ISSN (Online) 2582-9327

IJARSCMT

International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 2, Issue 12, December 2021

Antibiofilm Activity of Essential Clove Oil Bacteria

Komal Bernwal

NSS College of Engineering, Palakkad, Kerala, India

Abstract: *Samples were collected from mouth by swabbing across the gingival and subgingival region, as well as from the roof and floor of the buccal cavity from individuals. Samples were first inoculated in Nutrient broth (HiMedia, India) and viable cells were enumerated on Nutrient Agar (HiMedia, India) after 48 h of incubation at 37 °C. Affer 48 hrs turbidity was observed. After 24 hrs well isolated colonies were obtained then prepare Nutrient agar slants for subculture the isolate. The biofilm forming bacteria was identified by using Christensen tube method. A loopful of test organism inoculated in test tubes containing 10 ml of trypton soy broth with 1 ml of 1% glucose solution and incubated all the tubes at 37°c for 48hrs. After the incubation the tubes were decanted and then washed with phosphate saline buffer, then stained it with crystal violet (1%) then wash the tubed with distilled water then air dried and then examine for identification of biofilm forming activity. In this method the results obtained according to biofilm production activity of bacteria like strong, weak and moderate. Escherichia sp, gives strong biofilm production activity. Antibiofilm activity of essential clove oil was done using Kirby-disc diffusion method. Different concentration of clove oil was prepared using DMSO. The zone of inhibition was measured using zone scale. Clove oil shows maximum antibiofilm activity against isolated biofilm producing bacteria. Clove oil show antibiofilm activity against B3, B7, B15, B21.*

Keywords: Biofilm, Clover oil, Antibiofilm, *Eschericha coli*

I. INTRODUCTION

Biofilms is the community of microorganisms living together in amorphous extracellular matrix composed of polysaccharides, extracellular DNA and proteins. In the nature we have found that biofilm can develop both on biotic and abiotic surfaces. A biofilm is an accumulation of microorganisms embedded in an exopolysaccharide matrix of microbial and host origin called Polysaccharide Intercellular Adhesin (PIA) (1). A biofilm is a matrix of microorganisms with extracellular substances (EPS) that can be formed on different surfaces. These surfaces can be animal tissue (meat, fish products), catheter, teeth, stainless steel, plastic, glass, teflon, rubber, wood, etc. Biofilm formation can be divided into five parts. First (1-2): reversible and irreversible attachment to the surface; 3: development of the extracellular matrix; 4: maturation of the biofilm and; 5: dispersion. In reversible stage, the bacteria attach to surfaces with van der Waals attraction forces, electrostatic forces and hydrophobic interactions. During this phase, bacteria can be removed from surfaces easily for example by rinsing. If bacteria interact with surfaces by dipoledipole, hydrogen, ionic or covalent bonding, removal will be more difficult (2). The finishes

ISSN (Online) 2582-9327
IJARSCMT
International Journal of Advanced Research in Science, Commerce, Management and Technology
Impact Factor: 5.781
Volume 2, Issue 12, December 2021

of stainless steel surfaces also influence bacterial attachment. The adherence is stronger to untreated or sandblasted surfaces than to electropolished area (3). After attachment the bacteria form microcolonies and produce a matrix called extracellular polymeric substance (EPS). The EPS protects bacteria within the biofilm (cells are more tolerant to stress factors) and is responsible for binding. It is composed of polysaccharides, proteins, nucleic acids, lipids (4). When biofilms consists of different microorganisms, the matrix is thicker and more stable than the matrix of single species biofilms. If the biofilm reaches the maximum, starvation, enzymatic degradation and increased fluid shear will appear. The cells from the top of the biofilm will disperse and colonize new surfaces. Biofilms can occur in different industrial and medical environments. 65-80% of infections are related to biofilms (5). Biofilms are surface associated multicellular communities in which cell are held together by means of a self-produced extracellular matrix.). A biofilm is a sessile form of bacterial existence on solid surfaces or air- liquid interfaces, in which bacteria multiply covered by self-produced biofilm matrix. The ability to form biofilms is a unique and universal feature of bacteria. Bacteria biofilms are known to be developed in host epithelial cells, bones, tooth, walls of blood vessel and 2 medical appliance. The process of biofilm formation occurs through a series of events leading to adaptation under diverse nutritional and environmental conditions. Biofims are formed by various different steps like attachment initially to a surface, formation of micro-colony, three dimensional structure formation and last is biofilm formation, maturation and detachment. Biofilms affect human life in different ways, such as food, environment and public health. Nearly all (99.9%) of microorganisms have the ability to form biofilm on a wide range of surfaces. Biofilms posing a great problem for public health due to its resistant nature to antibiotics and disease associated with indwelling medical divices. Biofilm forming bacteria Nearly all (99.9%) of micro-organisms have the ability to form biofilm on a wide range of surfaces i.e. biological and inert surfaces (6). When micro-organisms bind to a surface, they produce extracellular polymeric substance (EPS) and form biofilm. Biofilm posing a great problem for public health due to its resistant nature to antibiotics and disease associated with indwelling medical devices (7). It is found that H. influenza has the ability to form biofilm in human body and can escape from human immune system. Biofilm forming capability has been reported in large number of bacterial species such as P. aeruginosa, S. epidermidis, E. coli spp, S. aureus, E. cloacae, K. pneumoniae (8,9,10,11).

In this study we report antibiofilm activity of essential clove oil against biofilm forming bacteria.

II. MATERIAL AND METHODS

2.1 Methods

A. Collection of Sample:

1. Sample Collection and Culture Condition

Samples were collected from mouth by swabbing across the gingival and subgingival region, as well as from the roof and floor of the buccal cavity from individuals. Samples were first inoculated in Nutrient broth (HiMedia, India) and viable cells were enumerated on Nutrient Agar (HiMedia, India) after 48 h of incubation at 37 °C. Six colonies with visually distinguishable morphologies

ISSN (Online) 2582-9327
IJARSCMT
International Journal of Advanced Research in Science, Commerce, Management and Technology
Impact Factor: 5.781
Volume 2, Issue 12, December 2021

were randomly selected for further studies. The viability of the isolated cultures was checked in Luria Bertani (LB) broth (HiMedia, India) and screened for biofilm formation.

2. Collection of Essential Clove Oil

The essential clove oil used in the present experiment obtained from local medical store.

B. Identification of Biofilm Forming Bacteria

1. Christensen Tube Method

This is a qualitative method for biofilm detection. A loopful of test organisms inoculated in 10 ml of trypton soy broth with 1% glucose in test tubes. Incubate the tubes at 37°c for 48 hrs. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Wash excess stain with distilled water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible thick film lined the wall and the bottom of the tube. (Christensen et al., 1982)

2. Antibiofilm Activity of Clove Oil (Kirby-Bauer disc diffusion method).

Disc diffusion method was done using Muller-Hinton Agar. Prepare cellulose (whatmann No. 1) filter paper discs. Different concentration of clove oil was prepared using DMSO (DimethylSuphoxide). Dip the paper disc in different concentration of clove oil and then placed these diffused paper disc on Muller -Hinton agar plates swabbed with different biofilm forming bacteria. The plates were incubated at 37°c for 24hrs. After 24hrs the zone of inhibition was measured using zone scale.

III. RESULT AND DISCUSSION

The total 4 samples were collected. These samples then isolated on Nutrient agar. The biofilm forming activity of these isolate were identified by tube method.

Table 1: Biofilm production activity of isolates from hospital samples

Isolates	Oral Cavity Sample	Biofilm production activity
B3	Gingival Region	Strong
B7	Subgingival region	Weak
B15	Roof of Buccal Cavity	Strong
B21	Floor of Buccal Cavity	Strong

These isolated bacteria were then tested for their ability to form biofilm in trypton soy broth using Christensen's tube method where enhanced growth was seen in some tube and some shows very poor growth in trypton soy broth. Then biofilm forming potential was visualized by Christensen's

ISSN (Online) 2582-9327

IJARSCMT

International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 2, Issue 12, December 2021

tube method using crystal violet stain and indicated the clear results of positive potential of isolated strains to form strong and weak biofilm.

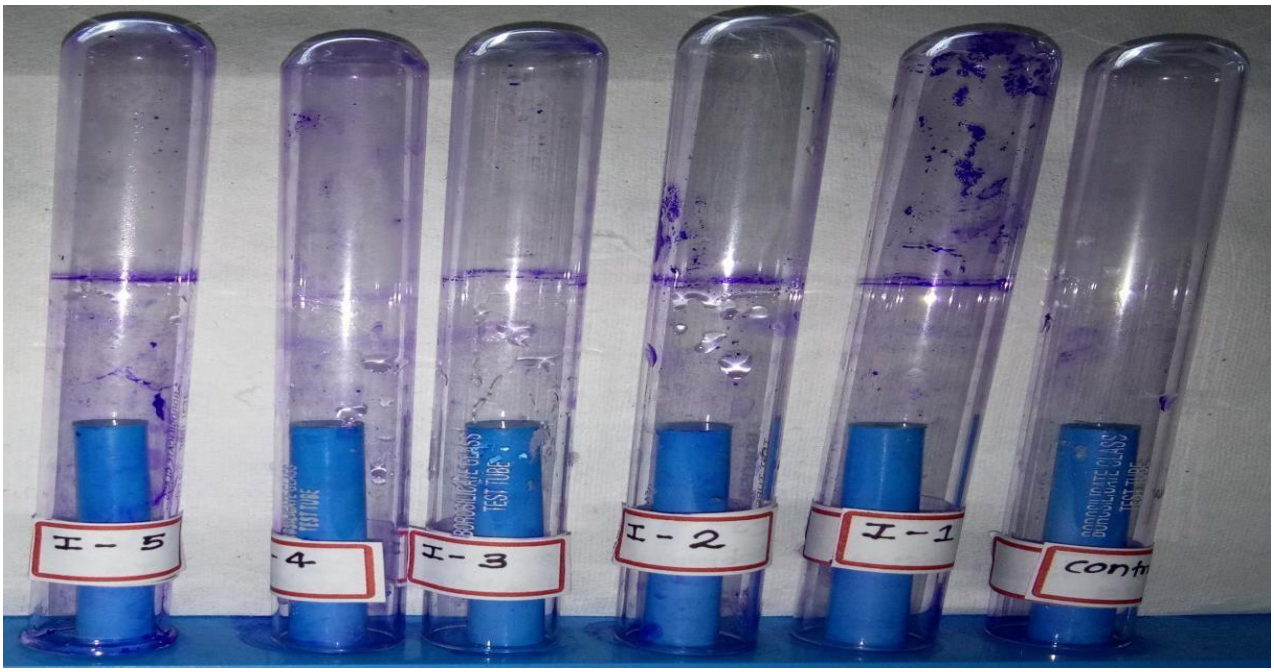

Figure 1: Biofilm activity of different isolates by Christensen tube method

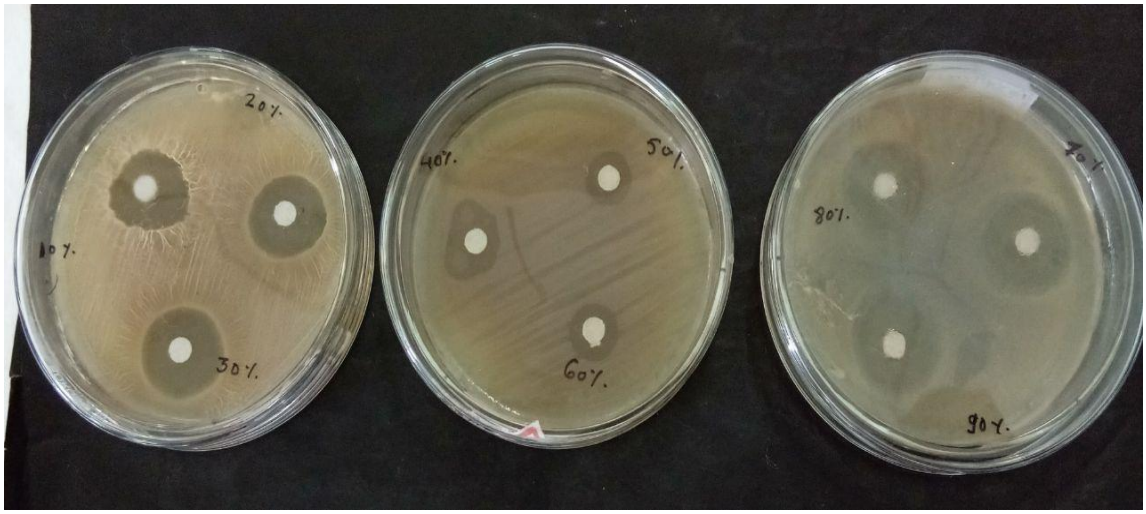

Figure 2: Antibiofilm activity of isolate 1

IV. DISCUSSION

Christensen tube method was used to detect the biofilm forming activity of the isolates. Similar finding were also noticed in present study as biofilm forming bacteria were isolated and identified in clinical isolates collected from hospitals. In the disc diffusion method performed to determine the antibiofilm activity of essential clove oil, shows inhibition zones between 13-15mm were identified against all the isolates at different concentration. The essential clove oil was found to have different properties of antibiofilm activity against all Gram negative and Gram positive strains Many plant compounds have been used for development of new antimicrobial agents. However, few plant

ISSN (Online) 2582-9327

IJARSCMT

International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 2, Issue 12, December 2021

extract have been investigated for their antibiofilm activity and some other studies have shown that antimicrobial agents can stimulate biofilm formation by different bacteria. *Escherichia coli sp.*, *Styphalococcus aureus sp., Styphalococcus epidermis sp., Proteus sp., Pseudomonas sp.*, *Klebsiella sp*. was generally known to cause nosocomial infection and may be inweding medical diveces and responsible for biofilm production (12,13).

Clove oil contains phenolic acids such as thymol, eugenol, carvacol are secondary plant metabolites that account for the antimicrobial activity of essential oils such as oregano, cinnamon and clove. The results have shown that the clove oil was more effective and created a zone of inhibition in bacterial strains. In case of clove oil, the measurments for zone of inhibition showed *E. coli* was more resistant. Eugenol in clove oil is 79.2%. The inhibitory activity of clove is due to the presence of several constituents (13,14,15).

Biofilm formation enables bacterial pathogens to colonize a wide variety of host niches and persist in harsh environments, making their eradication particularly difficult Biofilm characteristics determine whether, to what extent, and which antimicrobial treatments may be Biofilm formation enables bacterial pathogens to colonize a wide variety of host niches and persist in harsh environments, making their eradication particularly difficult Biofilm characteristics determine whether, to what extent, and which antimicrobial treatments may be effective. The age and composition of the biofilm are the major factors influencing the susceptibility of the resident microorganisms. As the biofilm matures, increased EPS accumulation, combined with the nutrient and oxygen gradients that affect cell metabolism and growth rates, result in reduced entry and activity of antimicrobial agents making biofilm- forming pathogens progressively more resistant to antibiotic regimens. Thus, novel strategies, designed to block a specific biofilm step without killing the bacteria, such as the use of antiadhesion agents, or using natural, bacterially produced signals to promote bacterial dispersal, are exciting avenues for exploration and ultimately the development of fast-acting, potent, and bioavailable treatment strategies.

V. CONCLUSION

This study concludes that essential clove oil has positive effects on antibiofilm formation by isolates B1, B7, B15, B21. This is because clove oil has high level of eugenol and eucalyptol components. Christensen tube method is quantitative and reliable method to detect biofilm forming microorganism. Essential oils are composed of numerous different chemical compounds and their antimicrobial and antibiofilm activity might be attributed to several different mechanisms. In the present study, essential clove oil have show antibiofilm activity on both gram positive and gram negative bacteria biofilm infection is difficult to treat .

To better understand and control biofilms on indwelling medical device, research must progress in several techniques for collecting should be developed quantification of the biofilm depends on the number of organisms recovered by contact with the agar surface.

ISSN (Online) 2582-9327

IJARSCMT

International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 2, Issue 12, December 2021

REFERENCES

[1]. Srey S, Jahid IK, Ha SD. Biofilm formation in food industries: a food safety concern. Food control (2013); 31: 572-588

[2]. Arnold JW, Bailey GW. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scaning electron and atomic force microscopy study. Poult Sci. (2000); 79: 1839-1845.

[3]. Simoes m, Simoes LC, Vieira MJ. A review of current and emergent biofilm control strategies. LWT-Food Sci Technol (2010); 43:573-83.

[4]. Coenye T, Nelis HJ. Review, In vitro and in vivo model systems to study microbial biofilm formation. Journal of Microbiological Methods (2010); 83:89-105

[5]. Sekhar S. Role of biofilm formation in the persistent colonization of Haemophilus influenza in children from northern India. J Med Microbial (2009); 58:1428-1432.

[6]. Khan S. Isolation of Shigella species and their resistance patterns to a panel of fifteen antibiotics in mid and far western region of Nepal. Asian Pacific J Tropical Dis (2014); 4:30-34.

[7]. Fux C. Survival strategies of infectious biofilms. Trends microbial (2005); 13:34- 40.

[8]. Ma L. Assembly and development of the Pseudomonas aeruginosa biofilm matrix. Plos pathogens (2009); 5:e1000354.

[9]. Parsek MR and Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis.

[10]. AnnuRev Microbiol (2003); 57:677-701.

[11]. Saharkhiz MJ, Motamedi M, Zomorodian K, Pakshir K, Miri R, Hemyari K. Chemical composition, Antifungal and Antibiofilm activities of the essential oil Mentha piperita L. ISRN Pharm article 718645: 6.

[12]. Sharma S, Singh S, James bond, Singh A, Rustagi A. Evaluation of antibacterial properties of essential oils from clove and eucalyptus. Asian J Pharm. clin res, vol 7 (2014); 5:291-294.

[13]. Afreenish Hassan, Javaid Usman, Fatima Kaleem, Maria Omair, Ali Khalid,Muhammad Iqbal. Evaluation of different detection methods of biofilm formation in the clinical isolates. (2011).

[14]. Costeron JW, Stewart PS, Greensberg EP. Bacterial biofilms: a common cause of persistant infections. Science (1999); 284:1318-22.

[15]. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods for biofilm formation in the clinical isolates. Braz J Infect Dis (2011); 15:305-11.

[16]. Lee JH, Park JH, Cho HS, Joo SW, Cho MH, Lee J. Anti-biofilm activities of quercetin and tannic acid against Staphylococcus aureus. Biofouling (2011); 29(5): 491-499.

[17]. Rewatkar AR, Wadher BJ. Staphylococcus aureus and Pseudomonas aeruginosa-Biofilm formation methods. IOSR-JPBS (2013); 5:36-40.