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Research article

Evaluation of effectiveness aqueous extract for some leaves of wild edible plants in Egypt as anti-fungal and anti-toxigenic

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ABSTRACT

Plants are one of a prefect source of natural effective compounds that have antimicrobial, and other activities. This study investigated the activity of the aqueous extract for three wild edible plants (*Sonchus oleraceus*, *Cichorium pumilum,* and *Portulaca oleracea*) at three concentrations (1.5, 2.5 and 5 mg/ml) as antifungal and antitoxigenic. Many functional groups such as alcohols, phenols, alkanes and alkenes, etc were appeared in aqueous extracts by Fourier Transform Infrared Spectroscopy (FTIR) analysis. Where an extract of *Portulaca oleracea* gave a greater total phenolic and flavonoids were 210.4 ± 1.15 and 36.7 ± 0.79 mg/mL, respectively, followed by *Sonchus oleraceus* (192.3 ± 2.11 mg/mL) and *Cichorium pumilum* (186.4 ± 2.18 mg/mL). The results indicated that increasing the concentration of the extract, the area of inhibition zone increased with all treatments, where the highest inhibition zone was observed using 5 mg/ml for *Portulaca oleracea* extract was 17.1 ± 1.7, 26.5 ± 1.5 and 22.8.±2.3 mm against *Aspergillus flavus, Aspergillus ochraceus* and *Aspergillus parasiticus*, respectively, while the lowest antifungal activity was marked with *Cichorium pumilum* extract with all tested fungi. The results have also indicated that the aqueous extract has inhibited formed of aflatoxin B_1 (AFB_1) and ochratoxin A (OTA), where the percentages of inhibition AFB_1 were 78.03, 68.8 and 81.7% after treated yeast extract sucrose (YES) media by 5 mg crude extract for extract *Sonchus oleraceus, Cichorium pumilum* and *Portulaca oleracea*, respectively. In contrast, the inhibitory effect against OTA at the same concentration was 77.5, 72.3, and 85.2% in the same order for plants. Finally, these plants provide an aqueous extract that contains many effective compounds that enable to play the role of antifungal and antitoxigenic.

1. Introduction

Aspergillus spp is ubiquitous fungi that usually inhabit in indoor air environments especially dust, soils and flotsam of the plants. *Aspergillus* contamination of food and feed cause's major economic problems worldwide, many *Aspergillus* species such as *A. flavus and A. parasiticus, A. nomius, A. bombycis and A. ochraceus* produce various mycotoxins, which aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) [1, 2, 3]. These are mostly produced by *A. flavus* and *A. ochraceus*, respectively, as well as they, are classified as carcinogens by the International Agency for Research on Cancer (IARC) [4]. These fungi grow with wide range temperature between 20 to 40 °C with a 10–20% of moisture content and 70–90% of relative humidity in the air, therefore associated with a tropical and subtropical climate, which includes Egypt [5]. Many extracts and compounds obtained from natural sources, such as plants, algae, microalgae and bacteria were screened for the ability to inhibit of growth of fungal and produced toxins [6, 7, 8]. Accordingly, A lot of studies reported that the natural sources for inhibitors of biosynthesis for AFB_1 and OTA were the common bioactive compounds in plant extract for example phenols, flavonoids, alkaloids, tannins, terpenes and resins which that all possess antifungal properties [9, 10, 11, 12]. Many wild plants grow in Egypt, which many Egyptians used to eat especially in rural areas, or use in treating certain diseases as digestive, purgative and emollient [13]. Three wild edible plants (*Sonchus oleraceus*) known as Sow Thistle and *(Cichorium pumilum* called common chicory) belonging to the Asteraceae family. *Portulaca oleracea* is an annual succulent in the family Portulacaceae called in Egypt "Rejlah" [14, 15]. Therefore, this study aimed to evaluate an aqueous extract for leaves of three wild edible plants as anti-fungal and anti-mycotoxins produced by *Aspergillus* spp including (*A. flavus*, A *ochraceus* and *A. parasiticus*). Through, examined of the impact of these extracts on the growth and the ability to produce AFB_1 and OTA.

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Table 1. The content of aqueous extract from phenolic, flavonoid and antioxidant.

Wild plants	Total phenolic (mg/mL)	Total flavonoids (mg/mL)	Antioxidant activity (%)
S.oleraceus	192.3 ± 2.11	25.1 ± 1.33	57.3
C. pumilum	186.4 ± 2.18	24.3 ± 1.53	53.8
P. oleracea	210.4 ± 1.15	36.7 ± 0.79	65.6

Fourier Transform Infrared Spectroscopy (FTIR).

2. Materials and methods

2.1. Plant material

The plants (*Sonchus oleraceus*, *Cichorium pumilum* and *Portulaca oleracea*) were collected from Kafr El-Sheikh government –Egypt. The fresh leaves plant were dried in an oven at 45–50 °C for 12 h, then ground to a fine powder and stored in plastic vials.

2.2. Fungal strains

Three fungal strains from *Aspergillus spp* were used in this study *A. flavus* (ATCC 28542), A *ochraceus* (ATCC 22947) and *A. parasiticus* (ATCC 26692).

2.3. Chemicals and solvents

Potato Dextrose Agar (PDA) and yeast extract agar were obtained from Sigma- Aldrich, Lyon, France. The standards of toxins (AFB_1 and OTA) and solvents were purchased from Sigma chemical Co. (St. Louis, MO).

3. Methods

3.1. Preparation of aqueous extract

Five grams of powder plants leaves were extracted by 100 ml of distilled water at room temperature for 24 h, then centrifuged at 3000 rpm for 15 min, and evaporated to near dryness, and the resulting viscous powder was dissolved to obtain stock solution.

3.2. Determination of total phenolic content

The total phenolic content in aqueous extract for leaves was determined using Folin – Ciocalteau method as follows: Briefly, 1 ml of extract in a volumetric flask was diluted with distilled water to 46 ml. 1 ml of Folin-Ciocalteu reagent was added and the contents of the flask were blended completely. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then mixture left for 2 h. The absorbance of mixture was measured at 760 nm. Total phenolic content was measured by plotting the calibration curve of a Gallic acid equivalent (GAE) standard [16].

3.3. Determination of total flavonoid content

The total flavonoid content was measured according to the method Yan-Hwa *et al,* 2000 [17]. as follows: 1 ml of extract was diluted with 4.3 ml of 80 % aqueous ethanol containing 0.1 ml of 10 % $Al(NO_3)_3$ and 0.1 ml of 1 M aqueous CH_3COOK. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. The total flavonoid content was measured by us using calibration curve of a quercetin equivalent (QE) standard.

3.4. Determination of antioxidant activities

Determination of DPPH radical scavenging activity: The antioxidant activity of extracts, based on the scavenging activity of the stable DPPH free radical was determined by the method described by Lee *et al*, 2015 [18].

3.5. Assay of antifungal activity

The antifungal activity was tested on the three strains of fungi using agar well diffusion technique Perez *et al*, 1990 [19]. The strains were cultivated on PDA slants at 28 °C for 7 days. Spores were harvested by adding 10 ml of sterile distilled water containing 0.05% Tween 20 and scraping the surface of the culture to free the spores. One mL of spore suspension was inoculated into each plate. Wells of 5 mm diameter was made on the PDA surface and filled with the three gradual with three concentrations of (1.5, 2.5 and 5 mg/ml), obtained from diluting the stock solutions were used. Wells containing with (100 µl pure solvent (water) were used as a negative control, while wells containing with Nystatin (1000 Unit/ml were considered as positive control. The

Table 2. FTIR spectrum of bond in the aqueous extract for *S. oleraceus.*

NO. of peak	Band position (cm^{-1})	bond and functional group
1	3913.82	O–H Hydroxy group
2,3,4	3882,3850,3780	O–H stretch, free hydroxyl alcohols, phenols
5	3421.1	N–H stretch aliphatic primary amine O–H stretch
6	1855.19	C=C asymmetric stretch
7	1614.13	–C=C– stretch alkenes Aromatic C=C stretching N–H bend 1° amines
8	1406.82	C–C stretch (in–ring) aromatics S=O stretching sulfate
9	1273.75	C–O stretching alkaylaryl ether C–N stretch aromatic amines
10	1119.48	C–O alcohols, carboxylic acids, esters, ethers C–N stretch aliphatic amines
11	930.485	O–H bend carboxylic acids
12	855.275	C–Cl stretch alkyl halides
13	612.288	-C=C–H: C–H bend alkynes C–Br stretch alkyl halides
14	546.72	C–Br stretch alkyl halides
15,16,17	444.619:411.728	C–Cl stretch alkyl halides

Table 3. FTIR spectrum of bonds in the aqueous extract for *C. pumilum.*

NO. of peak	Band position (cm^{-1})	bond and functional group
1	3913.82	O–H stretching of Water; N–H stretching of protein amide A
2,3,4,5	3886.83:3720.01	O–H stretch, free hydroxyl alcohols, phenols
6.7.8	3697.84:3658.3	Free OH stretch
9	3430.74	N–H stretch (1°, 2° amines, amides) O–H alcohol
10,11	2973.7:2927	C–H stretchalkanes methyl and methylene groups O–H stretch alcohol N–H stretch amine salt
12	2075.03	N=C=S stretching isothiocyanate
13	1904.36	C=C=C stretching allene
14,15	1855.19: 1811.79	C=O stretch acid halide
16,17	1781.9:1758.76	C=O stretch ester, carboxylic acid
18	1624.73	–C=C– stretch alkenes Aromatic C=C stretching N–H bend 1° amines
19	1507.1	N–O stretch nitro compounds C–C stretch (in–ring) aromatics
20	1406.82	S=O stretching Sulfate sulfonyl chloride
21	1271.109	C–O stretching Alkyi, Aryl ether C–N stretch aromatic amines
22	1120.44	C C–O stretching aliphatic ether secondary alcohol
23	1049.09	S=O stretching sulfoxide
24,25,26	617.109:545.756	C–Br stretch alkyl halides
27,28	491.759:416.549	C–Cl stretch alkyl halides

inoculated plates were incubated at 27 °C for 48 h, and then the antifungal activity was assessed by measuring the zone of inhibition (mm). The results average was calculated from at least three replicates for each treatment.

3.6. Control of producing AFB_1 and OTA by aqueous extract in YES medium

The yeast extract sucrose (YES) culture medium (2% yeast extract and 15% sucrose/liter distilled water) was used in this experiment. YES was poured into 250 ml Erlenmeyer flask and autoclaved at 121 °C for 15 min, then cooled and inoculated with spores suspension of *A. flavus* and *A. ochraceus* both separately. Gradual concentrations of (1.5, 2.5 and 5 %) from aqueous extract were added to YES medium and incubated at 28 °C for 14 days. After the end of the incubation period, the AFB_1 was extracted then determined using HPLC as following: AFB_1 was extracted from YES medium by chloroform (20 ml twice with 10 ml YES media) then homogenization for 3 min in a separation funnel. Finally filtration of the chloroform phase, then evaporation to dryness to use with HPLC.

3.7. Determination of AFB_1 by HPLC

The HPLC system consists of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Watres 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze 2. A phenomenex C_{18} (250 × 4.6 mm i.d.), 5 μm from Waters corporation (USA). The mobile phase consists of (water/methanol/acetonitrile (240:120:40v/v/v) with flow rate of 1.0 ml/min. The fluorescence detector was operated at wavelength of 360 nm and 440 for excitation and emission, respectively. Concentrations of AFB_1 in samples were calculated individual for each sample through using compare peak area for sample with standard with 20 μl as injection volume for both

Table 4. FTIR spectrum of bonds in the aqueous extract for *P. oleracea.*

NO. of peak	Band position (cm^{-1})	bond and functional group
1	3912	O–H stretching of Water; N–H stretching of protein amide A
2,5	3897.84:3657.34	O–H stretch, free hydroxyl alcohols, phenols
6,7,8	3697.84:3685.3	Free OH sharp
9	3429.78	N–H stretch (1°, 2° amines, amides) O–H alcohol
10	2925	C–H stretchalkanes methyl and methylene groups
11	2075	C ≡ C Alkynes
12,13,14	1879.29:1855.19	C=O Aldehydes C=O stretch non conjugated Acid halid
15	1625.7	–C=C– stretch alkenes Aromatic C=C stretching N–H bend 1° amines
16	1508	N–O stretching nitro compound
17	1406.82	O–H bending carboxylic acids
18	1319.07	O–H bending phenol
19	1271.82	C–O stretching Alkyi, Aryl ether
20	1044.26	S=O stretching sulfoxide
21	581.433	C–Cl stretch halocompound
22	535.15	C–I stretch halocompound
23	451.261	C–Br stretch alkyl halides

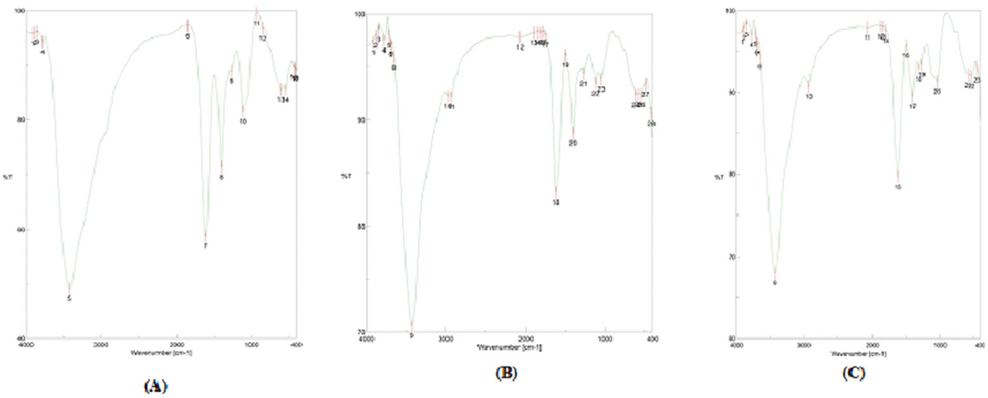

Figure 1. FTIR spectrum of aqueous extract for *S. oleraceus.* (A), *C. pumilum* (B) and *P. oleracea* (C).

standard solutions and sample extracts [20]. The percentage of inhibition was calculated as following equation. The percentage of inhibition $AFB_1 = \frac{C-T}{C} \times 100$.where: C is concentration of toxin in the positive sample that inoculated by spores of fungus only. T is concentration of toxin in the sample containing spores of fungus and aqueous extract (treatment sample).

3.8. Extraction and determination of OTA

Ten milliliters of YES medium was filtered, and extracted with 20 ml of chloroform. The chloroform phase was filtered and concentrated then dryness under a nitrogen to dry film, it was dissolved in 1 ml water/acetonitrile (3: 1 v/v) and mixed well by vortex for 30 s. The mixture was used for HPLC analysis. The HPLC system consisted of Waters Binary Pump Model 1525, Model Waters 1500 Rheodyne manual injector and Waters 2475 Multi- Wavelength Fluorescence Detector (Waters Pacific Pte Ltd, Science Park Road, Singapore). The data workstation with software Breeze TM 2 phenomenex C_{18} column (250 9 4_6 mm i.d.), 5 lm from Waters Corporation (Milford, MA). An isocratic system with acetonitrile: water: acetic acid (55: 43: 2) was used with flow rate of 1.0 ml/min. The injection volume was 20μl for both standard solutions and sample. The fluorescence detector was operated at wavelength of 335 nm and 465 nm for excitation and emission, respectively. OTA concentrations in samples were calculated individual for each sample through using compare peak area for sample with standard [21]. The percentage of inhibition was calculated as previously mentioned.

3.9. Statistical analysis

General Linear Model procedure of the SPSS ver. 18 (IBM Corp, NY) was used to statistically analysed. The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio. All statements of significance were depended on the probability of P-value $\leq$ 0.05 was considered to be statistically significant.

4. Results and discussion

4.1. Total phenols and flavonoids in the aqueous extract

Data recorded in Table 1 shown an aqueous extract of *P. oleracea* has a greater total phenolic and flavonoids were 210.4 ± 1.15 and 36.7 ± 0.79 mg/mL, respectively, followed by *S. oleraceus* (192.3 ± 2.11 mg/mL) and finally *C. pumilum* (186.4 ± 2.18 mg/mL). On the other hand DPPH free radical scavenging activity was ranged 53.8–65.6% with three plants extract. These compounds affected by many factors such as water, air, soil, elevation, geographical variation, plant growth stage and differences between species, as well as extraction methods [22, 23, 24].

4.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis of bonds and functional groups in aqueous extract for three plants were presented in Tables 2, 3, and 4. The results indicated that many functional groups appeared in the aqueous leaves extract with the FTIR analysis such as alcohols, phenols, alkanes, carboxylic

Table 5. The inhibition zone (mm) of aqueous extract for three plants.

Type of plant	Concentration (mg/ml)	Fungi*			Mean with plant
		A. flavus	*A. ochraceus*	*A. parasiticus*	
S. oleraceus	**1.5**	4.4 ± 0.6	7.1 ± 0.36	6.6 ± 0.7	**6.05 ± 1.3**
C. pumilum		-	3.1.±1.2	3.5 ± 0.51	**2.24 ± 1.8**
P. oleracea		7.4 ± 1.3	8.5 ± 0.5	9.6 ± 2.1	**8.5 ± 1.6**
Mean with type of Fungi		**3.9 ± 3.3**	**6.2 ± 2.4**	**6.6 ± 2.8**	
S. oleraceus	**2.5**	5.5 ± 0.75	9.8 ± 1.9	11.3 ± 1.5	**8.9 ± 2.9**
C. pumilum		3.5 ± 1.5	4.5 ± 1.3	6.2 ± 0.76	**4.7 ± 1.6**
P. oleracea		11.6 ± 1.3	18.7 ± 1.5	14.7 ± 2.5	**14.9 ± 3.4**
Mean with type of Fungi		**6.8 ± 3.2**	**10.9 ± 6.3**	**10.7 ± 4.01**	
S. oleraceus	**5**	13.7 ± 1.4	22.8 ± 2.2	20.5 ± 1.8	**19.01 ± 4.4**
C. pumilum		6.2 ± 0.77	9.3 ± 1.5	13.3 ± 0.65	**9.6 ± 3.2**
P. oleracea		17.1 ± 1.7	26.5 ± 1.5	22.8.±2.3	**22.1 ± 4.4**
Mean with type of Fungi		**12.3 ± 4.3**	**19.5 ± 7.9**	**18.8 ± 4.5**	

N = 3; Mean ± SD (Std. Deviation); -: No inhibition.

Table 6. ANOVA analysis of the effect of concentration of aqueous extract, type of plant and fungi strains on a growth.

Source	SS	df	MS	*F*	*P.*
Intercept	9238.414	1	9238.414	4504.848	0.00000
Plant	1282.385	2	641.1925	312.6591	0.00000
Con.	1781.679	2	890.8395	434.3924	0.00000
Fungi	356.5913	2	178.2956	86.94076	0.00000
Plant * Con.	142.4493	4	35.61231	17.36532	0.00000
Plant * Fungi	49.61148	4	12.40287	6.047904	0.000427
Con * Fungi	62.10741	4	15.52685	7.571224	0.000064
Plant * Con * Fungi	59.6137	8	7.451713	3.633614	0.0019
Error	110.7417	54	2.050772		
Total	13083.59	81			

SS, sum of squares; df, degree of freedom; MS, mean square; *P*, probability at confidence 0_95.

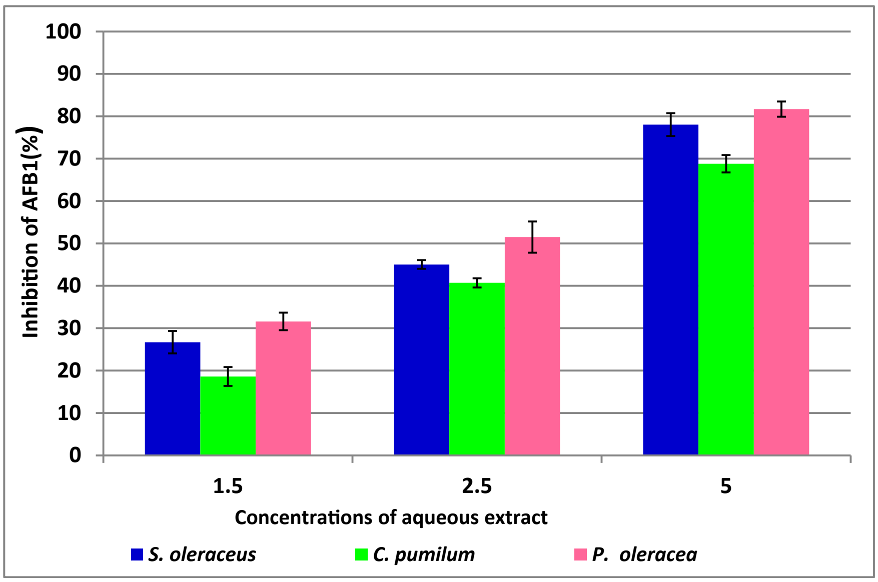

Figure 2. The percentages of inhibition of AFB_1 produced by *A. flavus* in YES media treated by aqueous extract.

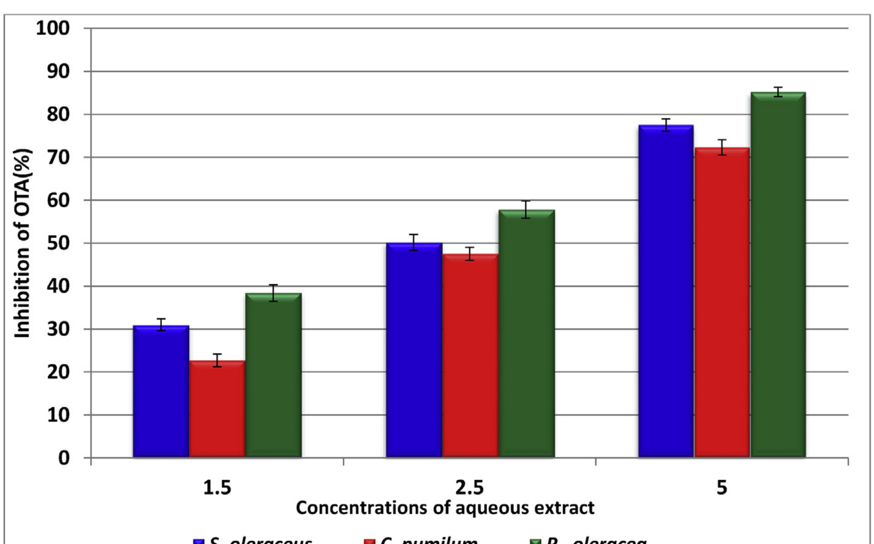

Figure 3. The percentages of inhibition of OTA produced by *A. ochraceus* in YES media treated by aqueous extract.

acids, aldehydes and alkenes. All the three plant extracts contains high absorbance at 3421 to 3430.74 cm^{-1} (OH stretching and N–H) and 1614.13 to 1624 cm^{-1} (C=O stretching) as shown in Figure 1. The general the extract of these plants contains phenolic and flavonoid compounds, which include more than 400 compounds, as well as other chemical constituents such as steroids, vitamins, minerals, fatty acids, alkaloids, saponins, etc. these compounds, especially flavonoids have a fifteen carbon, which consists of two phenyl rings bound together by three carbon atoms to form an oxygenated heterocycle [25, 26].

4.3. Effectiveness of aqueous extract as antifungal

In generally, data presented in Table 5 showed the inhibition zone for *P. oleracea* extract give higher inhibition compared with *S. oleraceus* and *C. pumilum*, as well as inhibition zone increased with an increase the concentration of the extract. In case *S. oleraceus* extract the results indicated that the *A. flavus*, *A. ochraceus* and *A. parasiticus* inhibition zone was ranged (4.4 $\pm$ 0.6 to 13.7 $\pm$ 1.4 mm), (7.1 $\pm$ 0.36 to 22.8 $\pm$ 2.2 mm) and (6.6 $\pm$ 0.7 to 20.5 $\pm$ 1.8 mm), respectively. In contrast, the only case that did not show any inhibitory growth was with *A. flavus* at 1.5 mg/ml from *C. pumilum* extract. *P. oleracea* extract recording the highest inhibition at in all concentrations and also against three strains was (7.4 $\pm$ 1.3 to 17.1 $\pm$ 1.7 mm) with A. *flavus*, while with *A. ochraceus* and *A. parasiticus* strains the inhibition zone were (8.5 $\pm$ 0.5 to 26.5 $\pm$ 1.5 mm) and (9.6 $\pm$ 2.1 to 22.8 $\pm$ 2.3 mm), respectively. The analysis of variance showed higher significant differences in inhibition zone with all strains as well as concentrations of aqueous extract, also there were significant differences between plants used in this study Table 6. Anti-fungal effect for flavonoids and phenolics were through several mechanisms of action such as they influential interfere with membrane functions by changing the permeability of cellular membranes, cell division and synthesis of RNA and protein which could lead eventually to the inhibition of growth, also oxidation of lipid in cell [27, 28].

4.4. Impact of aqueous extract on AFB_1 and OTA produced in YES medium

The results displayed in Figures 2 and 3 show the percentages of inhibition AFB_1 and OTA produced by *A. flavus* and *A. ochraceus* in YES medium, respectively, which were treated by aqueous extract for three plants. The preliminary data reflected that the percentages of inhibition of AFB_1 by aqueous extract of *S. oleraceus* were 26.7, 45.03 and 78.03% at 1.5, 2.5 and 5 mg concentration of extract, respectively. On the other hand at the same above concentrations extract of *P. oleracea* gave the highest inhibition for AFB_1 was 31.6, 51.5 and 81.7%. While inhibitory effect against OTA at 5 mg/100ml was (77.5, 72.3 and 85.2%) for extract *S. oleraceus, C. pumilum* and *P. oleracea*, respectively. The obtained results showed that the extract of *C. pumilum* in all treatments gave the lowest values for reduce of toxin.

The antitoxigenic activity for these plants may be due to several of the activation compounds were identified such as 2-Pentadecanone, Luteolin-O-glucuronide, n-hexadecanoic acid and hydroxycoumarins in the aqueous extract of *S. oleraceus* [29, 30, 31]. As well as extract of *C. pumilum* has sesquiterpene, caffeic acid, cichoriin, esculin, umbelliferone, scopoletin and 6,7- ihydroxycoumarin, flavone derivatives (cichoric acid, chlorogenic acid, apigenin, quercitin) [31, 32, 33]. Many studies reported that aqueous extract of *P. oleracea* has some alkenals, coumarins, including hexanal, 2 hexanal and 2-Pentenal inhibited fungal growth [34, 35, 36]. The inhibitor effect of these active compounds for producer of AFB_1 and OTA depend on many mechanisms including, the effect of these compounds on specific sites within the cell of the fungus, for instance effect on the expression pathways of the gene responsible for producing the toxin, in addition block the action of enzymes responsible for the biosynthesis process, as well as the induction of mitochondrial dysfunction, because of the mitochondria are responsible for providing acetyl-CoA which is a main precursor for AFB_1 biosynthesis in the fungal. Aqueous extract of *P. oleracea* contains five flavonoids (, apigenin, myricetin, kaempferol, quercetin and luteolin) with many chemical constituents such as steroids, vitamins, minerals, fatty acids; alkaloids, saponins, etc. have been isolated from the plant. In addition to clerodane diterpene portulene, rutin, apigenin and glycolflavones. The inhibitory effect for these substances may be due to sotpping the synthesis of enzymes responsible for biosynthetic pathway for AFB_1 and OTA [37, 38, 39].

5. Conclusion

The aqueous extract used in this study is a good source of many effective compounds that are easy to use with many products such as pharmaceutical products (soap or shampoo) and bakery products. This extract can be obtained in an easy, environmentally friendly, safe, and low-cost way *P. oleracea* extract had the greatest effect as antifungal and antitoxigenic.

Declarations

Author contribution statement

Tarek A. El-Desouky: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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