

Efficacy of Bark (*Juglans regia* L.) Extracts Against Periodontitis Bacteria: an *In Vitro* Study

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Abstract

Juglans regia is one of the medicinal plants widely applied in many applications due to its pharmacological value and desirable characteristics of its parts. Hence, there is a motivation of medicinal and cosmetics applications. In this paper, the crude aqueous extracts from *Juglans regia* bark were screened for in vitro antibacterial properties against clinical isolates of Periodontitis bacterial causative agents (*Granulicatella adiacens*, *Staphylococcus sciuri*, and *Kocuria* spp. The antibacterial test was carried out using the Kirby Bauer method. The tested extract from this medicinal plant with the different concentrations (100 mg/ml, 250 mg/ml, 500 mg/ml) were screened. The standard antibiotics Ciprofloxacin (5 µg/ml) and Cefotaxime (30µg/ml) were used as controls. The extract of 250 mg/ml being more effective in action as compared to the others. Furthermore, *Kocuria* spp showed the most isolate affected by the extract. This research has revealed the active inhibitory effect of bark extract against all the tested isolates. This extract contains active chemical components that contribute to biological activity thereby assisting to combat bacterial infections. However, many studies need to be carried out to identify the responsible constituents for growth inhibition.

Keywords: *Juglans regia* bark, aqueous extract, antibacterial activity, Periodontitis.

Introduction

The demand is driven recently to use alternatives natural compounds instead of chemical ones such as plant extracts from medicinal and aromatic plants in addition to wild plants in defending diseases caused by different microbes fungal, bacterial, viral, nematode and insect^{1,2}. The inclination towards the use of natural materials as a substitute for the artificial ones has increased dramatically due to some consequences of the latter such as undesirable side effects, complexity, the emergence of the medicine resistance trait and cost benefits, competition by manufacturers³⁻⁷. Many

traditional medicines are made by grounded the leaves or bark, and the derivative mixture may contain hundreds of potentially active molecules. Identifying such molecules and examined their effectiveness is well established as antioxidant materials⁸, anti-tumor⁹, anti-inflammatory^{10,11}, in addition to the antimicrobial activity¹². *Juglans regia* tree normally known as walnut is considered as one of the important medicinal plants that have proven to be effective in therapeutic and beauty fields¹³. Related parts of *Juglans regia* such as shells, kernel, and seed, bark have been examined by many researchers in various studies¹⁴. The bark of *Juglans regia* locally known as Deirum in Asia and middleeast countries. This medicinal part can be utilized as a tooth cleaner, cosmetic product for coloring the lips by women and the cosmetic industry¹⁵. *Juglans regia* bark is available in different sizes and shapes, fibrous and characterized

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with resinous, scented features¹⁶. Furthermore, Walnut (*Juglans regia* L.) bark has been determined as a valuable medicinal source to treat a range of diseases and its activity as anti-inflammatory, antioxidant, blood purify, anticancer, depurative, diuretic, and laxative activities has been revealed¹⁷. The chemical composition of *Juglans regia* bark mainly compromise from various therapeutic components such as β -sitosterol, ascorbic acid⁵, juglone, folic acid, gallic acid, regiolone, and quercetin-3- β -D-arabinoside, flavonoids, pectic substances, sterols and phynols¹⁸. *Juglans regia* L. bark is well recognized as a bioactive compound against *fungi Aspergillus niger, Alternaria alternate, Trichoderma vierns and Fusarium solani Candida albicans*¹⁹. The inhibition activity has been established not only as antifungal but also, its application as an antibacterial agent has been confirmed²⁰. The extract of *Juglans regia* bark exhibited a broad-spectrum antimicrobial activity in a dose-dependent manner against some pathogenic bacteria such as Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans, Streptococcus salivarius*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*)²¹. A wide range of oral pathogenic bacteria is applied in many antimicrobial studies using natural products from medicinal plants for the promise to discover the potential replacement remedies to the synthetic antibiotics. Hence, this study was carried out to evaluate the antibacterial activity of aqueous extract of *Juglans regia* bark against three species of periodontitis bacteria.

Materials and methods

Collecting plant samples and preparing them for extraction

The stem bark was obtained from the local market, to be prepared by grinder and the grounded parts were kept at room temperature. The stock extracts solution was prepared from *Derum* (*J regia* L.) by dissolving (1gm) in 1mL to obtain dilution 500,250,100 mg / mL aqueous extract and then filtered by Whatman filter paper.

Specimens Collection

In this study, 20 samples were collected from the Collage of Dentistry, University of Kufa, from patients with periodontitis. Samples were collected from patients with cotton rolls and carefully cleaned with sterile cotton. For each site, 2 paper points (30-40) were inserted into the periodontal pocket for 30 seconds, the pocket depth was equal to or exceeding (3.5-7) mm. It was then placed in a sterile container with saline (2 ml) and inoculated on the agar plates.

Antimicrobial assay

The isolated bacterial strains of periodontitis were diagnosed by the Vitek 2 system for identity and they were: *Granulictaella adiacens, Staphylococcus sciuri* and *Kocuria* spp. One colony of each isolate was inoculated in a brain heart infusion broth (BHI) and incubated for 3 hours at 37 °C to obtain culture turbidity with standard turbidity (0.5 McFarland standard). On Muller Hinton agar spread 0.1ml of the culture with a sterile swab, dry at room temperature for (10-15) minutes. Inhibitory activity was detected by the agar well diffusion method, after sterilizing the cork borer, four wells were made on the surface of the culture media with a diameter of 10 mm then add (100 μ l) to each well in different concentrations of *derum* extract. antibiotic discs were applied at the center such as ciprofloxacin (5 μ g) and Cefotaxime (30 μ g) for comparison with the plant extract, after which the plate was incubated for 18-24 hours at a temperature of 37 °C. The diameter of the inhibition zones was recorded. The experiment was repeated twice and the average of four replicates was calculated.

Statistical Analysis

The data gathered and exported to a Microsoft Excel spreadsheet where descriptive statistics were performed. The data was analyzed processed using SAS version 9.1. Two-way ANOVA was also carried to determine if there was any interaction between the effect of extracts concentration and the pathogenic bacteria. $P \leq 0.05$ is considered significant in both tests (Tukey test). Further-

more, the analysis was done to find the difference between the means (4 replicates). One-way ANOVA (Analysis of variance) was carried out to demonstrate statistical difference using the varying zones of inhibition when the extracts from *Juglans regia* L. bark was used against the isolates included in this study.

Results

The results of an in vitro antibacterial assay of the aqueous extract of bark (*Juglans regia*) against a group of a clinically significant panel of prederontitis causative bacteria at different concentrations of 100 mg/ml, 250 mg/ml, and 500 mg/ml are demonstrated in this study. Figure 1 demonstrated that isolate *Granulicatella adiacens* exhibited significant inhibitory response ($P < 0.05$) at the average concentration level (250 mg/ml), however, this isolate did not show significant effect ($P >$

0.05) when the highest concentration (500 mg/ml) was used. Regarding *Staphylococcus sciuri* isolate, there is a high response ($P < 0.05$) at the average concentration level (250 mg/ml), which reveals that the two isolates were in parallel response. However, a concentration of 100 mg/ml of the bark extract is established to inhibit the growth of *Staphylococcus sciuri* isolate significantly ($P < 0.05$).

Respecting the third isolate *Kocuria spp*, the manner of action was slightly different, as thereby with an increase in the concentration of the bark extract, there was a high inhibition activity ($P < 0.05$) between 100 mg/ml, 250 mg/ml, whereas, there were no statistically significant differences accompanied ($P > 0.05$) between both concentrations (250 mg/ml and 500 mg/ml) Figure 3.

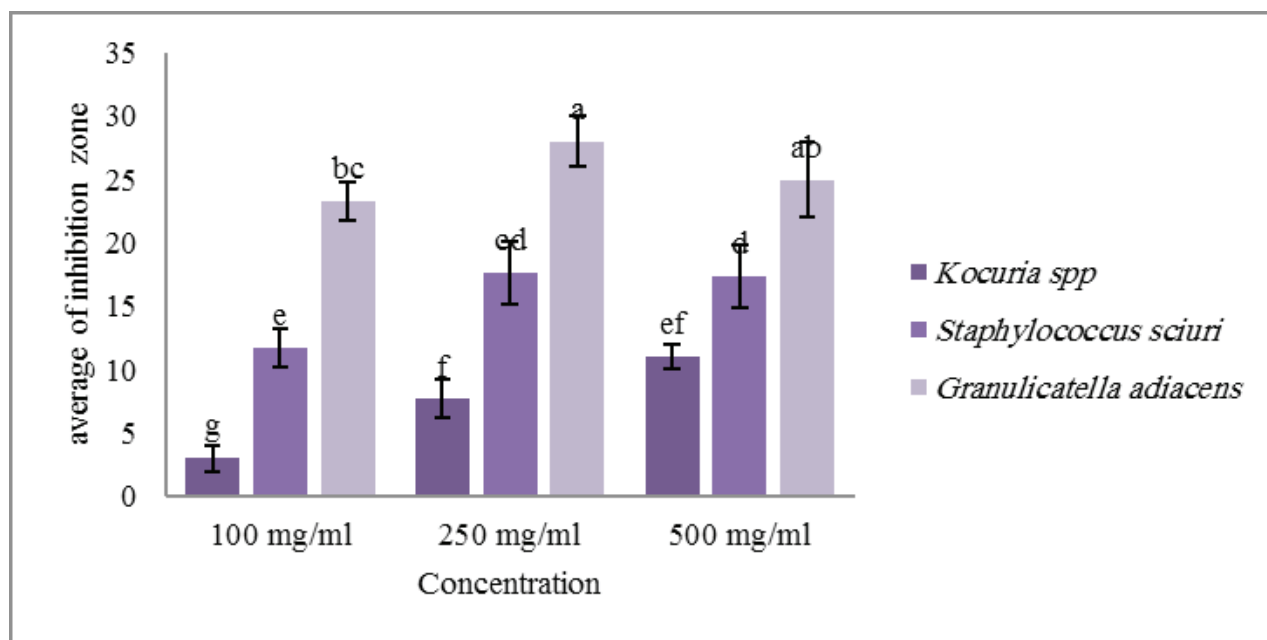


Fig. 1 The inhibitory effect of three different concentrations of bark *Juglans regia* against three treated isolates. The error bars represent \pm standard deviation (SD). Bars with different letters demonstrate significant differences (ANOVA, two-way, Tukey, $P < 0.05$, $n = 4$).

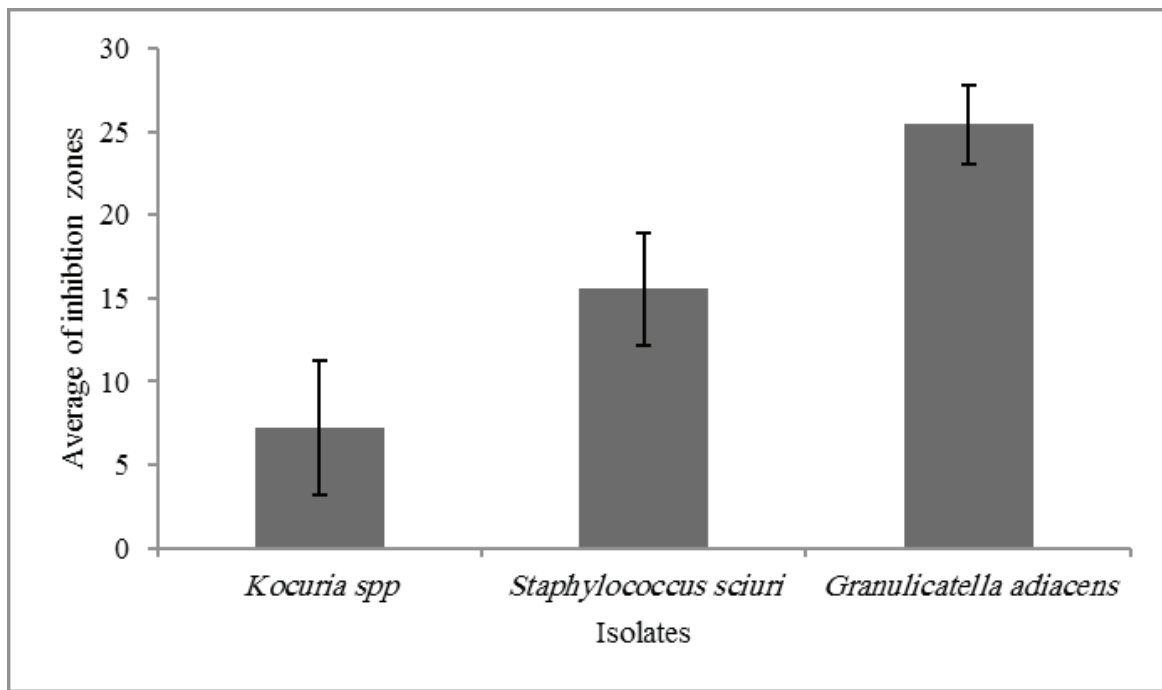


Fig. 2 The response of the average treated isolates (*Granulicatella adiacens*, *Staphylococcus sciuri* and *Kocuria spp.*) towards the average concentrations of aqueous bark extract of *Juglans regia L.*. The error bars represent \pm standard deviation (SD). Bars with different letters demonstrate the significant differences (ANOVA, one way, Tukey, $P < 0.05$, $n = 7$).

The finding also revealed that the concentration of 100 mg/ml and 250 mg/ml had a significant antibacterial effect against the tested bacteria ($P < 0.05$) as shown in **Figure 3**. However, the inhibition effect was approximately similar continued to be in the same manner in the case of 500 mg/ml and there were no significant differences were recorded ($P > 0.05$).

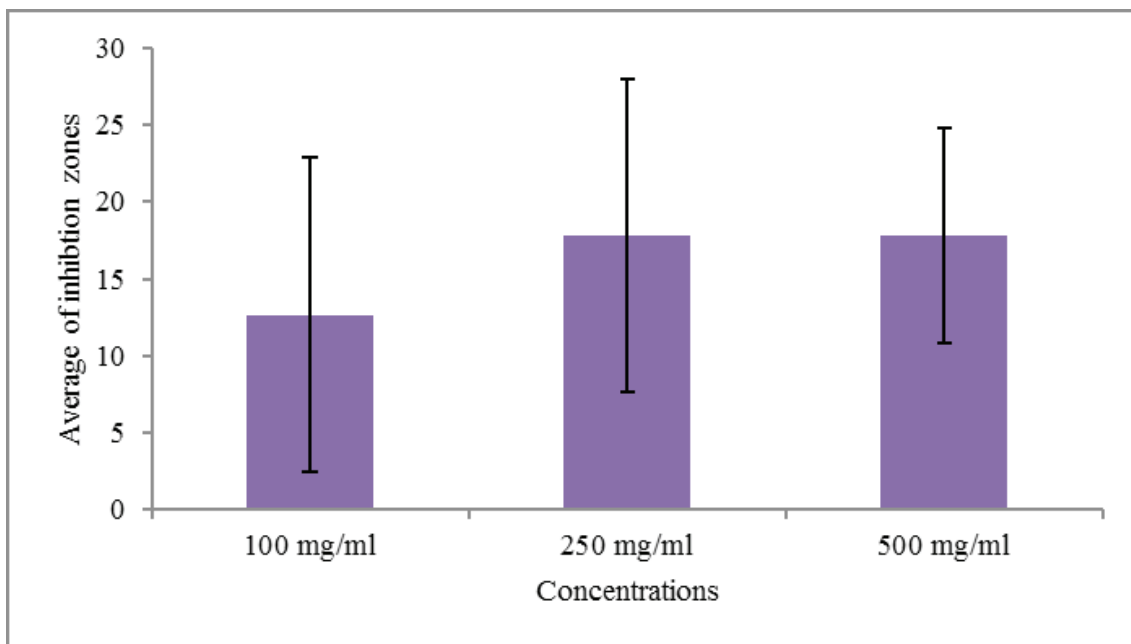


Fig. 3 Inhibition rate for three different concentrations of the bark extract of *Juglans regia L.* as average isolates. The error bars represent \pm standard deviation (SD). Bars with different letters demonstrate the significant differences (ANOVA, one way, Tukey, $P < 0.05$, $n = 7$).

The inhibition zones caused by the standard antibiotics were also observed in different degrees of inhibition based on the sensitivity of the tested bacteria. As can be noticed from **Table 1** there was a significant antigrowth activity of both antibiotics used 30mm

of Ciprofloxacin and 38mm of Cefotaxime against *Granulicatella adiacens* isolate, whereas the lowest inhibition zones were detected on *Kocuria spp.* The data suggested that this bacteria was not highly sensitive neither to the bark extracts nor to the typical antibiotics.

Table 1 Average of inhibition zones of bark extract on tested isolates. The presented values are the average of 4 replicates of inhibition zones (mm). Average of inhibition zones of the standard antibiotics are shown.

Isolates	Control		Concentration (mg/ml)			Average of inhibition zones (mm)
	Ciprofloxacin (5 µg/ml)	Cefotaxime (30µg/ml)	100	250	500	
Granulicatella adiacens	30	38	23	28	25	Average of inhibition zones (mm)
Staphylococcus sciuri	25	20	11.67	17.67	17.3	
Kocuria spp	10	8	3	7.67	11	

Discussion

As an adaption of bacteria that cause infection to withstand antibiotics, the possibility for antimicrobial resistance to cause a global health crisis emerges large^{3,7}. This issue has led the need for the development of new medicines. Scientists and policymakers have transformed their efforts and working together to conceive new antibacterial agents naturally to overcome such a threat⁸.

Periodontitis one of the bacterial infections mostly associated with bacterial plaque ²¹. Some people avoid using the commercial available mouth rinses products due to the harmful chemicals and colors; alcohol ²⁰. Therefore, the current research was carried out to investigate the antibacterial activity of bark (*Juglans regia* L.) Aqueous extracts against clinical bacterial isolated from patients with periodontitis. From the finding, the bark of *Juglans regia* extract showed a considerable level of antibacterial activity against examined clinical isolates. Generally speaking the extract from *Juglans*

regia L. bark proved to be significantly active against *Granulicatella adiacens* at (250mg/ml) concentration. The second susceptible strain was *Staphylococcus sciuri* that being affected by the extract with a diameter of inhibition (17.67 mm) at (250mg/ml) concentration, whereas the inhibition zones were increased gradually by increasing the concentrations from 100 mg/ml to 500 mg/ml in the case *Kocuria spp.* Interestingly, the concentrations used showed variances in antibacterial growth, particularly, at the concentration ranged from 250 mg/ml -500 mg/ml, they were slightly comparable in activity against all the examined bacteria. Therefore, it is worth noting that it would have been better to select concentrations within these two ranges to demonstrate the accurate efficacy of the extract because the overlap between concentrations has led to indefinite the ideal concentration of inhibition. Comparatively, the zones of inhibition formed by standard antibiotics utilized were varied, the data suggested that the sensitivity of the tested isolates was incompatible. The aqueous extract had significantly antibacterial action against some oral

bacteria such as *Staphylococcus aureus*, *S. salivarius*, and *S. sanguis* compared to control. However, there was no observed activity on *S. mutans* when compared with the antibacterial agent Erythromycin²⁰. The activity of *Juglan regia* bark extract improved by the synthesis of nanoparticles materials, this synthesized particles showed significant inhibition against *Streptococcus mutans*, which is the main causative agent for dental cavities. The nanoparticles also showed promising antibiofilm action by inhibiting the glucosyltransferase enzyme¹⁶. Study indicated that the ethanolic extracts of *Juglan regia* bark exhibited more efficient antimicrobial activity against oral microbes included yeast and bacteria in comparison to the aqueous extract²¹. The findings of this study obviously showed that the aqueous extracts of *Juglans regia* bark considerably inhibited the growth of the examined oral bacteria, and available reports are agreeable with the current outcomes. The antibacterial characteristic of the medicinal plant might be associated with metabolites such as phenolic compounds, terpenoid, alkaloids, flavonoids, and steroids. The bark of *Juglans regia* contains ketones, sterol, and flavonoid^{8,29}.

Conclusion

Plants of pharmacologically active constituents have been used by traditional therapists to treat a lot of infectious diseases. In compliance with the findings; the extract has exhibited desirable antibacterial activity against all the tested isolates. This study consolidates the use of natural products as medicines and confirms the antibacterial potentials of the bark of *Juglan regia* (Walnut plant). The results strongly support the traditional use of this plant (*Derum*) as a preventive remedy for various microbial diseases of soft and hard tissues in the oral cavity. And the possibility of using it as an oral rinse as a prevention or as a treatment against oral pathogenic bacteria. Undoubtedly, the extract contains chemical substances that take part in metabolic activities, thereby helping to fight against bacterial infections. However, further biochemical, chemical and pharmacological investigations must be carried to identify and examine the purified bioactive components that are responsible for the antimicrobial activity and their mechanism of

action.

Conflict of Interest: None

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Ethical Clearance: Not required

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