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Effect of Oak Bark and Walnut Bark Extracts on Fungi Causing Gum and Tooth Infections

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Abstract: This study aimed to evaluate the antifungal activity of aqueous extracts of oak bark and walnut bark against fungi associated with oral infections. Samples were collected from patients attending dental clinics in Al-Qadisiyah Governorate and were cultured and identified according to their cultural and microscopic characteristics. The isolated fungi included Penicillium parasiticus, Aspergillus flavus, A. niger, Fusarium solani, Rhizopus stolonifer, Candida albicans, and Geotrichum candidum. Aqueous extracts of oak bark and walnut bark were prepared and tested at concentrations of 6.25, 12.5, and 20 mg. The results showed variable inhibitory effects according to fungal species, extract type, and concentration. The combined oak bark and walnut bark extract produced marked inhibition against some isolates, whereas Rhizopus stolonifer and Fusarium solani showed comparatively greater resistance. The findings indicate that oak bark and walnut bark extracts have potential antifungal activity against selected oral fungal isolates and may warrant further investigation as natural sources of antifungal compounds.

Keywords: Oral Fungi, Gingivitis, Bark of Oak and Walnut.

Research Paper

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INTRODUCTION

Medicinal plants currently hold a significant place in agricultural and industrial production; they serve as the primary source of plant-based medicinal drugs or provide the active ingredients used in pharmaceutical preparations.

Oak bark is considered a medicinal product with a long history of use. The utilized part is the bark—specifically, the outer layers of the plant's trunks and the outer layers of the roots of woody plants. The oak is a massive tree belonging to the Fagaceae family and the genus *Quercus*. In the spring, the oak tree produces small, yellowish-green flowers, but it does not bear fruit until it reaches the age of twenty. The lifespan of an oak tree ranges from 200 to 400 years.

It is a large tree, reaching a height of up to 25 meters. Its trunk is solid, and its bark is hard and fissured, allowing it to be stripped away from the wood.

The sturdy oak tree comes in two main varieties: the "summer oak," characterized by short leaf stalks, and the "winter oak," which has long leaf stalks; however, the leaves themselves are similar in appearance—firm with serrated edges. The tree produces hard, acorn-like fruit that ripens and falls to the ground in October. A hot sitz bath (30°C) prepared from a decoction of oak bark—taken for ten minutes—is used to treat rectal and uterine prolapse; for rectal prolapse in children, the decoction can be applied as a compress or administered via small rectal enemas. Vaginal discharge in women is treated with an oak bark decoction douche, a remedy particularly useful in cases of uterine cancer. It is also effective for treating skin chafing in children, bedsores (such as those on the heels, buttocks, or shoulders caused by prolonged immobility due to illness or paralysis), and frostbite on the toes during winter.

Powdered bark is sprinkled over eczema and other skin lesions to treat them. A decoction of the bark—prepared by boiling four tablespoons in a liter of water for fifteen minutes—is used hot for enemas and vaginal douches. Oak bark decoction is also added to bathwater to treat nerve weakness associated with aging or resulting from scrofula; this is done by boiling half a kilogram of bark in three liters of water for fifteen

minutes, then adding the strained liquid to the bath. It also treats diarrhea in children suffering from scrofula or abdominal lymph node tuberculosis.

This decoction serves as an effective general tonic for both children and the elderly. It is used to treat bedwetting, hemoptysis (coughing up blood), hematemesis (vomiting blood), various forms of gastrointestinal bleeding, hemorrhoidal bleeding, and excessive menstrual bleeding. Additionally, the oak bark decoction is used as a gargle for oral inflammation, bleeding gums, and tonsillitis. Drinking oak bark decoction is also said to cure stomach cancer. Walnut bark is also utilized; the part employed is the black-colored bark, which is the same part of the plant from which medicinally used oil is extracted.

Walnut bark is also used as a medicinal plant material. The bark contains several phytochemical constituents that may contribute to biological activity. In the present study, walnut bark was investigated together with oak bark for its possible inhibitory effect on fungi associated with oral infections.

Both oak bark and walnut bark have been traditionally used for medicinal purposes, and plant-derived compounds may possess antimicrobial and antifungal properties. The present study therefore focused specifically on the activity of aqueous extracts prepared from these two types of bark.

Previous studies have reported antifungal activity for several plant extracts against pathogenic fungi. These findings support the investigation of plant-derived extracts as potential sources of compounds with antifungal activity against selected oral fungal pathogens (Maziere *et al*., 2025; Davidova *et al*., 2024).
Aspergillus flavus, *A. fumigatus*, *Penicillium* spp., *Candida albicans*, *Fusarium pedrosii*, *Alternaria* spp., and *Rhizopus* sp.

Plant extracts have recently garnered interest as sources of natural products; compared to antibiotics, they offer protective properties when used as therapeutic alternatives against various pathogens. Therefore, this study aimed to:

1. Determine the inhibitory effect of oak bark and walnut bark extracts against selected oral pathogenic fungi.
2. Evaluate the potential of these medicinal plant extracts as natural alternatives for inhibiting selected oral fungal pathogens.
3. Compare the effects of oak bark and walnut bark extracts with the reference antimicrobial agents used in the study.
4. Determine the effective concentrations of the two extracts and their effects on the growth of selected oral fungi.

MATERIALS AND METHODS

1: Collection of Specimens

A number of samples related to oral infections were obtained and identified by the researcher using taxonomic keys. The samples were collected from patients of various ages visiting clinics in Al-Qadisiyah Governorate between January 2016 and March 2017. Swabs were taken from infected patients using sterile oral swabs, and samples were also collected from contaminated teeth, with two replicates for each sample. The samples were then transported to the laboratory for the necessary analyses.

1- Preparation of Culture Media Used in the Study:

1-1- Preparation of Sabouraud Dextrose Agar (SDA):

The medium was prepared according to the method described by Emmons (1974) by dissolving 40 g of dextrose, 10 g of peptone, 20 g of agar, and 0.05 g of the antibiotic chloramphenicol. The volume was then adjusted to 1000 mL using distilled water. The mixture was placed in a clean glass flask and sterilized in an autoclave at 121°C and a pressure of 1 atm for 30 minutes. (Note: The antibiotic was added to the medium after sterilization). This medium was previously used by Al-Mousa (1997) for the isolation of opportunistic fungi.

Additionally, a commercially prepared SDA medium was used by dissolving 65 g in one liter of sterile distilled water, followed by sterilization in an autoclave under the same conditions mentioned above.

2-2- Potato Dextrose Agar (PDA)

The medium was prepared in the laboratory using the following ingredients: 200 g of potatoes, 20 g of dextrose, and 20 g of agar. Alternatively, a commercially prepared medium was used by dissolving 39 g in one liter of sterile distilled water; the medium was then sterilized in an autoclave at 121°C and a pressure of 1 atm for 30 minutes.

In both cases (2-1 and 2-2), the media were poured into Petri dishes and prepared for inoculation and sample subculturing.

Culturing Fungal Isolates:

After the fungal swabs were obtained and brought to the laboratory, they were cultured on Sabouraud Dextrose Agar (SDA)—a medium specific for pathogenic fungi—using three replicates for each swab. The inoculated plates were incubated at 37°C for 3–7 days. The fungi observed on the plates were as follows:
*Penicillium parasiticus, *Aspergillus niger, *Rhizopus stolonifer, *Fusarium solani* *Aspergillus flavus*,*Candida albicans*,*Geotrichum candidum*

They were identified based on their morphological and microscopic characteristics.

Collection of Plant Samples:

Plant samples were obtained from a local herbalist's shop and brought to the laboratory. They were cleaned of impurities and stored in bags until use (Qutb, 1981).

Preparation of the Aqueous Extract:

After cleaning the two plant materials of impurities, a 50 g sample of each was weighed and pulverized using an electric blender. The samples were then placed in flasks, and 500 ml of boiling distilled water was added to each. The mixtures were processed using a rotary evaporator at 50°C until a viscous liquid was obtained.

Chemical screening for active compounds in oak and walnut bark

1- Detection of Tannins:

Mix 10 g of the plant powder with 50 ml of distilled water and heat to boiling; then, filter the solution and allow the filtrate to cool. Divide the filtrate into two portions: add 1% lead acetate to the first portion—the presence of tannins is indicated by the formation of a gelatinous precipitate; add 1% ferric chloride to the second portion—the presence of tannins is indicated by the appearance of a bluish-green color (Shihata, 1951).

2- Detection of Alkaloids:

The method described by Fahmy (1933) was adopted for the detection of alkaloids; it entails boiling 10 g of plant powder with 50 ml of distilled water acidified with 4% hydrochloric acid. Filter the solution after cooling and test 0.5 ml of the filtrate on a watch glass with each of the following reagents:

1. Dragendorff's reagent: yields an (orange) precipitate.
2. Mayer's reagent: yields a (white) precipitate.
3. Wagner's reagent: yields a (brown) precipitate.
4. Detection of glycosides:

The method of Al-Shaikhli *et al*., (1993) was followed, as described below:

One milliliter (1 ml) of the plant extract was placed in a test tube, and 2 ml of previously prepared Benedict's reagent was added. The mixture was then placed in a boiling water bath for 5 minutes; a positive result (indicating the presence of glycosides) was evidenced by the appearance of a red color. To confirm the result, equal volumes of Fehling's reagent and the aqueous plant powder extract were mixed and placed in a boiling water bath for 10 minutes; the formation of a red precipitate indicated a positive result.

4- Detection of Phenols:

The method described by Harborne (1973) was followed by adding 3 ml of the plant extract to 2 ml of a 1% ferric chloride solution; the appearance of a bluish-green color indicated the presence of phenols.

5- Detection of Flavones:

The method described by Jaffer *et al*., (1983) was adopted, as follows:

Solution (A) was prepared by dissolving 10 g of plant powder or extract in 5 mL of 95% ethyl alcohol, followed by filtration. Solution (B) was prepared by adding 10 mg of 50% ethyl alcohol to 10 mL of 50% potassium hydroxide. Upon mixing equal volumes of both solutions, the appearance of a yellow color indicates the presence of flavones.

6- Detection of Resins:

The method described by Shihata (1951) was followed: 50 mL of 95% ethyl alcohol was added to 5 g of plant powder or extract. After boiling in a water bath for two minutes, the solution was filtered. Then, 100 mL of distilled water acidified with concentrated hydrochloric acid was added to the filtrate; the appearance of turbidity indicated the presence of resinous substances.

7- Detection of Saponins:

There are two methods for detecting saponins, as described by Shihata (1951):

1. Vigorously shaking the aqueous solution of the plant sample powder in a test tube; the appearance of a dense foam that persists for several minutes indicates the presence of saponins.
2. Adding 5 ml of mercuric chloride to 1.5 ml of the aqueous plant extract; the appearance of a white precipitate indicates a positive result.

Statistical Analysis: The results were analyzed using a Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

3-1- During the study, seven common fungal species belonging to seven genera were isolated and identified; these appeared in the culture plates despite the variation in the timing of swab collection. A brief description of each species follows:

Table 1: Cultural and Microscopic Characteristics of the Isolated Pathogenic Fungi

Fungal Species	Cultural Characteristics on PDA Medium	Cultural Characteristics on SDA Medium	Microscopic Characteristics
Penicillium parasiticus	Bright green colony, 7–8 in diameter.	Colony diameter ranges from 2–6 cm and is bluish-green in color.	Hyaline, septate hyphae; conidiophores are brush-shaped; conidia are spherical or oval.

Fungal Species	Cultural Characteristics on PDA Medium	Cultural Characteristics on SDA Medium	Microscopic Characteristics
Aspergillus flavus	Bright golden-yellow, clearly visible, with a smooth appearance.	Colony diameter ranges from 5–7 cm; conidial structures/spores are produced; the colony is dark olive in color.	Large conidial heads; spherical in shape, with a spherical, rough/echinulate surface.
Aspergillus niger	Colony diameter is 9 cm, clearly visible and black in color; the colony base is yellow and the colonies are black, with black granular margins.	Colony diameter ranges from 7–9 cm and is black in color; the colony base is yellow, while the colonies are black, with black granular margins.	Large conidia with a club-shaped appearance, borne on septate conidiophores; conidia are oval and echinulate.
Fusarium solani	Colony diameter reaches 3–5 cm after 5 days; colonies are white, cottony, with a yellow base.	Colony diameter reaches 3 cm after 5 days; the colony is pale green and cottony in appearance.	Conidia are small, oval and elongated. Large conidia are borne on short, branched conidiophores and are curved at one end, while the other end is oval-tipped. Chlamydospores occur singly or in pairs.
Rhizopus stolonifer	Colony diameter reaches 9 cm and the colony is gray to black, with a woolly appearance.	Colony diameter reaches 6 cm after 4 days and is dark brown in color.	Sporangia are clearly visible, thick-walled and spherical; chlamydospores are spherical or oval. Sporangiophores are clearly visible and bear sporangia.

Note: The table includes the five fungal species whose cultural and microscopic descriptions are present in Table (1) of the uploaded research. The other two isolated fungi, Candida albicans and Geotrichum candidum, are listed elsewhere in the research, but their detailed characteristics are not provided in the extracted Table (1), so I have not added unsupported descriptions.

2- Detection of Active Constituents in (Oak and Walnut) Bark

Table 2: Phytochemical Compounds Detected in Oak Bark and Walnut Bark Extracts

Active Compounds	Oak Bark Extract	Walnut Bark Extract
Saponins	+	+
Alkaloids	+	+
Glycosides	+	+
Phenols	+	+
Tannins	+	+
Resins	+	+
Flavonoids	−	+

The table above reveals the presence of several active compounds in the extracts of oak and walnut bark. The presence of these substances within the plant's composition is highly significant, as one or more of them can affect microorganisms in general, and specifically those responsible for certain pathological infections (Davidova *et al*., 2024).

These substances in the extracts act against certain microorganisms—such as fungi—by inhibiting their biological activity, a finding consistent with the work of Reddy *et al*., (2000) (Al-Qadi & Osama, 2001).

3-2 – Effect of Aqueous Extracts of Oak Bark and Walnut Bark on Selected Oral Fungi:

Table 3: Effect of Aqueous Extracts on Aspergillus niger

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
2.1	5.3	6.25	Oak bark
	* 1.2	12.5	
	* 0	20	
4.5	4.2	6.25	Walnut bark
	6.3	12.5	
	3.1	20	
0.4	1.2	6.25	Oak bark + Walnut bark
	0	12.5	
	0	20	

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
1.5	2.5	6.25	Drug (1) Metronidazole
	2.1	12.5	
	* 0	20	
1.1	* 3.4	6.25	Drug (2) Spiramycin
	* 0	12.5	
	* 0	20	
9	9	-	Con.
	9	-	
	9	-	

Mean colony diameters were calculated from three replicates.

The results indicate a predominance of Aspergillus species, particularly A. niger and A. flavus, among the fungi evaluated. Table (3) shows that A. niger was variably affected by the tested oak bark and walnut bark extracts. The inhibitory response depended on extract type and concentration, and the combined extract showed a stronger inhibitory effect at the higher concentrations.

This finding is consistent with the results reported by Mustafa (1995).

When oak bark and walnut bark extracts were combined at the same concentrations, clear inhibition of colony growth was observed. The stronger effect of the combined treatment suggests that constituents present in the two bark extracts may contribute to the observed antifungal activity.

The drug Metronidazole demonstrated an effect on *A. niger* fungal colonies; beyond merely inhibiting colony growth, it also impacted the mycelium, as microscopic examination revealed that the hyphae were affected by the drug.

As for the drug Spiramycin, colony diameters measured 3.4, 0, and 0, indicating clear inhibition; all tested concentrations (6.25, 12.5, and 20) resulted in significant differences regarding the inhibition of colony growth. This finding aligns with the work of Dismukes and Como (1994), who demonstrated that antifungal drugs affect fungal growth due to the presence of chemical groups that interfere with and inhibit hyphal development.

In both cases, they were compared with control treatments in which no concentration was used and which had a mean diameter of 9 cm.

Table 4: Effect of aqueous extracts on the fungus *A. flavus*

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
3.7	5.5	6.25	Oak bark
	4.3	12.5	
	* 1.4	20	
4.1	7.1	6.25	Walnut bark
	3.2	12.5	
	*2.2	20	
2.7	5.2	6.25	Oak bark + Walnut bark
	3.1	12.5	
	*0	20	
1.2	2.3	6.25	Drug (1) Metronidazole
	1.5	12.5	
	*0	20	
0.7	2.2	6.25	Drug (2) Spiramycin
	* 0	12.5	
	* 0	20	
9	9	-	Con.
	9	-	
	9	-	

* The mean colony diameter is derived from the average of three replicates.

The results in Table (4) indicate concentration-dependent inhibition of A. flavus by the tested oak bark and walnut bark extracts. The combined extract produced greater inhibition at the highest concentration, while the reference drugs also showed inhibitory activity.

The colony diameters were 5.2, 3.1, and 0, respectively. With the use of the drug Metronidazole, the concentration of 20 showed significant differences compared to the concentrations of 6.25 and 20, where the colony diameters were 2.3, 1.5, and 0, respectively. In

the case of the drug Spiramycin, the concentrations of 12.5 and 20 showed highly significant differences compared to the concentration of 6.25, where the colony diameters were 2.2, 0, and 0, respectively.

These scientific findings align with the observations of Buckley (1989) regarding the ability of antifungal agents to destroy human-pathogenic fungi by inhibiting their vital biological activities.

The inhibitory effects observed with the aqueous oak bark and walnut bark extracts may be related to their phytochemical constituents, including tannins, phenolic compounds, saponins, alkaloids, glycosides, and resins detected in the present study. However, the specific compound responsible for the observed activity cannot be established from the present experiment alone.

The inhibitory effect observed with the aqueous oak bark and walnut bark extracts may be associated with their phytochemical composition. Further chemical and mechanistic studies are required to identify the compounds responsible for inhibition of A. flavus.

Table 5: Effect of aqueous extracts on the fungus *Penicillium parasiticus*

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
2	6	6.25	Oak bark
	* 0	12.5	
	* 0	20	
2.1	5.2	6.25	Walnut bark
	*1.3	12.5	
	* 0	20	
0	* 0	6.25	Oak bark + Walnut bark
	* 0	12.5	
	* 0	20	
0.7	2.3	6.25	Drug (1) Metronidazole
	* 0	12.5	
	* 0	20	
0	* 0	6.25	Drug (2) Spiramycin
	* 0	12.5	
	* 0	20	
9	9	-	Con.
	9	-	
	9	-	

* The mean colony diameter is derived from the average of three replicates.

Analysis of the results in Table (5) revealed that the oak bark and walnut bark extracts showed marked inhibitory effects against P. parasiticus, particularly at 12.5 and 20 mg. The combined extract produced complete inhibition under the tested conditions.

When the same concentrations of oak bark and walnut bark extracts were combined, the treatment produced complete inhibition of fungal colony growth, with mean colony diameters of 0, 0, and 0 cm, respectively.

These findings indicate that P. parasiticus was sensitive to the tested oak bark and walnut bark extracts, either individually or in combination. The present experiment does not establish the specific mechanism of inhibition; therefore, mechanistic explanations require further investigation.

Regarding the use of the agent Metronidazole, it demonstrated a clear effect at the same concentrations, resulting in mean colony diameters of 2.3, 0, and 0, respectively.

The results for both agents can be attributed to their content of azole compounds—specifically clotrimazole—which directly affect fungal infections, particularly those caused by *Aspergillus*, *Penicillium*, and *Candida* species. This finding aligns with the conclusions reached by Al-Duboon (1998) and Maziere *et al.,* (2025).

Table 6: Effect of aqueous extracts on the fungus *Rhizopus stolonifer*

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
2	8	6.25	Oak bark
	6.5	12.5	
	5.1	20	
2.1	9	6.25	Walnut bark
	9	12.5	

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
	7.2	20	
0	7.3	6.25	Oak bark + Walnut bark
	5.4	12.5	
	* 2.1	20	
0.7	8.2	6.25	Drug (1) Metronidazole
	4.5	12.5	
	* 1.5	20	
0	5.6	6.25	Drug (2) Spiramycin
	5.2	12.5	
	3.1	20	
9	9	-	Con.
	9	-	
	9	-	

The mean colony diameters were derived from the average of three replicates.

Analysis of Table (6) revealed comparatively greater resistance of R. stolonifer to the individual oak bark and walnut bark extracts at the tested concentrations. In contrast, the combined extract showed a clear inhibitory effect at 20 mg.

Regarding the mixture of the two extracts, a clear effect was observed; a highly significant difference was noted at a concentration of 20, where the mean colony diameter was 2.1 cm compared to the control treatment. This finding is consistent with Pia and Platt (1995).

Regarding the use of the drug Metronidazole, the fungus resisted low concentrations (6.25 and 12.5), resulting in colony diameters of 8.2 and 4.5, respectively, compared to the control treatment. However, inhibition was observed at a concentration of 20, where the colony diameter was 1.5; this represented a highly significant difference compared to the control and the other concentrations tested. In contrast, the use of the drug Spiramycin showed no effect on colony diameters, as the fungus was resistant to it.

The observed resistance may be related to differences in the susceptibility of R. stolonifer to the tested extracts. The present study did not investigate the molecular or enzymatic mechanisms of resistance, so the exact mechanism cannot be determined from the available data.

Table 7: Effect of aqueous extracts on the fungus *Fusarium solani*

LSD	Mean Colony Diameter	Concentration (mg)	Extract Type
5.2	7.1	6.25	Oak bark
	5.5	12.5	
	3.2	20	
6.5	8	6.25	Walnut bark
	6.4	12.5	
	5.2	20	
4	4.6	6.25	Oak bark + Walnut bark
	4.1	12.5	
	3.5	20	
0.4	1.4	6.25	Drug (1) Metronidazole
	* 0	12.5	
	* 0	20	
0	* 0	6.25	Drug (2) Spiramycin
	* 0	12.5	
	* 0	20	
9	9	-	Con.
	9	-	
	9	-	

* The mean colony diameter is derived from the average of three replicates.

Analysis of the results in Table (7) showed that the plant extracts—whether from walnut bark, oak bark, or a combination of both—did not exhibit any inhibitory effect on the fungus *F. solani*. Although some inhibition was observed upon visual inspection of the culture plates, the statistical analysis revealed no significant impact of these extracts compared to the control treatment, where the mean colony diameters were

(7.1, 5.5, 3.2), (8, 6.4, 5.2), and (4.6, 4.1, 3.5), respectively.

Accordingly, F. solani showed comparatively low susceptibility to the tested plant extracts under the experimental conditions. The present data do not establish the cellular or molecular basis of this response.

In contrast, the fungus was significantly affected by the drug Metronidazole—indeed, at all tested concentrations—with mean colony diameters of 1.4, 0, and 0, respectively, compared to the control treatment.

Regarding the use of the agent Spiramycin, it affected the mean colony diameters (0, 0, 0) by 100% at all concentrations compared to the control treatment. These results suggest that both agents impacted the viability of the fungus *F. solani* by inhibiting growth and—in some instances—disrupting the mycelium, rendering it vulnerable despite its inherent resistance to certain chemicals. This finding is consistent with the work of Bhatnager and Arar (1992).

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