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Original article

Antimicrobial Evaluations of *Juglans Regia* Bark Against Oral Pathogenic Microorganisms

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ABSTRACT

Oral pathogenic microorganisms significantly contribute to various diseases, impacting both oral and systemic health, including chronic periodontitis and dental caries. Natural plants, including Juglans regia (walnut tree), have gained momentum for their therapeutic potential due to their antibacterial properties, offering a promising avenue to combat antibiotic resistance. This study fundamentally aimed to validate the traditional use of Juglans regia bark in maintaining oral health; it sought to evaluate the antibacterial and antifungal effects of Juglans regia bark. The methanolic extracts of Juglans regia bark, imported from China, were prepared using the maceration technique, various microorganisms procured from the American Type Culture Collection (ATCC), including Gram-positive bacteria (Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA)), Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and the local fungal strain Candida albicans. Antimicrobial activity was assessed using the agar well diffusion assay at concentrations of 250 mg/ml and 125 mg/ml, with chlorhexidine and phenol serving as reference standards. Minimum inhibitory concentrations (MIC) were determined using the microdilution technique, with serial dilutions ranging from 125 mg/ml down to 3.9 mg/ml. At 250 mg/ml, Escherichia coli exhibited the largest zone of inhibition (27mm), followed by Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans (25mm, 23mm, and 25mm, respectively). At 125 mg/ml, Candida albicans showed the best results (22mm). The lowest effective inhibitory concentration (MIC) for C. albicans was 15.6 mg/ml, while S. aureus and P. aeruginosa also showed equal MICs.

Introduction

Oral pathogenic microorganisms play a crucial role in the development of various diseases, significantly impacting both oral and systemic health. Although chronic periodontitis, for instance, is characterized by inflammation leading to the loss of tooth attachment, which can result in systemic issues such as cardiovascular diseases and diabetes complications [1]. Nevertheless, the biofilm formed by these microorganisms is essential in the pathogenesis of periodontal disease, where different species such as *Streptococcus*, *Actinomyces*, *Neisseria,* and *Lactobacillus* species cooperate to create a disease-provoking microbiota [2]. Moreover, pathogenic bacteria can enter the bloodstream, linking oral health to systemic diseases and highlighting their significance in overall health. On the other hand, opportunistic pathogens as *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli (E. coli),* which thrive in individuals with compromised immune systems and poor oral hygiene, further exacerbate oral microbial diseases [3]. Additionally, the presence of cariogenic bacteria, primarily *Streptococcus mutans,* in dental plaque contributes to dental caries, a prevalent condition caused by acid production from fermentable carbohydrates [4]. Thus, understanding the role of these microorganisms is vital for developing effective preventive and therapeutic strategies to mitigate their impact on oral and systemic health.

Recently, natural plants as Centipede cunninghami, Aloe vera, and Neem have been recognized for their therapeutic potential in treating periodontal diseases due to their antibacterial and anti-inflammatory properties [5]. Their extracts work by inhibiting bacterial growth and reducing gum inflammation, alongside other beneficial ingredients like coenzyme Q10 and folic acid [6-8]. Nowadays, medicinal plants offer a promising avenue for overcoming the persistence of antibiotic resistance in oral pathogenic bacteria [9, 10]. For instance, polyphenols can complex with macromolecules, inhibiting their functions and thereby helping to offset resistance mechanisms; on the other hand, flavonoids and terpenoids disrupt microbial membranes, which not only provides a direct antimicrobial effect but also enhances the permeability of bacterial membranes, making them more susceptible to antibiotics. Additionally, alkaloids serve as inhibitors of efflux pumps, a common bacterial resistance mechanism. The integration of these phytochemicals into treatment regimens could significantly improve outcomes against drug-resistant bacteria, particularly in cases where conventional antibiotics fail.

As research continues to uncover the potential of these natural compounds, they may play a crucial role in addressing the demanding public health challenge of antibiotic resistance [10-14]. However, *Juglans regia*

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(Figure 1), commonly known as the walnut tree, is notable for its bark, which possesses various medicinal and ecological properties [15]. Although the bark is rich in phenolics and flavonoids, contributing to its antibacterial, antioxidant, anti-inflammatory, and anticancer activities [15,16]. Additionally, it has been studied for its heavy metal accumulation capabilities (Figure 2), revealing significant concentrations of metals like copper and lead, which can indicate environmental health [17]. Nevertheless, tannins present in the *J. regia* bark give the material a reddish-brown stain and help in the precipitation of proteins [18]. Some volatile compounds like gallic acid and saponins, which are responsible for the soapy texture and bitter taste, are also present. It also contains ash and has a mild abrasive effect as it is tough and fibrous [19]. Yet, one of the most important compounds in the *J. regia* bark is the organic compound called juglone. Juglone is formed from glucose and has a distinct phenyl group. Due to this compound, the bark of this plant holds numerous medicinal benefits. It acts as an antifungal, antibacterial, antiviral, antiparasitic, antioxidant, and antitumor agent [20-22].

Figure 1: Juglans Regia (walnut tree) and Extract Features.

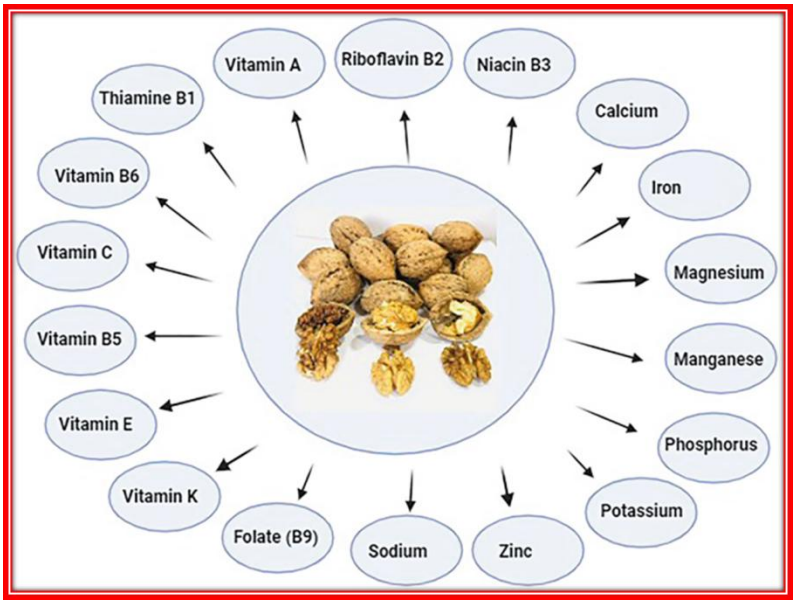

Figure 2: Vitamins and metals that are incorporated in Juglans Regia [23]

The antibacterial effect of Juglone was due to disrupting the cell membrane through various mechanisms, inhibiting DNA synthesis, interfering with energy production, and hindering bacterial proteins' function [24]. Tannins *J. regia* bark, on the other hand, bind to bacterial proteins, causing them to clump and lose their functionality. This multi-pronged approach likely contributes to the bark's efficacy against diverse pathogens [25]. In contrast, while the bark shows promising bioactivity, the potential toxicity of some compounds, such as juglone, necessitates careful evaluation. Further research is essential regarding heavy metals accumulated within the *J. regia* bark, warranting further toxicity investigation to balance its use in traditional medicine with safety [26].

In Libya, *J. regia* bark was used traditionally as a topical application to enhance gum and tooth health, in addition to natural colouring of lips [27]. In other countries *J. Regia* bark had been used contrarily as a topical home remedy for dermal inflammation and excessive perspiration of the palms and soles. It is also a common home remedy for the treatment of eczema [28]. The ethanolic extract found in it gives it excellent antioxidant properties. Some parasitic infections have also been cured with the help of J. regia bark extracts [28]. Therefor this study fundamentally aimed to prove and validating the traditional use of *J. regia* bark in maintain oral health by preventing pathogenic infections microorganisms through evaluating the

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antibacterial and antifungal effects of Juglans regia bark against common oral pathogens, determine the minimum inhibitory concentration (MIC) of Juglans regia bark extract, and compare the effectiveness of plant extract with commercially available chlorohexidine as mouth wash.

Material and methods

Chemicals, reagents, and cultures

All chemicals' reagents used throughout this study for either identification and/or antibiotic susceptibility test were obtained from Sigma-Aldrich company, U.K., unless otherwise mentioned. All cultures were of the purest available grade BDH U.K. unless otherwise indicated; all bacterial growth media were obtained from Oxoid Company.

Plant Collection

Juglans regie bark used in this study was imported from China, commercially obtained from Tripoli markets. The best time to collect Juglans regia bark is in the spring or early summer. from China, commercially obtained from Tripoli markets. The best time to collect Juglans regia bark is in the spring or early summer.

Plant extract

In this study, the Methanol 96% was used to extract active constituents from Juglans regie bark and measured its weight, which was 9.708g. Using the maceration technique, the yielded extract was 2.036g.

Anti-microbial activity

Microorganisms

The microorganisms employed in the current study were procured from the American Type Culture Collection (ATCC). Deep freeze-stored cultures were employed for all bacterial strains and reactivated using brain heart infusion broth. Strains of Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213), *Methicillin-resistant Staphylococcus aureus* (ATCC. 35126), Gram-negative bacteria *Escherichia coli* (ATCC.25922), *Pseudomonas aeruginosa* (ATCC. 24846), and a fungal local strain *Candida albicans* were obtained from Tripoli University Hospital.

Agar Well Diffusion Assay

In this study, Mueller-Hinton agar plates were inoculated with 0.1 ml of 10^6 CFU/ml bacterial suspension, while Sabouraud dextrose agar plates were inoculated with corresponding fungal strains. Following the creation of wells in the agar, 50 microliters of extract at concentrations of 250mg/ml and 125 mg/ml were added, and the zones of inhibition were measured after incubation at 37 °C for 18 hours, with chlorhexidine and phenol serving as reference standards.

Table 1: Muller-Hinton agar (MHA) medium

Ingredients	Gram/Litre
Beef Extract	gm 2.00
Acid Hydrolysate of Casein	gm 17.50
Starch	1.50 gm
Agar	17.00 gm
Distilled Water	ml 1000

Table 2: Sabouraud's dextrose agar (SDA) medium

Ingredients	Amount
Dextrose	gm. 40.0
Peptone	gm. 10.0
Agar	20.0 gm.
Distilled Water	1000 ml
pH	5.6

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MIC assay

The minimum inhibitory concentration (MIC) of any given compound is characterized as the lowest concentration that entirely prevents observable growth (turbidity in liquid media). MIC values were ascertained utilizing the microdilution technique. Solutions of the extract of Juglans regie bark were formulated in phosphate buffer at a concentration of 125 mg/ml. From this stock solution, serial dilutions of the extracts (125, 62.5, 31.25, 15.6,7.8 and 3.9mg/ml) were prepared to determine the MIC. All assessments were conducted in triplicate.

Results

The obtained results indicated that *Escherichia coli* showed the best results at a concentration of 250mg/L with a zone of inhibition of 27mm compared to *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Candida albicans,* 25, 23, and 25mm, respectively. At a concentration of 125mg/ml, the zone of inhibition slightly decreased, but *Candida albicans* demonstrated the best results with a zone of inhibition of 22mm, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli,* 21, 19, and 20, respectively. The bark extract of J. regia did not affect *MRSA* (Table 1). J. regia did not affect *MRSA*. Table 1

Table 3. Zone of inhibition of J. regia bark in the cup-cut method.

Microorganisms	Zone in mm of 250mg /ml	Zone in mm of 125 mg /ml	Phenol 5%	0.12% chlorohexidine
Pseudomonas aeruginosa	25 mm	19mm	24mm	*23mm*
Escherichia coli	27mm	20mm	30mm	25mm
Candida albicans	25mm	22mm	35mm	20mm
Staphylococcus aureus	23mm	21mm	32mm	20mm
MRSA	0mm	0mm	29mm	12mm

The result of the MIC of J. regia bark against the test microorganisms has shown the lowest effective inhibitory concentration. Of J. regia bark extract against C. *albicans* 15.6mg/ml, and such extract reveals equal MIC against both *S. aureus and P. aeruginosa*. However, 62.5mg/ml is accordingly considered the MIC of *E. Coli.*

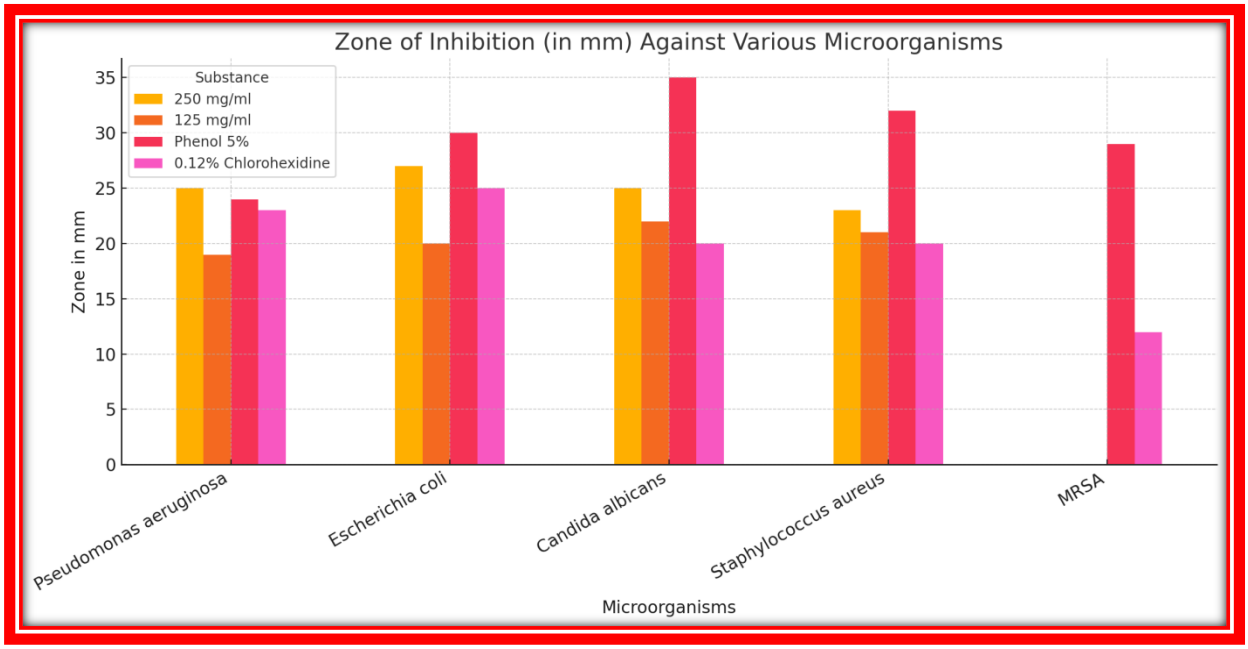

Figure 3. Zone of inhibition of J. regia bark in the cup-cut method.

Table 4: MIC results of J. regia bark extract

Microorganisms	*E. Coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
MIC	62.5mg/ml	31.25mg/ml	31.25mg/ml	15.6 mg/ml

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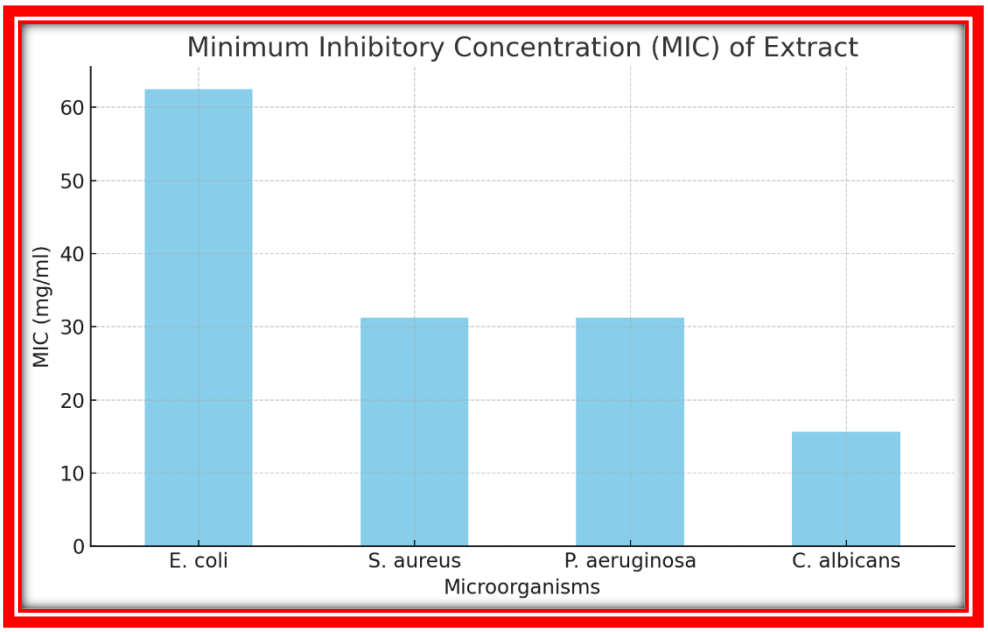

Figure 4: Zone of inhibition of J. regia bark in cup-cut method.

Discussion

In this study, the effect of methanolic extracts of J. regia bark on selected microorganisms was investigated using a cup-cut assay method, at concentrations of 250 mg/ml and 125 mg/ml, which exhibited significant antimicrobial activity, compared to phenol as a control and chlorohexidine 0.12%. This study reveals that the antimicrobial effect of J. regia bark increases with the increase in concentration. However, Zakavi, Faramarz, et al., a study published in 2013 in Iran, advocate the results obtained by this study as they find the high concentrations of ethanol of J. regia bark exhibit significant antimicrobial effects against *Streptococcus mutans* and *Staphylococcus aureus*. Moreover, the effect was concentration-dependent, with ethanolic extracts showing larger zones of inhibition compared to aqueous extracts [29]. Nevertheless, a study conducted in 2020 in Kurdistan found that the minimum inhibitory concentrations (MIC) for alcoholic and aqueous extracts of J. regia were 14mg/ml and 18mg/ml, respectively, equivalent to the MIC obtained by this study, 15mg/ml of the methanolic extracts [30].

Other research highlighted the capacity of J regia extracts to act against both gram-positive (e.g., *Bacillus cereus, Bacillus subtilis, S. aureus*) and gram-negative bacteria (e.g., *Pseudomonas aeruginosa, Escherichia coli, Klabsiella pneumoniae*) [21]. Further study emphasized the potential of J. regia as an alternative to antibiotics to prevent bacterial resistance [29]. This study augments the significant antimicrobial activity of methanolic extracts of J regia against both bacterial and fungal species, supporting the potential use of J. regia in improving oral hygiene and as an alternative to traditional antibiotics.

Conclusion

The current study confirms the antimicrobial potential of J.regia bark extracts, validating its traditional use as a preventive remedy for various microbial diseases, including dental caries and periodontal disease. These findings indicate that J. regia bark could serve as a valuable source of natural antifungal and antibacterial lead compounds for pharmaceutical applications. The study supports the growing interest in utilizing natural remedies over synthetic medications, highlighting the potential of J. regia as an effective alternative to chemotherapy. Further research is required to isolate and characterize the specific bioactive compounds responsible for the detected antimicrobial effects. In vivo studies and clinical trials are necessary to confirm the efficacy and safety of these extracts for practical use. Such investigations could pave the way for developing new natural antimicrobial agents, thereby advancing the field of phytotherapy and promoting sustainable healthcare solutions. for chemotherapy. Further research is required to isolate and characterize the specific bioactive compounds responsible for the detected antimicrobial effects. In vivo studies and clinical trials are necessary to confirm the efficacy and safety of these extracts for practical use. Such investigations could pave the way for developing new natural antimicrobial agents, thereby advancing the field of phytotherapy and promoting sustainable healthcare solutions.

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Conflict of interest. Nil

References

1. Abate BB, Kasie AM, Reta MA, Kassaw MW. Neonatal sepsis and its associated factors in East Africa: a systematic review and meta-analysis. Int J Pediatr. 2020;65:1623-33.
2. Popova C, Dosseva-Panova V, Panov V. Microbiology of periodontal diseases. A review. Biotechnol Biotechnol Equip. 2013;27(3):3754-9.
3. Thomas C, Minty M, Vinel A, Canceill T, Loubieres P, Burcelin R, et al. Oral microbiota: A major player in the diagnosis of systemic diseases. Diagnostics (Basel). 2021;11(8):1376.
4. Daboor SM, Masood FSS, Al-Azab MS, Nori EE. A review on streptococcus mutans with its diseases dental caries, dental plaque and endocarditis. Int J Med Res. 2015;2(2):76-82.
5. Cock IE. Medicinal and aromatic plants–Australia. In: Encyclopedia of Life Support Systems. UNESCO; 2011.
6. Thakare R, Rathod D, Rai M. Role of medicinal plants and their metabolites for the management of plant pathogens. In: Sustainable crop disease management using natural products. Wallingford, UK: CABI; 2015. p. 131-43.
7. Kumar P, Karam N, Choudhury D. Assessment of the Phytochemical Constituents and Metabolites in the Medicinal Plants and Herbal Medicine Used in the Treatment and Management of Skin Diseases. In: Herbal Medicine Phytochemistry: Applications and Trends. Springer; 2023. p. 1-41.
8. Malcangi G, Patano A, Ciocia AM, Netti A, Viapiano F, Palumbo I, et al. Benefits of natural antioxidants on oral health. Antioxidants (Basel). 2023;12(6):1309.
9. Álvarez-Martínez FJ, Barrajón-Catalán E, Micol V. Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. Biomedicines. 2020;8(10):405.
10. Abdallah EM, Alhatlani BY, de Paula Menezes R, Martins CHG. Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. Plants (Basel). 2023;12(17):3077.
11. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019;18:241-72.
12. Al Aboody MS, Mickymaray S. Anti-fungal efficacy and mechanisms of flavonoids. Antibiotics (Basel). 2020;9(2):45.
13. Yan Y, Li X, Zhang C, Lv L, Gao B, Li M. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics (Basel). 2021;10(3):318.
14. Cushnie TT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents. 2014;44(5):377-86.
15. Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (Juglans regia) chemical composition and research in human health. Crit Rev Food Sci Nutr. 2016;56(8):1231-41.
16. Catanzaro E, Greco G, Potenza L, Calcabrini C, Fimognari C. Natural products to fight cancer: A focus on Juglans regia. Toxins (Basel). 2018;10(11):469.
17. Dogan Y, Unver MC, Ugulu I, Calis M, Durkan N. Heavy metal accumulation in the bark and leaves of Juglans regia planted in Artvin City, Turkey. Biotechnol Biotechnol Equip. 2014;28(4):643-9.
18. Boulfia M, Lamchouri F, Toufik H. Chemical analysis, phenolic content, and antioxidant activities of aqueous and organic Moroccan Juglans regia L. Bark extracts. Chem Biol Chem. 2020;16(9):1328-39.
19. Khattak P, Khalil TF, Bibi S, Jabeen H, Muhammad N, Khan MA, et al. Juglans regia (walnut tree) bark in dentistry: walnut tree bark in dentistry. In: Proceedings of the 2022 International Conference on Biological and Medical Sciences; 2022. p. 152-6.
20. Medic A, Jakopic J, Solar A, Hudina M, Veberic R. Walnut (J. regia) agro-residues as a rich source of phenolic compounds. Biomolecules. 2021;10(6):535.
21. Bourais I, Elmarrkechy S, Taha D, Mourabit Y, Bouyahya A, El Yadini M, et al. A review on medicinal uses, nutritional value, and antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticancer potential related to bioactive compounds of J. regia. Food Rev Int. 2023;39(9):6199-249.
22. Barekat S, Nasirpour A, Keramat J, Dinari M, Meziane-Kaci M, Paris C, et al. Phytochemical composition, antimicrobial, anticancer properties, and antioxidant potential of green husk from several walnut varieties (Juglans regia L.). Antioxidants (Basel). 2022;12(1):52.
23. Quratul-ain S, Siddiqui MB. Medicinal and Nutritional Importance of Juglans regia Linn. on Human Health. In: Ansari MA, Shoaib S, Islam N, editors. Medicinal Plants and their Bioactive Compounds in Human Health: Volume 1. Singapore: Springer; 2024. p. 981-97.
24. Rahman MM, Rahaman MS, Islam MR, Hossain ME, Mithi FM, Ahmed M, et al. Multifunctional therapeutic potential of phytocomplexes and natural extracts for antimicrobial properties. Antibiotics (Basel). 2021;10(9):1076.
25. Smith AH. The effect of condensed tannins on bacterial diversity and metabolism in the intestinal tract [dissertation]. [Urbana-Champaign (IL)]: University of Illinois at Urbana-Champaign; 2003.

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26. Azhar N, Khan M, Riaz M, Hussain M, Dar M, Shaheen H, et al. Measurement of toxic metal contamination, phenolic and flavonoid contents, anticancer, antimicrobial and antioxidant activities of dietary and medicinal plant Juglans regia L. J Chem Soc Pak. 2018;16(3).
27. Eswayah A, Labyad N, Aleanizy F, Belaid A, Alqahtani F, Alfassam H. Antimicrobial and Anti-inflammatory properties of Juglans regia Leaves. Oman J Ophthalmol. 2019;35:1756-9.
28. Jaiswal BS, Tailang MJ. Juglans regia: A review of its traditional uses phytochemistry and pharmacology. Int J Adv Pharm Res. 2017;7(9):390-8.
29. Zakavi F, Golpasand Hagh L, Daraeighadikolaei A, Farajzadeh Sheikh A, Daraeighadikolaei A, Leilavi Shooshtari Z. Antibacterial effect of Juglans regia bark against oral pathologic bacteria. Int J Dent. 2013;2013:854765.
30. Abdullah ZG, Swilaiman S, Ahmad SH, Darogha SN. Antibacterial effect of Juglans regia against dental caries Streptococcus mutans. Pak J Med Health Sci. 2020;14:1313-7.

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