

Antibacterial effect of juglans regia l bark extract at different concentrations against human salivary microflora

Tuqa Aldawood¹, Alaa Alyousef^{2,*}, Shima Alyousef³, Nora Aldosari⁴, Sara Hussam⁵, AlaaAlhadad⁶, Fikrat Bhaian⁷, Dalia Sharaf Eldeen⁸, Nishanth Sayed Abdul⁹

¹⁻⁷Dental Intern, ⁸Assistant Professor, ⁹Lecturer, Dept. of Riyadh College of Dentistry and Pharmacy, Riyadh, Kingdom of Saudi Arabia

*Corresponding Author:

Email: alaa.a.lyousef@student.riyadh.edu.sa

Abstract

Objective: To evaluate and assess the anti bacteriogenic effect of the extract of bark of Derum (*Juglans regia* L.) at different concentrations on the salivary microflora.

Materials and Method: This study is an experimental in vitro study designed to collect the salivary samples from the patients. All tests were performed under sterile conditions.

Results: The results of the antimicrobial assay of the aqueous and acetone extracts of Derum (*J regia* L.) at different concentrations of 150 µg, 200 µg, 250 µg, and 300 µg showed that the aqueous extract had significant inhibitory effect on the growth of oral microbial flora compared to the acetone extract.

Conclusion: This study supports the use of natural products as medicines and confirms the antibacterial potentials of the plant *J Regia* L. (Walnut)

Keywords: *Juglans Regia* L., Dental caries, Antibacterial.

Introduction

Researchers have postulated that chewing stick helps in cleaning the teeth and periodontal tissue. Firstly, by their mechanical action which remove dental plaque and food debris and secondly by their chemical effect on teeth, gingiva, and/or antimicrobial properties due to the presence of active ingredients with chemotherapeutic effects.⁽¹⁾ Recently the World Health Organization (WHO) has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene. Such sticks are effective, inexpensive, commonly available, and contains many medical properties.⁽²⁾ Many reports suggest the effectiveness of traditional herbs against microorganisms. Plant- derived medicines have been a part of traditional health care system and the antimicrobial properties of plant derived compounds are well documented. *Juglans regia* L. has been used in traditional medicine from ancient times. *J regia* L. stem bark contains chemical constituents namely, β -sitosterol, ascorbic acid, juglone, folic acid, gallic acid, regiolone, and quercetin-3- α -L-arabinoside.^(3,4) The antibacterial properties of this plant material may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids.⁽⁵⁾ It is reported that leaves from *J regia* L. contain monoterpenes and sesquiterpenes; and the bark contains ketones like juglone, regiolone, sterol and, flavonoid.⁽⁶⁾

Some studies have demonstrated the antimicrobial activities of walnut products. However, information about *J regia* L. leaves is almost non-existent.⁽⁷⁾ Green walnuts, shells, kernels and seeds, bark, and leaves are

used in the pharmaceutical and cosmetic industries.^(8,9) The health benefits of walnuts are usually attributed to their chemical composition. Walnuts are a good source of essential fatty acids and tocopherols.⁽¹⁰⁾ *J regia* L. leaves are also used as a traditional medicine in China and Europe and have shown various health benefits for the treatment of skin inflammations, venous insufficiency, and ulcers. Moreover, the researches in pharmacology and therapeutics have shown that *J regia* L. leaves have hypoglycaemic, antioxidative, antimicrobial, and antihypertensive effects.^(11,12) Antifungal, antibacterial, and antioxidant activities of this plant have been described in the past.⁽¹³⁻¹⁵⁾ It can prevent gum disease and dental caries.^(16,17) Furthermore, the use of a chewing stick leads to a greater mechanical and chemical cleaning of oral tissues as compared to a standard toothbrush.⁽¹⁸⁾ The aim of this study is to evaluate and assess the anti bacteriogenic effect of the extract of bark of Derum (*Juglans regia* L.) at different concentrations on the salivary microflora.

Material and methods

This is an experimental in vitro study designed to collect the salivary samples from the patients attending the dental clinics in Riyadh colleges of dentistry and pharmacy with no history of using antiplaque agents for the past six months and having three to four mild dental caries. A total of fifty male and female patients aged between 18-30 years having dental caries of three or more teeth were selected. The saliva samples were collected from the patients with sterile cotton tipped

Swabs placed in the floor of mouth. It was then placed in a sterile container with saline (2 ml) and inoculated on the agar plates. The paper disc diffusion method was employed. Samples of each acetone and aqueous extracts (30 mg) were dissolved in respective solvents (1ml). Sterile 5 mm diameter filter paper discs were impregnated with these extracts of different concentrations ranging from 100 µg to 400 µg per disc.

The salivary flora were inoculated on nutrient broth and incubated for 24 hours at 37±0.1 °C. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with these test samples were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37±0.1 °C for 48 hours. After incubation all the plates were observed for

zones of inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions.

Results

The results of the antimicrobial assay of the aqueous and acetone extracts of *Derum* (*J regia* L.) at different concentrations of 150 µg, 200 µg, 250 µg, and 300 µg are presented in Tables 1 to 4. Aqueous extract had significant inhibitory effect on the growth of oral microbial flora compared to the acetone extract. The efficacy of acetone extract and aqueous extract on salivary microflora is displayed in Fig.1&2. Aqueous extract exhibited zones of inhibition against most of the tested samples whereas acetone extract exhibited zones of inhibition against selected samples. A concentration of 300 µg /disc with the average zone of inhibition 16.50 mm in aqueous extract and 14.25 mm in acetone extract is found to inhibit the growth of salivary microbial flora of the in vitro test samples of saliva.

Table 1: Effect of aqueous extract of *Derum* on salivary microflora at different concentrations

| Agar plates with saliva | ZONE 1(150 µg) | ZONE 2(200 µg) | ZONE 3(250 µg) | ZONE 4(300 µg) |
|---------------------------------|----------------|----------------|----------------|----------------|
| 1 | 10 | 12 | 11 | 11 |
| 2 | 9 | 11 | 12 | 13 |
| 3 | 11 | 13 | 16 | 21 |
| 4 | 15 | 15 | 19 | 21 |
| Average zone of inhibition (mm) | 11.25 | 12.75 | 14.5 | 16.5 |

Table 2: Aqueous extract of *Derum* with zones of inhibition

| Plate number | Concentration | Average zone of inhibition(mm) |
|--------------|---------------|--------------------------------|
| 1 | 150 µg | 11.25 |
| 2 | 200 µg | 12.75 |
| 3 | 250 µg | 14.5 |
| 4 | 300 µg | 16.5 |

Table 3: Effect of acetone extract of *Derum* on salivary microflora showing at different concentrations

| Agar plates with saliva | ZONE 1 (150 µg) | ZONE 2 (200 µg) | ZONE 3 (250 µg) | ZONE 4 (300 µg) |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|
| 1 | 11 | 12 | 16 | 13 |
| 2 | 13 | 12 | 11 | 11 |
| 3 | 14 | 15 | 13 | 19 |
| 4 | 15 | 16 | 16 | 14 |
| Average one of inhibition (mm) | 13.25 | 13.75 | 14 | 14.25 |

Table 4: Acetone extract of *Derum* with zones of inhibition

| Plate number | Concentration | Average zone of inhibition mm) |
|--------------|---------------|--------------------------------|
| 1 | 150 µg | 13.25 |
| 2 | 200 µg | 13.75 |
| 3 | 250 µg | 14 |
| 4 | 300 µg | 14.25 |

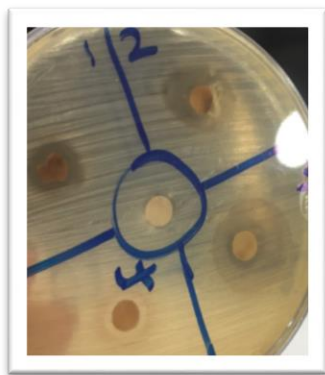


Fig. 1: Acetone extract

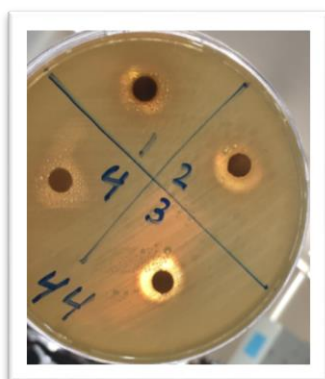


Fig. 2: Aqueous extract

Discussion

To date no studies were reported in the Kingdom of Saudi Arabia (KSA) on the antibacterial effects of extract of *Derum* bark despite the use of different components of *Derum* as a cosmetic material for lips and as a tooth brush among Saudi population. Therefore, the present in vitro study was aimed at investigating the antibacterial effect of the bark of *Derum* (*J. regia* L.) extract at different concentrations against oral salivary microflora of dental caries patients in KSA. A study conducted for antimicrobial activity of *Derum* has showed that acetone extract was more effective than the aqueous extract against cariogenic bacteria.⁽⁶⁾ On the contrary, the present study showed that the aqueous extract has a greater effect compared to the acetone extract but both had antibacteriogenic effect to an extent. However, the findings of current study was similar to that of a previous study which reported that the aqueous extract of this plant inhibited in vitro growth of *Streptococcus mutans* after rinsing than with the alcoholic extracts of bark of *J. regia* L. on in vitro salivary samples.⁽¹⁹⁾

Similar in vitro studies were conducted by using green hull of *J. regia* L. to study its antibacterial and antioxidant properties and reported that the ethanol extract showed maximum antibacterial activity of green

hull of this plant compared to other extracts.⁽²⁰⁾ Another study stated that the *J. regia* L. contains chemicals like ascorbic acid, juglone, folic acid, gallic acid, regiolone that are involved in the antibacterial, antioxidant, and antifungal activities of this plant. This plant has greater potential for treating more severe cases such as periodontitis and gingivitis due to its antibacterial effect on different microbial flora particularly, gram positive organisms.⁽²¹⁾ Further in vivo studies with larger sample are recommended to confirm the role of *J. regia* L. as a natural remedy and as a preventive medicine for various oral lesions.

Conclusion

This study supports the use of natural products as medicines and confirms the antibacterial potentials of the plant *J. regia* L. (Walnut). The results strongly support the traditional use of this plant (*Derum*) as preventive remedy for various microbial diseases of soft and hard tissues in the oral cavity. These plants contain chemical substances that take part in metabolic activities thereby helping to fight against bacterial infections.

Acknowledgement

The authors are thankful to the staff of department of microbiology, Riyadh colleges of dentistry and pharmacy, Riyadh, KSA.

References

1. Eid MA, Selim HA, Al-Shammery AR. The relationship between chewing sticks (Miswak) and periodontal health. Part I. Review of the literature and profile of the subjects. *Quintessence International*.1990;21(11).
2. Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. *International Dental Journal*.1995;45(3):218-22.
3. Evans CE, Banso A, Samuel OA. Efficacy of some nupel medicinal plants against *Salmonella typhi*: an in vitro study. *Journal of Ethnopharmacology*.2002;80(1):21-4.
4. Yelne M, Sharma P, Dennis T. Database on medicinal plants used in Ayurveda. Central Council for Research in Ayurveda & Siddha, New Delhi.2002;4.
5. Smith E, Williamson E, Zloh M, Gibbons S. Isopimaric acid from *Pinus nigra* shows activity against multidrug-resistant and EMRSA strains of *Staphylococcus aureus*. *Phytotherapy Research*.2005;19(6):538-42.
6. Deshpande RR, Kale AA, Ruikar AD, PANVALKAR PS, KULKARNI AA, DESHPANDE NR, et al. Antimicrobial activity of different extracts of *Juglans regia* L. against oral Microflora. *International Journal of Pharmacy and Pharmaceutical Sciences*.2011;3(2):200-1.
7. Qa'dan F, Thewaini A-J, Ali DA, Afifi R, Elkhawad A, Matalka KZ. The antimicrobial activities of *Psidium guajava* and *Juglans regia* leaf extracts to acne-developing organisms. *The American Journal of Chinese Medicine*.2005;33(02):197-204.
8. Pereira JA, Oliveira I, Sousa A, Valentão P, Andrade PB, Ferreira IC, et al. Walnut (*Juglans regia* L.) leaves: phenolic compounds, antibacterial activity and

- antioxidant potential of different cultivars. *Food and Chemical Toxicology*. 2007;45(11):2287-95.
9. Stampar F, Solar A, Hudina M, Veberic R, Colaric M. Traditional walnut liqueur-cocktail of phenolics. *Food Chemistry*. 2006;95(4):627-31.
 10. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology*. 2006;29(4):203-10.
 11. Cheniany M, Ebrahimzadeh H, Vahdati K, Preece JE, Masoudinejad A, Mirmasoumi M. Content of different groups of phenolic compounds in microshoots of *Juglans regia* cultivars and studies on antioxidant activity. *Acta Physiologiae Plantarum*. 2013;35(2):443-50.
 12. Girzu M, Carnat A, Privat A-M, Fialip J, Carnat A-P, Lamaison J-L. Sedative effect of walnut leaf extract and juglone, an isolated constituent. *Pharmaceutical biology*. 1998;36(4):280-6.
 13. Saimbi C, Shubh N, Kapoor K, Kaushal S. Clinical effect of *Juglans regia* on developing dental plaque. *Journal of the International Clinical Dental Research Organization*. 2009;1(3):1.
 14. Isanga J, Zhang G-N. Biologically active components and nutraceuticals in peanuts and related products. *Food Reviews International*. 2007;23(2):123-40.
 15. Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A. Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral *Candida* strains. *European journal of clinical microbiology & infectious diseases*. 2010;29(1):81.
 16. Almas K. The effect of *Salvadora persica* extract (miswak) and chlorhexidine gluconate on human dentin: a SEM study. *J Contemp Dent Pract*. 2002;3(3):27-35.
 17. Halawany HS. A review on miswak (*Salvadora persica*) and its effect on various aspects of oral health. *The Saudi Dental Journal*. 2012;24(2):63-9.
 18. Malik AS, Shaukat MS, Qureshi AA, Abdur R. Comparative effectiveness of chewing stick and toothbrush: A randomized clinical trial. *North American journal of medical sciences*. 2014;6(7):333.
 19. Jagtap A, Karkera S. Extract of *Juglandaceae regia* Inhibits Growth, In- vitro Adherence, Acid Production and Aggregation of *Streptococcus mutans*. *Journal of pharmacy and pharmacology*. 2000;52(2):235-42.
 20. Sharma P, Ravikumar G, Kalaiselvi M, Gomathi D, Uma C. In vitro antibacterial and free radical scavenging activity of green hull of *Juglans regia*. *Journal of Pharmaceutical Analysis*. 2013;3(4):298-302.
 21. Zakavi F, Golpasand Hagh L, Daraeighadikolaei A, Farajzadeh Sheikh A, Daraeighadikolaei A, Leilavi Shoostari Z. Antibacterial effect of *Juglans regia* bark against oral pathologic bacteria. *International journal of dentistry*. 2013;2013.