In Vitro Antibacterial Activity of Bark Extracts of *Juglans regia* L. Against Oral Pathogens

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Abstract

Dental diseases are among the most common health problems treated with traditional remedies. The plants used in dental care possess antibacterial activity. In the view of this an effort was made to evaluate the antimicrobial activity of the ethanolic and aqueous extracts of bark of *Juglans regia*, against oral bacteria, *Streptococcus mutans* (MTCC 890) and *Staphylococcus aureus* (MTCC 737). Minimum inhibitory concentration (MIC) 500 µg/ml for ethanolic and aqueous extract were used for this purpose. Different dilutions of these extracts were used to have concentration of 500 µg/ml, 1000 g/ml, 1500 µg/ml, 2000 µg/ml and 2500 µg/ml. In well diffusion method the ethanolic extract at higher concentration 2500 µg/ml showed maximum zone of inhibition 15mm against *S.mutans* and 12 mm against *S.aureus* however aqueous extract at same concentration showed maximum zone of inhibition 10 mm against *S.mutans* and 11 mm against *S.aureus.* The results of *in vitro* antimicrobial assay proved that ethanol solvent was more successful in the extraction of phytochemicals from plant samples than aqueous solvent, as ethanol extract showed higher antimicrobial activity than aqueous extract against both the test pathogens. Phytochemical screening of alcoholic extract showed the presence of different types of compounds like alkaloids, phenolics, tannins, flavonoids, steroids, saponins and resins which may contribute for the antimicrobial action of the above medicinal plant. The results of our study may provide the basis for using natural antimicrobial substance for oral hygiene purposes.

Keywords: *Juglans regia*; bark; antibacterial activity; *Streptococcus mutans*, *Staphylococcus aureus*

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INTRODUCTION

Juglans regia L. is one of the medicinal plants, commonly known as English walnut, and is a deciduous tree belonging to the family Juglandaceae. It is native plant of central Asia, including parts of China, Iran, and Afghanistan, but is now widely cultivated throughout the world for its valuable wood and nutritious nuts. Because of its medicinal properties it is used in Jammu, Ladakh, and Kashmir as anthelmintic, gum problems, liver ailments, skin diseases, tooth cleaning, toothache, constipation, sore throat, against hair fall, joint pain, skin eruptions, anemia, rheumatism, mouth and ulcers; and as antiseptic [1]. Its barks, leaves, and husk are used as dyes and in folk medicine as herbal remedies for several diseases [2]. Dental diseases are among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of herbal remedies for the discovery of novel bioactive compounds that might serve as leads for the development of potent drugs. It appears when demineralization factors overcome the demineralization capabilities of the saliva reservoir, resulting in mineral imbalances within the tooth surface [3].

Among the many plants used as chewing sticks, the stem bark of *Juglans regia* L.(Juglandaceae) is very popular and is used as a tooth cleaning device by the people living in all of temperate Europe and in the Himalayas. Antimicrobial agents are usually incorporated into hygiene products for the treatment and prevention of plaque and gingivitis [4]. High concentrations of ethanoic and aqueous extracts have had antimicrobial effects against *S. sanguis, S. mutans, S. salivarius* and *staphylococcus aureus* with significant difference in contrast to control [5]. The use of natural products could help to overcome bacterial resistance to the antimicrobials that are currently employed in clinical therapy [6]. Here is a continuous need of new antimicrobial components due to rapid appearance of multiple drug-resistance bacteria [7]. Herbal medicine has been the first choice, if not the only, method of health and oral care in large populations, mainly in developing countries. Based on the popular belief that chewing sticks can efficiently clean the teeth, several investigators have directed their research to explore their antibacterial properties [8]. The different extracts of the different parts of walnut have been used and found to be significantly effective in improving the various pathological conditions in rats as well as human beings due to the presence of a variety of photochemical in it [9]. Finely powdered bark is used as an ingredient for the treatment of bleeding gums as mouth rinse [10]. It can also be used in cosmetics due to the presence of juglone for dentifrices, toothpowders, mouth rinses, deodorants and chewing gums [11].

In our investigation efforts have been made to discover plants possessing antimicrobial agents, as plaque-control for the prevention of dental caries and periodontal diseases. We have been published isolation of five unreported phytoconstituents named benzjuglansoic acid, naphthjuglansoic acid, juglans benzoate, regiaoleate and regiapalmitate and their structures have been elucidated as 2-methoxy-benzyl-n-benzoicacid,1-methoxy-2-n-octanylnaphthyl-9-oic acid, o-undecanyl-n-heptacanyl-2-hydroxy-benzoate, n-heneicosanyl-9-octadeconoate, n-heptyl triacont-12'-enyl hexa decanoate. In addition, β-sitosterol, β-sitostero-β-D-glucoside, stigmasterol-β-D-glucopyranosiode and β-sitosterol- β-D-glucuronopyranoside from the bark of this plant [12]. In the present paper, we report the antibacterial effect of aqueous and ethanolic extracts of bark of *J. regia* against oral pathogens viz., *S. mutans* and *S.aureus*.

MATERIALS AND METHODS

Collection and Authentication

The barks of *J. regia* were collected from Sopore Kashmir, authenticated by Dr. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi A voucher specimen No. PRL/JH/05/24 is deposited in the herbarium section of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction

The barks of *J. regia* were dried in an oven at 45^{o}C for 2-3 days and coarsely powdered. The ground bark was extracted with ethanol and water in a Soxhlet apparatus. The ethanol and aqueous extracts were concentrated under reduce pressure to yield a dark red viscous mass.

Preliminary phytochemical screening of *J. regia*

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents. The qualitative chemical tests give the general idea regarding the nature of chemical constituents of crude drugs. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds,

flavonoids, proteins and amino acids, saponins, sterols, acidic compounds, mucilages and resins [13].

Antibacterial activity

Microorganisms and growth media

Staphylococcus aureus (MTCC 737) and *Streptococcus mutans* (MTCC 890) were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. Maintained on nutrient agar and brain heart infusion medium slants at 4ºC.

Preparation of sample solution

The concentrated ethanoic and aqueous extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) of *J. regia*, were dissolved in1 ml of dimethyl sulphoxide (DMSO), an inert solvent, which was also used as control. Different dilutions of these extracts were used to have concentration of 500 µg/ml, 1000 g/ml, 1500 µg/ml, 2000 µg/ml and 2500 µg/ml. Standard drug, Amikacin (30 µg/disc) was used against *S. aureus* and chloramphenicol (30 µg/disc) against *S. mutans* and DMSO were used as negative controls, respectively.

Procedure

Minimum inhibitory concentration (MIC) of each extract was done by cup and plate method [14]. The inoculum of the *S. aureus* and *S. mutans* were spreaded over nutrient agar medium and brain heart infusion medium, respectively by garden culture method [15]. Five cavities of bore size (6 mm) were bored in solid medium and 100 µl of test drug was poured in each cavity. Standard drug, Amikacin (30 µg/disc) was used against *S. aureus* and chloramphenicol (30 µg/disc) against *S. mutans* were placed aseptically in a seprate petri dish. The plates were kept at room temperature for one hour to diffuse the drug in surrounding medium and then incubated at 37°C for 24 h. The diameter of the zone of inhibition formed around the cavities and disc of standard antibiotic after overnight incubation was accurately measured.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the standard of agar dilution method described in the NCCLS guidelines [16]. It was determined for the ethanol extract recorded the highest antimicrobial activity. The lowest concentration of active principle that prevented microbial growth was considered to be the MIC. The test organisms were separately seeded in the agar medium. The wells (10 mm in diameter) were cut from the agar and 0.1 ml of extract solution (different concentrations) was transferred into them. After 24 h incubation period, the plates were examined and the inhibition zones were determined.

Determination of antibacterial activity

Antibacterial activity was done by cup and plate method. The inoculum of the *S. aureus* and *S. mutans* were spreaded over nutrient agar medium and brain heart infusion medium, respectively by garden culture method. Five cavities of bore size (6 mm) were bored in solid medium and 100 µl of test drug was poured in each cavity. Standard drug, Amikacin (30 µg/disc) was used against *S. aureus* and chloramphenicol (30 µg/disc) against *S. mutans* were placed aseptically in a seprate petri dish. The plates were kept at room temperature for one hour to diffuse the drug in surrounding medium and then incubated at 37°C for 24 h. The diameter of the zone of inhibition formed around the cavities and disc of standard antibiotic after overnight incubation was accurately measured [17, 18].

Statistical analysis

Results from representative experiments are shown. The disc diffusion method was carried out in triplicates and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Extraction

The ground bark (3 kg) was extracted with ethanol then with water in a Soxhlet apparatus. Both extracts were concentrated under reduce pressure to yield a dark red viscous mass of ethanol (375 g, 12.5%) and aqueous (425g, 14.16%).

Qualitative phytochemical analysis

Phytochemical screening of alcoholic extract showed the presence of different types of compounds like alkaloids, phenolics, tannins, flavonoids, steroids, saponins and resins which may contribute for the antimicrobial action of the above medicinal plant.

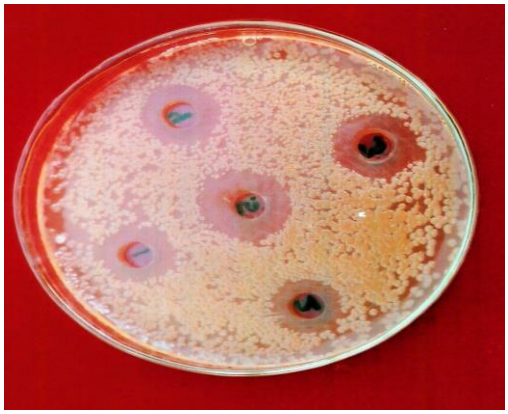
Antibacterial activity

The results showed that in case of control disc no zone of inhibition was seen so far as our study is concerned 1% DMSO, as solvent is having no effect on the tested organisms. Ethanolic extract of *J. regia*, exhibited better antibacterial activity against *S. mutans* in compared to standard drug chloramphenicol. The data of this study clearly indicated that ethanoic extract of the bark significantly inhibited the growth of the tested

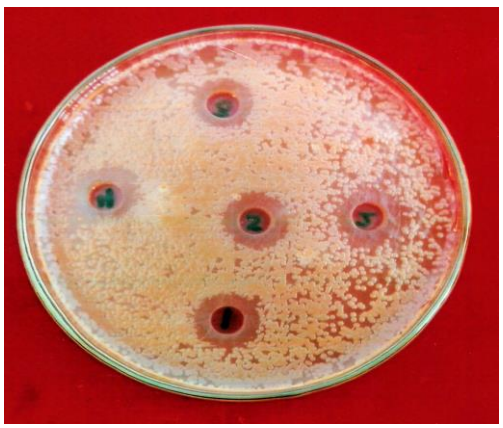
oral bacteria, and those reports are compatible with our finding (Table 1and Fig 1 &2). The antibacterial property of the plant material may be due to the presence of phenolic compounds, terpenoid, alkaloids, flavonoids, and steroids.

Table-1: Antibacterial activity of plant extracts

Drug	Extract	Concentration (µg/ml)	Diameter of zone of inhibition (mm)	
			S. aureus	***S. mutans***
J. regia	Ethanolic	**500**	05	10
		1000	09	11
		1500	10	12
		2000	11	13
		2500	12	15
	Aqueous	**500**	04	04
		1000	05	06
		1500	07	08
		2000	09	09
		2500	10	10



1. 500 µg/ml; 2. 1000 µg/ml; 3. 1500 µg/ml; 4. 2000 µg/ml; 5. 2500 µg/ml
Fig.1: Antibacterial activity of alcoholic extract of *J. regia* against *S. mutans*



1. 500 µg/ml; 2. 1000 µg/ml; 3. 1500 µg/ml; 4. 2000 µg/ml; 5. 2500 µg/ml
Fig. 2: Antibacterial activity of alcoholic extract of *J. regia* against *S. aureous*

CONCLUSION

The findings of the present work have revealed that the bark of *J. regia* possess good antibacterial activity. Further studies are required to find these effects in order to replace synthetic medications with natural remedies. In conclusion, it is very likely that the synergistic action of this extract and conventional antibiotics will be more effective against the oral pathogens.

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Conflict of Interest

The Authors declare no conflict of interest in this work.

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