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

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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 acqueous 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

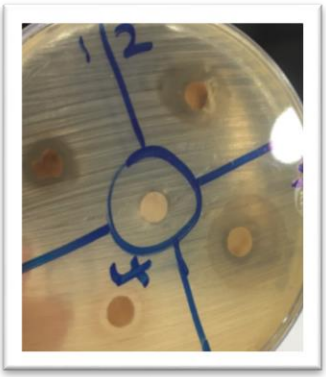

Fig. 1: Acetone extract

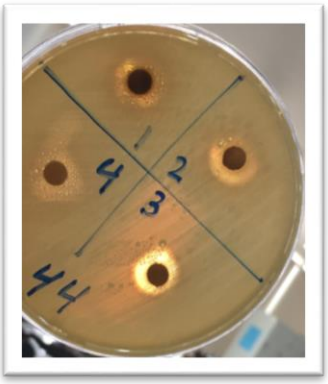

Fig. 2: Aqueous extract

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.

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