

Antimicrobial effect of *Juglans regia* bark with commonly used antibiotics against initial colonizers of Plaque and Caries: A comparative study

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Abstract

Introduction: There is a progressive increase in the patients of dental caries and periodontitis in the recent years. The main emphasis is laid on avoiding the accumulation and inducing the elimination/suppression of caries-causing microbes in the oral cavity. A variety of antimicrobial agents are incorporated into oral hygiene products but these products come up with their own disadvantages. Natural products prove to be biocompatible with medicinal properties.

Aims and Objectives: The objective of the study is to assess the antimicrobial activities of *Juglans regia* bark against dental caries and plaque. Also, antibacterial activity of *Juglans regia* bark is compared with oral rinses and systemic antibiotics.

Material and Methods: The study sample consisted of 50 subjects and was divided into two groups. Group 1 consisted of 25 samples without caries and Group 2 also included 25 samples with caries. Both samples were in the age group 18–22 years. Plant samples of *Juglans regia* bark from Kashmir and Himachal were collected. Extracts containing both varieties were prepared and antibacterial activities of both groups were determined. Comparison of *Juglans regia* bark with antibiotics and mouthwashes was also done.

Results and Conclusion: *J. regia* bark of both the varieties showed zone of inhibitions. Ethanol variety showed larger zone of inhibition than distilled water variety of both plaque and caries groups. Chlorhexidine showed satisfactory antimicrobial activity while Betadine showed none. Amoxicilline was effective but Metronidazole showed no activity against both groups. Bark of *J. regia* proved to be very beneficial against both plaque and caries bacteria without any side effects as both mouthwashes and antibiotics are harmful and cannot be used daily.

Keywords: Antimicrobial, caries, *Juglans regia*, oral rinse, plaque

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INTRODUCTION

Oral health is an integrated part of general health and an open growth system which offers diverse habitats, wherein different species of micro-organisms can prosper. The primary requisite for any group of microbes to flourish in an oral cavity is their ability to adhere to the tooth surfaces, and they can cause caries and periodontitis.^[1] After the eruption of baby teeth in the oral cavity, a true biofilm consisting of a variety of micro-organisms termed *dental plaque* or *bacterial plaque* is formed.^[2] The second most prevalent bacterial infection in the oral cavity is dental caries after periodontal diseases. Overgrowth of *Streptococcus mutans* is recognized as the primary cause of the disease.^[3-5] Antimicrobial agents such as Chlorhexidine, triclosan, diethylene glycol are usually incorporated into oral hygiene products for the treatment of caries and periodontitis. These products come up with their own disadvantages also as they have to be used daily.^[6]

MOTHER NATURE acts a boon to the above problems as natural products are an unending source of chemical compounds which are often useful in pharmaceuticals and agro-ecosystem developments. The traditional medicinal plants are capable of treating various diseases.^[7] *Juglans regia*, known as Akhrot in India, is a member of the Juglandaceae family. The bark is resinous and scented.^[8] Walnut (*Juglans regia* L.) bark has been had anti-inflammatory, antioxidant, antibacterial, antiplaque, antifungal, anthelmintic, blood purify, anticancer, depurative, diuretic, and laxative activities.^[9-12] So in our department of oral pathology and microbiology a study was carried out comparing the antimicrobial efficacy of Akhrot (*Juglans regia*) bark with commonly used mouthwashes and antibiotics.

AIMS AND OBJECTIVES

1. To evaluate the antimicrobial activities of ethanolic and aqueous extracts of *Juglans regia* bark against plaque and caries colonizers.
2. Comparison of antibacterial activity of *Juglans regia* bark with commonly used oral rinses and systemic antibiotics

MATERIALS AND METHODS

Collection of plant samples

Two varieties of *Juglans regia* (Akhrot), viz. Kashmir variety and Himachal variety, were collected.

- The bark was scratched from the trunk of the tree using sharp sickle. The bark samples were free from any visible contamination, diseases, healthy and fresh. [Figure 1]

- They were thoroughly washed and shade dried at room temperature for five weeks. Then, they were grinded to powder. [Figure 2]
- The dried powder was stored in airtight bottles at room temperature for further use.

Preparation of the aqueous and ethanolic plant extracts

- Ethanolic extract was prepared from grinded *Juglans regia* bark powder (100 g) and 500 ml of ethanol (100%) using a Soxhlet apparatus.
- Aqueous extract was prepared from grinded *Juglans regia* bark powder (100 g) and 500 ml of distilled water using a Soxhlet apparatus. The extracts were filtered by Whatman filter paper and then concentrated in water bath. The residues obtained were stored in a freezer at 4°C until future use. [Figure 3]

Collection of microbial samples

The present study was conducted in the Department of Oral Pathology and Microbiology, Himachal Institute of Dental Sciences, Paonta Sahib. A total of 50 subjects were included in the present study. These 50 subjects were divided into two groups:

Group 1- comprised of 25 subjects without caries, age range varied from 18 to 22 years.

Group 2- comprised of 25 subjects with caries, age range varies from 18 to 22 years.

Group 1-Dental plaque samples-

Dental plaque samples were collected from students of a HIDS Paonta Sahib. They were evaluated and selected as per the following criteria -

Inclusion criteria for plaque sample

- Age group 18 to 22
- DMFT index = 0



Figure 1: *Juglans Regia* bark scratched from the plant



Figure 2: *Juglans regia* bark in powdered form.



Figure 3: Armamentarium for plant extract apparatus -autoclave, weighing machine, soxhlet apparatus

Exclusion criteria for plaque samples

- Subjects with a carious lesion
- Oral diseases
- Orthodontics appliances
- On antibiotic therapy for past 1 month or any other immune-compromised condition
- Was not using any kind of mouthwash for past 1 month.
- Presence of any oral mucosal lesion
- Any systemic disease like diabetes, hypertension
- Was not on any type of anticancer therapy or any kind of radiation exposure

All the subjects were informed on the purpose of the study, and a written consent was obtained from all the subjects. Before the collection of plaque sample subjects were advised not to brush their teeth one night before and not to eat or drink for two hours. Plaque samples were collected early morning from inter-proximal sites of lower central incisors with sterile curettes including both supra-gingival and sub-gingival plaque. The curette was placed buccally against the tooth surface sub-gingivally and withdrawn coronally.

Dental caries samples

Dental caries samples were collected from patients reporting at the OPD of HIDS Paonta Sahib with visible active carious lesions.

Inclusion criteria for caries sample

- Age group 18 to 22
- At least one visible carious lesion and less than six caries' lesions
- Only pit and fissure caries were selected for sample collection.

Exclusion criteria for caries samples

- Any other type of caries like smooth surface caries or root caries, etc.
- Presence of root stumps
- Oral diseases
- Orthodontic appliances
- On antibiotic therapy for past 1 month or any other immune-compromised condition
- Was not using any kind of mouthwash for past 1 month
- Presence of any oral mucosal lesion
- Any systemic disease like diabetes, hypertension
- Was not on any type of anticancer therapy or any kind of radiation exposure.

All the subjects were informed on the purpose of the study, and a written consent was obtained from all the subjects. Age ranges between 18 and 22 years. Before the collection of caries, samples subjects are advised not to brush their teeth one night before and not to eat or drink two hours before sample collection. Caries samples were collected from the active carious lesion. A water-cooled sterile diamond bur was used to collect samples of carious lesions. Food debris was removed from carious lesions with the help of a sterile spoon excavator. Local anaesthesia was delivered where necessary; the carious teeth were isolated with a rubber dam.

Determination of antimicrobial activities

Inoculum preparation

The medium was prepared by dissolving brain heart infusion broth (HiMedia Laboratories Pvt. Ltd) in distilled water and autoclaving at 121°C for 15 minutes. Plaque and caries samples were inoculated in autoclaved brain heart infusion broth and incubated at 37°C for 24 h.

Turbidity produced was adjusted to match 0.5 McFarland standard (10^8 cfu/ml). [Figure 4]

Agar well diffusion method

100 µl of microbial inoculums formed on the broth were aseptically introduced by micropipette and spread by using L-shaped spreader on surface of brain heart infusion agar plates. [Figure 5] Antimicrobial activity of ethanolic and aqueous stem bark extracts was tested by using the agar well diffusion method.

OBSERVATION AND RESULTS

Group: 1

Plaque samples were collected from the group 1 with curettes and stored in test tube-containing brain heart infusion broth (BHI broth) for 24 hours in an incubator.

These plaque samples were inoculated by micropipettes on BHI agar plates. Wells of 7 mm diameter were prepared with the help of borer in the culture plates, and various experimental extracts along with antibiotics solutions and mouth rinses were added to each well. These plates were again incubated for 24 hours. Comparisons of zone of inhibition were made between the antimicrobial agents and extracts of *Juglans regia* bark. These values were then statistically analysed.

Our finding showed that different systemic antibiotics (Cefixime, Amoxicilline and Metronidazole) showed a different zone of inhibition. Cefixime and Amoxicilline showed larger zone of inhibition, i.e. antimicrobial activity against plaque bacteria, but Metronidazole showed the smaller zone of inhibition. [Figure 6a], [Graph 1].

The zone of inhibition as recorded in our study in decreasing order is as follows- Cefixime > Amoxicilline > Metronidazole

In case of commonly used mouth rinses Chlorhexidine showed the larger zone of inhibition on BHI agar plates. Betadine showed smaller values, but surprisingly Listerine showed no zone of inhibition, i.e. no antimicrobial activity. A zone of inhibition in decreasing order is as follows: Chlorhexidine > Betadine > Listerine [Graph 2].

Extracts of *Juglans regia* bark showed larger zone of inhibition, i.e. good antimicrobial activity. When compared to different extracting solvent, *Juglans regia* barks showed the larger zone of inhibition when ethanol was used as an extraction solvent and smaller zone of inhibition when distilled water was used as an extraction solvent. Kashmir variety of *Juglans regia* showed the larger zone of inhibition as compared to Himachal variety of *Juglans regia*. [Figure 6b], [Graph 3].

Table 1: Zone of inhibition in mm against plaque bacteria with commonly used systemic antibiotics

Antibiotics	No of plaque samples	Minimum value	Maximum value	Mean	Std. Deviation
Cefixime	25	14	32	22.88	5.003
Amoxicilline	25	10	20	14.24	3.479
Metronidazole	25	0	1	0.24	0.436

Table 2: Zone of inhibition in mm against plaque bacteria with commonly used mouth rinses

Mouth rinses	No of plaque samples	Minimum value	Maximum value	Mean	Std. Deviation
Chlorhexidine	25	8	14	10.72	2.092
Listerine	25	0	0	0.00	0.000
Betadine	25	1	5	2.76	1.128

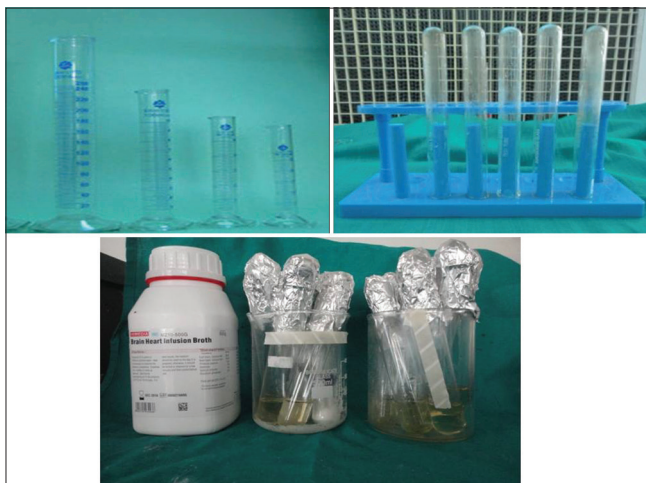


Figure 4: Armamentarium for Inoculum preparation - Measuring cylinder, test tubes, brain heart infusion broth, test tubes containing brain heart infusion broth.



Figure 5: Armamentarium for Agar Well diffusion method -Brain heart infusion plates, L shaped spreader, micropipette, different mouth wash and antibiotics used in study.

A zone of inhibition in decreasing order: Kashmir Ethanol (KE) > Himachal Ethanol (HE) > Kashmir Distilled water (KD) > Himachal Distilled water (HD).

Two extracting solvents were used, ethanol and distilled water. Ethanol alone showed the zone of inhibition in few plaque samples, but distilled water showed no zone of inhibition. [Figure 6c], [Graph 4].

Different comparisons were made to compare the value of the zone of inhibitions between the antimicrobial agents and *Juglans regia* extracts [Graph 5 and Table 5].

Group: 2

Caries samples were collected from the group 2 with carbide bur and stored in a test tube containing brain

heart infusion broth (BHI broth) tubes for 24 hours in an incubator.

These caries samples were inoculated by micropipettes on BHI agar plates. Wells of 7 mm diameter were prepared with the help of borer in the culture plates, and various experimental extracts along with antibiotic solutions and mouth rinses were added to each well. These plates were again incubated for 24 hours. Comparisons of zone of inhibition were made between the antimicrobial agents and extracts of *Juglans regia* bark. These values were then statistically analysed.

Table 3: Zone of inhibition in mm against plaque bacteria with extracts of *Juglans regia* bark from Kashmir and Himachal varieties

<i>Juglans regia</i> extracts	No of plaque samples	Minimum value	Maximum value	Mean	Std. Deviation
KE	25	5	13	8.48	1.735
HE	25	4	12	8.28	1.926
KD	25	2	10	6.24	1.739
HD	25	3	9	6.04	1.620

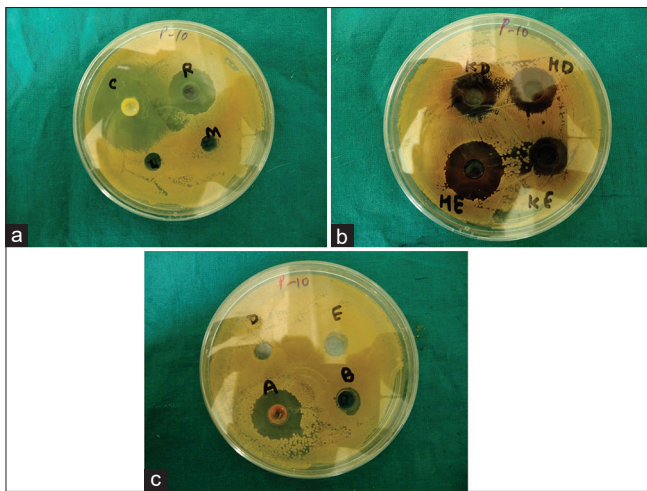
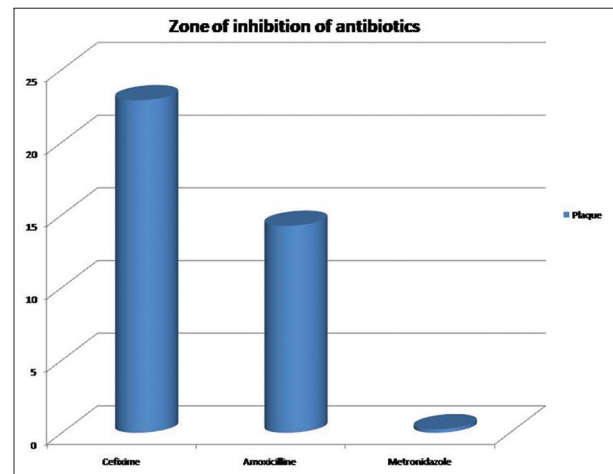
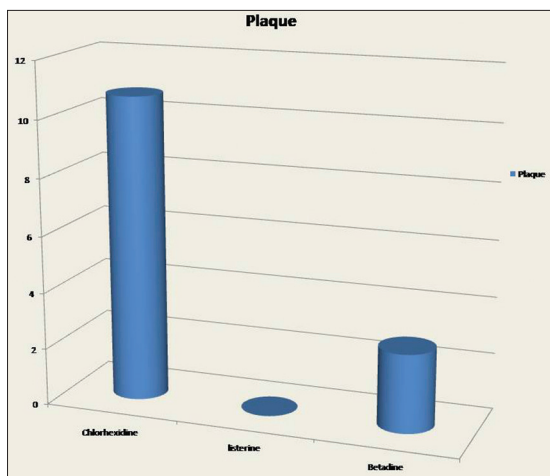


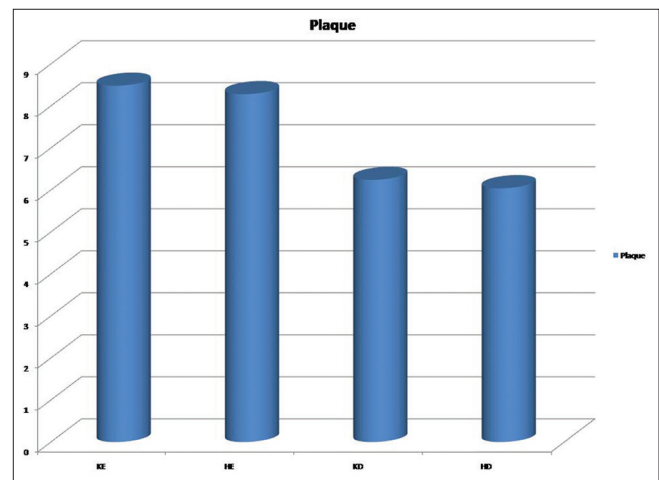
Figure 6: Culture Plate showing Zone of inhibition formed by C-Cefixime, R- Rexidine, L-Listerine, M-Metronidazole. Zone of Inhibition formed by (a) KD (Kashmir distilled water). (b) HD (Himachal distilled water). (c) Himachal Ethanol and Kashmir Ethanol. Zone of inhibition formed by D-Distilled water, E-Ethanol, A-Amoxicillin, B-Betadine.



Graph 1: Zone of inhibition of systemic antibiotics against plaque bacteria



Graph 2: Zone of inhibition of commonly used mouth rinses against plaque bacteria.



Graph 3: Zone of inhibition of *Juglans Regia* bark extracts against plaque bacteria.

Our finding showed that different systemic antibiotics showed different zone of inhibition. Cefixime and Amoxicilline showed larger zone of inhibition, i.e. antimicrobial activity against caries bacteria, but Metronidazole showed the smaller zone of inhibition. A zone of inhibition in decreasing order- Cefixime > Amoxicilline > Metronidazole [Figure 6a], [Graph 6].

In case of commonly used mouth rinses, Chlorhexidine showed the larger zone of inhibition on BHI agar plates. Betadine showed smaller values, but surprisingly Listerine showed no zone of inhibition, i.e. no antimicrobial activity against caries bacteria. A zone of inhibition in decreasing order- Chlorhexidine > Betadine > Listerine [Graph 7].

Table 4: Zone of inhibition in mm against plaque bacteria with extracting solvents of *Juglans regia* bark

Solvents used in extracts	No of plaque samples	Minimum value	Maximum value	Mean	Std. Deviation
Distilled water	25	0	0	0.00	0.000
Ethanol	25	1	3	1.76	0.597

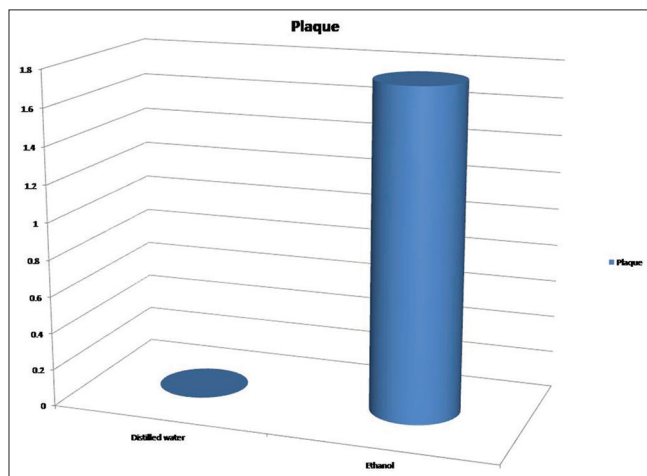
Table 5: ANOVA test

	Sum of squares	Df	Mean square	F	P
Between groups	12810.677	11	1164.607	250.918	0.000
Within groups	1336.720	288	4.641		
Total	14147.397	299			

Analysis of variance (ANOVA) was performed and shows significance values ($P < 0.05$) of the data

Table 6: Zone of inhibition in mm against caries bacteria with commonly used systemic antibiotics

Antibiotics	No of caries samples	Minimum value	Maximum value	Mean	Std. Deviation
Cefixime	25	10	28	17.44	5.083
Amoxicilline	25	6	16	11.20	3.416
Metronidazole	25	0	0	0.00	0.000



Graph 4: Zone of inhibition of extraction solvents against plaque bacteria.

Extracts of *Juglans regia* bark showed larger zone of inhibition, i.e. good antimicrobial activity. When compared to different extracting solvents, *Juglans regia* barks showed larger zone of inhibition when ethanol was used as an extraction solvent and smaller zone of inhibition when distilled water was used as an extraction solvent.

Himachal variety showed larger zone of inhibition in the ethanol extract as compared to Kashmir variety, but in distilled water extract Kashmir variety showed larger as compared to Himachal variety. A zone of inhibition in decreasing order- HE > KE > KD > HD [Figure 6b], [Graph 8].

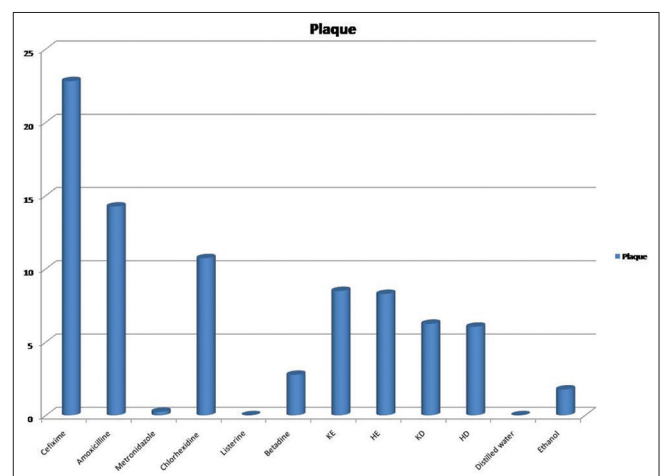
Two extracting solvents were used, ethanol and distilled water. Ethanol showed zone of inhibition in few plaque samples, but distilled water showed no zone of inhibition. [Figure 6c], [Graph 9].

Different comparisons were made to compare the value of the zone of inhibitions between the antimicrobial agents and *Juglans regia* extracts. [Graph 10 and Table 10]

Group 1 and Group 2- Comparisons of antimicrobial activity of antimicrobial agents and extracts of *Juglans regia* bark, between caries bacteria and plaque bacteria. [Graphs 11-15 and Tables 11-14].

DISCUSSION

Oral cavity offers diverse habitats, wherein different species of micro-organisms can prosper. These micro-organisms can cause the plaque and caries.^[13] A plaque has been implicated as the chief culprit in the etiopathogenesis of the dental caries and periodontal diseases.



Graph 5: Zone of inhibitions of antibiotics, mouth rinses, *Juglans regia* extracts and solvents used in extracts against plaque bacteria.

Dental caries is a bacterial disease process which produces organic acids as a by-product of their metabolism of fermentable carbohydrates.^[5] Different approaches are

Table 7: Zone of inhibition in mm against caries bacteria with commonly used mouth rinses

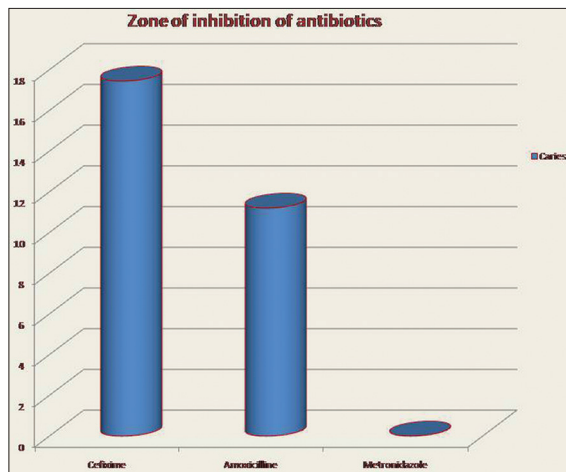
Mouth rinses	No of caries samples	Minimum value	Maximum value	Mean	Std. Deviation
Chlorhexidine	25	4	14	9.00	3.512
Listerine	25	0	0	0.00	0.000
Betadine	25	1	4	1.72	0.737

Table 8: Zone of inhibition in mm against caries bacteria with extracts of *Juglans Regia* bark from Kashmir and Himachal varieties

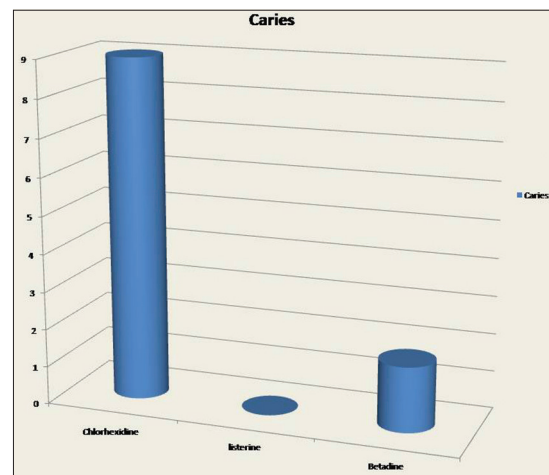
<i>Juglans regia</i> extracts	No of caries samples	Minimum value	Maximum value	Mean	Std. Deviation
KE	25	6	10	7.88	1.013
HE	25	5	12	7.96	2.131
KD	25	6	8	6.64	0.757
HD	25	4	8	6.36	1.551

tried to prevent the growth, adhesion and multiplication of these plaque and caries micro-organism. These approaches broadly include mechanical control (toothbrush, dental floss) and chemical control (mouthwashes, antibiotics).^[13-15]

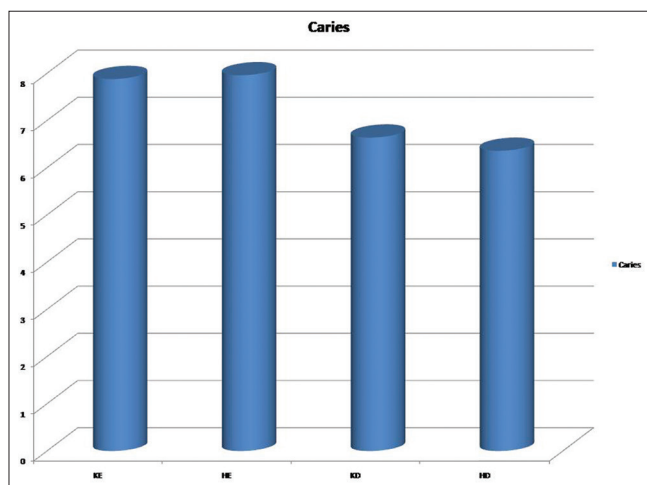
Chemical plaque control methods are extensively researched in the current era and new products are rising each day. But these products have their own advantages and disadvantages. The answer to these disadvantages is MOTHER NATURE. Natural products have been a major source of drugs for the centuries. In the few last decades, there has been an exponential growth in the field of herbal medicine. Plants produce chemical compounds as a part of their normal metabolic activities. Some of these metabolites are the source of curing health ailments. *Juglans regia* (Akhrot) is a medicinally important plant of family Juglandaceae. All parts of the plant have medicinal properties and are used in folk medicines since long. *Juglans regia* bark and leaves are commonly used to clean the teeth in many



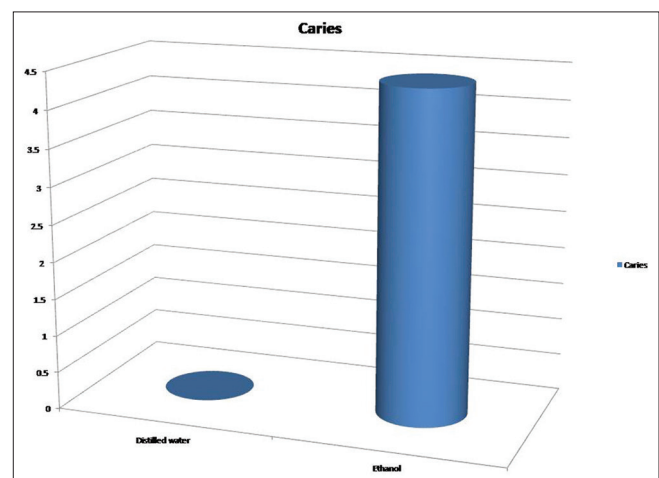
Graph 6: Zone of inhibition of systemic antibiotics against caries bacteria.



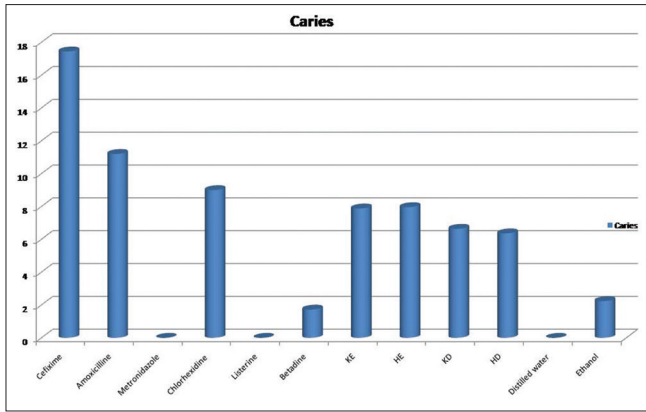
Graph 7: Zone of inhibition of commonly used mouth rinses caries bacteria



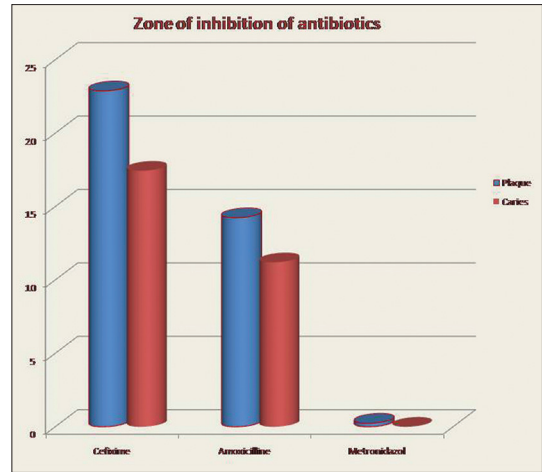
Graph 8: Zone of inhibition of *Juglans Regia* bark extracts against caries bacteria.



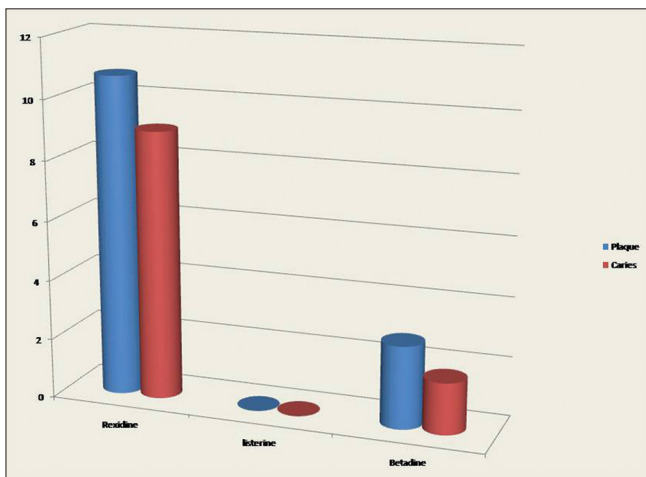
Graph 9: Zone of inhibition of extract solvents against caries bacteria.



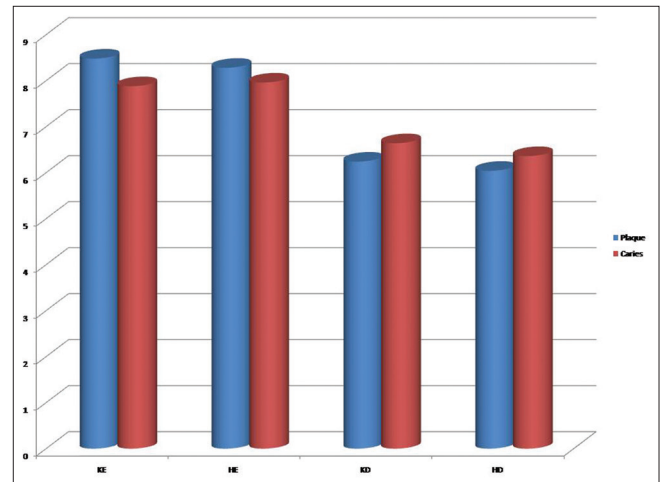
Graph 10: Zone of inhibitions of antibiotics, mouth rinses, *Juglans regia* extracts and solvents used in extracts against caries bacteria.



Graph 11: Zone of inhibition of systemic antibiotics against plaque and caries bacteria.



Graph 12: Zone of inhibition of commonly used mouth rinses against plaque and caries bacteria



Graph 13: Zone of inhibition of *Juglans regia* bark extracts against plaque and caries bacteria.

Table 9: Zone of inhibition in mm against caries bacteria with extracting solvents of *Juglans regia* bark

Solvents used in extracts	No of caries samples	Minimum value	Maximum value	Mean	Std. Deviation
Distilled water	25	0	0	0.00	0.000
Ethanol	25	1	4	2.24	1.012

Table 10: ANOVA test

	ANOVA				
	Sum of squares	Df	Mean square	F	P
Between groups	7877.050	11	716.095	143.331	0.000
Within groups	1438.880	288	4.996		
Total	9315.930	299			

Analysis of variance (ANOVA) was performed and showed significance values ($P < 0.05$) of the data

countries, in India, especially in the rural area of north India (Kashmir, Uttarakhand and Himachal Pradesh).^[9]

In a study done by Vishesh Upadhyay *et al.*^[12] on *Juglans regia* bark extract, all extracts show the antifungal activity. Pandita Nancy, *et al.*^[11] did the study on *Juglans regia* bark

extracts against the caries bacteria (*Streptococcus mutans*, *Streptococcus sobrinus*, *Actinomyces viscosus*). They found the antimicrobial activity and conclude that *Juglans regia* can be used for improving oral hygiene and can be included in the list of products for maintaining oral hygiene.^[11,12]

In the present study, we evaluate the antimicrobial activities of ethanolic and aqueous extracts of *Juglans regia* bark against plaque and caries colonizers and the comparison of its antimicrobial activity with commonly used oral rinses and systemic antibiotics.

Plant bark samples were collected from the Kashmir and Himachal Pradesh and authenticated by the National Institute of Pharmaceutical Education and Research S.A.S. Nagar (NIPER). These bark samples were then air-shade dried and grinded to form a powder.^[16] These dried, powdered plant samples were then stored in airtight container for further use.

Table 11: Comparisons of zone of inhibition between antimicrobial agents (systemic antibiotics, mouth rinses, *Juglans regia* extracts) and extracting solvents with extracts of Kashmir variety of *Juglans regia* bark extracts (ethanolic) against plaque and caries samples

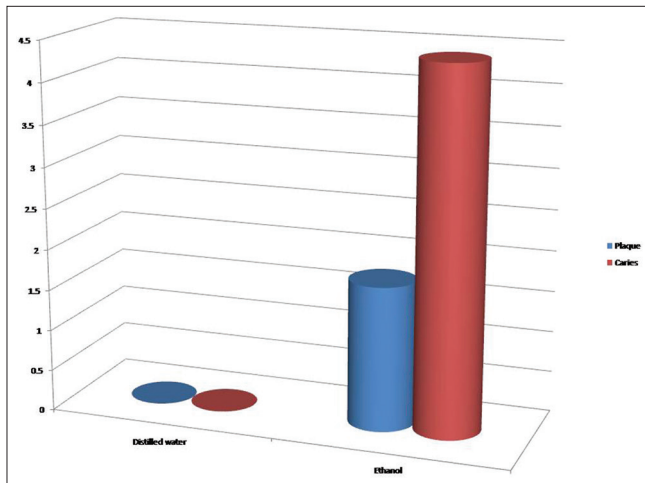
Antimicrobial agents		Plaque		Caries	
		Mean difference in plaque sample	P	Mean Difference in plaque sample	P
Kashmir ethanol (KE)	Cefixime	-14.400*	0.000	-9.560	0.000
	Amoxicilline	-5.760*	0.000	-3.320	0.000
	Metronidazole	8.240*	0.000	7.880	0.000
	Rexidine	-2.240*	0.015	-1.120	0.832
	Listerine	8.480*	0.000	7.880	0.000
	Betadine	5.720*	0.000	6.160	0.000
	Distilled water	8.480*	0.000	7.880	0.000
	Ethanol	6.720*	0.000	5.640	0.000
	HE	0.200	1.000	-0.080	1.000
	KD	2.240*	0.015	1.240	0.719
	HD	2.440*	0.004	1.520	0.407

*The mean difference is significant at the 0.05 level

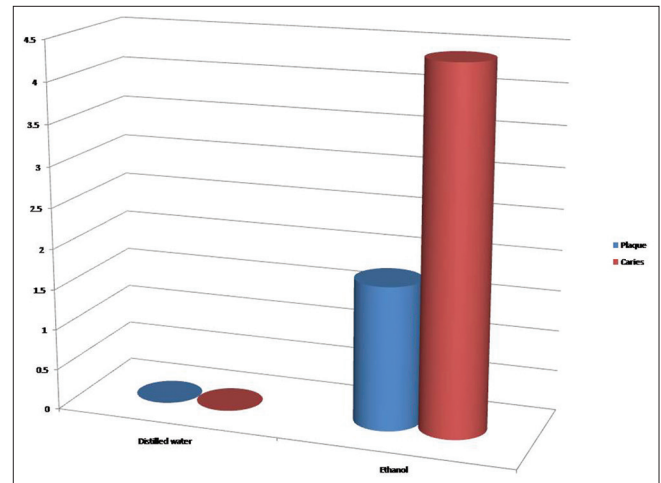
Table 12: Comparisons of zone of inhibition between antimicrobial agents (systemic antibiotics, mouth rinses, *Juglans regia* extracts) and extracting solvents with extracts of Himachal variety of *Juglans regia* bark extracts (ethanolic) against plaque and caries samples

Antimicrobial agents		Plaque		Caries	
		Mean difference in plaque sample	P	Mean difference in caries sample	P
Himachal ethanol (HE)	Cefixime	-14.600*	0.000	-9.480	0.000
	Amoxicilline	-5.960*	0.000	-3.240	0.000
	Metronidazole	8.040*	0.000	7.960	0.000
	Rexidine	-2.440*	0.004	-1.040	0.891
	Listerine	8.280*	0.000	7.960	0.000
	Betadine	5.520*	0.000	6.240	0.000
	Distilled water	8.280*	0.000	7.960	0.000
	Ethanol	6.520*	0.000	5.720	0.000
	KE	-0.200	1.000	0.080	1.000
	KD	2.040*	0.043	1.320	0.632
	HD	2.240*	0.015	1.600	0.325

*The mean difference is significant at the 0.05 level



Graph 14: Zone of inhibition of extract solvents against plaque and caries bacteria.



Graph 15: Zone of inhibitions of antibiotics, mouth rinses, *Juglans regia* extracts and solvents used in extracts against plaque and caries bacteria.

The broth was prepared from- Measured 37.0 gram of brain-heart infusion media in 100 ml of distilled water. This broth was used to culture the plaque and caries samples (HiMedia Laboratories Pvt. Ltd. Mumbai)

Plaque samples are direct samples of the bacteria colonizing teeth.^[17] The outcome of microbiological sampling depends on the used technique. The commonly used sampling devices were discussed by Tanner and Goodson (1986).

Table 13: Comparisons of zone of inhibition between antimicrobial agents (systemic antibiotics, mouth rinses, *Juglans regia* extracts) and extracting solvents with extracts of Kashmir variety of *Juglans regia* bark extracts (distilled water) against plaque and caries samples

	Antimicrobial agents	Plaque		Caries	
		Mean difference in plaque sample	Significance	Mean difference in caries sample	Significance
Kashmir distilled water (KD)	Cefixime	-16.640*	0.000	-10.800	0.000
	Amoxicilline	-8.000*	0.000	-4.560	0.000
	Metronidazole	6.000*	0.000	6.640	0.000
	Rexidine	-4.480*	0.000	-2.360	0.012
	Listerine	6.240*	0.000	6.640	0.000
	Betadine	3.480*	0.000	4.920	0.000
	Distilled water	6.240*	0.000	6.640	0.000
	Ethanol	4.480*	0.000	4.400	0.000
	KE	-2.240*	0.015	-1.240	0.719
	HE	-2.040*	0.043	-1.320	0.632
	HD	0.200	1.000	0.280	1.000

*The mean difference is significant at the 0.05 level

Table 14: Comparisons of zone of inhibition between antimicrobial agents (systemic antibiotics, mouth rinses, *Juglans regia* extracts) and extracting solvents with extracts of Himachal variety of *Juglans regia* bark extracts (distilled water) against plaque and caries samples

	Antimicrobial agents	Plaque		Caries	
		Mean Difference in plaque sample	P	Mean Difference in plaque sample	P
Himachal distilled water (HD)	Cefixime	-16.840*	0.000	-11.080	0.000
	Amoxicilline	-8.200*	0.000	-4.840	0.000
	Metronidazole	5.800*	0.000	6.360	0.000
	Rexidine	-4.680*	0.000	-2.640	0.002
	Listerine	6.040*	0.000	6.360	0.000
	Betadine	3.280*	0.000	4.640	0.000
	Distilled water	6.040*	0.000	6.360	0.000
	Ethanol	4.280*	0.000	4.120	0.000
	KE	-2.440*	0.004	-1.520	0.407
	HE	-2.240*	0.015	-1.600	0.325
	KD	-0.200	1.000	-0.280	1.000

*The mean difference is significant at the 0.05 level

They described the sampling tools as ‘dental-approved’ devices. Sampling that uses curettes, scalers, paper points, barbed broaches, etc., was reported. Loomer (2004)^[18] reported that paper point samples differ from curette samples and that curette collects plaque from the entire pocket whereas paper points collect plaque from the outer layer of the plaque. Nowadays, paper point and curettes are most commonly used for the collection of plaque samples.^[18] Hazem Mohammed^[19] did a study in comparison with curette and paper point sampling of plaque bacteria as analysed by real-time PCR and found that, curettes collect more amount of plaque.^[19]

In the present study, we use the curettes to collect the plaque samples. Both supra- and sub-gingival plaque samples were collected from the lower anterior teeth. Curettes were placed sub-gingivally and move toward the incisal edge of the tooth. These plaque samples were then cultured in the already prepared brain heart infusion broth in a test tube for 24 hours at 37^o c in an incubator.

Caries samples were collected from the carious tooth by sterile round carbide bur, and these samples were then

cultured in the BHI broth as described in the plaque sample. Antimicrobial activity was performed by using the agar well diffusion method.

According to our study, we found that all the extracts (ethanolic and distilled water) of *J. regia* bark (Kashmir and Himachal) showed zone of inhibitions, i.e. showed antimicrobial activity against plaque and caries bacteria as shown in chart-13.

In comparison, in case of plaque bacteria, the zone of inhibition with ethanolic extracts of *J. regia* bark (Kashmir and Himachal) was larger with mean values of 8.48 mm and 8.28 mm, respectively, and similarly distilled water extracts of *J. regia* bark (Kashmir and Himachal) showed slightly smaller zone of inhibitions with mean values 6.24 mm and 6.04 mm, respectively [Table 3].

Similar results were also found by Zakavi Farmariz *et al.*,^[16] and Vishwsh Upadhyay *et al.*,^[12] they did a study on different extracts of *Juglans regia* bark and found that, the ethanol and aqueous extracts of *Juglans regia* bark showed the antimicrobial activity.^[16,12]

Similarly, the zone of inhibitions against caries bacteria ethanolic extracts of *J. regia* bark (Kashmir and Himachal) showed larger zone of inhibitions with mean values of 7.88 mm and 7.96 mm, respectively, and similarly distilled water extracts of *J. regia* bark (Kashmir and Himachal) showed slightly lower zone of inhibitions with mean values 6.64 mm and 6.36 mm, respectively.

The zone of inhibitions of extraction solvents against plaque and caries bacteria we found that ethanolic extracts of both Himachal and Kashmir verity showed the larger zone of inhibition as compared to the distilled water extracts of both Himachal and Kashmir verity [Table 8].

This difference in antimicrobial activity of the ethanol extract and distilled water extract is due to the antimicrobial effect of ethanol itself. Ethanol shows antibacterial activity which is due to the fact that ethanol act by precipitating the bacterial protein and dehydration of bacterial protoplasm.^[20]

We found that antibiotics (Cefixime and Amoxicilline) showed the highest values of the zone of inhibitions with the mean value of 22.88 mm and 14.24 mm, respectively, and Metronidazole showed lower values with a mean value. 24 mm against plaque bacteria [Table 1].

Similarly, mean values of zone of inhibition by antibiotics (Cefixime and Amoxicilline) were 17.44 mm and 11.20 mm, respectively, and Metronidazole shows. 0 mm against caries bacteria [Table 6].

These values clearly indicate that Cefixime and Amoxicilline showed antimicrobial activity against plaque and caries bacteria, but Metronidazole does not show any antimicrobial activity against plaque and caries bacteria.

This difference of antimicrobial activity is due to the fact that Metronidazole is selectively toxic to anaerobic micro-organism,^[20] but most of the initial colonizer of plaque and caries are aerobic.^[15]

In case of mouth rinses, we found that Chlorhexidine and Betadine showed zone of inhibitions with the mean value of 10.72 mm and 2.76 mm, respectively, and Listerine showed no zone of inhibition with the mean value. 0 mm against plaque bacteria [Table 2].

Similarly, mean values of zone of inhibition by Chlorhexidine and Betadine were 9.0 mm and 1.72 mm, respectively, and Listerine shows. 0 mm against **caries** bacteria [Table 7].

These values clearly indicate that Chlorhexidine showed very good antimicrobial activity against plaque and caries

bacteria's and Betadine showed very less activity, but Listerine does not show any antimicrobial activity against plaque and caries bacteria.

Chlorhexidine inactivates micro-organisms with a broader spectrum than other antimicrobials (e.g. antibiotics) and have a quicker kill rate than other antimicrobials (e.g. povidone-iodine). It has both bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria) mechanisms of action, depending on its concentration. Chlorhexidine kills by disrupting the cell membrane.^[15]

Chlorhexidine is considered a safe and effective antimicrobial and antiseptic for the reduction of plaque and caries. The antimicrobial activity of 0.12% Chlorhexidine has been extensively investigated.^[15] Betadine shows antimicrobial activity due to its action by iodinating and oxidizing microbial protoplasm.^[20] The findings of the present study are consistent with the literature in case of Chlorhexidine and Betadine.

Listerine mouth rinse is a combination of essential oils (eucalyptol, menthol, thymol and methyl salicylate), which has been proven efficacious for the reduction of dental plaque. However, in this study Listerine did not exert inhibitory effects against plaque and caries bacteria.^[21]

In our study, we used two solvents ethanol and distilled water. Ethanol showed zone of inhibition but distilled water showed no zone of inhibition. Mean value of zone of inhibition of ethanol was 1.76 mm against plaque and 2.24 mm against caries [Tables 4,9].

When we compared the zone of inhibition of Juglans regia bark with systemic antibiotic, we found that systemic antibiotic Cefixime and Amoxicilline showed the larger zone of inhibition but Metronidazole showed no or smaller zone of inhibition as shown in chart-15. These systemic antibiotics have comparatively good antimicrobial activity against plaque and caries bacteria, but have well-known side effects such as diarrhoea, lose or frequent stools, abdominal or stomach pain, agitation, black, tarry stools, bleeding gums itch.^[20] We cannot use antibiotics on a daily basis for the oral hygiene due to their side effects and increased number of antibiotic-resistant bacteria. To overcome these disadvantages of systemic antibiotics against plaque and caries bacteria, natural product such as Juglans regia bark may be the best choice. Natural products have least side effects as compared to antibiotics.

Similarly, we compared the zone of inhibition of Juglans regia bark extracts with oral rinses and found that

Rexidine (Chlorhexidine) showed larger zone of inhibition and Betadine showed smaller zone of inhibition. Listerine showed no inhibition zone (chart-15). The data of this study clearly indicated that extracts of Juglans regia bark significantly inhibited the growth of the plaque and caries bacteria.

The side effects of Chlorhexidine include brown discoloration of the teeth, restorative materials and dorsum of the tongue. There is taste perturbation. There can be oral mucosal erosion, which is an idiosyncratic reaction and is dose-dependent. The bitter taste is difficult to mask.^[22] Side effects of Betadine include irritation of the lining of the mouth and throat and side effects within the body if excessive amounts of iodine are absorbed. So we can say that commonly used mouth rinses also have some side effects and limitation of uses. The answers to these disadvantages are natural products, i.e. Juglans regia.

CONCLUSION

The ordinary belief of the people in the area regarding the specific plants against the diseases is highly valuable. Plants can be used safely as compared to antibiotic because of their low cost and less side effects. The results of plant extracts against the bacteria confirmed the great potential of medicinal plants for the production of bioactive compounds which are useful in primary health care. We conclude that ethanolic extract of Juglans regia bark has the antibacterial effects against the plaque and caries bacteria with no side effects as compared to antibiotics and other mouth rinses. The natural products should be explored more for its antimicrobial activity and isolation of the active ingredient should be carried out to extensively study such products and its activity.

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Conflicts of interest

There are no conflicts of interest.

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