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To Isolate, Identify & Check Effect of Medicinal Plants on Biofilm Forming Oral Pathogen

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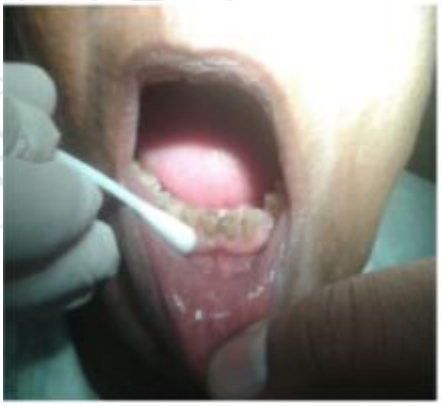
Abstract: ***The aim of this study was to investigate the oral infection causing bacteria and check affect of plant extract on bacteria by antimicrobial activity. Worldwide 60-90% children and nearly 100% adults suffering by dental cavities, severe periodental disease is found in 15-20% of middle aged adults, about 30% people aged 65-74 suffering from tooth loss. A total of 28 samples were collected out of that 50 bacterial isolates were isolated and from that 38 isolateswere identified as biofilm producing bacteria by tube assay & microtiter plate assay. The bacteria found were Staphylococcus aureus, Streptococcus viridans, Streptococcus mutans, Pseudomonas aeruginosa, Lactobacilli spp., Veillonella spp. causes skin and soft tissue infection, periodontal abscess and gum infection, soft tissue infection, gum and tooth infection, chronic periodentitis respectively. The bacterial isolates were identified and with the help of methanol plant extract antimicrobial activity were checked. To check affect on oral infection causing bacteria by antimicrobial activity. In conclusion, Plant extract might be suitable treatment of oral infection causing bacteria.***

Keywords: Oral bacteria, Plant extraction, Antimicrobial activity

1. Introduction

The study of bacteria in oral cavity, that can cause infection in or around the mouth. That is known as oral infection. All types of microbes living in mouth. The human oral cavity contain a numbers. of different bacteria with number of different habitats, including the teeth, tongue, gingival soft & hard palates, etc colonized by bacteria[1].Three types of oral infection occurs in human oral cavity (1) Dental plaque (2) Dental carries & (3) Abscess.These are most common cause of oral infection[2],[3],[4].The bacteria which were present in mouth- *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Lactobacilli spp*., *Veillonella spp*. causes skin and soft tissue infection, periodontal abscess and gum infection, soft tissue infection, gum and tooth infection, chronic periodentitis respectively[5].Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections. Infectious processes in which biofilms have been implicated with formation of dental plaque in oral cavity.For many years we referred to the sticky substance formed on our teeth as plaque. The new word for this substance on our teeth is biofilm.Biofilm is an association of bacteria that forms a unity on the surface. Bacteria live in biofilms wherever there is water, including oceans, sewers, water, pipes and of course the oral cavity. Biofilms are very sophisticated systems and dental plaque is a classic biofilm [6]. The natural treatment of dental infection involves using a series of natural remedies that can prevent it or reduce its symptoms, Among the natural remedis, to cure infection we have used the following; *Piper betle*[Pan], *Azadirachtaindica*[Neem], *Eucalyptus globules* [Nilgiri], *Zingiberofficinale*[Ginger], *Ocimum sanctum* [Tulsi][7],[8].Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries [9]. Leaves of the neem have been used in the treatment of gingivitis and periodontitis [10]. Neem has also showed better efficacy in the treatment of oral infections and plaque growth inhibition in treating periodontal disorders.*Eucalyptus globules* leaves that contain the ingredients used for therapeutic purposes, such as the essential oil. It is usually collected from the dried leaves. Dental use of oil: For treatment of gum diseases as antiplaque agent for bleeding gums for halitosis Stomatitis.[11] The medicinal power of ginger can cure many diseases. *Ocimum Sanctum*widely used in the treatment of several systemic diseases because of its anti-microbial property.[12] However, studies documenting the effect of Tulsi on oral disease causing organisms are rare.

2. Materials & Methodology



Collection of sample from patient's oral cavity by using sterile swab

The sample were collected from patient's oral cavity. The sample were obtained using sterile swab and added in sterile trypticase soy broth. After the enrichment the sample were cultured on trypticase agar plate [Himedia]. The selective medium was used to identify various oral infection causing bacteria. Different colonies were used for physical characterization of isolates[13]. For confirmation, Biochemical tests for various isolates were carried out[14]. To identify the biofilm producing isolates, We were using tube assay[15][16] and microtitre plate assay.[17] The

isolates were checked for antimicrobial against medicinal plant extract. [18],[19],[20].

3. Results & Discussion

3.1 Results

During the study, A total 28 samples were processed. The sample collected from different dental clinic around surat city. Total 51 samples were collected from BAPS hospital (Surat), Paliba clinic (Khergam), Shree clinic (Kim), Poojan dental clinic and Akshar Dental clinic (Killa-pardi) respectively. A total of 38 bacterial strains were isolated from 28 patient's sample in which Gram negative anaerobic bacteria were predominantly observed, (*Veillonella spp.*) and the most frequently isolated bacteria were *Staphylococcus aureus*, *Streptococcus viridans*group, *Streptococcus mutans*and also recorded *Pseudomonas aeruginosa*the Gram negative bacteria.[Figure No:1]

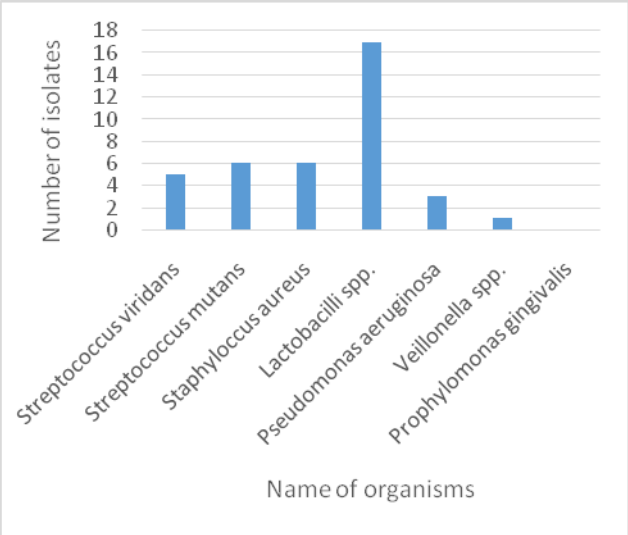


Figure 1: Numbers of bacterial strains isolated from oral cavity

In the Study, The isolates were obtained during primary isolation by four flame method. Total 50 isolates were found by using trypticase agar plate. To identify anaerobic or aerobic bacteria selective media were used. Like*Streptococcus viridans* group, *Streptococcus mutans, Lactobacilli spp., Staphylococcus aureus, Pseudomonas aeruginosa, Veillonella spp., Prophylomonasgingivalis* were used Mitissalivarius agar medium, Man Rogasa agar medium, Mannitol salt agar medium, Kings B base agar medium, Veillonella agar medium, and wilkinschlgren anaerobic agar medium respectively.

The isolates number 7B,7D,7E,8Agave large, mucoid, gummy colonies blue in color of *Streptococcus viridans* groupon Mitissalivarius agar plate. [Figure No:2] Isolates number 12A,13C,16D,19A,22B,23B,25A,27A,28E gave convex, opaque,pale blue colonies of *Streptococcus mutans*on Mitissalivarius agar plate.[Figure No:3] Isolates number7C,8C,8D,8E,15B,14B,16B,17A,17B,17C,20C,20D, 22A,23C,26A,26C,27D,28A,28D gavelarge, rhizoidal, convex, opaque colonies of *Lactobacillispp.* on Man Rogasa agar plate. [Figure No:4] Isolates number5C,17D,18A,18B,19C,21A,21B,24A gave Circular, convex, golden yellow colour colonies of *Staphylococcus aureus*onMannitol salt agar plate. [Figure No:5] Isolates number 13A,16A,16C gave small, undulate,blueish green color colonies of *Pseudomonas aeruginosa* on Kings B base agar plate. [Figure No:6] Isolates number 14A,15A gave diamond shaped, butyrous, grayish white color colonies of *Veillonellaspp* on Veillonella agar base plate.[Figure No:7] Isolates number 23A,24B,27C,28B,28C gave grayish-white color colonies of *Prophylomonasspp* on Wilkins chalgren anaerobic agar plate. [Figure No:8]

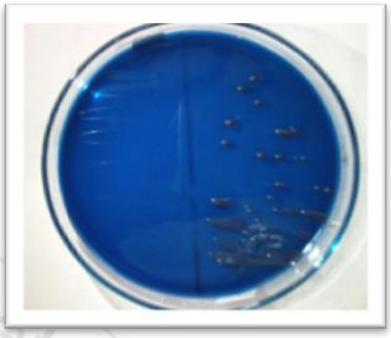


Figure 2: Mitissalivarius agar plate,*Streptococcus viridans* group

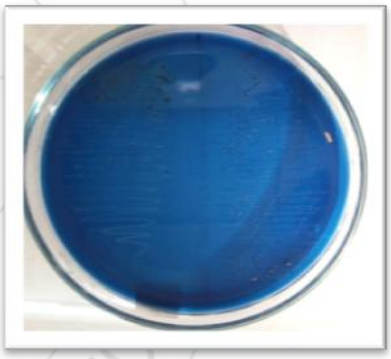


Figure 3: Mitissalivarius agar plate,*Streptococcus mutans*

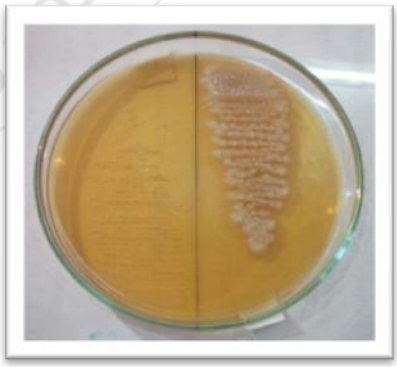


Figure 4: Man Rogasa agar plate, *Lactobacilli spp.*

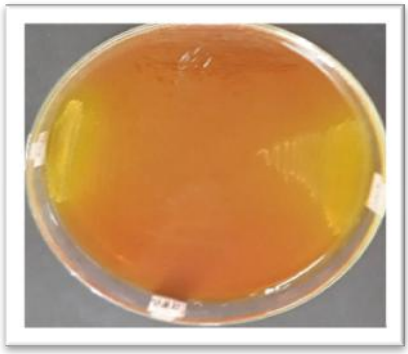


Figure 5: Mannitol salt agar plate,*Staphylococcus aureus*

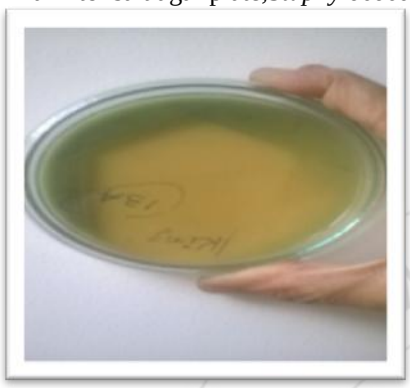


Figure 6: Kings B base agar plate,*Pseudomonas aeruginosa*

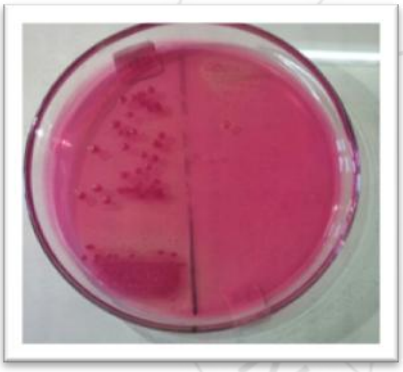


Figure 7: Veillonella agar base plate,*Veillonellaspp.*

Figure 8: Wilkins chalgren anaerobic agar plate,*Prophylomonasspp.*

For confirmation of *Streptococcus viridans* group, *Streptococcus Mutans* and *Staphylococcus aureus* blood agar plate were used it givesthe α, β, γ – haemolysis. [Figure no:9] In which α Haemolysis, β haemolysis, and γ haemolysis were found for 4 isolates, 2 isolates, and 11 isolates respectively.

Figure 9: Blood agar plate

Table 1: Result of blood agar plate of isolates

Reaction	Isolates Number
α-haemolysis	7E,17D,18B,19C
β-haemolysis	5C,24A
γ-haemolysis	13C,12A,27A,16D,18A, 18B,7B,19A,7D,8A,28E,23B,25A,21A,21B

Confirmation of Various biochemical test were carried out for identify of isolates.Toidentify isolates, perform the confirmatory biochemicals test, Like catalase test, oxidase test, bile esculin hydrolysis test, mannitol fermentation test, citrate utilization test, indole test, methyl red test,Voges-Proskauertest,nitrate reduction test, H_2S production test, Urea utilization test were carried out as per standard method. [Table No:2]

Table 2: Numbers of bacterial strains confirmed by biochemical test

Isolate No.	ProbableIdentify		Isolate No.	ProbableIdentify
5C	*Staphylococcus aureus*		17A	*Lactobacillus spp.*
7B	*Streptococcus viridans*		17B	*Lactobacillus spp.*
7C	*Lactobacillus spp.*		17C	*Lactobacillus spp.*
7D	*Streptococcus viridans*		17D	*Stapylococcusaureus*
7E	*Streptococcus viridans*		18A	*Staphylococcus aureus*
8A	*Streptococcus viridans*		18B	-
8C	*Lactobaciilus spp.*		19A	*Streptococcus mutans*
8D	*Lactobacillus spp.*		19C	-
8E	*Lactobacillus spp.*		20C	*Lactobacillus spp.*
12A	*Streptococcus mutans*		20D	*Lactobacillus spp.*
13A	*Pseudomonas aeruginosa*		21A	*Staphylococcus aureus*
13C	*Streptococcus viridans*		21B	*Staphylococcus aureus*
14A	*Veillonella spp.*		22A	-
14B	*Lactobacillus spp.*		22B	*Streptococcus mutans*
15A	-		23A	-
15B	*Lactobaciilus spp.*		23B	*Streptococcus mutans*
16A	*Pseudomonas aeruginosa*		23C	*Lactobacillus spp.*
16B	*Lactobacillus spp.*		24A	*Staphylococcus*

				aureus
16C	*Pseudomonas aeruginosa*		24B	-
16D	*Streptococcus mutans*		25A	*Streptococcus mutans*
17A	*Lactobacillus spp.*		26A	*Lactobacillus spp.*
17B	*Lactobacillus spp.*		26C	*Lactobacillus spp.*
17C	*Lactobacillus spp.*		27A	-
17D	*Stapylococcusaureus*		27C	-
18A	*Staphylococcus aureus*		27D	*Lactobacillus spp.*
18B	-		28A	*Lactobacillus spp.*
19A	*Streptococcus mutans*		28B	-
19C	-		28C	-
20C	*Lactobacillus spp.*		28D	-
20D	*Lactobacillus spp.*		28E	-

After confirmation 38 isolates were further processed to check the biofilm formation by tube assay and microtitre plate assay. We obtained 35 isolates positive by tube assay [Figure No: 10] and 0 non adherent (O.D.<ODc),18 weakly adherent (ODc<OD<2×ODc), 15 moderately adherent (2×ODc<OD<4×ODc), 4Strongly adherent (4×ODc<OD), total 38 isolates by microtitre plate assay.[Figure No: 11]

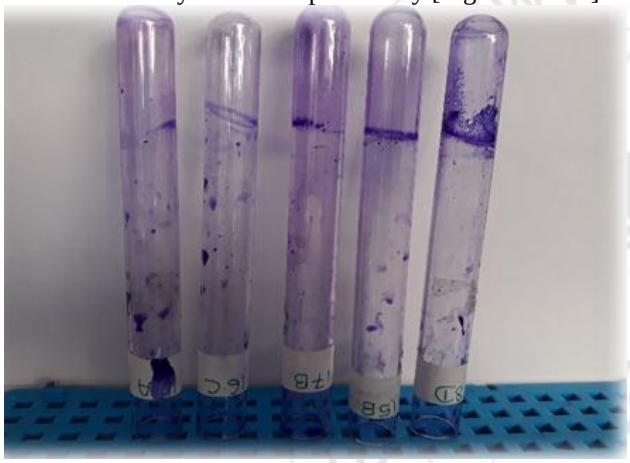


Figure 10: Biofilm formation by tube assay

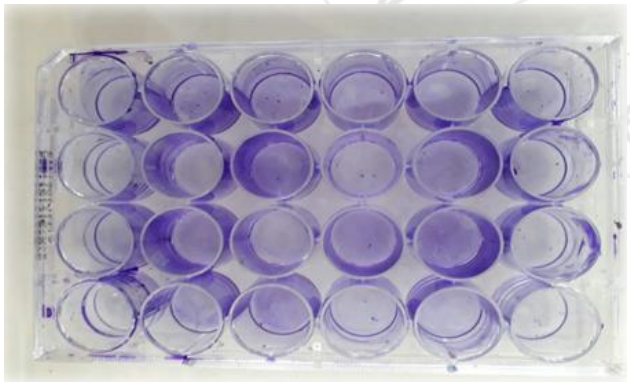


Figure 11: Biofilm formation by microtitre plate assay.

The 38 biofilm forming isolates were checked for antimicrobial test against the medicinal plant extract by using methanol extraction method. Some are susceptible and some are resistant towards plant extract as shown in figure No. 12

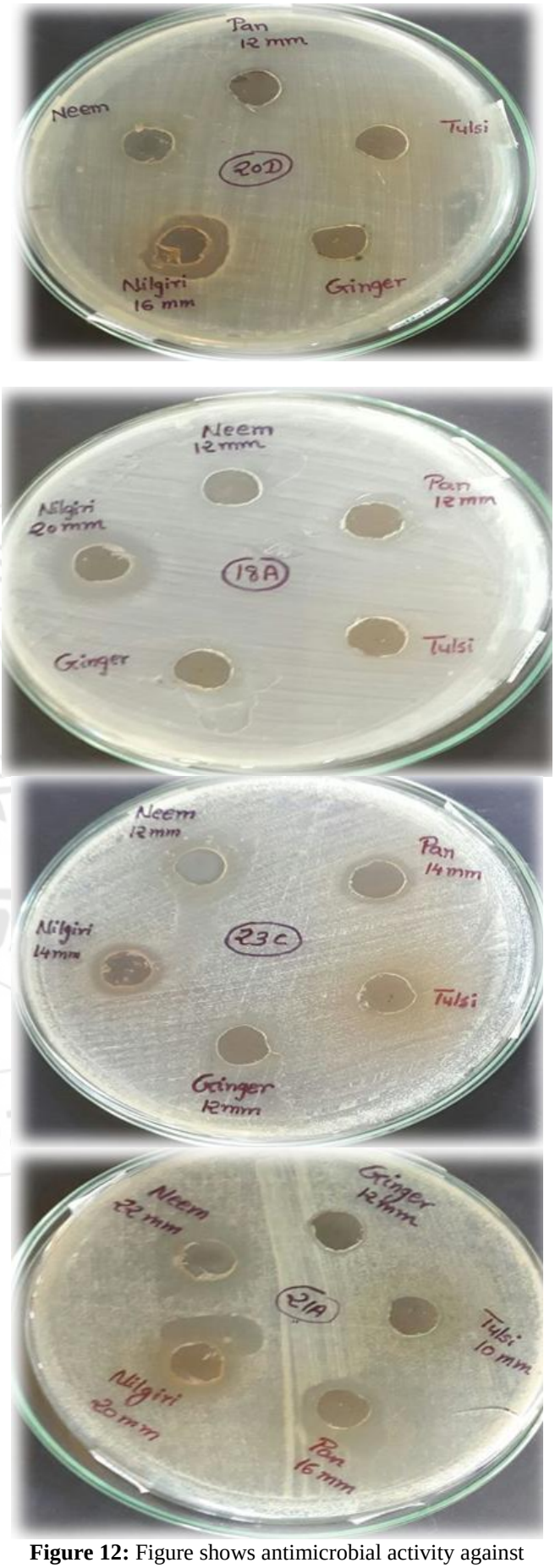


Figure 12: Figure shows antimicrobial activity against medicinal plant

Total 38 biofilmproducing isolates-*Streptococcus viridans, Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa, Lactobacilli spp., andVeillonella spp.* bacterial isolates show resistant as well as intermediate effect and also *Staphylococcus aureus*show susceptible effect against the plant extract by antimicrobial activity.

4. Discussion

The study highlights the screening and to check the antibacterial activity of oral biofilm forming pathogen by using medicinal plant extract. Total 38 isolates- biofilm forming were confirmed by tube assay and microtitre plate assay,which were further used to check the effect of medicinal plant extract.Oral bacterial species mostly lack an environmental niche and are found almost exclusively within the mouth [21]. In 2006,mathur et al., work on Formation of biofilm which observed by tube and concluded on the basis of attachment of biofilm. Microtitre plate concluded as non adherent, weakly adherent, Moderately adherent, strongly adherent[22]. In our study we have identified total 35 isolates by biofilm formation, In which0 non adherent,18 weakly adherent,15 moderately adherent,4 Strongly adherent. The bacteria which present in the samples were identified by morphological and biochemical characteristics.The isolated bacteria were subjected to antimicrobial assay by disc diffusion method against aqueous and methanol extract. Both aqueous and methanol extract showed inhibitory effect against the tested bacteria.Methanol extract exhibited larger zones of inhibition against all the isolated organisms. Betel leaves extracts showed more activity compared to neem leaves extracts.The identified bacteria used for antimicrobial assay by disc diffusion method against the methanol. Betel leaves, neem leaves and *Eucalyptus globules* leaves extract exhibitedlarger zone of inhibition compared to *Ocimum sanctum* and *Zingiberofficinale* leaves extract, work done by R Salam et al in 2014 [23].LinchuKuruvilla, 2012 were used the sample to characterized based on their macroscopic, microscopic,biochemical and physiological characteristics.Antibacterial effect of leaves of five medicinal plants *Piper betle, Areca catechu, Eucalyptus globules, Zingiberofficinale* and *Azadirachtaindica* were carried out in solvent systems(acetone, ethanol, chloroform, methanol and water)by disc diffusion method. Among the five plants extracts, three of them(Piper betle, Eucalyptus globules, Azadirachtaindica) were more effective in producing antibacterial property[24]. LinchuKuruvilla used five plant for antibacterial effect.In our case we have used five medicinal plant *Piper betle, Eucalyptus globules, Zingiber officinale, Azadirachta indica* and *Ocimum sanctum* & were carried out in one solvent system methanol instead of others by disc diffusion method. In that *Piper betle, Eucalyptus globules, Azadirachtaindica*were most effective against the identified isolates.

5. Conclusion

During the study, A total 28 samples were processed. From that samples, Total 50 bacterial strains were isolated by primary screening. Total 38 isolates confirmed by biochemical test. Those were further used to check biofilm formation by tube assay and microtitre plate assay, so we obtained 38 biofilm producing isolates. The *Streptococcus viridans, Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa, Lactobacilli spp., and Veillonella spp.* bacterial isolates were resistant as well as intermediate effect and also *Staphylococcus aureus* show susceptible effect against the plant extract by antimicrobial activity.Therefore, it has a potential for application using as a medicines as such or in combination with other. Betel leaves, neem leaves and *Eucalyptus globules* leaves extract showed the highest activity compared to *Ocimum sanctum* and *Zingiberofficinale* leaves extract.

Use of medicinal plant instead of antibiotics because the synthetic drug causes side effects, So natural plant compound based on the new development of drug, it could be useful for new drug with minimal side effects. Possible that plant extract may take a role as an adjuvant to the use of antibiotics or as a replacement of current antibiotics to treat the opportunistic infection.

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