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RESEARCH ARTICLE

HERBAL ANTAGONISM TO THE PATHOGENIC MICROBES RESPONSIBLE FOR ORAL INFECTION.

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Abstract

The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries: ***Streptococcus mutans*** and ***Staphylococcus*** **species** among them. These organisms can produce high levels of lactic acid following fermentation of dietary sugars, and are resistant to the adverse effects of low pH. These bacterial strains, most notably ***S. Mutans*** can be inherited by a child from a caretaker's kiss or through feeding premasticated food. Dental Caries (DC), periodontitis and Oral Candidiasis (OC) are widespread disorders. According to the World Health Organization, 60-90% of worldwide school-children has dental caries. These microbes are highly responsible for causing dental caries are treated in vitro with the help of guava leaf extract prepared in laboratory conditions and then checked and summarized. The microbes were first isolated using selective media and fresh pure cultures were prepared which were further cross checked performing staining procedure as well as biochemical test. These pure cultures were then treated with the hexane and ethanol extractives of guava (*Psidium guajava*) leaves. Preparative Thin Layer Chromatography (Prep TLC) is a useful technique for the purification of small quantities of sample. Because it allows for rapid separation of a number of components in a reaction mixture, it is especially useful for obtaining a profile of the products of a test reaction or the components of natural extracts.

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Introduction:-

Dental caries is the scientific term for tooth decay or cavities. It is caused by specific types of bacteria namely ***Streptococcus mutans*** and ***Staphylococcus*** species[1]. They produce acid that destroys the tooth's enamel and the layer under it, the dentin.They build up on the teeth in a sticky film called plaque. This plaque also contains saliva, bits of food and other natural substances. It forms most easily in certain places likecracks, pits or grooves in the back teeth ,between teeth,around dental fillings or bridgework, near the gum line. Bacteria collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a biofilm. Some sites collect plaque more commonly than others, for example sites with a low rate of salivary flow (molar fissures). Early caries may not have any symptoms. Later, when the decay has eaten through the enamel, the teeth may be sensitive to sweet, hot or cold foods or drinks.[2]The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel. This is referred to as a white spot lesion, an incipient carious lesion or a "microcavity".

As the lesion continues to demineralize, it can turn brown but will eventually turn into a cavitation ("cavity"). Before the cavity forms, the process is reversible, but once a cavity forms, the lost tooth structure cannot be regenerated. A lesion that appears dark brown and shiny suggests dental caries were once present but the demineralization process has stopped, leaving a stain. Active decay is lighter in color and dull in appearance. Once the decay passes through enamel, the dentinal tubules, which have passages to the nerve of the tooth, become exposed, resulting in pain that can be transient, temporarily worsening with exposure to heat, cold, or sweet foods and drinks. Dental caries can also cause bad breath and foul tastes. In highly progressed cases, infection can spread from the tooth to the surrounding soft tissues. Complications such as cavernous sinus thrombosis and Ludwig angina can be life-threatening.[3] ***Candida albicans*** is the most widely spreaded fungal pathogen in humans. It the pathogen is able to kill the host if the infection is systemic .Two important factors that affect the pathogenicity of *C. albicans* are the ability to switch between yeast growth and filamentous growth, i.e. the hyphal switch, and the ability to form biofilms which enables *C. albicans* to adhere to the surface of substrates. Infection caused by *C.albicans* is a serious threat for immune compromised patients. Now-a –days microbial species are very much prone to antibiotics resistance. So herbal remedies are the alternative sources. *Psidium guajava* is used as folk medicine for tooth swelling and pain in rural Bengal[5]. Antimicrobial activity of *Psidium guajava* was studied against above mentioned test organism[6].

Materials and Method:-

Microbial sample collection:-

Microbial sample isolation was performed by taking two sets of oral sample collected in the form of swab and kept in sterile container. To the oral swab sample 9ml distilled water was poured to make the 10^{-1} dilution. After serial dilution from 10^{-8} dilution colonies were collected after streak plate method. The pure culture preparation on made over high mediamannitol Salt Agar (selective for Staphylococcus sp.) blood agar (selective for growth of Streptococcus sp.). Single colony was inoculated in nutrient broth after identification by observing colony characterisation and biochemical tests[6]. After 18 hours incubation the pure culture was stored in refrigerator at 4°C. Pure culture of ***S. mutans*** and ***C. albicans*** were collected from R.G Kar Medical College, Kolkata.

Preparation of plant extractives :-

1 kg of medium matured Guava leaf (*Psidiumguajava* L.) was taken and air dried for 21 days. The material was then powdered and soaked in to obtain extracts from two different sets of solvent.

Hexane extracts:-

Hundred five grams of dry ground plant leaf sample to be added in 1 Litre of hexane in sterile bottles and rotated with constant agitation (200 rpm) overnight at 20^0C in a temperature controlled bioshaker for 72 hours. The hexane fraction was separated using sterile cheese cloth and filter through sterile Whatman filter paper (no. 2).The extractive was concentrated under rotary evaporator. After concentrating the solvent 6 ml oily substances was stored in 4ºC.

Ethanol extract:-

The residual plant sample after hexane extraction was dried at 40^0 C overnight in an oven. Then 1L of ethanol to be added to dried residue and agitated (200 rpm) for 7 days at 25^0C in a temperature-controlled bioshaker. The ethanol

fraction was separated using sterile cheese cloth and filter through sterile Whatman filter paper (no. 2).The extractive was concentrated under rotary evaporator. After concentrating the solvent they are stored in 4ºC..

Fractionation of Ethanol extract:-
Preparative Thin Layer Chromatography (Prep TLC) analysis was performed for the separation of the components in the ethanolic extractive. From the sample analysis two major bands Rf = 0.9 and Rf=0.76 were found and separated.

Antimicrobial assay:-
The antimicrobial activity of the hexane extractive which contains mostly the non polar phytochemicals and the essential oil was evaluated against the microbial sample. Similarly the ethanol extractive was also evaluated against the microbial sample ***Streptococcus mutans*** and ***Staphylococcus*** species and ***C.albicans.***

The component mixtures found from p- TLC also subjected for antimicrobial activity. Antimicrobial susceptibility was performed by the well-diffusion method using plant extractive and mixed components of plant extractives that obtained from p-TLC.

Results:-

Table: 1

Extractives	**Avr. Zone of Inhibition for *Staphylococcus* sp.(cm)**	**Avr. Zone of Inhibition for *Streptococcus mutans*.(cm)**
Crude extract: n-hexaneext (100 µl)	-	-
n-hexane ext (200 µl)	1.54	1.85
n-hexaneext (300 µl)	2. 15	2.66
n-hexane ext (400 µl)	2.85	3.15
Control	2.54	3.0
Crude extract: Ethanol ext(100 µl)	1.75	1.86
n-hexane ext (200 µl)	2.34	2.45
n-hexane ext (300 µl)	2.9	3.18
n-hexane ext (400 µl)	3.66	3.90
Control	2.54	3.00

Table: 2

Extractives	**Avr. Zone of Inhibition for *Staphylococcus* sp.(cm)**	**AvrZone of Inhibition for *Streptococcus mutans*.(cm)**
Upper fraction of Ethanolextrat obtained from TLC: (20 µl)	1.86	2.38
(50 µl)	3.54	3.24
(100 µl)	4.00	3.82
Control	2.54	3.00
Lower fraction of Ethanol extract obtained from TLC:		
(20 µl)	1.24	1.85
(50 µl)	2.48	2.42
(100 µl)	2.95	2.85
Control	2.54	3.00

(Control was Metronidazole. The concentration was 1mg/ml)
No inhibitory zone was found against ***C.albicans*** when extractives were tested.

Discussion:-

The present work demonstrates the antimicrobial potential of ***Psidium guajava*** leaves extract by using various solvents. The results indicate that Ethanol is better than-hexane for the extraction of the antibacterial properties of guava. The results also indicate that the plant extracts have no antifungal effect on the pathogenic yeast *C.albicans*, showing that they do not contain active ingredients against the organisms. The observed inhibition of Gram positive bacteria, ***Streptococcus mutans*** *and* ***Staphylococcus sp***, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using Ethanol or n-hexane as the solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, *P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against dental caries caused by ***Streptococcus mutans*** and ***Staphylococcus sp***.

Conclusion:-

The inhibitory effect of crude extract of the guava leaf (both ethanol extract and n-hexane extract) seems significant against ***Staphylococcus sp*** and ***Streptococcus mutans.*** The inhibitory effect of the same crude extract however shows a reasonable degree of antagonism to establish as a good antibacterial property against ***Staphylococcus sp.*** and ***Streptococcus mutans***

Now coming to the fractionated extractive obtained by TLC of ethanol extractive, the upper fraction shows high degree of inhibition comparable to a broad spectrum antibiotic metronidazole. In case of lower fraction of the same extractive obtained by TLC it shows moderate degree of inhibition as the zone of inhibition decreases comparable to a broad spectrum antibiotic metronidazole (1 mg/ml).

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Data Sharing Statement:-
We cannot share any unpublished data with other laboratory or person.

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