Anti- cariogenic efficacy of Ficus racemosa extract using disc diffusion method - An In vitro study

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Introduction:

Dental caries is reported as the most common widespread and infectious condition, especially among young children [1] in the world. It still continues to be a major health problem in developing countries because of the poor socio economic condition and the lack of awareness. [2] In a study conducted in Chennai, India it was found that Dental health was left neglected in children whose parents had secondary education, who reside in a suburban population and who have not visited the dentist for more than 3 years.[3] In a study done in Chidambaram, India an increased prevalence of dental caries was found in the ages between 9 and 11. There is a large population which still remain ignorant about the effects of poor oral health.[4] World Health Organization has said that 60- 90% of school children worldwide have experienced caries, and being more prevalent in Asian and Latin American countries. [5] In a study conducted in South India, it was estimated that the caries prevalence is 78%.[6]

The development of caries is influenced by the host, agent and environmental factors. The etiology of dental caries is that caries occurs when bacteria in the dental plaque ferment dietary carbohydrate, which produces an acid, thus lowering the pH and dissolves the hydroxyapatite of dentine and enamel leading to caries formation. [7] The oral micro flora is home to diverse bacterial populations. Micro organisms inhibit the oral cavity, some of which are responsible for the occurrence of caries. It was found that Streptococcus mutans, Lactobacilli and Actinomyces are the components of normal microbial flora of the oral cavity which play an important role in the pathogenesis of dental caries and an increased number of these microorganisms is associated with increased frequency of caries. [8-10] There are several predisposing factors which can cause the oral micro flora to accumulate as well as cariogenic bacteria which cause dental caries. The breakdown of carbohydrates by the oral micro flora result in a fermentation process and the production of an acidic environment, which is favourable for the cariogenic bacteria to thrive and demineralization of sound tooth structure leading to the development of dental caries.

Disruption of the dental plaque and preventing the accumulation of the microbial population reduces the occurrence of caries by disrupting the process of development of caries. This is achieved by proper brushing habits and proper maintenance of oral hygiene. Also, increasing the brushing time increases the disruption of plaque to an extent likely to cause a significant increase in oral health benefits.[11] Knowledge regarding brushing habits and frequency of brushing is essential to maintain a good oral hygiene. The lack of knowledge among parents about the initiation of brushing habit and the frequency of brushing might result in te development of inadequate oral hygiene.[12] The successful development of vaccines against oral diseases is a matter of great importance to ensure safety along with effective protection.

Hence, Dental caries a bacterial driven, generally chronic, site-specific, multifactorial, dynamic disease process that results from the imbalance in the physiologic equilibrium between tooth mineral and plaque fluid.[13]

The demands of herbal medicines are on the rise because they have potent pharmacological activity and their economic values are found to be beneficial to people.[14] India has an abundant diversity of medicinal plants and is rightly termed as the botanical garden of the world. [15]Various studies have been conducted with numerous herbal products against cariogenic organisms like Streptococcus mutans. Pooja Agarwal et al. studied the antimicrobial activity of various concentrations of Tulsi extract (Ocimum tenuiflorum) against Streptococcus mutans and it shows a positive anti microbial activity against the bacteria.[16] Rajalakshmi Rakshanaa et al. carried out a study on the antibacterial efficacy of herbal mouthwash against oral microbes and found out that the inhibitory effect against Streptococcus mutans was significant.[17] Nantiya Joycharat et al. studied the anti Streptococcus mutans efficacy of Thai herbal formula used as a remedy for dental caries. Ficus religiosa was proven to inhibit the growth of Streptococcus mutans.[18] Prashant GM et al. investigated the efficacy of neem extracts (Azadirachta indica) among 4 types of Streptococcus and the results showed that Neem extracts produced the maximum zone of inhibition on Streptococcus mutans. [19] Gianmaria F. Ferrazzano et al. Conducted a study on the Antimicrobial Properties of Green Tea Extract Against Cariogenic oral micro flora. The results showed a statistically significant reduction in colony counts of mutans streptococci and lactobacilli which shows the efficacy of green tea extracts against cariogenic bacteria.[20] Mohammad Bagher Rezvani et al demonstrated the synergistic effect of honey and cinnamon against Streptococcus mutans. The results of the study show that a profound synergistic effect of honey and cinnamon was observed against Streptococcus mutans.[21] Hence it is seen that many herbal products are effective in preventing caries by preventing the growth of Streptococcus mutans.

Ficus racemosa, commonly known as fig tree, is proposed to have certain action against cariogenic bacteria. It is known as atti maram in tamil, and it belongs to the Moraceae family and is native to South East Asia, Indian subcontinent and Australia. Ficus racemosa is an evergreen, deciduous tree with dark green coloured leaves. The fruits are found in small clusters on short or long braches which are leafless and arise from the main trunk. The bark, leaves, stem, roots and the fruit extracts are used widely to treat diseases in herbal medicine. The fruit of this tree is used to treat constipation; the leaves are used for Diabetes as the ethanolic fruit extracts reduced the blood glucose level within 2 weeks in experimental studies in rats, to lower cholesterol level, and skin diseases like eczema; the fruits and leaves are also used to make medicine. The roots are used as a treatment option for gout. Ripe fruits of Ficus racemosa are used as an antidote against venoms and to treat poisoning. The fruits are powdered and used to treat asthma effectively.[22]

Ficus racemosa has been widely studied for various properties like reducing fever, anti inflammatory, properties against micro organisms. [23] The leaves of Ficus racemosa were found to be effective against Castor oil- induced diarrhoea. [24] Also, Ficus racemosa is found to lower blood glucose levels. The hypoglycemic effect of the extract of bark of Ficus racemosa is stronger on diabetic patients.[25] Ficus racemosa stem bark extracts possesses moderate anticholinesterase activity. This indicates the potential in the development of natural therapeutics for Alzheimer's disease and other related problems from Ficus racemosa. [26] Ethanolic extracts of Ficus racemosa exhibits a steady anti oxidant state and it does not alter the cell cycle delay caused by radiation. Also, it was stated that ethanolic extracts of Ficus species proved to have a higher reactivity level against microbes, worms and also hepatic damage when compared with standard drugs.[27] The bark of Ficus racemosa was found to have anti diuretic action.[28] The methanolic stem extracts of Ficus racemosa were shown to have antitussive activity.[29] The aim of this study is to determine the anti- cariogenic efficacy of Ficus racemosa extracts, and thus help to determine if it can be used effectively to prevent dental caries.

Materials & Methods:

The study was conducted following approval by the Instuitutional Scientific Review Board. Ethanolic fruit extract of Ficus Racemosa were collected from Hosur, Tamil Nadu and were authenticated by Green Chem lab, Bengaluru, India. Bacterial strain used were Streptococcus mutans (ATCC 25175). The organisms were obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai.

The plant extract 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic Soy Broth (TSB). From the stock solution various concentrations were prepared, viz., 5mg/100µl 10mg, 15mg, 20mg /ml respectively in to wells of micro plates.100µl of these concentration was taken and the plates were incubated at 37°C for 24hrs.The pooled extracts were concentrated and extracts were loaded into sterile readymade discs (Hi-media, MUMBAI) in different volumes of 15mg/ml, 20mg/ml and 25mg/ml / disc respectively and allowed to dry for 24 hours at room temperature. Mueller Hinton agar plates were spread with 100µl of actively growing broth cultures of the respective bacteria and are allowed to dry for 10 min. The sterile readymade discs loaded with each extract individually (5mg/ml and 25mg/ml/ disc respectively) were imposed on the inoculated plates. The plates were then incubated at 37°C for 24 hours. The development of the zone of inhibition around the around the extract loaded disc was recorded.[30-32] (Fig 1)

Fig 1: Disc diffusion assay of Ficus racemosa fruit extract against Streptococcus mutans showing zone of inhibition.

Result and Discussion

The study shows that when Ficus racemosa is tested at various concentrations against S.mutans, the maximum zone of inhibition is noted at 20mg/ml and 25 mg/ml. Eventually the other concentrations also showed the zone of inhibition against the organism tested**.** (Table 1)

Table 1: Zone of inhibition formed around Ficus racemosa ethanolic fruit extracts in (mm)

5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
12	14	20	22	24

Anti cariogenic evaluation of Ficus racemosa against S.mutans -Disc Diffusion assay depicted by Zone of inhibition in (mm)

Fruits of Ficus racemosa are reported to contain sterols, triterpenoids, flavonoids, glyco- sides, tannins, carbohydrates (Deshmukh et al., 2007), β-sitosterol, gluanol acetate, hentriacontane, tiglic acid of taraxasterol, lupeol acetate (Singhal & Saharia, 1980; Nguyen et al., 2001; Chandra et al., 1979; Merchant et al., 1979), gallic acid, ellagic acid (Rao et al., 2008) and α-amyrin acetate (Narender et al., 2008).[33] The inhibition zone formed around the discs of ethanolic fruit extracts of Ficus racemosa when introduced into the growth of Streptococcus mutans is attributed to the presence of secondary metabolites which include alkaloid, glycoside, steroid, tannin, terpenoid and flavaniod in the extract. [34]

Antimicrobial susceptibility testing requires a medium to culture the organism to be investigated and a provision to study the effect of the drug or plant extract used, for which Agar disc diffusion method was used in this study. Introduced in 1940[35], this method is the standard official method in clinical and microbiological laboratories. Many standards are published by the Clinical and Laboratory Standards Institute (CLSI) which have to be followed for testing of yeast and bacteria. [36, 37] The principle is that agar is inoculated with the test bacterium and when the antibiotic- impregnated disc is introduced, the antibiotic diffuses through the agar medium, thereby produces an antibiotic concentration gradient. This concentration gradient is high at the edge of the disc and gradually decreases as the distance from the disc increases. The gradient is present from the edge of the disc up to the point where the antibiotic disc does not affect the growth of the organism, and it continues to row uninterrupted. A clear zone is thus seen around the antibiotic disc if the growth of the organism is inhibited by the disc. The zone of inhibition and is measured in mm.

This method of analysis is chosen due to its simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided[38-41].This has been proved by studies conducted using various plant extracts done by Geetha R. V. et al who studied the antibacterial property of different types of tea extracts against S. mutans and Lakshmi et al. who studied the antibacterial activity of extracts from Acacia catechu leaf extracts against E. faecalis and S. mutans. [42-43.]

Various factors influence the type of medium used to study the organism, such as the rate of growth of the organism, the rate of diffusion of the antibiotic, and the activity of the agent. In this study, Mueller Hinton Agar plates were used to grow the organism Streptococcus mutans.

Inoculum density can also alter the development of inhibition zones. If the inoculum is too light, thee will be a larger inhibition zone although the sensitivity of the organism is unchanged. Relatively resistant strains may then be reported as susceptible. Conversely, if the inoculum is too heavy, the zone size will be reduced and susceptible strains may be reported as resistant. Hence in this study optimal results were obtained with an inoculum size that produces near confluent growth.

Culture plates if left at room temperature for longer periods than the required time after being seeded, the growth of the organism will take place even before the discs are applied. Hence a reduction in the zone diameter will be seen which will be in turn be reported as if the strain is being resistant. In this study, the discs were kept for 24 hours at 37^0C and this was maintained to have effective growth in optimal zone. If the temperature is decreased, then the time required for effective growth is prolonged and larger inhibitory zones develop around the discs. At higher temperatures, the culture is viable to change.

If the media is very thin, then excessively large inhibition zones may be formed and if the media is very thick, then an excessively thin inhibition zone will be formed. Proper spacing between the discs is needed to prevent overlapping of the inhibition zones or deformation near the edge of the plates. Preformed plates with predetermined agar thickness was used measuring 10 cm and loaded with different volumes of the plant extract was used to obtain accurate results.

Stringent measure were taken when interpreting the results. Plates in which the test bacteria as isolated colonies or less than semi-confluent growth were not recorded, hence to increase the accuracy of the test results. Distorted zones of inhibition were also rejected.

The influence of various factors like inoculum density and pH of the medium were avoided by taking adequate measures. The zone of inhibition of the growth of the organism Streptococcus mutans was measured in mm as measured from the discs containing the extracts of Ficus racemosa.

The results of the present study showed that Ficus racemosa is most effective against S. mutans at conentrations of 20 mg/ ml and 25 mg/ ml. It is due to active ingredient only at a higher concentrarion. Karthick Auswin et al. studied the inhibition of superoxide dismutase using herbal compounds using herbal compounds to treat oral diseases caused by Streptococcus mutans and found a positive inhibition of superoxide dismutase.[44] Hiram Sony et al. studied the anti cariogenic activity of 12 medicinal plants against 6 oral pathogens in invitro condition. The results stated that only Ficus racemosa leaf extract has shown activity against Lactobacillus acidophilus among the 12 medicinal plants included in the study. [45] Krishna Murti et al showed that Ficus racemosa extract showed maximum inhibition against Staphylococcus aureus.[46] In this study it was seen that the antimicrobial activity of extracts of Ficus benghalensis and Ficus racemosa against three bacterial strains such as Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia.

Conclusion

Ficus racemosa is a herb which possess lots of medicinal value to treat many diseases. The study suggests that this extract possess significant anticariogenic efficacy and could be used in clinical trials for exploring it to commercial use.

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