International Journal of Education and Science Research Review
E-ISSN 2348-6457
July –August - 2021, Volume-8, Issue-4 P-ISSN 2349-1817
www.ijesrr.org Email- editor@ijesrr.org

Anti-biofilm activity of combination medicinal plant extract against upper respiratory tract infection

AMRISH
Research Scholar
Biotechnology
Sri Satya Sai Universitu Bhopal,M.P.

DR. SYED SAHAB AHMAD
Guide Name

Abstract

Bacterial biofilms are common in nature and pose a serious threat to global health. Biofilms commonly create significant contamination and seriously disrupt the event of anti-infection blockage, making bacterial disease treatment difficult. Antimicrobials, bacteriophages, and majority detection inhibitors are among the most often utilized traditional therapies for biofilms. Regardless, these treatments are insufficient for the safe and effective treatment of biofilms. Anti-infection drugs frequently cause blockage in treated bacteria, and antibacterial peptides are easily degraded by proteases, reducing their viability. These findings show that there is still room for progress in the management of biofilms. Customary drug medicines have been found to have significant inhibitory impacts on biofilms, as per developing proof. This overview summed up and examined the sufficiency parts of normal and regular biofilm-battling drug medicines, as well as contrasting instruments. The advantages and disadvantages of those treatments have been distinguished and deciphered, and we've inferred that consolidating solutions with conventional prescriptions will be a more sensible biofilm-battling system. This study presents an expected structure for the headway of ant biofilm specialists and gives remarkable motivations to the treatment of bacterial biofilm messes.

Keywords: *Anti-biofilm, Combined, medication, respiratory tract infection (RTI).*

1. INTRODUCTION

Bacterial infections have posed a severe threat to worldwide public health, and the discovery and spread of drug-resistant bacterial strains has only added to the problem's severity. Bacterial biofilms acquire antibiotic resistance and act as virulence factors in human infections. Microbial cells generate bacterial biofilms that adhere to live or inanimate surfaces. These cells stick together and are encased in an extracellular polymeric matrix that they manufacture themselves. Bacteria form biofilms in greater than 90% of cases. The resilience of bacteria in mature biofilms is substantially higher than that of planktonic bacteria, which is not conducive to the treatment of bacterial illnesses. Antibiotic therapy is presently the most widely used traditional treatment for bacterial infections. Phage treatment, quorum sensing (QS) system inhibitors, monoclonal antibodies, immunomodulators, and micro biome-based therapeutics, antibiotic potentiates, and antisense nucleic acid methods are examples of atypical conventional therapies. To ensure that a sufficient concentration of antibiotics is achieved at the affected site, high-dose effective and highly penetrating antibiotics are frequently

chosen for the treatment of infections caused by biofilms. Antibiotic efficacy has been gradually declining as bacterial resistance to antibiotics has increased. Nontraditional therapies have some drawbacks, such as the necessity for diagnostic specificity prior to usage, high cost, and infusion reaction requirements. Many factors, including quorum sensing and other systems that differ between species, can cause a shift in behavior. When a cell switches modes, it undergoes a phenotypic shift in behaviour that involves the up and down regulation of a large number of genes. Biofilms are created by a variety of microorganisms, including pathogens, and serve as a way for them to shield themselves against antimicrobial agents. As a result, biofilm development is acknowledged as a critical pathogenicity component for both opportunistic and "true" infections. The biofilm matrix, which functions as an efficient barrier to antimicrobial agent penetration, is responsible for microbes' antibiotic tolerance. Several mechanisms have been proposed to explain the phenomenon of biofilm resistance, including delayed antimicrobial penetration into the extracellular matrix of the biofilm, slowing the rate of growth of organisms within the biofilm, or other physiologic changes caused by organism interaction with a surface. Antimicrobials or other compounds that have been shown to penetrate and kill biofilm organisms, as well as treatments that disrupt or target specific components of the biofilm matrix, will be used in effective treatment regimens. More research is needed to better understand the role of biofilms in infection and how in vitro or in vivo biofilms respond to various treatments.

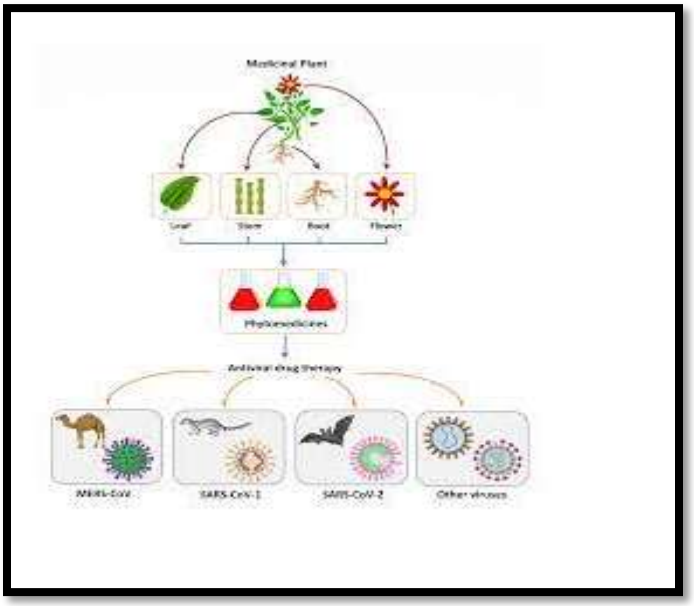

Figure: 1 Infections of the upper respiratory tract

1.1 Infections of the upper respiratory tract

Upper respiratory tract infection (URTI) is one of the most frequent medical diseases that people face on a daily basis around the world (Spurling, 2013). In a developed country, upper respiratory tract infection is a big problem. Although there have been a number of comparable illnesses with similar or overlapping clinical manifestations under each category of sickness, distinguishing the damaged respiratory mucosal portion

requires judgement. Various URTI signs and symptoms Stuffy and runny nose, sneezing, coughing, sore throat, fever, vomiting, irritability, loss of appetite, and watery eyes have all been reported. Cooper et al., 2001; Fondell et al., 2011; Lee et al., 2000; Cooper et al., 2001).

1.2.1 Types of Uris

URTIs can be defined as a set of illnesses that mostly affect the upper respiratory tract (Call et al., 2005). (Table1.1.2.1).

Table 1.2.1 Upper respiratory tract infection in humans

S. No	Infection	Features
1	Pharyngitis	Acute inflammation of the throat, resulting in pain on swallowing and swollen, red pharyngeal mucosa
2	Common cold	Self-limiting rhinitis, causing nasal discharge, nasal obstruction, discomfort and sneezing.
3	Bronchitis	Cough and sputum production; can be acute or chronic.
4	Pneumonia, community and hospital-acquired	Occurs prior to or immediately after hospital admission; cough, chest signs and fever. Occurs in vulnerable patients in hospital; onset gradual and symptoms unreliable for diagnosis
5	Chronic	Insidious onset, prolonged course; usually diagnosed by radiological findings

1.3 Biofilms made up of microbes

1.3.1 Biofilms have a unique nature

In nature, micro-organisms exist as both planktonic, free-floating cells or in a community commonly referred to as a biofilm. A biofilm is a community of cells attached to either a biotic or abiotic surface enclosed in a complex exopolymeric substance (EPS) (Costerton et al., 1999, Mah and O'Toole, 2001 and Hugo and Russell,

2004). Where biofilms are problematic, the term microbial fouling or bio-fouling may be used. It refers to the undesirable formation of a layer of living micro-organisms and their decomposition products as deposits on surfaces in contact with liquid media (Kumar and Anand, 1998). Attachment occurs to surfaces that are preconditioned with nutrients sufficient for the growth and survival of the microorganisms. Almost any surface is susceptible to microbial colonization as long as nutrients are available.

2. REVIEW OF LITERATURE

Silva et al., 2009, Sudbery et al., 2004 Vulva vaginal Candidiasis may affect up to 75 % of women. Candida infections are the fourth most common hospital-acquired infection in India and second most in the United States of America (Field et al., 2006; Hasan et al., 2009). Candida albicans can exist in various morphological forms like yeast, hyphae and psedohyphae and chlamydospores (Sudbery et al., 2004). Hyphae are the filamentous tube like structures without constriction at the septal junctions consider as virulence factor of C.albicans. Hyphae have invasive property that can promote tissue penetration.

Heitman et al., 2007 The use of antifungal agents has increased from many years for the treatment of a variety of diseases caused by fungi. There are eight different targets for antifungal therapy like ergosterol synthesis, chitin synthesis, ergosterol synthesis, glucan synthesis, squalene epoxidase, nucleic acid synthesis, protein synthesis, andmicrotubules synthesis.

Sobelet al., 2010 Azole antifungals are widely used to treat candida infections. It consist of imidazole and triazole derivatives such as Ketoconazole, Fluconazole, voriconazole etc., that block the synthesis of ergosterol in the cytoplasmic membrane.

Johnson et al., 2010 Fluconazole is effective against oropharyngeal and vaginal candidiasis and is effective at very low concentrations and shows very less side effects.

Heitman et al., 2010 Morpholins and allylamines inhibit the conversion of lanosterol to ergosterol, while Echinocandins are glucan synthesis inhibitors. Flucytosine was first developed as an anticancer agent but later on it was developed as an antifungal agent and today it is used in adjunctive therapy with Amphotericin.

Mitchell et al., 2012 Biofilms are microbial communities containing a dense network of yeast and filaments embedded inside a exo-polymeric matrix which makes it resistant against chemotherapeutic agents (Desai et al., 2014). Biofilms are not just a mixture of yeast and filaments but it shows different developmental phases differing from planktonic growth mode.

Wagner et al., 2009; Campbell et al., 2012 Combination therapy is considered as an effective approach to improve the efficacy of therapy in the treatment of invasive infections (Mukherjee etal., 2005).Combination therapy is very useful and effective since they may increase both the rate and degree of microbial killing (Doble et al., 2008). Another important reason for using combined drugs is that each drug may have different mechanism of action.

Chen et al., 2013 Caspofungin, an Echinocandin has the ability to interfere with fungal cell wall biosynthesis by inhibiting beta-1, 3-D-glucan synthase. Caspofungin is effective against resistant strains of C. albicans.

Caspofungin is used in the treatment of invasive candidiasis(Heitman et al.,2007). To increase the efficacy of caspofungin towards biofilm of C. albinos, caspofungin is combined with Diclofenac. Diclofenac is an anti-inflammatory drug which inhibits hyphae development and biofilm formation. Susceptibility of C.

3. BACTERIAL BIOFILM FORMATION AND PATHOGENIC CHARACTERISTICS

3.1. Biofilm formation

Bacterial biofilms are organized by microbial aggregates that live in extracellular polymeric matrices that are irreversibly attached to the surface of an object, living body or tissue and are difficult to remove unless deterred and inhibited quickly. The biofilms consist of 5–35 % cell volume, and the extracellular matrix is composed of 97 % water, 2 % protein and polysaccharide, and 1 % DNA and RNA. As shown in Fig. 1, biofilm formation is a dynamic cyclic process in four stages involving bacterial attachment and cohesion, micro colony formation, biofilm maturation, and bacterial dispersion. Planktonic bacteria reach the attachment surface through Brownian motion, hydrodynamic motion and active swimming motion. They use the interaction of their own pili, adhesins carried on flagella, electrostatic action, hydrophobicity and other physical and chemical actions to provide adhesion force between the attachment surfaces.

3.2. Pathogenic mechanisms

Diseases caused by bacterial biofilms are associated with the formation of biofilms on the surface of diseased tissues and the continuous release of free bacteria, causing persistent infection and chronic inflammation. The structural characteristics and properties of bacterial biofilms make them less susceptible than planktonic bacteria to antibiotics and the body's immune system.

4. TRADITIONAL MEDICINAL PLANTS

In many countries such as India and China, natural products have been used in traditional medicines for thousands of years, and have shown promise as a source of components for the development of new drugs (Newman et al., 2003).

4.1.1 Synergistic plant extracts

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Plant compound aid in treating microbial infection effectively without the risk of developing drug resistance. However, plant-derived antimicrobials are less potent Hence; it becomes apparent that plants adopt a different paradigm – synergy – to combat infections (Hemaiswarya et al., 2008).

4.1.2 Synergistic Treatment

Combinations plant extracts contain a myriad of compounds in complex matrices in which most of the constituents are responsible for enhanced efficacy in treating the microbial infection (WHO, 2001). The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases.

Table 4.1.3. Selected plants and their application

Scientific Name	Common Name	Application
Leucas Aspera	Thumbai Cough	Wound Healing
Vitex Negundo	Nochi	Cough, Medicinal Use
Gymnema Sylvestre	Cherukurinja Anti-Diabetic	And Cough

4.1.4 Classification

Kingdom:	Plantae
Order:	Lamiales
Family:	Lamiaceae
Genus:	Leucas
Species:	L. asper

Figure: 4.1 Leucas aspera (Wild.) Linn

4.2.1 Streptococcus pyogenes

Streptococcus pyogenes owes plays a major role as a pathogen as a pathogen to its ability to colonize and rapidly multiply and spread in its host while evading phagocytosis and confusing the immune system. Acute diseases associated with Streptococcus pyogenes occur chiefly in the respiratory tract, bloodstream, or the skin.

4.2.2 Mechanism of Biofilm forming

Several steps are required for biofilm formation: (I) Initial attachment to a surface, (II) Formation of micro colonies, (III) Maturation of micro colonies into EPS-encased three-dimensional biofilms, and (IV) Detachment and dispersion of single cells from the matrix (Davey, 2003; Stoodley, 2002).

5. MATERIALS AND METHOD

5.1 Culture and Collection

About 30 throat swab samples were obtained from pharyngitis patients, attending Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. S. pyogenes MTCC 1924 (IMTECH, Chandigarh, India) was used as reference strain. S. pyogenes was isolated from the throat swab samples using Streptococcus Selection Agar (SSA) (Himedia, India). All the isolates were tested for their biofilm forming characteristics by observing the slime formation in routine media and β-haemolysis using Blood Agar (Himedia, India).

5.2 Detection of Biofilm forming Bacteria

Biofilms were allowed to grow on round coverslips (Nunc, Wiesbaden, Germany) placed in 24-well polystyrene cell culture plates (Greiner Bio-One). Plastic surfaces of the coverslips were used uncoated or were coated with human fibronectin (Roche), human fibrinogen (Sigma), human collagen types I and IV (Biomol), or human laminin (Sigma) at a concentration of 50 µg /ml_1 overnight at 4°C.

6. CONCLUSIONS

C.albicans biofilms are resistant to most of the antifungal drugs than the planktonic forms. Candida barbicans biofilms are difficult to eradicate. Commercial antifungal agents including fluconazole and amphotericin are widely prescribed but they are not very effective in clinical situations Overall, increased cost and drug resistance has put limitations on the use of antifungal drugs. New and effective methods are therefore urgently needed. Combination therapy might be a valid alternative method to find better drugs to cure life threatening infections associated with biofilms of C.albicans. To limit the use of high concentrations of antifungal drugs, we have explored the use of drug combinations against biofilms formed by C.albicans.

Our study suggest the use of lemongrass oil components with antifungal drugs as a strategy for the prevention of mature biofilm of Candida albicans and also for avoidance of side effects associated with high concentrations of antifungal drugs. To confirm the practical utility of these combinations, in vivo studies are necessary. Nerol, citral, linalool, β-ionone, terpinolene, 1, 8 cineol, geraniol and geranyl acetate in combination with fluconazole showed synergistic interaction against developing biofilms of two strains of C.albicans and brought down the MIC of fluconazole by four fold. Fluconazole when combined with geraniol, nerol and β-ionone showed synergistic interaction against mature biofilms and reduced fluconazole MIC by two fold. We hypothesize that the anticandida activity of lemongrass oil components may be due to membrane damage as well as inhibition of oxidative phosphorylation and inhibition of respiratory chain function.

7. REFERENCES

1) Alem, M. A., & Douglas, L. J. (2004). Effects of aspirin and other nonsteroidal antiinflammatory drugs on biofilms and planktonic cells of Candida albicans. Antimicrobial Agents and Chemotherapy, 48 (1), 41-47.

2) Al-Fattani, M. A., & Douglas, L. J. (2006). Biofilm matrix of Candida albicans and Candida tropicalis: chemical composition and role in drug resistance. Journal of Medical Microbiology, 55 (8), 999-1008.

3) Ashraf A, Yousri F, Taha N, El-Waly OA, (2015).Ramadan AE, Ismail E, Hamada R, Khalaf M, Refaee M, Ali S, Madyn A. Effect of Some Non- steroidal Anti-Inflammatory Drugs on Growth, Adherence and Mature Biofilms of Candida spp. American Journal of Microbiological Research. Jan 23;3(1):1-7.

4) Bachmann, S. P., Ramage, G., VandeWalle, K., Patterson, T. F., Wickes, B. L., & LópezRibot, J. L. (2003).

5) Antifungal combinations against Candida albicans biofilms in vitro. Antimicrobial Agents and Chemotherapy, 47(11), 3657-3659.

6) Baillie GS, Douglas LJ (2000) Matrix polymers of Candida albicans biofilms to antifungal agents. Journal of Antimicrobial Chemotherapy 46:397–403 12. Ben Khedir S, Mzid M, Bardaa S, Moalla D, Sahnoun Z, Rebai T.

7) (2016). In Vivo Evaluation of the Anti-Inflammatory Effect of Pistacia lentiscus Fruit Oil and Its Effects on Oxidative Stress. Evidence-Based Complementary and Alternative Medicine. Dec 14; 2016.

8) Bink, A., Kucharíková, S., Neirinck, B., Vleugels, J., Van Dijck, P., Cammue, B. P., & Thevissen, K. (2012). The non-steroidal anti-inflammatory drug Diclofenac potentiates the in vivo activity of caspofungin against Candida albicans biofilms. Journal of Infectious Diseases, 206(11), 1790-1797.

9) Bink, A., Pellens, K., Cammue, B. P. A., & Thevissen, K. (2011).Anti-biofilm strategies: how to eradicate Candida biofilms. Open Mycology Journal, 5, 29-38.

10) Bishop, S. C., Burlison, J. A., Blagg, J., & Brian, S. (2007). Hsp90: a novel target for the disruption of multiple signaling cascades. Current Cancer Drug Targets, 7(4), 369-388.

11) Bonhomme J, d'Enfert C. (2013). Candida albicans biofilms: building a heterogeneous, drug-tolerant environment. Current Opinion in Microbiology .Aug 31; 16(4):398-403.

12) Borjihan, H., Ogita, A., Fujita, K. I., Hirasawa, E., & Tanaka, T. (2009). The vacuoletargeting fungicidal activity of amphotericin B against the pathogenic fungus Candida albicans and its enhancement by allicin. The Journal of Antibiotics, 62(12), 691-697.

13) Agnihotri, A.K., S. Khatoon, M. Agarwal, A.S. Rawat, S. Mehrota and P. Pushpangadan 2004. Nat Prod Sci, 10 (4):168 – 72. Akiyama H., S.Morizane, O.Yamasaki, T.Oono and K. Iwatsuki. 2003.

14) Assessment of Streptococcus pyogenes microcolony formation in infected skin by Confocal Laser Scanning Microscopy, J Dermatol Sci.32:193-9. Alade, P.I. and O.N. Irobi, 1993.

15) Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. Journal of Ethnopharmacology, 39(3):171-174. Al-Ani NFI., 2008.