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The inhibiting effect of *Azadirachta indica* against dental pathogens

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ABSTRACT

The present study was carried out to evaluate the antimicrobial properties of neem extract against three bacterial strains causing dental caries using disc diffusion method. The pathogenic bacteria such as Streptococcus mutans, Streptococcus salivarious and Fusobacterium nucleatum were isolated from dental caries. The organic extracts of neem were prepared using different solvents such as petroleum ether, chloroform, ethanol and distilled water and were screened for its antimicrobial activity. Among the four extracts of neem, petroleum ether and chloroform extract showed strong antimicrobial activity against S. mutans with inhibition zone of 18 mm at 500 µg concentrations. Chloroform extract of neem showed strong activity against Streptococcus salivarius with inhibition zone of 18 mm. The third strain Fusobacterium nucleatum was highly sensitive to both ethanol and water extract of neems with inhibition zone of 16 mm. The results demonstrate that the chloroform extracts of neem has a strong antimicrobial activity and suggest that it can be useful in the treatment of dental caries.

Keywords: dental caries, phytochemicals, bioactive compounds, *Streptococcus* sp, Neem, disc diffusion.

INTRODUCTION

Dental caries is a multifactorial human disease that has widely affected many populations all over the world. Bacterial plaque plays the primary role in the pathogenesis of the disease. Dental plaque is a general term for the diverse microbial community (predominantly bacteria) found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque is an example of a biofilm; current researches are showing that the properties of bacteria associated with a surface in a biofilm can be markedly different than those of the same cells growing in liquid broth (planktonic cells). Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest threat of disease [1].

Oral health influences the general quality of life and poor oral health is linked to chronic condition and systemic diseases. The association between the oral disease and the oral micro biota is well established of more than 750 species of bacteria that inhabit the oral cavity [2].

Several agents are commercially available, these chemicals can alter oral micro biota and have undesirable side effect, such as vomiting, diarrhea, and tooth staining. Hence the search for unconventional product continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. However 80% of the world's population use plant as their primary source of medication [3] in view of the fact that antibiotics are sometimes associated with adverse side effects to the host including hypersensitivity, immunosuppressive and allergic reactions, it is of interest to develop alternative antimicrobial drugs such as medicinal plants for treatment of infectious diseases. The plant extract or phytochemicals that hinder the growth of

oral pathogens, diminish the progress of dental plaque, manipulate the adhesions of bacteria to surface and reduce the symptoms of the oral diseases. Clinical studies that have investigated the safety and worth of such plant dried medicines [4].

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been broadly used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally multifaceted. More than 140 compounds have been isolated from diverse parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been verified to exhibit immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties [5]. Wolinsky *et al*., [6] have reported that the active components from a bark containing neem stick have appeared to inhibit virulence factors of oral streptococci related with dental plaque formation. From other studies, it has been noted that neem can be regarded as a valuable plant source for the validation of its use in habitual medicine and for contemporary drug development.

Almas [7] compared the compare the effectiveness of antimicrobial activity of Neem and Arak chewing stick's aqueous extracts at various concentrations. Chewing twigs of the mango or neem tree is a widespread way of cleaning the teeth in the rustic and semi-urban residents. These twigs are also believed to own medicinal properties. Prashant *et al*., [8] conducted a study to evaluate the antimicrobial effects of these chewing sticks on the microorganisms *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis* which are involved in the development of dental caries. Vanka *et al*., [9] studied the antibacterial effect of Neem mouthwash against salivary levels of *Streptococcus mutans* and *Lactobacillus* has been experienced over a period of 2 months. Chetan *et al.,* [10] study has been conducted to uncover the phytochemicals present in the methanolic and aqueous extracts of leaves of *Epipremnum aureum* Linn. and to evaluate the antimicrobial effect of the extracts against clinical isolates of bacterial and fungal strains.

Conventional drugs usually provide effective antibiotics therapy for bacterial infections; however, there is an increasing problem of antibiotics resistance and undesirable side effects. Hence we need new solutions for such problems. So we evaluated the antibacterial activity of neem extracts against dental pathogens by disc diffusion method.

MATERIALS AND METHODS

Collection of clinical sample

Seven dental plaque samples were collected from the adult patients in Sri Mohambika hospital, Kulasekaram, Kanyakumari district.

Isolation and identification of dental pathogens

The dental plaque sample was inoculated on blood agar plates and incubated for 18-24 hours at 37^{o}C streak plate technique and the pathogens were isolated and identified by Bergey's manual [11].

Processing of neem

The neem leaf was collected, cleaned, shade dried and ground into powder using a blender. The resulting powder was then stored at room temperature in a clean, air-tight container.

Preparation of neem extract

Four solvents such as petroleum ether, chloroform, ethanol and distilled water were used to prepare the extracts. 50gm of neem leaf powder was added in separated sterile conical flask with 50ml of each solvents, the content was mixed well and kept in bioshaker for 24 hours. After 24 hours, the content in each flask was filtered using Whattman no.1filter paper and then concentrated by evaporation.

Preparation of disc

The discs were prepared by sterile filter paper dried in an oven to remove moisture. The extracts were applied on the dried filter paper disc by micropipette to obtain disc containing 100µg, 200µg, 300µg, 400µg and 500µgof extract concentration in each disc.

Antibacterial assays

Antibacterial activity of extracts was evaluated by disc diffusion method. A 100 µL of diluted bacterial suspension (5 x 10^6 cfu mL-1) of test bacterial strains was spread on the surface of Muller Hinton agar. Then sterile disc containing 100 µg, 200 µg, 300 µg, 400 µg and 500 µg of each extracts was placed onto the surface of agar plate. For negative control, discs were impregnated with respective solvent. Plates were incubated at 37^{o}C for 24 h and diameters of inhibition zones (mm) were determined.

RESULTS AND DISCUSSION

The dental pathogens for this study were isolated from the dental plaque samples were identified as *Streptococcus mutans, Streptococcus salivarius* and *Fusobacterium nucleatum.* The antibacterial activity of neem extracts against *Streptococcus mutans* was shown in table 1. Petroleum ether and chloroform extracts of *Azardirachta indica* showed higher activity (18mm) at 500µg concentration. Ethanol extract showed lowest activity (14mm) against this pathogen.

Table 1. Antibacterial activity of neem extract against *Streptococcus mutans*

Extract	Concentration of Neem extract (mm)				
	100	200	300	400	500
Petroleum ether	9.66±0.57	13.66±0.47	14.66±0.57	17.66±0.57	18.33±0.57
Chloroform	9.66±0.47	11.66±0.47	12.33±0.47	14.33±0.47	17.66±0.47
Ethanol	8.33±0.47	10.66±0.47	12.33±0.47	12.66±0.47	13.66±0.47
Distilled water	9.00±0.81	11.66±0.47	11.66±0.47	13.66±0.47	16.00±0.81

Table 2. Antibacterial activity of neem extract against *Streptococcus salivarius*

Extract	Concentration of Neem extract (mm)				
	100	200	300	400	500
Petroleum ether	8.33±0.47	11.66±0.47	12.66±0.47	15.66±0.47	16.33±0.47
Chloroform	11.66±0.47	13.66±0.47	14.33±0.47	15.66±0.47	18.66±0.47
Ethanol	8.33±0.47	8.66±0.47	10.33±0.47	13.00±0.81	15.66±0.47
Distilled water	10.33±0.47	10.66±0.47	11.66±0.47	13.66±0.47	15.66±0.47

Table 3. Antibacterial activity of neem extract against *Fusobacterium nucleatum*

Extract	Concentration of Neem extract (mm)				
	100	200	300	400	500
Petroleum ether	8.33±0.47	8.33±0.47	8.66±0.47	11.66±0.47	13.00±0.81
Chloroform	8.33±0.47	8.66±0.47	11.33±0.47	11.66±0.47	14.33±0.47
Ethanol	8.66±0.47	9.33±0.47	11.66±0.47	13.66±0.47	15.66±0.47
Distilled water	8.33±0.47	8.33±0.47	11.33±0.47	13.00±0.81	15.33±0.47

Vanka *et al*., [12] studied on acetone extract of *Azardirachta indica* showed maximum inhibitory activity against *Streptococcus mutans* with 22mm. Whereas the chloroform, ethanol and methanol extracts were with sensible inhibitory effect on all tested organisms. *Azadirachta indica* mouth wash is reported to inhibit growth of *S. mutans* and carious lesions.

The isolate *Streptococcus salivarius* was highly sensitive to the chloroform extracts (18mm) of *A. indica* and it gives comparable sensitivity pattern for all the other extracts (16mm) were given in table 2. Bhuaiyan *et al*., [13] made a study on antibacterial property of crude neem bark on *Streptococcus* and were found that neem was effective towards dental diseases.

The sensitivity pattern for the pathogen *Fusobacterium nucleatum* was depicted in table 3. It was sensitive to water extract as well as ethanol extract (16mm) of *Azardirachta indica* followed by the other two extracts such as petroleum ether and chloroform. Khan *et al* [14] evaluated the antimicrobial properties of *A. indica* leaves against *Micrococcus albus, Staphylococcus aureus, Proteus vulgaris and Pseudomonas aerogenosa* causing dental carries. The anti-microbial activities of petroleum ether extract, chloroform extract and methanol extract of neem leaves are

checked by disc diffusion method. All the leaf extracts exhibited significant inhibition. Comparative study of the results obtained from both the methods indicates that the chloroform Extract shows better antimicrobial activity against desired strains.

The methanol extract of neem leaf was tested for its antibacterial, antisecretory and antihemorrhagic activity against *Vibrio cholerae*. *A. indica* extract had significant antibacterial activity against the multi-drug-resistant *Vibrio cholerae* of serotypes O1, O139 and non-O1, non-O139 [15].

Other plant extracts such as *Prosopsis africana, T. chebula, T. glaucescens* etc were also inhibits the growth of the dental pathogens. The study about those plants were done by the scientists and already reported as, the inhibitory effects produced by the aqueous and ethanol extracts of *Prosopis Africana* on *Streptococcus mutans* was not significantly different (P>0.05) [16]. Some phenolic compounds isolated from *F.carica* exhibit anticaries activity either due to growth inhibition against mutans Streptococci or due to the inhibition of glucosyltransferases [17].

The acetonic extract of *T.chebula* was more potent against *S.mutans* compared to other tested extracts. The acetone and ethanol extracts of fruits of *T. chebula* showed greater antimicrobial activity than the corresponding water and methanolic extracts [18]. Both ethanol and aqueous extracts of *Terminalia glaucescens,* the tested chewing sticks had inhibitory effect on clinical isolate of *Streptococcus mutans*. The effect exhibited by ethanol extract was significantly higher than that produced by aqueous extract [19]. Musa *et al*., [20] studied the antibacterial activity of various plants from Igaland against bacteria including dental pathogens. The extracts of *Ficus racemosa* showed no activity against *Streptococcus* sp [21] whereas our plant extract showed better activity against *Streptococcus* sp. Kwasi *et al.,* [22] observed no sign of toxicity was detected during the experimental period. Rabbits treated with various doses of the crude extracts of neem leaves for 14 days had progressive weight gain.

CONCLUSION

Plants contain phytochemicals such as alkaloids, tannins, essential oils and flavanoids which have pronounced antimicrobial activity. This underlies the use since antiquity of herbs to improve oral hygiene and prevent tooth decay, gum disease and periodontitis. The neemstick is an under estimated tool for dental hygiene which is only beginning to be explored in controlled clinical studies. From this study the village dispensary, Neem can play a key role in the future of dental hygiene.

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