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Evaluation of Natural Antifungal Compounds from *Artemisia afra* and its Effectiveness on Prevention of Aflatoxins

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Abstract

Artemisia afra is a medicinal plant with antimicrobial activities, known to treat tuberculosis, measles, cough, and influenza. In consequence, its applications have increasingly become pivotal to control various microbes which cause different diseases. This study therefore, evaluated the chemical composition and the antifungal activity of *A. afra* essential oil. The extraction of essential oil was done using steam distillation. The essential oil chemical composition was analysed by GC-MS. The analysis revealed 19 compounds from *A. afra*. The main compounds were borneol (71.529%), terpinen-4-ol (8.582%), β-thujone (3.283%), β-terpineol (2.590%), α-terpineol (1.791%), chrysanthenone (1.507%), myrtenal (1.393%), eucalyptol (1,8-cineole) (1.263%), *cis*-sabinene hydrate (1.172%) and *trans*-carane, 4,5-epoxy (1.088%). The growth and aflatoxin production inhibitions against *A. flavus* were determined via antifungal and antiaflatoxigenic bioassays of the *A. afra* essential oil at the concentration range of 10–50 µL/mL using a poisoned-food method. The quantification of the aflatoxins production was carried out using high-performance liquid chromatography. The antifungal activity of *A. afra* essential oil was effective against *A. flavus* with growth inhibition of 99.97–99.99% and controlled aflatoxins production by 99.25–99.63%. The results indicated that *A. afra* essential oil can become an alternative natural fungicide for the control of *A. flavus* growth and aflatoxins production.

Keywords: *Artemisia afra*; essential oil; *A. flavus*; aflatoxins; GC-MS.

Introduction

Plants possess therapeutic compounds with beneficial pharmacological effects. Generally, such plants (for example neem, basil, *Aloe*, lemon grass, and ginger) are designated as medicinal plants (Namdeo 2018). Medicinal plants are the richest sources of compounds of different classes such as alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogen, tannins, resins, lactones and volatile oils (Ezeonu and Ejikeme 2016), that act as antimicrobials, antioxidants, anti-inflammatories, antidepressants, cytotoxic or sedative (Loi et al. 2020) and have been applicable in treating various diseases.

Artemisia afra grows in thick, bushy areas, usually leafy with tall hairy and ridged stems up to 2 m high and as low as 0.6 m (More et al. 2012). It is commonly known as "African wormwood" in English, "Mhlonyane" in Zulu (Patil et al. 2011), and

"Fivi" in Kiswahili (Shauri 1991). *A. afra* is one of the popular medicinal plants in Africa employed to treat a variety of ailments including coughs, colds, headaches, chills, dyspepsia, loss of appetite, gastric derangement, colic, croup, whooping-cough, gout, asthma, malaria, diabetes, bladder and kidney disorders, influenza, tuberculosis, convulsions, fever, heart inflammation, and rheumatism in addition to serving as a purgative (Van Wyk 2008, Liu et al. 2009). Recently, a concoction from *A. afra* has been claimed to treat COVID-19 in Tanzania and Madagascar (WHO 2019). The *A. afra* extract is reported to possess antibacterial, antifungal, antioxidant and anti-inflammatory properties (Liu et al. 2009).

Extraction is the main method for separating compounds from plant materials by using selective solvents in standard extraction procedures (Stalikas 2007, Gahlot et al. 2018). During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity (Pandey and Tripathi 2014). Polar solvents such as water are used in the extraction of polar compounds (phenols, terpenoids, tannins, and saponins). The choice of solvent depends on the toxicity of the solvent, quantity of compounds to be extracted, availability of solvent and potential health hazard of the extractant (Pandey and Tripathi 2014). The extraction efficiency is influenced by the nature of compounds, temperature, the extraction method used, sample particle size, and the solvent used (Stalikas 2007). Various techniques such as HPLC, GC-MS, TLC, HPTLC, paper chromatography and NMR can be used to separate, identify, determine and quantify the compounds present in plants (Ingle et al. 2017, Feng et al. 2020). The compound composition in *A. afra* is influenced by the harvesting season/months, soil type (Abad et al. 2012), the choice and stage of drying conditions (Asekun et al. 2007), the geographical location (Liu et al. 2009), chemotype or subspecies, choice of plant part or genotype (Viljoen et al. 2006), extraction method (Abad et al. 2012) and plant ontogeny (Padalia et al. 2011). Compounds present in *A. afra* have shown antimicrobial activities against *Trypanosoma brucei*, *Aspergillus niger*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Abad et al. 2012, More et al. 2012). Due to the alarming increase in the rate of food spoilage and contamination by microorganisms, extensive research work has to be done to eliminate this serious problem (El-Soud et al. 2015). Some of these microorganisms are *A. flavus* and *A. parasiticus* which produce secondary metabolites known as aflatoxins that are potentially harmful to crops, animals and humans (Saleem et al. 2017, Kaale et al. 2021). The aflatoxins B_1, B_2, G_1 and G_2 are the major four toxins amongst at least 18 structurally related toxins (Massomo 2020). Aflatoxin B_1 is particularly important since it is the most toxic and potent hepato carcinogenic natural compound (Makun et al. 2012). *A. flavus* causes a broad spectrum of diseases in humans, ranging from hypersensitivity reactions to invasive infections (El-Soud et al. 2015). Fortunately, natural products, particularly essential oils can participate effectively against these microorganisms in particular *A. flavus* growth and aflatoxins production (El-Soud et al. 2015). Therefore, this study was carried out to evaluate the antifungal activity of *A. afra* essential oil against *A. flavus* and aflatoxins production.

Materials and Methods

Collection of plant materials

Artemisia afra Jacq. leaves were collected in the wilderness of Lushoto in the Tanga region, Tanzania (altitude: 1677 m. GPS 4030.364 S, 38015'237 E) in July 2020. The plant species was identified in the field and confirmed at the Herbarium of the Department of Botany, University of Dar es Salaam where voucher specimen FMM 3976 of *A. afra* is preserved.

Chemical reagents

The chemical reagents used in this study were of analytical grade during extraction and HPLC grade during extraction of aflatoxins and HPLC analysis, purchased from chemical suppliers. These included

methanol (b.p. 64.6 °C, Loba Chemie India, CAS No. 67-56-1), dichloromethane (b.p. 36.6 °C, Loba Chemie India, CAS No. 75-09-2), anhydrous sodium sulphate (Loba Chemie India, CAS No. 7757-82-6), trifluoroacetic acid (TFA) (b.p. 72.4 °C Loba Chemie India, CAS No. 76-05-1), acetonitrile (b.p. 82 °C Merck KGaA, CAS No. 75-05-8), potassium bromide (Loba Chemie India, 7758-02-3) and nitric acid (b.p. 83 °C Loba Chemie India, CAS No. 7697-37-2).

Method of extraction: Steam distillation

Artemisia afra fresh leaves samples were washed thoroughly with tap water to remove dust and any other foreign materials. The steam distillation method system comprised a 2 L flask containing distilled water, a glass column containing the plant materials, a heating mantle, and a condenser was used (Božović et al. 2017). Samples of *A. afra* (380 g) were placed in a glass column, with steam passing over the materials to release molecules of the volatile oils. The steam contained essential oils, which passed through the condenser and collected. After extraction, *Artemisia afra* distillates were separated by dichloromethane and excess water was removed by anhydrous sodium sulphate. The final essential oil obtained was packed in amber glass bottles and stored at 4 °C for further analysis.

Identification of compounds in the essential oil of *Artemisia afra* using GC-MS

Gas chromatography-mass spectrometry (GC-MS) was used for qualitative analysis of constituents of *A. afra* essential oil. GC-MS analyses were performed using GC-MS-QP2010 Shimadzu (Japan), with an AOC-20i/s auto-sampler. The GC separation was performed with the Restek-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The oven temperature program was 40 °C to 200 °C and held at 40 °C for 4 min. The temperature was increased to 200 °C for 4 min at the rate of 20 °C/min. The injection temperature was 250 °C with split injection mode. The flow rate of carrier gas helium (99.99%) was 1.21 mL/min. The ion source temperature and interface temperature in MS were 230 °C and 300 °C, respectively from which the GC chromatograms and MS spectra were obtained. The GC chromatograms were used for the determination of the abundance of the individual constituents while MS spectra were used for the identification of the constituents by matching the mass spectra of the constituents to those recorded in the NIST Mass Spectral Library with the associated database (NIST 11) and available literature. The identification done was based on a similarity search between 85% to 97% that resulted from comparing MS chromatograms of compounds in the library and those from the samples. Consequently, disappearing peaks or compounds that had lower than 85% similarity were not identified. Blank control samples containing only solvents and reagents that were used in dissolving the essential oil for GC-MS analysis was run in parallel with the samples to check for any sources of contamination.

Fungal strain

The aflatoxin-producing fungal strain *A. flavus* (S-TZFHKW11T-2), generously supplied by the International Institute of Tropical Agriculture (IITA), Mikocheni, Dar es Salaam, was used throughout the study.

Antifungal and antiaflatoxigenic activities of *Artemisia afra* essential oil against *A. flavus*

Artemisia afra essential oil obtained was used to determine the antifungal and antiaflatoxigenic activities against *A. flavus* using Sabouraud dextrose broth (SDB) (30 g/1000 mL) (Hi-Media, India) as a growth medium and HPLC-UV for detecting and quantifying aflatoxins.

Growth inhibition bioassay

The mycelial dry weight determination method was used for growth inhibition bioassay as previously described by Sharma and Tripathi (2008), Mostafa et al. (2011) and Jantapan et al. (2017) with some modifications. Twenty mL of liquid media was dispensed into conical flasks (100 mL) and sterilized by autoclaving for 15 minutes

at 121 °C and 1.03 bar. The sterile hot broth was allowed to cool before mixing with essential oil. Serial dilutions were made from the original stock of broth containing essential oil to obtain the following concentrations: 10 µL/mL, 25 µL/mL and 50 µL/mL. Ten (10 µL) of a spore suspension (10^5 spores/mL) from a toxigenic *Aspergillus flavus* was then inoculated in each flask. The flasks (100 mL) were then incubated at 28 °C for 7 days in the dark. After the incubation period, the growth of the aflatoxigenic fungi *A. flavus* in all flasks was visually examined. The mycelia from each flask were filtered in pre-weighed filter papers (Whatman no. 1) and the filtrate was used for antiaflatoxigenic bioassay. The collected mycelia on the filter paper were washed with sterile distilled water and dried in the oven overnight at 70 °C. The dried mycelia were weighed after cooling at room temperature using a desiccator. Fungal growth inhibition (%) based on dry weight (mg) was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Control weight - Treated sample weight}}{\text{Control weight}} \times 100$$

Antiaflatoxigenic bioassay

The effectiveness of *A. afra* essential oil in controlling aflatoxins production was evaluated by the method described by Gandomi et al. (2009) and Jantapan et al. (2017) with some modifications. The aflatoxins in the filtrate obtained after mycelia filtration from the Sabouraud dextrose broth were extracted with a mixture of methanol and distilled water (80:20). The mixture sample was shaken for 30 minutes in a water bath shaker. The extract was then re-filtered with a micro-filter syringe into the vials. The eluted sample was derivatized by adding 50 µL trifluoroacetic acid (TFA) (Asis et al. 2002). The derivatized eluate was homogenised with a vortex vigorously for 30 seconds before high-performance liquid chromatography (HPLC) analysis.

The levels of individual aflatoxins in the samples were determined using reverse phase HPLC (Agilent Technologies HPLC, Germany, Series 1260 Infinity II) with UV-detector (G7114A Series DEACX10455) and auto-sampler (G7129A Series DEAEQ21126). The mobile phase was prepared by mixing 200 mL of distilled water, 50 mL of acetonitrile and 250 mL of methanol, H_2O/ACN/MeOH (40:10:50). The mobile phase was derivatized by adding 59.6 mg of potassium bromide and 50 µL of nitric acid. 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 with a UV detector at a wavelength of 365 nm.

For each sample, the running time was 6 minutes and 0.8 mL/min mobile phase flow rate. 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 (0.5 µg/mL) was run parallel with the vials for analysis by comparison. The following formula was employed to determine the aflatoxins concentrations from each vial:

$$[\text{Sample (µg/mL)}] = \frac{\text{Peak area of sample} \times [\text{Standard (µg/mL)}] \times \text{Volume of extraction solvent}}{\text{Peak area of standard} \times \text{Volume of the extracted sample}}$$

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

The control of aflatoxins production exhibited by essential oil was measured by considering the total concentrations of individual aflatoxins of the treatments and control after 7 days of incubation period using the following formula:

$$\text{Antiaflatoxigenic activity (\%)} = \frac{[\text{Control (µg/mL)}] - [\text{Treatment (µg/mL)}]}{[\text{Control (µg/mL)}]} \times 100$$

A blank control sample containing 80% MeOH 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.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using Minitab 17 (2020, Pennsylvania, USA) and statistical significance was considered when $p \leq 0.05$. The means produced by the data were tested with Tukey's Honestly Significant Difference (Tukey HSD) test. In this test, the concentration of essential oil expressed in µL/mL was tested against the inhibition activity expressed in percentage. All data are expressed in mean ± standard deviation.

Results and Discussion

GC-MS analysis of *Artemisia afra* leaves essential oil

The GC-MS analysis of *A. afra* leaves essential oil (yielded 0.47% v/w) revealed 19 compounds (Table 1 and Figure 1). The major compounds identified were borneol (71.529%), terpinen-4-ol (8.582%), β-thujone (3.283%), β-terpineol (2.590%), α-terpineol (1.791%), chrysanthenone (1.507%), myrtenal (1.393%), eucalyptol (1,8-cineole) (1.263%), *cis*-sabinene hydrate (1.172%) and *trans*-carane, 4,5-epoxy (1.088%).

Table 1: Percentage composition of compounds identified in the essential oil of *A. afra*

S/N	Compounds	Retention time (min)	Peak area	Percentage composition
1.	Eucalyptol (1,8-cineole)	4.974	60595	1.263
2.	*cis*-Sabinene hydrate	5.497	56255	1.172
3.	β-Terpineol	5.952	124306	2.590
4.	β-Thujone	6.054	157558	3.283
5.	α-Thujone	6.215	20786	0.433
6.	Camphene	6.261	26311	0.548
7.	Chrysanthenone	6.331	72303	1.507
8.	*trans*-Carveol	6.642	40185	0.837
9.	*trans*-Carane, 4,5-epoxy	6.806	52206	1.088
10.	Borneol	6.973	3432631	71.529
11.	Terpinen-4-ol	7.116	411868	8.582
12.	α-Terpineol	7.303	85939	1.791
13.	Camphor	8.235	22510	0.469
14.	*cis*-Verbenol	8.432	40015	0.834
15.	Bornyl acetate	8.627	46816	0.976
16.	α-Bisabolol	8.998	33647	0.701
17.	Myrtenal	9.221	66831	1.393
18.	α-Cadinol	13.110	13727	0.286
19.	Juniper camphor	13.316	34445	0.718

The results have shown that compounds such as artemisia alcohol, artemisia ketone and artemisyl acetate were not detected in this study as reported in previous studies (Viljoen et al. 2006, Liu et al. 2009, Patil et al. 2011). Compounds identified in this study have different percentage compositions compared to previously reported studies on the analysis of oil from *A. afra* (Mwangi et al. 1995, Chagonda et al. 1999, Viljoen et al. 2006, Asekun et al. 2007, Vagionas et al. 2007, Patil et al. 2011). This could be due to the influence of the following factors on the oil composition as reported by various reports, such as geographical location, harvesting seasons/months, fertilizer, soil type, chemotype or subspecies, choice of plant part or genotype, and drying methods (Abad et al. 2012, Liu et al. 2009, Oyedeji et al. 2009). For example, in the essential oil of *A. afra* from Mbeya in Tanzania camphor (46.2%), α-thujone (15.2%), artemisia ketone

(7.4%) and 1,8-cineole (4.2%) were the major compounds (Vagionas et al. 2007). Mwangi et al. (1995) reported that 1,8-cineole (67.4%), terpinen-4-ol (6.5%), and borneol (5.1%) were found to be the major constituents in the essential oil of *A. afra* from Kenya. The report of *A. afra* Ethiopian species showed that artemisyl acetate (35.5%), yomogi alcohol (13.5%) and artemisia ketone (13.2%) were the major compounds (Asfaw and Demissew 2015). According to More et al. (2012), the monoterpenes content of some *Artemisia* species such as borneol, camphor, chrysanthenone, myrtenal and eucalyptol (1,8-cineole) varied seasonally with high content in July (4.18%) and the lowest during May (0.97%). Chagonda et al. (1999) analysed oils from wild and organically cultivated plants of *A. afra* from Zimbabwe and the major components of the oils indicated large variations between cultivated and wild populations. The oil of wild populations contained artemisia ketone (6.3–41.9%), 1,8-cineole (0.1–27.9%) and camphor (8.5–27.1%), whereas the oil of the cultivated populations mainly contained artemisia ketone (32.1–34.8%), camphor (21.8–24.4%), 1,8-cineole (10.9–15.7%), borneol (14.2–19.1%) and camphene (3.0–5.6%).

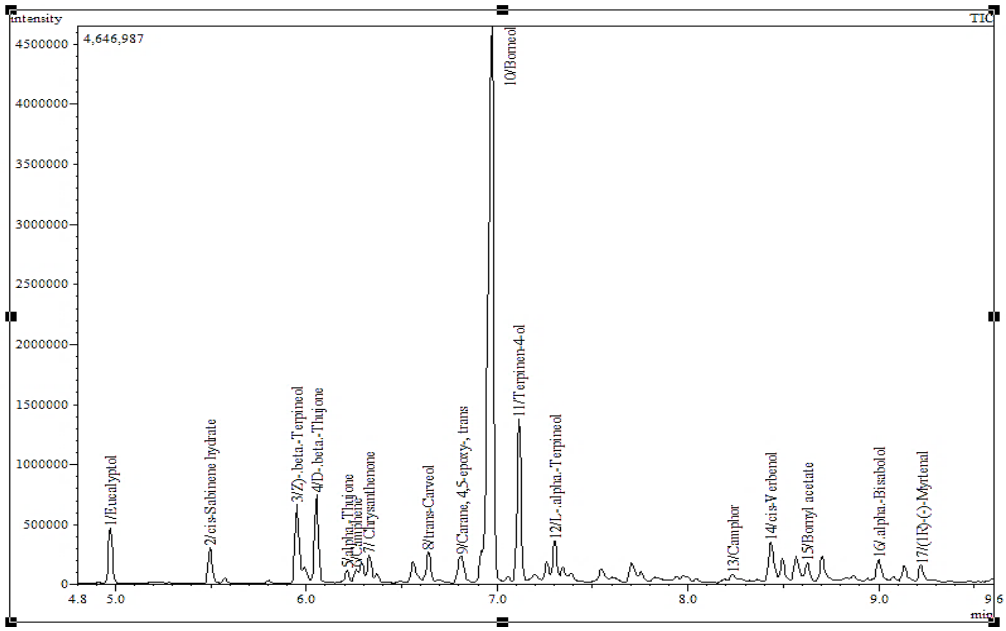

Figure 1: GC chromatogram for *Artemisia afra* essential oil.

The impact of drying methods on the composition of the essential oil of *A. afra* was studied by Asekun et al. (2007), they found that the oil extracted from fresh plants contained Artemisia ketone (6.9%) which was absent in the oil obtained from air and sun-dried plants. Oyedeji et al. (2009), studied the α-thujone content isolated from essential oil obtained by hydro-distillation of fresh and dried twigs of *A. afra* plants and found that the concentration of α-thujone increased significantly in the dry leaves when compared with the fresh leaves.

Growth inhibition of *A. afra* essential oil against *A. flavus*

After identifying the compounds present in the essential oil of *A. afra*, the growth inhibition against *A. flavus* was investigated. The antifungal activity of *A. afra* essential oil after 7 days of incubation period against *A. flavus* growth is presented in Table 2. The essential oil from *A. afra* was able to inhibit

the *A. flavus* growth by 99.97% at a concentration of 10 µL/mL. In addition, the results showed that there was no significant difference ($p > 0.05$) in *A. afra* essential oil in inhibiting *A. flavus* growth at concentrations of 10, 25 and 50 µL/mL.

Table 2: Effects of *A. afra* essential oil on *A. flavus* growth, values are expressed as mean ± SD. Mean values with the same letters are not significantly different ($p > 0.05$)

Extract type	Concentration (µL/mL)	Growth inhibition (%)
Control	0	0
Essential oil	10	99.97 ± 0.01^{a}
Essential oil	25	99.98 ± 0.01^{a}
Essential oil	50	99.99 ± 0.006^{a}

Essential oils are recognized to inhibit the growth of *A. flavus*. For instance, Gundidza (1993) reported that *Artemisia afra* essential oil at the concentration of 10 µL/mL inhibited *A. flavus* growth by 38.20%. In addition, Mohan et al. (2016) noticed that *Artemisia maritima* at a concentration of 10 µL/mL inhibited *A. flavus* growth by 35.40%. Furthermore, Soares et al. (2015) reported that *Artemisia vulgaris* essential oil at the concentration of 10 and 15 µL/mL inhibited *A. flavus* growth at 63.93% and 88.58%, respectively.

The effect of essential oil in fungal growth might be due to its chemical compositions and its ability to form charge transfer complexes with electron donors in the fungus cell (Allameh et al. 2011, Abd El-Aziz et al. 2015). The essential oil can lead to interference with the amino acids involved in germination (Abd El-Aziz et al. 2015). Alpsoy (2010) reported that there is a relationship between the chemical structures of the most abundant compounds in the essential oil and their antifungal activities. However, there is evidence that minor components have a critical part to play in the antimicrobial activities, possibly by a synergic effect with other components (Alpsoy 2010). For example, the major and minor compounds identified in *Artemisia* species essential oils are signalled to have potent inhibitory effects at a broad spectrum against the microorganisms tested (Juteau et al. 2002, Kordali et al. 2005, Wenqiang et al. 2006, Çetin et al. 2009). Nevertheless, the essential oil of four organs (fruits, stems, leaves and flowers) of the *Scabiosa arenaria* Forssk plant in Tunisia contained β-thujone and chrysanthenone, which exhibited antimicrobial activities (Besbes et al. 2012). β-Terpineol showed a significant antimicrobial activity as reported by George et al. (2006) and constituted a major compound found in the essential oil of rhizomes of *Amomum cannicarpum. trans*-Carane, 4,5-epoxy and myrtenal were among the compounds identified in the essential oil of *Cyperus rotundus* rhizomes oil, which showed good antimicrobial activity against *Aspergillus niger* and *Candida albicans* (Eltayeib and Ismaeel 2014).

The mode of action of essential oils and their constituents against fungal growth involves the inhibition of enzymes that control energy or structural compounds production, impairing ergosterol metabolism, disrupting cell wall and cell membrane, coagulating the cytoplasm and hence damage of cellular organelles and leakage of macromolecules and the lysis, inhibiting cytoplasmic and mitochondrial enzymes, and altering the osmotic and the redox balance (Ghannoum and Rice 1999, Ben Miri et al. 2020, Loi et al. 2020). Due to their lipophilic nature, it allows them to pass through the cell wall and cytoplasmic membrane damage while disrupting various layers of polysaccharides, fatty acids and phospholipids (Basak and Guha 2018, Loi et al. 2020). For instance, Kumar et al. (2020) investigated the mode of action of *Artemisia nilagirica* essential oil, whereby the fungal cells treated with 1.4 µL/mL volatile oil exhibited deformity, shrinkage and detachment of plasma membrane from the cell wall. At the same dose, *A. nilagirica* essential oil completely inhibited ergosterol

synthesis in the cell membrane of *Aspergillus flavus*. Ergosterol is the predominant component of the fungal cell membrane, it is essential to preserve cell membrane functionality as cholesterol does in animal cells (Ghannoum and Rice 1999, Loi et al. 2020). The absence or reduced presence of ergosterol in fungal membranes results in osmotic and metabolic instability of the fungal cell, compromising reproduction and infectious activity (Nazzaro et al. 2017).

Inhibition of aflatoxins production in *A. flavus* by *A. afra* essential oil

Table 3 shows the inhibitory effects of *A. afra* essential oil on aflatoxin production by *A. flavus* exposed to different *A. afra* essential oil doses, 10–50 µL/mL. The results showed that essential oil from *A. afra* is significantly strong at inhibiting aflatoxin production in *A. flavus*. Likewise, there was no significant difference ($p > 0.05$) in *A. afra* essential oil in inhibiting aflatoxin production at concentrations of 10, 25 and 50 µL/mL.

Table 3: Effects of *A. afra* essential oil on aflatoxins production, values are expressed as mean ± SD. Mean values with the same letters are not significantly different ($p > 0.05$)

Extract type	Concentration (µL/mL)	AFB_1 (µg/mL)	AFB_2 (µg/mL)	Total aflatoxins (µg/mL)	Antiaflatoxigenic activity (%)
Control	0	7.40	0.24	7.64	0
Essential oil	10	0.06	0.00	0.06	99.25 ± 0.13^a
Essential oil	25	0.05	0.00	0.05	99.30 ± 0.12^a
Essential oil	50	0.03	0.00	0.03	99.63 ± 0.11^a

Some studies have described the use of essential oils in inhibiting *A. flavus* to produce aflatoxins. For example, Kumar et al. (2020) reported that *Artemisia nilagirica* essential oil inhibited aflatoxin B_1 produced by *Aspergillus flavus* at a concentration of 1 µL/mL. In another experiment, *A. nilagirica* volatile oil at a concentration of 0.16 µL/mL completely inhibited aflatoxin B_1 produced by *Aspergillus flavus* (Sonker et al. 2015). The inhibition of aflatoxin production by essential oils is related to the downregulation of aflatoxin biosynthesis genes (Xiang et al. 2020). For instance, Xiang et al. (2020) have shown that two regulatory genes, *aflR* and *aflS*, and four structural genes for aflatoxin biosynthesis clustered in the genome of *A. flavus* were found to be downregulated by cinnamon, oregano, and lemongrass essential oil, which were consistent with the observation that aflatoxin B_1 production was reduced in all samples.

Moreover, the antiaflatoxigenic actions of essential oils and their constituents may be related to the inhibition of the ternary steps of aflatoxins biosynthesis involving lipid peroxidation and oxygenation (Alpsoy 2010, El-Soud et al. 2015). It has also been reported that essential oils can decrease the damaging effects of aflatoxins in two different ways. Firstly, essential oils reduce the DNA binding activity of aflatoxins. Secondly, aflatoxins cause an increase in reactive oxygen species (ROS) and essential oils react with ROS. Therefore, essential oils protect the cells from the harmful impacts of aflatoxins (Alpsoy 2010).

Conclusion

In this study, 19 compounds were successfully identified in the essential oil extracted from *A. afra* growing in Lushoto, Tanga Region of Tanzania. Qualitative comparison with literature data showed that the compounds identified in the essential oil of *A. afra* were similar to those reported elsewhere, although there were differences in the chemical composition. Furthermore, the study has shown that *A. afra* essential oil can be used as a potential source of natural fungicide against *A. flavus* growth and controlling of aflatoxins production. This study recommends further research that will enable the isolation, identification and characterization of specific compounds that facilitated the antifungal and antiaflatoxigenic activities of *A. afra* essential oil.

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Conflict of interest
The authors declare that there is no conflict of interest regarding this work.

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