

Comparison of Antimicrobial Activities of Methanol Extracts of *Juglans regia* against *Staphylococcus aureus*, & *Streptococcus mutans* with Ciprofloxacin: *InVitro*

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Received 6/5/2012 – Accepted 20/6/2012

الخلاصة

يعتبر التسوس من بين مجموعة المشكلات السنية المزمنة والخطيرة ايضا. وهو مرض مايكروبي مؤدي الى تكلس انسجة الاسنان وان البلاك البكتيري يلعب دور اساسي في الامراضية. في هذا البحث تم مقارنة الفعالية البكتيرية لخلاصة قشور السيقان لنبات الجوزالميثانولية ضد اثنين من بكتريا الموجبة لصبغة كرام (العنقوديات الذهبية والمسبحيات الميوتنسية). اجري الاختبار لفعالية خلاصة النبات على البكتريا المعزولة من نماذج اللعاب لمرضى يعانون من تسوس الاسنان والتهاب اللثة وباستخدام طريقة التنافذ بالحفر ومقارنة منطقة التثبيط مع المضاد البكتيري سبروفلوكساسين.

تم في هذه الدراسة تقييم الفعالية البكتيرية لخلاصة قشور السيقان لنبات الجوزالميثانولية ضد اثنين من بكتريا الموجبة لصبغة كرام (العنقوديات الذهبية والمسبحيات الميوتنسية). وكانت منطقة التثبيط ٨،٧ ملم و ٧،٢ ملم للمسبحيات الميوتنسية والعنقوديات الذهبية على التوالي ، بينما كانت منطقة التثبيط للمضاد البكتيري سبروفلوكساسين ١٥،١ ملم و ١٥،٥٢ ملم و ١٥،١ ملم ضد كل من المسبحيات الميوتنسية والعنقوديات الذهبية على التوالي.

ABSTRACT

Among several dental problems caries process (cavitation) is deadly serious and chronic too. It is an irreversible microbial disease of calcified tissues of teeth. Bacterial plaque plays the primary role in the pathogenesis of the disease. This work compares the antibacterial activities of methanol extracts of stem bark of *Juglans regia* against two Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus mutans*). The efficacy of the plant extracts has been assessed by testing on salivary samples of patients suffering from dental carries and gingivitis by antimicrobial assay was carried out using well diffusion method and compared the zone of inhibition of the extracts with ciprofloxacin (antibacterial).

The present study, antibacterial activities (zone of inhibition in mm) of methanol extracts of bark stem of *Juglans regia* against *S. mutans*, *S. aureus* was evaluated. methanolic bark extract of *Juglans regia* was effective in inhibiting the two bacteria with zone of inhibition ,7.2mm and 8.7mm against *S. mutans*, *S. aureus* respectively while the inhibition zone of Ciprofloxacin 15mm and 15.52mm ,15.1mm against *S. mutans*, *S. aureus* respectively.

`Key words: *Juglans regia*, medicinal plant, antimicrobial activity, well diffusion

INTRODUCTION

The advancement in biological and engineering research is bringing a medical revolution to dentistry. Among several dental problems caries process (cavitation) is deadly serious and chronic too. It is an irreversible microbial disease of calcified tissues of teeth, characterized by demineralization of inorganic portion of teeth and destruction of organic substance of teeth (1). It was established that mutans group of Streptococci are the key agents causing dental caries (2).

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity, and the plant extracts were found to have potential against microorganisms (3, 4).

Over the past few decades, there has been much interest in natural products as sources of new antimicrobial agents. Different extracts from traditional medicinal plants have been tested (5). Many reports show the effectiveness of traditional herbs against microorganisms. As a result, plants have become one of the bases of modern medicine (6).

There is a continuous need of new antimicrobial components due to rapid emergence of multidrug-resistant pathogens and explosive dreadful infectious diseases (7). Plants are natural source of antibacterial agents. Plant-derived medicines have been a part of our traditional health care system, and the antimicrobial properties of plant derived compounds are well documented. Herbal medicines are more effective and less harmful as they have negligible side effects. They exhibit low mammalian toxicity and can be handled (6, 8). In Ayurvedic system of medicines *Juglans regia* is reported to have potent activity for dental complaints. The juice of the green husks, boiled with honey, is a good gargle for a sore mouth and inflamed throat. A piece of the green husks put into a hollow tooth, eases the pain. Decoction of the stem bark is useful in dental complaints (9). Antifungal, antibacterial and antioxidant activities of this plant have also been described by (10, 11). The health benefits of walnuts are usually attributed to their chemical composition. Walnuts are a good source of essential fatty acids and tocopherols (12).

In the present investigation, the methanolic stem bark extracts of *Juglans regia* were evaluated for antimicrobial activity against common human pathogen

MATERIALS AND METHODS

Preparation of methanol extract:

Twenty five grams of the material was soaked in 100 ml of methanol and allowed to stand for 24 hrs followed by boiling until the volume was reduced to one-third. The extracts were obtained by filtration and stored in a refrigerator at 4°C .

Inoculums preparation:

The tested bacteria firstly isolated from saliva patients suffering from dental carries and gingivitis on mitis salivarius + bacitracin medium and identification by API-20 Strep. system (bioMérieux, France) for *S. mutans*, and on Brain Heart Infusion agar for *S. aureus* (13). Seven colonies of the strains were inoculated to Brain Heart Infusion broth and

incubated at 37°C for 22–24 h. The turbidity was adjusted with sterile broth to correspond to the 0.5 McFarland standards.

Agar well diffusion method

Antibacterial activity of methanolic stem bark extracts were tested using agar well diffusion method (14). The microbial inoculum was standardized at 0.5 McFarland. 200µl of bacteria were aseptically introduced and spread using cotton swabs on surface of Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate. 50µl of the methanolic stem bark of *J. regia* were introduced into the wells in the plates. A negative control well was too made with 50µl of the extracting solvent (methanol). Ciprofloxacin was used as positive control. Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37°C for 24 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. Finally the diameter of the zone of inhibition were recorded and expressed in mm (15).

Statistical analysis: The results were calculated as mean diameter of zone of inhibition in mm \pm standard deviation (mean \pm SD). By ANOVA analysis.

RESULTS AND DISCUSSION

In the present study, antibacterial activities (zone of inhibition in mm) of methanol extracts of bark stem of *Juglans regia* were studied against two gram positive bacteria *S. mutans*, *S. aureus* was evaluated. methanolic bark extract of *Juglans regia* was effective in inhibiting the two bacteria with zone of inhibition ,7.2mm and 8.7mm against *S. mutans*, *S. aureus* respectively while the inhibition zone of Ciprofloxacin 15mm to 15.52mm against *S. mutans*, *S. aureus* respectively Table (1) and (Figure 1 &2).

Table-1: Inhibition zone (mm) of methanolic bark extract of *Juglans regia* against *S. mutans* compared with Ciprofloxacin.

Agent or inhibitor	The mean of inhibition zone mm averages \pm SD)		<i>Strept.</i>
	<i>mutans</i>	<i>Staph. aureus</i>	
<i>jugules reja</i> L.	7.2 \pm 2.1		8.7 \pm 2.7
Ciprofloxacin.	15.52 \pm 3.10		15.1 \pm 3

*=significant at 0.05

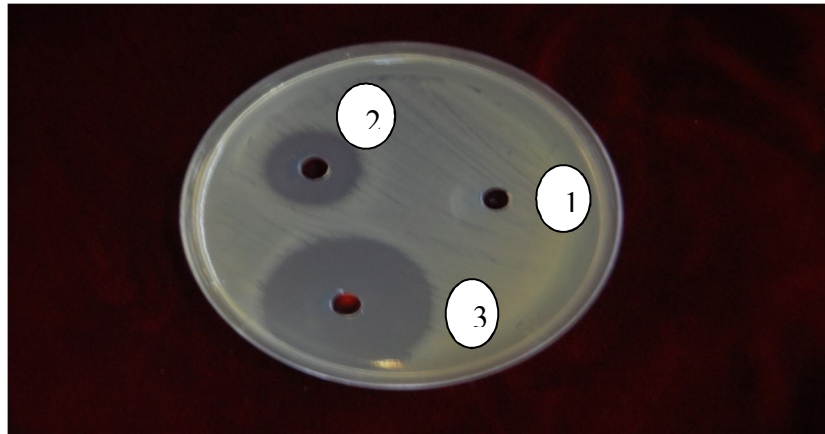


Figure-1: Inhibition zone (mm) of methanolic bark extract of *Juglans regia* against *S. mutans* compared with Ciprofloxacin.
1=negative control. 2= *Juglans regia*. 3= Ciprofloxacin.

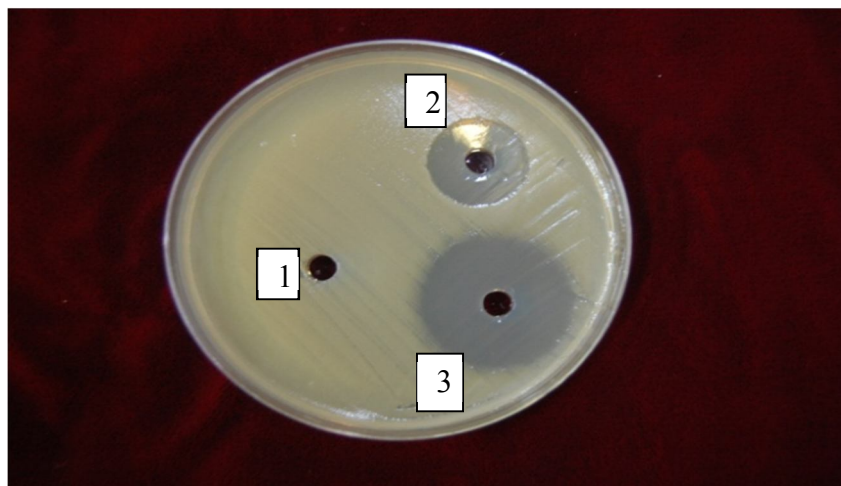


Figure-2: Inhibition zone (mm) of methanolic bark extract of *Juglans regia* against *S. aureus* compared with Ciprofloxacin.
1=negative control. 2= *Juglans regia*. 3= Ciprofloxacin.

Antimicrobial activities of various other parts of *Juglans regia* have already been studied with different microorganisms. (16) reported the growth inhibition effect of *Juglans regia* bark extract against gram positive (*S. aureus* and *S. mutans*), gram negative (*E. coli* and *P. aeruginosa*) and pathogenic yeast (*C. albicans*). (17) studied the antimicrobial activity of *Juglans regia* leaf extracts, in which they reported the zone of inhibition ranged from 15.8–17.6 mm against *P. acnes*, 11.3–15.7 mm against *S. aureus* and 12.9–15.5 mm against *S. epidermidis* by disc diffusion method. The chemical composition, antioxidant potential and antimicrobial activity were studied in the fruits of six walnuts (*Juglans regia*) cultivars Franquette, Lara, Marbot, Mayette, Mellanaise and Parisienne) produced in Portugal. Their

antimicrobial activities were checked against gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*), revealing activity against the different tested microorganisms(18,19). Antibacterial properties of the plant material may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids (20). It is reported that leaves from *J. regia* L. contain monoterpenes and sesquiterpenes, and the bark contains ketones like juglone, regiolone, sterol and flavonoid(21,22).

Methanol extract was found to be more effective of the extracts as anti-microbial against the oral microflora. This study has confirmed the antimicrobial potentials of the plant, thus supporting its folklore application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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