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Phytochemical characterization and antifungal potentials of *Melia azedarach* Linn leave aqueous extract to inhibit aflatoxins biosynthesis in food during storages

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Abstract

The current study was aimed to assess the chemical compositions of *Melia azedarach* Linn (Chinaberry) leaves aqueous extracts. Additionally, the extracts were also tested to investigate its antifungal potentials against *Aspergillus flavus* and *Aspergillus parasiticus*. Leaf extract of *M. azedarach* was obtained by maceration technique, subsequently analyzed using UV-Visible Spectrophotometer, Fourier Transform Infrared (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS). The total phenolic and flavonoids contents were; 67.5 ± 0.4 mg GAE/g DW and 12.7 ± 0.2 mg QE /g DW respectively. The presence phytochemicals were confirmed from various functional groups recorded in FT-IR spectra. The results were further validated through GC-MS analysis where a total of 18 compounds were identified with seven major compounds; namely 1-Butanol, 3-methyl-, acetate (11.53%), followed by coumaran (10.04%), (R, S)-2-propyl-5-oxohexanal (7.07%), 10-octadecenoic acid, methyl ester (5.16%) and 5,7-Octadien-2-one, 3-acetyl (3.06%). The extract exhibited antifungal activities against two major aflatoxin-producing fungi, *A. flavus* and *A. parasiticus*. The aqueous extract (31.25 to 500 mg mL^{-1}) was active to inhibit the spore germination, mycelial growth, biomass production and aflatoxin biosynthesis. Spore germination was significantly reduced, with maximum inhibition of 83% against *A. flavus* and 85% against *A. parasiticus* at 500 mg mL^{-1}. Mycelial growth and fungal biomass were markedly declined with increasing trend in extract concentration. The recorded biomass inhibition was

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73.2% and 76.9% respectively against *A. flavus* and *A. parasiticus*. The extract also significantly suppressed the aflatoxin production in the selected fungal strains at higher concentrations, exceeded from 75% with respect to aflatoxins B_1, B_2, G_1, and G_2. The findings suggest that *M. azedarach* leaves extract is a valuable source of bioactive compounds possessing strong antifungal and anti-aflatoxigenic properties and could be considered as a promising natural alternative for controlling aflatoxins contamination in agricultural food sectors.

Keywords: aflatoxins; flavonoids; mycelial growth inhibition; phenols; spore germination inhibition

Introduction

Aflatoxins (AFs) are extremely toxic secondary metabolites synthesized by species of Aspergillus genus like *Aspergillus nomius*, *A. parasiticus* and *A. flavus*. These metabolites could expand in a natural way in foods products like wheat, rice, maize, beans and many others. Presently, there are more than twenty diverse groups of AFs, while the most significant types are G1, G2, B1 and B2 (Ponzilacqua *et al*., 2018). Among the mycotoxins, AFs are considered as more carcinogenic, severely affecting human being when utilized in polluted food. The *A. parasiticus* and *A. flavus* are the main species responsible to produce AFs in food stuffs. The poisonous *A. flavus* species synthesize AFs; B1 and B1, whereas toxigenic *A. parasiticus* can synthesize; G1, G2, B1 and B2. The degree of toxicity of AFs displayed in animals is in the order of: AFs B1>AFsG1>AFsB2>AFsG2 (Zahija *et al*., 2023). Maximum doses of AFs contamination can cause aflatoxicosis or acute toxicity in animals which could be lethal if left unchecked. Chronic contamination might, leads to hepatic carcinoma, dysfunction of the immune system, reproductive system destruction and growth impairment (Tian *et al*., 2023). As per literature reports worldwide financial losses due to AFs exposure are expected per year up to 100M $ (Tian *et al*., 2023).

Plant based compounds are used to ensure food safety for customers. These non-synthetic materials are sustainable and ecofriendly with low ecosystem hazards. Such substances might be applied to minimize the level of contamination in food during storage, pre-harvest and in the field. However, it needs precise formulations to assure the quality control, homogenous distribution and effectiveness of the product (Cadenillas *et al*., 2024). In this regard, herbs essential oils and extracts have been studied as growth inhibitors of fungi and recognized as secure substitute in the food products for mycotoxins inhibition. Various aqueous extracts of plants contained chemical substances or phytochemicals that stop the AFs production, which have raised the attention of researchers these days (Ponzilacqua *et al*., 2018).

Currently numerous techniques have been used to prevent or limit the fungi growth and consequently the AFs biosynthesis in crops during storage, post-harvest, pre-harvest and in field conditions. Several of these techniques generally have been considered as good agriculture practices, however the role of climate change, that cannot be managed, might powerfully decrease their effectiveness. Apart from this, synthetic fungicides are applied prominently to inhibit the development of toxic fungi; however, its toxic side effects and the rising targeted fungicides/pesticides resistance needs substitute techniques to inhibit AFs pollution in the crops now a days (Cadenillas *et al*., 2024). Aflatoxins inhibition and detoxification is necessary for food security. The so far in use techniques can be categorized as biological, chemical and physical methods. Every technique has their own limitations, however, being as curing method it should be secure and non-influenced by the climatic conditions to ensure the nutrition ingredients of the targeted crops, plants or grains (Velazhahan *et al*., 2010). Furthermore, customer insists for healthier and more sustainable foodstuff products have compelled the food industry to substitute synthetic additives with non-synthetic or natural origin substitutes (Tian *et al*., 2023). Perfectly, such substances would be derived from plants, being associated with low side effects for human (Zhou

et al., 2015). In this connection *M. azedarach* plant is selected due to its easy, affordable and abundant mass availability in Pakistan.

The *M. azedarach* belongs to Meliaceae family, generally known as Mahaneem, Persian lilac, white cedar, Umbrella tree and Chinaberry. This plant is commonly found in Australia, South-East Asia, Tropical and subtropical countries, and the North-West region of Himalaya of Pakistan and India (Jaafar *et al*., 2016; Abbas *et al*., 2017). The *M. azedarach* is traditionally used as a medicinal plant and possess anti-inflammatory, analgesic, antifertility, antifungal, antibacterial, antimalarial, antiviral, anticancer, antioxidant, stomachic, astringent, antilithic, diuretic and anti-parasitic properties (Antara and Amla, 2012; Abbas *et al*., 2017; Shrestha *et al*., 2021). The important and potential uses of this plant as described literature have compelled us to investigate some unexplored aspects of this plant therefore, the current study was carried out to investigate the biochemical compounds in *M. azedarach* leaves aqueous extracts (MALAE) using UV-Visible Spectroscopic, FT-IR and Gas Chromatography-Mass Spectroscopy analysis. Additionally, the antifungal potential using mycelium growth inhibition, spore germination, spore germination inhibition, fungi dry weight inhibition and anti-aflatoxigenic activities against *A. flavus* and *A. parasiticus* were also determined.

Materials and Methods

Collection of plant leaves

The fresh and mature leaves of *M. azedarach* were obtained from Experimental Research Farm of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar-Khyber Pakhtunkhwa-Pakistan (Latitude: 34°0' N. Longitude: 71°35' E). These plants were not sprayed with any synthetic pesticides during the entire growing season. The expert plant taxonomist of PCSIR-Peshawar Dr. Hina Fazal, Senior Scientific Officer identified the plant as *Melia azedarach* given Voucher #10822(PES) submitted in the Herbarium of PCSIR-Peshawar, Pakistan.

Preparation of plant extract

The collected leaves were rinsed and washed with running tap H_2O, dried in Air Cabinet Dryer (England) at 40 °C for 24 hours. The plant materials were then powdered using grinder Mill (Standard Model No.3 Wiley Mill USA). The powder sample 50 g was taken in a sterilized Erlenmeyer flask and added it to 200 mL distilled water. The flask was enclosed in aluminium foil and store for five days at 28 ± 01 °C. The flask mixture was shaken strongly at four hours gaps to assure maximum extraction from the plant sample. After five days the mixture was filtered Whatman No. 1 filter paper (Whatman International Ltd. Maidstone- England). This process was repeated three times to obtain a maximum extraction. The obtained filtrate was afterward evaporated at 45 °C using Vacuum Rotary Evaporator (Büchi R-200, Switzerland; B-490 Heating Bath- Buchi) for 24 hours. The final concentrated extract was further evaporated using vacuum oven to remove the remaining water. The final plant extract was stored in a sterilized and clean Amber Bottle with tight cap and stored at 10 °C using Cooled Incubator (Memmert-Germany) for further experiments.

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC were determined in the *M. azedarach* leaves aqueous extract (MALAE) applying Folin-Ciocalteu assay (Bonface *et al*., 2020). The optical density (absorbance) of the reaction blend and control was noted at 765 nm using Hitachi U-2900 UV-Vis Spectrophotometer (Tokyo- Japan). The TPC was quantified as mg Gallic acid equivalent (GAE) per gram of sample dry weight. Using the standard protocol of aluminium chloride assay the total flavonoid (TFC) in the *M. azedarach* leaves aqueous extract was determined (Bonface *et al*., 2020). The optical density of the blank and sample was calculated at 415 nm through U-2900-Hitachi UV-Vis Spectrophotometer (Tokyo-Japan). The calibration curve was drawn at the concentration of 60, 50,

40, 30 m, 20 and 10 mg mL $^{-1}$) using quercetin as positive control. The outcome was measured as mg quercetin equivalent (QE) per gram of the sample dry weight.

Fourier Transform Infrared (FT-IR) analysis

The kinds of functional groups (chemical bonds) present in substances are categorized applying

Fourier transform infrared (FTIR) spectrophotometer was used. For the results of IR spectrum, the FTIR Prestige -21 Shimadzu Japan. The *M. azedarach* leaves aqueous extract was investigated from 400-4000 cm^{-1} and working at 4/cm resolution with a ten number of scans using IR solution software.

Gas chromatography-mass spectrometry analysis

The biochemical compounds present in the *M. azedarach* leaves aqueous extract were identified and quantified using GC-MS. The GC-MS analysis was carried out through GC-MS (Shimadzu, Japan QP2010) with autosampler. Helium gas was used as carrier gas using Colum DB-5 (30 m x 0.25 mm internal diameter and 0.25 µm thickness) at a linear velocity (30 cm s^{-1}). The oven was set to ramp temperature for three minutes to 60 °C and afterward at a rate of 3 °C/min to 210 °C. The detector and injector temperatures were adjusted to 270 °C and 300 °C respectively. The ionization energy was 70 eV, the time of scan was 1s and the mass range was 40-300 amu. The unknown compounds were identified by comparing their GC retention time (RT) with those of known substances and comparing their mass spectra (MS) with published reports, with those of standard MS from the NIST library (NIST 05) of known compounds.

Antifungal assay

Cultivation and maintenance of fungal strains

Fungal species *A. flavus* and *Aspergillus parasiticus* were collected from Mycotoxin Research Section of PCSIR Laboratories Complex Peshawar. These fungi were grown on Potato Dextrose Agar (PDA) Petri plates and PDA slants and kept for seven days at 27 °C in an incubator (Memmert-Germany). After incubation PDA plates and slants copies were kept in a cooled incubator at -40 °C for in future experiments (Neycee *et al*., 2012).

Mycelium growth inhibition assay

M. azedarach leaves aqueous extract was dissolved in 50% dimethyl sulphoxide (DMSO) to produce a final concentration of 31.25, 62.5, 125, 250 and 500 mg mL^{-1}. From each concentration of the extract 2 mL of sample was poured into sterilized petri dishes. Then 18 mL of molten streptomycin sulphate-PDA media was added to these petri dishes. The inoculated petri dishes were rotate in clock and anti-clock wise direction on Laminar Air Flow Cabinet bench to completely mix the contents prior to solidification. The mycelial disc of each fungus (seven day old) was cut (5mm) using a sterile Cork-borer and kept upside down on the centre of petri dishes. The petri dishes lacking plant extracts were designated as controls. The reference standard fungicide Benlate (8.5m mg mL^{-1}) was used to compare its efficiency with *M. azedarach* leaves aqueous extract. The petri dishes were kept in an incubator (Memmert-Germany) for seven days at 27 °C. The mycelial growth diameter (mm) of each tested fungus were after incubation. The plant extract fungicidal toxicity was measured as percentage % of mycelial growth inhibition using the formula described in equation 1:

$$Fp = F1 - F2 / F1 \times 100 \quad (1)$$

Where: Fp= Mycelial growth inhibition %; F1 = Control mycelial growth and F2 = Treatment mycelial growth.

Spore inhibition and germination assay

The fungi spores were obtained via sterile distilled H_2O possessing tween 80 (0.1%) from the Petri Plates of PDA grown fungi at 28 °C for seven days. The spore's concentrations were bringing to 1.45 x 10^6 spores per milliliter. 20mL of the sterilized PDA media was mixed individually with 31.25, 62.5, 125, 250 and 500 mg mL

$^{-1}$ then 1.45×10^6 spores/mL. The media containing the fungal spores was later dispenses into 10cm petri plates. The plates were placed in an incubator (Memmert-Germany) for solidification for four days at 28 ± 2 °C. The germination reduction percentage of spores was calculated by applying the formula given below in equation 2

$$\text{Spore Germination Inhibition (\%)} = [(N_c - N_t / Nc] \times 100 \quad (2)$$

Where, *Nc*: Spores number in Control; *Nt*: Spores number in treatment.

Dry weight method

In this method the sterilized 5mL of Potato Dextrose Broth (PDB) in test tubes was amended with 20 µL lively grown mycelium of tested fungi. The spore concentration was maintained to 1×10^6 spores/mL and this was achieved from seven days old fungi culture. In five test tubes, 20 µL (31.25, 62.5, 125, 250 and 500 mg mL $^{-1)}$ of *M. azedarach* extracts in DMSO (v/v) were poured, whereas one test tube was designated as negative control means without extracts. All the test tubes were placed in an incubator (Memmert-Germany) for seven days at 25 °C. After incubation the mycelia of fungi were isolated through filtration applying Whatman® No.1 filter paper (Whatman International Ltd. Maidstone- England). To obtain steady weight of fungal mycelia the filter paper was dried in an oven (DAIHAN scientific Co. Ltd. Korea) at 60 °C temperature. The fungi inhibition growth was measured by scrutinize the sample and control mycelial dry weight. The growth inhibition percentage was measured using the below equation 3 (Kumari *et al*., 2021):

$$\text{Inhibition Growth \%} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \quad (3)$$

Aflatoxin biosynthesis inhibition

For the determination of aflatoxin biosynthesis inhibition, the fungus 1mL spore's suspension was shifted into 250 mL conical flask possessing PDB and *M. azedarach* extract at 500, 250, 125, 62.5 and 31.25 mg mL $^{-1}$ concentrations respectively. The flasks were incubated at 28 °C for five days without agitation. Afterward the liquid culture was filtered in the course of Whatman® No.1 filter paper (Whatman International Ltd. Maidstone- England). The mycelium of fungi was washed with distilled H_2O, and then dried in an oven (Memmert Germany) for 72 h at 50 °C followed by the measurement of constant dry weight. Aflatoxin extraction was achieved by taking 5 mL of fluid culture from each flask, filtered through a 0.2 µm syringe filter' and finally mixed with chloroform (5 mL). The organic portion was received in 25 mL glass beaker and after evaporation in an air circulated oven dryer at 40 °C the residue obtained. The residue was re-dissolved in methanol (500 µL) and stored in dark glass vial at -20 °C for further research work (Yazdani *et al*., 2013).

Detection and quantification of aflatoxins

Thin-Layer Chromatography (TLC) and fluorescence spectroscopy techniques were used to detect and quantify aflatoxins respectively (Yazdani *et al*., 2013). The silica gel 60 F254 (20×20 cm, Merck) TLC plate was used for TLC analysis. The mobile phase comprising Toluene: chloroform: acetone (15:75:10 v/v) was used. The test sample 20 µL and aflatoxins standards B1, B2, G1 and G2 with 01 µg/mL concentrations were marked on TLC plates at room temperature in a TLC tank. The spots developed on TLC plates were observed under UV light at 365 and 254 nm.

Quantitative assay of AFs was measured using spectrofluorophotometer (Shimadzu, Japan). The AFs which were previously isolated from fluid culture were diluted with methanol and concentrations were measured using a linear calibration curve. Calibration curve was produced with a range of standards AFs at 10-300µg mL^{-1}. The emission and excitation wavelength were 435 and 365 nm respectively. This technique produces a detection limit of 5 ppb AFs (Yazdani *et al*., 2013). The inhibition of AFs displayed by *M. azedarach* leaves aqueous extract was calculated by applying below formula given in equation 4:

$$\text{Aflatoxin inhibition (\%)} = [\text{Control}] - [\text{Treatment}] / [\text{Control}] \times 100 \quad (4)$$

Statistical analysis

All the results obtained were expressed as means ± standard deviations (n = 3). Statistical significance between means was evaluated at 95% confidence level ($p < 0.05$). One-way ANOVA, Tukey HSD (post-hoc) and t-tests between fungi (Welch) were performed. The Statistical Package for the Social Sciences (SPSS) (2012, Statistics 22, IBM, Armonk, NY) was used to analyzed the data.

Results

The *M.azedarach* leaves collected, processed and used for extraction. The TPC and TFC analysis of *M. azedarach* leaves extract is shown in Figure 1. The results show that the total phenolic content was 67.5 ± 0.4 mg GAE/g DW and total flavonoid content was 12.7 ± 0.2 mg QE /g DW. The FTIR Chromatogram of *M. azedarach* leaves extract is shown in Figure 2. The FTIR chromatograms revealed the presence of spectra 3321.42 cm^{-1} (functional group O-H stretching and alcohol), 2937.59 cm^{-1} (functional group C-H stretching and alkane), 2848.86 cm^{-1} (functional group C-H stretching and class of compound alkane), 1600.92 cm^{-1} (functional group C = C stretching and conjugated alkene). The spectrum of IR absorption bands in the region 1564.27 cm^{-1} can be due to aromatic ring and lactone. The observed band 1417.68 cm^{-1} (functional group C-C Stretching and aromatic compounds), the spectrum of IR absorption bands in the region 1238.30 cm^{-1} correspond to functional group C-O stretching and alkyl aryl ether), the band at 1149.57 cm^{-1} (C-O stretching and class of compound ester), the peak value 1097.50 cm^{-1} (functional group C-O stretching and primary alcohol) and the wave number 1031.92 cm^{-1} corresponds to C-N Stretching and Aliphatic amines.

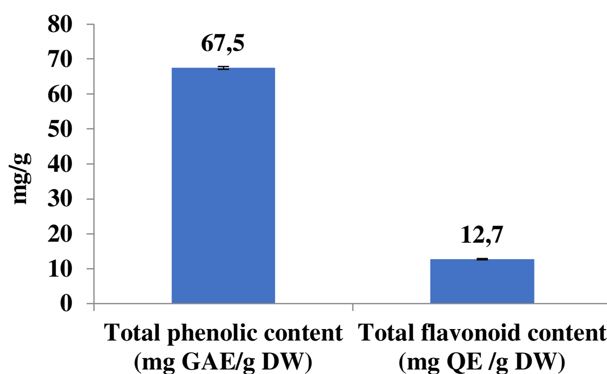

Figure 1. Analysis of TPC and TFC Contents of MALAE

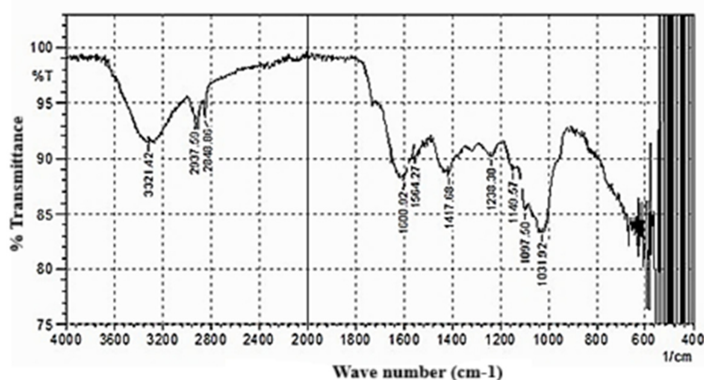

Figure 2. FTIR chromatogram of MALAE

In the present study, a total of 18 different phytochemicals have been analyzed in the aqueous extract of the leaves of *M. azedarach* (Chinaberry). The identified compounds of the extract, their retention time (RT), area and percentage concentration are given in Table 1 and the chromatogram graph (Figure 3) showed peaks with individual compounds. From observations and comparison with the mass spectrum of each constituent with NIST and Wiley library, these were confirmed to be 1-Butanol, 3-methyl-, acetate (11.53%), Coumaran (10.04%), (R,S)-2-Propyl-5-oxohexanal (7.07%), 10,Octadecenoic acid, methyl ester (5.16%), 5,7-Octadien-2-one, 3-acetyl- (3.06%), 7-Oxabicyclo[4,1,0] heptane, 1-methyl-4-(2-methyloxiranyl)- (2.78%), Pentadecanoic acid, 14-methyl-methyl ester (2.69%), n-Hexadecanoic acid (2.69%), 2-Decene-1-ol (2.43%), Pentadecanoic acid (1.87%), Octadecanoic acid, methyl ester (1.74%), 2-cyclohexene-1-ol,3-methyl- (1.64%), Cyclohexanone, 4-hydroxy- (1.13%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (1.06%), 3',5'-Dimethoxyacetophenone(1.06%), Phenol, 3,4,5-trimethoxy- (1.03%), Tetradecanoic acid (0.70%) and Cyclohexaneethanol, 2-methylene- (0.35%).

Table 1 GC-MS Analysis of MALAE

ID#	Compound	Retention time	Area	Concentration (%)
1	1-Butanol, 3-methyl-, acetate	5.849	217051	11.53
2	(R,S)-2-Propyl-5-oxohexanal	6.192	133136	7.07
3	Coumaran	8.540	188910	10.04
4	2-Decene-1-ol	11.774	45718	2.43
5	Cyclohexanone, 4-hydroxy-	16.452	21188	1.13
6	Tetradecanoic acid	16.834	13178	0.70
7	3',5'-Dimethoxyacetophenone	16.963	19999	1.06
8	Phenol, 3,4,5-trimethoxy-	17.906	19300	1.03
9	Pentadecanoic acid	21.287	35274	1.87
10	7-Oxabicyclo[4,1,0]heptane, 1-methyl-4-(2-methyloxiranyl)-	21.466	52248	2.78
11	5,7-Octadien-2-one, 3-acetyl-	21.709	57606	3.06
12	Cyclohexaneethanol, 2-methylene-	23.208	5629	0.35
13	2-cyclohexene-1-ol,3-methyl-	23.388	30897	1.64
14	Pentadecanoic acid, 14-methyl-methyl ester	25.386	50721	2.69
15	n-Hexadecanoic acid	25.386	50721	2.69
16	9,12-Octadecadienoic acid, methyl ester, (E,E)-	27.976	19892	1.06
17	10,Octadecenoic acid, methyl ester	28.094	97168	5.16
18	Octadecanoic acid, methyl ester	28.588	32823	1.74

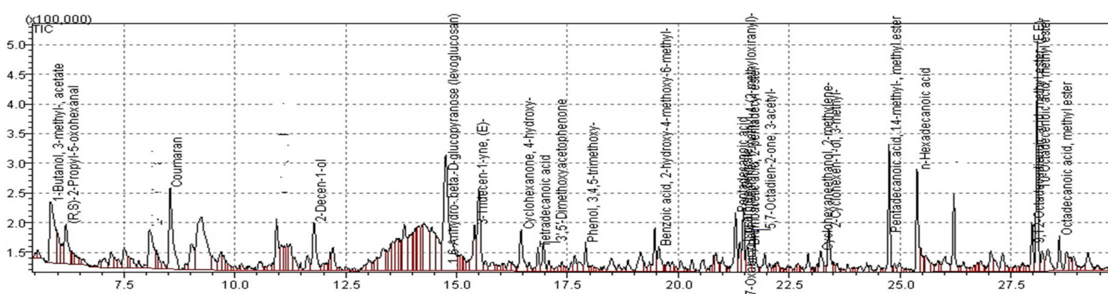

Figure 3. GC-MS chromatogram of MALAE

The botanical extracts could possess antifungal properties, which can be determined through scrutinized the fungal colony growth inhibition. This is usually quantified by applying measurement of colony diameter (mm) and % prevention measurement. The maximum inhibition colony diameter and maximum inhibition percentage displayed higher antifungal activities. The toxicological effect of the MALAE on the mycelial growth of *A. flavus* and *A. parasiticus* was assessed by measuring colony diameters and calculating the percentage inhibition using the extract concentrations ranging from 31.25 to 500 mg mL^{-1} (Figure 4). A concentration-dependent reduction in mycelial growth was observed for both fungal species. In *A. flavus*, the inhibition percentages increased was significantly ($p < 0.05$) from 39.74% at 31.25 mg mL^{-1} to 73.08% at 500 mg mL^{-1}. Similarly, *A. parasiticus* showed the inhibition values rising from 41.3% to 76.00% was significantly ($p < 0.05$) inhibited. Overall, the results demonstrated that *M. azedarach* leaves aqueous extract effectively inhibits the mycelial growth of both fungal species in a dose-dependent manner, with *A. parasiticus* showing slightly higher sensitivity to the extract compared to *A. flavus*.

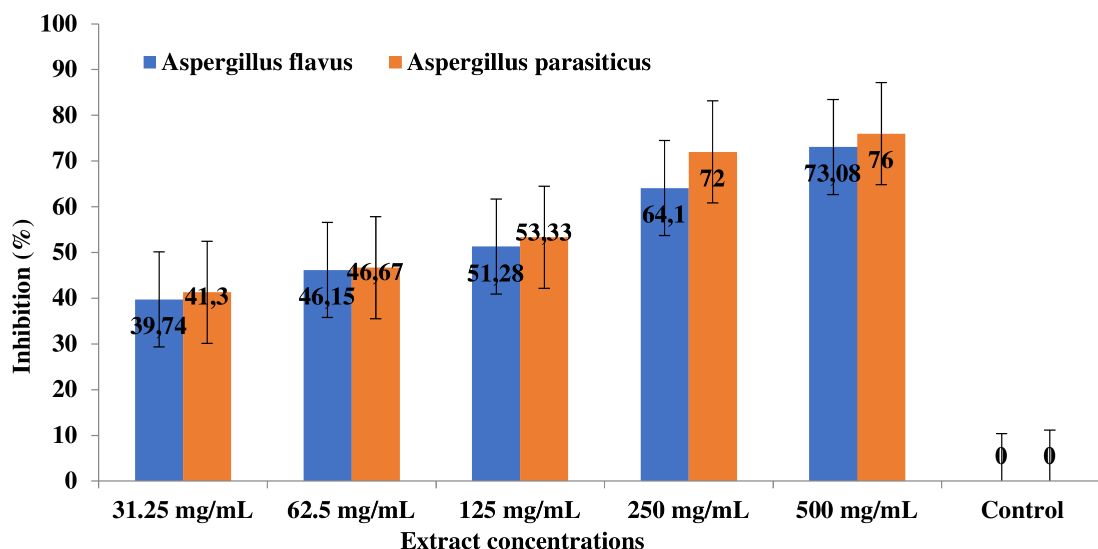

Figure 4. Toxicological effect of MALAE on the mycelial growth of fungi
Data is presented as mean ± SD

The effect of *M. azedarach* leaves aqueous extract on the spore germination of *A. flavus* and *A. parasiticus* was evaluated at various concentrations ranging from 31.25 to 500 mg mL^{-1}, utilizing distilled water as the control (Figure 5). A concentration-dependent inhibition of spore germination was observed in both fungal species. In the case of *A. flavus*, the spore germination percentage decreased progressively from 62% at 31.25 mg mL^{-1} to 17% with 500 mg mL^{-1}, corresponding to an increase of inhibition from 38% to 83%. Similarly, *A. parasiticus* exhibited reduced germination from 58% to 15% at same concentration gradient, with spore germination inhibition increasing from 42% to 85%. The highest antifungal activity was recorded at 500 mg mL^{-1} for both fungal species, showing 83% and 85% inhibition of spore germination for *A. flavus* and *A. parasiticus*, respectively significant ($p < 0.05$). In contrast, the control treatment exhibited 100% spore germination and no inhibition, confirming the effectiveness of the extract. These findings suggest that *M. azedarach* extract exhibited noteworthy antifungal activities, with increasing concentration of dosages resultant in improved inhibitory potential on fungal spore germination.

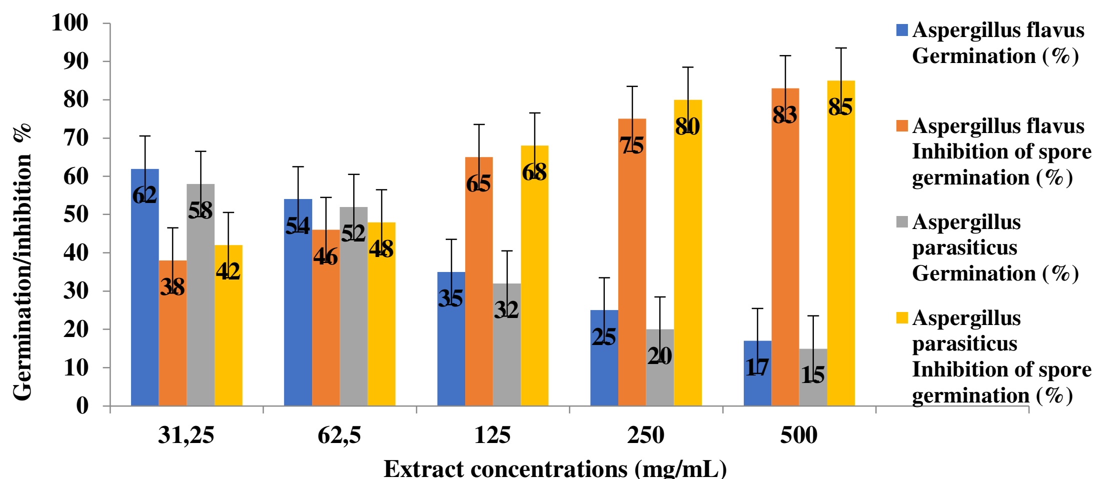

Figure 5. Effect of MALAE on spore germination and inhibition
Data is presented as mean ± SD

The effect of *M. azedarach* leaves aqueous extract on the biomass production of *A. flavus* and *A. parasiticus* was evaluated at various concentrations ranging from 31.25 to 500 mg mL^{-1} (Table 2).

Table 2. Effect of *M. azedarach* leaves aqueous extract on biomass of tested fungi.

Extract Concentrations	*Aspergillus flavus*		*Aspergillus parasiticus*	
	Biomass (mg)	I%	Biomass (mg)	I%
31.25	240 ± 0.2^{c}	41.46	210 ± 0.1^{d}	46.15
62.5	230 ± 0.4^{e}	43.90	200 ± 0.4^{f}	48.72
125	185 ± 0.2^{g}	54.88	160 ± 0.2^{h}	58.8
250	140 ± 0.3^{i}	65.85	110 ± 0.3^{j}	71.8
500	110 ± 0.5^{k}	73.2	90 ± 0.5^{l}	76.9
Control	410 ± 0.2^{a}	00	390 ± 0.2^{b}	00

Results represents a replicate of three (n=3) ± Standard Deviation (SD). Superscripts (a–l) designate significant differences ($p < 0.05$, Tukey's HSD test) across both fungi and all treatments. Means with different letters are significantly different; means sharing the same letter would not differ significantly

A consistent and concentration-dependent reduction in fungal biomass was observed for both species compared to the untreated control. In *A. flavus*, biomass decreased from 240 ± 0.2 mg at 31.25 mg mL^{-1} to 110 ± 0.5 mg at 500 mg mL^{-1}, with the corresponding inhibition percentages ranging from 41.46% to 73.2%. Similarly, *A. parasiticus* showed a biomass reduction from 210 ± 0.1 mg to 90 ± 0.5 mg over the same concentration range, with inhibition percentages increasing from 46.15% to 76.9%. The control group, which received no extract treatment, exhibited the highest biomass values: 410 ± 0.2 mg for *A. flavus* and 390 ± 0.2 mg for *A. parasiticus*, confirming the absence of growth inhibition under untreated conditions. One-way ANOVA indicated a significant effect of concentration on biomass for both fungi (A. flavus: $F (5,12) = 327024.19$, $p < 0.001$; A. parasiticus: $F (5,12) = 352271.19$, $p < 0.001$). Tukey's HSD post-hoc tests showed that each treatment concentration produced biomass values significantly dissimilar from control ($p < 0.05$). Independent (Welch) t-tests comparing the two species at each concentration are reported in the results table. Linear regression of % inhibition versus dosage displayed strong positive relationships for both species (A. flavus: $p = 0.0166$, slope = 0.0677, $R^2 = 0.8875$; A. parasiticus: $p = 0.0231$, slope = 0.0664, $R^2 = 0.8606$), steady with a dose–response. These results further support the antifungal efficacy of extract, indicating strong suppression of fungal biomass accumulation in a dose-responsive manner. Remarkably, *A. parasiticus* exhibited slightly greater sensitivity to the given extract as compared to *A. flavus*.

The impact of extract on a AFs production by *A. flavus* and *A. parasiticus* was examined at varying concentrations ranging from 31.25 to 500 mg mL^{-1} (Table 3).

Table 3. Effect of *M. azedarach* leaves aqueous extract on Aflatoxins production

Extracts Concentrations (mg mL^{-1})	*A. flavus*		*A. parasiticus*			
	Aflatoxins (µg mL^{-1})		Aflatoxins (µg mL^{-1})			
	B1	B2	B1	B2	G1	G2
500	90 ± 0.1^f	55 ± 0.1^f	80 ± 0.1^f	45 ± 0.5^f	20 ± 00^f	12 ± 0.1^f
250	100 ± 0.2^e	60 ± 0.3^e	100 ± 0.1^e	50 ± 0.1^e	25 ±0.1^e	15 ± 0.5^e
125	120 ± 0.4^d	70 ± 0.5^d	175 ± 0.5^d	80 ± 0.3^d	35 ± 0.3^d	27 ± 0.3^d
62.5	135 ± 0.6^c	110 ± 0.1^c	190 ± 0.2^c	100 ± 0.2^c	45 ± 0.2^c	40 ± 0.1^c
31.25	200 ± 0.3^b	150 ± 0.4^b	260 ± 0.1^b	120 ± 0.8^b	55 ± 0.4^b	45 ± 0.4^b
Control	370 ± 0.1^a	210 ± 0.2^a	355 ± 0.6^a	180 ± 0.5^a	75 ± 0.1^a	55 ± 0.2^a

Each value represents an average of triplicate (n=3) and ±Standard Deviation (SD). Superscripts (a–f) are Tukey HSD groupings for each toxin separately. Means with different letters are significantly different at p < 0.05. Statistical tests: one-way ANOVA across concentrations (per toxin), followed by Tukey's HSD. Grouping (superscripts) was derived from Tukey results and displayed as compact letters

The data revealed a marked concentration-dependent reduction was significantly (p < 0.05) in all six major aflatoxins (B_1, B_2, G_1, and G_2) produced by both fungal species. In *A. flavus*, aflatoxin B_1 levels decreased from 200 ± 0.3 µg mL^{-1} at 31.25 mg mL^{-1} to 90 ± 0.1 µg mL^{-1} at 500 mg mL^{-1}, while B_2 was reduced was significantly (p < 0.05) from 150 ± 0.4 µg mL^{-1} to 55 ± 0.1 µg mL^{-1}. The control sample showed the highest production of B_1 and B_2 at 370 ± 0.1 µg mL^{-1} and 210 ± 0.2 µg mL^{-1}, respectively. Similarly, *A. parasiticus* showed a significant decline in aflatoxin production with increasing extract concentration. Aflatoxin B_1 levels decreased from 260 ± 0.1 µg mL^{-1} at 31.25 mg mL^{-1} to 80 ± µg mL^{-1} at 500 mg mL^{-1}, while B_2 dropped from 120 ± 0.8 µg mL^{-1} to 45 ± 0.5 µg mL^{-1}. Additionally, aflatoxins G_1 and G_2 were reduced from 55 ± 0.4 µg mL^{-1} and 45 ± 0.4 µg mL^{-1} at the lowest concentration to 20 ± 0.0 µg mL^{-1} and 12 ± 0.1 µg mL^{-1}, respectively, at 500 mg mL^{-1}. The control group produced the highest levels of aflatoxins across all categories, with *A. parasiticus* showing 355 ± 0.6 µg mL^{-1} of B_1, 180 ± 0.5 µg mL^{-1} of B_2, 75 ± 0.1 µg mL^{-1} of G_1, and 55 ± 0.2 µg mL^{-1} of G_2. One-way ANOVA revealed a highly significant effect of *M. azedarach* leaves water extract on AFs synthesis by both *A. parasiticus* and *A. flavus* (p < 0.001). Tukey's HSD post-hoc comparisons showed that all tested dosages (31.25-500 mg mL^{-1}) significantly minimize AFs B1 and B2 levels in *A. flavus* relative to the untreated, with the highest inhibition showed at 500 mg mL^{-1}. Likewise, in *A. parasiticus*, the extract evidently reduced the synthesis of AFs B1, B2, G1, and G2 in a dose-dependent way, with the sturdiest destruction observed at the maximum dosage. Independent Welch's t-tests additional established significant variances between each treatment group and the control (p < 0.05), supporting the dose-dependent inhibitory activity of the extract. The results obtained clearly demonstrate that *M. azedarach* aqueous extract significantly suppresses aflatoxin biosynthesis in a dose-dependent manner.

The prevention outcome of *M. azedarach* leaves aqueous extract on aflatoxin synthesis through *A. flavus* and *A. parasiticus* was evaluated across a range of dosages. The inhibition percentage (%) of each aflatoxin type (B_1, B_2, G_1, G_2) was measured as described in Figure 6. A clear concentration-dependent increase in aflatoxin prevention was observed in both fungal species. At the maximum dosage (500 mg mL^{-1}), *A. flavus* showed highest prevention of 75.7% for B_1 and 73.81% for B_2, while *A. parasiticus* displayed 77.46% and 75% inhibition for B_1 and B_2, respectively. In addition, the G-group aflatoxins were also significantly suppressed, with *A. parasiticus* showing 73.3% and 78.2% inhibition for G_1 and G_2, respectively, at concentration of 500 mg mL^{-1}. The % inhibitions slowly turn down with decreasing dose concentration. For instance, at 125 mg mL^{-1}, *A. flavus* exhibited 67.57% and 66.67% inhibition for B_1 and B_2, respectively, while *A. parasiticus* showed 50.70% and 55.55% was significantly (p < 0.05) inhibited for the same aflatoxins. The lowest inhibition was

observed at 31.25 mg mL^{-1}, with values ranging between 18.18% and 45.94% across all aflatoxins tested. These findings reinforce the earlier results, highlighting the ability of *M. azedarach* aqueous extracts to significantly inhibit aflatoxin biosynthesis in both *A. flavus* and *A. parasiticus*, with stronger effects observed at higher concentrations.

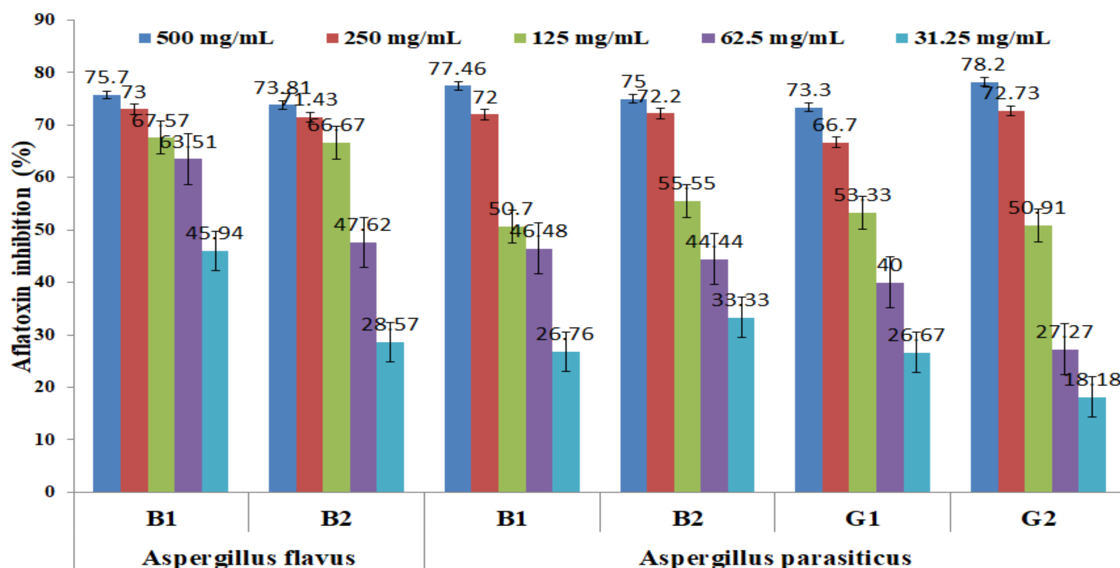

Figure 6. Effect of MALAE on Aflatoxins inhibition
Data is presented as mean ± SD

Discussion

Bioactive compounds composition, identification and quantification

A number of phenolic substances and other secondary metabolites with diverse composition are distributed in different plants species (Bonface *et al.*, 2020). The phenolic content values of *M. azedarach* identified and quantified in our study are different to those already reported in the literature studies. The reported TPC in the *M. azedarach* extract was 82.54 mg GAE/g DW (Munir *et al.*, 2012), is higher than those of the present study. In other literature findings, the extracts of *M. azedarach* TPC was 7.828 mg GAE/g DW (Bonface *et al.*, 2020), comparatively lower than those of the current study conducted. The secondary metabolites of *M. azedarach* disclosed the occurrence of glycosides, flavonoids, steroids, triterpens, triterpenoids, coumarin, phenols, cinnamic acid, benzoic acid, gum, tannins, saponins, resins and alkaloids (Ahmed *et al.*, 2008; Jaafar *et al.*, 2016). The flavonoids play its role in various functions of the plants and the main actions long been recommended to a compound as secondary anti-free radical defense system in tissues of plants while depiction to dissimilar stresses (Bonface *et al.*, 2020). The flavonoids contents (FC) vary extensively, as for TPC and the ranking order was significantly correlated ($p < 0.05$). The results of the current study were found different to those in the literature. According to published research work TFC 16.99 mg QE/g DW and 0.532 mg QE/g DW was accounted respectively in the *M. azedarach* extracts (Munir *et al.*, 2012; Bonface *et al.*, 2020). The differences in TPC and TFC might be outcomes of various factors which have been pointed out in the previous finding in the literature. Such dissimilarities in the TPC and TFC concentration might be accounted to the occurrence of secondary metabolites at contradictory concentrations and additional factors. These factors include genotypes, maturity of the plants, solvents polarities, methods of extractions, different seasons, phytogeographical areas differences and harvesting times could alter the

concentrations of TPC, TFC and other secondary metabolites of medicinal plants (Mpofu *et al*., 2006; Sreelatha and Padma, 2009; Charoensin, 2014; Vyas *et al*., 2015; Bonface *et al*., 2020).

FTIR Analysis

The bioactive components in the plants samples as functional groups are categorized accredited on the peak value (wave number) in the area of infrared radiation with transmittance percentage (%T) or absorbance applying FTIR spectrum. The leaves of *M. azedarach* aqueous extract observed a characteristic absorption peaks values (Figure 2) and verified the occurrence of alcohol, alkane, conjugated alkene, aromatic ring and lactone, aromatics compounds, alkyl aryl ether, ester, primary alcohol and aliphatic amines compounds. These compounds were found with medium and strong appearance in bending and stretching functional groups. In common, it is known that FTIR assay helps to discover the functional groups of active substances support on the peak wave number in infrared radiation area (Sownthariya and Shanthi, 2022). FTIR chromatogram of the leaves extract of *M. azedarach* showed the functional groups of the secondary metabolites as aliphatic amines, ester, ether compounds, aromatics molecules, aldehyde, alkanes and alcohol (Zishan *et al*., 2024). In many plants the alkanes are generally present in the epicuticular and cuticle wax (Sownthariya and Shanthi, 2022). FTIR is recognized as a useful tool for the identification and characterization of functional groups (chemical bonds) or compounds occurred in unidentified blend of plant extracts (Zishan *et al*., 2024). The leaves acetone extracts of *M. azedarach* observed a distinctive absorption band and verified the occurrence of halo compounds, primary alcohol, ether, alkyl aryl, conjugated alkene group, aldehyde, alkane and alcohol with medium and powerful peak in bending and stretching chemical bonds (Sownthariya and Shanthi, 2022).

GC-MS analysis

Despite the modernization of characterization techniques, FTIR and GC-MS remain as extensively used methods in the determination and identification of phytochemicals in plant samples. The GC-MS is verified technique for quantifying and identifying bioactive compounds in plants extracts. The quantitative secondary metabolites assay of *M. azedarch* extract found the occurrence of various bioactive compounds belonging to diverse organic groups. Saturated and unsaturated fatty acids were the major plentiful hydrocarbon substances make out in this extract. Various species of plants applied fatty acids as protective system against pathogenic microbes. The primary goal of fatty acid is to intermingle with the electron transport chain. Moreover, they inhibit enzyme function and reduce nutrition absorption (Desbois and Smith, 2010).

The 1-Butanol, 3-methyl-acetate is an alcoholic based molecule present in the *Aegle marmelos* leaves reported for antimicrobial properties (Mujeeb *et al*., 2014). The methanol maceration extracts of *Christia vespertilionis* leaves found 24.321% 1-Butanol, 3-methyl-, acetate and displayed antimicrobial characteristics (Izzah *et al*., 2021). Our study shows the presence of this compound in the concentration of 11.53% in MALAE and examines its antifungal properties as well anti-aflatoxins inhibition potential.

The methanolic extract of *Barleria cristata* GC-MS profiling identified and quantified numerous substances along with (R, S)-2-Propyl-5-oxohexanal. The occurrence of a number of bioactive secondary metabolites in the leaves of *B. cristata* recognized their utilization by conventional practitioners for a broad spectrum of diseases. According to literature published reports, identification of a precise bioactive compounds and utilizing them to biological system will create a plenty of outcomes (Harini *et al*., 2021).

The coumaran was isolated from *Lantana camara* leaves and found to be strong biofumigant, which could be utilized as biopesticide (Rajashekar *et al*., 2014). The current study shows that the Coumaran compound found in the *M. azedarach* leaves extract was 10.04% and the current results authenticate the antifungal and anti-aflatoxigenic activities.

The essential oil of *Coriandrum sativum* L. leaves have possessed numerous bioactive molecules including 2-Decen-1-ol (17.01%) recognized to possess antioxidant, antifungal and antibacterial properties

(Gurning *et al*., 2020). In current investigations this compound was found 2.43% in *M. azedarach* leaves extract, which showed antifungal and anti-aflatoxin activities.

The cyclohexanone and cyclohexane are utilized as co-solvent and solvent in diverse pesticide products, comprising agriculture crop, turtf, garden, yard and outdoor products (US EPA, 2015). This compound was also found to be 1.13% in the *M. azedarach* leaves extract in our study. The developments of fungicides from plants extracts have shown potential as sustainable and environment friendly alternative compared to synthetic fungicides. The compounds found in the *M. azedarach* leaves extract revealed the antifungal, pesticidal and as a solvent property.

The most important biological activities of myristic acid (Tetradecanoic acid) were reported to be immune-modulating, anti-parasite, anti-viral, anti-cancer and anti-fungal (Javid *et al*., 2024). The current study shows that Tetradecanoic acid was found in the leaves crude extract of *M. azedarach*, which verified its antifungal and aflatoxins inhibition potential.

Limited literature reports are available about the antimicrobial activities of 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z) and hexadecanoic acid, methyl ester, so outlying its occurrence has been reported in several therapeutic plants comprising M. *azedarach* species (Gopalakrishnan and Udayakumar 2014; Jahirhussain *et al*. 2015). The existence of hexanedioic acid, dimethyl ester (Omoruyi *et al*., 2014), 1, 4-benzenedicarboxylic acid (Ezhilan and Neelamegam, 2012) and 2-Heptanol (Swamy *et al*., 2015) has been reported in diverse plants with antimicrobial activities. As the present study disclose the occurrence of these compounds in the *M. azedarach* leaves extract and further elaborate the antifungal activities against *A. flavus* and *A. parasiticus* as well as inhibition of aflatoxins.

The *M. azedarach* leaves methanol extract showed the potential antifungal activities. The extract was further analyzed by GC-MS assay. The screen out compounds were 2- heptanol (0.6%); hexanedioic acid, dimethyl ester (1.3%); hexanedioic acid, dimethyl ester (2.3%); 7-methyl-Z-tetradecen-1-ol acetate (2.5%); vitamin E (2.7%); cyclopropaneoctanoic acid, 2-[2-[(ethylcyclopropyl) methyl] cyclopropyl] methyl-, methyl ester (2.8%); hexadecanoic acid, methyl ester (12.3%); 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-(34.8%) and phytol (36%) (Sana *et al*., 2017).

Pentadecanoic acid (C15:0) is a vital un-even-chain fatty acids with wide range of bioactivities to protect liver, immune and cadiometabolic health. Additionally, C15:0 has antimicrobial activities, such as it inhibits the growth of pathogenic fungi and bacteria. Conclusively, these wide spectrum activities of C15:0 verify its function as vital ingredients in nutrition to assist physiological health for long-term (Venn *et al*., 2023). The compound of pentadecanoic acid14-methyl, methyl ester displayed antimicrobial and antifungal activities in literature study (Akpuaka *et al*., 2013). This compound was also identified in a concentration of 2.69% in the *M. azedarach* leaves in current research work confirms its role as antifungal and AFs inhibition. In another literature findings; it has been reported that methanol extract of *M. azedarach* leaves *contain* total phenolic content, and *total* flavonoid contents with potential bioactivities (Mwamatope *et al*., 2020)

The main abundant secondary metabolite screen out in the GC-MS analysis is saturated fatty acid n-hexadecanoic acid which has antifungal and antioxidant activities against *Alternaria alternata* (Youssef *et al*., 2021; Cho *et al.,* 2010). The *n*-hexadecanoic acid possessed substantial antimicrobial activities against *K. pneumoniae, E. coli, B. subtilis* and *S. aureus* at maximum concentration 50 µg/mL having 11.93, 11.10, 10.96 and 11.93mm zones of inhibition respectively (Ganesan *et al*., 2024). The occurrence of n-hexadecanoic acid in *Rosa indica, Labisia pumila, Cyrtocarpa procera, Kielmeyera coriaceam Allium nigrum, Carissa congesta* and *Benincasa hispida* has been reported for antimicrobial activities (Mickymaray *et al*., 2016). The main target of n-hexadecanoic acid activity is the microbial cell membrane. Additionally, it slows down enzyme production, reduce cellular energy production and finally leads to destroy the microbial cells. Because of its elevated degree of effectiveness and safety, this antimicrobial compound is strongly recommended (Shaaban *et al*., 2021). The essential oil and organic extracts of *Allium roseum* exhibited antifungal activities against *Fusarium oxysporum,*

Rhizoctonia solani, Pythium ultimum, Alternaria solani, Botrytis cinerea and *Fusarium solani*. The antifungal activities might be accredited due to the presence of of hexadecanoic acid, recognized for its potent antifungal activities (Rouis-Soussi *et al*., 2014). The current study reported 2.69% Hexadecanoic acid in the *M. azedarach* leaves extract and confirm the antifungal activities against tested aflatoxigenic fungi. In another study *M. azedarach* fruit extracts by GC-MS screening showed a mixture of six (06) compounds of fatty acids namely octadecanoic acetate, octadecadiena, ethyl linoleate, tetradecanoic acid ethyl ester, pentadecanoic acid and cyclodecane (Abbas *et al*., 2017). Our current investigation explains the amount of these compounds along with other related ingredients in *M. azedirach* leaves as 2.69% (Pentadecanoic acid) and 0.70% (Tetradecanoic acid) which showed potential antifungal activities.

It has been recognized that n-Hexadecanoic (palmitic) acid ($C_6H_{32}O_2$) was the main plentiful substance with diverse biological properties i.e. anesthetic, cytoprotective, antisecretory, anti-inflammatory, fibrinolytic, antimutagenic, antihypoxic, sclerosing, antiseborrheic, antieczematous and antimicrobial (Karthikeyan *et al*., 2019; Hrichi *et al*., 2022). The current study reports the n-Hexadecanoic in the MALAE with the antifungal potential which is clear from the obtained results. The GC-MS analysis of *Gossypium* seed powder showed 9, 12-octadecadienoic acid as an abundant phytochemical substance and the ethanolic extract of this plant exhibited antibacterial and fungicidal activities especially against *A. flavus* and *Aspergillus niger*. The antimicrobial activities of *Gossypium* seed powder could be accredited to the occurrence of bioactive compounds in it (Krishnaveni *et al*., 2014). This study observed the presence of 9, 12-Octadecadienoic acid with a concentration of 1.06% in Persian lilac leaves aqueous extract and support the antifungal and anti-aflatoxigenic activities.

The unsaturated fatty acid methyl ester (10, Octadecenoic acid and methyl ester) have the abilities to enhance the immunity, decrease blood cholesterol, possess antioxidant, antifungal and antibacterial activities (Asghar and Choudahry, 2011). The fatty acid methyl ester (Octadecanoic acid methyl ester) has reported antimicrobial activities (Belakhdar *et al*., 2015). The current study regarding GC-MS profiling of the *M. azedarach* leaves extract support the occurrence of 10, Octadecenoic acid, methyl ester (5.16%) and octadecanoic acid, methyl ester (1.74%) and corroborates exhibited antifungal activities against the *Aspergillus flavus* and *A. parasiticus* as well as its aflatoxins inhibition.

Various fatty acids have been revealed to stimulate or inhibit the sporulation and growth of plants pathogenic fungi (Aly *et al*., 2011). Furthermore, Liu *et al*., (2008) stated that the hexadecanoic acid (saturated fatty acid) exhibited powerful antifungal activities as compared to unsaturated ones such as oleic acid. It is recommended that the hexadecanoic acid (fatty acid) may be a substitute technique against phytopathogens to control its growth and minimize the compensation (Liu *et al*., 2008).

Antifungal activities

The antifungal and fungistatic activities of *M. azedarch* flower, leaves, fruit seed, seed kernels were recognized in the previous literature findings (Carpinella *et al.,* 2003; Jabeen *et al*., 2008; Antara and Amla, 2012; Javaid and Rehman, 2011; Neycee *et al*., 2012; Sen and Batra, 2012) against a wide range of plant pathogenic fungi such as *Rhizoctonia solani, Fusarium oxysporum*, Geotrichum spp., Sclerotium spp., Trichoderma spp., *Sclerotinia sclerotiorum*, *Fusarium verticillioides*, *Fusarium solani*, *Diaporthe phaseolorum* and *A. flavus, A. niger, R. stolonifer, Macrophomina phaseolina*, *Diaporthe phaseolorum* and *Ascochyta rabiei* and Sclerotium *rolfsii*. According to reported literature study the antimicrobial activities of *M. azedarach* extracts are due to the occurrence of phenolic and flavonoids substances previously characterized by researchers (Akacha *et al*., 2016a; Akacha *et al*., 2016b).

Regarding the previous literature work *A. parasiticus* showed susceptibility to all phenolic acids when exposed for 10 days (Loran *et al*., 2022). The phenolic acid could destroy the porosity of the fungi membrane

through intermingle with lipid bilayers, results in distorted homeostasis in the course of seepage of intracellular ingredients and growth retardation (Sung and Lee 2010). The fungistatic activities of *M. azedarch* might be accredited due to various antifungal compounds such as pinoresinol, 4-hydroxy-3- methoxycinnamaldehyde, vanillin and hydroxycoumarin scopoletin (Carpinella *et al*., 2003; Carpinella and Ferrayoli, 2005). Five different bioactive compounds have been isolated from *M. azedarach* leaves such as 3-5 dimethoxy benzoic acid, benzoic acid, ursolic acid, β- amyrin and β-sitosterol, which showed fungistatic activities against *Ascochyta rabiei* (Jabeen *et al*., 2011). The phenolic compounds could interact with the membrane of fungi, which might in turn have impact on development of fungi leading to minimize the dry weight of mycelial. The phenolic acid in the external layers of grains, have been accounted fungal development and mycotoxin synthesis in *Aspergillus* species. Though, great variations in the comeback of poisonous molds to phenolic substances are displayed in the previous findings regarding mycotoxins synthesis by fungal species (Loran *et al*., 2022).

Anti-aflatoxins potential of M. azedarach extract

The application of plants aqueous extracts is a smart choice compared with essential oil which required particular instrument to be isolated. In short words, the utilization of herbal extracts has been magnetizing higher consideration in the particular society due to its potentially low cost, renewable, biologically safe, ecofriendly and biodegradable nature (Ponzilacqua *et al*., 2018). Polyphenols like terpenes, tannins, flavonoids and phenols are active compounds that control fungi growth and AFs synthesis. Furthermore, certain active compounds with anti-oxidant properties might hamper AFB1 synthesis by regulating the oxidative stress around the fungi (Cadenillas *et al*., 2024).

Up to date, various phenolic compounds that could minimize the AFs biosynthesis in *A. flavus* have been discovered. Curcumin control AFs production through stir up oxidative burst in the cells of fungi, whereas flavonoids with -OCH_3 or -OH functional groups at point 6 in ring A and site 4′ of ring B restrain AFs synthesis by *A. flavus* (Tian *et al*., 2023). A sturdy association between antioxidant activity, polyphenols content and aflatoxin B1 inhibition has been reported in literature (Cadenillas *et al*., 2023a). These findings are in close agreement with various literature reported studies representing that botanical extracts with noteworthy anti-radicals activity, like *Curcuma longa* (IC_{50DDPH}: 74 mg mL^{-1}) (Behiry *et al*., 2022), *Illicium verum* (IC_{50DPPH}: 5 mg mL^{-1}) (*Aly et al*., 2016) and *Uncaria tomentosa* (IC_{50DPPH}: 13 mg mL^{-1}) (Cadenillas *et al*., 2023b), could possess potential aflatoxin-B1 inhibition activity. This potent bioactivity might be connected to the existence of anti-radical compounds in the extracts like condense tannins. Actually, the research carried out displayed that condensed tannins in the aqueous extracts of *Mimosa tenuiflora* were potent inhibitor of the AFB1 (Hernandez *et al*., 2021). Extract with minimum quantity of tannins (10mg/g DM), along with other other polyphenols compounds are accountable for inhibition of AFB1 (Cadenillas *et al*., 2023a). According to this hypothesis illustration, it was demonstrated that the effectiveness of polyphenols derived from tea on inhibition of AFB1, documented that flavonoid (quercetin) and gallic acid (hydrolysable tannins) were strongly efficient to inhibit the AFs synthesis (Zhou *et al*., 2015). The *Trachyspermum ammi* (Ajowan) seed extracts exhibited up to 65% degradation of AFB1 (Velazhahan *et al*., 2010). The mechanism involved in the antimycotoxin and antifungal activities of polyphenols compounds, comprised alteration in fungi. This alteration lead to membrane disturbing functioning and permeability, lessening oxidative stress and oxidases inhibition, as well as down-regulation of the translation of vital genes associated in the production of mycotoxins (Ahmed *et al*., 2022).

Naturally occurring flavonoids could repress AFs biosynthesis; but, the shape-activity correlation stays uncertain. Thirty-six (36) naturally occurring flavonoids were examined against *A. flavus*, both in in-situ (on maize kernels) and in-vitro, to evaluate their shape-activity correlation and biological activities. Production of AFs (IC values: 10.85-20.09 μg mL^{-1}), and the translation of associated genes i.e. (*aflR, aflQ, aflK* and *aflD*) were reported to be powerfully prevented (Tian *et al*., 2023). The condensed tannins prevented AFB1

biosynthesis is due to a down-regulation of the interior regulators (*aflS* and *aflR*) of aflatoxin B1 genes cluster (Cadenillas *et al*., 2024).

Conclusions

Herein, the aqueous extract of *M. azedarach* was investigated for its phytochemical's composition along with potential antifungal activities against *A. flavus* and *A. parasiticus*. The extracts by virtue of its rich phytochemical composition played a key role in AFs biosynthesis inhibition by inhibiting fungal growth which would open a door to the valorization of certain agricultural by-products rich in these bioactive compounds. The findings suggest that *M. azedarach* extracts could be effectively used for the inhibition of *A. flavus* and *A. parasiticus*; the major aflatoxins producing fungal strains. In other words, study confirms the potential of *M. azedarach* leaf extract as a natural fungicide and aflatoxin inhibitor to be used in post-harvest protection of agricultural commodities. However, these findings need validation, first to find the responsible antifungal compounds in the selected plant extract, then their isolation in pure form and the underlying precise antifungal mechanism(s).

Authors' Contributions

Conceptualization: MZ and JA; Data curation: AS and MS; Formal analysis: MI and NG; Funding acquisition: MZ, RU and AA; Investigation: AS, AA, RU; Methodology: JA; Project administration: JA; Resources: JA; Software: AS; Supervision: JA; Validation: MI and NG; Visualization: RU, AA and NG; Writing - original draft JA, AS, MS, MI and MZ; Writing - review and editing: JA, MI, MA and MZ.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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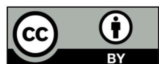

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