

Research Article

Use of Natural Dietary Spices for Reclamation of Food Quality Impairment by Aflatoxin

Misgana Negera and Alemayehu P. Washe 

Department of Chemistry, Hawassa University, P.O.Box: 05, Hawassa, Ethiopia

Correspondence should be addressed to Alemayehu P. Washe; alemayehup@yahoo.com

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Certain natural dietary spices, in addition to their use in food flavoring and preservation, have the ability to detoxify or degrade a wide range of chemical carcinogens. In the present study, aqueous extracts of judiciously selected natural dietary spices were evaluated for their ability to degrade aflatoxin B1 (AFB1). A total of 9 spices including garlic (*Allium sativum*), ginger (*Zingiber officinale*), black cumin (*Nigella sativum*), clove (*Syzygium aromaticum*), sacred basil (*Ocimum basilicum*), lemon grass (*Cymbopogon citratus* L. (DC) Stapf), thyme (*Thymus schimperi*), fenugreek (*Trigonella foenum-graecum*), and lemon traditionally used by the Ethiopian Community for food flavoring and preservation were considered. Aflatoxin degradation efficacy of the spice extracts was studied through determination of the toxin in extract-treated and nontreated samples using LC-MS/MS. The degradation was characterized by electrochemical methods based on the characteristic oxidation peak of phenolic hydroxyl resulting from the degradation of the toxin after treatment with the extracts. Of the various spices, garlic showed the highest 35.8% (30 min) to 61.7% (1 hr) in spiked and 46.7% (30 min) to 68.3% (1 hr) and real-sample (contaminated maize) treatment reduction followed by lemon 34.2% (30 min) to 56.0% (1 hr) in spiked and 41.1% (30 min) to 60.6% (1 hr) in real-sample treatment at 25°C. The level of reduction was higher in real-sample treatment than that in spiked. This suggests adsorptive contribution by the matrix in addition to the chemical degradation. The current findings revealed nonobvious benefits of the use of natural dietary spices as effective solution for AFB1 degradation and decontamination of food.

1. Introduction

Food quality impairment by food-borne pathogens is among major public health problems across the world [1]. Some of these pathogenic problems are associated with mycotoxins that are elaborated by certain species of fungi. Among the most important mycotoxins, aflatoxins (AFs) are considered to be the most toxigenic. AFs are secondary metabolites mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* [2, 3]. These fungi can infect crops before or after harvest and produce aflatoxins. Aflatoxins consist of a group of approximately 20 related compounds, although only aflatoxins B1 (AFB1), B2 (AFB2), G1, and G2 are commonly encountered in feed and food commodities [4]. Among the AFs, aflatoxin B1 (AFB1) is the most toxic and can be a significant risk to human and can cause significant economic losses.

Numerous studies have reported that AFB1 can induce liver cancer in many species of laboratory and wild animals, including subhuman primates, and is a potent hepatocarcinogen in humans [5–9]. The genotoxicity of AFB1 is linked to its epoxidation reaction in the liver of hosting animal. The epoxide form is highly reactive and can bind to DNA and albumin in the blood serum, forming adducts and hence causing DNA damage [10]. Consequently, aflatoxins affect protein synthesis and can lead to suppression of immune responses [10–12]. In addition to the health effects, the economic consequences of aflatoxin are also profound. Crops with unacceptable levels of aflatoxins often have to be destroyed. Alternatively, contaminated food crops are sometimes diverted into animal feed, which in turn can lead to reduced growth rates, illness, and death of the animals. Moreover, animals consuming aflatoxin-contaminated feeds

can produce meat and milk that contain the toxin residues and another toxic biotransformation product, aflatoxin M1 (AFM1) [13].

Considering the socioeconomic impact of aflatoxin, different countries and organizations have stipulated the maximum permissible level of AFs in food and feed commodities. The US Food and Drug Administration (USFDA) sets total aflatoxins below 20 ppb in interstate commerce food and feed and limits AFM1 below 0.5 ppb for the sale of milk [14]. The Commission Regulation of the European Union (EU) No 165/2010 stipulated 8 $\mu\text{g}/\text{kg}$ of AFB1 in food subjected to sorting or physical treatment before human consumption, and the corresponding 2 $\mu\text{g}/\text{kg}$ of AFB1 for direct human consumption and 0.05 $\mu\text{g}/\text{L}$ for AFM1 in milk [15].

Risk of human exposure to aflatoxin contamination of food and feed commodities is a major concern in Ethiopia due to the occurrence of the toxin in different kinds of grains and dairy products [13, 16–18]. The prevalence of aflatoxin contamination of food and feed commodities in Ethiopia was attributed to the climatic conditions (temperature, humidity, etc.) that are favorable for the proliferation of the aflatoxigenic fungi [16]. Other factors such as the predisposing pre- and postharvest factors including frequent seasonal drought causing soil and water stress, lack of resistant varieties, harvesting methods, and storage facility are also contributing to the common incidence of aflatoxin in this country. The problem is exacerbated by, *inter alia*, lack of awareness about aflatoxins and the risks associated with them in the value chain actors including farmers, traders, and consumers. Farmers often feed left-over moldy grains to livestock and commonly use different mixed concentrate feeds such as brewery by-product (“atela”), wheat bran, noug cake, maize grains, and silage to increase production. However, these products are susceptible to contamination with AFB1 [17, 18]. Thus, humans are exposed since the toxins or their biotransformation products accumulate in the value chain products. That is why the Comprehensive Africa Agriculture Development Programme (CAADP) recently set aflatoxins as a high-priority research area, establishing the Partnership for Aflatoxin Control in Africa (PACA) [19]. Despite the progresses in some areas, very limited scientific information is available on control strategies. Particularly, community-based, low-cost, safer, and easy to implement at a larger scale approaches are yet to be developed. Therefore, conduct of studies aiming at practical solutions to the detoxification of AFs is of paramount importance to design effective strategies for aflatoxin control.

The scientific methods reported to date on AF control have focused on three approaches: prevention of contamination of food and feed by the fungi that elaborate the toxins (mainly *A. flavus* and *A. parasiticus*), decontamination of the toxins from contaminated foods and feeds, and inhibition of aflatoxin absorption in the gastrointestinal tract [20]. Although prevention can be considered as the most rational and economic approach, its implementation is difficult in tropical areas where favorable environmental and climatic conditions promote the fungal

growth. In addition, aflatoxins are extremely durable and unavoidable under most conditions of storage, handling, and processing of foods or feeds [21]. In this context, degradation or detoxification of aflatoxin is the most promising route to decontaminate foods already contaminated with the toxins. Since the two key sites for toxicity of AFB1 are a double bond in furan ring and a lactone in coumarin ring, either removal of the double bond or opening up of the lactone ring can be taken as possible sites of degradation and detoxification [20].

Various physical, chemical, and biological methods have been proposed for the detoxification of aflatoxins [22–24]. Nevertheless, all these methods have their own drawbacks. To be successful, however, a detoxification strategy must ensure the Food and Agriculture Organization criteria such as destroy, inactivate or remove the mycotoxins, not produce or leave toxic and/or carcinogenic residue, and not alter significantly the nutritional and technological properties of the product and must be technically and economically feasible [25].

Natural plant extracts are of interest as a source of safer or more effective alternative to biological agents for aflatoxin detoxification. The use of plant products for aflatoxin control has been reported by several authors [24]. However, much emphasis has been given to inhibition of the plant extracts against the growth of the aflatoxigenic fungi. In addition, many of these plants are not suitable to be used in foods, as the resultant products cannot be consumed by humans. In this context, the use of natural dietary spices provides an attractive opportunity as a community-based (suitable for large-scale implementation), safer, cost-effective, and practical method for aflatoxin control.

In the present study, aqueous extracts obtained from judiciously selected dietary natural spices traditionally used by the Ethiopian Community for food flavoring and preservation were evaluated for their ability to degrade AFB1.

2. Materials and Methods

2.1. Chemicals and Materials. All the reagents and standards used in this study were of analytical grade. Aflatoxin B1 standard was purchased from ACROS, New Jersey, USA, in a crystalline form. Stock solution of AFB1 (2000 $\mu\text{g}/\text{L}$) was prepared in methanol and stored at 4°C. The working solutions of AFB1 standard were freshly prepared by diluting the stock solution with phosphate buffer solution (1.7 mM, pH = 7). Methanol and acetonitrile were used for TLC analysis and sample preparation. Mobile phases for LC-MS were of HPLC grade and were purchased from VWR International (Leuven, Belgium). Monobasic potassium phosphate and sodium phosphate dibasic were obtained from Sigma Aldrich (St. Louis, MO, USA), while acetic acid, ammonia, nitric acid, and potassium bromide were from Merck (Darmstadt, Germany). In all analytical steps, MQ water produced by Direct-Q3 water purification system was used (Millipore, Molsheim, France). Liquid chromatography tandem mass spectrometry (LC-MS/MS) Agilent Technologies 1260 HPLC and Triple Quad 6460 series was used for AFB1 quantification.

2.2. Sample Selection and Collection. Selection of spices samples was based on their large-scale use as edible food flavoring and preserving products and reported phytochemical compositions and ability to inhibit the effects of a wide range of chemical carcinogens [26–28]. A total of 81 (3 samples of each spices \times 3 markets \times 9 kinds of spices) spices samples were randomly collected from local retail traders around Hawassa city, Southern Nations, Nationalities, and Peoples Region of Ethiopia. Samples were collected during January to March 2017. The collected samples were made up of nine (\times 9 samples each) different kinds of spices (garlic (*Allium sativum* L.), ginger (*Zingiber officinale Roscoe*), black cumin (*Nigella sativa*), thyme (*Thymus vulgaris* L.), fenu-greek (*Trigonella foenum-graecum*), clove (*Syzygium aromaticum* L.), lemongrass (*Cymbopogon citrates* (DC.) Stapf.), sacred basil (*Ocimum santicum*), and lemon (*Citrus limon* (L.))) commercialized in three different markets (Aroge Gebeya, Addisu Gebeya, and Dato Gullit) near Hawassa city. The samples were randomly purchased by picking three samples of each spice from each market. Maize samples were collected from local farmers around Hawassa city. All samples were washed with distilled water and dried in an oven at 65°C for 72 hr. Then the dried material of each sample was ground to fine powder and stored in plastic bags at 4°C until the beginning of the analysis.

2.3. Screening and Preparation of Maize and Spice Extracts. Screening of the collected maize and spices samples for the presence or absence of AFs was carried out using TLC against AFs standards and according to the Association of Analytical Communities Official Method of Analysis (AOAC, 1995). Firstly, 25 gm of each of the spices and maize powder samples was added into a 250 ml Erlenmeyer flask. Then, 100 ml of methanol and water (85:15) was added, stoppered, and protected with foil. The mixture was vigorously shaken for 30 min and filtered through Whatman No. 1 filter paper. For cleanup, 20 ml of the filtrate solution was transferred into a 125 ml separating funnel containing 20 ml of 10% NaCl solution. Then, 12.5 ml of n-hexane was added and shaken for one minute. The phase was allowed to separate. The upper phase (organic) was discarded, and the lower phase (aqueous) was drained into the second 125 ml Erlenmeyer flask. To the aqueous phase, 12.5 ml of chloroform was added and shaken for one minute. Phases were allowed to separate. The lower phase was allowed to pass through a bed rock of sodium sulphate into the 250 ml Erlenmeyer flask, and the upper phase was discarded. After chloroform extraction, the extract was concentrated by evaporating on a steam bath and dissolved in 10 ml of methanol and shaken very well. Finally, screening was carried out by spotting the samples and AFB1 standard on TLC. Diethyl ether: methanol: water in 96:3:1 was used as the solvent system for plate development. The spots were visualized under a long wavelength UV lamp (365 nm) to determine the presence or absence of AFB1 in the samples based on the bluish fluorescent characteristics of the toxin. Only clean (not contaminated with aflatoxin) spices were considered for further experiments. However, the

contaminated maize samples were considered for the treatments after the determination of initial loads of the toxin using LC/MS/MS. For preparation of aqueous extracts of the spices, 20 g of dry powder plant material from each plant species was soaked in sterile PBS (10 ml of 2 mM PBS/g of plant material) and kept on a shaker for 24 hr. Then, the mixtures were filtered through muslin cloth followed by Whatman No. 41 filter paper to remove leaf debris and obtain a clear filtrate, respectively. The filtrate was kept at 4°C and used for detoxification studies.

2.4. Testing Aflatoxin Degradation by Spice Extracts. Two types of samples, namely, standard AFB1 samples (T1) and maize samples naturally contaminated with AFB1 (T2), were considered for the treatment as shown in Figure 1. The latter samples are regarded herein as real samples. In the case of T1, 100 ng of AFB1 was added into 500 μ l (50 mg/L crude) of plant extract in PBS in a microcentrifuge tube and incubated at 37°C for 24 h in an incubator. A similar procedure was used for real-sample treatment. Prior to the treatment, however, the contaminated maize samples were powdered, homogenized, and divided into two portions—portion 1 and portion 2. From both portions, nearly 17 gm was suspended in a 500 μ l of plant extract and incubated under same conditions. After incubation, the aflatoxin in the mixture was extracted with same volume (500 μ l) of chloroform. The chloroform fraction was evaporated on a heat block at 60°C, and the residue was dissolved in 10 μ l of methanol and analyzed by LC-MS/MS. The mobile phase consisted of water: acetonitrile: methanol: fatty acid (60:25:15:0.1 v/v). The column temperature was maintained at 35°C. The total operation time was 20 min with the flow rate of 0.5 mL·min⁻¹. MS conditions were as follows: capillary current 450 nA, gas flow was 10 L·min⁻¹, and 50 ESI nebulizer used for ionization. The AFB1 levels were determined at the beginning ($t = 0$) and during ($t \neq 0$) the exposure time. A brief outline of the overall procedure including the treatments is shown in Figure 1.

The efficacy of each spice extract was evaluated in terms of percent degradation of the toxin in treated (T1 and T2) vis-à-vis nontreated (T1C and T2C) samples using the following formula:

$$\% \text{ Degradation} = \frac{C_0 - C_t}{C_0} \times 100\%, \quad (1)$$

where C_0 is the concentration of AFB1 at the beginning (after mixing the samples with the extract) and C_t is the residual levels of AFB1 corrected for the matrix effect after incubation for a period of time t . Method validation was carried out by spiking known amounts of AFB1 standard in the powdered maize samples.

2.5. Electrochemical Characterization of Degradation. Electrochemical experiment was carried out using BAS CV 50W, which was connected to Dell Pentium personal computer. The standard buffer solution of pH = 12 has been used in this study. Glassy carbon (GC) as working electrode, Ag/AgCl as reference electrode, and platinum rod as counter

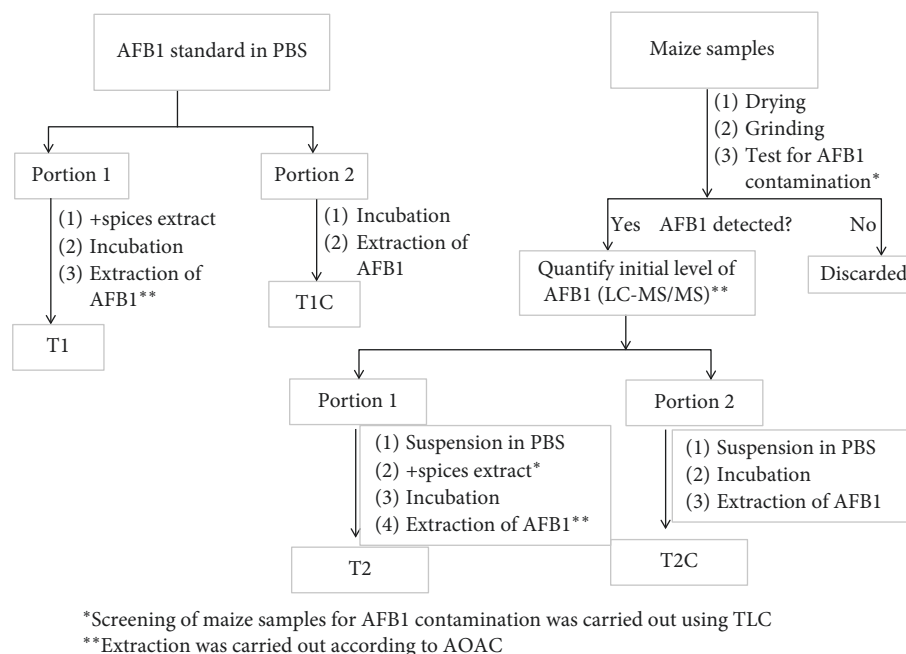


FIGURE 1: A schematic of the overall procedure followed for the determination of efficacy of the spices against aflatoxin B1.

electrode were used for recording cyclic voltammetry (CV). The GC was polished with alumina suspensions and washed with water prior to measurement. CVs were recorded between 0 and 1.5 V at a scan rate of 50 mVs^{-1} , after incubation of $100 \mu\text{g}\cdot\text{L}^{-1}$ AFB1 standards with garlic extract for 48 hr at 40°C .

2.6. Phytochemical Basis and Mechanistic Insights. Qualitative screening of various secondary metabolites was performed according to Ngbele [29].

2.6.1. Test for Alkaloid. One milliliter of 1% HCl was added to 3 ml of the extract in methanol in a test tube. The mixture was heated for 20 min, cooled, and filtered. Then, 1 ml of filtrate was tested in 0.5 ml of Dragendorff's reagent. Formation of reddish brown precipitate is marked as positive test.

2.6.2. Test for Flavonoids. Three milliliters of extract was poured to the beaker. A piece of magnesium ribbon and 3 drops of concentrated hydrochloric acid were added to the mixture. A red coloration indicated the presence of flavonoids.

2.6.3. Test for Phenols. To test the presence of phenol, 3 ml of extract was mixed with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.6.4. Test for Terpenoids. Five millimeters of the extract was dissolved in methanol with 2 mL of chloroform. Then, 3 mL

of concentrated H_2SO_4 was added. A reddish brown coloration indicates positive result for the test.

2.7. Statistical Analysis. All measurements and assays in this study were carried out in triplicate. Statistical analysis of the data was carried out using Student's *t*-test and one-way ANOVA to test whether there is significant difference ($P = 0.05$) between the reduction in the level of AFB1 in the two or more treatments vis-à-vis the presence or absence of extract.

3. Results and Discussion

3.1. Characterization of AFB1 Degradation by the Extracts Using LC-MS/MS. For LC-MS/MS quantification, standard concentrations of AFB1 in the range of $1\text{--}10 \mu\text{g}\cdot\text{L}^{-1}$ were used to construct the calibration curve. For LC-MS/MS quantification, a linear calibration curve with a regression equation of $y = 1244.80x$ and $R^2 = 0.997$ was observed using standard concentrations of AFB1 in the range of $1\text{--}10 \mu\text{g}\cdot\text{L}^{-1}$. The validation of LC-MS/MS data was then carried out with determination of % recoveries and coefficient of variation (% CV). The recoveries were recorded by spiking powdered maize samples 3.5 ng/g of AFB1 standard and determining the recovered amount. Simultaneously, %CV corresponding to triplicate measurements was tabulated. The recorded % recovery ranged between 86.4 and 89.7% with %CV of 2.4%. The residual levels of AFB1 in T1 and T2 were determined in $\mu\text{g}\cdot\text{L}^{-1}$ and $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The initial level of AFB1 contamination in the contaminated maize samples was $7.47 \mu\text{g AFB1/kg}$ maize. This level is higher than the limits of the EU. It is interesting to see whether the treatments by the edible spice extracts can reduce this level to below permissible value. For the treatment, $200 \mu\text{g}\cdot\text{L}^{-1}$ of standard AFB1 (T1)

and 250 μg AFB1/kg maize (nearly 17 gm of maize containing 7.47 μg AFB1/kg in 500 μL) (T2) were prepared in PBS. Figures 2(a) and 2(b) show the residual levels of AFB1 in the two treatments (T1 and T2) as a function of exposure time to 50 $\text{mg}\cdot\text{L}^{-1}$ and 50 $\text{mg}\cdot\text{kg}^{-1}$ of the extract, respectively. The reduction in AFB1 levels ranged from 6.5% to 35.8% (T1) and 17.3% to 46.7% (T2) after 30 min exposure at 25°C. Therefore, the 30 min exposure was sufficient to observe a significant ($P = 0.05$) difference between the treated and nontreated samples. Of the various spices, garlic showed the highest 35.8% (30 min) to 61.7% (1 hr) in T1 and 46.7% (30 min) to 68.3% (1 hr) in T2 reduction followed by lemon 34.2% (30 min) to 56.0% (1 hr) in T1 and 41.1% (30 min) to 60.6% (1 hr) in T2 at 25°C. In the current experiment, relatively the least reduction is observed with thyme. Nevertheless, all the studied extracts could reduce AFB1 levels to certain extents compared to nontreated samples after 24 hr exposure at 25°C. Therefore, garlic and lemon were considered for further study on the effect of concentration on the rate of AFB1 decrease. As expected, the rate of decrease of AFB1 levels in both T1 and T2 increased with the concentration of the spice extracts (Figures 2(c) and 2(d)). The efficacy of the extract varied significantly with the type of the spices. Also, a slight difference was observed between the efficacies of the spices in T1 and T2, which could be most likely due to the matrix effect.

This result suggested that dietary spices can be used to effectively control the level of AFB1 in food preparations. The current result is also consistent with the findings of previous studies [30, 31] in which the observed reductions of aflatoxin B1 contents of contaminated foods varied from 56% to 95%. Vijayanandraj et al. [32], showed 58.1, 55.8, 36.4, and 33.6% reduction of AFB1 by *Trigonella foenum-graecum* (fenugreek), *Curcuma longa*, *Syzygium aromaticum*, and *Ocimum basilicum*, respectively. Rastegar et al. [33] reported up to 93.1% reduction of AFB1 by the roasting process incorporating lemon juice and/or citric acid. Proctor et al. [31] reported that increased spice extract concentration, exposure time, and temperature significantly increased the rate of reduction of AFB1. The observed degradation of AFB1 by the extracts suggested the existence of water soluble phytochemicals like phenolics and alkaloids in the plant materials [27, 32]. The highest activity of garlic could thus be attributed to such chemicals and their relative abundance. More recently, Panda et al. [34] reported 74.7% and 70.2% reduction of AFB1 in vitro and in rice, respectively, through exposure to aqueous extract of *Ocimum tenuiflorum* (sacred basil) at high temperature (85°C/4 h). Direct detoxification was also reported by other authors [35, 36] who showed the detoxification of the toxin by allyl isothiocyanate from oriental mustard. As a result, the current findings are consistent with the existing literature and add values to it by lending credit to the indigenous knowledge on the use of dietary spices for aflatoxin control in line with their application to food flavoring and preservation.

3.2. Characterization of AFB1 Degradation by the Spices. Whether the observed decrease of AFB1 after treatment with spice extracts is due to adsorptive removal or degradation to

a different compound is verified using LC/MS/MS. In this case, the fate of a characteristic peak on the chromatogram and molecular base ion in the mass spectrum specific to AFB1 is used as a basis to decipher the mechanism of AFB1 reduction by the spice extracts. Briefly, 100 ng of AFB1 standard was mixed with 100 ml of aqueous extract of garlic and incubated at 25°C. After overnight exposure, the AFB1 level in the mixture was analyzed by lc-ms/ms. Figures 3(a) and 3(b) show the selected ion chromatogram and molecular base ion peak in the mass spectrum, respectively, of AFB1 obtained before treatment and Figures 3(c) and 3(d) after treatment with aqueous extract of garlic. In the AFB1 mass spectrum, the most abundant fragment eluting at 7.5 min in the positive ionization mode was the protonated molecule $[M + H]^+$ at m/z 313 specific to AFB1 [37]. When AFB1 was incubated with the aqueous extract of garlic, the peak on the chromatogram and molecular base ion peak at m/z 313 specific for AFB1 had disappeared confirming almost complete degradation of AFB1 by the extract. Similar result has been reported by previous authors [32].

The aflatoxins toxicity data demonstrated that the presence of double bond in the terminal furan and lactone rings are key factors for the toxic and carcinogenic activities of AFB1. The disappearance of AFB1 in response to treatment by natural spice extracts thus indicated degradation of the toxin, and this could be most likely due to cleavage of the lactone ring to phenolic and acidic groups.

3.3. Electrochemical Characterization of AFB1 Degradation.

The disappearance of characteristic peaks in the chromatogram and selected base ion peak in the mass spectrum led us to propose structural changes in the AFB1 occurring most likely in the lactone ring leading to phenolic and acidic groups. Since a degradation product bearing the phenolic group is electroactive unlike aflatoxin B1, it can serve as a basis for electrochemical characterization of the degradation. The cyclic voltammetric behavior of a solution of AFB1 standard in 0.1 M phosphate buffer ($\text{pH} = 12$) before and after treatment with citric acid and garlic extract was studied. When AFB1 in PBS was treated with citric acid, a discernible anodic peak (curve (B) in Figure 4(a)) was observed between 800 and 1000 mV. The absence of this peak in other control solutions (AFB1 in 0.1 M KNO_3 (A), citric acid in 0.1 M KNO_3 (C), and PBS (D)) suggested the peak to be attributed to the product of the treatment. Similarly, when AFB1 in PBS was treated with garlic extract anodic peak, though diminished, curve (C) in Figure 4(b)) was observed between in the same region. The negative scan did not show any peak. These results reinforced our hypothesis and confirmed the successful application of cyclic voltammetry for characterization of AFB1 detoxification.

The findings are in agreement with the reports by other authors. In 1976, Cucullu and his coworkers identified the ammoniation product of aflatoxin B1 as dihydro-4-hydroxy-6-methoxyfuro [38, 39] benzofuran and nonfluorescent phenol similar to aflatoxin D1 that lacks the cyclopentenone ring with a molecular weight of 206 [40]. The conversion of AFB1 into AFD1 in the presence of citric acid was also

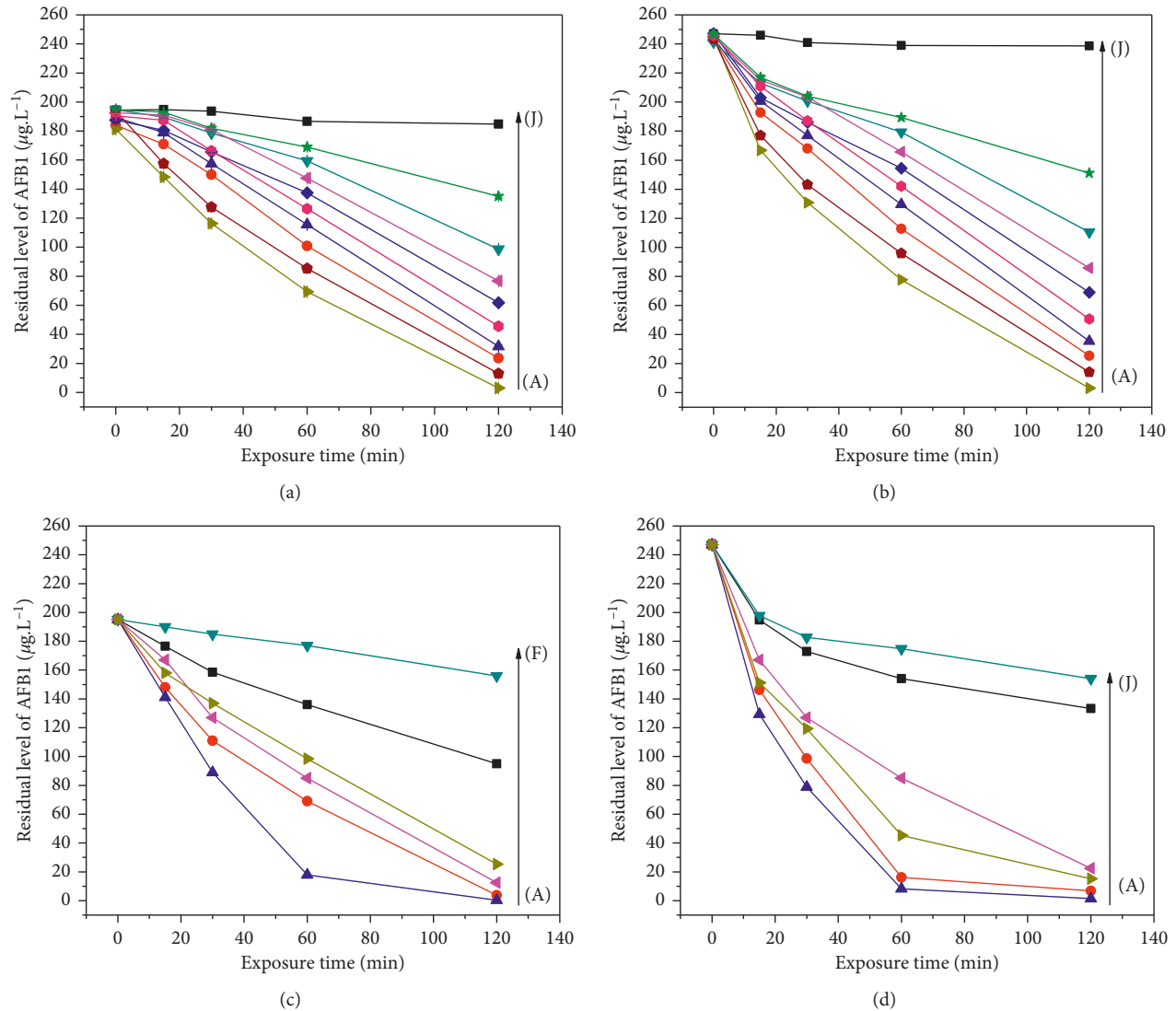


FIGURE 2: Effect of exposure time when the different aqueous extracts (A: garlic, B: lemon, C: sacred basil, D: black cumin, E: lemongrass, F: ginger, G: clove, H: fenugreek, I: thyme, and J: control) are incubated with standard AFB1 (a) and AFB1-contaminated maize sample (b); effect of concentration (A: 50 mg/L, B: 10 mg/L, and C: 1 mg/L garlic and D: 50 mg/L, E: 10 g/L, and F: 1 mg/L lemon) on standard (c) and AFB1-contaminated maize samples (d).

reported [41]. The reduced toxicity of AFD1 has been associated with the open lactone ring in AFD1 structure [42].

3.4. Correlation between Efficacy against AFB1 and the Aflatoxigenic Fungi. Most of the previous studies on the use of herbal, medicinal, and aromatic plants extracts for aflatoxin control focused on the ability to inhibit the growth of the principal aflatoxigenic fungi (*A. flavus* and *A. parasiticus*), and consequently, the production of aflatoxins in culture media and food products. In the present study, the spices were tested for their ability to degrade aflatoxin, not against its production by the aflatoxigenic fungi. We examined whether a good correlation exists between the efficacy of selected spices against aflatoxin (current study) and production of AFB1 by *A. flavus* and *A. parasiticus*. The result is shown in Table 1.

Although a good correlation can be observed with certain spices such as garlic, lemon, and ginger, it is difficult to draw a general conclusion. Thyme, for instance, was found to be the most efficient growth-inhibiting agent of the two fungi as reported by several authors [42], but it had the least activity in reducing AFB1 as observed in the current study.

3.5. Phytochemical Basis and Mechanistic Insights. In order to support the insights regarding molecular basis for the degradation of AFB1 by plant extracts, we conducted qualitative screening of the studied spices for classes of compounds they elaborate. The results are indicated in Table 2. The presence of the envisaged classes of compounds including alkaloids and other compounds containing amine or hydroxyl functional groups strongly supported the proposed mechanism that the degradation is attributed to the

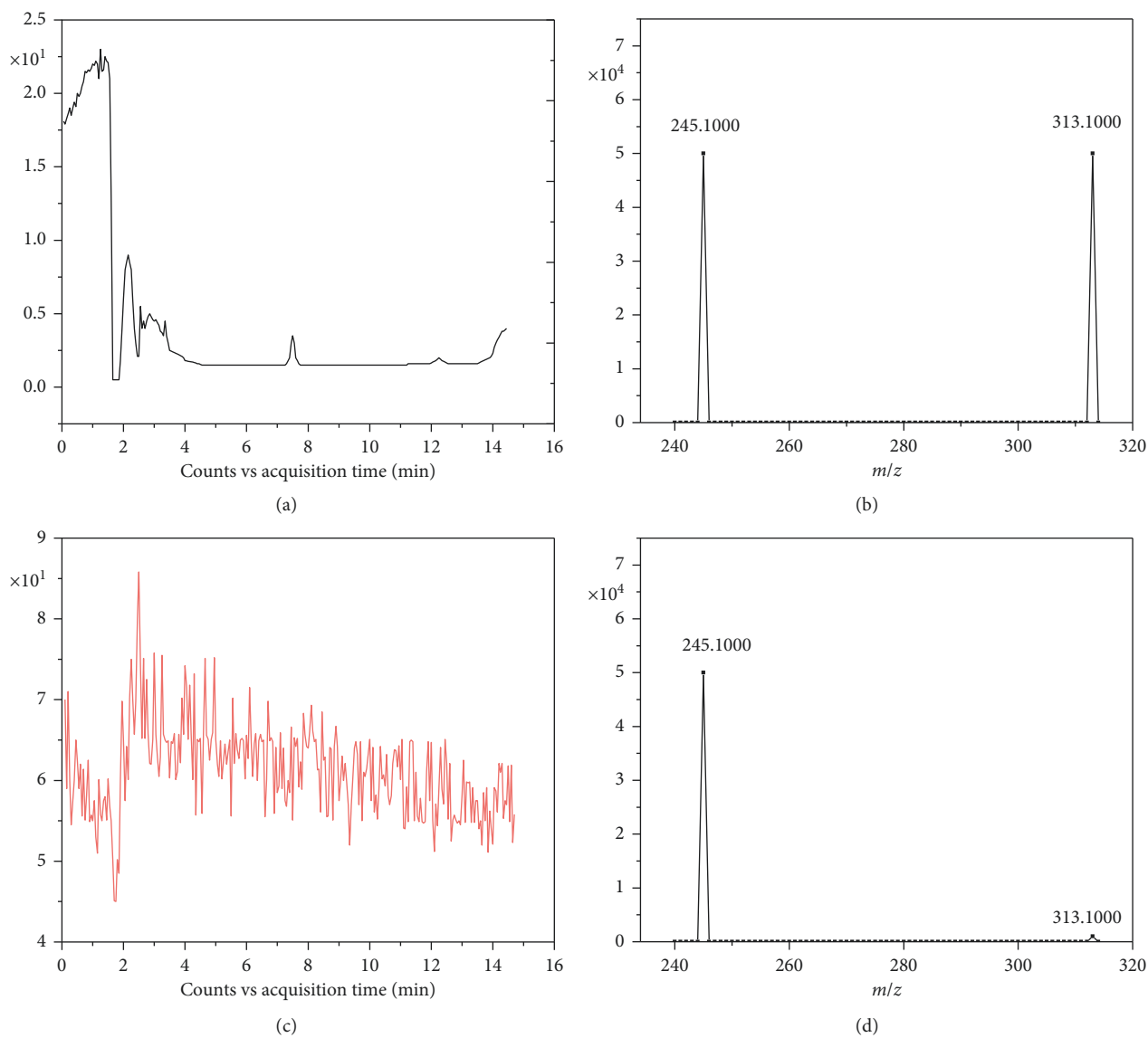


FIGURE 3: (a) Chromatogram of AFB1-contaminated sample before treatment, (b) mass spectrum of selected ion (molecular base ion peak) of AFB1 before treatment, (c) chromatogram of AFB1-contaminated sample after treatment with garlic extract for 24 h-min at 25°C, and (d) mass spectrum of selected ion (molecular base ion peak) of AFB1 after treatment.

presence of such water-soluble compounds in the plant extracts as active principles. Several authors reported a similar finding on detoxification of aflatoxin by plant products [32, 38, 47]. Vijayanandraj et al. [32] reported alkaloids as active principles for the observed detoxification of aflatoxin by the leaf extract of *A. vasica*. Alkaloids were suggested as active principles because other potential classes of compounds such as phenolics and terpenoids were not detected in the leaf extract. In other words, phenolics and terpenoids may also have the detoxification activity if present in the plant extract. Other studies on the degradation of aflatoxin by chemical agents revealed structural alterations in aflatoxin molecules after detoxification. Ciegler et al. [39, 48] observed hydroxydihydro-aflatoxin B1 as the product of detoxification. The same authors identified the ammoniation product of aflatoxin B1 as dihydro-4-hydroxy-6-methoxyfuro [2,3-b] benzofuran, a nonfluorescent phenol

similar to aflatoxin D1 that lacks the cyclopentenone ring with a molecular weight of 206. Velazhahan et al. [38] suggested the modification of lactone ring structure of AFG1 as the mechanism of detoxification of aflatoxin G1 by the seed extract of ajwain (*T. ammi*). Other workers reported molecular breeding of crops with an ability to degrade the aflatoxin as an alternative strategy for the management of aflatoxin contamination in agricultural commodities [49, 50]. Molecular breeding of the natural dietary spicy plant can be even more interesting. Hence, it can be concluded that natural dietary spices can be used as alternative agents for degradation of aflatoxin.

4. Conclusions

The efficient degradation of AFB1 by aqueous extracts of selected natural dietary spices has been successfully

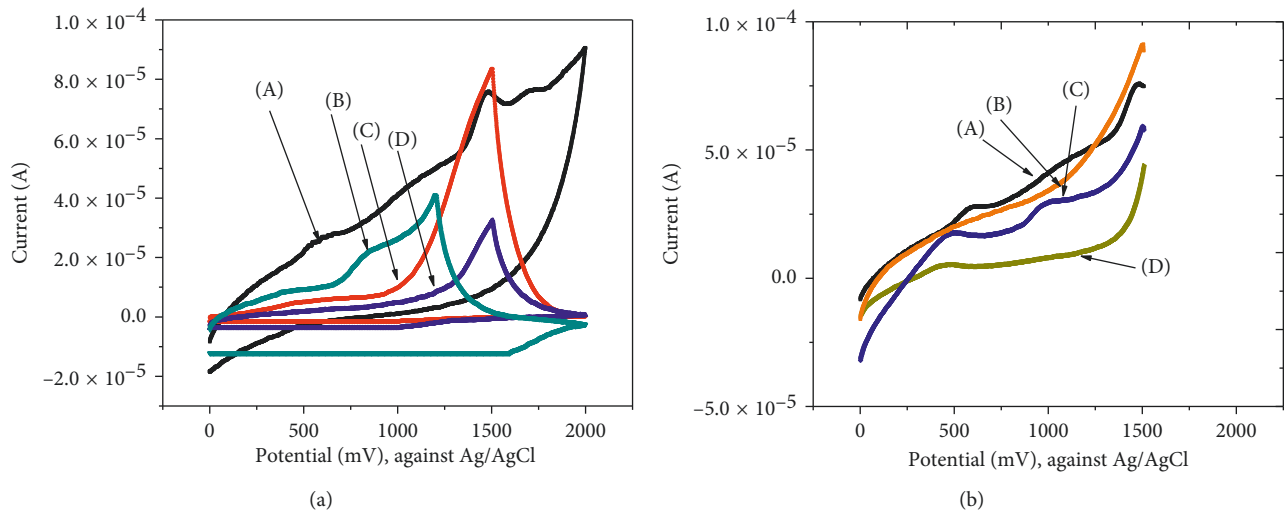


FIGURE 4: (a) Cyclic voltammogram of (A) AFB1 in 0.1 M KNO₃, (B) AFB1 incubated with citric acid for 60 min at 28°C, (C) citric acid in 0.1 M KNO₃, and (D) PBS (scan rate of 50 mV/s; phosphate buffer pH = 12). (b) Cyclic voltammogram of (A) AFB1 in methanol, (B) garlic extract in 0.1 M KNO₃, (C) AFB1 incubated with garlic extract for 60 min at 28°C, and (D) phosphate buffer (scan rate of 50 mV/s; phosphate buffer pH = 12).

TABLE 1: Comparison between different reported plant extracts for detoxification of aflatoxin with current work.

Plants name	Scientific name	Current result (% reduction of AFB1)	Reported result (inhibition of AFB1 production)	Reference
Cumin	<i>Cuminum cyminum</i> (L.)	—	89.0 (ELISA)	[32]
Clove	<i>Syzygium aromaticum</i> (L.)	49.54 (LC/MS/MS)	36.4 (ELISA)	[32]
Basil	<i>Ocimum basilicum</i>	—	90.14 (HPLC)	[32]
Lemongrass	<i>Cymbopogon citratus</i>	53.84 (LC/MS/MS)	91.5% (HPLC)	[43]
Lime	<i>Citrus aurantifolia</i> (L.)	—	66 (HPLC)	[44]
Lemon	<i>Citrus lemon</i> (L.)	90.02 (LC/MS/MS)	—	
Ajwain	<i>Trachyspermum ammi</i>	—	91.98 (ELISA)	[45]
Vasaka	<i>Adhatoda vasica</i> nees	—	98.3 (ELISA)	[41]
Garlic	<i>Allium sativum</i>	93.20 (LC/MS/MS)	54.2 (HPLC)	[46]
Thyme	<i>Thymus vulgaris</i>	51.22 (LC/MS/MS)	79.1 (HPLC)	[46]
Ginger	<i>Zingiber officinalis</i>	57.75 (LC/MS/MS)	79.1 (HPLC)	[46]
Black cumin	<i>Nigella sativa</i>	40.32 (LC/MS/MS)	—	
Fenugreek	<i>Trigonella foenum-graecum</i>	55.72 (LC/MS/MS)	—	

TABLE 2: Phytochemical test results of eight tested spices.

Spices	Garlic	Ginger	Clove	Fenugreek	Thyme	Black cumin	Lemon	Lemongrass	Sacred basil
Alkaloids	+	+	—	+	—	+	+	+	+
Flavonoids	—	+	+	—	+	—	+	+	+
Phenols	+	—	+	+	+	+	+	+	—
Terpenoids	+	+	+	+	—	—	—	+	+

demonstrated using LC-MS/MS. The mechanism of reduction of AFB1 by the spice extracts was investigated using LC-MS/MS and electrochemical methods. Among the studied spices, garlic showed the maximum (61.7%) degradation of AFB1 followed by lemon (56.0%) during 1 hr exposure of AFB1 standard to the spice extracts at 25°C. Chemical transformation of the toxin parent compound to another compound, most likely lacking lactone ring, is proposed as a possible mechanism of AFB1 degradation based on the results from LC-MS/MS and electrochemical characterization of the mechanism of AFB1 reduction by the plant extracts. The

present study has thus lent scientific credit to the nonobvious benefits of indigenous knowledge of using natural spices for food flavoring and preservation to control aflatoxin. Further studies on the degradation products and structural elucidation of the active principle in the efficient spices and other similar plant products for aflatoxin reduction are needed.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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