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ANTIBACTERIAL EFFECT OF PHYTOCHEMICALS AGAINST ISOLATED PATHOGENIC MICROORGANISMS ASSOCIATED WITH DENTAL CARIES IN HUMAN TEETH

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ABSTRACT:
Oral infections caused by pathogenic bacteria are a common health issue, necessitating the development of effective antibacterial agents. Phytochemicals derived from various plant sources have gained attention due to their potential antimicrobial properties. The present study was mainly focused on the isolation and identification of oral pathogens and furthermore potential applications phytochemicals in oral health care. Caries sample were obtained arbitrary from 50 children's patients of Government Dental Hospital College, Nagpur in the age group of 4 to 14 years & examined for isolation of various oral pathogens on Mitis Salivarius agar. Among 50 Samples, different 26 isolates were identified on the basis of Morphological, Cultural & Biochemical basis. Antimicrobial activity of solvents extracts of different plants were determined by zone of inhibition using agar well diffusion method. *Tinospora* exhibited antimicrobial properties against *S. mutans* with the maximum activity at 2% concentration. It could be formulated as mouthwash and used to prevent plaque in patients at high risk for caries and gingivitis.

Keywords :- Dental caries, Well diffusion, phytochemicals & antibacterial.

INTRODUCTION :

Dental caries is a globally prevalent and ubiquitous oral health and public health concern. 1-3 It can affect children at a very early age, 4-6 and will certainly afflict most individuals in adolescence and throughout adulthood.dental caries remains a prevalent disease in society today. Dental caries is a multi-factorial oral condition with a complex etiology. The interplay between dental plaque, constituents of the diet, and the host tissue, as well as genetic and environmental factors, have each increasingly been recognized for their importance in the pathogenesis of dental caries.[1]

Dental caries is the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. The signs of the carious demineralisation are seen on the hard dental tissues, but the disease process is initiated within the bacterial biofi lm (dental plaque) that covers a tooth surface. Moreover, the very early changes in the enamel are not detected with traditional clinical and radiographic methods. Dental caries is a multifactorial disease that starts with microbiological shifts within the complex biofilm and is aff ected by salivary flow and composition, exposure to fl uoride, consumption of dietary sugars, and by preventive behaviours (cleaning teeth). The disease is initially reversible and can be halted at any stage, even when some dentine or enamel is destroyed (cavitation), provided that enough biofilm can be removed. Dental caries is one of the most common preventable childhood diseases; people are susceptible to the disease throughout their lifetime. It is the primary cause of oral pain and tooth loss.It can be arrested and potentially reversed in its early stages, but is often not self-limiting and without proper care, caries can progress until the tooth is

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destroyed.[2] Therefore, physicians and other health-care providers should be familiar with dental caries and its causes.[2-3] Dental caries is the predominant cause of tooth decay. Although the affl iction is not life threatening, it causes nagging pain and thus possess physical as well as psychological discomfort. The economic burden of the disease is also very high. If treatment were available, the costs of dental caries alone would exceed the total healthcare budget for children in many low-income countries. The earliest detectable stage is the appearance of a white spot on the tooth. This may later advance in steps to form a lesion, cavitations, and then to complete tooth loss. The cavities caused in dental caries are usually painless until they damage the vascular system of the tooth. Sweets often cause cavities and dentists fill them. However, whether caries is the natural fate of the tooth or there is a particular preventable factor that leads to it has been a topic of intense debate. Nevertheless, it is clear that the condition is complex and multifactorial.[4] The oral cavity is a complex, dynamic system, inhabited by over 700 different bacterial species. Under normal conditions, these bacterial communities live in symbiosis without causing harm to the host. However, a change in environmental or stress signal can tip the equilibrium toward pathogenic bacteria leading to oral diseases, such as dental caries, gingivitis, and periodontitis.[5] Dental caries is one of the most common and costly diseases in the world, and although rarely life threatening it is a major problem for health service providers.[6] The present study focus on isolation of bacterial strains from dental carries and evaluate the antimicrobial activity of some medicinal plant extract against isolates.

METHODOLOGY :

Dental Caries samples were collected from school going children of age group up to 15 years from Government Dental Hospital, Nagpur. Dental plaques from all the patients were picked up through the spoon esclavator and transferred it into the 2 ml of sterile saline water. Samples were inoculated on various specific media such as Mitis Salivarius Agar, Blood agar and Congo red Agar. Identification of bacteria was done on the basis of morphological, cultural and biochemical characteristics. The leaves of *Murraya koengii*, *Tinospora cordifolia* and *Moringa oleifera* were collected from the local area of pipla (Nagpur). The extraction process was carried out using soxhlet apparatus. Accurately weighed 15 g of powered leaf sample was extracted with 250 ml of solvent (Water and Methanol). The well diffusion was used to investigate the antimicrobial activity of *Murraya koengii, Tinospora cordifolia* and *Moringa oleifera*.

RESULT AND DISCUSSION :

Collection of Samples & Identification

In the present study, 50 samples were collected and out of that 26 samples were found positive The sample were collected from the government hospital Nagpur. All positive samples were process further for identification of bacterial strains on the basis of morphological, cultural and biochemical characteristics. which shown in following table

In the present study total 50 sample was collected among this 26 isolated sample was found the sample was collected from the carries active patient which is under the 15 age group. The highest caries scores were observed in the first primary molars (D's) for the primary dentition and the first permanent molars (6's) for the permanent dentition Regarding caries experience, significantly reduced microbial diversity was observed on teeth with high caries compared to caries-free teeth.in which the 5 bacterial strains are isolates i.e. *Streptococcus mutans, Streptococcus salivarious, Staphylococcus pasteuri, Kocuria varians and Kocuria rosea* . The same results were observed

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by Reyam Abdul Khuder Mohammed et. Al and others The majority of bacteria isolated from patients with tooth caries were Streptococci, which were isolated at 53.5%, followed by S. aureus, which were isolated at 33.8%, and another Staphylococci were 12.7%. In conclusion, Streptococcus spp. was the main bacteria that isolated from patients with tooth caries at age from 13-65 years.[7-9]

Antimicrobial activity

In the present study evaluate the antibacterial activities of the crude extracts obtained from the leaves of *Murraya koengii, Tinospora cardifolia* and *Moringa oleifera* using different solvents like methanol, cold water and hot water against oral pathogens. The antibacterial efficacy of the extracts of Murraya koengii , Tinaspora cordifolia and Moringa oleifera leaves was quantitatively evaluated on the basis of inhibition zone in mm (Table 3) following the disc diffusion method. Methanol and cold water extract have shown better activity than the standard drug. Hot water extract was less effective against oral pathogens. Methanol extract was more effective against oral pathogens. The preliminary phytochemical screening revealed the presence of Tannis, alkaloids, and flavonoids in some of the extracts methanol, Cold water as shown in Table 3. The methanol extract shows the presence of Alkaloids and Tannis in Moringa koengii , Tinospora cardifolia and Moringa oleifera. Cold water extract shows the presence of alkaloids Murraya koengii and Tinospora cardifolia. Falvnoids presence of Tinospora cardifolia and Moringa koengii. Tannis presence of cold water extract .

The present study concludes that the *Murraya koengii* may serve as a potential source of bioactive compounds in the of tooth decay. The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is required today. In the present vitro study it was shown that aqueous as well as methanol extracts of Curry leaves inhibit growth of oral pathogens. Which is main casual factor for Dental caries? Previous studies where this plants extracts has shown promising results against the pathogens used in our study.*Kunjal S. Mistry, Zarna Sanghvi et. al.and other* studied were showed that the Azadirchta indica, Minusops elengi, Tinospora cardifolia, and cholorohexidine Gluconate on common endodontic pathogens like Streptococcus mutans, enterococcus faecailis and Staphylococcus aureus. The agar diffusion test was used to check the antimicrobial activity of the Methanolic extracts of the medicinal plants along with chlorohexidine Gluconate. All the plants extracts showed considerable antimicrobial activity against selected endodontic pathogens. Chlorohexidine was also the most consistent of all the medicaments tests, showing inhibitory effect aginst all the tree pathogens at all the selected concentrations.[10-13]

CONCLUSION:

Prevalence of dental caries was high and found public health problem. Socioeconomic status, educational level, and poor oral hygiene practices were associated factors for dental caries. Health promotion about oral hygiene and integration of services are supremely important for the prevention of the problem of dental caries. The study was concluded that the percentage of male child has a highly carries active patient as compare to female. The total sample was collected 50 and out of this the positive sample of female was 12 and positive sample of male was 14. In this study the 5 different bacterial strains were isolated and they are identified on the basis of cultural, morphological and biochemical characteristics. *Streptococcus mutans, Streptococcus salivarious, Staphylococcus pasteuri, Kocuria varians and Kocuria rosea* were confirmed on the basis of

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above characteristics. According to the present study, *Tinospora* exhibited antimicrobial properties against *S. mutans* with the maximum activity at 2% concentration. It could be formulated as mouthwash and used to prevent plaque in patients at high risk for caries and gingivitis. However, more research is required to test its antimicrobial properties in the oral environment through in vivo studies and also the adverse effects if any on oral mucosal cells and teeth.

Further in vivo studies are needed to observe the effects of curry leaves extracts on salivary pH, dental enamel and other oral soft tissues to enable the preparation of chemical plaque controlling agents like mouth washes.

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Table No.1 Morphological And Cultural Characteristic of Bacterial Isolates

Sr. No.	Isolates sample	Morphological characteristic	Cultural Chacteristics					
			Shape	Size	Margine	Elevation	Colour	Texture
1	S1 P4	Gram Positive	Round	Small	Entire	convex	Grey	Slimy, mucoid
2	S2 P1 G	Gram positive	Round	Small	Entire	convex	Grey	Shiny,slimy,mucoid
3	S2 P1 B	Gram positive	Round	Small	Entire	Flat	Black	Slimy, mucoid
4	S2 P4 G	Gram positive	Round	Medium	entire	convex	Grey	Slimy, mucoid
5	S2 P4 B	Gram positive	Round	Small	entire	Flat	Black	Slimy, mucoid
6	S2 P8 G	Gram positive	Round	Small	entire	convex	Grey	Slimy, mucoid
7	S2 P8 B	Gram positive	Round	Medium	entire	convex	Black	Slimy, mucoid
8	S2 P5 LG	Gram positive	Round	Medium	entire	convex	Lightgrey	Metallic shiny,Slimy, mucoid
9	S2 P5 DG	Gram positive	Round	Medium	entire	convex	Dark grey	shiny,Slimy, mucoid,smooth
10	S2 P7	Gram positive	Round	Small	entire	Flat	Black	Slimy, mucoid and smooth
11	S2 P2 GB	Gram positive	Round	Medium	Entire	convex	Greyish black	Slimy, mucoid and smooth
12	S2 P3 BB	Gram positive	Round	Small	Entire	Flat	Black	Slimy, mucoid and smooth
13	S3 P1 GB	Gram positive	Round	Small	Entire	convex	Greyish black	Shiny,Slimy, mucoid and smooth
14	S3 P3 NB	Gram positive	Round	Small	Entire	convex	Nevy blue	Shiny,Slimy, mucoid and smooth
15	S3 P2 NB	Gram positive	Round	Small	Entire	Convex	Nevy blue	Slimy, mucoid and smooth
16	S3 P4 G	Gram positive	Round	Small	Entire	convex	Grey	Slimy, mucoid and smooth
17	S3 P5 G	Gram positive	Round	Medium	entire	convex	Grey	Shiny,Slimy, mucoid and smooth
18	S3 P5 BB	Gram positive	Round	Small	entire	Flat	Black	Slimy, mucoid and smooth
19	S3 P6 LG	Gram positive	Round	Small	entire	convex	Light grey	Slimy, mucoid and smooth
20	S4 P2 GB	Gram positive	Round	Medium	entire	convex	Greyish black	Slimy, mucoid and smooth
21	S4 P4 G	Gram positive	Round	Medium	entire	convex	Grey	Slimy, mucoid and smooth
22	S4 P4 B	Gram positive	Round	Small	entire	Flat	Black	Slimy, mucoid and smooth
23	S4 P2 B	Gram positive	Round	Small	Entire	Flat	Black	Slimy, mucoid and smooth
24	S4 P1 B	Gram positive	Round	Small	Entire	Flat	Black	Slimy, mucoid and smooth
25	S4 P6 G	Gram positive	Round	Small	Entire	convex	Grey	Slimy, mucoid and smooth
26	S4 P5 B	Gram positive	Round	Small	Entire	Flat	Black	Slimy, mucoid and smooth

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Table No.2. Isolated positive samples of oral pathogens

Sr. No.	Name of Bacteria	Isolates
1	***Streptococcus mutans***	S2 P5 DG, S2 P7, S2 P2 GB S2 P3 BB, S3 P1 GB, S3 P3 NB S1 P4, S3 P5 G
2	***Streptococcus salivaris***	S4 P5 B, S2 P1 G, S4 P6 G S3 P6 LG, S4 P4 B
3	***Staphylococcus pasteuri***	S4 P1 B, S2 P1 B, S2 P8 B S2 P5 LG, S4 P2 GB
4	***Kocuria varians***	S3 P2 NB, S3 P4 G, S4 P4 G S4 P2 B,S3 P5 BB
5	***Kocuria rosea***	S2 P4 G, S2 P4 B,S2 P8 G

Table No. 3 Antimicrobial activity of various phytochemicals against isolated Oral pathogens

Sr.No.	Isolates	Extracts								
		Hot						Methanol		
		Az	Mu	Ri	Ci	Ib	Psi	Mu	Mo	Ti
1	S3P6	-	-	-				19mm(S)	15mm(I)	13mm(I)
2	S2P7	-	-	-				15mm(I)	18mm(S)	17mm(S)
3	S4P5	-	-	-				19mm(S)	12mm(I)	11mm(I)
4	S2P4	-	-	-				21mm(S)	15mm(I)	16mm(I)
5	S4P2B	-	-	-				17mm(S)	14mm(I)	14mm(I)
6	S4P1B	-	-	-				26mm(S)	13mm(I)	12mm(I)
7	S3P2NB	-	-	-				17mm(S)	12mm(I)	12mm(I)
8	S3P5G	-	-	-				25mm(S)	-	-
9	S2P8	-	-	-				-	-	-
10	S4P6	-	-	-				17mm(S)	-	-
11	S3P4G	17mm	-	12mm				-	25mm	-
12	S2P4G	-	-	-				-	-	-

(Abbrevation:- S- sample , P- patient,B- Blue, G- green, NB- Nevy Blue, Resistance (R), intermidiate (I), sensitive (S), Mu -*Murrrya koengii*, Mo-*Moringa oleifera*, Ti-*Tinospora cardifolia*, Az-*Azadirachta indica*, Ri-*Ricinus Communis*, Ci- *Citrus Lemon* Ib-*Indian Bael*
Psi- *Psidium Guajava)*

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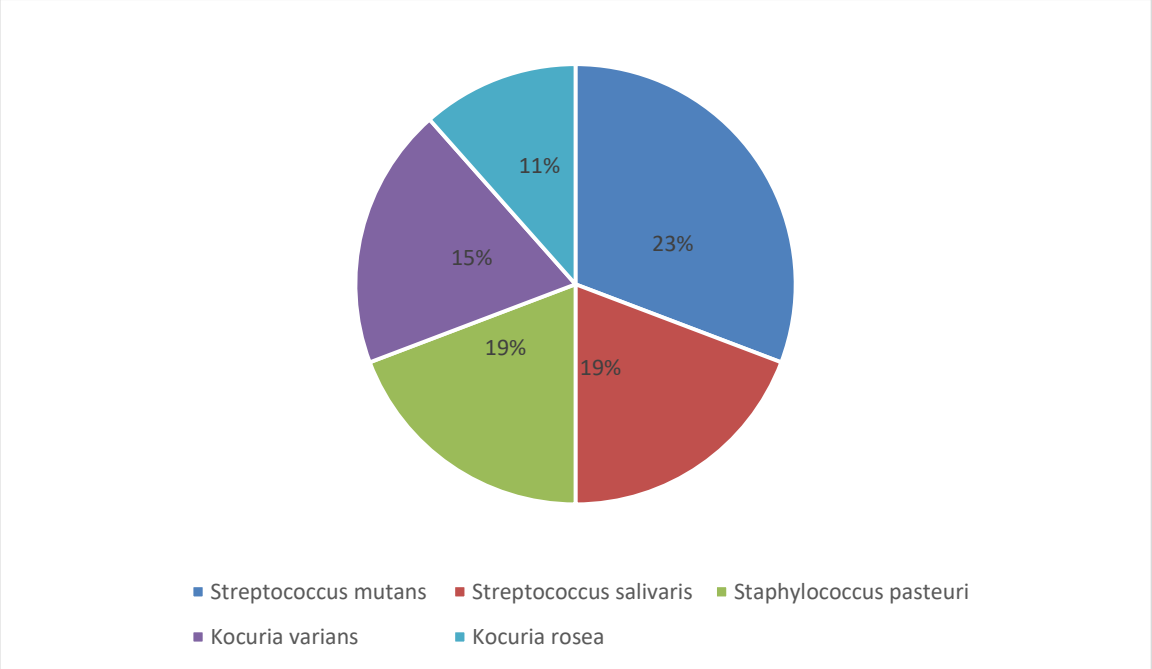

Figure 1 Percentage Distribution of 26 isolate into 5 different bacterial strains

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