IJPSR (2020), Volume 11, Issue 1 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 April 2019; received in revised form, 08 August 2019; accepted, 13 August 2019; published 01 January 2020

A REVIEW ON ANTI-BIOFILM INHIBITOR FROM PLANT ESSENTIAL OILS

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Keywords:

Microbial biofilms, Antibiofilm activity, Essential oils and Analgesic effect

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ABSTRACT: Bacteria predominantly remain in a self-produced polymeric matrix, adherent to an inert or living surface. This microenvironment community of bacteria is known as biofilm. Commonly visualized as a slimy layer, a number of unique features distinguish biofilms from their planktonic counterparts. Formation of biofilms depends on the extracellular signals, mechanical, biochemical, environmental conditions and genetic factors in bacteria. The attachment of biofilms is an intriguing process which is regulated by several characteristics such as substratum, cell surface, and growth medium. These biofilms cause various infections and are of at most importance when concerned about public health. One of the challenges faced during antibacterial drug development is to create compounds to counter-attack the biofilm infections. In this study, antibiofilm activities of five essential oils (EUGENOL; CINNAMALDEHYDE; PIPERIDINE; LIMONENE) were discussed. This review consisting of explicit evidence proved that plants are indeed a wonderful source to provide naturally occurring compounds for development of therapeutic and preventive agents against infections caused by biofilms.

INTRODUCTION: Biofilm is defined as a thick layer of prokaryotic organisms that have been aggregated to form a colony or community. The colony attaches to a surface with a slime layer, thereby protecting the micro-organisms. There are numerous factors why biofilms are developed, and all of which promote growth, the survival of the microorganisms [1]. One strong replacement proves to be the look for naturally crop up element of plant origin capable of inhibiting biofilm development. From decades, plant distillate and their biologically concentrated active compounds have been a source for rich natural products, which have a major role in inhibition and curing diseases, source to maintain a balance human health.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(1).14-24

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(1).14-24

Furthermore, they are widely accepted due to the perception that they are safe and have a long history of use in folk medicine to cure diseases and illnesses since ancient times.

Biofilm Structure: The study shows that the transport and transformation processes in biofilms influenced by the biofilm structure, has been keenly investigated in the microscopic level using electrode and various biochemical tests. The biofilm is highly arranged which has been characterized by increase of density of the biofilm, decrease of biomass and variation of porosity with biofilm depth. As per the biofilm depth, both effective diffusivity for dissolved oxygen and effectiveness factor rate decrease. For the arranged or stratified biofilm structure, the substrate and space in biofilms affect the biofilm thickness. According to various biofilm thicknesses, the density and porosity tend shows different data. After analyzing all this information regarding biofilms, it not only bridges the gap between mathematical modeling and external observation of biofilms, it is useful in obtaining a clearer description of biofilms [2]. Studies regarding biofilm matrices and their related infections are the most viewed part with respect to clinical diseases. Most organisms which may be pathogenic or not, inhibit these biofilms for their survival and attach themselves to the substrate or surface present in such matrix. Such films act as home base for such various dangerous infections spread throughout and also allows to evolve and disseminate over time.

The microorganisms present in such biofilms develop their survival methodologies to adapt for various environmental conditions like growth regulation, population heterogeneity, and proteolysis systems, *etc.* The ability of pathogenic organisms is high to resist and respond to a variety of immunological activities. Generally, biofilms are a heterogeneous group of microorganisms which are present inside a coagulated matrix which consists of polysaccharide, protein, and DNA. Factors such as bacterial defense mechanisms, area for colonization, community cooperation benefits and mode of growth acts as a catalyst for biofilm formation. This complex matrix acts as a protective layer or barrier for the organisms present in the biofilm. But within a biofilm, single species or multiple species of organisms inhibit for survival. Such microorganisms can also vary the pH of environment which proves to be an important factor in biofilm formation [3].

Staphylococcus aureus (*S. aureus*), *P. aeruginosa*, *Acinetobacter baumannii* are some of the important organisms which thrive on medical devices and they are found to be 1000 times more resistance to antibodies as compared to other organisms.

Biofilm Properties: Usually the biofilm favors to develop in very high humid satiation in the exterior of leaves and liquid. They may also develop on solid surfaces which are immersed or open to a damp, where biofilm may possess a different kind of microorganisms. Based on the Rotating Biological Contactor instrument, various properties of the biofilm-like special distribution of biofilm density, population of bacteria, rates of biochemical reaction were measured [8]. In comparison with the biofilm depth, density and number of heterotrophs increases according to it but per unit biomass of intrinsic reaction rates did not change much in variation with depth of it [4]. Cells present in biofilms are responsible for sensing and interacting with their surroundings through the matrix. The condition of the cells change with respond to environment interface, are determined by physicochemical properties of the cells in matrix. These bio and physicochemical properties are defined by the structure and composition of the cells.

They help to modulate the properties such as detachment process and resistance to antibiotics. The matrix usually consists of only 10% bacteria and rest of dry mass of biofilm. Where the matrix is central key for response and signaling both in physical and chemical environment.

Biofilm Habitat: During the course of years, many researchers focused mainly on free-floating bacteria which existed freely in the environment, following the similar footsteps of Louis Pasteur and Robert Koch. In the 1970s, many scientists began to see the big picture of life forms of bacteria, by considering the existence of biofilms, which forms the majority component of bacterial biomass in the environment. After the 1970s, they deeply studied and found out how much complex can a bacterial biofilm community can exist [5].

A famous molecular biologist from the Montana State University shared that studying biofilms with heterogeneous groups proved to be more difficult than with homogenous groups. In this paper published in Nature Reviews, He states that biofilm culture has proved to be much more difficult which included factors such as diffusion of liquid through a biofilm and forces acting upon it. According to Stoodley, such difficult parameters to work with biofilms forced many scientists to deter from it. In shallow water rivers, constituting hard material substrates consist of epileptic biofilms which is the main site of primary production. In benthic food web functioning, the contribution of biofilms is of much significance, and its contribution can be noted in areas of biogeochemical processes like decomposition and nutrient retention. Epileptic biofilms are influenced by external disturbances such as floods, thermo dependent bacterial degradation of mat and they form a very much unlikely stable habitats.

Though most of the bacteria residing in biofilms in aquatic and soil environments, they carry out their usual biochemical processes, and they are responsible for prolonged pulmonary and urinary infections among humans. The infections caused by the biofilms are considered highly problematic because of their resistance to antimicrobials and their extreme diverse settings. So, many research works are carried out in this field to produce some productive results by considering the factors which control the activities of biofilms [6].

Earlier studies have shown that the development of biofilms is characterized by various environmental factors such as flow conditions under various habitats, oxygen and nutrient availability and hydrodynamic shear stress which explains about the attachment of cells to surfaces and consequently detachment of cells from biofilms. The growth of biofilms also changes the organic and inorganic nature of the environment. Examples like decrease in oxygen and nutrient content from surrounding environment and increase in surface friction between them. From these occurrences, it is highly important to study biofilms and environment interaction with multi-disciplinary approaches since they react with the environment in a very diverse and complex manner.

Biofilms consist of densely packed microorganisms which grow on surfaces considered to be living or nonliving, with polymers secreted by them. Based on the study conducted, biofilms are very much complex in nature, and their physical structure reveals that they are coordinated and act as cooperative groups [7].

Biofilms can be vaguely described as a group of organisms which attach themselves to substances which are identical in the manner as same as embedded in a matrix of EPS (Extracellular polymeric substances). Communication of bacteria within a biofilm is done by a process called quorum sensing which releases the chemical called pheromones. Many factors contribute to biofilm formation such as surface resins, motility of bacteria, nutrient availability. As biofilms are resistant to antimicrobials, very high concentrations of such agents are added to stop the growth of organisms in biofilms. In humans, more than 80% of infections are caused by biofilms.

Biofilm formation is made possible when it reacts to cellular recognition of various attachment sites on surface and even when it is visible to planktonic cells to concentrations of antibiotics. Phenotypic type of behavior occurs when a microorganism changes itself from being single to a member of a group. According to Marshall Pathogenesis, the bacterial pathogens cause chronic disease for which biofilm bacteria forms a major part [8]. By using low doses of antibiotics, the growth of persisted cells can be shunned because they are main reason for causing infections. There is found to be a major surge in research and analyses external biofilms. Such biofilms pose a major threat to industrial environments. They can cause corrosion in pipes internally which is difficult to rectify, and formation of biofilms on floors can make sanitation difficult [8].

Medical conditions like the dental plague, chronic infections, peritonitis, and urinary infections. The most commonly formed biofilms include bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus viridians,* and *Escherichia coli*. Methods to detect biofilm production include Tissue Culture Plate (TCP), Tube method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination.

Biofilm Formation Mechanisms: Several studies in this research field describe the formation of biofilms. Three stages of the biofilm life cycle: attachment, maturation, and detachment are just communities of bacteria embedded in extracellular polymeric matrix, and they are associated with numerous infections as they are resistant to antimicrobial treatments, and in some other cases they proved to be impossible to eradicate it. Attaching themselves to both biotic and biotic surfaces, it grows larger through cell division because of the availability of nutrients in it. And if such nutrients are prevented from reaching the cells, it may detach from the surface and return to a planktonic state. The biofilms which have been matured may show a different case of adhesion, growth, and detachment as shown in **Fig. 1**.

The aggregate of microbial on tooth surfaces was studied by Antoine van Leeuwenhoek. Heller showed that the growth and activity of marine microbes were improved depending upon the surface to which they were attached. Zobell found that more microbes were attached to solid surfaces during a study on marine bacterial populations [9].

Bacteria create their colony based upon various parameters such as nutrient content of soil, water pipe systems and living organisms. It is due to the fact that colonies are formed where a constant source of nutrient is available to protect them from environmental conditions [21]. This forms the main reason for biofilms to imbibe resistance against amoebae, host body, bacteriophage, *etc.* Due to this reason, biofilm engineering has become one of the important areas of research [23].

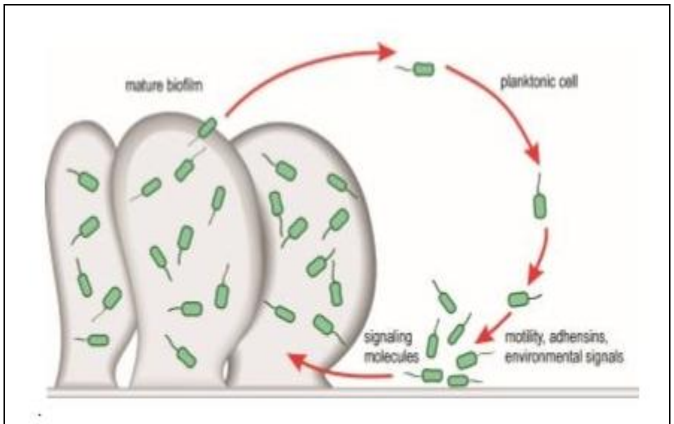

FIG. 1: MECHANISM OF BIOFILM DEVELOPMENT

Attachment: The suitable attachment area of microbes in aquatic environments. Various parameters which affect the bacterial adhesion are covalent bonds, Van Der Waals interactions, electrostatic interactions, acid-base interactions. Hence the adhesion created by microbes is dynamic and reversible and if they are disturbed by external factors, they return to their natural state. Bacterial mobility, cell membrane proteins, extracellular polysaccharides, and signaling molecules play an important role in biofilm formation. The mobility of bacteria can be distinctively activated by flagella and fimbriae, both being type of protein growth on cell surface. Flagellum helps the bacteria to float in liquid medium whereas Fimbria is responsible for movement on substrate surface. Some species of bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* showed that flagella and fimbriae are important for bacterial mobility. Flagellum is responsible for establishing contact between the biofilm and the surface while the other is responsible for formation of microcolonies [10].

Maturation: The attachments for bacteria were regulated by extracellular appendages like type IV pili, and the flagellum is given in **Fig. 1**. After biofilm attachment, maturation comprises of two parts: adhesive process and disruptive process. The adhesive process is associated with microbial multiplication and disruptive process with formation of water channels in three-dimensional structures. Due to this type of formation, increased production of EPS and antibiotic resistance development are strongly correlated with biofilm bacteria [13]. After a certain point of time, various microbes present in biofilm can protect themselves from various antibodies, and their resistance can be as much as 1000 times as that of in planktonic state.

The nature of constituents of EPS matrix is highly complex, and it usually comprises of polysaccharide biopolymer along with other substances like lipids, nucleic acids, *etc.* This matrix forms the base of the intercellular space of bacterial communities in biofilms. This complex structure depends upon various factors such as bacterial species, environmental conditions. Intercommunication of bacteria within a biofilm is done by means of a process called quorum sensing. This has been closely associated with the formation of biofilm with release of autoinducers (AI) into the surrounding environment. When bacterial population is low, autoinducers do not reach the threshold concentration needed to induce gene expression. The induction or repression of quorum sensing-dependent genes requires that the bacteria secreting the AI be in sufficient numbers for the AI concentration to reach a critical threshold [11]. Auto inducers, being small molecules are transported out of their cell into the environment.

The chemical composition of autoinducers vary between microorganisms, but they are classified into three types auto inducing oligopeptides, acyl-homoserine lactones, and a novel signal molecule named autoinducer-2 (AI-2). Each serves a different purpose in its specific nature. When AHL enters the cell, it binds to the transcriptional active-tor at the N-terminus and AHL binding causes conformational changes that expose an oligerimization and DNA binding domain at the C terminus. Detailed analysis of specific organism of biofilm *P. aeruginosa* was found to have several developmental stages, in which each stage comprises of a phenotype. In a premature biofilm, each stage was biologically different from other stages, but in a biofilm which is matured, all the stages exist simultaneously. Five distinct phases can be identified from *P. aeruginosa* biofilm maturation and they are reversible adsorption, irreversible attachment, maturation I, maturation II and dispersion.

The interaction between the planktonic bacteria and substrate surface forms the initial stage in biofilm formation. And this process is reversible adsorption because the microbes only stay for some time on the attached medium and then detach from it. It usually lasts for a few minutes [12]. The microorganisms attach themselves to a surface during the second stage and lose their involuntary movement. So, this attachment cannot be made reversible. For time duration of 2 h of next stage, the establishment of colonies takes place. In between the time period of these two stages, the result determined by the protein analysis shows that some biochemical changes took place, in which it was further examined and found that there exist some differences in the number of proteins gathered. The third stage in biofilm formation is maturation I. In this stage, extracellular polysaccharides substances are produced. The colonies formed by microbes increase and become multi-layered of thickness up to 10 µm. This stage lasts for three days. In this next stage, Maturation II, size of colonies increases up to thickness of 100 µm.

This stage lasts for six days. In between maturation I and maturation II stages results related to protein expression proven that there seems to be a small variation between the two. And some of their configurations is directly related to phenotypic modulation of bacterial cell. Dispersion is the last stage in biofilm formation. During this stage, the structures of colonies change because bacterial cells regain their mobility and detach themselves. This process takes place importantly to provide better access to nutrients to biofilms [7]. Water channels between colonies are formed in this stage. It lasts long for 9 to 12 days. Protein expression in the dispersion phase and planktonic cells are the same, which shows that few bacteria may come back to planktonic phenotype [11].

Detachment: After the formation of colonies by the proliferation of sessile cells, nature cells have a strong tendency to colonize the surface entirely. In general, various factors contribute to the detachment of biofilms and they are physical, environmental and extracellular secretion. This detachment can be further divided into active and passive types. Active cell dispersion is initiated by the microbes themselves, whereas passive detachment which is mediated by external forces. The detachment mechanism comprises of three parts: erosion, plugging, seeding in environment. Continuous release of microbes from the upper layers of biofilm which occurs by some sort of nutrient limitations is the process called erosion. The successive rate of loss of biofilm occurs usually at a later stage of biofilm formation. Seeding is the release of large number of microbes from the inside of biofilm community. The microbes in such colonies will have a shell-like type of structure with an inner cavity [13].

Essential Oils: The essential oils are well-known for their antibacterial properties and their wide range of inhibition against different pathogenic organism have been evaluated largely. A high amount of information and usage demonstrated that oral hygiene are favored by commercially available essential oils and have a long term benefit by daily usage when compared to products with chlorhexidine.

Berberis: Naturally plants are able to produce by-product which acts as a defense against various microorganisms. A chemical named berberine is alkaline medicinal plants isolated from *Berberis aristata*, *Berberis aquifolium*, *Berberis vulgaris*, *Coptis chinensis*, *Coptis japonica*, *Hydrastis canadensis*, *Phellodendron amurense,* and *Phellodendron chinense* schneid is given in **Fig. 2**. Berberine has large useful properties like anti-diabetic, anti-diarrhoeal, anti-microbial, immuno-stimulating, hypotensive and anti-inflammatory properties. Evaluation of anti-bacterial activities has been focused on bacteriostatic or reaction of berberine on other species. Among the research carried out in this field, it is found that berberine has less inhibitory action against Gram-negative bacteria and it is woke more against *S. aureus* and *S. epidermidis*. The reaction of berberine with human cells is very weak in both *in-vitro* and *in-*

vivo experiments [14]. For the treatment of various infections, the pharmacological treatment by phytochemicals proves to be an inexpensive and rapid method. Due to antibiotic resistive properties present in bacteria, it is necessary that the combination of antibiotics must be used to treat against such staphylococcal infections. The enriched activity of anti-biotics with natural products has more positive results against microbes which are harder to destroy it.

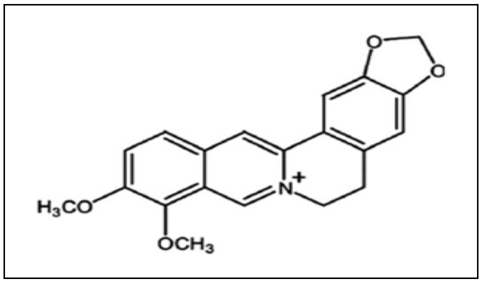

FIG. 2: CHEMICAL STRUCTURE OF BERBERIS

Staphylococcus epidermidis, is one among the main reason of medical material related problems due to its attachment and ability to form biofilm on biomaterial surfaces. Even though, the activity of berberine on planktonic bacteria has been studied the inhibition rate of berberine on biofilm formation hasn't possessed proper report till this date. In this investigation, berberine is observed as bacteriostatic in particular for *S. epidermidis* and that sub-minimal concentration of berberine inhibits and blocks the *S. epidermids* biofilm formation. Though the mechanisms of killing and inhibition of bacteria from biofilm formation are not explained completely, but from the studied data indicates a potential property of berberine can be used as a therapeutic agent for preventing the infections that are caused by biofilm formation.

The anti-mycotic activity of the plant alkaloid berberine (BBR), alone and in combination with antifungal azoles, work against planktonic and biofilm Candida cultures. Berberine against inhibit various Candida species in the following order of susceptibility: *C. krusei* > *C. kefyr* > *C. Glabrata* > *C. Tropicalis* > *C. parapsilosis* and *C. albicans*. *C. tropicalis*. Synergism between BBR and MCZ or FLC whilst neither BBR nor MCZ alone minimally inhibits the biofilm formation of *C. albicans*, their combination reduced biofilm formation by 91%, of reduction was established in the surface area coverage. The BBR/MCZ combination also worked well against the metabolic activity of pre-formed C. albicans biofilms. Berberine exhibits a combined effect with commonly used antimycotic drugs work against C. albicans, either in planktonic or in biofilm growth phases [24]. It has been observed that berberine as a capacity to bind with the nucleic acids and inhibits enormous multiplication of cells also evident by its DNA and protein folding kinetics [24]. Therefore, the berberine may be utilized as antimicrobial drug to work against *E. coli*. Future trails can be concentrated on minimizing the economic losses in animal husbandry by assessing the antimicrobial capacity of berberine hydrochloride to work against many varieties of drugs that resist the enter pathogens [15].

Berberine as the efficiency to work against certain endodontic oral pathogens formed by various species of biofilm on teeth. The tested solution showed a significant inhibition when compared with saline. But bactericidal efficiency was very much effective when combined with NOCl56. Periprosthetic infection is commonly caused by *Staphylococcus epidermidis* forms biofilm. The study was to examine the efficiency of berberine on *S. epidermidis* attachment and biofilm formation on titanium alloy surface, which is very much useful in the area of orthopedic joint prostheses. From investigation, the higher concentrations of berberine are much effectively worked against the development of *S. epidermidis* biofilm on the titanium surface and these results suggest that berberine is a potential agent for treating the Periprosthetic infection [16].

Cinnamonum: Many biofilms is involved in various microbial infections. The investigation says that *S. aureus* is a prevalent pathogen stance second-most position in ICUs and it is the root cause of infections in women, by causing more complications in urinary infectious treatments due to microorganism with biofilm formation. Microorganisms are protected by biofilm from predator and extracellular aggression. Certain antibiotics resist the activity of bacterial biofilm population. But the increased resistance causes problematic to use antimicrobial drugs. The results show medical plants are alternative to treat diseases. Among various classes of essential oil, the botanical origin plant extract that has the antimicrobial ability are obtained from species of the genus Cinnamonum (Lauraceac) such as *Cinnamonum cassia* and *Cinnamonum zeylanicum* is illustrated in **Fig. 3**. *C. zeylanicum* is originated from some areas of Ceylon and India and also known by "Cinnamon-of-Ceylon" [5]. Due to its many medicinal qualities this variety is used as folk medicine and also as aromatic, digestive, hypertensive [17]. Essential oils are obtained from various parts of the plant with each parts of different chemical composition with [E] Cinnamaldehyde (65-78%). *C. cassis* most popularly known as "Cinnamon-china" is a perennial tree origin to southern China and also spread widely in southern and eastern Asia. Western medicine, *C. cassia* is used primarily in various bacterial and fungal related infection. Its trunk bark consisting of 70 to 90% (E)-cinnamaldehyde. The various studies, in addition, says that the essential oil also contains antifungal activity against *Candida albicans*.

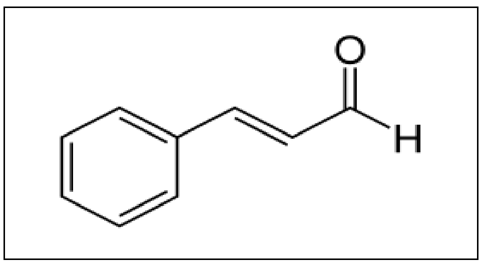

FIG. 3: CHEMICAL STRUCTURE OF CINNAMONUM

In the Cinnamonum species, (E)-Cinnamaldehyde compound **Fig. 3** is the most abundant essential oils (E)-Cinnamaldehyde contains phenylpropanoid that has reported as an agent for antimicrobial activity against various microorganism. A study results in Iran using *C. zeylanicum* stalks is filtered by hydro distillation. It possesses 17 compounds in the filtered essential oil. The most of the component in a group were cinnamaldehyde (80.42%). In an investigation evaluated that chemical constitution of the essential oil of *C. cassia* bark is derived from the lower part of the stems possesses most of the identified compounds observed belonged to the hydrocarbon and oxygenated fractions of various stages. The major component identified was (E)-cinnamaldehyde [18]. The constituents that determine the yield and composition of the essential oils are large. In some special cases, it is hard to isolate these constituents from each other as they are bonded together and influence one another. These include seasonal variations, in the part of organ used in the plant, and in the development stages of the plant, as well as its geographic and genetic origin. The study shows in planktonic form, both essential oils and (E)-cinnamaldehyde work against the growth of all bacteria assayed. (E)-cinnamaldehyde possesses both bacteriostatic and bactericidal activity and work against S.aureus and *S. epidermidis*.

But in contrast, (E)-cinnamaldehyde as no bactericidal property against *S. pyogenes* and *P. aeruginosa E. coli* bacteria was observed. As a result, shows that the essential oil of *C. cassia* possesses 85.06% (E)-cinnamaldehyde and in addition, it also has antimicrobial activity against strains of *P. aeruginosa*, *E. coli* and *S. aureus*. The results showed a strong inhibitory effect. A studypossess an anti-bacterial activity against *E. coli* and *S. aureus* by cinnamaldehyde. In a study, it was shown that cinnamaldehyde possesses morphological changes; the root cause was the increase of nucleic acid and protein levels in the cell suspension, which indicates that the cell membrane was damaged.

According to the results of the concentrations of the substances as a capacity to reduce the growth S. pyogenes biofilm shows that the (E) cinnamaldehyde concentration was eight times stronger than that of EOCc and four times stronger than that of EOCz. In this case, it may be suggested that other components of the essential oils also participate synergistically in the anti-biofilmation. A similar property is observed on biofilms of *P. aeruginosa*. However, the concentration of the essential oils remains the same against Staphylococcus [19]. The *C. zeylanicum* obtained commercially as an essential oil was able to work against the biomass and numerous viable *P. aeruginosa* biofilm developing cell. Studies have shown that trans-cinnamaldehyde work against *E. coli* biofilm development in the fragments of urinary catheter areas. Biofilm inhibition was tested with *E. coli*, and trans-cinnamaldehyde was used to treat infection. All most all the concentration assays work against E. coli biofilm development in urinary catheter fragments.

Another species of Cinnamonum also inhibit anti-biofilm activity; the essential oil secretes from the trunk bark of *C. burmannii* inhibit plank tonic cell development and the formation of *S. aureus* and *P. aeruginosa* biofilms. These results conclude that, the 50% planktonic cell development was inhibited by these essential oils [20].

Pipredinen: Pipredinen consisting of more than the variety of species, plant genus Mentha is known for various activities like antimicrobial, antiviral and insecticidal activity is given in **Fig. 4**. Due to aromatic and stimulant nature of mint oil, it is used aggressively in the pharmaceutical industry. *Mentha piperita*, is a member of plant genus Mentha, is widely known as peppermint and its oils are used as a flavoring agent in various products like digestive tablets, toothpaste *etc*. *Mentha arvensis*, widely called as pudina is widely harvested in India and its neighboring countries like Nepal, Bangladesh, Sri Lanka *etc*. These plants used in the form of medicine helps in controlling digestion, skin diseases. The properties of extracts of *M. arvensis* were found based upon the experimentation on uropathogens and enteric pathogens. *M. arvensis* and *M. paperita* showed activities of anti-bacterial and anti-biofilm in recent studies against pathogens.

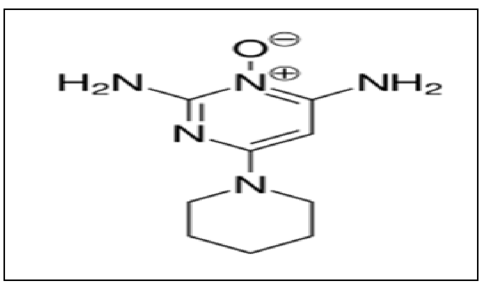

FIG. 4: CHEMICAL STRUCTURE OF PIPREDINEN

A. actinomycetemcomitans, being a gram negative capnoic, *coccobacillus* is aetiological agent of aggressive behaviour and also to chronic periodontitis. Its dissemination of Aggregatibacter actinomycetemcomitans into various oral sites produces deadly infections infection like endocarditis, pneumonia and abscess in neck part. Aggregatibacter actinomycetemcomitans is widely identified and its activity may seem to be pseudo in nature but it produces oral biofilms. Eliminating this bacterium seems to be somewhat complex as the combination of antibiotics and mechanical treatment is usually employed. But during the course of treatment, it's resistance to antibiotics possess a challenge when treating such infections. Studies in this area have been done various researchers particularly in antimicrobial resistance of Aggregatibacter actinomycetemcomitans. Based on studies, oils of Mentha prove to be strong resistor to A. actinomycetemcomitans. One of the native behaviors of bacteria is biofilm formation. And this biofilm formation is somewhat responsible for antibiotic resistance activity and causing infection. During the present course of study, concentration of oils was kept at 100 ml/ml. This study is used to analyze anti-biofilm activity of Mentha essential oils against A. actinomycetemcomitans and no previous reports were published related to this study. The results from this study showed the uses of oils from such plants which can be used in oral care products. Herbal agents who include anti-biofilm activity will be helpful to prevent orodental colonization.

Limonene: Limonene [1-methyl -4 - (1-methyl-ethenyl) cyclohexane], a monocyclic monoterpene, is seen in the citrus fruit rinds is shown in **Fig. 5**. The typical orange juice contains 100 ppm of limonene. But the usage of limonene has been regarded as safe by the Code of Federal Regulation in the USA as a flavoring agent. Its properties are considered useful since can dissolve cholesterol in gallstones. The current research shows properties of limonene against species of the genus *Streptococcus*. By using surface coating and physiological assays, influence of organisms and mechanism of anti-biofilm activity can be studied. By means of PCR, the results were validated. An active ingredient of limonene was proved against broad anti-biofilm species.

The biofilm inhibition of limonene was assessed against *Streptococcus mutans* and *Streptococcus mitis*. Determination of MBIC was derived, by conducting a biofilm inhibition assay with high concentration (80%) of limonene against Streptococcus pyogenes SF370. The results showed an increase biofilm activity of limonene at MBIC concentration. Clinical isolates were more potent in biofilm formation [21], its activity was tested against five isolates of Streptococcus pyogenes. Limonene showed a remarkable anti-biofilm activity (75 - 95%) against all the clinical isolates tested at 400 mg/ml. A hypothesis was present stating that due to its oily nature, it may change the surface structure and making the host unsuitable biofilm formation. To prove this statement, an assay was performed, in which the ability of pathogens to form biofilms was eliminated.

When dental implants are used, in which most of it is made of titanium and stainless steel, the inability to form biofilm by *Streptococcus mutans* can be assessed and limonene can be used against it. Since limonene shows surface-oriented anti-biofilm activity, the influence of limonene on the extracellular phenomenon of the organisms was studied.

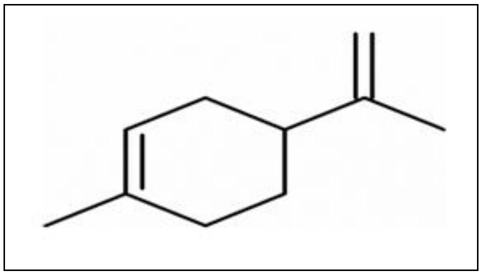

FIG. 5: CHEMICAL STRUCTURE OF LIMONENE

Eugenol: Clove essential oil (EO) is aromatic and volatile substance extracted from *Syzygium aromaticum* (L.), which is widely used in the food and beverage industry as a flavoring agent. And its application can also be found in cosmetics, medical therapy, *etc*. Due to its properties like antioxidant, anti-inflammatory, anti-microbial. It was also commented as a "Generally Regarded As Safe" chemical by the US Food and Drug Administration. Eugenol (4-allyl-2-methoxyphenol) is illustrated in **Fig. 6**, the important constituent in clove EO being eugenol, is found to possess anti-microbial activity against gram-negative and gram-positive bacteria. It is notable that eugenol shows strong inhibition activity against anaerobic oral bacteria, *e.g*. *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Streptococcus mutans*. In inclusion, eugenol possess high zone of inhibition in Zinc Oxide Eugenol (ZOE), a dental product was introduced in the market 1890s for its analgesic and antiseptic effects [22]. Various essential oils were found to work against EHEC biofilm development, but with broad various efficiencies. Remarkable, major 75% these four essential oils, namely bay, cinnamon bark, clove, and pimento berry oil work against EHEC biofilm development.

No growth reduction of EHEC cells above 30% at OD 620 nm was observed at 0.005% (v/v) as compared with untreated controls. Reported that cinnamon bark oil possesses anti-biofilm activity against EHEC, but for the first time that bay, clove and pimento berry oils have been resulted to have anti-biofilm activity. Previously, eugenol was found to inhibit biofilm formation by Staphylococcus aureus, *Candida albicans* [38], Listeria monocytogenes and EHEC1418, and to work against quorum sensing of *Pseudomonas aeruginosa* [25]. The present result shows anti-biofilm activity against EHEC by eugenol-rich oils (bay, clove, and pimento berry oils) and thereby illustrating explicit details of constituent and its active chemical structural relationships and mechanism involved in it.

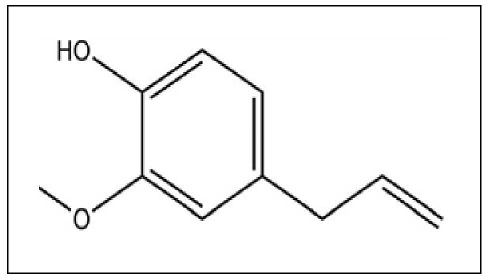

FIG. 6: CHEMICAL STRUCTURE OF EUGENOL

In the existing study shows, essential oil and eugenol derivatives, undergo activities which reduce the development of biofilm and virulence of EHEC. Additionally, eugenol having PLGA coatings showed higher activities of prevention of EHEC biofilm formation in rugged and solid surfaces. From these conclusions, it can be suggested that derivatives of eugenol and its essential oils prove to be useful against anti-virulence strategies against infections of EHEC. Periodontitis, a serious inflammatory disease, damaged tissues in gingival area and might lead to loss of tooth. The most common periodontal pathogens are gram-negative bacteria *Porphyromonas gingivalis*. Treatment for periodontitis involves surgical and non-surgical therapy along with antibiotic treatment. Bacteria present in periodontal areas can be reduced by means of antibacterial agents like tetracycline, metronidazole.

The outermost layer of Gram-negative bacteria behaves not only as a barrier and maintains the cell. The cell membrane is an important site of anti-microbial action. For example, anti-bacterial agents that interact with cell membrane can affect the overall permeability of cell, alter the membrane potential and structure [23]. Bacteria in biofilm are more resistant to drugs than their planktonic counterparts. It is found that phenolic blueberry extract inhibited anaerobic periodonto pathogen *Fusobacterium nucleatum* at concentrations that did not inhibit bacterial growth. It has resulted that eugenol at sub-MIC concentrations can inhibit the biofilms of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Enterococcus faecalis* [24].

In the research, eugenol at sub-MICs work against *P. gingivalis* biofilm development and at wider concentrations removed pre-developed biofilms. The results validated that eugenol reduced the mass of biofilm in an independent mode rather than as an incidental result of growth inhibition. And apparently, biofilms were more sensitive at the initial stage of formation. Hence, the progression of periodontitis might be slowed down and *P. gingivalis* develop resistance to eugenol not easily. Eugenol from CLEO displayed antibacterial activity against *P. gingivalis*. On the other side, eugenol inhibited growth of *P. gingivalis* associated with membrane damage and also reduces biofilm development of *P. gingivalis*. Both the oils clove and eugenol as wide range of applications in food industry as flavorings, but also plays a central role in gums or toothpaste as effective antibacterial additives for periodontitis prevention.

CONCLUSION: In the twenty years of research, there have been many studies and explosion to discover molecules with the ability to inhibit biofilm formation. As related review all through this analysis, there is some considerable evidence that withdraws plant products, essential fractions, phytochemicals components or various natural chemical from plants have the potential to be formed as inhibitory agents or remedy to work against these biofilm-based infections.

Future studies are required to provide a pathway for rational experiment in clinical levels by confirming both safety and capacity of these strong active agents of plant compound *in-vitro*. The current development and discovery of novel agents aim to bring down the expenses and reduces the risk of building up a resistance to antibiotics by incorporating the novel molecules alone or in fusion with present antimicrobial drugs.

There are various problems that are required to be resolved under clinical trial for the use of anti-biofilm agents. Still, large agents that are responsible for the action of anti-biofilm mechanisms is unknown. The compounds that show their antimicrobial efficiency are required to be studied under various *in-vitro* or *in-vivo* conditions. There is a need to increases the active agent ability to work against biofilm. These active agents under remedial strategy may serve as modifier in fusion with familiar anti-biotics and thus their level of usage and pharmacodynamics must be optimized to the lowest level. Finally, more extensive classes of plant molecules to be explored that increases the level of developing novel anti-biofilm agents.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors have no conflicts of interest.

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How to cite this article:
Anusriha S and Ponnarmadha S: A review on anti-biofilm inhibitor from plant essential oils. Int J Pharm Sci & Res 2020; 11(1): 14-24. doi: 10.13040/IJPSR.0975-8232.11(1).14-24.