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Chemical Composition and Bioactive Potential of Extracts from *Diospyros capricornuta* F. White against *Aspergillus flavus* and *Aspergillus parasiticus*

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Abstract

Diospyros capricornuta is an endemic species widely distributed along the coast of Tanzania that is used as food condiments and traditional medicine. The chemical compositions of *Diospyros capricornuta* leaves, stem-bark, and root-bark extracts; and their bioactive potentials against *Aspergillus flavus* and *Aspergillus parasiticus* were investigated. The leaves, stem-bark, and root-bark samples of *D. capricornuta* were extracted using Soxhlet apparatus and the resultant extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). A total of 14 compounds were identified from the extracts, whereby 2,4-di-*tert*-butylphenol was the most abundant compound in all extracts. The growth and aflatoxin production inhibitions against *A. flavus* and *A. parasiticus* were determined via antifungal and antiaflatoxigenic bioassays of the extracts at the concentrations of 0.0, 62.5, 125.0, and 250.0 µg/mL using a poisoned-food method. The High-Performance Liquid Chromatography (HPLC) technique was used to quantify the aflatoxins after bioassays to evaluate aflatoxin inhibitions. The stem-bark extracts at the highest dose of 250.0 µg/mL inhibited aflatoxin production by *A. flavus* for over 99% and *A. parasiticus* for over 94%. Overall, the results show that the leaves, stem-bark, and root-bark extracts of *D. capricornuta* are potential inhibitors against *A. flavus* and *A. parasiticus*-the producers of aflatoxins.

Keywords: *Diospyros capricornuta*, Growth inhibitions, Aflatoxin inhibitions, *Aspergillus flavus, and Aspergillus parasiticus*.

Introduction

Aspergillus flavus and *Aspergillus parasiticus* are the principal aflatoxin producers which have attracted the attention of mycotoxin researchers since the discovery of aflatoxins in the early 1960s (Sargeant et al. 1961). When they colonize food crops, these fungi produce aflatoxins as secondary metabolites in response to harsh climatic conditions of high temperature, humidity, and persistent droughts. Such compounds are well-known human and animal carcinogens which are unfortunately, the main food contaminants associated with liver cancer and aflatoxicosis in the tropical regions (Azziz-Baumgartner et al. 2005, Liu and Wu 2010). Recently, studies have focused on the phytochemical approach using medicinal plants to search for safe antiaflatoxigenic agents which could be used as alternative food preservatives to inhibit the aflatoxin-producing molds from producing aflatoxins and contaminating food crops, particularly in storage (Bluma and Etcheverry 2008). The focus has emerged from the current growing concern that petrochemically-derived

antiaflatoxigenic tablets used to protect cereal crops in storage are considered toxic and environmentally unfriendly (Da Cruz Cabral et al. 2013). In addition, the *Aspergillus* species have been reported to develop resistance against the pesticides; consequently, farmers indiscriminately use them such that they jeopardize food safety via unacceptable accumulation of pesticide residues in the stored food crops (Da Cruz Cabral et al. 2013).

Traditionally, medicinal plants have been used since ancient times as sources of natural and safe medicines to treat various human ailments. Many people depend on medicinal plants for their primary healthcare needs partly because they cannot afford contemporary medicines and pharmaceutical drugs (Sawadogo et al. 2012). Nowadays, investigations on these medicinal plants have contributed to the discovery and development of biodegradable pharmaceutical drugs, which are used against bacteria and fungi with less or no microbial resistance (Da Cruz Cabral et al. 2013). There is enough evidence that extracts of medicinal plants have strong antimicrobial properties against microbes such as aflatoxin-producing fungi (Selvi et al. 2003, Sánchez et al. 2005, Gorran et al. 2013, Kaale et al. 2020). In principle, bioactive compounds from the medicinal plant extracts have been reported to be "generally recognized as safe" (GRAS) by the United States Food and Drug Administration, renewable, biodegradable, and versatile to control aflatoxin contaminations (Njoki et al. 2017, Ponzilacqua et al. 2018).

The medicinal plants of the genus *Diospyros* which are predominantly distributed along the coast of East Africa have long been used as sources of traditional medicines to treat inflammations, leprosy, bacterial infections, and fungal infections (Mallavadhani et al. 1998, Dzoyem et al. 2011). Some of such *Diospyros* species include *D. usambariensis, D. mafiensis, D. kirkii, D. magogana* and *D. capricornuta*, which grow well as shrubs along the coast of East Africa (White 1988). Of particular interest, studies have reported high biological activities of *Diospyros* spp. against *A. flavus*, *A. parasiticus*, and *A. niger* (Mmongoyo et al. 2017, Mbunde et al. 2019).

Specifically, *D. capricornuta*, locally called *Mkondekonde*, has been used as a food condiment by the *Zaramo* people of Pwani Region in Tanzania. Stem and root barks of *D. capricornuta* like other plant species of the genus *Diospyros* have been used by traditional healers to treat common human ailments such as fever, inflammations, and skin fungal infections (Mallavadhani et al. 1998). Despite enormous evidence of the antimicrobial potential of *Diospyros capricornuta* (Mallavadhani et al. 1998), the chemical compositions and bioactive potential of its leaves, stem-bark, and root-bark extracts against *A. flavus* and *A. parasiticus* have not been reported. The present study aimed to determine chemical compositions as well as the bioactive potential of *D. capricornuta* leaves, stem-bark, and root-bark extracts against two aflatoxin-producing fungi *A. flavus* and *A. parasiticus*.

Materials and Methods

Collection of plant materials

The leaves, stem-bark and root-bark samples of *D. capricornuta* were collected on 08th July 2020 from the Pugu forest area (S 06°53´28.4, E 039° 05´56.3) in Pwani Region, Tanzania. A voucher specimen, FM 3975, was deposited at the Botany Department Herbarium of the University of Dar es Salaam for future reference. The samples were dried under shade for 5 days and pulverized using a laboratory grinding mill (Model 4, Martha R. Thomas Company, Philadelphia, USA). The powders of leaves, stem-bark, and root-bark samples were then packed separately in glass jars and stored at -4 °C before solvent extraction.

Extraction

The powders of stem bark (35 g), and root bark (35 g) of *D. capricornuta* each dissolved in 200 mL methanol were extracted separately using the Soxhlet apparatus for 6 hours for each sample at 65 °C. The stem-bark and root-bark extracts were then filtered separately and each filtrate was concentrated

using the rotary evaporator (BIOBASE RE 100-PRO, Qingdao, China). The concentration of the filtrates yielded 12.04 g of the stem-bark and 14.76 g of the root-bark extracts. The Soxhlet extraction of the leaves (35 g) gave a supernatant methanolic extract which was mixed with 5% (0.5 g) of activated carbon charcoal and placed on a magnetic stirrer for 24 hours to remove chlorophyll pigments (Gupta et al. 2015). Then, it was filtered to obtain a methanolic extract solution which was thereafter completely evaporated using a rotary evaporator and 0.45 g of leaf methanolic extract was obtained. Finally, these methanol-free leaf, stem-bark and root-bark extracts of *D. capricornuta* were used for GC-MS analyses.

GC-MS analysis

A GC-MS–QP 2010 (Shimadzu, Japan) with an AOC-20 i/s auto-sampler was used for determining the chemical compositions in *D. capricornuta* leaves, stem-bark, and root-bark extracts. The GC separation was performed with a Restek-5MS capillary column (30 m × 0.25 mm × 0.25 µm). All analyses were carried out at 90 to 280 °C running temperature. The oven temperature was held at 90 °C for 4 min and increased to 280 °C for 16 min at the rate of 11 °C/min. The total running time was 20 min in splitless mode. The flow rate of carrier gas helium was 1.21 mL/min. The ion source and interface temperatures in MS were 230 and 300 °C, respectively. Identification of the compounds was performed by peak integration method with Mass Spectral Library and Search Software (NIST 11). The similarity searches between 85 to 97% were obtained by comparing MS chromatograms of compounds in the library with those from the samples of leaves, stem-bark, and root-bark extracts of *D. capricornuta*. The peaks of compounds below 55000 absorption intensity were not integrated. Consequently, the peaks of compounds that had similarity searches of lower than 85% were not identified. Blank control samples containing only the solvents and other chemicals that were used in the extraction for GC-MS analyses were run in parallel with the samples to check for any contamination.

Fungal strains

Wild type fungal strains of *Aspergillus flavus* (S-TZFHKW11T-13) isolated from sorghum and *Aspergillus parasiticus* (TZG207C-14) isolated from groundnuts were used throughout the study. The strains were generously donated by the International Institute of Tropical Agriculture (IITA) at Mikocheni in Dar es Salaam, Tanzania.

Growth inhibition bioassays

The poisoned food method was used for antifungal bioassays as previously described (Matasyoh et al. 2011, Rani et al. 2013, Balamurugan 2014). Potato Dextrose Agar (PDA) (39 g/1000 mL) was used as the growth medium. The agar was sterilized by autoclaving for 15 min at 126 °C and 103 KPa and allowed to cool before mixing it with various concentrations of the extracts. The leaves, stem-bark and root-bark extracts at the following masses; 62.5, 125, and 250 mg, of each extract were dissolved in 1 mL of dimethyl sulfoxide (DMSO) to obtain stock concentrations of 62.5, 125, and 250 mg/mL, respectively. A micropipette was used to pipette 20 µL from each extract stock concentration into a 90 mm agar plate. This was mixed with 20 mL of PDA to obtain the new concentrations (doses) of the leaves, stem-bark, and root-bark extracts on the plate at 62.5, 125 and 250 µg/mL, respectively. The molten agar, mixed with the extracts at different doses was left to solidify for 15 minutes before center-inoculation with fungal spores. Then, the plates were center-inoculated with 10 µL fungal spores (10^5 spores/mL) of *A. flavus*, and *A. parasiticus* suspended in deionized water. The plates were observed after every 24 hours for 3 consecutive days. The growth inhibition caused by each extract dose was measured by considering the colony diameter readings after 72 hours incubation period, using the following equation:

$$\text{Growth inhibition (\%)} = \frac{\text{control diameter - treatment diameter}}{\text{control diameter}} \times 100\%$$

Inhibitions of aflatoxin production in *A. flavus* and *A. parasiticus*

The antiaflatoxigenic bioassays against *A. flavus* and *A. parasiticus* were conducted with some modifications as previously reported (Reddy et al. 2009). The broth growth media were prepared using Sabouraud Glucose Agar (SGA). This was done by dissolving 65 g of SGA in 1000 mL of distilled water and sterilizing by autoclaving the mixture for 15 minutes at 126 °C and 103 KPa. Then, the hot broth growth media were allowed to cool to 50 °C before mixing with the extracts. Under the same sterile environment, the broth growth medium (20 mL) was transferred into a sterilized bottle (100 mL). To 20 mL of the broth, 20 µL of each concentration of each extract type was added. The new concentrations (doses) of the extracts obtained were 62.5, 125, and 250 µg/mL. Then, 10 µL of fungal spores (10^5 spores/mL) of *A. flavus* and *A. parasiticus* suspended in deionized water were inoculated in each 100 mL bottle. The bottles were stoppered and incubated at room temperature for 7 days.

The aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2 were extracted as previously described by Asis et al. (2002) with some modifications. After 7 days of incubation, the broth agar was filtered into a sterilized 100 mL conical flask to remove mycelia. The filtrate (5 mL) was mixed with 20 mL of 80% methanol in 100 mL conical flasks and placed in a water bath shaker for 30 min and re-filtered with Whatman no.1 filter paper into 100 mL sterilized conical flasks.

The clean-up of broth samples was performed by passing the filtrate through the Aflastar immunoaffinity column and washed with 20 mL of distilled water. The toxins were eluted using 500 µL of methanol HPLC grade three times and collected in 1.5 mL amber glass vials. Derivatization was performed by adding 50 µL of trifluoracetic acid (TFA) to amber glass vials before analysis with HPLC (Asis et al. 2002).

Quantification of aflatoxins

The levels of aflatoxins in the samples were determined using the HPLC facility of Agilent Technologies HPLC, Germany (Series 1260 Infinity II) with UV-detector (G7114A Series DEACX10455) and autosampler (G7129A Series DEAEQ21126). The mobile phase was prepared by mixing 200 mL of distilled water, 50 mL of acetonitrile, and 250 mL of methanol to achieve analytical conditions of H_2O/ACN/Me-OH (40:10:50, v/v). The mobile phase was derivatized by adding 59.6 mg of potassium bromide and 50 µL of nitric acid to 500 mL of the mobile phase. All reagents (manufactured by Blulux Laboratory Pvt Ltd, Faridabad India) used for HPLC analysis were HPLC grade. A chromatographic Poroshell 120 Eclipse EC-C18 column (4.6 × 100 mm, 4 µm of particle size) was used in the analysis of individual aflatoxins. The wavelength used for HPLC analysis was 360 nm. For each sample, the run time was 6 min and 0.8 mL/min liquid flow rates. A standard sample containing all four aflatoxins: AFB_1 (2 µg/mL), AFB_2 (0.5 µg/mL), AFG_1 (2 µg/mL) and AFG_2 (2 µg/mL) was run parallel with the vials for analysis by comparison. The following equation was employed to determine aflatoxin concentration in each vial (Herzallah 2009):

$$[\text{Sample}] = \frac{\text{Peak area of sample} \times [\text{Standard}] \times \text{Volume of extraction solvent}}{\text{Peak area of standard} \times \text{Volume of extracted sample}}$$

whereby, the volume of extraction solvent used was 20 mL, and the volume of extracted sample was 5 mL.

A blank control sample containing 80% methanol that was used in the extraction of aflatoxins in the broth samples was run to check for any solvent contamination. All experiments were performed in triplicates.

The aflatoxin inhibition exhibited by each extract was measured by using the following equation:

$$\text{Aflatoxin inhibition (\%)} = \frac{[\text{control}] - [\text{treatment}]}{[\text{control}]} \times 100\%$$

Statistical analysis

One-way analysis of variance (ANOVA) using Minitab 17 (2020, Pennsylvania, USA) was used to determine the level of significance ($p \leq 0.05$) of the growth and aflatoxin production inhibitions with increasing concentrations. All data are expressed as means ± standard deviations.

Results

GC-MS analysis

The GC-MS-guided determination of the chemical composition of the leaves, stem-bark, and root-bark extracts afforded a total of 14 compounds (Tables 1-2). Of these, only one compound, phthalic acid dibutyl ester, was unique to stem-bark and root-bark extracts of *D. capricornuta*, but could not be found in the leaves extract. However, the remaining 13 compounds were common across all the three plant parts: leaves, stem-bark, and root-bark.

Table 1: Chemical composition of leaves methanolic extracts of *D. capricornuta*

Compound	Retention time (min)	Peak height (intensity counts)	% Composition
1-Dodecene	4.025	9514	2.110
1-Tridecene	6.007	24479	5.430
Octahydro-1H-indole	6.271	66697	14.794
2,4-Di-tert-butylphenol	7.102	89707	19.898
1-Tetradecene	7.732	46991	10.423
1-Heptadecene	9.238	57737	12.806
Diisobutyl phthalate	10.107	29797	6.609
Dibutylphthalate	10.422	24182	5.364
1-Nonadecene	10.59	52506	11.646
1-Heptacosanol	11.823	26312	5.836
Behenic alcohol	12.958	9023	2.001
Bis(2-ethylhexyl) phthalate	13.799	12480	2.768
n-Tetracosanol-1	14.007	1420	0.315

Table 2: Chemical composition of stem-bark and root-bark methanolic extracts of *D. capricornuta*

Compound	Retention time (min)	Peak height (intensity counts)	% Composition	
			Stem-Bark	Root-Bark
1-Dodecene	4.023	12622	2.538	2.307
1-Tridecene	6.009	25512	5.129	5.443
Octahydro-1H-indole	6.268	16479	3.313	2.541
2,4-Di-*tert*-butylphenol	7.104	100397	20.184	21.833
1-Tetradecene	7.733	51261	10.306	10.376
1-Heptadecene	9.237	70888	14.251	13.932
Diisobutyl phthalate	9.789	18969	3.814	4.177
Phthalic acid dibutyl ester	10.106	38359	7.712	7.530
Dibutyl phthalate	10.42	33353	6.705	6.094
1-Nonadecene	10.589	61322	12.328	12.698
n-Nonadecanol-1	11.822	36008	7.239	6.746
Behenic alcohol	12.955	12627	2.539	2.501
Bis(2-ethylhexyl) phthalate	13.797	17083	3.434	3.362
n-Tetracosanol-1	14.003	2530	0.509	0.460

Of particular interest, 2,4-di-tert-butylphenol was found most abundant at 19.9, 20.1, and 21.8% in leaves, stem-bark, and root-bark, respectively, amongst all the compounds of D. capricornuta obtained in this study. On the other hand, while octahydro-1H-indole was the second most abundant at 14.8% in leaves, 1-heptadecene was the second most abundant in 14.2 and 13.9% in stem-bark and root-bark extracts, respectively (Table 1).

Growth inhibitions against *A. flavus* and *A. parasiticus*

Weak growth inhibitory activities exhibited by all types of extracts are presented in Table 3. Though weak, they are significantly different between the two fungal strains of *A. flavus* and *A. parasiticus* ($p < 0.05$). Notably, the extracts inhibited the vegetative growth of *A. parasiticus* more than that of *A. flavus* suggesting that the former fungal strain is more susceptible to all extracts and doses than the latter fungal strain. Increasing extract doses increased the vegetative growth inhibition in both *A. flavus* and *A. parasiticus*. The greatest inhibition (45%) was acquired at 250 µg/mL of leaves extract against *A. parasiticus* and the weakest inhibition (7%) was at 62.5 µg/mL of leaves extract against *A. flavus*. However, there was no significant difference in vegetative growth inhibitions against *A. flavus* and *A. parasiticus* across all types of extracts ($p = 0.584$).

Table 3: Growth inhibitions of *D. capricornuta* extracts against *A. flavus* and *A. parasiticus*

Extract type	Concentration (µg/mL)	Growth inhibition (%) †	
		A. flavus	*A. parasiticus*
Leaves	62.5	6.77 ± 0.41^{g}	26.74 ± 0.34^{c}
Leaves	125	14.63 ± 0.21^{f}	34.89 ± 1.35^{b}
Leaves	250	16.67 ± 0.41^{f}	44.67 ± 0.23^{a}
Stem-bark	62.5	13.24 ± 0.80^{f}	26.67 ± 0.34^{c}
Stem-bark	125	20.13 ± 0.61^{e}	26.59 ± 0.35^{c}
Stem-bark	250	22.64 ± 0.22^{de}	28.28 ± 0.71^{c}
Root-bark	62.5	13.64 ± 0.34^{f}	26.74 ± 0.13^{c}
Root-bark	125	20.13 ± 0.61^{e}	34.33 ± 1.74^{b}
Root-bark	250	25.51 ± 0.43^{cd}	36.12 ± 0.49^{b}

† Means that share similar Tukey letters indicate that they are not significantly different from each other.

Inhibitions of aflatoxin production in *A. flavus* and *A. parasiticus*

Table 4 shows the inhibitory effects of leaves, stem-bark, and root-bark extracts on aflatoxin production by *A. flavus* and *A. parasiticus* exposed to different doses. The data of the present study showed that all extracts were significantly strong at inhibiting aflatoxin production in *A. flavus* and *A. parasiticus* ($p = 0.029$). However, the stem-bark extracts were the most potent at inhibiting aflatoxin production against *A. flavus* (> 98%) even at lower doses of the extract. When the *A. parasiticus* was exposed to similar doses of the stem-bark extract, the inhibition of aflatoxin production ranged from 78 to 94%. On the other hand, the root-bark extract doses had shown stronger inhibitions by 93–97% against *A. parasiticus*. The leaf extract showed moderate aflatoxin production inhibitions against both fungal strains at all doses except 250 µg/mL at which only *A. flavus* showed strong inhibition by 97%. Comparatively, the data demonstrate that *A. flavus* was more vulnerable to inhibitory effects of the extracts than *A. parasiticus*. From dose-activity considerations, the inhibitory effects on aflatoxin production across the two fungal strains significantly increased with an increase in the doses of the extracts ($p = 0.049$).

Table 4: Antiaflatoxigenic activities of *D. capricornuta* methanolic extracts against *A. flavus* and *A. parasiticus*

Extract type	Concentration (µg/ml)	Aflatoxin inhibition							
		A. flavus		*A. parasiticus*					
		Aflatoxin B_1 concentration (µg/mL)	Aflatoxin inhibition (%) †	AFB_1	AFB_2	AFG_1	AFG_2	Total aflatoxin concentration (µg/mL)	Aflatoxin inhibition (%) †
Negative control	0	20.4	0	8.64	0.88	52.88	0.76	63.16	0
Leaves	62.5	8.44	58.63 ± 0.20 ^{l}	3.76	0.4	22.44	0.04	26.64	57.88 ± 0.07 ^{l}
Leaves	125	3.12	84.58 ± 0.12 ^{h}	3.72	0	14	0	17.72	71.96 ± 0.10 ^{k}
Leaves	250	0.44	97.84 ± 0.20 ^{b}	2.88	0	9.92	0	12.8	79.50 ± 0.35 ^{i}
Stem-bark	62.5	0.4	98.04 ± 0.39 ^{b}	2.2	0	11.2	0.12	13.52	78.53 ± 0.12 ^{j}
Stem-bark	125	0.24	98.74 ± 0.10 ^{a}	1	0	3.2	0	4.2	93.41 ± 0.07 ^{e}
Stem-bark	250	0.2	99.01 ± 0.03 ^{a}	0.84	0	2.64	0	3.48	94.47 ± 0.03 ^{d}
Root-bark	62.5	8.72	57.19 ± 0.11 ^{l}	1.08	0	2.84	0.16	4.08	93.63 ± 0.06 ^{e}
Root-bark	125	2.48	87.64 ± 0.20 ^{g}	0.76	0	1.12	0	1.88	97.02 ± 0.07 ^{c}
Root-bark	250	1.84	91.11 ± 0.12 ^{f}	0.6	0	1.12	0	1.72	97.27 ± 0.02 ^{c}

† Means that share similar Tukey letters indicate that they are not significantly different from each other.

Discussions

In recent years, there has been a growing interest amongst researchers to search for fungal inhibitors from indigenous herbs which could be applied to manage aflatoxigenic fungal proliferations and aflatoxin contaminations of agricultural crops. This motive comes from the concerns that synthetic fungicides are carcinogenic, residual in nature, and prohibitively expensive (Da Cruz Cabral et al. 2013). Furthermore, the motive comes from the urge of discovering alternative mitigation strategies against the negative effects of aflatoxins produced by *A. flavus* and *A. parasiticus* on the animal and public health (Da Cruz Cabral et al. 2013). The present study aimed to determine the chemical composition profiles of *D. capricornuta* methanolic extracts of leaves, stem-barks, and root-barks; and to investigate their potentials against aflatoxigenic fungi.

In the determination of the chemical composition of the extracts, it can be noted that 2,4-di-*tert*-butylphenol was the most predominant and principal compound across the extracts. The reason for this predominance is not clear. However, since *D. capricornuta* is a tropical plant confronted by natural enemies such as herbivores, bacteria, and fungi, the high abundance of 2,4-di-*tert*-butylphenol in all extracts could be reasoned that this phenolic component is biosynthesized by the plant as a defence chemical against its natural enemies (Sytar et al. 2018). Actually, 2,4-di-*tert*-butylphenol in collaboration with other compounds are responsible for the observed anti-aflatoxigenic activities. These findings are consistent with the findings of Bankole (1997), Nogueira et al. (2010) and Da Cruz Cabral et al. (2013) who reported that phenolic compounds and essential oils can suppress both vegetative growth and aflatoxin production by *A. flavus* and *A. parasiticus*.

Considering the susceptibility of the two fungal species to extracts of *D. capricornuta* in the laboratory conditions, it is noted that the mycelial growth of *A. parasiticus* is more susceptible to all extracts than that of *A. flavus*. However, the extracts inhibited aflatoxin production more effectively in *A. flavus* than in *A. parasiticus*. Arguably, this sends the message that weak inhibition of vegetative growth of a certain fungal strain does not necessarily mean weak inhibition of aflatoxin formation. This is consistent with Mmongoyo et al. (2017) who found out that although extracts and diosquinone from *D. mafiensis* weakly suppressed fungal growth, they inhibited aflatoxin production strongly even at lower doses. Similarly, the *D. capricornuta* stem-bark methanolic extracts strongly inhibit toxin production in *A. flavus*, particularly at the dose of 250 µg/mL by over 99% and *A. parasiticus* by 94%. This phenomenon of inhibitors from the herbal extracts strongly inhibiting fungal toxin production without severely inhibiting their mycelial growth has been reported (Bhatnagar and McCormick 1988, Jayashree and Subramanyam 1999, Mmongoyo et al. 2017). Since the aflatoxigenic fungi produce aflatoxins via a complex biochemical system called aflatoxin biosynthesis (Yu et al. 2004), the mode of action of the extracts to inhibit the fungi to produce aflatoxins is associated with their inherent abilities to block aflatoxin biosynthesis and stop aflatoxin production completely (Holmes et al. 2008). Thus, such extracts are potential assets that could be used to protect food crops, particularly in storage, from both fungal spoilage and aflatoxin contaminations. The medicinal plant, *D. capricornuta*, and many other species belonging to the genus *Diospyros* have been in use for centuries by indigenous communities along the coastal regions of Tanzania as sources of traditional medicines from the leaves, stem-barks, and root-barks to treat human ailments such as leprosy, diarrhoea, and fungal skin infections (Mallavadhani et al. 1998). In this respect, the use for protecting food crops against aflatoxigenic fungi may be safe. Actually, the small-scale farmers may sustainably use chips or powders of leaves, stem-barks, and root-barks as cheap preservatives to preserve their cereal crops in storage (Bankole 1997, Mmongoyo et al. 2017). According to Da Cruz Cabral et al. (2013), the complex combination of compounds in the extracts interacting with the fungi may be helpful to offset the issue of fungal resistance against synthetic fungicides. In future perspectives, systematic bioassay-guided isolation and purification works of the methanolic extracts of *D. capricornuta* leaves, stem-barks and root-barks must be pursued to discover anti-aflatoxigenic compounds for drug development to replace the modern petrochemical-based antifungals. Such active compounds could be used to better understand the mechanism of inhibition of mycelial growth and aflatoxin formation. This is necessary for the endeavours to discover inhibitors that can completely shut down the aflatoxin biosynthesis pathway.

Conclusions

In conclusion, this study confirms that the leaves, stem-bark, and root-bark extracts of *D. capricornuta* are potentially bioactive against the producers of aflatoxins- the *A. flavus* and *A. parasiticus*. They inhibit mycelial growth and very strongly aflatoxin production by over 99% in *A. flavus* and by over 94% in *A. parasiticus* at 250 µg/mL of stem-bark extract alone. The inherent potencies of the extracts of *D. capricornuta* evaluated in this study show that their applications as natural inhibitors from herbs to protect crops in storage from fungal spoilage and aflatoxin contaminations may be considered. It is hoped that the findings of this study will be useful to economically-challenged indigenous farmers along the coast of Tanzania to mix chips and powdered products of the *D. capricornuta* with their cereal crops to control the producers of aflatoxins during storage. Since indigenous communities like the *Zaramo* have used it for centuries for herbal medicines and food condiments, mixing chips and powdered material with cereal food crops to prevent post-harvest fungal deterioration should be safe and affordable.

Declaration of Competing Interest:
Authors declare no conflicts of interest.

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