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Research Article

ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF *JUGLANS REGIA* L. AGAINST ORAL MICROFLORA

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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. There is a continuous need of new antimicrobial components due to rapid emergence of multidrug-resistant pathogens and explosive dreadful infectious diseases. 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 easily. In Ayurvedic system of medicines *Juglans regia* L. is reported to have potent activity for dental complaints.

Present study deals with evaluation of the effect of acetone and aqueous extracts of *J. regia* L., a traditional medicinal plant. The efficacy of the plant extracts has been assessed by testing on salivary samples of patients suffering from dental carries. Antimicrobial assay was carried out using disc diffusion method. Acetone extract was found to be more effective as anti-cariogenic medicine. Chlorhexidine was used as a standard.

Keywords: *Juglans regia* L., Dental caries, Disc diffusion method, Chlorhexidine

INTRODUCTION

It is well known and world-wide accepted that oral cavity is the mirror of total body health. 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. Giants of dental research W. D. Miller, G. V. Black put forward Chemicoparasitic theory (1889) stating pivotal role of oral microorganisms(bacteria) present in the mouth and their interaction with the retained food particles to produce substances capable of dissolving enamel.[1] It was established that mutans group of Streptococci are the key agents causing dental caries.[2] According to Dr. Keyes and Fitzgerald dental caries was an infectious process of tooth with interplay of plaque, tooth and diet[3]. A wide spectrum of antibacterial medicines is used to treat these infections. But these drugs can sometimes give rise to numerous adverse orofacial manifestations, particularly dry mouth, taste disturbances, oral mucosal ulceration, and/or gingival swelling[4]. There is a continuous need of new antimicrobial components due to rapid emergence of multidrug-resistant pathogens and explosive dreadful infectious diseases. Plants are natural source of effective antibacterial agents. Recent reviews indicate that there is a great potential to find compounds leading to the production of new antibiotics from plant source[5].

The vast biodiversity of Indian forests provides several plants, which are mentioned in Ayurveda for dental care. *Juglans regia* L., the royal species from family Juglandaceae, has been used in traditional medicines from ancient times. All parts of the plant: root, stem, bark, leaves, seeds, seed oil are medicinally important being depurative, anthelmintic, laxative, detergent, astringent and diuretic and exhibit antimicrobial activity to a greater extent[6]. Some extracts of the leaves show anticancer activity[7]. 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[8]. The species is also utilized in the treatment of tuberculosis and tuberculosis of cervical glands.[9]Antimicrobial activities of leaves, fruits, seeds and bark of the plant against gram+ve and gram-ve strains are reported in literature. It suggests that *J. regia* L. can definitely be the remedy for dental caries. The antibacterial properties of the plant material may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids[10]. It is reported that leaves from *J. regia* L. contain monoterpenes and sesquiterpenes, and the bark contains ketones like juglone, regiolone, sterol and flavonoid[11]. Hence the stem bark extracts of *J. regia* L.are tested for antimicrobial activity study against the microbes present in the saliva samples of patients suffering from dental caries.

MATERIALS AND METHODS

Collection and Identification of plant material

The plant material (Stem bark) of the species was collected from local market. Authentication was performed at Agharkar Research Institute, Pune, Maharashtra, India. Its voucher specimen No. is 14319.

Preparation of extracts

Air shade dried powdered bark material (10gm) was extracted using acetone and distilled water (50 ml) separately by soaking it for 24 hours at room temperature. The solvents were removed under reduced pressure to obtain crude extracts of acetone (JA, 14.4%) and water (JW, 18%).

Criteria for selection of patients

Patients in mixed dentition period in age group of 6–12 years are selected. They should have good general health with no history of antibiotic therapy and use of chemical anti plaque agents prior to six months of study initiation. They should have three or more than three teeth with caries.

Microbial flora

The saliva samples from the dental caries patients were collected using sterile cotton tipped swabs placed in the floor of the mouth. It was then placed in a sterile container with saline (2 ml) and was used to inoculate on the agar plates.

Anti-microbial assay

The paper disc diffusion method was employed. Samples of each acetone and aqueous extracts (30mg) were dissolved in respective

solvents (1ml). Sterile 5mm 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. Chlorhexidine was used as positive control.

RESULTS AND DISCUSSION

The results of the antimicrobial assay of the aqueous and acetone extracts of *J. regia* L. are presented in **Table No. 1** and **2**. Acetone extract had significant inhibitory effect on the growth of microorganisms. The length of incubation (72 hours) produced no significant effect on the degree of inhibition. The efficacy of acetone extract and aqueous extract on salivary flora is displayed in **Fig.1** and **2** Acetone extract exhibited zones of inhibition against all the tested samples; whereas aqueous extract is active for selected samples with comparatively smaller zones of inhibition. The influence of solvent for extraction on the inhibitory capacity of the extract on the test organism has been reported by Al-Bayati and Sulaiman [12].

A concentration of 250 µg /disc is found to inhibit the growth of most of the test samples of saliva.

The inhibition of salivary microbial flora in this study has confirmed the occupancy of the bioactive principle in the plant material. The study indicates that an active molecule must be present in the acetone as well as aqueous extracts of the plant material. Thin layer chromatographic technique proves that acetone extract is composed of altogether different components than aqueous extract. Acetone being semi polar solvent depicts semi polar components while water being polar makes an appearance of polar components.

Table 1: Effect of Aqueous extract on micro-organisms showing Average zones of inhibition (*mm)

Sr. no	Concentration	Avg. Zone of inhibition (mm)
1	150 µg	1.5
2	200 µg	1.6
3	250 µg	1.76
4	300 µg	1.3
5	control	2

***** Zones of inhibition are represented excluding the diameter of disc

Table 2: Effect of acetone extract on micro-organisms showing Average zones of inhibition (*mm)

Sr. no	Concentration	Avg. zone of inhibition (mm)
1	150 µg	1.3
2	200 µg	3.3
3	250 µg	4
4	300 µg	5.3
5	Control	6

***** Zones of inhibition are represented excluding the diameter of disc

Fig. 1: Aqueous extract

Fig. 2: Acetone extract

CONCLUSION

Acetone extract was found to be more effective of the extracts as anti-microbial against the oral micro flora. 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.

Emergence of multidrug-resistant strains, significant side effects of existing antibacterial drugs and their limited options are unsolved problems even today. The search for alternative, superior medicines leads us to the promising source of natural products. Plants contain chemical substances that take part in the metabolic activities thereby helping to fight the bacterial infections.

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