



REVIEW OF RESEARCH

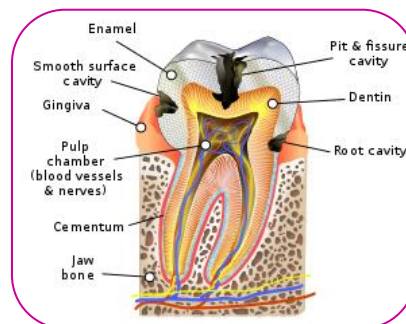
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ISOLATION OF DRUG RESISTANT BACTERIA FROM DENTAL CARRIES AND STUDY OF EFFECT OF DIFFERENT PLANT EXTRACT ON IT

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ABSTRACT:-

Dental caries is considered as a major public health problem globally due to its high prevalence & significant social impact. The WHO reports 60-90% of school children worldwide are affected by dental caries. *Streptococcus mutans* is alpha haemolytic, Gram positive, facultative anaerobic spherical shaped bacterium. It is a part of normal flora of oral cavity. It can produce acid from sugar in saliva which promotes erosion of tooth enamel. It is most common cause of dental caries. It has a polysaccharide coat (glycocalyx) that allows it to stick on teeth and also damage heart valves. In recent years, drug resistance among human pathogenic bacteria has been commonly reported throughout the world. Bacteria become resistant to antibiotic by different ways. The resistance of numerous bacterial pathogens to many antibiotics continues to increase globally. So there is a continuous demand of new drugs. In the present study antibacterial activity of *Allium sativum*, *Cinnamon zeyanicum*, *Oscimum sanctum* & *Syzyium aromaticum* was studied against MDR *Streptococcus mutans* isolated from dental caries. Antibiotic susceptibility test of the clinical isolates was done by using modified Kirby-Bauer disc diffusion method in accordance with the guidelines of the clinical & laboratory standards institute. Interpretation of resistance was based on the NCCLS criteria. The antibacterial activity of aqueous plant extract was tested at different concentrations by agar well diffusion method. The most common pattern of multiple drug resistance of isolates of *S. mutans* observed was ampicillin-ciprofloxacin-amoxicillin-rifampicin-vancomycin. It was found that *A. sativum* showed highest antibacterial activity against *S. mutans* followed by *C. zeyanicum*, *S. aromaticum* & *O. sanctum*. It was also observed that antibacterial activity increases with the increase in concentration of plant extract.

KEY WORDS: Antibacterial activity, Dental caries, Disc diffusion method, Multi- drug resistant, Plant extracts, *Streptococcus mutans*.

INTRODUCTION:

Streptococcus mutans is a Gram-positive, facultative anaerobic spherical bacterium. It can metabolize carbohydrates and are considered to be the principle etiological agent of dental caries. It has a polysaccharide coat (glycocalyx) that allows it to stick on teeth and also damage heart valves [1]. Dental caries is an infectious disease which results in localized dissolution and destruction of calcified tissues of the crown and root surfaces of the tooth. It is considered as a major public health problem globally due to its high prevalence and significant social impact. The World Health Organization reports 60-90% of school children worldwide have experienced caries, with the disease being the most prevalent in Asian and Latin

American countries [2]. Laboratory investigations demonstrated the ability of the species to produce the lactic acid which causes dental caries. *Streptococcus mutans* present in mouth is more prevalent in dental caries subjects than *Streptococcus sobrinus* [3] and has been demonstrated to be a causative specialist for dental caries because of its capacity to fermentable sugar into organic acids. These acids can cause a fall in pH, which can lead to an increase of enamel solubility that is dental caries. [4, 5, 6]. The indiscriminate use of antibiotics has led to an increase in antibiotic resistance among microorganisms [7, 8]. Bacteria become resistant to antibiotic by different ways. R-plasmid often contains genes for resistance to several antibiotics [9]. Plasmid can be transferred between bacterial cells in a population and between different but closely related bacterial populations [8].

The application of plants as medicine perhaps dates back to prehistoric period. The application of plants therefore is as old as 4000 to 5000 B.C. In India earliest references of curative properties of plants appear in Rig-Veda which is said to be written between 3500 to 1600 B. C. The rural population in different parts of the world is more disposed to traditional way of treatment [10, 11]. It is estimated that about 80 % of the rural population in developing Asian nation depend on home care and traditional medicine for major therapies [12]. The problem of drug resistance could overcome by herbal drugs. Due to this reason now a day the demand of herbal products as therapeutic agents is increasing all over the world. Keeping this view in mind in the present study an attempt was made to isolate MDR *Streptococcus mutans* from dental caries & to study effect of plant extract on it.

MATERIALS AND METHODS

Isolation of MDR *Streptococcus mutans* from dental caries

Streptococcus mutans was isolated from dental caries by using specific media. MSA, MSBA & Blood agar media were used for its isolation. Isolates of *Streptococcus mutans* were identified by using different morphological, cultural & biochemical characters. Antibiotic susceptibility testing was carried out by Kirby-Bauer's disk diffusion method [13] for drug susceptibility according to National Committee for Clinical Laboratory standards (NCCLS) [14]. The Muller Hinton agar plates were smeared evenly using with isolates of *Streptococcus mutans*. This was then impregnated with antibiotic discs using sterile forceps & then gently pressed down onto the agar. Plates were kept at low temperature and then incubated at 37 °C for 24-48 hrs. Antibiotics used in this study were Amoxycillin (30mcg), Ampicillin (10mcg), Ciprofloxacin (5mcg), Co-trimoxazole (1.25mcg), Erythromycin (15mcg), Gentamycin(10mcg), Methicillin (5mcg), Nalidixic acid (30mcg), Rifampicin (5mcg) and Vancomycin(30mcg) supplied by Hi-Media Laboratories, Mumbai .

Preparation of aqueous herbal extract

Fresh and good quality of bulb, fruits, leaves & buds of *Allum sativum*, *Cinnamon zeyanicum*, *Oscimum sanctum* & *Syzium aromaticum* respectively were procured from the local market. After cleaning the plant materials were chopped into small pieces with sterile knife. Then crushed in mortal and pestle by adding few ml of sterile distilled water. Aqueous extract of above plant bulb, fruits, leaves and buds (5% w/v) were prepared separately. Then it was filtered & tested by using agar well diffusion method.

Antibacterial testing of herbal extract

A 0.1ml of suspension of isolates of *Streptococcus mutans* was thoroughly mixed with sterile molten nutrient agar and poured into sterile Petri plates under aseptic conditions. After solidification, plates were used for making of well by using flamed cork borer. 0.5 ml of single plant extract was added in each well. Plates were kept at low temperature for diffusion then incubated at 37 °C for 24- 48 hrs. After incubation, zones of inhibition were measured & noted.

RESULT AND DISCUSSION

50 isolates of *Streptococcus mutans* were isolated from the samples of dental caries by using MSA, MSBA media and identified according to morphological, cultural & biochemical characters. Hemolytic pinpoint colonies were developed on blood agar after incubation under anaerobic condition. Isolates of *Streptococcus mutans* were Gram-positive, non motile, cocci, oxidase -ve, sorbitol +ve, catalase -ve, mannitol +ve, sucrose +ve, growth in 4% NaCl +ve. [1, 14, 15, 16]. 10 isolates of *Streptococcus mutans* showed antibiotic resistance to one or more antibiotic. The most common pattern of multiple drug resistance of isolates of *Streptococcus mutans* observed was ampicillin-ciprofloxacin-amoxicillin-rifampicin-vancomycin. The MAR index of each isolate was calculated by using Eq. (1). The antibacterial activity of plant extract on isolates of *Streptococcus mutans* was studied, zone of diameter were measured & noted.

The MAR index of each isolate was calculated by using following formula:

$$\text{MAR Index} = \frac{\text{No. of Antibiotics to which the isolate was resistant}}{\text{Total no. of antibiotics tested}} \quad (1)$$

Table 1: Percent resistance of *Streptococcus mutans* isolates against individual antibiotic

Sr. No.	Antibiotics	No of isolate showing resistance	Percent resistance
1.	Amoxycillin (30mcg)	04	40
2.	Ampicillin(10mcg)	06	60
3.	Ciprofloxacin(5mcg)	05	50
4.	Co-trimoxazole(1.25 mcg)	00	00
5.	Erythromycin(15mcg),	00	00
6.	Gentamycin(10mcg),	00	00
7.	Methicillin (5mcg)	00	00
8.	Nalidixic acid (30mcg)	00	00
9.	Rifampicin (5mcg),	04	40
10.	Vancomycin(30mcg)	01	10

Table 2: Antibacterial activity of plant extract on isolates of *Streptococcus mutans*

Sr. No	Isolate of <i>S. mutans</i>	Zone Diameter in mm			
		<i>A. sativum</i>	<i>C. zeyanicum</i>	<i>O. sanctum</i>	<i>S. aromaticum</i>
1.	ISM01	+++	+	+	---
2.	ISM02	++	++	---	++
3.	ISM03	+++	+	---	+
4.	ISM04	+	+++	+	---
5.	ISM05	++	---	---	+
6.	ISM06	+++	+	+	---
7.	ISM07	+++	++	+	---
8.	ISM08	++	---	---	++
9.	ISM09	+	++	+	++
10.	ISM10	++	++	+	++

(--: no antimicrobial activity, +: antimicrobial activity)

It was found that *A. sativum* showed highest antibacterial activity against *S. mutans* followed by *C. zeylanicum*, *S. aromaticum* & *O. sanctum*. It was also observed that antibacterial activity increases with the increase in concentration of plant extract. Antimicrobial activity of *Allium sativum* depends on the allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) after crushing or cutting garlic, which is responsible for its antimicrobial effects [17]. The antimicrobial activity of *C. zeylanicum* is attributed to the presence of cinnamaldehyde which is the predominant active component found in cinnamon oil [18]. *Oscimum sanctum* contains enormous antimicrobial substances like Eugenol, ursolic acid and carvacrol which are responsible for its antimicrobial activity [19, 20]. The antimicrobial activity of *S. aromaticum* is due to oil called oelum caryophylli which is a mixture of hydrocarbon and eugenol (a phenol) [21]. It has antibacterial & antifungal activity [22]. The indiscriminate use of antibiotics has led to an increase in antibiotic resistance among microorganisms. Hence plants can be used for treatment of serious infections caused by MDR bacteria.

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