Bacterial Biofilm Inhibitor

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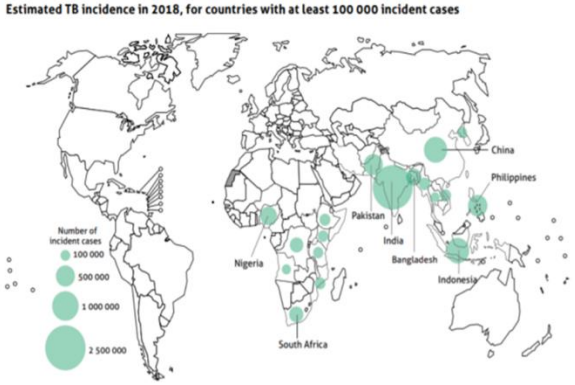
Abstract **- Tuberculosis is a major threat to the health of millions of populations. *Mycobacterium tuberculosis* and various microorganisms like bacteria, fungi remain in a self-produced polymeric matrix, adherent to an inert or living surface. This micro environment community of bacteria is known as biofilm. 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 respect natural products having powerful antimicrobial effects remain important participants. The objective of this study aimed to investigate the anti-mycobacterial activity of the phytochemicals. Phytochemicals are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids.**

Index Terms **- Tuberculosis, Biofilm, Phytochemicals, Extracts.**

INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). It typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB). About a quarter of the world's population is infected with M. tuberculosis and thus at risk of developing TB disease**.** Worldwide, around 10 million people fall ill with tuberculosis (TB) each year. TB is one of the top 10 causes of death, and the leading cause from a single infectious agent (Mycobacterium tuberculosis). The disease can affect anyone anywhere, but most people who develop TB (about 90%) are adults, the male: female ratio is 2:1, and case rates at national level vary from less than 50 to more than 5000 per 1 million populations per year. Globally, an estimated 1.7 billion people are infected with M. tuberculosis and are thus at risk of developing the disease. Globally in 2018, an estimated 10.0 million (range, 9.0– 11.1 million) people fell ill with TB equivalent to 132 cases (range, 118–146) per 100 000 population. Most of the estimated number of cases in 2018 occurred in the WHO South-East Asia Region (44%), African Region (24%) and Western Pacific Region (18%); smaller proportions of cases occurred in the WHO Eastern Mediterranean Region (8.1%), Region of the Americas (2.9%) and European Region (2.6%).The 30 high TB burden countries3accounted for 87% of all estimated incident cases worldwide, and eight of these countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).



Currently, the world as a whole, most WHO regions and many high TB burden countries are not on track to reach the 2020 milestones of the End TB Strategy. Globally, the average rate of decline in the TB incidence rate was 1.6% per year in the period 2000−2018, and 2.0% between 2017 and 2018. The cumulative reduction between 2015 and 2018 was only 6.3%. The global reduction in the number of TB deaths between 2015 and 2018 was 11%.Global targets and milestones for reductions in the burden of TB disease have been set as part of the Sustainable Development Goals (SDGs) and WHO's End TB Strategy. SDG includes a target to end the global TB epidemic by 2030. WHO End TB Strategy includes targets of a 90% reduction in TB deaths and an 80% reduction in the TB incidence rate between 2015 and 2030, with 2020 milestones of a 35% reduction in TB deaths and a 20% reduction in TB incidence rates .[1]

Mycobacterium Tuberculosis:

M. tuberculosis is an extraordinary paradigm of intracellular pathogens that does not possess classical virulence factors. Indeed, it can persist in the host during long-term latency without causing significant damage or transmission unless the host immunity is compromised, e.g., when the host is treated with TNF-α blockers or co-infected by human immunodeficiency virus type-1 (HIV-1).[2] *Mycobacterium tuberculosis* is a species of pathogenic bacteria in the family Mycobacteriaceae and it is the causative agent of tuberculosis. It is first discovered by Robert Koch in 1882. *Mycobacterium tuberculosis* is an obligate aerobe. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence. [3]

Bacteria that live various environmental conditions naturally form biofilm as the survival strategy to protect the cell from an harsh environment. Bacteria are generally studied in the research laboratories as single cell suspensions called as planktonic cultures, however, in nature, bacteria primarily exist as a community encased in a self-produced extracellular matrix called as biofilms. Bacterial biofilms are associated with a number of infections such as endocarditis, cystic fibrosis, pneumonia, infectious kidney stones, inner ear infections and many hospital-acquired infections from catheters and ports. It is believed that the extracellular polymeric substance (EPS) could act as a barrier for antibiotic penetration and thus may contribute to the drug tolerance observed in biofilms. A number of Mycobacterial species are known to for biofilms including *Mycobacterium tuberculosis (Mtb), Mycobacterium smegmatis (Msm), Mycobacterium avium* and *Mycobacterium ulcerans*. [4]

Pathogenesis of TB

Infection occurs when a person inhales droplet nuclei containing tubercle bacilli that reach the alveoli of the lungs. These tubercle bacilli are ingested by alveolar macrophages; the majority of these bacilli are destroyed or inhibited. A small number may multiply intracellular and are released when the macrophages die. If alive, these bacilli may spread by way of lymphatic channels or through the bloodstream to more distant tissues and organs (including areas of the body in which TB disease is most likely to develop: regional lymph nodes, apex of the lung, kidneys, brain, and bone). This process of dissemination primes the immune system for a systemic response. [5]

Biofilm

Bacterial biofilms are major global health concern due to having capability to tolerate antibiotics. Biofilms are immobile colonize microbial communities and grow on medical implants like sutures, catheters, dental medical implants as well as plants and animal tissues through by self-produced extracellular polymeric substances. [6] Biofilm can be defined as it is a thick layer and aggregation of prokaryotic organism in the form of colony and communities. [7] These biofilms are the self-produced extra polymeric matrix which forms the environment of the bacterial microorganism. Biofilm consists bacterial cells and extra polymeric substance but the important ingredients that are present with in a biofilm matrix have drawn the attention of many scientific leaders all over the globe. Van Leeuwenhoek first observed and discovered microbial biofilm.[8] This biofilm matrix is also commonly referred as glycocalyx, the extracellular substances are typically polymeric substances and commonly comprise a matrix of complex polysaccharides, proteinaceous substances and glycopeptides, lipids, lipopolysacharides and other materials that serve as a scaffold holding the biofilm together. [9] Cells are those attached to the surface called sessile cells while the free swimming or

free-floating cells or counterparts called planktonic cells.

Biofilm Formation

Formation of biofilm done by the three stages:

Attachment of planktonic cells (Reversible or irreversible): The initial attachment of the planktonic cells to the surface by weak Van Der wall forces between two cells. Various parameters which affect the bacterial adhesion are covalent bonds, electrostatic interactions, acid-base interactions. This is called reversible because it can be detached. When these cells transforming free floating cells to sessile cells structure and their attachment is strong to the surface, interaction/attachment within the cells. Some extra polymeric substance secreted by the cells to make the strong adhesion to the surface which is responsible for the formation of the biofilm. Flagellum and others responsible for formation of contact between the biofilm and the surface and formation of colonies. The cells that attach irreversibly to surfaces (i.e., those not removed by gentle rinsing) will begin cell division, form microcolonies, and produce the extracellular polymers that define a biofilm. [10]

Maturation:

After attachment to a surface a biofilm grows in a three-dimensional manner. Based on this complex biofilm formation, two characteristics are strongly correlated with biofilm bacteria: the increased production of EPS and the development of antibiotic resistance. Bacterial colony can be up to 1000 times greater than bacteria in a planktonic state . Bacteria communicate with one another using small molecules, part of a process called quorum sensing (QS). QS has been intimately associated with biofilm formation with the release of small molecules called autoinducers (AI) or pheromones into the surrounding environment. In a mature biofilm, more volume is occupied by the loosely organized glycocalyc matrix (75-95%) than by bacterial cells (5-25%). [11]

Detachment and dispersal:

The final stage of the biofilm formation nvolves the release and disperse of the mature cells and so the same process starts. Biofilm is regarded as the primary source infections. Disperse of planktonic cells accelerate the formation of the new biofilms. Factors like mechanical force, nutrient scarcity and accumulation of waste products, pH alterations, and termination of biofilm building materials enable the detachment and dispersal of the mature biofilms in to the environment. [12]

PHYTOCHEMICALS

The emergence of bacterial resistance to various antibiotics has increased the rate of infectious diseases worldwide, accounting for more than 41% of the global disease. The emergence of multidrug-resistant organisms has forced the scientist to search for new antimicrobial substance from various sources including medicinal plants. Herbal extracts are found to be more efficient, safer and better-quality drugs with improved antibacterial and antifungal activities.: The majority of current infectious diseases are almost untreatable by conventional antibiotic therapy given the advent of multidrug-resistant bacteria. The degree of severity and the persistence of infections are worsened when microorganisms form biofilms. Therefore, efforts are being applied to develop new drugs not as vulnerable as the current ones to bacterial resistance mechanisms, and also able to target bacteria in biofilms. Natural products, especially those obtained from plants, have proven to be outstanding compounds with unique properties, making them perfect candidates for these much-needed therapeutics.

Nature continues to inspire the discovery of novel compounds with interesting structures and biological activity. These naturally derived compounds have served as scaffolds for the development of a plethora of synthetic therapeutic agents. Currently, many groups are focusing their research on the discovery of novel compounds capable of inhibiting biofilms.[13]

The research on phytochemicals and use of phytochemicals is increasing more because of the harmful side effects of the synthetic compounds. [14]

Herbal Extracts Against Myobacterium Tuberculosis

The individual n-hexane extracts of *Costusspeciouss*, *Cymbopogoncitrus and Tabernaemontana* coronaria showed the anti-TB activity with MIC of 100-200µg/ml and MBC of 200µg/ml. [15]

Thymol and carvacrol also shows the antimycobacterial activity with the MIC estimation of 0.78 and 2.02µg/ml against *M. tuberculosis* and carvacrol introduced MIC of 2.02 and 5.20µg/ml

individually [18]. Adelina Jimenez-Arellanes and Mariana Meckes found in their study that the hexane extracts of *Artemisia ludoviciana (A. ludoviciana)*, *Chamaedoratepejilote (Ch. tepejilote)*, *Lantana hispida (L. hispida)* and *Malvaparviflora (M. parviflora)* were active against *M. tuberculosis* at 200 µg/mL. *J. communis* hexane extract showed activity against *M. tuberculosis* at 100 µg/mL. [16]

Phenylpropanes as Cinnamaldehyde and cinnamic acid shows the antibacterial activity against M. Tuberculosis with the MIC of 8.16 and 3.12µg/ml. [17]

Phytomolecule plumericin extract from *Plumeria(Apocynaceae)* demonstrated action against delicate as four MDR strains of *Mtb* with MIC estimations of 0.0015 to 0.002 mg/mL and MBC (minimum bactericidal concentration) estimations of 0.003–0.004 mg/mL.

Bioactive compounds indicate a favorable position over rifampicin and isoniazid by being profoundly showing the dynamic activity against the MDR strains. [18]

A study done on the crude extract, diospyrin and 7-methyljuglone obtained from the plant, exhibited minimum inhibitory concentrations (MIC) of 8.0, 8.0, and 0.5 µg ml^{-1}, respectively, against *M. tuberculosis* H37 Rv (ATCC 27294), a drug-sensitive strain. Minimum inhibitory concentrations (MICs) of 7-methyljuglone against drug-resistant strains of *M. tuberculosis* ranged from 0.32 to 1.25 µg/ml. The concentration of 7-methyljuglone that effected a 90% reduction of growth of *M. tuberculosis* .[19]

A study found that the methanolic extract of *Kingiodendronpinnatum Rox. Hams, Humboldtiabrunonis Wall, Derrisscandens Benth and Ceasalpiniamimosoides Lamk* completely inhibited the growth of *M. tuberculosis* at the concentration of 50µg/ml. [20]

Crude extract and active constituents of *Morindacitrifolia Lin* noni fruit such as flavonoid, scopoletin, antraquinon and alkaloids shows the anti-mycobacterial property against M. tuberculosis H37Rv strain. At various doses (p value=0,000). Crude extract (59.00±60.513), alkaloids (64.83±49.356), anthraquinones (69.50±50.396), and flavonoid (72.92±58.728) showed the highest anti-tubercular activity in inhibiting the growth of *Mycobacterium tuberculosis* strain H37Rv. The minimum inhibitory concentration was found at a dose of 40 mg/ml. [21]

Bhunu, B Mautsa R & Mukanganyama S found in their experiment the *Parinari curatellifolia* ethanol extract, dichloromethane extract and water extract were the only extracts that effectively inhibited biofilm formation in *M. smegmatis*. Combining the ethanol extract with kanamycin enhanced the effect of the ethanol extract in terms of inhibition of biofilm formation. *Parinari curatellifolia* leaves contain phytochemicals that have the potential to be used both as antimycobacterial and anti-biofilm formation compounds. [22]

Lippia javanica (Verbenaceae) is an aromatic herb that occurs all over Mozambique. This compound was tested against *Mycobacterium tuberculosis*, it was found to exhibit a minimum inhibitory concentration of 50 µg/mL against sensitive strain of *M. tuberculosis*, H37Rv reference strain (27294). [23]

The methanol extract of *Alstoniascholaris* and *Mucunaimbricata* in murine model shows the antibacterial property. Female BALB/c mice were infected with the *Mycobacterium tuberculosis* $H_{37}Rv$ suspension. Histopathology study showed cells such as lymphocytes, epithelioid, Langhans giant cell, and fibrous tissue proliferation in lungs, depletion of lymphocytes in the spleen. The data indicate that methanol extract of *A. scholaris* has potential antimycobacterial activity, and the synergistic group consisting of rifampicin and *A. scholaris* could be a rational choice for the treatment of TB. [24]

80% of methanol extracts of *P. stellatum and O. integrifolia* and 80% of methanol and acetone extracts of *P. Americana* had anti-tuberculosis activity ($p<0.001$) against M.tuberculosis H37Rv. [25]

Phytochemicals Inhibitory Effects on other Bacterial Species

Many plant-derived natural products possessed antimicrobial and anti-biofilm functions *In -vitro*. A variety of molecules derived from natural plants or medicinal herbs extract as well as the underlying mechanisms in anti-biofilm function were identified. The anti-biofilm effects of natural products are mainly relying on the following aspects, the inhibition of formation of polymer matrix, suppression of cell adhesion and attachment, interrupting ECM generation and decreasing virulence factors production, thereby blocking QS network and biofilm development.

Reserpine, being found extensively in the plants of genus *Rauwolfia,* was effective at biofilm inhibition of *Klebsiella pneumoniae* at 0.0156 mg/mL, which was 64-fold lower than its MIC. [26]

Zhao et al. found that tetrandrine inhibited *Candida albicans* biofilm formation in a dose-dependent manner. Tetrandrine can break down the maintenance of 60% mature *C. albicans* biofilm at 32 mg/L. The results also indicated that tetrandrine may inhibit hyphal growth through the Ras1p-cAMP-PKA pathway which plays an important role in promoting hyphal growth. [27]

The highest (66%) anti-biofilm activity against *Bacillus* sp. Mcn4 was observed with *T. absinthioides* and *L. divaricate* extracts. The highest (68%) anti-biofilm activity against *Staphylococcus* sp. Mcr1 was observed with *L. chilense* extract. *T. minuta*, *T. absinthioides*, and *L. divaricata* showed percentages of anti-biofilm activity of between 55% and 62%. The anti-adherence effects of *T. minuta* and *L. chilense* observed in *Bacillus* sp. Mcn4 reflected a difference of only 22% and 10%, respectively, between anti-adherence and biofilm inhibition [28]

An ethanolic extract rich in ellagic acidobtained from the root of *R. ulmifolius* used to inhibit the *S. aureus* biofilm formation. On studying results demonstrate that extract 220D-F2 from the root of *Rubus ulmifolius* can be used to inhibit *S. aureus* biofilm formation to a degree that can be correlated with increased antibiotic susceptibility without toxic effects on normal mammalian cells. [29]

Cranberry fruit is a rich source for polyphenols. Studies have reported that a non-dialysable cranberry fraction enriched in high molecular weight polyphenols inhibits biofilm formation and prevents the attachment and colonization of human pathogens, especially cariogenic and period onto pathogenic bacteria, to host tissues. [30]

Researcher found in a study that Xylitol and farnesol have potency to inhibit the biofilm formation by the Staphylococcus aureus (SA). [31] Farnesol also shows the anti-biofilm activity against the *B. pseudomallei* . [32]

Allicin comes from a precursor substrate Allilin. Allicin inhibited the biofilm formation of Staphylococcus epidermidis and prevent the adherence of this bacteria to medical devices. [33]

A study on the *L. nobilis* L showed that the composition of *L nobilis L* consist the anti-biofilm activity against the Staphylococcus aureus . MTT assay revealed that active compositions displayed an excellent antibiofilm activity with eradication percentages ranging from 79.6 ± 2.27 to 95.2 ± 0.56. [34]

CONCLUSION

In the period of antibiotic resistance "superbugs" the formation of new antibacterial is significant and herbal extracts are an attractive source for new drugs and also herbal extracts have less side effects as compared to synthetic drugs. In the present paper, we have reviewed the various phytochemicals against mycobacterium species and phytochemicals effect on bacterial biofilm formation. Herbal extracts shows the inhibitory effects with lower (MIC) and also shows the synergistic effect with anti-tubular drugs. Thus, combining target specific properties of anti-TB drugs with multiple health benefits of medicinal plants could be a positive way out for the management of tuberculosis and associated side effects.

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