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Original Article

Antifungal Activity of Phenols Compound Separated from *Quercus infectoria* and *Citrullus colocynthis* against Toxic Fungi

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Abstract

Penicillium expansum is one of the most harmful post-harvest fungal pathogens. *Aspergillus flavus* is a saprotrophic fungal organism with broad distribution, producing mycotoxins that are toxic to humans and animals. This study aimed to investigate the antifungal activity of phenolic alcohol extract for the dry plants Oak (*Quercus infectoria* Oliv) and Bitter Melon (*Citrullus colocynthis* (L.) Schrad). Three concentrations of phenolic alcohol extract of Oak and Bitter Melon (100, 200 and 300 mg/mL) have been prepared against two fungi, *Penicillium expansum* and *Aspergillus flavus.* The results showed that all three concentrations of phenolic extracts gave antifungal activity, and the percentage inhibition of diameter growth (PIDG) increased with increasing concentrations. The *C. colocynthis* extract gave the highest average of PIDG (38.29%), followed by *Q. infectoria* with an average of PIDG (34.13%) against *P. expansum* and *A. flavus.* The *A. flavus* fungus experienced more potent inhibition, with an average of PIDG (49.05%), than *P. expansum*, with an average PIDG of (23.37%). The results showed that the *C. colocynthis* extract gave the highest PIDG (70.7±3.90), followed by *Q. infectoria* with PIDG (31.1±3.335) at a concentration of (300 mg/mL) on *P. expansum.* While the results for phenolic extracts of *C.colocynthis* and *Q. infectoria* on *A. flavus* showed that the antifungal activity of *C. colocynthis* extract had the highest PIDG (72.09±4.10) followed by *Q. infectoria* with PIDG (62.49±3.63) at a concentration of (300 mg/mL). We concluded that the phenolic extracts of *Q. infectoria* galls and *C. colocynthis* fruit showed inhibitory activity against two toxin-producing fungi, *P. expanisum* and *A. flavus.*

Keywords: Phytotherapy, Plant extract, *Quercus, Citrullus, Penicillium, Aspergillus flavus*

1. Introduction

Decaying of vegetables and considerable economic losses in the grapes impute a group of phytopathogenic fungi that play a role in the food rotting process.

Penicillium expansum is one of the most harmful post-harvest fungal pathogens that produce patulin and many other toxic metabolites such as citrinin, roquefortine C and chaetoglobosins (1). It often contaminates apple and pear products, juices, oranges in the citrus industry and grapes. However, it is the underlying agent of "blue rot disease". Additionally, these species are responsible for systemic mycosis called "penicilliosis" in immunocompromised patients, and patulin pollution makes up a significant health risk for children who consume large quantities of fruit juices (2).

Aspergillus flavus is a saprotrophic fungal organism with broad distribution producing mycotoxins that are toxic to humans and animals, causing "Aspergillosis" and can also cause diseases in economically important crops, such as maize and peanuts (3).

Quercus infectoria Oliv is a small tree or a shrub belonging to the *Fagaceae* (*Quercaceae*) family. Galls of *Quercus infectoria* are known by different local

names, such as Oak. The plant is found in Turkey, Syria, Persia, Cyprus and Greece. Phytochemical analysis of a hydroalcoholic extract of fruits of *Q. infectoria* exhibits the presence of tannins, flavonoids, saponins, triterpenes and anthraquinones cumarines (4). The gall also contains gallic acid (about 2-4%), ellagic acid, sitosterol, methyl betulate, methyl linolate, starch, calcium oxalate nyctanthic, roburic and syringic acids with central nervous system active component (5).

Citrullus colocynths (L.) Schrad belongs to *the Cucurbitaceae* family, assorted as a drought-tolerant plant species usually found in all hot arid areas. In addition, it is found in the Mediterranean region and in India, where typically utilized as traditional medicine. Vegetative and generative modes quickly propagate it in summer (6). *C.colocynths* is used in treating disorders like inflammation of joints, pimples and boils, constipation and bowel upset, glucose level regulation, urticarial and hepatitis. *C. colocynths* contains a complex mixture of phenolic acid and glycosides varying wildly in composition (7).

Therefore, this study was designed to investigate the antifungal activity of phenolic alcohol extract for the dry plants Oak (*Quercus infectoria* Oliv) and Bitter Melon (*Citrullus colocynthis* (L.) Schrad).

2. Materials and Methods

2.1. Samples Collection

Fifteen samples have been collected from food material from Babylon and Najaf Province markets to isolate the toxin-producing fungi. The samples were transferred to the advanced mycology laboratory, Faculty of Science, Kufa University, for diagnosis and study. Dried plant parts were purchased from the local markets of Babylon and Najaf Province, *Q. infectoria* galls and *C. colocynthis* fruit, which were investigated in the advanced Botanical laboratory at the Faculty of Science, the University of Kufa after cleaning and foreign substances isolation. An electrical grinder crushed them, and then the powder was collected in nylon bags and kept in the laboratory at room temperature until use.

2.2. Culture Media

The Culture media has been prepared as recommended by the Manufacturing Company and sterilized by Autoclave at 121°C for 15 minutes in 15pis\ inch^2. This method has been used to isolate and grow the fungi (8).

2.3. Preparation of Phenolic Compounds Extracts

The phenolic compounds were obtained from *Q. infectoria* galls and *C. colocynthis* fruit by taking 100 grams of each plant and placed in a glass beaker, then adding 400 mL of 2% acetic acid by using a reflex condenser; the phenolic compound was extracted in a water bath at 70°C and for 8 hours. After accomplishing the extraction with a solution is left cooling. Then it is filtered and put in a separation funnel, adding an equal volume of n-propanol and an adequate amount of NaCl until it gets saturation. It forms two layers upper one contains phenol, while the lower layer is neglected. Then, the upper layer was concentrated by a rotary evaporator, and the dried material was put in a refrigerator at 4°C until the usage time. The concentration was prepared by dissolving (100, 200 and 300 mg/mL) of alcoholic extracts with 1 mL of 10% Dimethylsulphoxide (DMSO). The final concentration for each solvent will be (100,200, and 300 mg/mL), which was used against fungi (9).

2.4. Study of the Antifungal Efficacy of Phenolic Compounds Extracts

The mixing method has been used with Sabouraud dextrose agar (SDA) to study the antifungal activity of phenolic extracts for three concentrations (100, 200 and 300 mg\mL). Then, relocated 0.1 mL from each concentration was to a Petry dish. The SDA medium was poured on it, and after that, dishes were left to be hardened, taking a disc with a diameter of 5 mL was taken by the sterile cork borer from each fungus, and the disc was placed on the surface of the culture medium. The petry dishes are incubated at a temperature of 25°C ± 2 for 7 days. The antifungal activity of the phenolic extracts is determined by measuring the diameter of the inhibition zone,

measured in millimetres (mm) by the ruler Mohammed AS (10).

Determination of the percentage inhibition of diameter growth

(PIDG)

Following the observation for MFC, the percentage inhibition of diameter growth (PIDG) values were determined according to the equation below:

PIDG (%)= Diameter of sample – Diameter of control×100

Diameter of control

2.5. Determination of the Percentage Inhibition of Diameter Growth (PIDG)

The percentage inhibition of diameter growth (PIDG) values was determined according to the equation below (11):

PIDG (%)= Diameter of sample–Diameter of control/diameter of the control×100

3. Results

3.1. Effect of *Q. infectoria* and *C. colocynthis* Phenolic Extracts on *P. expansum* and *A.* flavus

The antifungal activity of two different phenolic extracts (*Q. infectoria* galls and *C. colocynthis* fruit) against the *P. expanisum* and *A. flavus* has been evaluated using a mixing method with the medium. The phenolic extract showed effective inhibition of fungal growth, and the percentage inhibition of diameter growth (PIDG) increased with increasing concentration compared with the control group. The results in table 1 showed that the phenolic extract of *C. colocynthis* followed by *Q. infectoria* gave highly significant inhibitory activity against *P. expanisum* and showed that the PIDG were (%70.7±3.90) and (%31.1±3.335) at a concentration (300 mg/mL) respectively. While a concentration (100 mg/mL) showed a lower effect with PIDG (%3.7±0.64) in each of the *C. colocynthis* and *Q. infectoria*. In the same table for the *C. colocynthis* extract, the highest percentage of inhibition was given to *P. expanisum*, an average (%29.96). While the effect of *Q. infectoria* extracts on *P. expanisum* was given lower percentage inhibition with an average (%16.78). The concentration (300 mg/mL) gave a highly significant percentage inhibition to *P. expanisum* with an average (%59.9) (Figure 1).

Table 2 showed phenolic extracts effects of *Q. infectoria* galls and *C. colocynthis* fruit against the *A. flavus* that gave significant differences among all concentrations compared with the control group. The highest PIDG of *C. colocynthis* extract at a concentration of 300 mg/mL (72.09±4.10) compared with other concentrations (200 and 100 mg/mL) with percentage inhibition (51.47±9.46) and (16.29±4.29) respectively. While the highest percentage inhibition of *Q. infectoria* extracts at a concentration of 300 mg/mL (62.49±3.63) compared with other concentrations (200 and 100 mg/mL) with percentage inhibition (39.28±6.0) and (52.68±3.08) respectively (Figure 2).

In the same table for the *Q. infectoria* and *C. colocynthis* extract, the highest percentage inhibition was against *A. flavus* at *Q. infectoria* with an average of 51.48%, followed by *C. colocynthis* with an average of 46.62%. The concentration (300 mg/mL) gave a highly significant percentage inhibition to *A. flavus* with an average (% 67.29) (Figure 2).

When the comparison of the best percentage inhibition of diameter growth of both *Q. infectoria* and *C. colocynthis* on *P. expansum* and *A. flavus*, which were chosen from Table 3, the result showed that the *C. colocynthis* extract had the maximum antifungal activity with highly significant (%38.29) on *P. expansum* and *A. flavus* in compare to *Q. infectoria* with an average of PIDG (%34.13). The *A. flavus* fungus was most affected by inhibition, with an average of PIDG (%49.05) compared with *P. expansum*, with an average of PIDG (%23.37) (Figure 3).

Table 1. PIDG of *Quercus infectoria* and *Citrullus colocynthis* extracts towards different concentrations of *Penicillium expansum*

Type of extract	Mean of PIDG (%)±SD			
	100 (mg/mL)	200 (mg/mL)	300 (mg/mL)	Mean b
Quercus infectoria	3.7±0.64	15.55±5.87	31.1±3.335*	16.78
Citrullus colocynthis	3.7±0.64	15.45±2.36	70.7±3.90**	29.96*
Mean a	3.7	15.5*	59.9**	
Control	0	0	0	
LSD	a=4.014 ab= 5.677 b=3.277			

Data are means of three replicates (n=3) ± standard deviation. Data is presented as highly significant***, slightly significant**, and significant* at $P \leq 0.05$ within different extract concentrations. LSD was applied to all the data

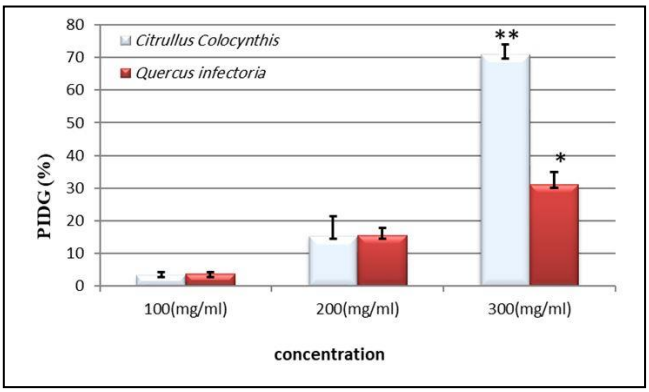

Figure 1. PIDG of *Quercus infectoria* and *Citrullus colocynthis* extracts against different concentrations of *Penicillium expansum*

Table 2. PIDG of *Quercus infectoria* and *Citrullus colocynthis* extracts towards different concentrations of *Aspergillus flavus*

Type of extract	Mean of PIDG (%)± SD			
	100 (mg/mL)	200 (mg/mL)	300 (mg/mL)	Mean b
Quercus infectoria	52.68±3.08**	39.28±6.0*	62.49±3.63***	51.48*
Citrullus colocynthis	16.29±4.29	51.47±9.46**	72.09±4.10***	46.62
Mean a	34.485	45.375	67.29*	
Control	0	0	0	
LSD	a=7.68 ab= 11.12 b=6.42			

Data are means of three replicates (n=3) ± standard deviation. Data is presented as highly significant***, slightly significant**, and significant* at $P \leq 0.05$ within different extract concentrations. LSD was applied to all the data.

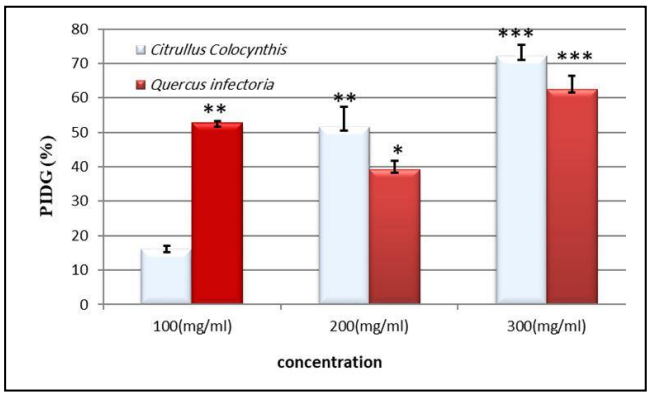

Figure 2. PIDG of *Quercus infectoria* and *Citrullus colocynthis* extracts towards different concentrations of *Aspergillus flavus*

Table 3. Comparison of the best percentage inhibition of diameter growth of both *Quercus infectoria* and *Citrullus colocynthis* on *Penicillium expansum* and *Aspergillus flavus*

Type of extract	Mean of PIDG (%)		Average b
	Penicillium expansum	***Aspergillus flavus***	
Quercus infectoria	16.78	51.48***	34.13
Citrullus colocynthis	29.96*	46.62**	38.29*
Average a	23.37	49.05**	
L.S.D	a= 3.18 ab=4.723 b=3.022		

Data are means of three replicates (n=3) ± standard deviation. Data is presented as highly significant***, slightly significant**, and significant* at $P \leq 0.05$ within different extract concentrations. LSD was applied to all the data

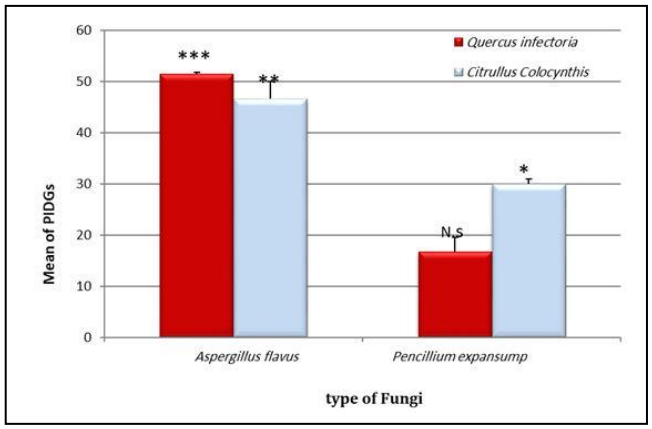

Figure 3. Comparison of the best percentage inhibition of diameter growth of both *Quercus infectoria* and *Citrullus colocynthis* on *Penicillium expansum* and *Aspergillus flavus* The values are reported as mean±standard deviation (n=3), highly significant***, slightly significant**, significant* NS: not significant at $P \leq 0.05$ by Two-way ANOVA

4. Discussion

The development of antimicrobial resistance for many pathogenic fungi discovers the drugs from natural sources as an important issue in public health. This study aimed to evaluate the antifungal activity of phenolic extracts of *Q. infectoria* galls and *C. colocynthis* fruit against the *P. expanisum* and *A. flavus.* The study revealed that the phenolic extract of *C.colocynthis* plant has antimicrobial activity against the *P. expanisum* and *A. flavus.* This is comparable with studies that attribute antifungal, antibacterial, and anti-inflammatory properties to *C.colocynthis* (12). Gurudeeban, Ramanathan (13) that alcoholic extract of *C.colocynthis* has antifungal activity against *A.fumigatus* and *A. flavus*. The biological activity of the plant extracts was mainly attributed to their functional groups, the chemical structures and the synergic interactions between the ingredients (14). The solvent extraction formulation and the concentration of the active compounds in the crude extracts affect its antifungal activities (15).

C. colocynthis fruit contains tannins, and flavonoids are abundant in its alcoholic extracts (16). Flavonoids and alkaloids have known antimicrobial activity (17). The cell wall damage and leakage of cellular material, thus causing the cellular death of microorganisms are attributed to the presence of that Phytochemical compounds (18). However, the resistance of some fungi to the plant extracts may be due to a fungal enzyme that leads to the decomposition of the active compound, removal of active substances, insensitivity of the site targeted by the molecule (19) or by mutations in the genes encoding fungicide targets (20).

The phenolic extract of *Q. infectoria* galls shows antifungal activity against the *P. expanisum* and *A. flavus.* The presence of phenols and flavonoids, particularly tannins in gall, are recorded in the preliminary phytochemical screenings (21). The antibacterial, antifungal and anti-inflammatory properties exhibited by this plant are attributed to the presence of these compounds. The *Q. infectoria* possesses many pharmacological actions such as anti-inflammatory, antiseptic, antibacterial, antiviral, antifungal activities, analgesic, antistomatitis, expectorant, sedative and antipyretic properties (22). The phenolic compound (flavonoids and tannins) works by a union with the protein and its deposition, altering the cell's nature and function and leading to the decomposition of the cell membranes. As a result, the components are expelled from the cell, and the cells will die (23). However, other mechanisms might be blocking enzymetic activity by protein binding or inhibition of DNA or RNA formation (24, 25).

In the present study, the phenolic extracts of *Q. infectoria* galls and *C. colocynthis* fruit showed inhibitory activity against two toxin-producing fungi, *P. expanisum* and *A. flavus.* Therefore, this study suggested that these plants may be considered a medicinal plant source for effective phenolic compounds that can treat some fungal infections.

Authors' Contribution

S. K., E. S., and R. S. contributed to the conception, design, and data interpretation. E. S. contributed to data analysis and revision of the manuscript's intellectual content.

Ethics

This article does not contain human or animal participants, or it does not require an applicant here.

Conflict of Interest

The authors declare that they have no conflict of interest.

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