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Evaluation of Phytochemical Analysis for Chia (*Salvia hispanica* L.) and Papaya (*Carica papaya* L.) Seed Extracts as Antioxidant, Antifungal and Anti-aflatoxins

Marwa A. Younos[1*], Engy M. Akl[2]

[1]Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt.
[2]Fats and Oils Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt.

Abstract
Bioactive compounds from vegetal sources are a potential source of natural biological activities. Therefore, this study aims to determine the polyphenolic compounds in chia (*Salvia hispanica* L.) seeds extract (CSE) and papaya (*Carica papaya* L.) seeds extract (PSE), and evaluate their antioxidant, antifungal, and anti-aflatoxigenic activities. The polyphenolic compounds of the ethanolic CSE and PSE were elucidated. Each of them was tested as antioxidant, antifungal, and anti-aflatoxins. The obtained data indicated that the chemical composition of chia and papaya seeds included 33.4 % and 18.4% of oil, 25.5 % and 31.5% of protein, and 12.4 % and 19.1 %of fiber respectively. CSE contained 9.55mg/g and 30.35 mg/g of phenolic compounds (PC) and flavonoid compounds (FC) respectively, while PSE contained 10.72 mg/g and 16.39 mg/g of PC and FC respectively. Both extracts exhibited the ability to scavenge the DPPH radical. However, it was noted that PSE was more efficient compared with CSE. In HPLC analysis, Ellagic acid and Vanillin were the major detected PC in CSE, while Caffeic and Chlorogenic acids were the major detected PC in PSE. On the other hand, both extracts reduced significantly the fungal growth and spore viability of all tested fungi and reduced the mycelial dry weight and aflatoxins production of *Aspergillus parasiticus* at all concentrations used compared with untreated control. Also, the reduction percentage was increased by increasing the concentration used. It can be concluded that CSE and PSE are potential sources of polyphenolic compounds with antioxidants, antifungal and anti-aflatoxigenic properties.

Keywords: Chia (*Salvia hispanica* L.) seeds, Papaya (*Carica papaya* L.) seeds, Polyphenolic compounds, Flavonoid compounds, Antioxidant, antifungal, anti-aflatoxigenic activities

1. Introduction

Chia, scientifically known as *Salvia hispanica* L. and belonging to the Lamiaceae family, is an herbaceous plant extensively utilized for both commercial and medicinal reasons. Originally, it was grown in tropical and subtropical regions, but it has now become a globally grown crop, particularly valued for its role as a significant dietary supplement. In today's context, dried chia seeds, renowned for their nutritional benefits, are incorporated into various food items such as fruit juices, smoothies, milk, yogurt, salads, soups, and baked products [1]. At present, researchers and scientists describe chia seeds as the valuable seed of the 21st century [2]. Due to its physiological and functional attributes, chia and its derived products have the potential to serve as a valuable component in creating healthier product alternatives [3]. The health-promoting properties of chia seeds can be attributed to their abundant chemical composition (notably their high levels of essential fatty acids, essential amino acids, polyphenols, bioelements, and vitamins). Various recent scientific studies not only outline the chemical composition of chia seeds but also validate their diverse biological activities and advantageous impacts on the body. These activities include antimicrobial, anti-inflammatory, antioxidant, anticoagulant, hypoglycemic, hypolipemic, hypotensive, hepatoprotective, cardioprotective, and immunostimulatory properties [4, 5]. Papaya, scientifically known as *Carica papaya* L., stands as a primary horticultural crop in tropical and subtropical

*Corresponding author e-mail: marwayounos@yahoo.com.; (Marwa A. Younos).
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areas and belongs to the Caricaceae family [6]. Its origins can be traced back to Southern Mexico and Costa Rica [7]. The papaya fruit holds significant nutritional and economic importance. Besides the fruit itself, the leaves, seeds, and peels of papaya encompass numerous bioactive elements. These by-products are abundant in phytochemicals, particularly phenolic compounds, renowned for their antioxidative properties. Papaya seeds, in particular, offer various nutritional and health advantages due to their richness in proteins, fibers, oils, alkaloids, flavonoids, tannins, saponins, anthraquinones, and benzyl isothiocyanate. The antioxidant capabilities of papaya seeds might play a role in the development of functional foods and nutraceuticals, utilizing these residual parts of papaya in the upcoming period [8]. Out of all the plant components, the seeds hold the most noteworthy medicinal benefits. Despite constituting approximately 7% of the fruit's weight, they are commonly disposed of. Studies have indicated that extracts from the seeds possess anticancer, hypolipidemic, antifertility, and anti-inflammatory properties [9]. The seeds are employed for the treatment of gastrointestinal nematode infections and have demonstrated anthelmintic properties [10]. Both the seeds and unripe fruit have displayed inhibitory effects against human enteric pathogens [11].

Both Chia and Papaya seeds are natural products known for their antifungal properties against various pathogenic fungal strains, including *Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus niger, Fusarium oxysporum,* and *Candida albicans* [12, 13]. Therefore, the present study aimed to evaluate the antioxidant capacity, and the antifungal efficiency as well as the anti-aflatoxigenic activity of the ethanolic extracts of chia (*Salvia hispanica* L.) and papaya (*Carica papaya* L.) seeds.

2. Materials and Methods

2.1. Source of plant materials

Chia (*Salvia hispanica* L.) seeds were obtained from the National Research Centre (NRC), Giza, Egypt, while Papaya (*Carica papaya* L.) seeds were purchased from Egyptian local markets during 2022.

2.2. Source of fungi

Five test organisms (*Alternaria alternata, Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum,* and *Penicillium expansum*) were obtained from Plant Pathology Dep., National Research Center (NRC), Egypt, and maintained on Potato Dextrose Agar (PDA) medium.

2.3. Chemicals

All chemicals were obtained from Sigma Chemical Co.

2.4. Determination of the chemical composition of chia and papaya seeds

The chemical composition of seeds was determined according to A.O.A.C. [14].

2.5. Preparation of ethanolic extracts of chia and papaya seeds

Twenty grams of seeds of each of the chia and papaya plants were sterilized with 1% sodium hypochlorite solution for 5 minutes at room temperature. Afterward, they were rinsed with distilled water, sun-dried for several days, and then oven dried at 50ºC for 24 hours. The dried seeds were ground into a coarse powder using a Thomas-Willey milling machine. They were then separately extracted using a mixture of absolute ethanol and water (80% v/v). The extraction process involved filtering the extracts through Whatman filter paper No. 1 (125 mm) and collecting the ethanol filtrates in two separate beakers. These filtrates were concentrated using a rotary evaporator at 40°C to obtain the crude extracts. The crude extracts were stored at 4ºC for further analysis [15].

2.6. Determination of total soluble phenolics

The quantification of phenolic compounds in CSE and PSE was conducted following the method outlined by Fu et al. [16]. A 200 µL of the sample was mixed with 3 mL of distilled water. Next, 2 mL of a 10% folin reagent was added to the mixture and thoroughly shaken for 5 minutes. A further 1 mL of 7.5% sodium carbonate was added and the mixture was shaken again. The resulting solution was kept in the dark for one hour, after which the absorbance at 765 nm was measured using a T80 UV vis spectrophotometer. The total phenolic content was determined by preparing a calibration curve with gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of the sample, following the methodology described by Zilic et al. [17].

2.7. Determination of total flavonoids

The measurement of soluble flavonoids in CSE and PSE was carried out based on the method described by Kanatt et al. [18]. The absorbance at 510 nm was recorded using a T80 UV-vis spectrophotometer. The total flavonoid content was determined by utilizing a calibration curve and expressed as milligrams of catechin equivalent (CE) per gram of the sample.

2.8. Evaluation of the antioxidant activity of CSE and PSE by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging

The DPPH radical-scavenging activity was determined following the procedure outlined by De Ancos et al. [19]. The reduction of the DPPH radical was measured at a wavelength of 517 nm. The results were expressed as the percentage inhibition of DPPH using the following equation:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

Where absorbance control is the absorbance of DPPH solution without extract

2.9. High-performance liquid chromatography (HPLC) analysis of phenolic compounds of CSE and PSE

To analyze the phenolic compounds, 1 mL of each tested extract was placed in a conical flask fitted with a stopper, and 20 mL of 2M NaOH was added. The flasks were then flushed with nitrogen gas and shaken for 4 hours at room temperature. Following this, the pH was adjusted to 2 using 6 M HCl. After centrifuging the samples at 5000 rpm for 10 minutes, the supernatant was collected. The phenolic compounds were then extracted twice using a 1:1 mixture of ethyl ether and ethyl acetate (50 mL each time). The organic phase was separated and evaporated at 45°C. The resulting samples were dissolved in 2 mL of methanol.

To conduct high-performance liquid chromatography (HPLC) analysis, an Agilent 1260 series instrument was employed along with an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) for effective separation. The mobile phase composition consisted of water (A) and acetonitrile containing 0.05% trifluoroacetic acid (B) at a flow rate of 0.9 mL/min. A linear gradient program was implemented as follows: 0 min (82% A), 0-5 min (80% A), 5-8 min (60% A), 8-12 min (60% A), 12-15 min (82% A), 15-16 min (82% A), and 16-20 min (82% A). The multi-wavelength detector was set to monitor the samples at a wavelength of 280 nm. For each sample solution, an injection volume of 5 µL was utilized. To ensure sample purity, all samples were filtered through a 0.45 µm Acro-disc syringe filter manufactured by Gelman Laboratory (MI) prior to injection. The identification of peaks was achieved by comparing their retention times and UV spectra to those of the standard compounds [20].

2.10. Evaluation of the antifungal activity of the ethanolic CSE and PSE *in vitro*

2.10.1. Effect of CSE and PSE on the mycelial growth of different fungi

The ethanolic CSE (chia seed extract) and PSE (papaya seed extract) were assessed for their effects on the mycelial growth of various fungi (*Alternaria alternata, Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum,* and *Penicillium expansum*) *in vitro*. Each sterilized extract was mixed separately with sterilized Potato Dextrose Agar (PDA) medium at different concentrations (0.5%, 1%, and 2% v/v) in sterilized Petri dishes. PDA medium without any plant extracts was used as a control. Each PDA plate was inoculated at the center with 5 mm-discs of 7-day old fungal colonies using a sterilized cork borer. The plates were then incubated at a temperature of 28 ± 2°C. Three plates were used for each treatment as replicates. After a 7-day incubation period, the diameter of each fungal colony was measured according to the method described in Singh et al. [21] and Younos and Abdel-Galil [22]. Medium-free extract was used as a control. The reduction percent of mycelial growth was calculated according to Jabeen et al. [23] by using a formula:

$$\text{R (\%)} = \frac{\text{(C - T)}}{\text{(C)}} \times 100$$

Where C represents the growth in the control group and T represents the growth in the treatment group.

2.10.2. Effect of CSE and PSE on the spore viability of different fungi

The effects of the ethanolic CSE and PSE on the viability of spores of different fungi (*Alternaria alternata, Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum,* and *Penicillium expansum)* were investigated using different concentrations (0.5%, 1%, and 2%). A 0.5 cm diameter disc of each fungal culture (7 days old) on PDA, was placed in the center of each Petri dish. The dishes were then incubated at a temperature of 28 ± 2°C for 5 days. To collect the spores produced, 9 mL of sterile water was poured over the fungal mycelium and separated using a drawing brush. The resulting spore suspension was filtered through a muslin cloth. The concentration of the collected spore suspension was adjusted to 1 $x10^2$ conidia/mL using a Haemocytometer slide. Each sterilized extract (CSE and PSE) was separately mixed with sterilized PDA medium at different concentrations (0.5%, 1%, and 2% v/v) in sterilized Petri dishes. PDA medium free of plant extracts was used as a control. Each PDA plate was then inoculated with 1 mL of spore suspension (containing 1 $x10^2$ conidia/mL), which was spread evenly over the plate, and incubated at 28 ± 2°C. Each treatment was replicated on three plates. After 48 hours of incubation, the proportion of germinated spores was calculated according to the method described by Meena and Mariappan [24].

2.10.3. Effect of ethanolic CSE and PSE on the dry weight of aflatoxigenic fungi (*Aspergillus parasiticus*)

The effects of the ethanolic CSE and PSE on the dry weight of aflatoxigenic fungi (*Aspergillus parasiticus*) were examined using different concentrations (0.5%, 1%, and 2%). Each sterilized extract (CSE and PSE) was separately mixed with 100 mL of Potato dextrose broth media in flasks, with the concentrations mentioned above. The control group consisted of Potato dextrose broth without any plant extracts. All flasks were then inoculated with 1 mL of a spore suspension containing 1 x 10^2 conidia/mL of *A. parasiticus* and incubated at a temperature of 28 ± 2°C for 14 days. Each treatment was replicated three times. After the incubation period, the cultures were filtered through pre-weighed Whatman filter paper No. 1. The dry weight of the mycelium was measured after drying at 70ºC for 24 hours in an oven [25]. The reduction percent of growth inhibition (R %) based on dry weight is calculated as:

$$R\% = \frac{(\text{Control weight} - \text{Sample weight})}{(\text{Control weight})} \times 100$$

2.11. Evaluation of the anti-aflatoxigenic activity of the ethanolic CSE and PSE *in vitro*

2.11.1. Effect of CSE and PSE on aflatoxins production *in vitro*

The effects of the ethanolic CSE and PSE on the reduction of aflatoxins produced by aflatoxigenic fungi (*Aspergillus parasiticus*) were examined using different concentrations (0.5%, 1%, and 2%). Each sterilized extract (CSE and PSE) was separately mixed with 100 mL of Yeast extract sucrose medium (YES) in flasks, with the concentrations mentioned above. The control group consisted of YES medium without any plant extracts. The flasks were then inoculated with 1 mL of a spore suspension containing 1 x 10^2 conidia/mL of *A. parasiticus* and incubated at a temperature of 28 ± 2°C for 14 days. At the end of the incubation period, the cultures were filtered through Whatman filter paper No.1 to remove the mycelial growth. To extract the aflatoxins, 25 mL of each culture filtrate and 10 mL of chloroform were thoroughly shaken for 15 minutes in a separating funnel and then filtered again separately. The organic phase was concentrated until dry. The concentration of aflatoxins produced was determined using HPLC equipment, following the method described by Kumar et al. [26]. The reduction of AF production (R %) was calculated as follows:

$$(R\ \%) = \frac{(\text{AFs conc. (control)} - \text{AFs conc. (treatment)})}{(\text{AFs conc. (control)})} \times 100$$

2.12. Statistical Analysis

The data collected in this study were analyzed using IBM SPSS Statistics software v.16 (USA). Statistical significance was determined using a one-way Analysis of Variance (ANOVA) test. A value of $p<0.05$ was considered to indicate statistical significance. The least significant difference (LSD) was calculated at a significance level of $P \leq 0.05$, following the method described by Gomez and Gomez [27].

3. Results

3.1. Determination of the chemical composition of chia and papaya seeds

The chemical composition of chia and papaya seeds as expressed in Fig (1) showed that they contain 33.4 % and 18.4% of oil, 25.5 %, and 31.5% of protein and 12.4% and 19.1% of fibre respectively.

3.2. Determination of total phenolic, flavonoid compounds content, and antioxidant activity of the ethanolic CSE and PSE

Total phenolic, flavonoid compounds and antioxidant activity of CSE and PSE were illustrated in Table 1. It showed that CSE contained 9.55mg/g and 30.35 mg/g of PC and FC respectively. It also showed that PSE contains 10.72 mg/g and 16.39 mg/g PC and FC respectively. It was noted that CSE and PSE are rich in flavonoids than phenolic. Both seeds extracts exhibited the ability to scavenge the DPPH radical. In contrast, PSE exhibited the scavenging ability more than CSE.

Table 1 Phenolic, flavonoid compounds and antioxidant activity of the ethanolic CSE and PSE

Plant extract	Phenolic compounds (mg/g)	Flavonoid compounds (mg/g)	DPPH scavenging activity %
Chai seeds Extract (CSE)	9.55 ± 0.2	30.35 ± 0.4	50 ± 0.5
Papaya seeds Extract (PSE)	10.72 ± 0.4	16.39 ± 0.3	64.6 ± 0.5

DPPH= 2, 2-diphenyl-1-picrylhydrazyl, Results are mean values of three replicates ± standard deviation

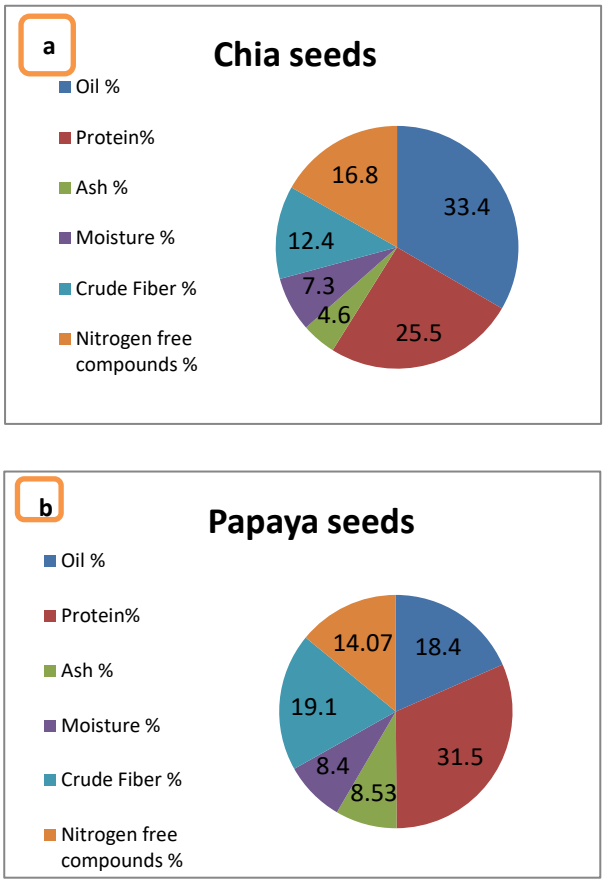

Figure 1. The chemical composition of: a- Chia seeds, b- Papaya seed

3.3. Determination of the polyphenolic profile of the ethanolic CSE and PSE

The qualitative and quantitative analysis of the main polyphenolic compounds present in CSE and PSE were demonstrated in Table (2) and Fig. (2). It was noticeable that the CSE characterized by high amount of ellagic acid, vanillin, gallic acid, cholorogenic acid On the other hand, the major phenolic compounds identified in PSE characterized by caffeeic acid , gallic acid, cholorogenic acid, quercetin, and ellgic acid and other phenolic compounds as shown in Table (2) and Fig. (2).

3.4. Antifungal activity

3.4.1. Effect of the ethanolic CSE and PSE on the mycelial growth of different fungi

The effect of the ethanolic CSE and PSE on the mycelial growth of the tested fungi (*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium oxysporum*, *Penicillium expansum*) was evaluated by employing various concentrations (0.5 %, 1 %, and 2 %). Data in Table (3) indicated that CSE and PSE reduced significantly ($P < 0.05$) the

Table 2 The polyphenolic profile of the ethanolic CSE and PSE

Phenolic compounds	Chia seed extract (CSE) (µg/g)	Papaya seed extract (PSE) (µg/g)
Gallic acid	10.37	27.22
Chlorogenic acid	19.11	33.58
Catechin	6.05	0.00
Methyl gallate	0.56	0.21
Caffeic acid	1.09	55.22
Syringic acid	0.71	2.27
Pyro catechol	1.21	1.17
Rutin	2.10	1.71
Ellagic acid	54.93	23.41
Coumaric acid	0.00	0.11
Vanillin	38.48	0.41
Ferulic acid	6.17	0.97
Naringenin	2.20	3.82
Daidzein	0.81	0.00
Quercetin	4.07	6.97
Cinnamic acid	0.78	0.90
Hesperetin	0.00	2.37

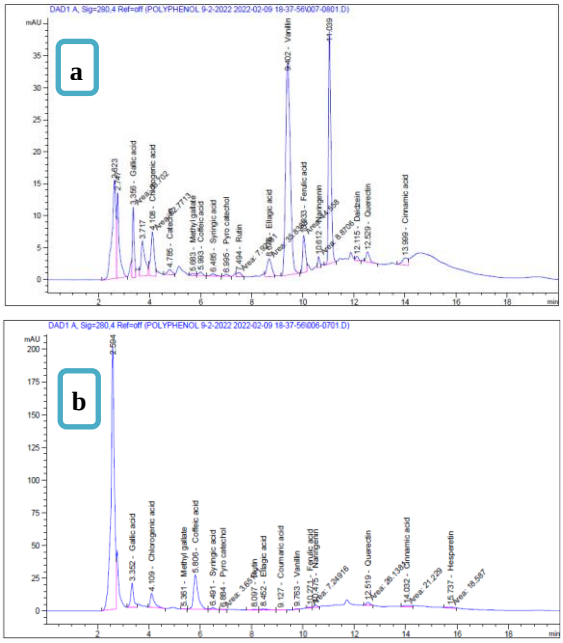

Figure 2. HPLC chromatograms of the polyphenolic profile of a- CSE, b- PSE

fungal growth of the tested fungi at all concentrations used compared with untreated control. Also, the growth inhibition was increased by increasing the concentration used. On the other hand, *A. flavus*, *A. parasiticus*, and *A. alternata* were more affected by CSE than PSE, where they recorded 84.00, 77.60 & 70.40%, 72.73, 50.00 & 40.91%, and 62.50, 56.25 &43.75 % reduction with CSE, and 82.40, 70.40 & 52.00 %, 72.73, 45.45 & 31.82%, and 59.38, 53.13 & 43.75% reduction for PSE at 2%, 1%, and 0.5% respectively, while each of *Fusarium oxysporum* and

Penicillium expansum were more affected by PSE than CSE, where they gave 62.22, 55.56 & 50.00%, and 46.88, 40.63 & 28.13% reduction with PSE, and 57.78, 51.11&47.78%, and 40.63, 25.00 & 12.50% reduction with CSE at the same concentrations respectively.

3.4.2. Effect of the ethanolic CSE and PSE on the spore viability of different fungi

The effect of the ethanolic CSE and PSE on the spore viability of the tested fungi (*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium oxysporum*, and *Penicillium expansum*) was evaluated by employing various concentrations (0.5 %, 1 %, and 2 %). Data in Table 4 indicated that CSE and PSE reduced significantly ($P < 0.05$) the spore viability of the tested fungi at all concentrations used compared with untreated control. Also, the reduction percentage was increased by increasing the concentration used. On the other hand, *A. flavus*, *A. alternata*, and *A. parasiticus* were more affected by CSE than PSE,

Table 3 Effect of the ethanolic CSE and PSE on the mycelial growth of different fungi

Plant extract	Conc.	*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Fusarium oxysporum*		*Penicillium expansum*		LSD 5%
		linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	
Chia seeds extract (CSE)	0.5 %	45.00 ± 0.35 ^{a}	43.75	18.50 ± 0.21 ^{a}	70.40	32.50 ± 0.23 ^{b}	40.91	23.50 ± 0.14 ^{a}	47.78	70.00 ± 0.27 ^{cd}	12.50	
	1 %	35.00 ± 0.21 ^{a}	56.25	14.00± 0.37 ^{a}	77.60	27.50 ± 0.16 ^{ab}	50.00	22.00 ± 0.18 ^{a}	51.11	60.00 ± 0.63 ^{bc}	25.00	0.969 A
	2 %	30.00 ± 0.14 ^{a}	62.50	10.00± 0.05 ^{a}	84.00	15.00 ± 0.30 ^{a}	72.73	19.00 ± 0.24 ^{a}	57.78	47.50 ± 0.52 ^{ab}	40.63	
Papaya seeds extract (PSE)	0.5 %	45.00 ± 0.27 ^{a}	43.75	30.00± 0.21 ^{b}	52.00	37.50 ± 0.41^{b}	31.82	22.50 ± 0.36 ^{a}	50.00	57.50 ± 0.12 ^{abc}	28.13	
	1 %	37.50 ± 0.32 ^{a}	53.13	18.50± 0.31 ^{a}	70.40	30.00 ± 0.62 ^{b}	45.45	20.00 ± 0.61 ^{a}	55.56	47.50 ± 0.23 ^{ab}	40.63	0.754 A
	2 %	32.50 ± 0.17 ^{a}	59.38	11.00± 0.20 ^{a}	82.40	15.00 ± 0.35 ^{a}	72.73	17.00 ± 0.24 ^{a}	62.22	42.50 ± 0.15 ^{a}	46.88	
Control		80.00 ± 0.42 ^{b}		62.50± 0.14 ^{c}		55.00 ± 0.13 ^{c}		45.00 ± 0.30 ^{b}		80.00 ± 0.39 ^{d}		0.896 B
LSD 5%		0.817 B		0.823 A		0.678 B		0.479 B		0.681 C		

R%= Reduction Percent, Results are mean values of three replicates ± standard deviation

Table 4 Effect of the ethanolic CSE and PSE on the spore viability of different fungi

Plant extract	Conc.	*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Fusarium oxysporum*		*Penicillium expansum*		LSD 5%
		Viable spores x10^2	R %	Viable spores x10^2	R %	Viable spores x10^2	R %	Viable spores x10^2	R %	Viable spores x10^2	R %	
Chia seeds extract (CSE)	0.5 %	84.00 ± 0.75 ^{c}	53.07	37.00 ± 0.48 c	71.32	50.00 ± 0.61^{b}	48.45	107.00 ± 0.72 d	52.44	210.00 ± 0.92 e	50.00	
	1 %	44.00 ± 0.67 ^{b}	75.42	21.00 ± 0.28 ^{b}	83.72	31.00 ± 0.42 ^{a}	68.04	95.00 ± 0.68 ^{cd}	57.78	120.00 ± 0.78 ^{c}	71.43	29.070 A
	2 %	21.00 ± 0.23 ^{a}	88.27	11.00 ± 0.15 ^{a}	91.47	20.00 ± 0.30 ^{a}	79.38	75.00 ± 0.47 ^{b}	66.67	65.00 ± 0.68 ^{a}	84.52	
Papaya seeds extract (PSE)	0.5 %	112.00 ± 0.64 ^{d}	37.43	49.00 ± 0.62 ^{d}	62.02	57.00 ± 0.68 ^{b}	41.24	100.00 ± 0.79 ^{cd}	55.56	150.0 ± 0.84 ^{d}	64.29	
	1 %	72.00 ± 0.58 ^{c}	59.78	32.00 ± 0.31 ^{c}	75.19	46.00 ± 0.58 ^{b}	52.58	86.00 ± 0.48 ^{bc}	61.78	83.00 ± 0.62 ^{b}	80.24	20.205 A
	2 %	36.00 ± 0.34 ^{ab}	79.89	14.00 ± 0.12 ^{ab}	89.15	28.00 ± 0.30 ^{a}	71.13	45.00 ± 0.32 ^{a}	80.00	51.00 ± 0.25 ^{a}	87.86	
Control		179.00 ±0.87 ^{e}		129.00 ± 0.91 ^{e}		97.00 ± 0.75 ^{c}		225.00 ± 0.85 ^{e}		420.00 ±1.02 ^{f}		65.298 B
LSD 5%		23.651 B		17.687 A		11.548 B		24.759 B		55.463 A		

R%= Reduction Percent, Results are mean values of three replicates ± standard deviation

where they recorded 91.47, 83.72 & 71.32 %, 88.27, 75.42 & 53.07 %, and 79.38, 68.04& 48.45 % reduction for CSE, and 89.15, 75.19 & 62.02 %, 79.89, 59.78 & 37.43 %, and 71.13, 52.58& 41.24 % reduction with PSE at 2 %, 1 %, and 0.5 % respectively. While each of *P. expansum* and *F. oxysporum* was more affected by PSE than CSE, where they gave 87.86, 80.24 & 64.29 %, and 80.00, 61.78& 55.56% reduction with PSE, and 84.52, 71.43& 50.00% and 66.67, 57.78 & 52.44 % reduction with CSE at the same concentrations respectively.

3.4.3. Effect of the ethanolic CSE and PSE on the mycelial dry weight of the aflatoxigenic fungi (*Aspergillus parasiticus*)

The effect of the ethanolic CSE and PSE on the mycelial dry weight of the aflatoxigenic fungi (*Aspergillus parasiticus*) was evaluated by employing various concentrations (0.5 %, 1 %, and 2 %). Data in Table 5 indicated that CSE and PSE reduced significantly ($P < 0.05$) the mycelial dry weight of the aflatoxigenic fungus at all concentrations used compared with untreated control. Also, the reduction percentage was increased by increasing the concentration used. On the other hand, the highest reduction percent was recorded with CSE, which recorded 77.23, 72.51 & 69.11 % at 2 %, 1 %, and 0.5 % respectively, while PSE gave 74.35, 66.23 & 23.56 % at the same concentrations respectively.

3.5. Anti-aflatoxigenic activity

3.5.1. Effect of the ethanolic CSE and PSE on aflatoxins production *in vitro*

The effect of the ethanolic CSE and PSE on aflatoxins production by the aflatoxigenic fungi (*Aspergillus parasiticus*) in the YES medium was evaluated by employing different concentrations (0.5 %, 1%, and 2%). Data in Table 6 and Fig.3 indicated that CSE and PSE reduced the aflatoxins production by the aflatoxigenic fungus at all concentrations used compared with untreated control. Also, the reduction percentage was increased by increasing the concentration used. On the other hand, the highest reduction percent of the total aflatoxins produced was recorded with CSE which recorded 77.04, 39.73& 10.90 % at 2%, 1%, and 0.5% respectively, where it reduced the total aflatoxins production from 57.447 µg/ mL in control (untreated) to 13.187, 34.625 & 51.184 µg/ mL for the same concentrations respectively. While PSE reduced the total aflatoxins production from 57.447 µg/ mL in control to 19.137, 35.027& 53.405 µg/ mL with 66.69, 39.03 & 7.04% reduction for the same concentrations respectively.

Table 5 Effect of the ethanolic CSE and PSE on the mycelial dry weight of the aflatoxigenic fungi (*Aspergillus parasiticus*)

Plant extract	Conc.	*Aspergillus parasiticus*		LSD 5%
		Mycelial dry weight (g)	R%	
Chia seeds Extract (CSE)	0.5 %	1.18 ±0.27 [a]	69.11	0.387 A
	1 %	1.05 ±0.17 [a]	72.51	
	2 %	0.87 ± 0.10 [a]	77.23	
Papaya seeds Extract (PSE)	0.5 %	2.92 ± 0.28 [b]	23.56	2.589 A
	1 %	1.29 ±0.19 [a]	66.23	
	2 %	0.98 ±0.23 [a]	74.35	
Control		3.82 ±0.36 [c]		2.484 B

R%= Reduction Percent

Table. 6 Effect of the ethanolic CSE and PSE on the reduction of aflatoxins production *in vitro*

AFs Type	Contro (µg/ml	Chia seeds extract (CSE)						Papaya seeds extract (PSE)					
		0.5%		1%		2%		0.5%		1%		2%	
		Conc. (µg/mL)	R%	Conc. (µg/mL)	R%	Conc. (µg/mL)	R%	Conc. (µg/mL)	R%	Conc. (µg/mL)	R%	Conc. (µg/mL)	R%
AFB_1	55.294	50.397	8.86	34.103	38.32	12.966	76.55	52.183	5.63	34.449	37.70	18.849	65.91
AFG_1	0.091	0.053	41.76	0.042	53.85	0.017	81.32	0.067	26.37	0.051	43.96	0.036	60.44
AFB_2	1.862	0.568	69.50	0.410	77.98	0.159	91.46	1.008	45.86	0.527	71.70	0.252	86.47
AFG_2	0.198	0.166	16.16	0.070	64.65	0.045	77.27	0.147	25.76	ND	100	ND	100
Total	57.447	51.184	10.90	34.625	39.73	13.187	77.04	53.405	7.04	35.027	39.03	19.137	66.69

ND =Not Detected, R%= Reduction Percent

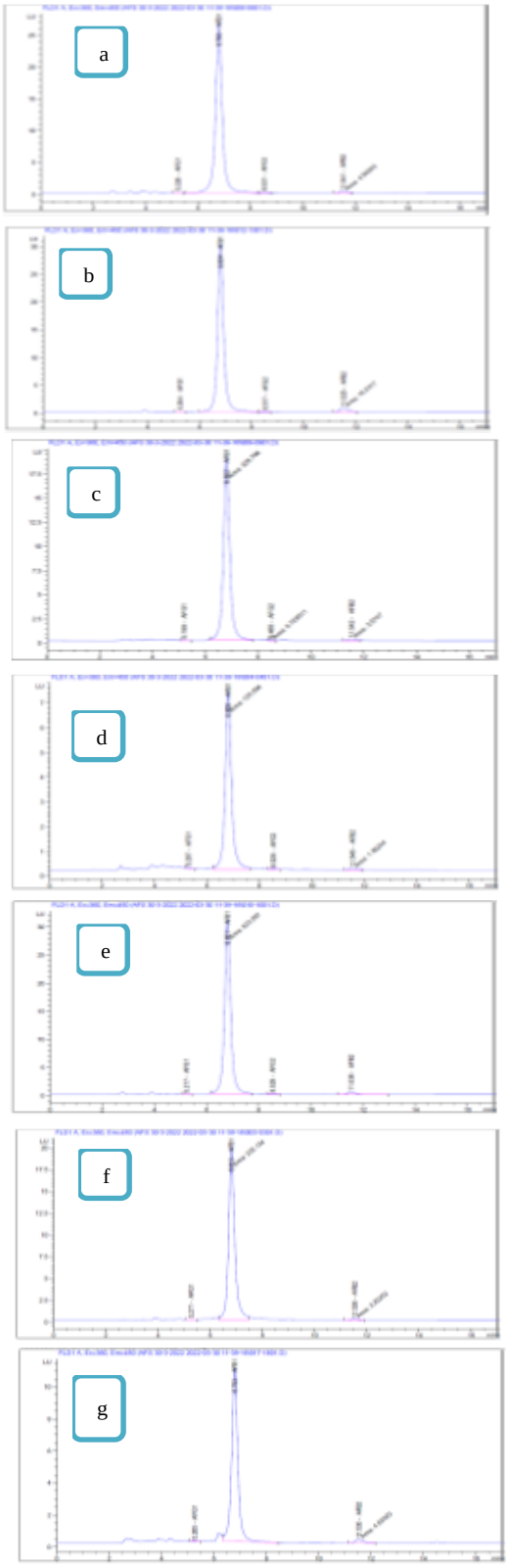

Figure 3. Chromatograms of aflatoxins (B1. G1, B2, and G2) produced by *Aspergillus parasiticus*, a- Control (untreated), b- CSE at 0.5%, c- CSE at 1%. d- CSE at 2%, e- PSE at 0.5%, f- PSE at 1%, and g- PSE at 2%.

4. Discussion

Studying the chemical structure of seeds is considered a primary step toward knowing their components and predicting their properties and activities. Analysis of the chemical composition of chia and papaya seeds showed that they contain 33.4 % and 18.4% of oil, 25.5 %, and 31.5% of protein, and 12.4% and 19.1% of fiber respectively. Chia seeds are rich in macro and micronutrients and dietary fiber, which are mostly present in the water-insoluble fraction, according to Ullah et al., [28]. Chia seeds were shown to have high levels of mucilage, lipids, proteins, ash, and nitrogen-free extract [29]. Similar to our results, Ngozika et al., &Sugiharto, [30; 31] stated that *Carica papaya* seed analysis revealed a high amount of fat, carbs, and protein all of which are important for building up the body and making it a suitable source of energy for malnourished people. Dietary fiber is included in seeds and aids in decreasing cholesterol and eliminating toxins from the digestive system [32]. Recent research indicates that several external factors, including geographic origin, climatic conditions, farming practices, and extraction methods, might affect the composition and concentration of important bioactive constituents in seeds [13].

Almost seeds contain phytochemical compounds such as phenolics and flavonoids that are released when treated with different polar solvents such as ethanol as in the current study. Phenolic and flavonoid compounds of CSE and PSE showed that they contain flavonoids more than phenolic compounds. Similar results were obtained by Masfufatun et al., [34], who mentioned that the papaya seed ethanolic extract phytochemical test revealed the presence of flavonoids, tannins, polyphenols, steroids, alkaloids, and saponins. According to Olcum et al., [35], *Carica papaya* seeds were found to be a rich source of phytochemicals, including flavonoids, tannins, alkaloids, glycosides, and phenols. The categories of phenolic compounds in the hydro methanol extract of chia seeds were quantified by Dib et al., [36]. Lower than our results, Scapin et al. [37] found that the concentration of flavonoids in the dry chai sample was 0.162 g QE/kg, whereas the concentration of phenolic compounds was 2,639 g GAE/kg. Because phenolic compounds exhibited OH groups, they have a major scavenging capability [38]. On the other hand, both chai and papaya seeds ethanolic extract exhibited the ability to scavenge the DPPH radical. However, it was noted that Papaya seeds extract more efficient (64.6 ± 0.5 %) compared with Chai seed extract (50 ± 0.5 %) this is might be attributed to that PSE is higher in total phenolic content (10.72) than CSE such as caffeic,

cholorogenic, galic, ellagic acids. There were a sustainable relationship between the content of phenolic compounds and antioxidant activity [39].

The aqueous extract from *C. papaya* seeds is safe and serves as a strong scavenger of free radicals, shielding Detroit 550 fibroblasts from the oxidative damage caused by H_2O_2 [40]. HPLC analysis of CSE was as the same as determined by Abdel-Aty et al., [41]. They found phenolic acids (gallic acid, p-hydroxybenzoic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, synapic acid, and cinammic acid) and flavonoids (apigenin, quercetin, chrysin). According to Ullah et al., [28]; Grancieri et al., [42], the main components of chia seeds that are related to health benefits are their phenolic acids, flavonols, isoflavonols. On the other hand, betacarotinoid, lycopene, benzyl isothiocyanate, benzylglucosinolate, betacryptoxanthin, chlorogenic acid, p-coumaric acid, quercetin-3-galactoside, protocatechuic acid, caffeic acid, quercetin, and other significant phytochemicals can be found in *C. papaya* seeds extract [43, 44]. All plant parts; including seeds, roots, tubers, leaves, and flowers, as well as various by-products, contain cholorogenic acids [45]. There are more than 5000 plant compounds according to Huang et al., [46]. Ellagic acid exhibited many benefits such as antimicrobial, antimutagenic, and human immunodeficiency virus (HIV) [47, 48, 49]. Because of its advantageous antioxidant characteristics, caffeic acid plays a part in the treatment or prevention of diseases like diabetes, cancer, and inflammation [50].

An assessment of the antifungal potential of different concentrations (0.5%, 1%, and 2%) of ethanolic CSE and PSE against *Alternaria alternata*, *Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum*, and *Penicillium expansum* showed a significant decrease ($P < 0.05$) in both the mycelial growth and spore viability of all tested fungi. Additionally, when compared with the untreated control, both CSE and PSE notably reduced the mycelial dry weight of the aflatoxigenic fungi (*Aspergillus parasiticus*) at all concentrations used. Moreover, the reduction percentage increased with higher concentrations. Our findings align with previous studies on this topic. Abd El-Zaher, [52] observed that the extract from *Carica papaya* seeds demonstrates a decrease in the survival rate of *A. flavus* cells as the concentrations of *C. papaya* seed extract increased. Güzel et al., [53] reported that the ethanol extract of chia seeds exhibited notable antifungal effects. Abdel-Hameed et al., [12] observed that both papaya seed extract and papaya seed oil exhibited antifungal activity against various pathogenic fungal strains, including *Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus niger, Fusarium oxysporum,* and *Candida albicans*. Motyka et al., [13] noted that extracts from different parts of chia (*Salvia hispanica*) such as seeds, leaves, flowers, sprouts, roots, and herbs displayed significant antifungal properties. The variations in the antifungal properties of ethanolic CSE and PSE may be attributed to the variations in their phytochemical compositions. The observed antifungal activity in these extracts may be linked to the active compounds found in the extracts, such as phenolic compounds (such as ellagic acid, ferulic acids, caffeic acid, and p-coumaric acid) which are known for their antimicrobial effects [54]. Furthermore, flavonoids (like catechin, kaempferol, apigenin, and quercetin) are recognized for their pivotal roles in eliciting antifungal effects [55]. The inhibition mechanism involving these compounds works by suppressing the actions of extracellular fungal enzymes (cellulase, pectinase, laccase, xylanase, etc.) and interfering with cellular membrane functions in pathogenic fungi [56]. These compounds also affect fungal growth by nutrient deprivation of substrates (such as metal complexation, and protein insolubilization) and impact fungal membranes (inhibiting oxidative phosphorylation) [57].

The assessment of the anti-aflatoxigenic effects of varied concentrations (0.5%, 1%, and 2%) of ethanolic CSE and PSE against *Aspergillus parasiticus* revealed that both CSE and PSE notably decreased the production of aflatoxins by the aflatoxigenic fungus (*A. parasiticus*) in the YES medium, at all concentrations compared to the untreated control. Furthermore, the reduction percentage increased as the concentration of the extracts increased. Notably, the highest reduction in the total aflatoxins produced was observed with CSE (77.04% at a concentration of 2%), compared to 66.69% with PSE at the same concentration. To the best of our knowledge, there are no reports available on the inhibitory activity of CSE and PSE on the biosynthesis of aflatoxins from *A. parasiticus*. In the present investigation, the anti-aflatoxigenic activity of these ethanolic extracts has been demonstrated for the first time. The anti-aflatoxigenic activity of CSE and PSE may be attributed to their phenolic contents (such as gallic acids, vanillic acids, and caffeic acids) and flavonoids. These findings align with Mallozzi et al., [58], who indicated that flavonoids have the potential to decrease aflatoxin production. In a similar vein, Wahdan, [59] highlighted the inhibitory effects of phenolic acids, such as vanillic and caffeic acids found in plant extracts, on the growth of *Aspergillus flavus* and *A. parasiticus*, along with their aflatoxin production. Moreover, Al-Rahmah et al., [60] supported this by explaining that phenolic compounds within plant extracts are crucial in

hindering the fungal growth and production of aflatoxins, by inhibiting one or more early steps in the AFB1 biosynthesis pathway. On the other hand, the inhibition of AF production may be explained in light of the findings of Holmes et al., [61], who suggested that phenolic compounds, which inhibit the production of aflatoxins, might work by influencing the environmental and physiological factors that regulate mycotoxin biosynthesis, or by altering the signal transduction pathways preceding the biosynthetic pathway. Additionally, according to Tian et al., [62], the dose-dependent reduction in aflatoxin content might occur due to the deactivation of norsolorinic acid and alteration in carbohydrate catabolic enzyme activity, which directly impacted sporulation inhibition and ultimately resulting in reduced aflatoxin biosynthesis.

5. Conclusion

The current study confirmed that the ethanolic extracts of chia and papaya seeds could be valuable sources of bioactive compounds with substantial biological activities including antioxidant, antifungal, and anti-aflatoxigenic. It was noted that CSE and PSE are rich in flavonoids than phenolic. Both seeds extracts exhibited the ability to scavenge the DPPH radical. In contrast, PSE exhibited the scavenging ability more than CSE. Caffeic and Chlorogenic acids were the major detected PC in PSE, while Ellagic acid and Vanillin were wealthy in CSE. It can be concluded that both extracts exhibited a significant impact on inhibiting the growth and spore viability of the mycotoxigenic fungi (*Alternaria alternata*, *Aspergillus flavus*, *A. parasiticus*, *Fusarium oxysporum*, and *Penicillium expansum*), and reduced the mycelial dry weight and aflatoxins production of aflatoxigenic fungi (*Aspergillus parasiticus*). Moreover, these extracts can be recommended as potentially effective and environmentally safer alternative fungicides.

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