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IN VITRO ANTIBACTERIAL ACTIVITY OF *EUGENIA JAMBOLANA* AGAINST *STREPTOCOCCUS MUTANS* CAUSING DENTAL PLAQUE FORMATION

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Keywords:
Dental caries, *Eugenia jambolana*, antimicrobial activity, agar well diffusion, Inhibition zone

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ABSTRACT

Streptococcus mutans is considered one of the most important cariogenic species of the human oral microbial flora. Total 150 samples were collected from Himachal institute of Dental sciences hospital Paonta sahib to check the prevalence of *S. mutans* in dental plaque. Total recovered isolates were 126 out of which 90 *Streptococcus mutans* were isolated. So the prevalence of recovered isolates was 60%. In the present investigation, the evaluation of current efficacy of 10 commercially available antibacterial drugs in India was carried out against *S. mutans* by Kirby-Bauer disc diffusion method. Of the 10 antibacterial drugs evaluated against the recovered isolate amoxicillin, ciprofloxacin and penicillin G were highly effective in terms of maximum diameter of growth inhibition zones followed by chloramphenicol. Five drugs namely, ofloxacin, tetracycline, erythromycin, chloramphenicol and gentamycin were found to be moderately effective against the three strains of *S. mutans*. Metronidazole and rifampicin were not effective against the bacteria as they did not show any inhibitory activity. Extracts of *Eugenia jambolana* showed antibacterial activities against *Streptococcus mutans* and showed the maximum zone of inhibition.

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. 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 (Scheie AAA,1994). Of the varied flora in the oral cavity, *Streptococcus mutans* has particular relevance because of its association with local infections such as dental caries (Ullman, R. F., 1988). *Streptococcus mutans* are the most cariogenic pathogens as they are highly acidogenic, producing short-chain carboxylic acids which dissolve hard tissues such as enamel and dentine (Shaw JH, 1987). Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases (van Gemert-Schricks MCM, 2008). The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by products of carbohydrate metabolism by *Streptococcus mutans*, a cariogenic bacterium. (LoescheWJ,1986). The demineralization occurs within a bacteria-laden gelatinous material called dental plaque that adheres to the tooth surfaces and become colonized by bacteria (Fujita K,2007). Secondary infections are caused by *Lactobacillus* species, and yeasts such as *Candida albicans* (Fujita K, 2007 & Radford JR,2007). Effective antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries. Antibiotics such as amoxicillin, penicillin, ciprofloxacin were highly effective in terms of maximum diameter of growth inhibition zones followed by chloramphenicol have been reported to effectively prevent dental caries in humans drugs namely, ofloxacin, tetracycline, erythromycin, and gentamycin were found to be moderately effective against the three strains of *S. mutans*. Metronidazole, and rifampicin were not effective against the bacteria as they did not show any inhibitory activity (Pranay Jain 2009). Erythromycin and chloramphenicol have been recommended as an alternative options for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures (Bagg J,1999 & Addy LD, 2005). *viridans* group *Streptococci* including *S. mitis*, *S. sanguis* and *S. mutans*, the most representative human cariogenic bacteria are moderately resistant to antibiotics (Venditti M,2005). These drawbacks justify further research and development of natural antibacterials that are safe for the host or specific for oral pathogens. The natural phytochemicals

could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other oral infections. As most of the oral diseases are due to bacterial infections and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacterias responsible for dental caries (Jonathan EK,2000).

Material and methods:

Sample collection:

In the present investigation, the total 150 dental plaque sample were collected from Himachal institute of dental sciences hospital Paonta Sahib. The sample were collected aseptically in sterile 50 ml Oakridge tubes and inoculated in nutrient broth for 24 hrs at 37^0C. Inoculated sample were streaked on Blood agar.

Isolation and identification of bacterial strains:

The isolates obtained were identified on the basis of colony morphology and biochemical reaction.

Antibiotic sensitivity tests:

The prevalence and antimicrobial sensitivity was tested by Kirby Bauer disc diffusion method, on Muller Hinton Agar. Ten several antibiotics were used for antibiotic sensitivity test. Standardisation of the technique controls variation in results and interpretation is based on comparison of inhibition zone with published criteria for zone diameter.

Collection of plant material:

Fresh fruits of *Eugenia jambolana* were collected from surrounding areas of Paonta Sahib (H.P.) and identified by Botanical survey of India, Dehradun.

Preparation of plant extract:

For the preparation of plant extract the fruits were dried under shade and stored into fine powder using electric blender. 50mg of dried powder sample was taken and extracted by Soxlet apparatus using distilled water, methanol and ethanol separately. The solvents were removed under reduced pressure in a rotary evaporator untill they become completely dry. Filtrates were preserved at 4^0 C.

Preliminary phytochemical analysis:

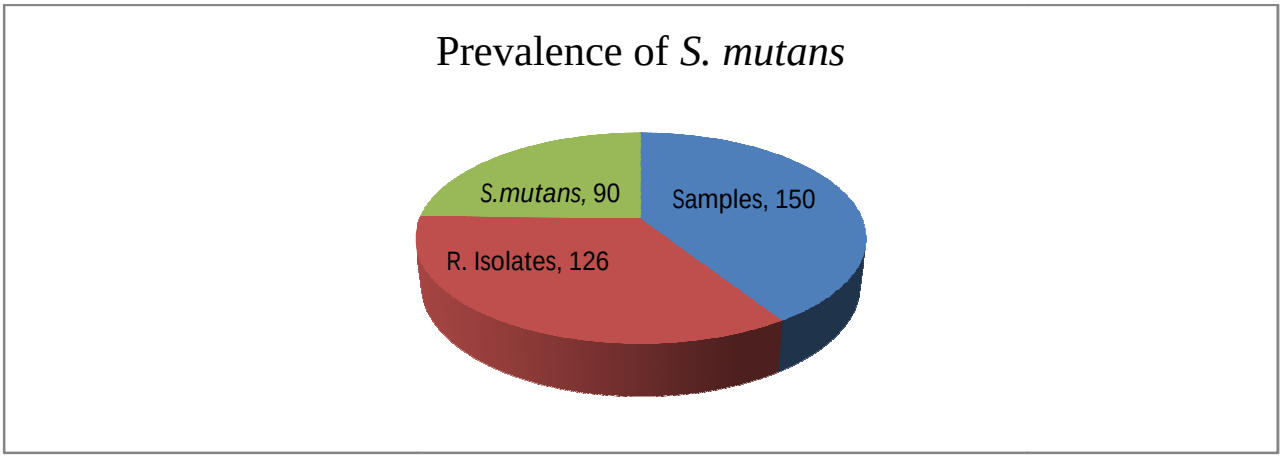
Phytochemical screening was carried out on the powered plant material based on standard protocol.

Determination of the antimicrobial activity:

From the dry filterate material, the 500mg/ml dilution of plant paste were prepared for antibacterial assay. The modified agar well diffusion method was employed to determine the antimicrobial activity of plant extracts. Three different extractions (Aqueous, methanol and ethanol) were taken. In agar well diffusion method, 100µl of the extracts (500mg/ml) were poured in to the wells. All the agar plates incubated at 37^0C. If antimicrobial activity was present on plates, it was indicated by an inhibition zone. The diameter of the inhibition zone were measured in millimeter after 24hrs. The experiments were conducted in triplicate for each test. The mean of triplicate result were taken.

Result and Discussion:

Total recovered isolates were 126 out of which 90 *Streptococcus mutans* were isolated during the study period in 150 dental plaque samples. So the prevalence of recovered isolates was 60%. The incidence of *S. mutans* isolates varies according to countries, regions or even hospitals. The prevalence rate of recovered isolate in the present study was much lower than those reported from Karnataka (87.37%) Hedge.P.P. (2005); Nigeria (85%) U.B.Owhe-Ureghe (2010); Nigeria (82%) E.O.Ophori (2010); Karnataka (74.7%) Beena Antony (2010). The prevalence rate of recovered isolate in the present study was much higher than those reported from Netherland (43%) F.J.M. Roester (1995).

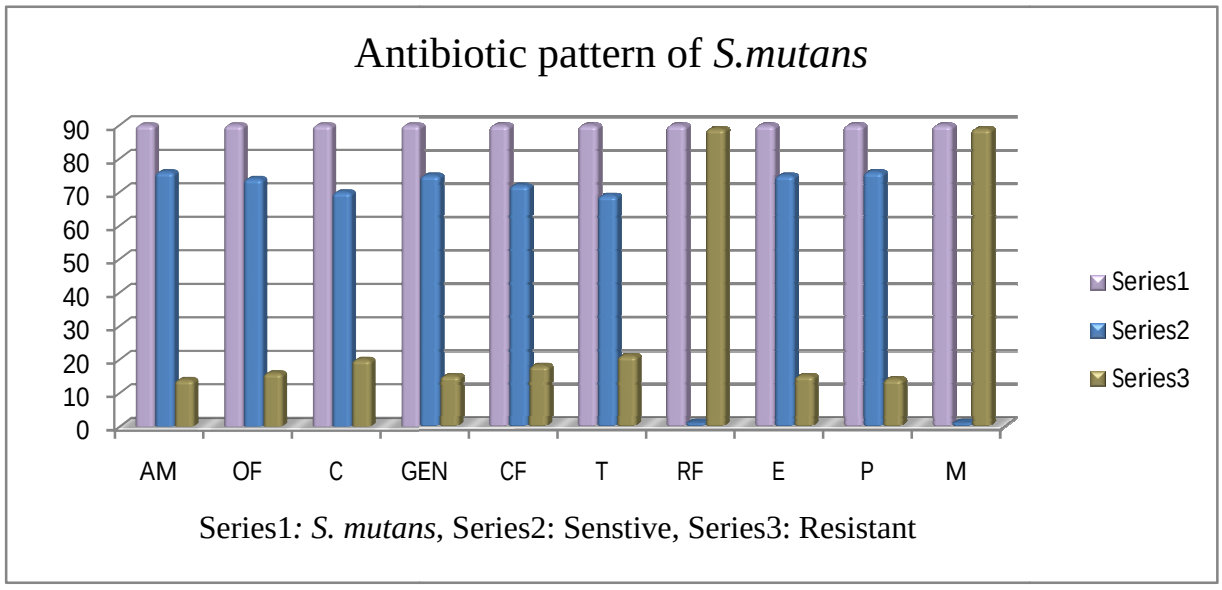


Antibiotic Sensitivity Test:

The antibacterial activity of 10 commercial drugs was assayed by Kirby-Bauer disc diffusion method. 80% of the recovered isolates were showed the sensitive activity against amoxicillin, ciprofloxacin, penicillin G, erythromycin, chloramphenicol, tetracycline, gentamycin and resistant activity against metronidazole, and rifampicin. The diameter of inhibition zones observed in 8 drugs namely amoxicillin (31mm), ciprofloxacin (30mm), penicillin G (29mm), erythromycin (28mm), tetracycline (26mm), chloramphenicol (23mm), gentamycin (23mm), ofloxacin (17mm), metronidazole and rifampicin showed no inhibition zones against the growth of *Streptococcus* strains, hence considered to be resistant against these drugs. Among the antibacterial drugs tested amoxicillin, penicillin, ciprofloxacin showed maximum zone of inhibition against *Streptococcus mutans. Streptococcus mutans* have been found to be most susceptible against amoxicillin as revealed by the data, the maximum zone of inhibition was found in amoxicillin (31mm). According to Pranay Jain and Ram Kumar Pundir study amoxicillin gave zone of inhibition 44-45mm (Pranay Jain,2009) and his study showed that the antibiotic Ciprofloxacin is resistant but as according to our study it was concluded that amoxicillin gave 31mm zone of inhibition and ciprofloxacin gave 30mm zone of inhibition. Our results from the present study substantiate the frequent use of broad spectrum amoxicillin and ciprofloxacin in dental practice by Al-Harooni and Skoog (AI-Haroni M,2007). This antibiotic is routinely prescribed as prophylaxis to the patients prior to massive dental procedures. It has been reported that the introduction of penicillin in the prophylactic treatment has reduced the infection, but the long-term use of penicillin could be compromised by the emergence of resistant strains. Erythromycin have been recommended as alternative options for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures. This antibiotic have not developed resistance against the strains of *S. mutans* as revealed by the zone of inhibition in the present study. Tetracycline show side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhea. But as effective in inhibiting the growth of *S. mutans* and hence should be recommended for use. Tetracycline's have few side effects but are not recommended for children or pregnant women because they can discolour developing teeth and alter bone growth. Gentamycin, an aminoglycoside, may lead to side effects which include damage to the ears and

kidneys. Metronidazole has been most frequently prescribed by the dental professionals. However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, metronidazole is used empirically in combination with one or more antibiotics, although the resistance to the drug may be associated with mobile genetic elements, aiding spread. It may be suggested from the present study that due to lack of appropriate knowledge of prescribing antibiotics for the treatment of dental caries on part of dental professionals, the microbial flora responsible for causing dental caries has developed resistance to the commercially available drugs. It may also be recommended that amoxicillin, ciprofloxacin and penicillin G are the most effective antibacterial drugs for the treatment of dental caries. Further investigation and education are required to attempt to slow resistance development and

lessen the future impact on antibiotic prescribing in dentistry.



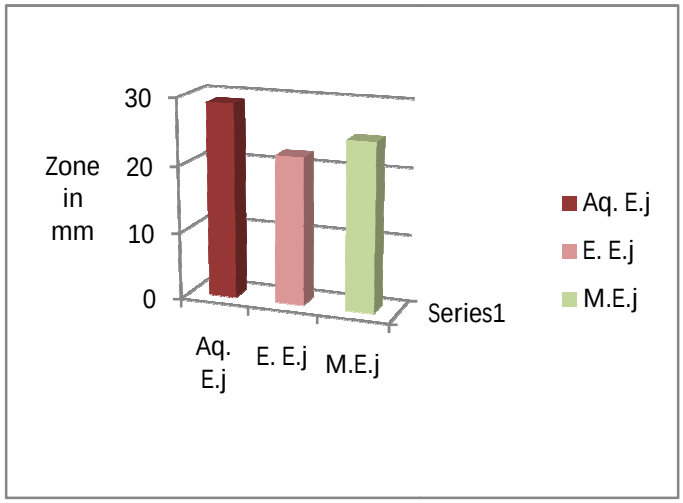
Series1*: S. mutans*, Series2: Senstive, Series3: Resistant

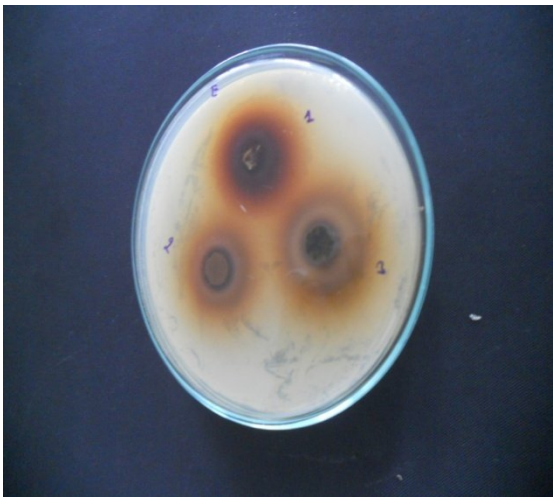
AM: Amoxicillin, OF: Ofloxacin, C: Chloramphenicol, GEN: Gentamycin, CF: Ciprofloxacin, T: Tetracycline, RF: Rifampicin, E: erythromycin, P: Penicillin G, M: Metronidazole.

Antimicrobial Activity of Medicinal Plants:

The results of antimicrobial activity of ethanol, methanol, acetonic and aqueous extracts of *E. jambolana* by agar well diffusion method revealed that all the three extracts of *E. jambolana* showed antimicrobial activity against dental caries causing bacteria i.e. *S. mutans* Highest mean

diameter of inhibition zone against *S. mutans* was produced by the aqueous extracts 29mm followed by methanolic extract 25mm against and ethanolic extract 22mm.





1 or Aq: Aqueous, 2 or E: Ethanol, 3 or M: Methanol, E.j or *Eugenia jambolana*

CONCLUSION:

Since all the tested extracts of *E. jambolana* were highly effective against the recovered isolates, purification and toxicological studies of the plant and *in vivo* trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental plaque causing bacteria. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *E. jambolana* should be emphasized for the control of dental plaque. It indicates that plants have the potential to generate herbal metabolites. The crude extracts demonstrating anti dental caries activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

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