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Biological control of *Zingiber officinale* extracts against pathogenic fungi isolated from juniper plant roots

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Abstract

In this study, the antifungal effect of aqueous and ethanolic extracts and crude juice of the ginger plant was evaluated against fungi isolates: *Aspergillus terreus*, *Fusarium poae*, *Penicillium crustaceum*, *P. wortmannii*. The results showed that ginger (aqueous, ethanolic extract, and crude juice) had a high level of inhibitory activity against *A. terreus*, with the inhibition rate equal to 91.6%. The ethanolic extract inhibited the growth of *F. poae* by 89.9%, but the aqueous extract and the crude juice inhibited the growth of *F. poae* by 61.01%, which was lower than the ethanolic extract. While the ethanolic extract and crude juice had a high inhibition efficacy against *P. crustaceum* fungus with the inhibition of fungal growth by 71.92% and 70.17%, respectively. It was higher than the aqueous extract in which growth was inhibited by 47.36%. Crude ginger juice had the highest effectiveness as it inhibited the growth of *P. wortmannii* fungi with 88.37%, followed by ethanolic extract with 69.76% and aqueous extract with 53.25%. The effect of ginger extracts on the dry weight of the study fungi was studied. The ethanolic extract of ginger recoded the lowest dry weight value in all fungi, followed by the crude ginger juice, while the aqueous extract showed less inhibitory effect against fungi compared to the previous extracts.\

Keywords: *Zingiber officinale*, Aqueous extract, Ethanolic extract, Crude juice.

Introduction

Plant diseases called Phytopathogens cause significant crop losses, despite advances in agricultural productivity (*1*). An excessive use of synthetic pesticides such as bactericides and fungicides have negative environmental consequences, such as soil and water pollution, and affects the efficiency of the control against pathogenic microbes over time (*2, 3*). This means that there is a need to consider the use of alternative natural biocides for the prevention of plant diseases caused by microbes that are effective, non-toxic, and beneficial to the environment (*4*). Plants are considered a therapeutic alternative for the majority of humans and herbal compositions account for up to 40% of total prescriptions. Several plant extracts and active components of medicinal plants have been suggested as potential sources of new medications due to their safety and few side effects (*5, 6*).

Ginger belongs to the Zingiberaceae family. There are approximately 20 species in the genus Ginger. The volatile oils of Zingerone, shogaol and gingerol make up one to three percent of the weight of crude ginger, giving ginger its distinct odor and flavor. Zingiberene is one of the most important components found in ginger. Smaller amounts of other sesquiterpenoids (-sesquiphellandrene, bisabolene, and farnesene) and a small monoterpenoid fraction (-phelladrene, cineol, and citral) have also been identified (*7*).

The history of ginger and its use in medicine have been documented since ancient times (*8, 9*). In vitro and in animals, gingerols have analgesic, sedative, antipyretic and antibacterial effects (*10, 11*). Ginger has a wide range of pharmacological properties that have been proven (*12*). Ginger is used to treat a variety of ailments in both humans and animals (*13*). Ginger was also used as a digestive aid, a nausea medication, and a treatment for baldness, toothaches, and respiratory problems, as well as a cholesterol-lowering plant (*14, 15*). Ginger extracts have anti-inflammatory and anti-cancer effects. In adult cancer patients, ginger supplements dramatically lessen the intensity of acute chemotherapy-induced nausea (*8*). The ginger extract has antifungal properties (*16*). In the study by (*17*) ginger root oils were shown to have an inhibitory effect against *Aspergillus niger* and *A. fumigatus*. The crude methanol extract of aromatic ginger showed strong innate resistance against *Fusarium*. Aromatic ginger can be used as an effective antifungal agent (*18*). The effect of ginger extract on *Candida albicans, Aspergillus spp.,* and *Penicillium spp.*, there was inhibition of the growth of the pathogenic fungi

(*19*). Thus, ginger can be used for medicinal purposes in pharmaceutical industries and can be used in the storage of materials to prevent fungi Active because it is a natural substance that is available and cheap. In a study (*20*) it was found that ginger can be used as a good antimicrobial and an important agent in suppressing the growth of plant pathogens and as a potential alternative to fungicides because ginger contains necessary chemical components such as essential oils.

Aspergillus terreus expands over the world and appears as contaminants in food storage places in many tropical and subtropical regions. *A. terreus* may cause infection in people with deficient immune systems (*21*). *Fusarium poae* causes serious problems in cereals due to its ability to produce mycotoxins (*22*). *Penicillium crustaceum* is produced mycotoxins and has been found to cause animal poisoning (*23*). *Penicillium wortmannii* is found in soil, air, and food. It aids in the decomposition of organic materials (*24*). It is one of the most important fungi discovered for its ability to spread huge numbers of spores in the environment which poses a major threat to humans, animals, and plants (*25*). The objective of this study was to examine the effects of aqueous, ethanolic, and crude juice ginger (*Zingiber officinale*) extracts on several phytopathogenic fungi.

Materials and methods

Plant sample:

Zingiber officinale rhizomes were collected at random from local markets and dried for two weeks at room temperature (25C°). The powder was then ground with an electric grinder and stored in a glass bottle until used to prepare the aqueous and ethanolic extracts. The crude juice was used directly without drying.

Fungal isolates used in the study:

The four fungi isolate *Aspergillus terreu*, *Fusarium poae*, *Penicillium crustaceum*, and *P.wortmannii* were isolated from the roots of the *Juniperus phoenicea* L. plant growing in the Libyan Al-Jabal Al-Akhdar region, which was state from severe deterioration, and symptoms appeared on the affected plants, and the broad crowns fell off, the leaves on the affected branches it turned yellow, then pale red, and finally brown. The tree's leaves can last two

months and then begin to fall off. Fungi were identified by morphological and microscopic characteristics (*26, 27*).

Preparation of extracts:

- **Aqueous extract:** Prepare the aqueous extract of ginger rhizomes according to the method (*28*), which comprised mixing 20 g of dry matter powder of the plant with 200 ml at a concentration of 10% of sterile distilled water and leaving the solution for 24 hours while stirring continuously by shaker in room temperature (25 C°) and filtered using sterilized Whatman No.1 filter paper; the extracted material was then concentrated in a rotary evaporator at 60C° and stored in sterile glass bottles to be tested against the pathogen fungi.
- **Ethanolic extract:** Prepare the ethanolic extract in the same way as the aqueous extract, but use ethyl alcohol 70% to replace water (*29*)**.**
- **Crude Juice preparation:** Mixing 10 g of peeled and chopped ginger a rhizome with 25 ml of sterile distilled water in blender electrophoresis and used directly in the inhibitory activity test for study fungi (*30*).

Detection of Some chemical compounds in aqueous, ethanolic extract, and crude juice of the ginger plant:

Some of the effective components in the aqueous and ethanolic extract and crude juice of ginger were detected using several reagents: reagent (Liebermann-Borcard) for turbines, ferric chloride For phenols, alcoholic potassium hydroxide 10% For flavonoids, Mayer's reagent for alkaloids, Aqueous lead acetate tannins (*31*).

Studying the effect of ginger (aqueous, ethanolic extracts, and crude juice) on the radial growth of the study fungi:

Fungi were inoculated on PDA plates in triplicates and incubated at 25C^{o} for 3 to 7 days to obtain young, actively growing colonies of fungi. 100µl of plant extract was mixed with 15ml of cooled (45C^{o}) molten PDA media, poured On to the plates, and allowed to solidify at room temperature for thirty minutes. A 6mm diameter mycelial disc was cut from the periphery

of 3 to 7-day-old cultures and inoculated onto the agar plates containing the plant extract aseptically. As for control, PDA plates containing no extract were used. The inoculated plates were incubated at 25C^{o} and colony diameter was measured and recorded after 7 days. The percentage of mycelia growth inhibition (MGI %) was calculated (*32, 33*).

Studying the biological activity of ginger (aqueous, ethanolic extracts, and crude juice) on the dry weight of study fungi:

The effect of the ginger extracts on the fungi growth in the potato dextrose liquid PDB . Conical flasks of 250 mm are used, 50 ml of the liquid medium is placed and various extracts are added to it. Then the flasks are inoculated with a 5 ml diameter disc of the fungi used in the study. As a control, a flask containing medium only and no extract was used. After 7 day incubation period, the fungal mycelia were filtered and washed twice with distilled water, then dried in an oven at 60C^{o} for 24 hours and with three replicates for each extract (*34*). Mycelia weight was determined using an electronic balance. The values of mycelium dry weight (MDW) were calculated to be g/flasks.

Statistical analysis:

The standard error (SE) was calculated using Past programs, and the data provided in this study were the means of three replications.

Results

The results of the ginger compounds (aqueous and ethanolic extract and crude juice) shown in Table 1 gave various responses during the initial tests. The positive detection of each compound gave the following: The flavonoids were yellow, the alkaloids were brown color reddish and the phenols did give the blue color in ethanolic extract and crude juice. The terpenoids were green and the tannins were white in aqueous and ethanolic extracts and did not give white sludge in the crude juice, so it was a negative result. Flavonoids, phenols, alkaloids, terpenoids, tannins, and soaps were found in aqueous and ethanolic ginger extracts and crude juice.

Table 1. Preliminary tests for basic chemical components in aqueous and alcoholic extract and fresh juice of the ginger plant

Ginger extracts	Alkaloids	Flavonoids	Phenols	Terpenoids	Tannins	Soaps

Aqueous	+	+	-	+	+	+
Ethanolic	+	+	+	+	+	+
Crude juice	+	+	+	+	-	+

The effects of ginger (aqueous and ethanolic extracts, as well as crude juice) on four fungal isolate growths (*Aspergillus terreus, Fusarium poae, Penicillium crustaceum*, and *P. wortmannii*) were tested in the PDA medium. The antifungal activity of the high extracts of aqueous, ethanolic, and crude juice in inhibiting radial growth against fungi tested, as shown in Table 2 and **Figures (1 and 2)**, as compared to fungi growth in the control treatment. *Aspergillus terreus* growth 6 cm, *F. poae* 5.9 cm, *P. wortmannii* 3. 4 cm, and *P. crustaceum* 7 .5 cm. In this study, ginger (aqueous, ethanolic extract, and crude juice) had a high inhibitory effect against the fungus *A. terreus.* The diameter of the growth was (0.5, 0.5, d 0.5an cm), respectively and ginger extracts had inhibitory activity on the *F. poae*, with diameters of growth being (2.3, 2.3, and 0.6 cm), respectively. On the other hand, the ethanolic extract and juice showed a strong inhibitory effect on *P. wortmannii* and *P. crustaceum*. The average growth diameter was (1.7, 1.6, 0.5, and 1.3 cm) respectively, which was higher than the aqueous extract.

Table 2 Show that the ethanolic and aqueous extracts and crude juice of ginger were screened for their activity against *A. terreus*, *F. poae, P. crustaceum,* and *P. wortmannii by* the mycelium dry weight method. Aqueous extract of ginger resulted in a decrease in the dry weight for *A. terreus* and a record 0.76 g / flask compared to control. The high effectiveness of ethanolic ginger extracts in lowering dry weight rates of *P. crustaceum* and *P. wortmannii* growth and recording 0.84, 0.77g / flask. The aqueous extract did not show any effect on *P. crustaceum*. The dry weight of mycelium was reduced by crude ginger juice. Inhibit *F. poae* growth and record 0.81g / flask.

Table 2. The effect of ginger (aqueous, ethanolic extracts, and crude juice) on the radial growth and the dry weight of study fungi (Mean±SE): The standard error

Ginger extract / Study fungi		*A . terreus*	*F . poae*	*P. crustaceum*	*P .wortmannii*
Control	Colony diameter (cm)	6.0 ±0.23	5 .9 ±0.26	5.7 ±0.20	4.3 ±0.11
	MDW (g / flask)	0.92 ±0.42	0.96 ±0.21	1.05 ±0.28	0.97 ±0.22
Aqueous	Colony diameter (cm)	0.5 ±0.05	2.3 ±0.08	3.0 ±0.20	2.0 ±0.14
	MDW (g / flask)	0.76 ±0.21	0.85 ±0.23	1.05 ±0.27	0.87 ±0.22
Ethanoic	Colony diameter (cm)	0.5 ±0.08	0.6 ±0.14	1. 6 ±0.20	1.3 ±0.03
	MDW (g / flask)	0.85 ±0.31	0.93 ±0.28	0.84 ±0.23	0.77 ±0.19
Crude juice	Colony diameter (cm)	0.5 ±0.06	2.3 ±0.15	1.7 ±0.20	0.5 ±0.14
	MDW (g / flask)	0.77 ±0.23	0.81 ±0.19	0.93 ±0.29	0.90 ±0.20

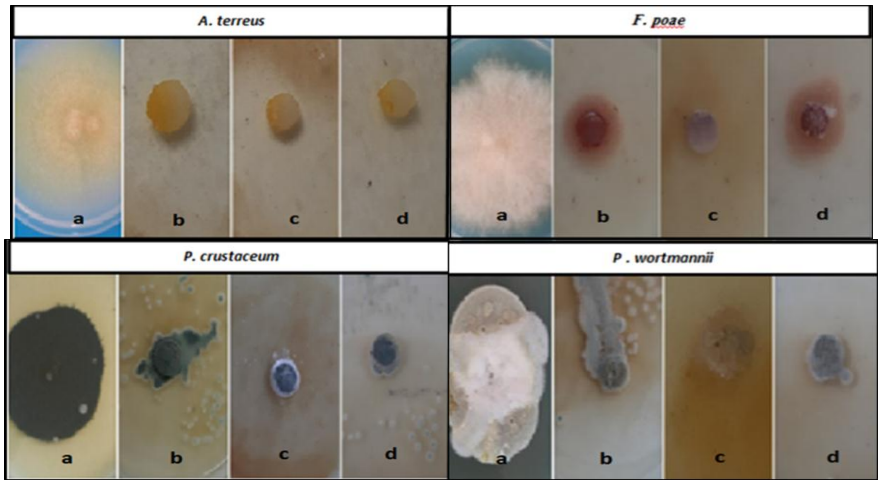
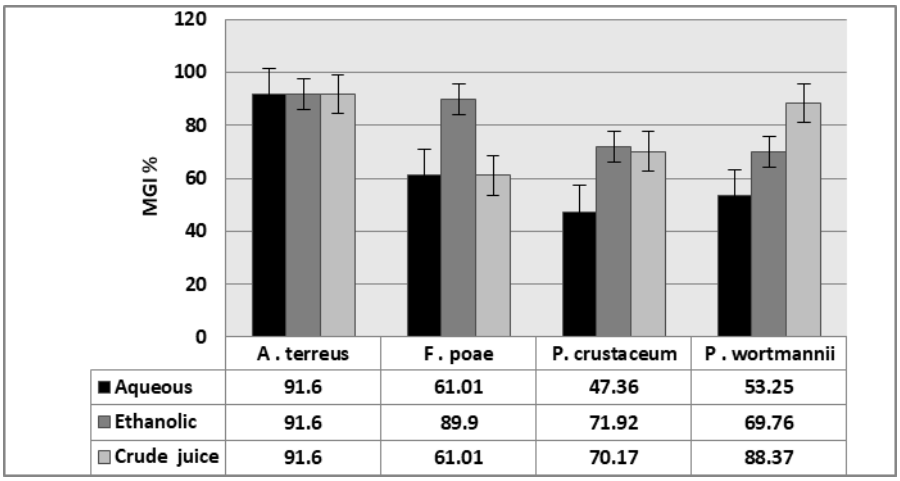


Figure: (1). shows the growth of the study fungi in the control (a) and the effect of ginger (aqueous b, ethanolic c extracts, and crude juice d) on the radial growth of the study fungi.



	A . terreus	F . poae	P. crustaceum	P . wortmannii
Aqueous	91.6	61.01	47.36	53.25
Ethanolic	91.6	89.9	71.92	69.76
Crude juice	91.6	61.01	70.17	88.37

Figure 2. The effect of ginger (aqueous, ethanolic extracts, and crude juice) on the percentage of mycelia growth inhibition (MGI %) of study fungi

Discussion

The results shown in Table 1 showed that aqueous and ethanolic ginger extracts and crude juice contain the following components: flavonoids, alkaloids, phenols, terpenoids, and tannins, and this is consistent with the results of most studies on the chemical composition of ginger (*35*, *36*). Many of the chemicals found in the ginger plant, such as shagelol and gingerol, are highly effective against microorganisms. Alkaloids play an important role in medicine and have a physiological effect on the organism (*37*). Phenolic aromatic compounds are the main active components of plants that have antimicrobial activities so that they have different modes of activity, such as cell wall degradation and disruption of the cytoplasmic membrane, diversity of fatty acid and phospholipid components, interference with DNA and RNA synthesis, and mitochondrial dysfunction in Eukaryotes (*38*).

Our results are in agreement with several researchers, such as (*39*) who discovered that some plant extracts, including ginger, have antagonistic activity against *Aspergillus sp.*, and (*40*) who reported that plant extracts can inhibit the germination of conidia and spores in a variety of fungal species.

Phenols, particularly gingerols and shogaols, are found in ginger extracts. Antifungal and antibacterial properties are found in plant species that contain phenols and volatile oils (*41*). Ginger extracts showed exhibited high inhibitory activity against the tested May be due to containing the following compounds: Zingiberine, Zingiberol, and Bisabolene (*42*). The high efficiency of ginger extracts could be attributed to the presence of ketones like gingerol and paradol in the rhizomes. Gingerol is a mixture of crystals gingerone, which is the main cause of ginger acidity and has a role in preventing microbe growth (*43*). The ethanolic extracts of ginger were efficient against a variety of fungi (*44*).

The crude ginger juice has inhibitory properties against *A.niger* and *Mycoderma spp.* The reason may be that the active ingredients in the extract were not affected due to the non-use of chemicals during the extraction and the ability of water to dissolve the plant and release its active substances (*45*). Severalresearchers indicated the inhibitory role of plant extracts towards *Aspergillus sp.* It is known for the production of ochratoxins and aflatoxin (*46, 47, 48*). In a study conducted by (*49*), ethanolic extracts of ginger plants were employed against seven species of fungi, including *Fusarium sp*., *Penicillium sp*., and *Aspergillusniger* and they proved to be highly effective against these fungi. The presence of caprylic acid, zingerone, shogaols, and gingerols in ginger ethanolic extract reduced the growth of *Aspergillus flavus*, *Penicillium oxalicum*, and *Rhizopus stolonifer*, *Mucor sp*. and *Scopulariopsis sp.* with zones of mycelia growth inhibition ranging from 30% to 100% (*41*).

Conclusion

Our study focused on the effect of ginger extract of its three types (aqueous extract, ethanolic, and crude juice) on four phytopathogenic fungi: *Aspergillus terreus*, *Fusarium poae*, *Penicillium crustaceum,* and *P. wortmannii.* The results showed that the antifungal effect is very clear. Therefore, ginger is considered safe for human consumption and contains active compounds that give it the ability to treat many human diseases.

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المكافحة البيولوجية لمستخلصات نبات الزنجبيل ضد بعض الفطريات الممرضة والمعزولة من جذور نبات العرعر

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المستخلص العربي

في هذه الدراسة تم تقييم التأثير المضاد للفطريات للمستخلصات المائية والإيثانولية والعصير الخام لنبات الزنجبيل ضد العزلات الفطرية الاتية: *Aspergillus terreus*, *Fusarium poae*, *Penicillium crustaceum*, *P. wortmannii*. أظهرت النتائج أن نبات الزنجبيل (المستخلص المائي و الإيثانولي والعصير الخام) كان له مستوى عال من النشاط المثبط ضد فطر *Aspergillus terreus* وكان معدل التثبيط متساوي 91.6٪. أدى المستخلص الإيثانولي إلى تثبيط نمو فطر *Fusarium poae* بنسبة 89.9٪ ، لكن المستخلص المائي والعصير الخام منع نمو *Fusarium poae* بنسبة 61.01٪ ، والتي كانت أقل من المستخلص الإيثانولي. بينما كان للمستخلص الإيثانولي والعصير الخام كفاءة عالية في تثبيط فطر *Penicillium crustaceum* بنسبة 71.92٪ و 70.17٪ على التوالي حيث كان أعلى من المستخلص المائي الذي ثبط نمو الفطر بنسبة 47.36٪. العصير الخام للزنجبيل كان ذات فعالية عالية حيث منع نمو فطر *P. wortmannii* بنسبة 88.37٪ ، يليه المستخلص الإيثانولي بنسبة 69.76٪ والمستخلص المائي بنسبة 53.25٪. تمت دراسة تأثير مستخلصات الزنجبيل على الوزن الجاف لفطريات الدراسة. سجل المستخلص الإيثانولي للزنجبيل أقل قيمة وزن جاف في جميع الفطريات ، يليه عصير الزنجبيل الخام ، بينما أظهر المستخلص المائي تأثيراً أقل تثبيطاً ضد الفطريات مقارنة بالمستخلصات السابقة.

الكلمات المفتاحية: الزنجبيل، المستخلص المائي، المستخلص الايثانولي، العصير الخام.