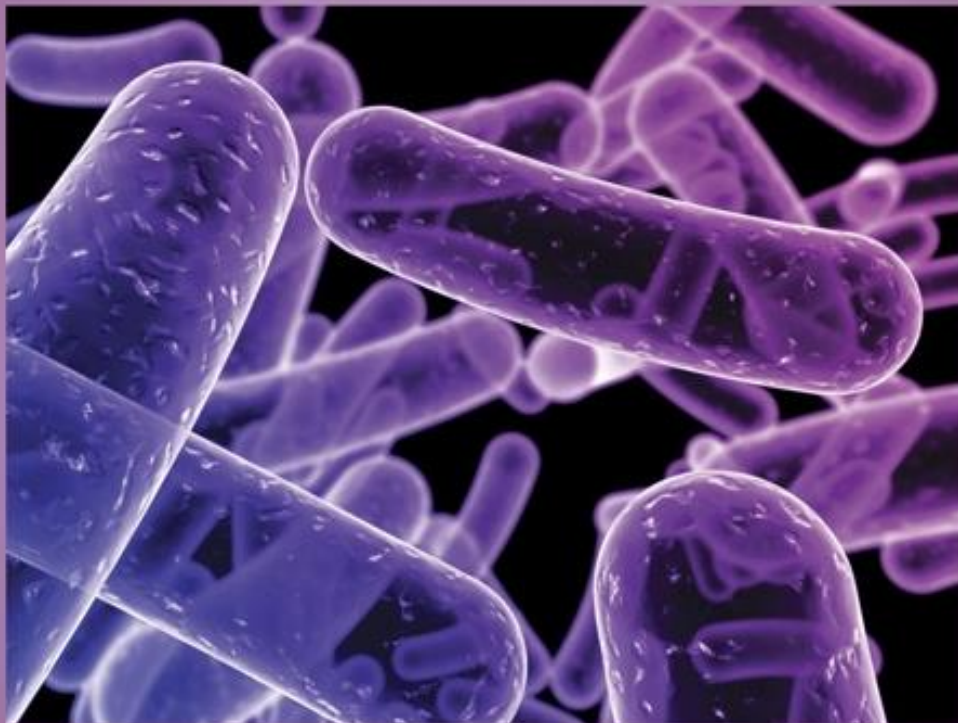
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

MICROBIOLOGY

G



ISSN
2090-0872

WWW.EAJBS.EG.NET

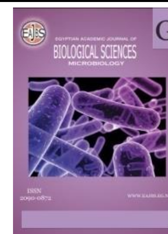
Vol. 16 No. 1 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.16 (1) pp.55- 72 (2024)*
DOI: 10.21608/EAJBSG.2024.353069

Egypt. Acad. J. Biolog. Sci., 16(1):55-72 (2024)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/



Assessment of Antifungal Activity of Orange and Pomegranate Peel Extracts Against Mycotoxigenic Fungi Associated Guava Fruits (*Psidium guajava* L.)

Marwa A. Younos
Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt.
*E.Mail: mh.younos@nrc.sci.eg

ARTICLE INFO
Article History
Received:2/4/2024
Accepted:3/5//2024
Available:7/5/2024

***Keywords*:**
Fruit Peel extracts, antioxidant activity, antifungal activity, mycotoxigenic fungi, guava fruits.

ABSTRACT

Using plant peel extracts as natural preservatives provides two advantages due to their ability to combat pathogens in food with antimicrobial properties and minimize agro-industrial wastes. So the main objective of the current study was to assess the effectiveness of the antifungal properties of ethanolic extracts of orange and pomegranate peels (OPE and PPE) against mycotoxigenic fungi-associated guava fruits. The occurrence of mycotoxigenic fungi was assessed. The polyphenolic compounds of the ethanolic OPE and PPE were elucidated. Each of their antioxidant and antifungal activities against isolated mycotoxigenic fungi was evaluated. The obtained data indicated that *Botrydiplodia theobromae* was the most abundant fungi. Test of mycotoxins production confirmed that *Alternaria alternata* (isolate No. 2) was found to produce 0.230 µg/ml of Alternariol toxin, while *Penicillium expansum* isolate No. (1) produced 4.260 of Patulin toxin. *A. parasiticus* isolates (No. 3& 7) produced Aflatoxins with a concentration of 0.012 and 0.020 µg/ml respectively. On the other hand, PPE contained the highest phenolic and flavonoid compounds contents and exhibited the highest antioxidant and antifungal activities. It could be concluded that the ethanolic PPE and OPE had significant antifungal effects against mycotoxigenic fungi attacking guava fruits.

INTRODUCTION

Fruits are essential for maintaining good health as they provide important vitamins and minerals needed for growth in our daily diet (Bankole 2004). Guava, a tropical fruit from the Myrtaceae family, is known for its high nutritional value and is even considered a "super fruit" (Joseph and Mini 2014). Guava fruit is rich in vitamins A, B1, B2, lycopene, lutein, zeaxanthine, dietary minerals, folic acid, copper, manganese and potassium (Rahman *et al.,* 2003). Fruits, in general, have high sugar content and their low acidity levels make them prone to fungal deterioration (Singh and Sharma 2007). The main culprits in fruit deterioration are microorganisms, particularly fungi, and bacteria, which secrete enzymes that degrade the cell walls (Ayanda *et al.,* 2013). Additionally, many fungal species can produce harmful mycotoxins, such as aflatoxins, ochratoxin, fumonisins, zearalenone, and deoxynivalenol, which can have a direct effect on human health (Pitt 2000).

Currently, synthetic fungicides are the primary method used to control postharvest fungal decay. However, concerns about chemical residues and the emergence of resistant strains of pathogens have resulted in an increased search for safer and more environmentally friendly alternatives (Mari *et al.,* 2014). Plant extracts have attracted particular interest due to their powerful antimicrobial properties and their perceived safety, as they are derived from natural sources, easily decomposable, and have low toxicity to the environment (Cabral *et al.,* 2013). By-products obtained from fruits and vegetables are rich sources of bioactive compounds and are renowned for their antimicrobial and antioxidant characteristics (Dilucia *et al.,* 2020). Among fruit and vegetable by-products, Pomegranate (*Punica granatum* L.) peel, which accounts for approximately 40-50% of the total fruit weight, is a significant by-product of pomegranate juice, jam, and jelly production (Gullon *et al.,* 2016). The pomegranate peel contains higher levels of phenolic acids, flavonoids, tannins, and other compounds compared to other parts of the fruit (Akhatar *et al.,* 2015). These bioactive compounds have been found to offer significant health advantages, including antioxidant and anti-cancer effects (Mushtaq *et al.,* 2015), as well as strong antimicrobial activity against harmful bacteria that cause spoilage and disease (Sun *et al.,*2021). Sweet orange (*Citrus sinensis* L.), which is widely consumed as fresh juice, constitutes about 70% of the overall global production and consumption of citrus fruits. Orange peel waste is generated in significant amounts, and it has the potential to have high economic value because of its high concentration of bioactive compounds such as polyphenols (flavonoids and phenolic acids), essential oils, carotenoids, ascorbic acid, dietary fiber, and trace elements (Sharma *et al.,* 2017). The polyphenolic extract obtained from orange peels has been discovered to have a range of positive effects, such as antioxidant, anti-cancer, and anti-inflammatory properties, and antimicrobial properties (Casquete *et al.,* 2015).

Hence, the aim of this study was to evaluate the antioxidant and antifungal activities of ethanolic extracts from orange and pomegranate peels against mycotoxigenic fungi commonly associated with guava fruits.

MATERIALS AND METHODS

Collected Samples:

Five samples of Guava fruits (*Psidium guajava* L.) were randomly gathered from four distinct locations in Egypt in 2023. Each sample was placed in a sterile polyethylene bag and transported to the microbiology laboratory for analysis within 24 hours of collection.

Plant Material Used:

Fresh oranges (*Citrus sinensis*, var. common Balady) and pomegranates (*Punica granatum*, var. Wonderful) were bought from local Egyptian markets in 2023. The fruits were cleaned with tap water, rinsed with sterilized distilled water, and peeled. The peels were air-dried for multiple days and then dehydrated in an oven at 40 °C for 24 hours (Dahham *et al.,* 2010). The powdered peels of all fruits were finely ground and stored at 4°C.

Isolation and Identification of Fungi Associated with Guava Fruits:

Naturally infected 5 guava fruit samples collected from each of the 4 different localities were washed with distilled water and then disinfected by immersing them in 70% ethanol for a duration of 2 minutes. After sterilization, the fruits were washed twice with sterile double-distilled water (5 minutes each) and left to dry for one hour in a laminar flow. A section of the spoiled guava fruit was delicately cut using a sterile blade, and the sliced portions were then placed on a sterile Potato dextrose agar (PDA) medium with 2% tetracycline to prevent bacterial growth. Each tested sample was replicated three

times. Incubation was carried out at a temperature of 28 ± 2°C for five days until fungal growth became visible. The growing fungi were isolated, counted, and documented. The total fungal count and fungal frequency percentage of naturally-occurring fungi were calculated as:

$$\text{The fungal frequency} = \frac{\text{The number of isolates of species}}{\text{The total number of fungal isolates}} \times 100$$

Pure cultures were microscopically identified based on cultural and morphological traits on specific media and following references such as Barent and Hunter (1977); Raper and Fennel (1965) for imperfect fungi genera and Singh (1991) for Aspergilli, Fusaria, and Penicillia at the Plant Pathology Dept., National Research Centre (NRC), Egypt. The total fungal count and the percentage of fungal frequency were recorded during the analysis.

Determination of Mycotoxins Produced by Fungi Isolated from Guava Fruits:

All isolated fungi (*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus parasiticus*, and *Penicillium expansum*) were undergone testing for mycotoxin production. Alternariol toxin production by *A. alternata* was achieved by cultivating it on a rice medium, followed by extraction according to the method outlined in Torres *et al.* (1998), and the quantity was determined using HPLC as detailed in Nawaz *et al.* (1997). Pure cultures of *A. flavus* and *A. parasiticus* were grown in 100 mL yeast extract sucrose medium to assess Aflatoxins production as described in Embaby *et al.* (2022); Munimbazi and Bullerman (1998), and extracted and quantified by HPLC as described in Kumar *et al.* (2010); Rubert *et al.* (2012). The production of Patulin by *P. expansum* isolates involved culturing them on Malt Yeast Extract Agar medium, and extracted according to the procedure described by Neri *et al.* (2010), and quantified by HPLC according to Christian (1990).

Preparation of Ethanolic Orange and Pomegranate Peel Extracts:

Three hundred and fifty grams of powdered orange and pomegranate peels were combined with 2 L of a solution consisting of 80% ethanol and water and then shaken for a duration of 3 days at room temperature (Klangpetch *et al.,* 2016). The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated by evaporating the solvents with a rotary evaporator (Mostafa *et al.,* 2018). Each extract was filter-sterilized (0.45 µm), stored in opaque bottles, and refrigerated at 4°C for further use.

Determination of Total Soluble Phenolics:

The phenolic compound content of OPE and PPE was assessed following the procedure outlined by Fu *et al.* (2014). Total phenolic content was quantified by utilizing a calibration curve established with gallic acid and reported as milligrams of gallic acid equivalent (GAE) per gram of the sample, as per the method described by Zilic *et al.* (2012).

Determination of Total Flavonoids:

The quantification of soluble flavonoids in OPE and PPE was carried out following the procedure outlined by Kanatt *et al.* (2011). The total flavonoid content was assessed by creating a calibration curve and reported as milligrams of catechin equivalent (CE) per gram of the sample.

Evaluation of the Antioxidant Activity of OPE and PPE by DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical-Scavenging:

The DPPH radical-scavenging activity was evaluated following the procedure outlined by De Ancos and colleagues (2002). The decrease in the DPPH radical was quantified at 517 nm. The outcomes were presented as the percentage of inhibition of the DPPH, calculated using the subsequent 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

High-performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds of OPE and PPE

High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1260 series system. Separation was performed on an

Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase comprised water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) flowing at a rate of 0.9 mL/min. A linear gradient program was employed for the mobile phase: 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). Detection was done at 280 nm using a multi-wavelength detector. Each sample solution was injected in a volume of 5 µl, and the column temperature was maintained at 40 °C. Prior to injection, all samples were filtered through a 0.45 µm Acro-disc syringe filter from Gelman Laboratory in MI. Peaks were authenticated by comparing their retention times and UV spectra with those of the standards (Kuyng *et al.,* 2006).

Evaluation of the Antifungal Activity of The Ethanolic Extracts of Orange and Pomegranate Peels *in vitro:*

Effect of OPE and PPE on the Mycelial Growth of Isolated Fungi:

The ethanolic extracts of OPE and PPE were evaluated for their impact on the mycelial growth of isolated fungi (*Alternaria alternata, Aspergillus flavus, Aspergillus parasiticus,* and *Penicillium expansum*) *in vitro*. Each sterilized extract was individually mixed with sterilized Potato Dextrose Agar (PDA) medium at various concentrations (0.5%, 1%, and 2% v/v) in sterilized Petri dishes. A control was maintained using a PDA medium devoid of plant extracts. In separate Petri dishes, 5 mm-discs containing 7-day-old mycelium of each fungus were inoculated in the center and incubated at a temperature of 28 ± 2°C. Three replicate plates were utilized for each treatment. Following a 7-day incubation period, the diameter of the fungal colonies was measured, in accordance with the methods outlined by Singh *et al.* (2014); Younos and Abdel-Galil (2023). A medium free of extract served as the control in the experiment. The reduction percent of mycelial growth was calculated according to Jabeen *et al.* (2013) by using a formula:

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

Where C = growth in control, T = growth in treatment

Effect of OPE and PPE on the Spore Viability of Isolated Fungi:

The effectiveness of the ethanolic extracts of OPE and PPE on the spore viability of isolated fungi (*Alternaria alternata, Aspergillus flavus, Aspergillus parasiticus,* and *Penicillium expansum*) was examined utilizing varying concentrations (0.5%, 1%, and 2%). A 0.5 cm diameter disc of each seven-day-old fungal culture on PDA was positioned at the center of each Petri dish and subsequently incubated at 28 ± 2°C for 5 days. To collect the generated spores, 9 mL of sterile water was poured over the developed fungal mycelium, separated with a drawing brush, and then filtered through a muslin cloth to obtain a spore suspension. The concentration of the collected spore suspension was adjusted to 1 x 10^2 conidia/mL using a Haemocytometer slide. Each sterilized extract was individually mixed with sterilized PDA medium at different concentrations (0.5%, 1%, and 2% v/v) in sterilized Petri dishes, with a PDA medium lacking plant extracts serving as the control. Every PDA plate was inoculated with 1 mL of spore suspension (containing 1 x 10^2 conidia/mL), evenly distributed across the plate, and incubated at 28 ± 2°C. Each treatment was replicated on three separate plates. After 48 hours of incubation, the percentage of germinated spores was calculated based on the method described by Meena and Mariappan (1993).

Statistical Analysis:

Data obtained in this research were analyzed utilizing software (IBM SPSS Statistics v.16, USA). Statistical significance was evaluated through a one-way Analysis of Variance (ANOVA) test. A p-value of less than 0.05 was deemed to be statistically significant. The Least Significant Difference (LSD) was computed at a significance level of $P \leq 0.05$ following

the method outlined by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Total Fungal Isolates Attacking Tested Guava Fruit Samples:

Isolation of microflora associated with guava fruit samples collected from four different locations resulted in 474 fungal isolates as shown in Table 1. On the other hand, data indicated that location B had the greatest number of fungal isolates (161 isolates 33.97%) followed by location A (121 isolates 25.53%), and location C (110 isolates 23.21%), while location D had the least fungal isolates which recorded 82 isolates (17.30%).

Table 1. Total fungal isolates attacking tested guava fruit samples

Samples		Location				Total
		A	B	C	D	
1	T.C	9	12	17	9	47
	%	1.90	2.53	3.59	1.90	9.92
2	T.C	6	22	33	8	69
	%	1.27	4.64	6.96	1.69	14.56
3	T.C	36	45	27	24	132
	%	7.59	9.49	5.70	5.06	27.85
4	T.C	38	31	17	26	112
	%	8.02	6.54	3.59	5.49	23.63
5	T.C	32	51	16	15	114
	%	6.75	10.76	3.38	3.16	24.05
Total		121	161	110	82	474
%		25.53	33.97	23.21	17.30	100

T.C= Total Count

Fungal Frequencies Associated with Tested Guava Fruit Samples:

The Identification of fungal species attacking guava fruit samples revealed the presence of eleven fungal species from nine different genera, as detailed in Table 2. These are *Alternaria alternata, Aspergillus niger, A. flavus, A. parasiticus, Botrydiplodia theobromae, Colletitricum gloeosporioides, Mucor* sp.*, Penicillium expansum, Phoma* sp.*, Rhizoctonia solani,* and *Rhizopus stolonifer*. On the other hand, *Botrydiplodia theobromae* exhibited the highest occurrence of fungi (34.18%), followed by *Rhizoctonia solani* (29.75%), *Colletitricum gloeosporioides* (13.29%), *Mucor* sp. (9.07%), *Rhizopus stolonifer* (7.38%), *Aspergillus niger* (1.90%), *A. parasiticus* (1.48%), *Penicillium expansum* (1.27%), *Alternaria alternata* (1.05%), and *A. flavus* (0.42%). A lower occurrence of fungi was noted with *Phoma* sp., which recorded 0.21%.

Determination of Mycotoxins Production:

Testing for mycotoxins produced by mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) gathered from guava fruits revealed that *Alternaria alternata* (isolate No. 2) from location A sample was found to produce 0.230 µg/ml of Alternariol toxin, while *Penicillium expansum* isolate No. (1) from the same location produced 4.260 of Patulin toxin. Aflatoxins were produced by *A. parasiticus* (isolate No. 3) from location B with a concentration of 0.012 µg/ml (0.007 µg/ml AFB2 and 0.005 µg/ml AFG2) and *A. parasiticus* (isolate No. 7) from location D produced 0.020 µg/ml (0.009 µg/ml AFB2 and 0.010 µg/ml AFG2). None of the *A. flavus* isolates was aflatoxins producer, as shown in Table 3.

Table 2. Fungal frequencies associated with tested guava fruit samples

Fungi		Location A	B	C	D	Total
Alternaria alternata	T.C	2	2	NF	1	5
	%	0.42	0.42	-	0.21	1.05
Aspergillus niger	T.C	3	4	1	1	9
	%	0.63	0.84	0.21	0.21	1.9
Aspergillus flavus	T.C	NF	2	NF	NF	2
	%	-	0.42	-	-	0.42
Aspergillus parasiticus	T.C	NF	6	NF	1	7
	%	-	1.27	-	0.21	1.48
Botrydiplodia theobromae	T.C	61	55	29	17	162
	%	12.87	11.6	6.12	3.59	34.18
Colletitricum gloeosporioides	T.C	53	2	7	1	63
	%	11.18	0.42	1.48	0.21	13.29
Mucor sp.	T.C	NF	7	20	16	43
	%	-	1.48	4.22	3.38	9.07
Penicillium expansum	T.C	2	2	NF	2	6
	%	0.42	0.42	-	0.42	1.27
Phoma sp.	T.C	NF	NF	NF	1	1
	%	-	-	-	0.21	0.21
Rhizoctonia solani	T.C	NF	75	36	30	141
	%	-	15.82	7.59	6.33	29.75
Rhizopus stolonifer	T.C	NF	6	17	12	35
	%	-	1.27	3.59	2.53	7.38
Total	T.C	121	161	110	82	474
	%	25.53	33.97	23.21	17.3	100

T.C= Total Count

Table 3. Determination of mycotoxin production

L	Mycotoxigrnic fungi	Isolate No.	Mycotoxins (µg/ml) Alternariol	Aflatoxins AFB_1	AFG_1	AFB_2	AFG_2	Total	Patulin
A	*Alternaria alternata*	2	0.230	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	1	-	-	-	-	-	-	4.260
B	*Alternaria alternata*	-	ND	-	-	-	-	-	-
	Aspergillus flavus	-	-	ND	ND	ND	ND	ND	-
	Aspergillus parasiticus	3	-	ND	ND	0.007	0.005	0.012	-
	Penicillium expansum	-	-	-	-	-	-	-	ND
C	*Alternaria alternata*	NF	-	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	NF	-	-	-	-	-	-	-
D	*Alternaria alternata*	--	ND	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	7	-	ND	ND	0.009	0.010	0.020	-
	Penicillium expansum	-	-	-	-	-	-	-	ND

L= Location, ND=Not detected, NF=Not Found

Assessment of Overall Phenolic and Flavonoid Content, As Well As Antioxidant Activity of OPE and PPE:

The total phenolic, flavonoid compounds and antioxidant activity of OPE and PPE were illustrated in Table 4. It showed that PPE contained 37.423 mg GAE / gram of total phenols and 17.750 mg CE / gram of total flavonoids. While OPE contained 2.130 mg GAE / gram of total phenols and 0.354 mg CE /gram of total flavonoids. On the other hand, both extracts

demonstrated the capacity to neutralize the DPPH radical. However, it was noted that PPE was more efficient compared with OPE, in which PPE had the highest antioxidant activity (38.47 mg TE/gram), while OPE recorded 0.06 mg TE/gram; this might be attributed to the phytochemical constituents of each extract.

Table 4. Phenolic, flavonoid compounds and antioxidant activity of the ethanolic OPE and PPE

Sample name	Total phenol mg GAE / gram	Total flavonoid mg CE / gram	DPPH mg TE/gram
Orange peel extract (OPE)	2.130 ± 0.31	0.354 ± 0.12	0.06 ± 0.02
Pomegranate peel extract (PPE)	37.423 ± 0.76	17.750 ± 0.17	38.47 ± 0.38

GAE= Gallic acid Equivalents, CE= Catechin Equivalents, TE= Trolox Equivalents

Determination of the Polyphenolic Profile of The Ethanolic Extracts of Orange and Pomegranate Peels:

The polyphenolic profile of OPE and PPE were detected using High-performance liquid chromatography (HPLC). Data indicated that Catechin (2366.25 µg/g), Ferulic acid (2017.14 µg/g), and Chlorogenic acid (1334.75 µg/g) were detected as major constituents in OPE, and other trace amounts of other phenolic compounds, while Gallic acid (9264.63 µg/g), Ellagic acid (4934.90 µg/g), and Catechin (4708.96 µg/g) were detected as major compounds in PPE, and other trace amounts of other phenolic compounds as shown in Table (5) and Figure (1).

Table 5. The polyphenolic profile of the ethanolic OPE and PPE

Phenolic compounds	Orange peel extract (OPE) (µg/g)	Pomegranate peel extract (PPE) (µg/g)
Gallic acid	69.45	9264.63
Chlorogenic acid	1334.75	0.00
Catechin	2366.25	4708.96
Methyl gallate	5.63	264.85
Caffeic acid	0.00	70.29
Syringic acid	190.90	54.36
Pyro catechol	52.76	0.00
Rutin	115.72	0.00
Ellagic acid	615.02	4934.90
Coumaric acid	6.02	0.00
Vanillin	46.59	22.80
Ferulic acid	2017.14	39.32
Naringenin	57.56	67.52
Daidzein	25.48	0.00
Quercetin	26.26	0.00
Cinnamic acid	0.00	0.00
Apigenin	8.42	290.13
Kaempferol	30.74	0.00
Hesperetin	0.00	0.00

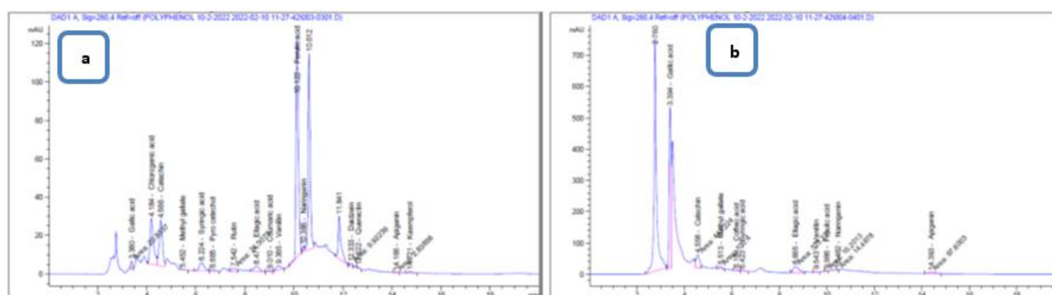


Fig.1 HPLC chromatograms of the polyphenolic profile of a- OPE, b- PPE

Determination of the Antifungal Activity of The Ethanolic Extracts Of Orange And Pomegranate Peels *in vitro:*

Effect of OPE and PPE on the Mycelial Growth of Mycotoxigenic Fungi:

The impact of OPE and PPE on the growth of isolated mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) was assessed by using different concentrations (0.5%, 1%, and 2%). Data in Table 6 indicated that OPE and PPE significantly decreased ($P < 0.05$) the fungal growth of the fungi tested at all concentrations compared to the control. Additionally, the growth inhibition significantly increased ($P < 0.05$) with higher concentrations used. Also, data confirmed that PPE had the highest antifungal activity. On the other hand, a higher reduction percent of the mycelial growth was detected with *A. parasiticus*, whereas PPE reduced significantly the mycelial growth of *A. parasiticus* with 82.67, 70.00 & 50.00 % reduction at 2, 1 & 0.5% respectively, while OPE recorded 80.00, 63.33& 50.00 % at the same concentration respectively, followed by *A. flavus*, whereas PPE gave 78.13, 75.00 & 56.25 % reduction, and OPE recorded 75.00, 68.75 & 56.25 % reduction at 2, 1 & 0.5% respectively. PPE reduced the mycelial growth of *A. alternata* with 63.81, 59.05 & 57.14 % reduction, while OPE recorded 62.86, 57.14 & 47.62 % reduction at 2, 1 & 0.5% respectively. While *P. expansum* mycelial growth was reduced by 68.75, 43.75 & 37.50 % reduction when treated with 2, 1 & 0.5% of PPE, and recorded 50.00, 31.25 & 18.75 % reductions with OPE at the same concentration respectively.

Table 6. Effect of the ethanolic OPE and PPE on the mycelial growth of mycotoxigenic fungi

Plant peel Extracts	Conc. %	*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Penicillium expansum*		LSD 5%
		linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	
Orange peel extract (OPE)	0.5	27.50 ± 0.34 a	47.62	35.00 ± 0.57 b	56.25	37.50 ± 0.61 d	50.00	65.00 ± 0.76 cd	18.75	1.002 B
	1	22.50 ± 0.27 a	57.14	25.00 ± 0.41 ab	68.75	27.50 ± 0.43 c	63.33	55.00 ± 0.34 bc	31.25	
	2	19.50 ± 0.10 a	62.86	20.00 ± 0.30 a	75.00	15.00 ± 0.27 ab	80.00	40.00 ± 0.10 ab	50.00	
Pomegranate peel extract (PPE)	0.5	22.50 ± 0.49 a	57.14	35.00 ± 0.67 b	56.25	37.50 ± 0.57 d	50.00	50.00 ± 0.82 bc	37.50	0.706 A
	1	21.50 ± 0.21 a	59.05	20.00 ± 0.43 a	75.00	22.50 ± 0.39 bc	70.00	45.00 ± 0.68 b	43.75	
	2	19.00 ± 0.15 a	63.81	17.50 ± 0.24 a	78.13	13.00 ± 0.27 a	82.67	25.00 ± 0.38 a	68.75	
Control		52.50 ± 0.64 b		80.00 ± 0.82 c		75.00 ± 0.74 e		80.00 ± 0.90 d		0.889 C
LSD 5%		0.551 B		0.975 A		0.933 A		0.855 C		

R % = Reduction Percent, Results represent the mean values of three replicates ± standard deviation. Variation in the letters within each column signifies significant variations at a P-value below 0.05.

Effect of OPE and PPE on the Spore Viability of Mycotoxigenic Fungi:

The impact of OPE and PPE on the spore viability of isolated mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) was evaluated by using various concentrations (0.5%, 1%, and 2%). Data in Table 7, indicated that OPE and PPE significantly lowered ($P < 0.05$) the spore viability of the tested fungi at all concentrations compared to the control. Additionally, the reduction percentage increased significantly ($P < 0.05$) with higher concentrations used. Also, data cleared that, PPE had the highest antifungal activity. Furthermore, the highest reduction percent was recorded with *A. parasiticus* and *P. expansum*, where PPE reduced the spore viability of *A. parasiticus* with 94.52, 86.76& 70.32% reduction, and OPE gave 89.50, 78.08 &67.12 % reduction at 2, 1 & 0.5% respectively. PPE reduced the spore viability of *P. expansum* with 93.98, 90.97 &81.94% reduction, and OPE recorded 85.81, 80.22 & 69.46 % reduction at 2, 1 & 0.5% respectively. The spore viability of *A. flavus* was reduced by 90.16, 80.33 & 71.04 % reduction when treated with PPE, and reduced by 85.79, 73.22 & 65.57 % reduction with OPE at 2, 1 & 0.5% respectively. The least reduction percent was recorded with *A. alternata*, in which PPE recorded 80.45, 72.63 & 45.25 % reduction, while OPE gave 76.54, 64.80 & 29.61 % reduction at 2, 1 & 0.5% respectively.

Table 7. Effect of the ethanolic OPE and PPE on the spore viability of mycotoxigenic fungi

Plant peel Extracts	Conc. %	*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Penicillium expansum*		LSD 5%
		Viable spores x10^2	R %	Viable spores x10^2	R %	Viable spores x10^2	R %	Viable spores x10^2	R %	
Orange peel extract (OPE)	0.5	126.00 ± 0.95 ^{d}	29.61	63.00 ± 0.86 ^{d}	65.57	72.00 ± 0.70 ^{d}	67.12	142.00 ± 0.96 ^{e}	69.46	22.673 B
	1	63.00 ± 0.78 ^{b}	64.80	49.00 ± 0.32 ^{c}	73.22	48.00 ± 0.57 ^{c}	78.08	92.00 ± 0.88 ^{d}	80.22	
	2	42.00 ± 0.59 ^{a}	76.54	26.00 ± 0.12 ^{ab}	85.79	23.00 ± 0.29 ^{ab}	89.50	66.00 ± 0.41 ^{c}	85.81	
Pomegranate peel extract (PPE)	0.5	98.00 ± 0.86 ^{c}	45.25	53.00 ± 0.37 ^{cd}	71.04	65.00 ± 0.64 ^{d}	70.32	84.00 ± 0.80 ^{d}	81.94	19.629 A
	1	49.00 ± 0.54 ^{ab}	72.63	36.00 ± 0.20 ^{b}	80.33	29.00 ± 0.24 ^{b}	86.76	42.00 ± 0.78 ^{b}	90.97	
	2	35.00 ± 0.44 ^{a}	80.45	18.00 ± 0.10 ^{a}	90.16	12.00 ± 0.18 ^{a}	94.52	28.00 ± 0.48 ^{a}	93.98	
Control		179.00 ± 1.02 ^{e}		183.00 ± 0.92 ^{e}		219.00 ± 0.85 ^{e}		465.00 ± 1.20 ^{f}		78.792 C
LSD 5%		23.086 C		24.299 B		30.621 A		65.593 A		

R % = Reduction Percent, Results represent the mean values of three replicates ± standard deviation. Variation in the letters within each column signifies significant variations at a P-value below 0.05.

DISCUSSION

Guava fruits, being rich in nutrients and possessing a low pH, provide favorable conditions for the growth of pathogenic fungi after harvest, leading to fruit deterioration (Wills and Golding 2015). By isolating fungi from tested 5 guava fruit samples collected from four different locations, a total of 474 fungal isolates were obtained, which included eleven different species: *Alternaria alternata, Aspergillus niger, A. flavus, A. parasiticus, Botrydiplodia theobromae, Colletitricum gloeosporioides, Mucor* sp., *Penicillium expansum, Phoma* sp., *Rhizoctonia solani,* and *Rhizopus stolonifer.* Several studies

have reported similar findings regarding the presence of fungal species causing post-harvest spoilage in guava fruits. For instance, Amadi *et al.* (2014) isolated fungi such as *Aspergillus parasiticus, A. niger, A. fumigatus, Fusarium oxysporum* and *Mucor* sp. from guava fruits. Ammar and El-Naggar (2014) identified *Alternaria alternata, A. raphani, Phoma* sp., *Colletotrichum dematium, Fusarium culmorum, F. sporotrichioides,* and *F. proliferatum* as causing rot in guava fruits. Embaby and Korkar (2015) detected *Aspergillus flavus, A. parasiticus, A. niger, Fusarium oxysporum, Botryodiplodia theobrome,* and *Rhizopus stolonifer* in guava fruit samples. This susceptibility to fungal diseases in fruits is attributed to factors such as their low pH, high moisture content, nutrient composition, and high levels of sugars, carbohydrates, proteins, fats, as well as low pH values and moisture content, which renders the fruits unfit for consumption (Singh and Sharma 2007).

Mycotoxigenic fungi pose significant threats to food safety on a global scale. These fungi not only cause food spoilage, resulting in loss and waste, but they also can produce toxic mycotoxins that can have serious health implications for humans and livestock (Jing *et al.,* 2014). Examination of mycotoxin production by mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) isolated from tested guava fruit samples indicated that isolate No. 2 of *Alternaria alternata* produced 0.230 µg/ml of Alternariol toxin, while isolate No. 1 of *Penicillium expansum* produced 4.260 µg/ml of Patulin toxin. *A. parasiticus* isolate No. 3 produced Aflatoxins at a concentration of 0.012 µg/ml, and isolate No. 7 of *A. parasiticus* produced 0.020 µg/ml of Aflatoxins. These findings align with similar results reported by Paster and Barkai-Golan (2008), who highlighted that *Aspergillus, Alternaria,* and *Penicillium* species are primary mycotoxigenic fungi responsible for infesting harvested fruits and vegetables, thereby contaminating the tissues with various mycotoxins such as Aflatoxins, Patulin, Trichothecenes, and Ochratoxin A. Ammar and El-Naggar (2014) also reported that *A. alternata*, associated with fruit rot in guava, produced *in vitro* significant amounts of Alternariol, Altenuene, Alternariol monomethyl ether, and Tenuazonic acid. Embaby and Korkar (2015) found that *Aspergillus parasiticus* isolates from Guava (*Psidium guajava* Linn.) samples were aflatoxins producers. According to Sajid *et al.* (2019), infection of fruits with *P. expansum* led to the formation of brown spots and was accompanied by the synthesis of patulin. Mycotoxin production is influenced by factors such as the variety of fruit or vegetables, location, climate, pre-harvest procedures, and harvesting techniques (Bora *et al.,* 2007). Additionally, the mycotoxigenic potential of fungi is influenced by the specific fungus species and strains, the composition of the substrate, and external factors like temperature and humidity (Fernández-Cruz *et al.,* 2010).

Fruit peels are rich in phenolic and flavonoid compounds, making them a subject of great interest and attention among researchers. The presence of -OH groups in the aromatic ring structure of these compounds contributes to their antioxidant potential (Khatiwora *et al.,* 2017).The assessment of overall phenolic and flavonoid levels in ethanolic extracts of orange and pomegranate peels (OPE and PPE) revealed that PPE had higher levels of total phenols (37.423 mg GAE/gram) and flavonoids (17.750 mg CE/gram), while OPE contained lower amounts of total phenols (2.130 mg GAE/gram) and total flavonoids (0.354 mg CE/gram). On the other hand, both OPE and PPE displayed the capacity to neutralize the DPPH radical. However, it was observed that PPE exhibited higher efficiency compared to OPE. These findings align with Hanafy *et al.* (2021), who reported that PPE exhibited the highest concentration of total phenolic compounds, flavonoids, and tannins, and

had the highest antioxidant capacity among the tested fruit peels (orange, pomegranate, and banana). Selahvarzi *et al.* (2021) discovered that the overall phenolic concentration in PPE was significantly higher compared to OPE, and confirmed that, PPE had a stronger antioxidant activity. El-Beltagi *et al.* (2022) also observed that ethanolic PPE showed elevated amounts of total phenolic compounds and total flavonoids compared to OPE. These variations in total phenolic and flavonoid content and the antioxidant properties can be attributed to factors such as the plant species and its origin, environmental conditions, seasonal differences, characteristics of the extraction solvent (such as polarity), and the degree of polymerization of flavonoid and phenolic compounds (Ştef˘anescu *et al.,* 2019). The higher antioxidant activity observed in PPE may be attributed to its higher content of phenols and flavonoids compared to OPE. This finding is consistent with Limei *et al.* (2022), who demonstrated that the DPPH radical scavenging capacity increased as the phenolic and flavonoid content increased.

Polyphenols found in fruit peels possess remarkable health-promoting activities, such as antimicrobial, antioxidant, antiviral, anti-allergic, anti-inflammatory, and anticarcinogenic effects, which help protect against diseases related to oxidative stress (Ma *et al.,* 2020). High-performance liquid chromatography (HPLC) analysis of the polyphenolic profile of OPE and PPE confirmed that OPE predominantly contained catechin (2366.25 µg/g), ferulic acid (2017.14 µg/g), and chlorogenic acid (1334.75 µg/g) as major constituents. On the other hand, PPE was characterized by high levels of gallic acid (9264.63 µg/g), ellagic acid (4934.90 µg/g), and catechin (4708.96 µg/g). These findings align with the research conducted by Ahmed *et al.* (2022) who reported that the most abundant polyphenolic compounds in Egyptian sweet orange peel extracts were gallic acid, vanillic acid, ferulic acid, sinapic acid, and syringic acid. Saparbekova *et al.* (2023) noted that PPE contains ellagic acid, gallic acid, punicalin, punicalagin, catechins and anthocyanins which contribute to its high antioxidant activity. On the other hand, different compounds were detected as major constituents in OPE and PPE by Benslimane *et al.* (2020), who identified peduncalagin, punigluconin, and punicalagin as the major polyphenolic compounds in ethanolic PPE. El-Beltagi *et al.* (2022) confirmed that naringin, hesperidin, vicenin II, rhoifolin, and neohesperidin were the major polyphenolic compounds found in the methanolic OPE. The disparities in the phenolic and flavonoid composition may be attributed to factors such as the plant species, the extraction method, and the solvent used, as these factors influence the extraction efficiency and compound solubility (Shabir *et al,* 2011). Additionally, various environmental, processing, and post-harvesting factors can influence the composition of the peels (Houston 2005).

Using natural products, such as plant extracts, to inhibit fungal growth is considered safe, effective, and environmentally friendly (Rasheed *et al.,* 2020). Among plant extracts, orange, and pomegranate peels have attracted interest as alternative antifungal agents because of their antioxidant and antimicrobial qualities, primarily linked to their elevated phenolic content (Quattrucci *et al.,* 2013; Sharma *et al.,* 2017). In an evaluation of the antifungal potential of ethanolic OPE and PPE at different concentrations (0.5%, 1%, and 2%) against mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) isolated from guava fruits, significant reductions ($P < 0.05$) were observed in the mycelial growth and spore viability of all tested fungi at all concentrations. Moreover, the magnitude of reduction increased with higher extract concentrations. Also, data confirmed that PPE exhibited the highest antifungal activity compared to OPE. Similar findings

were reported by Glazer *et al.* (2012) who found that aqueous PPE inhibited the mycelial growth of *A. alternata*. Nicosia *et al.* (2016) demonstrated that PPE exhibited strong fungicidal activity, particularly against the germination of conidia of *Penicillium expansum* and *P. digitatum*. In addition, Sadhasivam *et al.* (2019) found that PPE exhibited a strong inhibitory effect on the growth and toxin production of mycotoxigenic fungi, specifically *Aspergillus flavus* and *A. parasiticus*. Hanafy *et al.* (2021) found that both PPE and OPE exhibited activity against *A. flavus* and *A. niger*, with inhibition ranging from 65% to 100% compared to the positive control. Hernández *et al.* (2021) observed that the polyphenolic OPE hindered the conidial germination and the radial growth of *Alternaria alternata*. Liu *et al.* (2021) demonstrated that the ethanol OPE reduced the mycelial growth of *A. flavus* by 32.31% after 7 days of incubation, with fungal inhibition increasing as the extract concentration increased. Shehata *et al.* (2021) confirmed that OPE exhibited the highest antimicrobial activity against *A. parasiticus* among the tested citrus peel extracts (sweet orange, lemon, tangerine, and grapefruit) at a concentration of 0.5 mg/mL. The significant antifungal activity of orange peel extract (OPE) and pomegranate peel extract (PPE) may be attributed to their high content of polyphenols, particularly gallic acid, syringic acid, ferulic acid, ellagic acid, catechin, kaempferol, quercetin and rutin, which have been reported as active antifungal compounds (Benguiar *et al.,* 2020; Hernández *et al.,* 2021).

CONCLUSIONS

The current study demonstrated the presence of various mycotoxigenic fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) on guava fruits, which can produce harmful mycotoxins posing risks to human health. Utilizing ethanolic extracts from orange and pomegranate peels can provide valuable bioactive compounds with significant biological activities, including antioxidant and antifungal properties. It can be concluded that both extracts exhibited a significant inhibitory impact on the growth and spore viability of mycotoxigenic fungi contaminating guava fruits. Moreover, using different plant extracts provides an environmentally friendly method for controlling fungal diseases in guava fruit cultivation, as these extracts are recognized for their safety, affordability, absence of residue concerns, and cost-efficiency. Therefore, they can serve as beneficial substitutes for synthetic substances.

Declarations:

Ethical Approval: This study received approval from the Medical Research Ethics Committee at the National Research Center., Egypt. (No. 08441223).

Conflicts of Interest: The author declares no conflicts of interest.

Authors Contributions: M.Y. was responsible for the study design, experiment execution, data analysis, and manuscript drafting.

Funding: No funding was received.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

Acknowledgements: The author expresses gratitude to the Food Toxicology and Contamination Department, and the Plant Pathology Department at the National Research Center (NRC), Egypt for their assistance and support during the course of this study.

REFERENCES

Ahmed, H.M.A., Abou-Gharbia, H.A., Attia, R.S., and Youssef, M.M. (2022). Peels of some citrus fruits as source of antioxidants for use with different vegetables oils. *Alexandria Journal of Food Science and Technology*, 19 (2): 25-33. https://doi.org/10.21608/AJFS.2022.286710.

Akhatar, S., Ismail, T., Fraternale, D., and Sestili, P. (2015). Review:

Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174: 417–425. https://doi.org/10.1016/ j.foodchem.2014.11.035.

Amadi, J.E., Nwaokike, P., Olahan, G.S., and Garuba, T. (2014). Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava L.*) in Awka Metropolis. *International Journal of Engineering and Applied Sciences*, 4 (10): 270–274.

Ammar, M.I., and El-Naggar, M.A. (2014). Screening and characterization of fungi and their associated Mycotoxins in some fruit crops. *International Journal of Advanced Research*, 2 (4):1216-1227.

Ayanda, O.I., Ajayi, A.A., and Olasehinde, G.I. (2013). Isolation, characterization and extracellular enzyme detection of microbial isolates from deteriorated apple (*malus domestica*) fruits. *International Journal of Biological and Chemical Sciences,* 7(2): 641–648. https://doi.org/10. 4314/ijbcs.v7i2.20.

Bankole, S.A., and joda, A.O. (2004). Effect of lemon grass (*Cymbopogon citratus* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology*, 3(1): 52–59. https://doi.org/10.5897/ AJB2004.000-2009.

Barent, H.L., and Hunter, B. (1977). Illustrated genera of imperfect fungi 3rd Ed. Burgess Publishing Company, Minnesota, 2412.

Benguiar, R., Yahla, I., Benaraba, R., Bouamar, S. and Riazi, A. (2020). Phytochemical analysis, antibacterial and antioxidant activities of pomegranate (*Punica granatum* L.) peel extracts. *International Journal of Biosciences*, 6: 35−44. http://dx. doi.org/10.12692/ijb/16.6.35-44.

Benslimane, S., Rebai, O., Djibaoui, R., and Arabi, A. (2020). Pomegranate peel extract activities as antioxidant and antibiofilm against bacteria isolated from Caries and Supragingival plaque. *Jordan Journal of Biological Sciences*, 13(3): 403 – 412.

Bora, G., Hebel, M., and Lee, K. (2007). In-situ measurement of the detachment force of individual oranges harvested by a canopy shaker harvesting machine. *Agricultural and Food Science*, 113761794.

Cabral, L.D.C., Pinto, V.F., and Patriarca, A. (2013). Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International Journal of Food Microbiology*, 166: 1–14. http://dx.doi.org/ 10. 1016/j.ijfoodmicro.2013.05.026.

Casquete, R., Castro, S.M., Martín, A., Ruiz-Moyano, S., Saraiva, J.A., Cordoba, M.G., and Teixeira, P. (2015). Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels. *Innovative Food Science* and *Emerging Technologies*, 31: 37e44. http://dx.doi.org/10.1016/ j.ifset.2015.07.005.

Christian, G. (1990). Hplc tips and tricks. Great britain at the iden press Oxford: pp 608.

Dahham, S.S., Ali, M.N., Tabassum, H., and Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, 9(3): 273–281.

De Ancos, B., Sgroppo, S., Plaza, L., and Cano, M.P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, 82(8): 790–796. http://dx.doi.org/10. 1002/jsfa.1093.

Dilucia, F., Lacivita, V., Conte, A., and Del Nobile, M.A. (2020). Review: Sustainable use of fruit and vegetable by-products to enhance food packaging performance. *Foods,* 9: 857. https://doi.org/ 10.3390/foods9070857.

El-Beltagi, H.S., Eshak, N.S., Mohamed, H.I., Bendary, E.S.A., and Danial, A.W. (2022). Physical characteristics, mineral content, and antioxidant and antibacterial activities of *Punica granatum* or *Citrus sinensis* peel extracts and their applications to improve cake quality. *Plants,* 11: 1740. https:// doi. org/10.3390/ plants11131740.

Embaby, E.M., Faiesal, A.A., and Younos, M.A. (2022). Control of the toxigenic fungi affecting fig fruits quality. *Egyptian Journal of Chemistry*, 65 (9): 339–347. http://dx.doi.org/ 10.21608/ EJCHEM.2022.111742.5090.

Embaby, E.S.M., and Korkar, H.M. (2015). Decay of Guava fruit (*Psidium guajava* Linn.) quality caused by some mold fungi. *International Journal of Agricultural Technology*, 11(3): 713-730.

Fernández-Cruz, M.L., Mansilla, M.L., and Tadeo, J.L. (2010). Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. *Journal of Advanced Research*, 113–122. http:// dx.doi.org /10.1016 /j.jare.2010. 03.002.

Fu, R., Zhang, Y., Guo, Y., Liu, F., and Chen, F. (2014). Determination of phenolic contents and antioxidant activities of extracts of *Jatropha curcas* L. seed shell, a by-product, a new source of natural antioxidant. *Industrial Crops and Products*, 58: 265–270. http://dx. doi.org/10.1016/j.indcrop.2014.04 .031.

Glazer, I., Masaphy, S., Marciano, P., Bar-Ilan, I., Holland, D., Kerem, Z., and Amir, R. (2012). Partial identification of antifungal compounds from *Punica granatum* peel extracts. *Journal of Agricultural and Food Chemistry*, 60: 4841–4848. http://dx.doi.org/ 10.1021/jf300330y.

Gomez, K.A., and Gomez, A.A. (1984). Statistical procedures for agricultural research. John wiley & sons.

Gullon, B., Pintado, M.E., Pérez-Àlvarez, J.A., and Viuda-Martos, M. (2016). Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (*Punica granatum*) flour obtained from co-product of juice extraction. *Food Control*, 59: 94–98. http://dx.doi. org/10.1016/j.foodcont.2015.05.0 25.

Hanafy, S.M., Abd El-Shafea, Y.M., Saleh, W.D., and Fathy, H.M. (2021). Chemical profiling, in vitro antimicrobial and antioxidant activities of pomegranate, orange and banana peel-extracts against pathogenic microorganisms. *Journal of Genetic Engineering and Biotechnology*, 19: 80 https: //doi.org/10.1186/ s43141 -021-00151-0.

Hernández, A., Ruiz- Moyano, S., Galván, A., Merchán, A.V., Nevado, F. P., Aranda, E., Serradilla, M. J., Córdoba, M., and Martín, A. (2021). Anti-fungal activity of phenolic sweet orange peel extract for controlling fungi responsible for post-harvest fruit decay. *Fungal Biology*, 125 (2): 143-152

https://doi.org/10.1016/j.funbio.2020.05.005.

Houston, M.C. (2005). Nutraceutical, vitamins, antioxidants and minerals in the prevention and treatment of hypertension. *Progress in Cardiovascular Diseases*, 47: 396-449. http://dx.doi.org/10.1016/j.pcad.2005.01.004.

Jabeen, N., Ahmed, M., Shaukat, S., and Salam, I. (2013). Allelopathic effects of weeds on Wheat (*Triticum Aestivum* L.) germination and growth. *Pakistan Journal of Botany,* 45(3): 807-811.

Jing, Li., Lei, Z., Li, L., Xie, R., Xi, W., Guan, Y., Sumner, L.W., and Zhou, Z. (2014). Antifungal activity of citrus essential oils. *Journal of Agricultural and Food Chemistry*, 62 (14): 3011–3033. https://doi.org/10.1021/jf5006148.

Joseph, B., and Mini, P. (2014). Review on nutritional, medicinal and pharmacological properties of guava (*Psidium guajava* L.). *Indian Journal of Science and Technology*, 7(5): 54-558.

Kanatt, S.R., Arjun, K., and Sharma, A. (2011). Antioxidant and antimicrobial activity of legume hulls. *Food Research International*, 44: 3182-3187. http://dx.doi.org/10.1016/j.foodres.2011.08.022.

Khatiwora, E., Adsul, V.B., Torane, R.C., Gaikwad, S., Deshpande, N.R., and Kashalkar, R.V. (2017). Spectroscopic determination of total phenol and flavonoid contents of citrus limon peel from north eastern region of India. *Journal of Drug Delivery and Therapeutics*, 7(1): 21-24. http://dx.doi.org/10.22270/jddt.v7i1.1368.

Klangpetch, W., Phromsurin, K., Hannarong, K., Wichaphon, J., and Rungchang, S. (2016). Antibacterial and antioxidant effects of tropical citrus peel extracts to improve the shelf life of raw chicken drumettes. *International Food Research Journal*, 23(2): 700–707.

Kumar, A., Shukla, R., Singh, P., and Dubey, N. (2010). Chemical composition, antifungal and anti-aflatoxigenic activities of *Ocimum Sanctum* l. essential oil and its safety assessment as plant-based antimicrobial. *Food and Chemical Toxicology*, 48: 539-54. http://dx.doi.org/10.1016/j.fct.2009.11.028.

Kuyng, H., Tsao, R., Yang, R., and Cui, S. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, 95: 466 - 473. http://dx.doi.org/ 10.1016/ j.foodchem.2005.01.032.

Limei, Y.U., Yingxian, W.U., Dongjie, L., Sheng, Z., Junmei, L., Chen, H., Feng. W. (2022). The kinetic behavior of antioxidant activity and the stability of aqueous and organic polyphenol extracts from navel orange peel. *Food Science and Technology, Campinas*, 42: e90621. https://doi.org/10.1590/fst.90621.

Liu, Y., Benohoud, M., Yamdeu, J.H.G., Gong, Y.Y., and Orfila, C. (2021). Green extraction of polyphenols from citrus peel by-products and their antifungal activity against *Aspergillus flavus*. *Food Chemistry: X*., 12: 100144. http://dx.doi.org/10.1016/j.fochx.2021.100144.

Ma, G., Zhang, L., Sugiura, M., and Kato, M. (2020). Citrus and health in The Genus Citrus. Amsterdam: Elsevier: 495–511. https://doi.org/10.1016/B978-0- 12-812163- 4.00024-3.

Mari, M., Di Francesco, A., and Bertolini, P. (2014). Control of fruit

postharvest diseases: old issues and innovative approaches. *Stewart Postharvest Review*, 1 (1): 1. http://dx.doi.org/10.2212/spr. 2014.1.1.

Meena, S., and Mariappan, V. (1993). Effect of plant products on seed borne mycoflora of *sorghum. Madras Agricultural Journal*, 80: 383-387.

Mostafa, A.A., Al-askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N., and Bakri, M.M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences,* 25(2): 361–366. https://doi. org/10.1016/j.sjbs. 2017.02.004.

Munimbazi, C., and Bullerman, L. (1998). High Performance Liquid Choromatographic method for the determination of moniliformin in corn. *Journal of AOAC International*, 81: 999-10. https:// doi.org/10.1093/jaoac/81.5.999.

Mushtaq, M., Sultana, B., Anwar, F., Adnan, A., and Rizvi, S.S. (2015). Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. *Journal of Supercritical Fluids*, 104:122–131. http:// dx. doi. org/ 10.1016/ j. supflu.2015. 05.020.

Nawaz, S., Scudamore, K.A., and Rainbird, S.C. (1997). Mycotoxins in ingredients of animal feeding stuffs: I. Determination of *alternaria* mycotoxins in oilseed rape meal and sunflower seed meal. *Food Additives & Contaminants: Part A*, 14: 249-262. http://dx.doi.org/10.1080/ 02652039709374522.

Neri, F., Donati, I., Veronesi, F., Mazzoni, D., and Mari, M. (2010). Evaluation of *Penicillium expansum* isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts. *International Journal of Food Microbiology*, 143: 109-117. http: //dx.doi.org/ 10.1016/ j. ijfoodmicro. 2010.08.002.

Nicosia, M.G., Pangallo, S., Raphael, G., Romeo, F.V., Strano, M.C., Rapisarda, P., Droby, S., and Schena, L. (2016). Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract. *Postharvest Biology and Technology*, 114: 54–61. https:// doi.org/10.1016/j.postharvbio.201 5.11.012.

Paster, N., and Barkai-Golan, R. (2008). Mouldy fruits and vegetables as a source of mycotoxins: part 2. *World Mycotoxin Journal*, 1(4): 385-396. http://dx.doi.org/10. 3920 /WMJ2008.x044.

Pitt, J.I. (2000). Toxigenic fungi: which are important? *Medical Mycology*, 38 Supplement 1:17-22. PMID: 11204142.http://dx.doi.org/10.10 80/ mmy.38.s1.17.22.

Quattrucci, A., Ovidi, E., Tiezzi, A., Vinciguerra, V., and Balestra, G.M. (2013). Biological control of tomato bacterial speck using *Punica granatum* fruit peel extract. *Crop Protection*, 46: 18–22.http://dx.doi.org/ 10.1016/ j. cropro. 2012.12.008.

Rahman, M., Begum, K., Begum, M., and Faruque, C.A.A. (2003). Correlation and path analysis in guava. *Bangladesh Journal of Agricultural Research*, 28: 93–98.

Raper, K.B., and Fennel, D.I. (1965). The Genus Aspergillus. Williams and Wilkins, Baltimore. Company. USA: 137–146.

Rasheed, U., Ain, Q.U., Yaseen, M., Santra, S., Yao, X., and Liu, B. (2020). Assessing the aflatoxins mitigation efficacy of blueberry

pomace biosorbent in buffer. gastrointestinal fluids and model wine. *Toxins (Basel)*, 12(7): 1–21. https://doi.org/ 10.3390/toxins 12070466.

Rubert, J., Soler, C., and Mañes, J. (2012). Application of an HPLC–MS/MS method for mycotoxin analysis in commercial baby foods. *Food Chemistry*, 133(1): 176-183. http: //dx.doi.org/10.1016/j.foodchem.2 011.12.035.

Sadhasivam, S., Shapiro, O.H., Ziv, C., Barda, O., Zakin, V., and Sionov, E. (2019). Synergistic inhibition of mycotoxigenic fungi and mycotoxin production by combination of Pomegranate peel extract and Azole fungicide. *Frontiers in Microbiology*, 10: 1919. http://dx.doi.org/10.3389/ fmicb.2019.01919.

Sajid, M, Mehmood, S., Yuan, Y., and Yue, T. (2019). Mycotoxin patulin in food matrices: Occurrence and its biological degradation strategies. *Drug Metabolism Reviews*, 51: 105–120. http://dx.doi.org/10. 1080 /03602532.2019.1589493.

Saparbekova, A.A., Kantureyeva, G.O., Kudasova, D.E., Konarbayeva, Z.K., and Latif, A.S. (2023). Potential of phenolic compounds from pomegranate (*Punica granatum* L.) by-product with significant antioxidant and therapeutic effects: A narrative review. *Saudi Journal of Biological Sciences*, 30: 103553. http://dx.doi.org/10.1016/j.sjbs.20 22.103553.

Selahvarzi, A., Ramezan, Y., Sanjabi, M.R., Mirsaeedghazi, H., Azarikia, F., and Abedinia, A. (2021). Investigation of antimicrobial activity of orange and pomegranate peels extracts and their use as a natural preservative in a functional beverage. *Journal of Food Measurement and Characterization*, 15: 5683–5694. http://dx.doi.org/10.1007/s11694-021-01141-z.

Shabir, G., Anwar, F., Sultana, B., Khalid, Z.M., Afzal, M., Khan, Q.M., and Ashrafuzzaman, M. (2011). Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of gold mohar [Delonix regia (Bojer ex Hook.) Raf. *Molecules*, 16: 7302-7319. http://dx.doi.org/10.3390/ molecules16097302.

Sharma, K., Mahato, N., Cho, M.H, and Lee, Y.R. (2017). Converting citrus wastes into value-added products: economic and environmentally friendly approaches. *Nutrition,* 34: 29e46. http://dx.doi.org/10.1016/j.nut.20 16.09.006.

Shehata, M.G., Awad, T.S., Asker, D., El Sohaimy, S.A., Abd El- Aziz, N.M., and Youssef, M.M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science*, 326–335. https://doi.org/10.1016/j.crfs. 2021.05.001.

Singh, D., and Sharma, R.R. (2007). Postharvest diseases of fruit and vegetables and their management. In: Prasad, D., Ed., Sustainable Pest Management, Daya Publishing House, New Delhi, India. http://dx.doi.org/10.1016/ B978-0-12-812698-1.00001-7.

Singh, K. (1991). An illustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins. First edition, Danish Government Institute of Seed Pathology for Developing Countries, Hellerup, Denmark: 1048753476.

Singh, S., Srivastava, S., Mishra, J., Raaj, R., and Sina, A. (2014).

Evaluation of some plant extract against predominant seed mycoflora of Mungbean *Vigna Radiata* (L.) Wilczek Seed. *Life Sciences Leaflets*, 51: 83- 89.

Ștef˘anescu, B.E., Szabo, K., Mocan, A., and Crişan, G. (2019). Phenolic compounds from five *Ericaceae* species leaves and their related bioavailability and health benefits. *Molecules,* 24 (11): 2046. https: //doi.org/10.3390/molecules2411 2046.

Sun, S., Huang, S., Shi, Y., Shao, Y., Qiu, J., Sedjoah, R., Yan, Z., Ding, L., Zou, D., and Xin, Z. (2021). Extraction, isolation, characterization and antimicrobial activities of non-extractable polyphenols from pomegranate peel. *Food Chemistry*, 351: 129232. http://dx.doi.org/10.1016/ j.foodchem.2021.129232.

Torres, A., González, H., Etcheverry, M., Resnik, S., and Chulze, S. (1998). Production of alternariol and alternariol mono-methyl ether by isolates of *Alternaria* spp. from Argentinian maize. *Food Additives & Contaminants: Part A*, 15(1): 56-60. http://dx.doi.org/ 10.1080/02652039809374598.

Wills, R.B., and Golding, J. (2015). Advances in postharvest fruit and vegetable technology: CRC press. (1st ed.). CRC Press. https:// doi.org/10.1201/b18489.

Younos, M.A., and Abdel-Galil, M.M. (2023). Fungi and their associated mycotoxins contaminating tomato fruits (*Solanum lycopersicum*) and potential control of fruit spoilage by essential oils. *Middle East Journal of Applied Sciences*, 13(4): 486-504. https://doi.org/10.36632/ mejas/2023.13.4.32.

Zilic, S., Serpen, A., Akıllıoglu, G., Jankovic, M., and Gökmen, V. (2012). Distributions of phenolic compounds, yellow pigments, and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. *Journal of Cereal Science*, 56: 652-658. https://doi.org/10. 1016/j.jcs.2012.07.014.