Manual of microbiological methods for the food and drinks industry. Gloucestershire, UK: CCFRA, Chipping Campden.

Ben Frejd, S. M., Chebil, S., & Milk, A. (2009). Isolation and characterization of ochratoxin A and aflatoxin B1 producing fungi infecting grapevines cultivated in Tunisia. African Journal Microbiology Research, 3, 523–527.

Bhargava, S., Dhabhai, K., Batra, A., Sharma, A., & Malhotra, B. (2012). Zingiber Officinale: Chemical and phytochemical screening and evaluation of its antimicrobial activities. Journal of Chemical and Pharmaceutical Research, 4(1), 360–364.

Billing, J., & Sherman, P. W. (1998). Antimicrobial functions of spices: Why some like it hot. The Quarterly Review of Biology, 73(1), 3–49. https://doi.org/10.1086/420058

Boon, H. S., Olatunde, F., & Zick, S. M. (2007). Trends in complementary and alternative medicine use by breast cancer survivors: Comparing survey data from 1998 and 2005. BMC Womens Health, 7, 1–4. https://doi.org/10.1186/1472-6874-7-4

Cao, J., Shujun, Z., Weijun, K., Meihua, Y., Li, W., & Shihai, Y. (2013). Molecularly imprinted polymer-based solid phase clean-up for analysis of ochratoxin A in ginger and LC-MS/MS confirmation. Food Control, 33, 337–343. https://doi.org/10.1016/j.foodcont.201201055

Chourasia, H. K. (1995). Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. Mycology Research, 99, 697–703. https://doi.org/10.1016/S0953-7562(98)00531-5

Christensen, C. M. (1963). Influence of small differences in moisture content upon the invasion of hard red winter wheat by Aspergillus restrict and A. repens. Cereal Chemistry, 40, 385–395.

Criseo, G., Racco, C., & Romeo, O. (2008). High genetic variability in non-aflatoxigenic A. flavus strains by using Quadruplex PCR-based assay. International Journal of Food Microbiology, 125, 341–343. https://doi.org/10.1016/j.ijfoodmicro.2008.04.020

Dwivedi, V., Shrivastava, R., Hussain, S., Ganguly, C., & Bharadwaj, M. (2011). Cytotoxic potential of Indian spices (Extracts) against esophageal squamous carcinoma cells. Asian Pacific Journal of Cancer Prevention, 12, 2069–2073.

El-Gali, Z. I. (2014). Detection of fungi associated with some species in original form. Global Journal of Scientific Research, 2, 83–88.

El-Hamaky, A. M., Atef, A. H., El Yazeed, H. A., & Refai, M. K. (2016). Prevalence and detection of toxigenic A. flavus, A niger and A. ochraceus by traditional and molecular biology methods in feeds. International Journal of Current Research, 8, 25621–25633.

El-Menshawi, B. S., Fayad, W., Mahmoud, K., El-Hallouty, S. M., El-Manawaty, M., Oloffson, M. H., & Linder, S. (2010). Screening of natural products for therapeutic activity against solid tumors. Indian Journal of Experimental Biology, 48, 258–264.

Elami, M., Hashemi, S. J., Pourbaksh, S. A., Shahsavandi, S., Mohammadi, S., Shooshatri, A. H., & Jahnshiri, S. J. (2007). Application of PCR on detection of aflatoxinogenic fungi. Archives of Razi Institute, 62, 95–100.

Esmaili, S., Hamzeloo-Moghadam, M., Ghaffari, S., & Mosaddegheh, M. (2014). Cytotoxic activity screening of some medicinal plants from south of Iran. Research Journal of Pharmacognosy, 1(4), 19–25.

Frisvad, J. C., Threne, U., & Samson, R. A. (2007). Mycotoxin producers. In J. Dijksterhuis, & R. A. Samson (Eds.), Food mycology: A multifaceted approach to fungi and food (p. 135). Boca Raton, FL: CRC Press.

Ghrebawy, Y. A., Shebany, Y. M., Hussein, M. A., & Maghraby, T. A. (2015). Molecular detection of mycobiota and aflatoxin contamination of chili. Archives of Biological Sciences Belgrade, 67(1), 223–234. https://doi.org/10.22929/ABS141010028G

Griessler, K., Rodrigues, I., Handl, J., & Hofstetter, U. (2010). Occurrence of mycotoxins in Southern Europe. World Mycotoxin Journal, 3, 301–309. https://doi.org/10.3920/WMJ2009.1198

Gull, I., Saeed, M., Shaukat, H., Samra, Z. Q., & Athar, A. M. (2012). Inhibitory effect of Allium Sativum and Zingiber Officinalis extracts on clinically important drug resistant bacteria. Annals of Clinical Microbiology and Antimicrobials, 27, 11–18. https://doi.org/10.1186/1476-0711-11-8

Hassan, M. M., Gaber, A., & El-Hallous, E. I. (2014). Molecular and morphological characterization of Trichoderma harzianum from different Egyptian Soils. Wulfenia Journal, 21, 80–96.

IARC (International Agency for Research on Cancer) (2002). Monograph on the evaluation of carcinogenic risks to humans, world health organization. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation (Vol. 82, pp. 171–175). Lyon, France.

Jeswal, P., & Kumar, D. (2015). Mycobiota and natural incidence of aflatoxins, ochratoxin A, and citrinin in indian spices confirmed by LC-MS/Ms. International Journal of Microbiology, 1–8. https://doi.org/10.1155/2015/242486

Jiang, Y., Zhang, L., & Rupasinghe, V. (2016). The anticancer properties of phytochemical extracts from Salvia plants. Botanics Targets and Therapy, 6, 25–44. https://doi.org/10.2147/BTTA.S98610

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., … Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspectives, 113, 1763–1767. https://doi.org/10.1289/ehp.7998

Luthra, P. M., Singh, R., & Chandra, R. (2001). Therapeutic uses of Curcuma longa (turmeric). Indian Journal of Clinical Biochemistry, 16(2), 153–160. https://doi.org/10.1007/BF02864854

Nematollahi-Mahani, S. N., Rezazadeh-Kermani, M. Q. A., Mehrabani, M., & Nakhaee, N. (2007). Cytotoxic effects of Teucrium polium on some established cell lines. Pharmaceutical Biology, 45(4), 295–298. https://doi.org/10.1080/13880200701214904
Omafuvbe, B. O., & Kolawole, D. O. (2004). Quality assurance of stored pepper *Piper guineense* using controlled processing methods. *Pakistan Journal of Nutrition*, 3, 244–249. https://doi.org/10.3923/pjn.2004.244.249

Rank, C., Nielsen, K. F., Larsen, T. O., Varga, J., Samson, R. A., & Frisvad, J. C. (2011). Distribution of sterigmatocystin in filamentous fungi. *Fungal Biology*, 115, 406–420. https://doi.org/10.1016/j.funbio.2011.02.013

Rao, S., Timsina, B., & Nadumane, V. K. (2014). Antimicrobial effects of medicinal plants and their comparative cytotoxic effects on Hepg2 Cell Line. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6, 101–105.

Rohlf, F. J. (2000). *NTSYS-PC numerical taxonomy and multivariate analysis system*, Version 2.1. Setauket, NY: Exeter Software.

Romeilah, R. M., Fayed, S. A., & Mahmoud, G. I. (2010). Chemical compositions, antiviral and antioxidant activities of seven essential oils. *Journal of Applied Sciences Research*, 6(1), 50–62.

Saleem, A., El-Said, A. H. M., Moharram, A. M., & Abdelnaser, E. G. (2013). Cellulolytic activity of fungal isolated from anise and cumin spices and potential of their oils as antifungal agents. *Journal of Medicinal Plant Research*, 7(17), 1169–1181.

Sethi, S., Dutta, A., La Gupta, B., & Gupta, S. (2013). Antimicrobial activity of spices against isolated food borne pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5, 260–262.

Shan, B., Cai, Y., Brooks, J., & Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Food Microbiology*, 117(1), 112–119. https://doi.org/10.1016/j.ijfoodmicro.2007.03.003

Smidling, D., Mitic-Culafic, D., Vukovic-Gacic, B., Simic, D., & Knezevic-Vukcevic, J. (2008). Evaluation of antiviral activity of fractionated extracts of Sage *Salvia officinalis* L (Lamiaceae). *Archives of Biological Science Belgrade*, 60, 421–429.

Stankovic, N., Comic, L., & Kocic, B. (2006). Microbiological correctness of spices on sale in health food stores and supermarkets in Nis. *Acta Facultatis Medicae Naissenis*, 23, 79–84.

Trucksess, M. W., Stack, M. E., Nesheim, S., Page, S. W., Albert, R. H., & Hansen, T. J. (1991). Immunoaffinity column coupled with solution fluorometry or liquid chromatography post column derivatization for determination of aflatoxins in corn, peanuts, peanut butter: Collaborative study. *Association of Official Analytical Chemists Journal*, 74, 81–88.

Zinedine, A., Soriano, J. M., Juan, C., Mojemmi, B., Moltó, J. C., Bouklouze, A., Mañes, J. (2007). Incidence of ochratoxin A in rice and dried fruits from Rabat and Salé area, Morocco. *Food Additives & Contaminants*, 24, 285–291.

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