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Antifungal Activity of Kiwa Peel Extract Against *Aspergillus* Species

Saad Ali Hussein Al-Akabi
Diyala Education Directorate, Ministry of Education, Diyala, Iraq

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Annotation: Background: The emergence of fungal resistance to the classical antifungal agents has driven a quest for new resources among natural products. Some by-products from the agro-food industry, such as fruit peels, are a good source of bioactive compounds that can have effects on microorganisms. In the present investigation, we analysed antifungal properties of Kiwa peel extract (KPE) toward five Aspergillus species. Method: Kiwa peel extract was extracted and in vitro tested against Aspergillus niger, Aspergillus flavus and Aspergillus terreus with the help of agar well diffusion method. Various concentrations of extract (62.5–1000 µg/mL) were tested. The antifungal activity was evaluated by measuring the size of inhibition zones, and possible significance was analysed with one-way ANOVA followed by Tukey post hoc test. A significance level of $P < 0.05$ was applied. Results: KPE had concentration-dependent, significant anti-Aspergillus activity against all species tested. Maximum zones of inhibition were observed with the highest concentration (1000 µg/mL), and they significantly differed from lower concentrations ($P < 0.0001$). A. niger and A. flavus were slightly more susceptible than A. terreus out of the tested fungi. Conclusion: Our results showed that the ethanolic extract from KIWA peels had strong antifungal activity against pathogenic Aspergillus species and this is expected to be attributed largely to its phytochemicals. Kiwa

peel, an agricultural waste material, can be considered as a potential natural antifungal agent, which is efficient and environmentally friendly. More experiments should be carried out to identify and examine the mechanisms of these active compounds in vivo.

Keywords: Antifungal activity; Kiwa peel extract; Aspergillus species; Plant-derived antifungals; Natural bioactive compounds; Phytochemicals.

Introduction

Aspergillus species are common filamentous fungi found in soil, air, foodstuffs, and indoor air environments. Many species among them, such as A. fumigatus, A. flavus and Angier have high medical and agricultural relevance. [1] They cause various diseases, including allergic responses to invasive aspergillosis in immunocompromised patients, and also are known for producing mycotoxins such as aflatoxin that threaten human health and animal productivity [2]. Antifungal therapy for aspergillosis is mainly based on synthetic antifungals (azoles and polyenes). Nevertheless, the widespread occurrence of antifungal resistance, as well as associated high treatment costs and toxic side effects, has hindered their utility and promoted a quest for new antifungal agents, especially from natural compounds [3]. In the past few years, plant extracts have been gaining more attention because of their antimicrobial effects against a wide range of pathogenic bacteria and fungi, biocompatibility, and low level of toxicity [4]. Peels of the fruits have been widely recognised as a valuable waste material, possessing several active phytochemicals such as phenolic acids, flavonoids, tannins, and organic acids with antimicrobial and antioxidant activities. The use of such waste also falls within the scope of sustainability and value addition to agro-industrial by-products [5]. Kiwifruit peel has been known to be rich in phenolic compounds and natural antioxidants, indicating antifungal potential against pathogenic fungi. Several investigations have shown that peel extracts of plants can be used to reduce fungal growth through destabilization of the cell wall, permeability increase, and disruption in enzymatic activity culminating in the death of fungal cells [6]. Thus, the study of Kiwa peel extract on antifungal activity against Aspergillus species may offer a new perspective for preparing new, efficient, eco-friendly, and nontoxic antifungal agents to offer an alternative means to combat fungal infections and contamination.[7].

Material

Isolation of *Aspergillus* spp. from soil samples

1. Collect soil: Using sterile tools, collect ~10–50 g of soil from the top 0–5 cm (or sample depth required by study) into sterile bags or tubes. Label clearly (site, date, depth).
2. Transport & storage: Keep samples cool (~4–10 °C) and process within 24–48 h. If delay >48 h, store at 4 °C up to a few days; avoid freezing, which may alter fungal recovery.
3. Optional pre-treatment (to reduce fast-growing bacteria and select for spore-forming fungi): air-dry soil at room temp for several hours or heat at 40–60 °C for 30–60 min — use cautiously as heat can alter recovery. [CLSI,2017] Baghdad Soil samples were collected from several sites in Baghdad, Iraq, including agricultural fields and public garden areas. Sampling coordinates were recorded for each location.

2. Media & reagents

- Sabouraud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA).
- ✓ Typical SDA: peptone 10 g/L, glucose 40 g/L, agar 15–20 g/L; adjust to pH ≈5.6.
- Optional selective supplements: chloramphenicol 50 µg/mL (stock 5 mg/mL in ethanol or DMSO), streptomycin 50 µg/mL.
- Malt Extract Agar (MEA) or Czapek-Dox for morphology/phylogeny (optional).
- Sterile 0.85% saline or PBS + 0.01% Tween-80 (for spore suspensions).
- Sterile loops/needles, inoculating loops, spreaders.
- Lactophenol cotton blue for mounts (microscopy).
- Cryovials + sterile 15–20% glycerol for −80 °C storage.

2.1 Identification of isolates

Initially, the grown colonies were grouped according to their general appearance. Slides were stained with lactophenol cotton blue stain and viewed under a compound microscope to characterise the morphology of the fungal isolates. Using established techniques and taxonomic keys, identification was based on morphological features, including growth pattern, hyphae, colony and medium color, surface texture, edge character, and aerial mycelium. The following sources served as the fungal isolates' classification and identification keys: [8]

2.2 Collection of Kiwi peels, and preparation of KPE

The kiwi fruit was grown by local market farmers in Iraq and Baghdad. The ones that had to be thrown out because they were damaged had been peeled and cleaned properly. The resultant kiwi peels were boiled in boiling water for ten minutes. The produced KPE extract was centrifuged using a Thermo Scientific Heraeus machine, and the supernatant was stored at -19 °C until it was required. After being sorted and collected, the solid leftover from discarded kiwi peels was dried in an oven at 50°C before being stored. Additionally, the remaining pulp was collected, centrifuged, and stored at -19°C [9].

2.3 Preparation of Kiwi Peel

1. **Collection and cleaning**

- Wash fresh kiwi fruits thoroughly under running water to remove dirt and contaminants.
- Peel the fruits using a sterile knife, keeping only the skin for extraction.

2. **Drying (optional)**

- **Air-dry** the kiwi peels at room temperature in a clean environment for 1–2 days **or** use a **hot-air oven at 40–45 °C** until completely dry.
- Grinding dried peels into small pieces increases extraction efficiency.

3. **Grinding**

- Blend fresh or dried kiwi peels into small pieces or a coarse powder using a blender or grinder.

4. **Solvent extraction**

- **Aqueous extract:** Soak 50 g of peel pieces in 200 mL distilled water.
- **Organic extract (ethanol or methanol):** Soak 50 g of peel pieces in 200 mL 70% ethanol or methanol.

- Seal the container and shake occasionally. Extract for **24–48 hours** at room temperature, or perform **reflux extraction** at 40–50 °C for faster results.

5. **Filtration**

- Filter the extract through cheesecloth or muslin to remove coarse particles.
- Further filter using Whatman No. 1 filter paper for a clear solution.

6. **Concentration**

- Concentrate the filtrate using a rotary evaporator at 40–45 °C under reduced pressure to remove solvent.
- For aqueous extracts, concentrate using a water bath or lyophilization.

7. **Storage**

- Store the concentrated extract in sterile, labelled vials at **4 °C** for short-term use or **−20 °C** for long-term storage.

8. **Preparation for antifungal assay**

- Dissolve the concentrated extract in a small volume of sterile distilled water or 10% DMSO to prepare desired test concentrations.
- Filter-sterilise using a **0.22 µm membrane filter** before adding to agar diffusion or broth assays.

2.4 Zone of Inhibition

The agar well diffusion method was used to assess the Kiwi Peel preparation's antibacterial activity. To produce a consistent lawn of growth, 100 µL of the bacterial suspension against Gram-positive and Gram-negative bacterial strains was evenly distributed across the surface of each sterile Mueller-Hinton agar plate. 50 µL of various concentrations of Preparation of Kiwi Peel (1000, 500, 250, 125, and 62.5 µgmL-1) were applied to each well after wells were punched into the agar using a sterile cork borer. Amoxicillin served as a positive control, and control wells were left untreated. For a whole day, the plates were incubated at 37 °C. Following incubation, a calliper was used to measure the zones of inhibition (diameter in millimetres).

2.5 Statistical analysis

Graph Pad Prism 6 was used for statistical analysis. The study utilised the unpaired t-test. Conclusions have been displayed using the standard deviation as the mean of three tests. (SD).

3. Results

Fungi were isolated from soil samples collected from a variety of locations in Diyala, Iraq, including public garden spaces and agricultural fields.

On PDA media, several fungal colonies were seen after 5–10 days of incubation at 28–35°C. The colonies had a variety of colors and morphological characteristics, such as compact white or yellow colonies that turned from white to pale yellow in the daylight and creamish-yellow in the dark, becoming more greyish in the dark, and thick, dark green colonies. Other colonies looked pale, brownish-grey, or light grey. As seen in Figure 1, the colony surfaces were smooth with radial furrows and varied in color from whitish to cream-colored to grayish-brown.

***3.1 Aspergillus niger*:** Colonies compact, felt with dark black to blue conidiophores and distinguished by the flattened conidia. Conidial heads radiate, tending to split into loose columns with age. Phialides borne on medullae

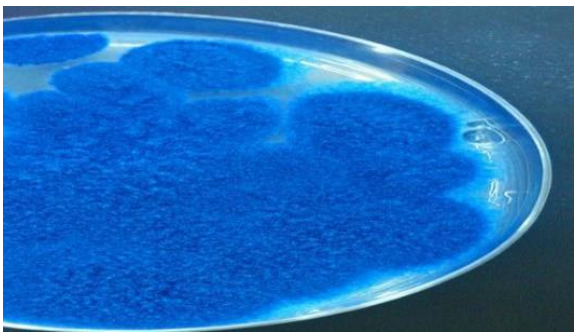


Figure (1): a- View of *Aspergillus niger* on PDA at 28 °C after 5 days of incubation.

A. ***flavus*****:** Colonies with a dense felt of yellow-green conidiophores. Conidial heads typically radiate. Phialides borne directly on the vesicle or on metulae

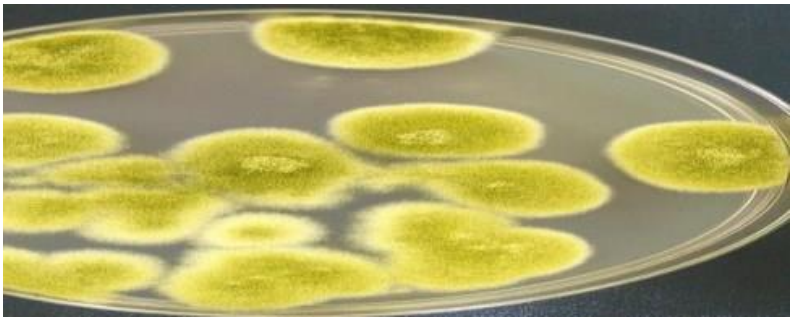


Figure (2): - View of *Aspergillus flavus* on PDA at 28 °C after 5 days of incubation

B. ***terreus*****:** Colonies dense felt of yellow-brown conidiophores, which are darker with age. Conidial heads compact, columnar. Phialides borne directly on metulae

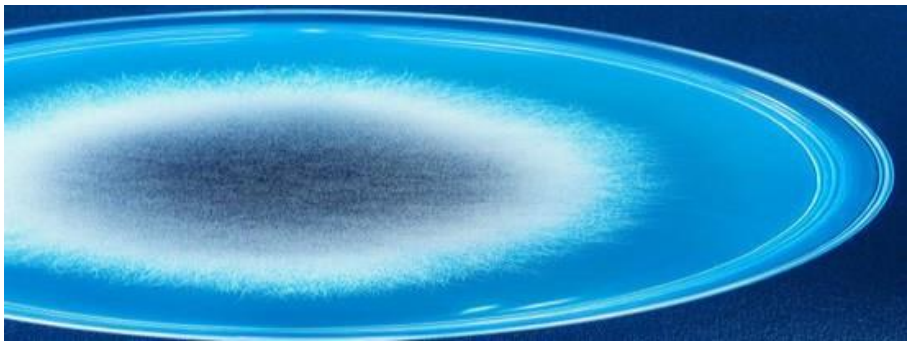
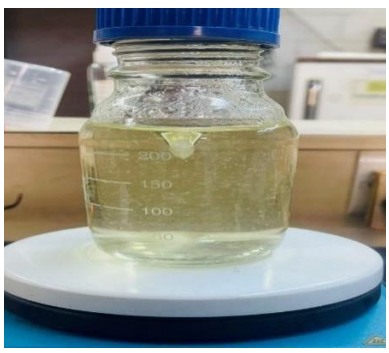


Figure (3): - View of *Aspergillus terreus* on PDA at 28 °C after 5 days of incubation

3.2 Kiwa Peel Extract



(a) (b)

Figure (4): a: Kiwa peel, b: Kiwa peel extract (KPE)

3.4 Anti-Candida Activities of KPE

1. Aspergillus niger

Table 1 shows that Kiwa peel extract (KPE) significantly inhibited the growth of Aspergillus niger at all tested concentrations. The mean zone of inhibition increased progressively from 11.0 ± 1.0 mm at 62.5 µg/mL to 22.7 ± 0.6 mm at 1000 µg/mL, indicating a clear dose-dependent antifungal effect. Statistical analysis revealed highly significant differences between concentrations ($P < 0.0001$). Tukey's multiple comparison test confirmed significant differences between low and high concentrations, demonstrating that increasing KPE concentration markedly enhances antifungal activity against A. niger.

Table 1. Antifungal activity of Kiwa peel extract against *Aspergillus niger* at different concentrations

Concentration µg/mL KPE	**Mean ± SD Zone of Inhibition**	**Sig.**	***p* Value**
62.5	11.0 ± 1.0a	**	<0.0001
125	13.7 ± 0.6b		
250	15.7 ± 0.67c		
500	18.3 ± 0.6d		
1000	22.7 ± 0.6d		
Tukey's multiple comparisons test	Below threshold?	Summary	Adjusted P Value
64 vs. **125**	Yes	**	0.0052
64 vs. **250**	Yes	**	<0.0001
64 vs. **500**	Yes	**	<0.0001
64 vs. **1000**	Yes	**	<0.0001
125 vs. **250**	Yes	*	0.0316
125 vs. **500**	Yes	**	<0.0001
125 vs. **1000**	Yes	**	<0.0001
250 vs. **500**	Yes	**	0.0052
250 vs. **1000**	Yes	**	<0.0001
500 vs. **1000**	Yes	**	<0.0001

2. Aspergillus flavus

As presented in Table 2, KPE exhibited strong antifungal activity against Aspergillus flavus. The inhibition zones increased from 12.7 ± 0.6 mm at 62.5 µg/mL to 22.7 ± 0.6 mm at 1000 µg/mL. All concentrations showed statistically significant inhibition compared to the control ($P < 0.0001$). No significant difference was observed between 125 and 250 µg/mL ($P > 0.05$), suggesting a temporary plateau in antifungal efficacy at intermediate concentrations. However, higher concentrations (500 and 1000 µg/mL) produced significantly greater inhibition, confirming a concentration-dependent response overall.

Table 2. Antifungal activity of Kiwa peel extract against *Aspergillus flavus* at different concentrations

Concentration µg/mL CS-Cu NPs	**Mean ± SD Zone of Inhibition**	**Sig.**	***p* Value**
62.5	12.7 ± 0.6a	**	<0.0001
125	15.3 ± 0.6b		
250	16.7 ± 0.67b		
500	19.7 ± 0.6c		
1000	22.7 ± 0.6d		

Tukey's multiple comparisons test	Below threshold?	Summary	Adjusted P Value
64 vs. **125**	Yes	**	0.0015
64 vs. **250**	Yes	**	<0.0001
64 vs. **500**	Yes	**	<0.0001
64 vs. **1000**	Yes	**	<0.0001
125 vs. **250**	No	ns	0.1020
125 vs. **500**	Yes	**	<0.0001
125 vs. **1000**	Yes	**	<0.0001
250 vs. **500**	Yes	**	0.0006
250 vs. **1000**	Yes	**	<0.0001
500 vs. **1000**	Yes	**	0.0006

3. Aspergillus terreus

Table 3 demonstrates that KPE also significantly inhibited Aspergillus terreus growth. The inhibition zones ranged from 13.7 ± 0.6 mm at 62.5 µg/mL to 21.3 ± 0.6 mm at 1000 µg/mL. Statistical analysis showed significant differences between most concentration levels ($P < 0.05$ to $P < 0.0001$). Although A. terreus exhibited slightly lower sensitivity compared to A. niger and A. flavus, the consistent increase in inhibition zones with increasing concentrations confirms the effectiveness of KPE against this species.

Table 3. Antifungal activity of Kiwa peel extract against *Aspergillus terreus* at different concentrations

Concentration µg/mL CS-Cu NPs	**Mean ± SD Zone of Inhibition**	**Sig.**	***p* Value**
62.5	13.7 ± 0.6a	**	<0.0001
125	15.7 ± 0.6b		
250	16.7 ± 0.67b		
500	19.3 ± 0.6c		
1000	21.3 ± 0.6d		
Tukey's multiple comparisons test	Below threshold?	Summary	Adjusted P Value
64 vs. **125**	Yes	*	0.0116
64 vs. **250**	Yes	**	0.0006
64 vs. **500**	Yes	**	<0.0001
64 vs. **1000**	Yes	**	<0.0001
125 vs. **250**	No	ns	0.2830
125 vs. **500**	Yes	**	0.0001
125 vs. **1000**	Yes	**	<0.0001
250 vs. **500**	Yes	**	0.0015
250 vs. **1000**	Yes	**	<0.0001
500 vs. **1000**	Yes	*	0.0116

Collectively, the results of all three tables confirm that **Kiwa peel extract possesses significant and concentration-dependent antifungal activity against different** *Aspergillus* **species**. The consistently low P-values indicate strong statistical significance and reproducibility. Variations in susceptibility among species may be attributed to differences in fungal cell wall structure and intrinsic resistance mechanisms.

4. Discussion

The fungicidal activity of KPE on Aspergillus species was successfully confirmed in the current investigation and is well represented by the quantitative data highlighted in Tables (1–3). Results: The data showed that KPE exerted a marked inhibitory effect on the growth of fungi

and such inhibition was found to be dose-dependent for all concentrations tested in comparison with control ($P < 0.05$). *Aspergillus niger*. KPE was found to be more and more effective towards A. niger as the concentration increased (Table 1). Inhibition zone was greater from 11.0 ± 1.0 mm at 62.5 µg/mL to 22.7 ± 0.6 mm at a concentration of 1000 µg/mL, with extremely significant differences ($P < 0.0001$). Tukey's multiple comparisons test showed that differences between nearly all concentration pairs were significant, especially at low and high concentrations, indicating a potent dose – effect relationship. These results indicate that A. niger is very sensitive to the bioactive compounds in KPE. [10]

Aspergillus flavus. The antifungal activity of KPE against A. flavus was quite similar to that exerted on A. parasiticus, as shown in table (2). Diameter of the inhibition zones ranged between 12.7 ± 0.6 mm at the lowest concentration and 22.7 ± 0.6 mm at a concentration of 1000 µg/mL. Most concentration groups were statistically different ($P < 0.0001$). But there was no significant difference between 125 µg/mL and 250 µg/mL ($P < 0.05$), indicating a plateau effect in the intermediate concentration. This may imply an adaptive response on behalf of the fungal or threshold concentrations of active agents that are necessary to reach maximal antifungal activity. [11]. *Aspergillus terreus* was a bit less sensitive to KPE (14%) as compared with A. niger and A. flavus, as presented in Table (3). However, a dose-dependent inhibition was evident; the size of inhibition zones was 13.7 ± 0.6 mm at a concentration of 62.5 µg/mL to 21.3 ± 0.6 mm at 1000 µg/mL. There were significant differences between the low and high concentrations ($P < 0.001$), indicating that KPE had antifungal activity against this strain. Reduced activity towards *A. terreus* could be due to known resistance mechanisms and differences in cell wall composition among these pathogens. [12].

Statistical significance and reproducibility. The uniformly low P-values (< 0.05 and < 0.0001) in all the tables show that KPE possesses statistically meaningful and repeatable antifungal activities. Triplicate readings (mean ± SD) have increased the reliability of the set of results, and minimized experimental variability.

The antifungal effect in the current study is probably due to the presence of phenolic compounds and flavonoids in kiwi peel. It is known that such compounds cause disturbances in the cell wall of fungal cells, which do not allow ergosterol synthesis, and generate oxidation stress resulting in the inhibition of growth. The same results are observed in (Perfect, 2017; Cowen et al., 2014; Hasan et al., 2024). inconclusive and different with plant extracts on peels against Aspergillus. [12] The findings reveal the promising of the Kiwa peel extract as a natural and environmentally friendly in vitro antifungal agent, especially given its origin as agricultural waste. This method provides a green solution as an eco-friendly substitute to synthetic antifungal drugs, and also represents a waste recycling perspective. In continuation, in vivo significance of the fraction was not determined and still more identification and studies of active compounds should be addressed. [13,14,1].

Conclusion

The current investigation has proved that extract obtained from Kiwa peel (KPE) can effectively suppress the growth of Aspergillus niger, Aspergillus flavus and Aspergillus terreus in a dose-dependent manner. The KPE showed significant inhibition at all the concentrations tested ($P < 0.05$) and this study confirms the efficacy of KPE as a natural antifungal agent. A niger and A flavus were also little more sensitive than A terreus among the tested species. Antifungal activity is associated with the bioactive of kiwi peel, especially phenolic and flavonoid compounds. This study indicates the potential of kiwi peel, an agricultural waste product, as a sustainable, environmentally friendly and low-cost source of antifungal activity. However, more research are encouraged to purify the active ingredients, and explore the possible mechanism of action and in vivo activity and safety.

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