

PHYTOCHEMICALS EVALUATION AND ANTIOXIDANT POTENTIAL OF *CYMOPOGON CITRATUS* EXTRACTS AND ITS INHIBITORY EFFECTS ON ASPERGILLUS SPECIES BIOMASS AND AFLATOXIN PRODUCTION

¹Javid Ali, ²Farida Munir, ³Aisha Ibrahim, ⁴Akhtar Aman, ⁵Hamid Hussain Afridi, ⁶Aroosa Zafar, ⁷Sayyad Ali, ⁸Imran Tariq

¹ Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar, Khyber Pakhtunkhwa.

²M.Islam College of Pharmacy Gujranwala, Punjab.

³M.Islam College of Pharmacy Gujranwala, Punjab.

⁴Shaheed Benazir Bhutto University Sheringal, Dir upper, KPK.

⁵Shaheed Benazir Bhutto University Sheringal, Dir upper, KPK.

⁶Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad.

⁶Cadson College of Pharmacy, Kharian, Punjab.

⁷Cadson College of Pharmacy, Kharian, Punjab.

⁸Punjab University College of Pharmacy, Allama Iqbal Campus, University of the Punjab.

¹javedali_14@yahoo.com, ²frdmnr331@gmail.com, ³aishaibrahim447@gmail.com, ⁴dramanrph@sbbu.edu.pk,

⁵hamid@sbbu.edu.pk, ⁶Arzafar@bs.qau.edu.pk, ⁷sayad.ali6@gmail.com, ⁸imran.pharmacy@pu.edu.pk

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Corresponding Author:

Aroosa Zafar*

Arzafar@bs.qau.edu.pk

Abstract

Lemongrass (*Cymbopogon citratus*) is a medicinal herb that could be a foundation of natural compounds with anti-radical, antifungal and anti-aflatoxigenic possessions. In this work, methanolic, ethanolic and aqueous lemongrass extracts were examined for their phytochemical content, antioxidant capacity, antifungal effect and ability to suppress aflatoxin formation. Preliminary qualitative screening detected alkaloids, saponins, tannins, flavonoids and glycosides, and the methanolic extract gave relatively stronger reactions for several of these groups. Antioxidant property was quantified with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique. At 500 µg/mL, the methanolic, ethanolic and aqueous extracts scavenged 80%, 75% and 70% of the radicals, respectively, compared with 98% for vitamin C. In the antifungal tests, biomass of *Aspergillus parasiticus* and *Aspergillus flavus* was suppressed in a concentration-dependent way. At 400 mg/mL, the extract of methanol was decreased growth by 77% and 74% in the two fungi, respectively, while the ethanolic extract gave 73% and 67% and the aqueous extract 68% and 64%. Suppression of aflatoxin production likewise rose with extract concentration. The strongest effect was seen with the methanolic extract against AFG₂ from *A. parasiticus* (87% at 400 mg/mL), followed by the ethanolic (83%) and aqueous (75%) extracts. Taken together, the results show that lemongrass extracts, especially the methanolic one, hold promise as antioxidant, antifungal and anti-aflatoxigenic agents.

Introduction

Aflatoxins (AFs) are highly carcinogenic to both animals and humans and cause liver cancer. Thus, toxigenic fungi and the mycotoxins they produce in foods and grain, that are stored long-term, are a true threat to humans and animals. *A. flavus* and *A. parasiticus* toxigenic strains were selected in this work due to their capability to produce high level of toxins and are known to colonize wheat seeds and other food commodities making them unfit for consumption (Al-Rahmah *et al.*, 2019).

Much is now being done to reduce the accumulation of AFs in stored food (Bankole and Joda, 2004) as it poses a risk to livestock and humans as well. The harm that chemical pesticides do to the environment and to human health is a pressing issue, and new fungicides that perform well and are environmentally friendly are needed. Hence the extracts of some ethnomedicinally important higher plants were screened for antifungal activity. These products from plants are not harmful to human health and can be degraded in the environment, which is beneficial (Al-Rahmah *et al.*, 2019). Previous research has also revealed plants as being antifungals that can be essential in controlling plant diseases, and that plants can be obtained locally, biodegradable, eco-friendly and cheap (Ahmad *et al.*, 2016).

Cymbopogon citratus (Lemongrass) belongs to the family Poaceae and is a popular herb for scented and medicinal uses. It is found in tropical and subtropical areas, especially in Southeast Asian nations like Thailand and India, and thrives in warm climates, being mainly grown in nations including India, Thailand and Brazil (Dwivedi, 2024). In traditional medicine, such as Ayurveda and traditional Chinese medicine, it has been recognized as a valuable remedy for digestive disorders, fever, and inflammation for a very long time (Dwivedi 2024). The plant is rich in carbohydrates, proteins, vitamins and minerals and has a small amount of fat (Muala *et al.*, 2021). The phytochemicals it contains like flavonoids, phenolic acids and terpenoids are gaining significant attention in contemporary medicine, and its integration into nutraceutical, cosmetics

and pharmaceutical applications are becoming more common (Dwivedi, 2024).

Phytochemicals are natural antioxidants with great interest as a replacement of the use of synthetic antioxidants in food and pharmaceutical products. The primary objective of the current work was to examine the phytochemical profile and anti-radical, antifungal and anti-aflatoxigenic properties of methanol, ethanol and water extracts of lemongrass.

Materials and Methods

Plant Collection

Lemongrass Leaves were procured from the Experimental Research Farm of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan and the botanical authentication was done by botanists of the Plant Taxonomy Section of PCSIR-Peshawar. The leaves at the Microbiology Laboratory were damaged and discarded and the remaining leaves were washed with 70% ethanol and distilled water to prevent contamination. The leaves were then dried in the shade at 25–29 °C for 5 days, till they become brittle. The dry leaves were grated in a Wiley Mill grinder (USA), Standard Model No. 3 with a sieve of 0.5 mm. The powder was stored in a clean airtight container and stored at 4 °C in a cooled incubator (Gallenkamp-England).

Plant extraction process

Extractions were performed in the Microbiology Research Section of PCSIR Laboratories Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan under the aseptic condition in a laminar air-flow chamber Lahore, Pakistan. Five hundred grams of powdered sample was weighed and transferred to four conical flasks and then the flasks were first sterilized by autoclaving for fifteen minutes at 121 °C. Separate samples of the product were dispensed into methanol and ethanol (200 mL each) and shaken on a BioLAB orbital shaker at 50 rpm for 10 min before standing for 72 h with occasional shaking for good mixing. The blend was sieved by Whatman No# 1 filter paper (England) and the liquid lipid fraction was removed. This was repeated four times using fresh solvent to obtain the highest possible yield and lowest marc. The pooled fractions from each solvent were dried by

evaporating in the emptiness Rotary Evaporator (Büchi Rotavapor R-200 with B-490 heating bath, Switzerland) after the final extraction. Methanolic and ethanolic fractions were pooled after the final extraction and dried by evaporating in the vacuum Rotary Evaporator (Büchi Rotavapor R-200 with B-490 heating bath, Switzerland). The dried extracts were kept in the dark bottles at 4 °C in the Gallenkamp (England) cooled incubator.

Aqueous Extraction (Decoction method) of Lemongrass Powder

Lemongrass powder was extracted using a powder to water ratio of 2–5 g/100 mL, temperature of extraction temperature was 85–95 °C and duration of extraction was five to ten minutes. Briefly, the Water Bath (Julabo, Germany) was heated to the required heat degree in the experimental plan, and a flask (conical) holding a determined quantity of H₂O (distilled) was put on Water Bath till the desired heat was achieved. The powder of *Cymbopogon citratus* was afterward poured into the conical flask and it was mixed at 150 rpm. At the end of extraction, the mixtures were instantly placed in snow H₂O to cool down to 28–30 °C and the mixture was filtered through Whatman No# 1 filter paper. This was done three times to enhance the recovery of water soluble constituents. The filtrates were combined and concentrated in vacuum Rotary Evaporator under condensed pressure and after drying the crude aqueous extract was kept stored in an airtight container at 4 °C until further experiments.

Phytochemicals Screening (Qualitative)

The lemongrass extracts were evaluated for phytochemicals screening using methods as described by Harborne (1987).

The antioxidant activity of lemongrass extracts (DPPH assay)

All the extracts, as well as the standard were prepared at concentrations of 50, 100, 200, 300, 400 and 500 µg/mL in 95% methanol. Vitamin C was used as standard and each solution was diluted with 1 mL of a 0.004% DPPH solution in 95% methanol. A blank was made using DPPH and 95% MeOH in a ratio of 1:95 mL. The mixtures were incubated in dark for 20 minutes then absorbance at 517 nm was measured applying UV-

1700, Shimadzu, Japan-UV-visible spectrophotometer. The percentage scavenging inhibition (I %) was measured with the using bellow formula.

$$\text{DPPH Radical Scavenging Activity \%} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A₀ = Control optical density, A₁ = Sample optical density

Sources of test fungi and preparation of conidia suspension

Test fungi *Aspergillus flavus* (A. *flavus*) and *Aspergillus parasiticus* (A. *parasiticus*) were procured from Microbiology Laboratory of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan. Pure culture was carried out on Potato Dextrose Agar (PDA) media with 60 µg/mL of chloramphenicol. Conidia suspensions were obtained by flooding the surface of the 7–10-day-old plate cultures with 10 mL of Tween 80 (0.05%) in autoclaved H₂O (distilled) and softly scraping the surface with a glass rod (bent) to release the conidia. To remove mycelial fragments the suspension was filtered through two layers of cheesecloth and the spores were precipitated for three minutes at 2000 RPM by centrifugation and re-suspended in Tween-80 (0.05%) in germ-free H₂O (distilled). Separately mould was diluted and the quantity of conidia was calculated in a Haemocytometer (Counting Chamber, Art#1280; Marienfeld, Germany), so as to achieve approximately 10⁶ conidia/mL. No.).

Antifungal Activities Determination of Lemongrass Extracts

The suppression activity of the lemongrass extracts was evaluated by adding 20 mL of Potato Dextrose Broth (PDB) to Erlenmeyer flasks (50-mL) containing the extracts at concentrations of 400, 300, 200, 100, 50 and 25 mg/mL and inoculated with a spore suspension of 1.0 × 10⁶ spores/mL. The flasks (Erlenmeyer) were shaken in a shaking incubator (rotational radius 26 mm) at 29 °C and 180 rpm. After five days, the mycelia were sieved, dehydrated at 70 °C to obtained persistent mass and weighed. All the steps were carried out in sterile environment using Laminar Air Flow Cabinet (Lahore-Pakistan), using sterile water as

the control. The culture filtrate was kept for AFs determination.

Samples Preparation for the AFs Determination.

Extraction: One-half of the test sample was blended in a Grinding Mill (Romer). The crushed material was well blend and the sample part was obtained from the ground material. Fifty grams (50g) of the ground sample was transferred to a conical flask (500 mL) containing H₂O (25mL), 25g of diatomaceous earth and 150mL of CHCl₃. The mixture was shaken for 30 min and then filtered through filter paper. Another 50 mL of CHCl₃ was picked and dried on a steam bath (Alim-un-Nisaa *et al.*, 2012).

Qualitative determination: Aliquots of 5, 10 and 15 µL of the sample were dotted immediately on a TLC plate 1.5 cm from the bottom edge, and 5 µL of the references were spotted twice as an internal standard. The plate was first flooded and dried in a half of a tank containing anhydrous ether. It was then developed again in the same direction with acetone-chloroform (1:9, v/v) (as necessary to change the R_f of the AFs). Finally, the spots of the test solution were inspected for the presence or absence of AFs (Alim-un-Nisaa *et al.*, 2012).

For quantifiable assay, 5, 4, 3, 2 and 1 µL were spotted on the silica gel-coated plates, and 5, 4, 3, 2 and 1 µL of the AFs reference were dotted beside each test solution on the same silica gel-coated plates. The fluorescence concentrations of the marks were matched and concentration of AFs were measured according to the bellow formula (Alim-un-Nisaa *et al.*, 2012);

$$\text{AFs } (\mu\text{g/kg}) = S \times Y \times V/W \times Z$$

Where:

Z = Sample extract volume in µL of obligatory to provide fluorescence strength match to that of

S = µL of AFs reference

S = AFs standard volume in µL of corresponding intensity to Z (µL of sample)

Y = AFs standards quantities in µg/mL

V = Solvents (Volume in µL) required to dilute final sample

W = original sample weight (g) confined in final extract

The AFs inhibition % was measured as the reduction in AFs in a treated sample relative to the untreated control, applying bellow formula.

$$\text{Inhibition } (\%) = C-T/C \times 100$$

Where: C = AFs concentration in the untreated control and T = AFs concentration in the treated sample.

Statistical Analysis

Data from triplicate experiments are expressed as mean ± standard deviation and were analyzed with SPSS Statistics 24.0 (SPSS, Inc., Chicago, IL, USA).

Results and Discussion

Results

The phytochemical screening of the extracts from the leaves of the *Cymbopogon citratus* exposed the occurrence of glycosides, flavonoids, tannins, saponins and alkaloids in all the three extracts: methanolic, ethanolic and aqueous (Table 1). The methanolic extract showed the highest level (+++) of glycosides and flavonoids while the extract of ethanol observed moderate level (++). The flavonoids were found in low concentration (+) and glycosides in moderate concentration (++) in the aqueous extract. Methanolic extract was moderate (++) while ethanolic and aqueous extracts were low (+) to alkaloids. The methanolic and ethanolic extracts contain moderate amount of (+ +) tannins and low amount of (+) saponins, while the aqueous extract contains low amount of (+) saponins and (+) tannins. In general, the methanolic extract displayed the maximum qualitative reactions among all the extracts, particularly the flavonoids and glycosides.

Table 1. Screening of Phytochemicals in Lemongrass Leaves Extracts.

Phytochemicals	Methanol	Ethanol	Aqueous
Alkaloids	++	+	+
Saponins	+	+	+
Tannins	++	++	+
Flavonoids	+++	++	+

Glycosides	+++	++	++
+ Symbol observes screening degree of the bioactive compounds occurred in extracts. + + + = large quantity, + + = average quantity and + = small quantity.			
The DPPH radical-inhibition activity of the <i>Cymbopogon citratus</i> leaves extracts increased with the extract dose concentration increases (Table 2). At 500 µg/mL the methanolic extract was the most active ($80 \pm 0.5\%$), ahead of the ethanolic ($75 \pm 0.5\%$) and aqueous ($70 \pm 0.5\%$) extracts. At 50 µg/mL, activity fell to $25 \pm 0.3\%$, $20 \pm 0.1\%$ and $10 \pm 0\%$, respectively. Vitamin C was the most active at all concentrations and had a maximum		activity value of $98 \pm 0.5\%$ at 500 µg/mL. The extract of methanol observed the highest antioxidant properties in all the cases, suggesting a relatively high potency. Significant difference in concentrations within a column ($p < 0.05$) is indicated by different superscript letters. To conclude, the methanolic extract was found to possess the maximum antioxidant activity among the extracts tested but still it was lower than the activity of vitamin C.	

Table 2. Lemongrass Leaves Extracts Antioxidant Activity (% Scavenging of DPPH).

Dose (µg/mL)	Extracts			Vitamin C
	Methanol	Ethanol	Aqueous	
500	80 ± 0.5^a	75 ± 0.5^a	70 ± 0.5^a	98 ± 0.5^a
400	60 ± 0.3^b	55 ± 0.1^b	50 ± 0.1^b	95 ± 0.1^b
300	50 ± 0.1^c	45 ± 0.2^c	40 ± 0.5^c	90 ± 0.4^c
200	42 ± 0.5^d	36 ± 0.5^d	30 ± 0.3^d	85 ± 0.2^d
100	31 ± 0.1^e	25 ± 0.3^e	20 ± 0.1^e	80 ± 0.5^e
50	25 ± 0.3^f	20 ± 0.1^f	10 ± 0^f	65 ± 0.1^f

Results are presented in a mean of triplicates $\pm$ standard deviation. Mean results with a dissimilar superscript letter in the similar column are significantly dissimilar at $p < 0.05$.

The methanolic extract of *Cymbopogon citratus* showed concentration-dependent antifungal activity against *A. parasiticus* and *A. flavus* (Table 3). When fungal biomass of *A. parasiticus* and *A. flavus* was compared at maximum dose (400 mg/mL) biomass for *A. flavus* reduced to 130 ± 0.5 mg (77% inhibition) and *A. parasiticus* biomass of condensed to 140 ± 0.1 mg (74% inhibition). At 25 mg/mL, inhibition fell to 30% and 24%, and biomass rose

to 390 ± 0.5 mg and 400 ± 0.2 mg. The mycelium mass production in untreated controls was highest: 560 ± 0.5 mg in case of *A. flavus* and 530 ± 0.1 mg for *A. parasiticus*. In general, the methanolic extract was more effective at higher concentrations and *A. flavus* was usually more susceptible as compared with *A. parasiticus*.

Table 3. Antifungal Activity of Lemongrass Methanol Extract.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>	
	Biomass (mg)	Inhibition (%)	Biomass (mg)	Inhibition (%)
400	130 ± 0.5	77	140 ± 0.1	74
300	150 ± 0.1	73	180 ± 0.2	66
200	210 ± 0.4	62	200 ± 0.5	62
100	280 ± 0.2	50	250 ± 0.6	53
50	320 ± 0.3	43	300 ± 0.7	43
25	390 ± 0.5	30	400 ± 0.2	24
Control	560 ± 0.5	00	530 ± 0.1	00

Mean values are presented as mean in triplicate $\pm$ standard deviation.

The ethanol extract of *Cymbopogon citratus* also observed concentration dependent inhibitory properties against *A. flavus* and *A. parasiticus* (Table 4). At 400 mg/mL, biomass was reduced to 152 ± 0.1 mg in *A.*

flavus and 175 ± 0.1 mg in *A. parasiticus*, matching to 73% and 67% inhibition. At 25 mg/mL, inhibition declined to 27% and 9%, and biomass increased to 410 ± 0.5 mg and 480 ± 0.8 mg, respectively. Biomass for the untreated control was 530 ± 0.1 mg for *A. parasiticus* and 560 ± 0.7 mg for *A. flavu*. In general, the ethanolic extract was more inhibitory to *A. flavus* than to *A. parasiticus* especially at low concentrations.

Table 4. Antifungal Potential of Lemongrass Ethanol Extract against Fungi.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>	
	Biomass (mg)	Inhibition (%)	Biomass (mg)	Inhibition (%)
400	152 ± 0.1	73	175 ± 0.1	67
300	172 ± 0.1	69	223 ± 0.3	58
200	232 ± 0.2	59	290 ± 0.5	45
100	300 ± 0.1	46	340 ± 0.2	36
50	340 ± 0.3	39	453 ± 0.7	14
25	410 ± 0.5	27	480 ± 0.8	09
Control	560 ± 0.7	00	530 ± 0.1	00

Results are presented as mean ($n=3$) $\pm$ standard deviation (SD).

The aqueous extract of lemongrass (*Cymbopogon citratus*) also showed concentration-dependent antifungal activity against *A. flavus* and *A. parasiticus* (Table 5). At 400 mg/mL, fungal biomass was lowered to 180 ± 0.1 mg in *A. flavus* and 190 ± 0.1 mg in *A. parasiticus*, giving 68% and 64% inhibition. Reducing the concentration to 25

mg/mL reduced the inhibition to 14% and 6% while biomass increased to 480 ± 0.6 mg and 500 ± 0 mg respectively. Biomass of the untreated controls were 560 ± 0.8 mg and 530 ± 0 mg respectively. In overall, the aqueous extract was more active against *A. flavus* than *A. parasiticus* and its effect was more at the highest Concentration tested.

Table 5. Antifungal Activity of Lemongrass Aqueous Extract against Fungi.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>	
	Biomass (mg)	Inhibition (%)	Biomass (mg)	Inhibition (%)
400	180 ± 0.1	68	190 ± 0.1	64
300	210 ± 0.5	62	250 ± 0.2	53
200	270 ± 0.2	52	325 ± 0.5	39
100	350 ± 0.6	37	390 ± 0.2	26
50	380 ± 0.2	32	485 ± 0	08
25	480 ± 0.6	14	500 ± 0	06
Control	560 ± 0.8	00	530 ± 0	00

Results are presented as mean ($n=3$) $\pm$ standard deviation (SD).

Table 6 shows that the methanolic extract of lemongrass was found to inhibit the biosynthesis of AFs by *A. parasiticus* and *A. flavus* in a concentration dependent manner. *A. flavus* was completely inhibited in the biosynthesis of AFB₂ and AFB₁ at 400 mg/mL. In *A. parasiticus*, the level of inhibition was 78% for AFB₁, 71% for AFB₂, 81% for AFG₁, and 87% for AFG₂. The extract dose, which is not more than 25 mg/mL, caused a

gradual decrease in inhibition: AFB₁ and AFB₂ had an inhibition of 5% and 9%, respectively, in *A. flavus*; AFB₁, AFB₂, AFG₁ and AFG₂ had an inhibition of 3%, 11%, 9% and 12%, respectively, in *A. parasiticus*. No inhibition seen in the untreated controls. In all, the highest anti-aflatoxigenic response was seen against the AFG₂ production by *A. parasiticus* at the highest concentration (87%).

Table 6. Inhibition (%) of AFs Using Lemongrass Methanol Extract.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>			
	AFB1	AFB2	AFB1	AFB2	AFG1	AFG2
400	82±1	83±1	78±1	71±2	81±1.5	87±1
300	70±1	75±2	62±0	64±0	43±1	71±2
200	52±2	55±1	46±1	50±1	38±2	54±0
100	36±1	38±1	26±1	36±1	28±12	37±1
50	21±0.5	26±0.5	12±0.5	25±0.5	17±0.5	21±0.5
25	05±0	09±0	03±0	11±0	09±0	12±0
Control	0±0	0±0	0±0	0±0	0±0	0±0

Results are presented as mean (n=3) ± standard deviation (SD).

The lemongrass (*Cymbopogon citratus*) ethanol extract also reduced the AFs biosynthesis by *A. flavus* and *A. parasiticus* in a concentration-dependent manner (Table 7). AFB₁ and AFB₂ produced by *A. flavus* showed inhibitory effect of 76% and 79%, respectively, at the highest concentration (400 mg/mL). For *A. parasiticus*, inhibition was 75% for AFB₁, 68% for AFB₂, 72% for AFG₁ and 83% for AFG₂. The effect

diminished gradually with decreasing concentration. AFB₁ and AFB₂ inhibited the growth of *A. flavus* by 3% and 5% respectively and AFG₁, AFG₂ inhibited the growth of *A. parasiticus* 0%, 7% and 6% and 8% respectively, at 25 mg/mL. No inhibition was observed in the untreated controls. The ethanol extract proved to be most effective at the highest concentration (83% against AFG₂ production by *A. parasiticus*).

Table 7. Inhibition (%) of AFs Using Lemongrass Ethanol Extract.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>			
	AFB1	AFB2	AFB1	AFB2	AFG1	AFG2
400	76±1	79±1	75±2	68±1	72±0.5	83±1
300	63±1	71±1	60±2	61±1	37±0.5	67±0.3
200	46±1	51±0.6	43±1	46±1	26±0.5	50±0.6
100	30±1	34±0	19±0.5	32±0.4	21±1	33±1
50	14±0	22±0.1	04±0	21±1	12±1	17±0.5
25	03±0	05±0	0±0	07±0	06±0	08±0
Control	0±0	0±0	0±0	0±0	0±0	0±0

Results values are presented as mean (n=3) ± standard deviation (SD).

The AFs production were inhibited by the aqueous extract of *Cymbopogon citratus* in a concentration-dependent manner (Table 8). AFB₁ and AFB₂ of *A. flavus* inhibited by 71% and 60%, respectively at 400mg/mL. In *A. parasiticus*, inhibition was 71% for AFs (B₁), 64% for AFs (B₂), 69% for AFs (G₁) and 75% for AFs (G₂). As the concentration of extracts decreased, so did the activity. At 25 mg/mL AFB₁ and AFB₂ inhibited *A. flavus* 2%,

and inhibited *A. parasiticus* 0%, 4%, 3% and 4% for AFB₁, AFB₂, AFG₁ and AFG₂, respectively. No inhibition was observed in the untreated controls. In general the highest inhibition was for production of AFG₂ by *A. parasiticus* (75% at 400 mg/mL). Of the three solvents, the aqueous extract gave a lower maximum inhibition than the methanolic and ethanolic extracts, based on the values reported.

Table 8. Inhibition (%) of AFs using Lemongrass Aqueous Extract.

Dose (mg/mL)	<i>Aspergillus flavus</i>		<i>Aspergillus parasiticus</i>			
	AFB1	AFB2	AFB1	AFB2	AFG1	AFG2
400	71±1	60±0.4	71±1.5	64±0.5	69±1	75±0.4
300	59±1	51±0	57±0	57±1	38±0.5	58±1
200	43±0	38±1	39±1	43±1	23±0	42±0.5

100	27±1	18±0.5	07±0	29±0.5	15±1	25±0.5
50	11±0	04±0	03±0	18±1	08±0	17±0
25	02±0	02±0	0±0	04±0	03±0	04±0
Control	0±0	0±0	0±0	0±0	0±0	0±0

Results are presented as mean (n=3) ± standard deviation (SD).

Discussion

Phytochemicals Compounds in Lemongrass Extracts

The results are consistent with the previous studies on bioactive phytochemicals derived from *Cymbopogon citratus* leaves. The study in Indonesia revealed that the *C. citratus* leaves showed the presence of flavonoids, tannins, saponins, alkaloids, phenolic compounds and steroids in the extract of ethanol. The highest total phenolic quantity (50.017 mg GAE/g extract) was obtained from the 30% ethanol concentration of ethanol. The result indicated that the higher yield obtained with the more polar 30% ethanol indicates that phenolics in lemongrass leaves can be recovered efficiently in a relatively polar solvent system. The antioxidant potential of the extract may be accredited in large part to the occurrence of phenolic compounds, especially flavonoids, which have the potential to provide a hydrogen atom or an electron to defuse free radicals. Based on these results, the leaves of *C. citratus* can be used as a good origin of non-synthetic antioxidants which can be applied to reduce the oxidative degradation of edible oils and other foods (Hasim *et al.*, 2015).

The results of bioactive compounds screening of ethanolic extract of Lemongrass leaves indicated the occurrence of the phytochemicals like tannins, flavonoids, saponins and essential oils, whereas no triterpenoids and alkaloids were detected. It is possible that the presence of alkaloids was not detected as they may be present in small quantities, and the performed screening technique was not complex enough to sense them (Sari *et al.*, 2023).

In one study of a lemongrass methanol extract, saponins were not detected, tannins and glycosides were detected, flavonoids were abundant and only slightly present alkaloids were detected (Owusu-Ansah *et al.*, 2023). A different analysis also confirmed the occurrence of flavonoids, glycosides, tannins alkaloids, but not saponin (Owusu *et al.*,

2021). The absence of saponins is similar with Otabor *et al.* (2019) who reported saponins in lemongrass whereas the presence of alkaloids, tannins, flavonoids and glycosides is in agreement with Ekpenyong *et al.* (2014). In the current study, glycosides were found in lemongrass, which is not in accordance with the reports of Shakur *et al.* (2017), Mgbemena (2010) and Zhou *et al.* (2016). These differences could be attributed to the extraction method as a polar solvent extracts most of the polar molecules (Owusu-Ansah *et al.*, 2023). Leaves of dried lemongrass have been reported to contain higher amount of phenols and flavonoids as compared to fresh lemongrass (Njar *et al.*, 2006). The methanolic extract resulted in higher number of phytochemical constituents than the ethanolic extract, also indicating that the method of extraction affects the detection of particular phytochemical constituents (Geetha and Geetha, 2014). No triterpenoids were found here, but perhaps they would be found in the ether or chloroform extract (Harborne, 1987).

The polarity and hydrogen-bonding ability of methanol make it a good solvent for diverse bioactive phytochemicals, particularly flavonoids and phenolic compounds. It can also be used to extract moderately less polar compounds like the non-glycosylated flavonoids and the terpenoids, while water extracts the highly water soluble compounds. Ethanol, in turn, is good for extraction of a combination of water soluble and fat soluble compounds. The bioactivity observed in methanolic extracts of plants can therefore be accredited to the easy extraction of flavonoids and other bioactive substances in the plant (Tazi *et al.*, 2024).

Antioxidant Activities of Lemongrass Extracts

Lemongrass's anti-radical properties are accredited to its high flavonoids and phenols compounds, which act a vital role in defusing the toxic free molecules and stopping oxidative stress-induced

diseases (Dwivedi, 2024). The antioxidant potential of the extract of lemongrass has been evaluated through DPPH and Ferric reducing antioxidant power (FRAP) assay. The extract of methanol exhibited the maximum % of DPPH hunting followed by the petroleum ether and hexane extracts (Yahya *et al.*, 2020). Lemongrass extracts have been reported to have high radical-scavenging properties, which makes them potential for formulating antioxidants (Dwivedi, 2024). The methanol extract of lemongrass was found to possess higher DPPH scavenging activity than the essential oil of lemongrass by Kusmardiyani *et al.* (2016). The high polarity index (5.1) of methanol may be related to its effectiveness as a DPPH scavenger compared to low-polar solvents such as petroleum ether and hexane. The flavonoids and polyphenols that methanol extracts well are free-radical scavengers, while the phenolic compounds are hydrogen-donating free-radical scavengers (Chew *et al.*, 2011). The ethanol extract of lemongrass had an IC₅₀ value of 56.2 µg/mL, whereas the IC₅₀ value of the methanol extract of lemongrass was 61.5 µg/mL (Dwivedi, 2024).

In another study, a 70% ethanol extract of lemongrass leaves at 10, 20, 30, 40 and 50 ppm gave mean inhibition of 7.08%, 14.01%, 25.19%, 31.64% and 37.68%, respectively (Fitria *et al.*, 2022).

The phytochemicals present in 70% ethanol extracts of lemongrass leaves were active compounds like phenolic compounds, saponins, steroids, tannins, alkaloids and flavonoids as revealed by the phytochemical test earlier (Shuhada *et al.*, 2018; Bhardwaj, 2020). The compounds possess potent antioxidant and free-radical hunting properties which can be calculated applying the DPPH techniques (Flieger and Flieger, 2020).

This indicates that the effect of the active compounds of lemongrass leaf extracts is to eliminate DPPH free radicals in the DPPH assay as the colour of the solution changes. The antioxidant activity is also due to phytochemicals such as polyphenols, flavonoids, saponins, alkaloids and tannins. The lemongrass leaf extract antioxidant properties was similar to the activity of a

lemongrass extract (Haerani *et al.*, 2019) and the citronella extract (Bayala *et al.*, 2020) with IC₅₀ values of 63.84 mg/kg and 58 mg/kg, respectively, both of which are classified as powerful antioxidants.

The antioxidant activity of lemongrass is believed to be due to the presence of phytochemicals substances in the leaves comprising phenols, tannins, flavonoids, alkaloids, and the presence of compounds containing numerous sulphide groups. Kanopa *et al.* (2012) mentioned that these compounds also can be used as antioxidants.

The anti-radical potential of lemongrass leaves has also been shown by their ability to inhibit the DPPH free radical. The 70% ethanol extract of lemongrass leaves showed maximum anti-oxidant activity with an IC₅₀ of 79.444 mg/L and this was attributed to anti-oxidant phytochemicals contained mainly in phenolic and flavonoid compounds that can donate hydrogen atoms or electrons for scavenging free radicals, reported by Hasim *et al.* (2015). The moderate activity of the ethanolic extract (70%) also indicates that a medium concentration may be more effective at extracting antioxidants from the *C. citratus* leaves than other concentrations. The results obtained are in agreement to the use of lemongrass leaves as a natural source of antioxidants in foods and other uses because it is cheap and readily available in nature.

Antifungal Activities of Lemongrass Extracts

The effectiveness of inhibiting *A. flavus* sporulation by Lemongrass leaf powder (10% w/w) was performed after 30 days of storage of maize grain by Daba *et al.*, 2025 which resulted in 53.52% inhibition. The antifungal activity of phenolic compounds isolated from Lemongrass leaves have been reported against *A. flavus* and *A. parasiticus* (Abu-Seif *et al.*, 2009).

The present findings are in harmony with previous studies on antifungal activity of aqueous extracts of lemongrass. Higher concentration (5 mg/mL) of water extract of *C. citratus* inhibited 70% of the biomass development of *Fusarium oxysporum* and 20 mg/mL totally suppressed the growth of the fungus, while the lower concentration (0.05 mg/mL)

suppressed 67% of spores germination and 77% of sporulation. This indicates that the antifungal activity of lemongrass is not exclusive to its essential oil and the polarity and water solubility of its phenolic constituents could act a vital job. The action also confirms the use of *C. citratus* aqueous extracts as natural source of antifungal compounds (Aourach *et al.*, 2021).

The extracts obtained from the lemongrass were tested against four root-rot fungi namely *Macrophomina phaseolina*, *F. oxysporum*, *Fusarium solani* and *Rhizoctonia solani*. The highest concentration used (50 μ L) of the n-heane extract was the most antifungal. However, in the field trials it was observed that 5% (w/v) lemongrass powder improved the length of root and shoot of brinjal which could be utilized for the control of the disease through botanical methods (Parveen *et al.*, 2022).

Earlier findings has also revealed antifungal activity in lemongrass leaf extracts obtained using various preparation methods. Fresh and dried leaves were extracted with cold water, hot water, ethanol and methanol and the extracts were experienced for action against *A. niger* and *Colletotrichum musae*. The methanol extract of dried leaves produced the highest inhibition zone against *A. niger* (10.90 mm) followed by methanol extract of fresh leaves (10.20 mm) and the least inhibition zone against *C. musae* (5.20 mm) by ethanol extract of fresh leaves. Such differences in activities can be attributed to the kind of solvent applied, the extraction temperature, and the physical form of the plant material, all of which can influence the recapture of the polyphenols substances, flavonoids and other antifungal components. These results also show that the extraction method significantly affects the anti-mould activity of the *C. citratus* extracts (Nyamath and Karthikeyan, 2018).

Generally, the antifungal activity of plant-derived compounds can be explained by a decrease in ergosterol, cell-membrane integrity disturbance, ion seepage increased and cell-membrane penetrability, mitochondrial structure disturbance, interfering with the enzymatic reactions of cell-wall biosynthesis, disruption of oxidated equilibrium,

suppression of carbohydrate metabolism, inhibition of mycotoxin biosynthesis genes, and changes in the molecular shape of mycotoxins (Jafarzadeh *et al.*, 2022).

Anti-aflatoxicenic Activities of lemongrass Extracts and Powder

Decline and AFs biosynthesis in the inoculated (*A. flavus*) shelled melon seeds were significantly reduced when ground lemongrass leaves were used at 10% (w/w). Farmers' stores were treated with essential oil and *C. citratus* powder at 0.5% (v/w) and 10% (w/w) respectively to see if there was any reduction in the number of visibly diseased seeds, the percentage level of *Aspergillus* infestation after 6 months storage, as well as the percentage germination after 6 months of storage compared to untreated seeds (Bankole and Joda, 2004).

Previous studies have reported that lemons grass (*Cymbopogon citratus*) was effective in controlling *A. flavus* and AFs in maize. Powdered leaves were more effective than whole leaves and 14% (w/w) powder did not allow any fungal growth or AFB₁ formation after 25 days of storage. Lemongrass water suspensions also reduced AFB₁ concentrations, but to a lesser extent, fungal growth. Lemongrass therefore, especially when in powder form, could be used as a natural remedy for *A. flavus* infestation and AFB₁ contamination of stored maize (Atanda and Olopade, 2013).

The present results are also in agreement with other reports on lemongrass essential oil that was fungicidal at 1.0 mg/mL and fungistatic at 0.6 mg/mL against *A. flavus* and completely inhibited the production of AFs at 0.1 mg/mL. The outcomes gained in the study was in support of the antifungal and anti-aflatoxicenic activity of lemongrass, but the study was conducted with methanolic extract (Paranagama *et al.*, 2003).

Cymbopogon citratus also has antifungal and anti-aflatoxicenic properties in stored food commodities. *C. citratus* powdered leaves and essential oil inhibited the growth of fungi causing decline and AFs biosynthesis in melon seeds inoculated with toxic moulds. The essential oil was found to have a dose-dependent effect: the doses of 0.1 mL/100 g seeds and 0.25 mL/100 g seeds

significantly decreased the reduction and AFs biosynthesis, whereas the doses 0.5 and 1.0 mL/100 g seeds inhibited the production of AFs totally. Deterioration of the fungus and AFs contamination were also lower in the presence of powdered leaves of 10 g/100 g of seeds (Bankole *et al.*, 2005).

The results also are in line with previous studies regarding the anti-moulds and anti-aflatoxigenic potential of lemongrass. Both powdered leaves and essential oil inhibited fungal deterioration caused by *Penicillium citrinum*, *A. flavus*, *A. niger* and *A. tamarii* in melon seeds and essential oil (EO) inhibited the production of AFs by toxigenic *A. flavus* in a concentration dependent way. The application of 0.1 and 0.25% (v/w) reduced deterioration and production of AFs, while 0.5 and 1.0% (v/w) completely suppressed the production of AFs. Similarly, the powdered leaves at 10% (w/w) caused significant reduction in fungal deterioration and the production of AFs. A lower fungal deterioration was observed in seeds inoculated with essential oil (0.5% v/w) as compared to the untreated seeds while the seeds inoculated with EO had a maximum germination rate and lower *Aspergillus* infestation after 6 months of storage. The results of study corroborate the antifungal and anti-aflatoxigenic apotential of *C. citratus* and indicate that the EO of *C. citratus* may be a natural way to reduce the growth of fungi and AFs production in a stored food commodity (Bankole and Joda, 2004).

It was also reported that use of neem and lemongrass leaf powders against the growth of toxic *A. flavus* in stored maize. The 10% (w/w) Lemongrass leaf powder showed 53.52% reduction in *A. flavus* sporulation after 30 days storage period. Though the effect was less than that of neem leaf powder, it demonstrated that *C. citratus* powder is an effective suppressor of fungal development in storage. In particular, neem powder at 5% (w/w) reduced AFs production, suggesting that use of plant-derived materials is an inexpensive means of reducing the growth of fungi and producing AFs in stored grain. The results agree with the activity observed here and confirm *C. citratus* as a natural

biocontrol agent of *A. flavus* and AFs contamination (Daba *et al.*, 2025).

The antifungal activity and antimycotoxigenic activity was significant when the lemongrass decoction was compared with essential oil. The essential oil was effective at much lower concentrations: 0.1% (w/v) inhibited growth of fungal hyphae and mycotoxin production by *A. flavus*, *A. parasiticus* and *A. ochraceus*; 0.3% completely inhibited growth of fungi and mycotoxin production by both *A. flavus* and *A. ochraceus*. Contrary to this, a higher concentration (1%) was required for the decoction to be effective. Thus, the EO was more active as compared with the aqueous decoction and the active antifungal and antimycotoxigenic components were more effective in the essential oil fraction than in the aqueous fraction (Abd-El Fattah *et al.*, 2010). The loss of aflatoxigenic capacity from mutant strains of *Aspergillus* has been correlated with the occurrence of abnormalities in conidiophores (Kale *et al.*, 1996). In general, the outcomes indicate that the biological properties may vary with the solvent extraction, doses, type of fungi, highlighting the need for optimization of extraction conditions to recover bioactive compounds from lemongrass. The continuous inhibition of both *A. flavus* and *A. parasiticus* in concert with decreased AFs production provides a basis for further study of the underlying mechanisms and the continuous research of compounds extracted from lemongrass as complementary natural agents to inhibit fungal contamination and AFs formation in food and agricultural products.

Conclusion

This study demonstrated the presence of phytochemical constituents in lemongrass (*Cymbopogon citratus*) extracts along with their antioxidant, anti-mould and anti-aflatoxin properties against *A. flavus* and *A. parasiticus*. The extract of methanol was utmost active in all the tests done, followed by the ethanolic and aqueous extracts. The anti-aflatoxin and antifungal effects were in general positive with increasing concentration, with maximum concentration tested (400 mg/mL) showing the highest level of

antifungal and anti-aflatoxigenic effects. The extract of methanol was the utmost effective extract in inhibiting AFs, achieving the highest inhibition (87%) against AFG₂ produced by *A. parasiticus* compared to the ethanolic and aqueous extracts which achieved 83% and 75% respectively. Grounded on these results, the extracts of lemongrass, particularly the methanolic extract, could potentially be used as sources of natural antifungal and anti-aflatoxigenic agents. Before they can be identified as natural preservatives, however, further research is required to identify the active constituents, effective and safe concentrations, and to validate performance in food systems.

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