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ANTIBACTERIAL EFFICACY OF CLOVE (*SYZYGIUM AROMATICUM*), HARAD *(TERMINALIA CHEBULA*) AND CHEWING STICKS AGAINST CARIOGENIC BACTERIA

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ABSTRACT

The increasing incidences of bacterial infection and gradual rise in resistance in bacteria against the available antibiotics, has high-lightened the need to find more alternative antibacterial agents from other sources. So, this study was planned to check the efficiency of aqueous and methanolic extracts of clove (*Syzygium aromaticum)*, harad *(Terminalia chebula)* and chewing sticks of neem *(Azadirachta indica),* kikar (*Acacia arabica*) and tahli (*Dalbergia sissoo*) on dental caries causing bacteria viz., *Streptococcus sp., Staphylococcus sp.* and *Lactobacillus sp*. These bacteria were isolated from 90 dental caries patients (54 males and 36 females, age group of 6-45 years) and identified with the help of standard morphological and biochemical tests. *Streptococcus* sp. was found to be most prevalent bacteria in dental caries patients followed by *Staphylococcus* sp. and *Lactobacillus sp.* Percentage of *Streptococcus* sp. and *Lactobacillus* sp. was more in males than females but the case was reverse for the *Staphylococcus* sp. Results showed that males were more prone to dental caries than females. Antimicrobial activity and minimum inhibitory concentration (MIC) of extracts was checked against the identified microorganisms by disc diffusion method. The methanolic extract of all the plants was found to more effective against the test bacteria as compared to aqueous extracts and minimum inhibitory concentration of methanolic extracts of all the plants was lower than the aqueous extracts. *T. chebula* methanolic extract was found to be most potent against the dental caries causing bacteria and extracts of *D. sissoo* was found least effective against the test bacteria.

Keywords: Dental Caries, Antimicrobial Activity, Clove Extract, Disc Diffusion Method

INTRODUCTION

Despite low mortality rate associated with dental diseases they have a considerable effect on self esteem, eating ability, nutrition and health both in childhood and older age. It remains one of the most widespread diseases of mankind. Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases. Dental caries is a pandemic, chronic, multifactorial, irreversible microbial disease of the calcified tissues of the teeth which affects individuals of almost all ages. Many factors such as tooth enamel or dentin, bacteria, diet, oral hygiene, salivary flow and time are responsible for dental caries **[1]**. Mainly three factors carbohydrate substrate, acid and oral microorganisms, destroys enamel and dentin which leads to dental cavity. Dental caries are caused by acidic metabolites of oral bacteria such *as Streptococci, Lactobacilli, Staphylococci, Actinomyces* and *Corynebacteria.* The saliva is unable to penetrate the thick build up of plaque and is therefore unable to neutralize the acid produced by the bacteria which results in tooth decay. Early cultures recognized the value of using spices and herbs for the treatment of various diseases. According to World Health Organization (WHO), about 80% of world population rely upon plant based traditional medicine for their primary healthcare need **[2]**. The demand of plant based therapeutics is increasing in both developed and developing countries due to the growing recognition that they are natural products, being non narcotic, having no side effects, easily available at affordable prices and sometimes the only source of healthcare available to the poor **[3]**. Despite several anticaries agents being available commercially, the search for an effective agent still continues. Natural products have shown to be a good alternative to synthetic chemical substances for caries prevention. It has been well documented that medicinal plants confer antimicrobial activity towards oral bacteria **[4]**. Clove (*Syzygium aromaticum***)** is oldest spices in the world and used from the ancient time as antimicrobial agent. *Terminalia chebula* (harad) has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine and always listed at the top of the list in Ayurvedic Materia Medica due to its extraordinary power of healing **[5]**. It is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. The literature survey of the folklore medicine reveals the use of dried fruit of *T. chebula* as

an anticaries agent **[6]**. One of the common traditional practices followed is use of herbal 'chewing sticks' instead of plastic bristle brushes to maintain oral health and hygiene. Chewing sticks may aid remineralization. If demineralization continues over time, enough mineral content may be lost so that the soft organic material left behind disintegrates, forming a cavity or hole. The best known examples of traditional chewing sticks used are neem (*Azadirachta indica*), kikar (*Acacia. arabica*), miswak *(Salvadora Persica*) and tahli (*Dalbergia sissoo*), the end of which is shredded and then used to massage the gums and clean the teeth. Various studies have shown that rural folk in different parts of India use stem, leaves and fibers of some plants for cleaning teeth, preventing and treating dental caries, gingival and periodontal diseases and other oral mucosal diseases **[7]**.

Recent natural remedies with the use of medicinal plants, which are good reservoirs of chemo-therapeutants can be, contributed as an alternative for antibiotic effects such as hypersensitivity reaction, supra infections, and teeth stainings. In the present clinical scenario globally, there is a great interest in the use of antimicrobial agents for prevention and treatment of dental diseases due to spread of antibiotic resistance **[8]**.

MATERIALS AND METHODS

90 root canal sample were collected from 54 male and 36 female patients (Age 6-45 years), from Dental Clinics and stored in eppendorf tubes containing nutrient broth for the isolation of *Streptococcus* sp., *Staphylococcus* sp. and *Lactobacillus* sp. because they have been implicated in dental caries **[9]**. Samples were cultured on nutrient agar, then sub-cultured on three different media i.e. nutrient agar, lactobacillus MRS agar & brain heart infusion agar and growth was observed after 24 hours. Bacterial isolates were identified using various morphological and biochemical tests such as Gram's staining, motility test, catalase test, nitrate reduction test and carbohydrate fermentation test following standard methods.

Preparation of Extracts

Cloves, harad fruits and chewing sticks (neem, kikar & tahli) were purchased from the local market. Extracts were prepared by washing cloves, harad fruits, chewing sticks carefully with distilled water and surface sterilized with mercuric chloride and again washed with distilled water 3-4 times. Cloves and harad fruits were then air dried at room temperature for some days and pulverized a fine powder using a sterilized mixer grinder and stored in airtight bottles. Chewing sticks were chopped into small pieces and air dried in an oven at 50°C.

These were then pounded with local pestle and mortar into fine powder. Aqueous and methanolic extracts were prepared by dissolving powders in distilled water and methanol (10gm/100ml), then incubated in shaker incubator for 48 hours at 37^0C and extracted using Whatman filter paper no.1. The extracts were then concentrated by evaporation at 40^0C and stored in air tight bottles **[10]**.

Antibacterial Activity: Disc Diffusion Assay

The antibacterial activity of extracts of clove, harad and chewing sticks was determined by disc diffusion method **[11]**. Petri plates were prepared with 20mL of sterile Mueller-Hinton agar (MHA) (Himedia). The test cultures (100μL of suspension containing 108 CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10minutes. The sterile 6mm disc (Himedia) impregnated with extracts (20 µl of 100 mg/ml extract) were placed on the surface of the medium and left for 30minutes at room temperature for compound diffusion. Negative control was prepared using respective solvent and penicillin (10 µg) was used as positive control. The plates were incubated for 24 hours at 37°C. The antibacterial activity was assessed by measuring the inhibition zone (millimetres).

Determination of Minimum Inhibitory Concentration

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 hours. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution in the Muller-Hinton broth to obtain concentrations from 100 mg/ml to 20 mg/ml. Standard antibiotic penicillin and respective solvent were placed as controls. A 10 µl of 10^7 (CFU) bacterial cultures were added to the tubes and were incubated at 37° C for 18 hours. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration **[12]**.

RESULTS AND DISCUSSION

The prevalence of dental caries was found to be more in males than females and maximum cases were present in age group 16-25 years as maximum samples (36 out of 90) were obtained from this age group followed by age group 26-35 years (32samples), age group 36-45 years (13samples) and age group 6-15 years (9samples) **(Table1)**.

Isolation and Identification of Dental Caries Causing Bacteria

After sub-culturing of 90 root canal samples on three different media, the bacterial isolates were further identified. Colony morphology, light microscopic properties and biochemical tests were taken into consideration in the identification of the isolated pathogens. *Streptococcus* sp. was found to be present in 70% of the total collected samples (90), *Staphylococcus* sp. in 65% samples and *Lactobacillus* sp. in 55% of the total samples collected **(Figure1)**. Prevalence of *Streptococcus* sp. and *Lactobacillus* sp. was higher in the male patients than female patients but the case was reverse for S*taphylococcus* sp. **(Figure 2)**. Prevalence of *Lactobacillus* sp. (55%) was found to be similar to previous study where it was 54% **[13]**. Percentage of isolated bacteria was found to be higher in this study than earlier study of Daniyan and Abalaka **[14]** and Hassan-Olajokun *et al.*, **[15]** but the percentage of *Streptococcus* sp. in the present study was found to be lower than previous study conducted by Schaeken *et al*., **[16]**.

Antibacterial Activity of Extracts

Results of antibacterial activity revealed that all the extracts of clove, harad, neem, kikar and tahli showed antibacterial activity against dental caries causing bacteria i.e *Streptococcuss* sp., *Staphylococcus* sp. and *Lactobacillus* sp. Aqueous extracts of all the tested plants exhibited lower antimicrobial activity as compared to methanolic extracts. Highest mean diameter of inhibition zones was produced by the methanolic and aqueous extracts of *T. chibula* against *Staphylococcus* sp. (23.46mm & 22.96mm respectively), *Streptococcus* sp. (22.32mm & 22.96mm respectively) and *Lactobacillus* sp. (21.82mm & 19.76mm). Lowest mean diameter of inhibition zones was produced by the methanolic and aqueous extracts of *D. sissoo* against *Streptococcus* sp. (10.96mm & 7.98mm respectively), *Staphylococcus* sp. (10.46mm & 8.52mm respectively) and *Lactobacillus* sp. (12.78mm & 9.46mm respectively) **(Figure 3 & 4)**. The positive bacterial control penicillin showed an antibacterial inhibitory zone of 27.32mm against *Streptococcus* sp., 34.66mm against *Staphylococcus* sp. and 25.65mm against *Lactobacillus* sp. Water and methanol (negative control) produced no observable zone against any of the tested microorganism. This study reveals that both the aqueous and methanolic extracts of tested plants have broad spectrum antibacterial activity against the dental caries pathogens as visualized by the formation of inhibition zones of bacteria thus having a great potential to be

developed as a herbal preparations to control dental caries.

Minimum Inhibitory Concentrations

Minimum inhibitory concentration of methanolic extracts of all the plants was lower as compared to aqueous extracts. Among the plant extracts tested, *T. chebula* methanolic extract was found to be most potent against the dental caries causing bacteria with MIC 20mg/ml for *Streptococcus* sp., 10mg/ml for *Staphylococcus* sp. and 15mg/ml for *Lactobacillus* sp. Extracts of *D. sissoo* was found least effective against the test bacteria **(Table 2)**.

The present results of antibacterial activity and MIC value of *T. chebula* against *Streptococcus* sp. were found to be in accordance with previous study conducted by Aneja and Joshi **[17]** where the antimicrobial activity and MIC values against *Streptococcus* sp. were 20mm and 25mg/ml respectively. The methanolic as well as aqueous extracts of *T. chebula* showed potential antibacterial activity **[18]**. The zones of inhibition of *Staphylococcus* (22.65mm), *Streptococcus* (21.32mm) and *Lactobacillus* sps. (29.97mm) with clove were quite high from the present study in earlier study that may be due to ethanol used for extraction instead of solvents used in the present study. Method of the clove extract preparation may also responsible for this variation **[5]**. *Staphylococcus aureus* was found more susceptible to ethanol and aqueous extracts among the tested organisms in an earlier study **[19]**. The chewing sticks used in India are usually from *Azadirachta indica* (Neem) and neem extracts show antimicrobial effects against *Streptococcus mutans* **[20]**. Use of chewing sticks reduced both the plaque index and bacterial count. *Acacia* is also used as an active constituent in toothpastes in India. The components of its bark and gum, mainly tannins, show antimicrobial and astringent effects. Plant tannins have the ability to reduce the attachment of *S. mutans* by binding to proline rich protein of the salivary pellicle or to the cell-surface lipoteichoic acid **[21]**. According to present study preparing an extract with organic solvent was shown to provide a better antibacterial activity, in accordance with the result obtained by Nair *et al*., **[22]**. The limited spectrum of antibacterial activity in the aqueous extracts may be due to polarity of antibacterial compounds make them more readily extracted by organic solvents as compared to aqueous extract; active compound may be present in insufficient amount in the aqueous extract to show activity with the dose level employed; and if the active principle is present in high quantities, there could be other constituents present in the extract exerting antagonistic effects of the bioactive compounds **[23]**.

Table 1: Distribution of Root Canal Samples According to Age Group and Sex

Age Group (Years)	Total No. of Sample	Male	Female
6-15	09	07	02
16-25	36	22	14
26-35	32	24	08
36-45	13	05	08

Table 2: Minimum Inhibitory Concentration of the Extracts (mg/ml)

Plant	Extract	Bacterial Organisms		
		Streptococcus sp.	*Staphylococcus* sp.	*Lactobacillus* sp.
Syzygium aromaticum	Aqueous Methanolic	35 30	30 20	25 20
Terminalia chebula	Aqueous Methanolic	25 20	20 10	20 15
Azadirachta Indica	Aqueous Methanolic	20 15	15 15	20 10
Acacia Arabica	Aqueous Methanolic	30 25	20 15	20 10
Dalbergia sissoo	Aqueous Methanolic	50 40	40 35	40 40

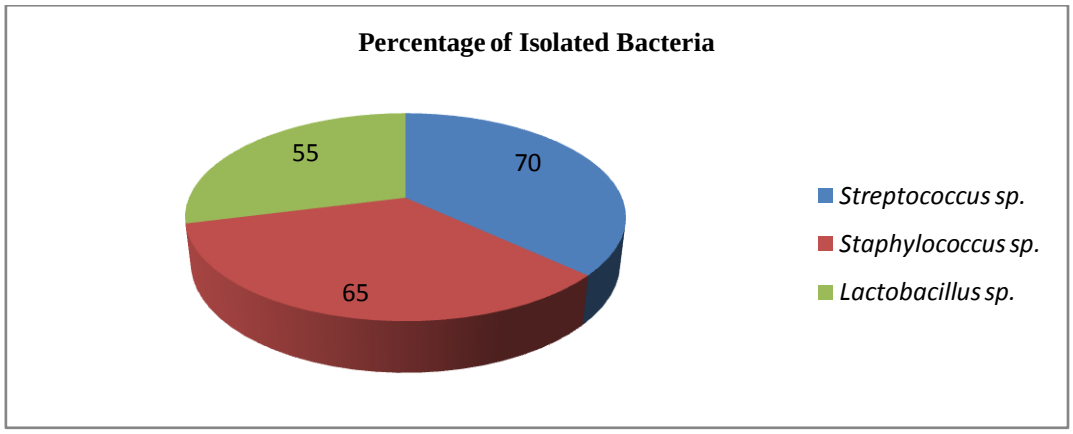

Figure 1: Percentage of Isolated Bacteria

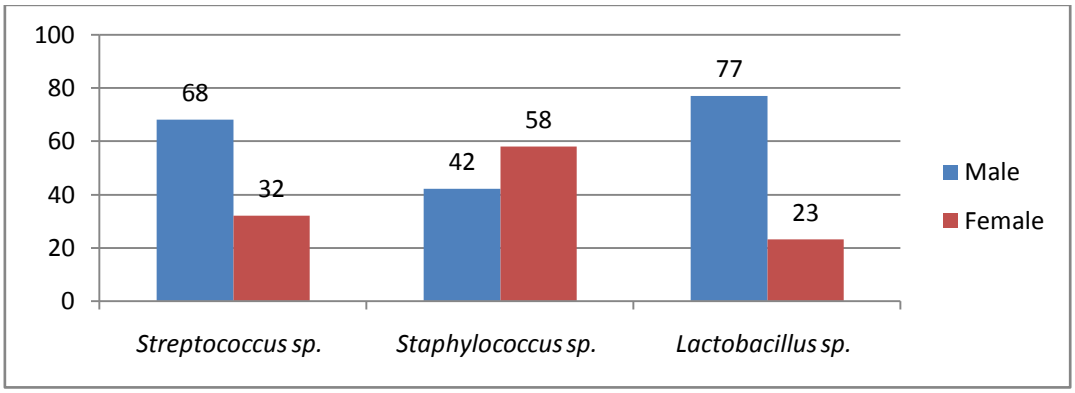

Figure 2: Percentage Distribution of Isolated Bacteria in Male and Female Patients

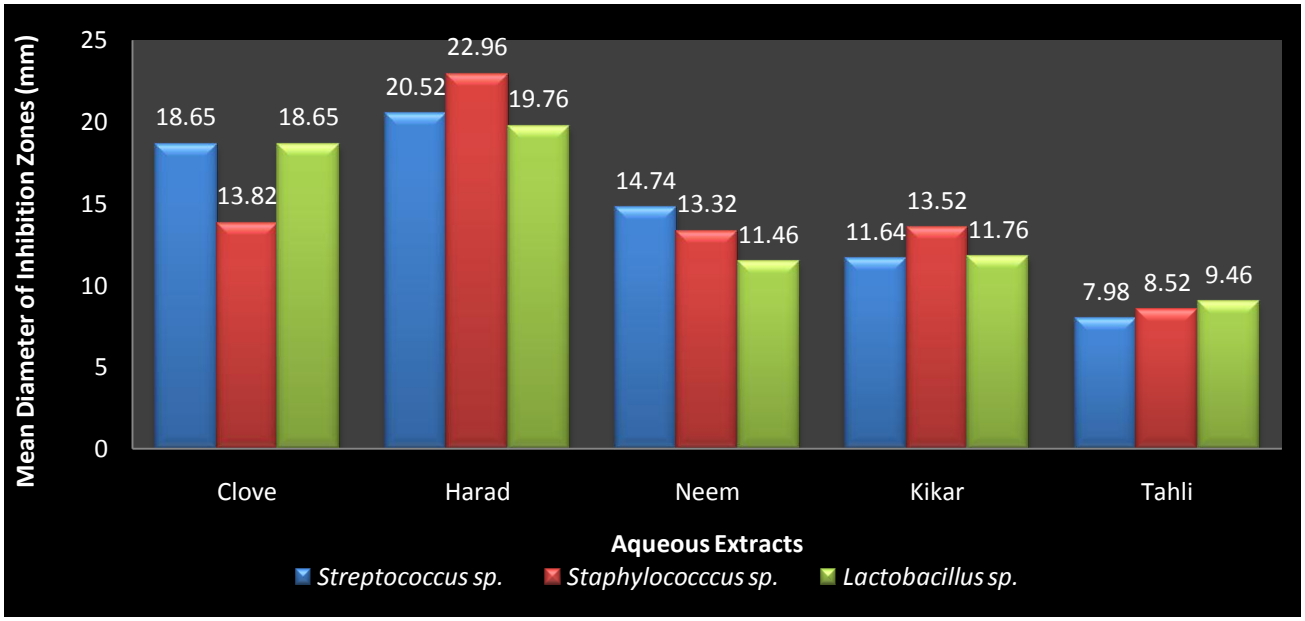

Figure 3: Comparative Antibacterial Activity of Aqueous Extracts

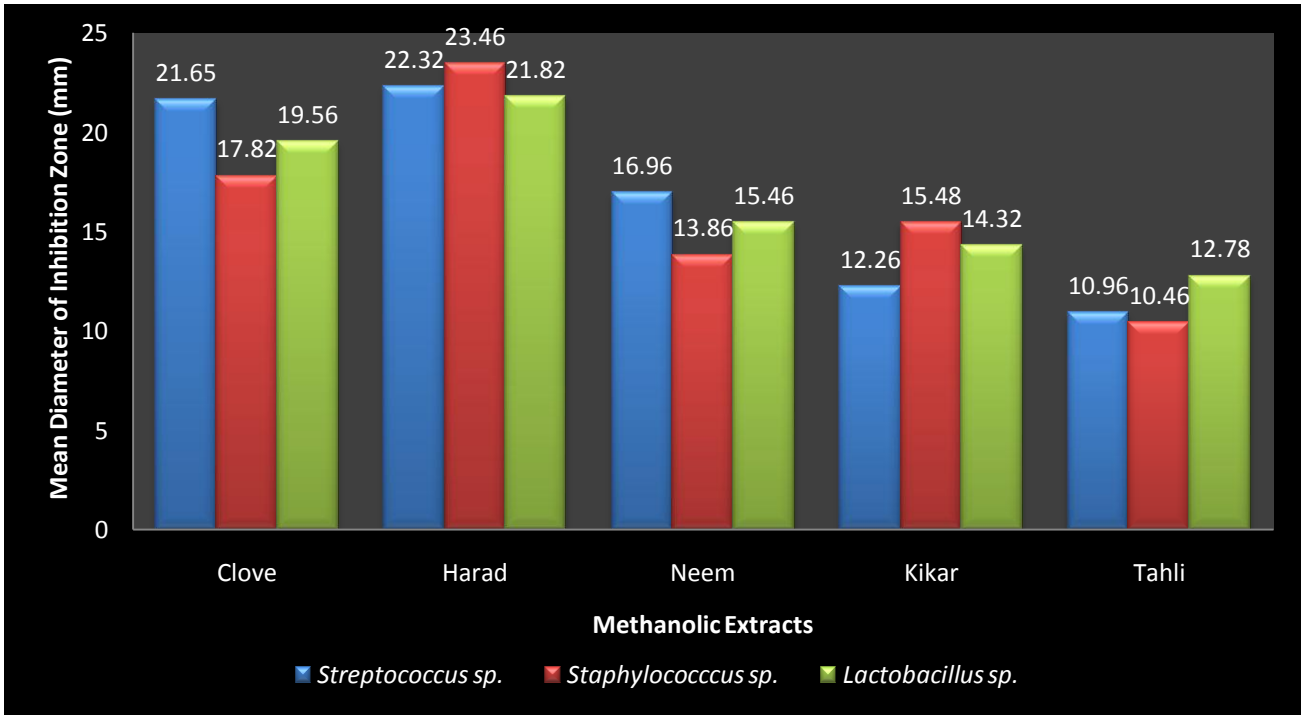

Figure 4: Comparative Antibacterial Activity of Methanolic Extracts

CONCLUSION

Results of this study reveal that dental caries were more prevalent in males than females and maximum patients were from age group 16-25 years. Tested extracts of all the plants exhibited growth inhibitory activity against dental caries causing bacterial strains evaluated. It is also observed that the methanolic extracts were more potent against *Streptococcus* sp., *Staphylococcus* sp. and *Lactobacillus* sp. than the aqueous extracts. Screening of various natural organic compounds and identification of active agents must be considered as a fruitful approach in the search of new herbal remedies for dental caries. These plant

extracts can be used in the toothpastes or toothpowders in order to prevent and control the dental diseases and can also be used to discover natural products that will lead to the development of new pharmaceuticals. Further study is needed for determination of active antimicrobial constituents and structural relationship which can help in the synthesis of effective and safe drugs; using spectroscopic techniques such as Nuclear magnetic resonance NMR, infra red spectrophotometry IR, mass spectrometery, UV and elemental analysis.

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