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RECENT CHALLENGES IN TUBERCULOSIS TREATMENTS: A REVIEW

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Abstract

Tuberculosis (TB) is one of the major leading causes for the death according to world health organization. The highest percentage of TB are recorded in Africa (72%), followed by India (27%), and China (9%). Multi-Drug resistant TB of rifampicin was stated in India, China, and Russian federation which represent around 50% of world cases. Therefore, studying the virulence factors of Mycobacterium tuberculosis (M. tuberculosis), early diagnosis of TB, and understanding the infection mechanism can aid for discovering of new potential therapeutic agents. To address TB disease problem and their side effect, metallic nanoparticles such as gallium, graphene oxide, titanium oxide, silver, and chitosan are used as new treatments. Furthermore, natural phytochemical compounds of medicinal herbs can be help in the treatment chest related diseases such as TB. Allicin, vaccine acetate, coumarin, piperine, and andrographolide, glabridin have known for their anti-mycobacterial properties through disruption bacterial colonization and replication. Different combating strategies can be solution for treatment this fierce disease.

Keywords : Mycobacterium tuberculosis, virulence factors, diagnosis, nanoparticles, phytochemical compounds.

Introduction

More than ninety species are documented in Mycobacterium genus. Two species are recognized as M. leprae and M. tuberculosis, which cause serious diseases in humans (Ryan and Ray 2004). The Greek prefix Myco reflects their mold like Growth nature. The cell wall of Mycobacterium is hydrophobic mycolic acids and peptidoglycan linked to arabinogalactan as polysaccharides. So, searching for new drugs requires the occurrence of defect in the biosynthetic pathway of the cell wall which plays an essential role in virulence (Bhamidi 2009).

M. tuberculosis was first discovered by Robert Koch 1982. These intracellular rods-shaped pathogenic bacteria can persist in the host for a long time without causing infection (Cooper 2009). Tuberculosis (TB) is caused by different associates of M. tuberculosis complex, such as M. africanum and M. tuberculosis, which are the sources of TB human infections. However, M. bovis,M. caprae and M. pinnipedii, are the cause of TB mammals' infections (Deloguet al., 2013).

According to World Health Organization (WHO), TB is one of major ten leading causes of mortality in the world. In 2017, Two hundred, and Thirty Thousand of children died from one million infected with tuberculosis disease. The highest percentage of Tuberculosis are recorded in Africa (72%), followed by India (27%), and China (9%). Even rifampicin, which is the most effective drug for treatment, 82% of new cases showed resistance against this drug. Multi-Drug resistant TB of rifampicin (MDR/RR TB) was reported in three countries (India, China, and Russian federation) which represent around 50% of world cases (WHO 2018).

Clinical behaviour of *M. tuberculosis*

Aerosolized droplets of M. tuberculosis, which are produced by the cough of infected persons, can transmit into a healthy person though inhalation. M. tuberculosis replicates originally in the alveoli of the lung then translocated into lung lymph nodes, and disseminate through the body. This pathogen can be latent in the host for a period of time without the appearance of TB clinical symptoms. Reactivation of the pathogen can occur in healthy or immunosuppressed persons, and also can appear in another organ instead of lungs. The symptoms of TB in case of lung infections are fever, loss of weight, cough, and damage of lung tissues. The interaction between the host immune response and pathogenic bacteria aid in understanding the bacterial pathogenesis. The consecutive pathogenic tactics starts with replication in the host macrophage and modify the host immune response to make conditions favourable for replications, then it should be able to persist in a dormant state for later reactivation (Glickman and Jacobs 2001).

Virulence factors of M. tuberculosis

The detection of virulence factors is desirable for well understanding of infection mechanism and for discovering of new potential therapeutic agents. Different virulence factors which is denoted in Fig. 1, and explained in details as follow: -

a. The Cell Wall

The cell wall structure's is the first strong impermeable barrier for drugs. The outer membrane of these bacteria resembles the outer membrane of gram-negative bacteria which comprises of inner mycolic acids and glycolipids & outer waxy layer. There is a thin layer of peptidoglycan between the outer and inner membrane which is associated

with arabinogalactan and lipoarabinomannan network, and bounded to mycolic layer (Hoffman et al., 2008; Zuber et al., 2008).

M. tuberculosis receptors for macrophage invasion

M. tuberculosis uses numerous receptors for macrophage infections such as mannose, complement, and FC receptors (Derem and Underhill, 1999). This pathogen locates in the vacuole of membrane, and maintains its survival inside the cell by altering the development of phagosome (Clemens and Horwitz 1995). Modifying the development of phagosome take place by the variation of the protein content and RabGTPase configuration (Clemens et al., 2000).In addition to, the elimination of ATPase proton with sequential acidification deficiency, and maintenance of TACO protein (Gatfield and Pieters, 2000). Till now, the relationship between modified macrophage responses and the infection that facilitates tissue damage and clinical symptoms are not well understood (Glickman and Jacobs 2001).

b. Virulence Proteins

ESXI is the main virulence factor for M. tuberculosis five type 7 which can translocate from the phagosome into cytosol of microphage (Abdallahet al., 2007). Also, other antigens such as ESAT-6, CFP-10, and two minor extremely immunogenic protein are secreted (Dielet al., 2011). M. tuberculosis needs iron and zinc for their growth and, ESX3 secretes some soluble factors involved in the optimum uptake for iron and zinc elements (Serafiniet al.,2009). The role of other antigens such as ESX2 and ESX4 is still unclear. Clarify the role and the purpose of the ESX secretion system is one of the major challenges of last years in the detection of TB pathogenicity (Delogu et al., 2013).

HBHA and PE_PGRS proteins on the surface of M. tuberculosis are in charge of bacteria adhesion to the host cells. The host defence response against HBHA relying on TB status, and the absence of PE_PGRS protein can prevent the bacteria from colonization in the lung tissue (Iantomasiet al., 2011&Zumbo et al., 2013). The sensitivity of M. tuberculosis to environmental factors such as low oxygen concentration and loss of nutrients activate the dormancy survival regulon (Dos hyproxia sensor) to be in a dormant state. In this state, bacteria stop their multiplications, trigger the anaerobic conditions, and stress proteins for acquisition their characteristic biological and immunological structures. For this reason, the bacteria can persist for long periods, and change into an active form by using resuscitating promoting factors (rpf), which generate a series of actions that endorses the bacterial growth (Kumar et al.,2007&Voskuil, et al.,2004).

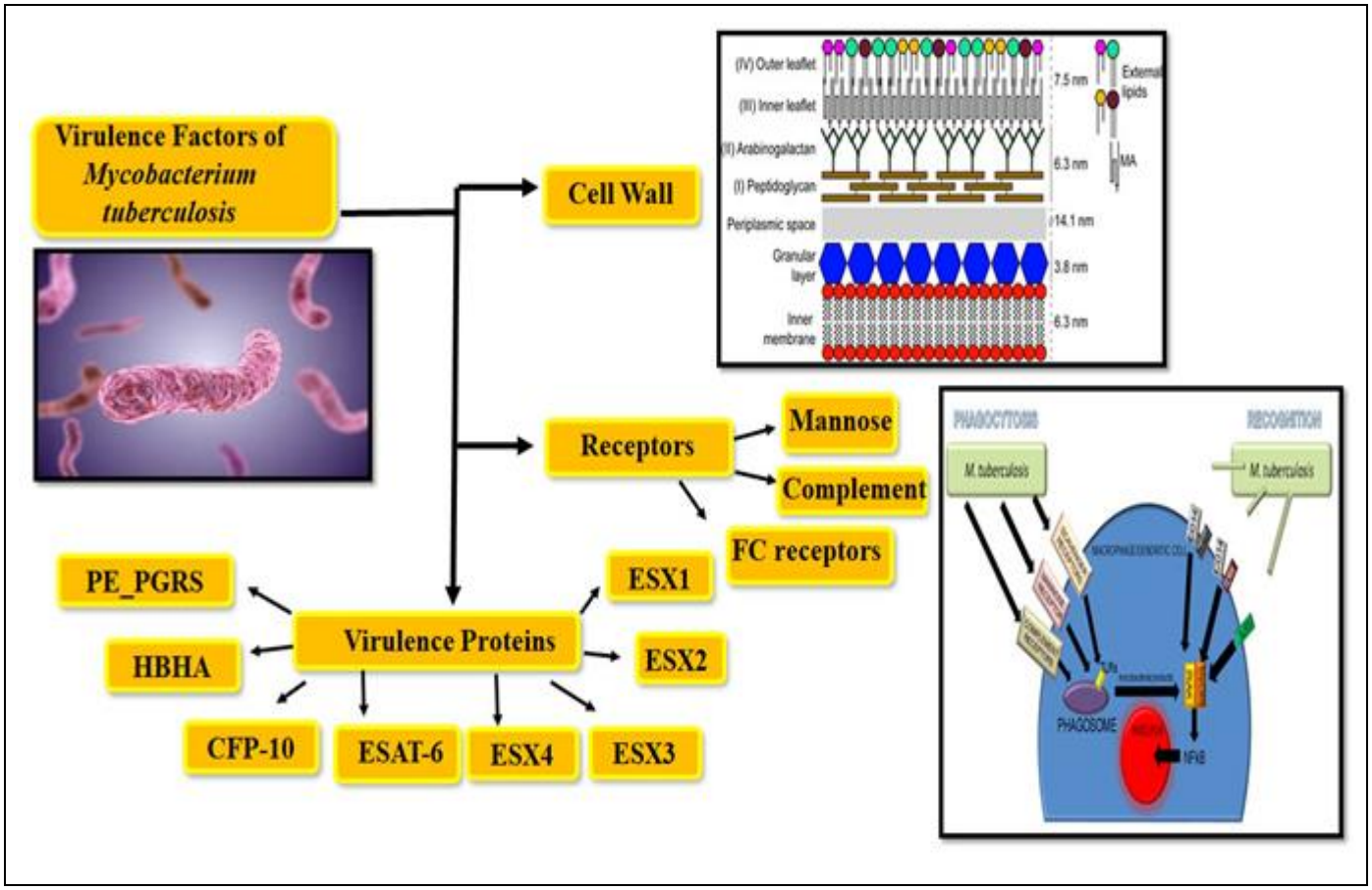

Fig. 2 : Virulence Factors of M. tuberculosis.

Determination of the temperature tolerance range of microemulsions made of single surfactant

As demonstrated previously, LGO and citral microemulsions made of single surfactant showed temperature sensitivity. Therefore, a second thermal evaluation was performed in the current investigation in order to determine a defined temperature range through which LGO and pure citral microemulsions made of single surfactant can persist and maintain their appearance as microemulsions. That attempt was fulfilled by visual observation of these microemulsions during slow and gradual cooling and heating cycles above and below the formulation temperature. Results of this evaluation showed that at the formulation temperature (25°C), the microemulsions made of single surfactant had bluish florescent and translucent appearance (Figure 3, sample a). Upon increasing the temperature gradually, the intensity of that bluish color increase until reaching 29°C -30°C at which the microemulsions completely lost their bluish appearance and became cloudy dispersion indicating the loss of the microemulsion characteristics (Figure 3, sample b). On the other hand, if the temperature is decreased gradually below 25°C, the microemulsions made of single surfactant becomes crystal clear and totally transparent (Figure 3, sample c), indicating the retention of the microemulsion character. Based on that result, the optimum range of temperature tolerance of LGO and citral formulated using single surfactant is preferably 25°C or below.

TB Diagnosis

1. **Sputum smear microscopy**

Acid Fast bacilli smear (AFB) is a cheap primary detection of M. Tuberculosis, but > 30% of patient outcomes are false-negative (Miorner et al., 1994). The WHO assesses that the test can only be used in 35% of patients to detect TB (Harris 2004). Several reasons for the false-negative result, one of which is the low accuracy examination, which requires a certain number of bacilli in one ml of sputum to be diagnosed reliably. Therefore, the analysis is not unique to *M. tuberculosis*, certain bacteria may also give false positive TB. In fact, extrapulmonary TB detection is restricted (Perkins 2000).

2. **Chest Radiography**

The abnormalities on chest X-rays indicate the infection of pulmonary tuberculosis where the pulmonary hollows and abrasions are look smaller compared with healthy people. Chest x-ray can check late stage of tuberculosis in the lungs only. Furthermore, the distortion in detection results may be appeared by presence of scars in lungs if the person past infected with TB and then cured from disease (Al-Zamel 2009).

3. **DNA Amplification Test**

This sensitive diagnostic assay can be used for both pulmonary and extrapulmonary TB. This technique may amplify DNA, but a certain number of bacilli should be included in the sample, and in the case of no pulmonary infections. Therefore, it is necessary to combine other tests with this assay and increase the accuracy of the test.

Different combating strategies Against *M. tuberculosis*

1. **Drug target by Nanoformulations**

Drug delivery systems are affecting on stimulation of the cells that they bind. Several studies have shown that the phagocytic absorption of particulate matter is often correlated with activation of the macrophage (Prior *et al.,* 2012). Sharma *et al.,* 2007 and, Yadav *et al.,* 2007 have reported that PLA-microparticles phagocytosis acts as an activation signal and reverses alternatives to classical mycobactericidal activation. When HIV -virus and TB persist in mononuclear phagocytes, the TNF-α will unable to employ its functional anti-mycobacterial action.

1. **Gallium Nanoparticles**

Gallium metal resembles iron metal and can bind to iron binding sites, once the binding occurs in enzyme, the enzyme become inactive. Also, the bacteria which have siderophore that chelate iron metal, cannot discriminate between iron and Gallium. All of these, causing disruption of bacteria leading to their death. Similarly, HIV requires iron metal for DNA replication, and viral cytopathogenicity. Thus, gallium could be easily replaced all essential iron factors and inhibit HIV replication (Savarino*et al.,* 2008; Drakesmith & Prentice 2008). Narayanasamy*et al.,* 2015 synthesize Gallium Nanoparticles that can persist in macrophage and facilitate the drug delivery to manage TB and HIV. They recorded that no toxicity for gallium on macrophage was observed for > 37 days.

2. **Graphene oxide Nanoparticles (GO-NPs)**

GO-NPs, which consist of carbon honeycomb and oxygen, can aid in the treatment of TB infections. It was confirmed by Park et al., 2015 and Zhang et al., 2011 that GO-NPs can gather in lungs without causing toxicity. Thick walled microorganism act as barrier for GO-NPs penetration However, GO-NPs controlled mycobacterium replication without affecting the cell integrity thereby shielding microorganisms from external surroundings, and preventing it from consuming their nutrients (Khanra et al.,2012; De Maio et al.,2019).

3. **Titanium Oxide Nanoparticles (TiO_2-NPs)**

TiO2-NPs have been applied for biological applications as cosmetic industry or antibiofilm as Streptococcus mutans and antibacterial activities. Also, TiO2-NPs were characterized by their poor solubility which reduced its toxicity. Spherical TiO2-NPs (16nm) were synthesized by using sol- gel method, and exhibited antimycobacterial activity against M. tuberculosis, M. bovis, and M. species.

By increasing the concentration, TiO2-NPs were affecting on biofilm formation, and reduced the metabolic activity of the bacteria up to three or four times. (Ramalingam et al., 2019).

4. Silver Nanoparticles (Ag-NPs)

Ag-NPs with an average particle size 50 nm have antimycobacterial activities against M. tuberculosis, M. bovis at concentrations values of 4-32 µg/ml, and 1-16 µg/ml, respectively (Selim et al., 2018).

5. Chitosan Nanoparticles (Ch-NPs)

Chitosan is natural constituent of external skeleton of insects, arthropods such as crustaceans, and Fungi (Youneset al., 2015; Vilaret al., 2016). Chitosan is known with their reactive functional group and gel formation, besides the antimicrobial, antifungal, even antitumor activities (Kong et al., 2010; Goya et al., 2009). Manipulation the surface of Ch-NPs attracted many researchers for pharmaceutical applications (Ahmed et al., 2016; Qi et al., 2014). Moreover, Ch-NPs improve the penetration of large particles through a mucosal surface (Sinai et al., 2010). Similarly, Wardanal et al., 2018 recorded that Ch-NPs have antimycobacterial effects against M. tuberculosis H37Rv with a MIC value of 1200 µg/mL whilst MBCs value of 2400 µg/mL by using the broth microdilution susceptibility method.

6. Plants derived prodrugs used for TB treatment

Worldwide, TB is the one of the primary causes of death. M. tuberculosis which accountable for TB. In absence of efficient therapeutic drugs for TB, beside the major side effects of anti-TB drugs are hepatotoxicity, ototoxicity, nephrotoxicity, skin rashes, fever, peripheral neuritis and rarely psychotic changes as well as increasing of Multi Drug Resistance TB has come to be a major threat, and therefore calls for a vital need to discover new efficient and nontoxic anti-M.tuberculosis and anti-TB treatment that will can improve therapeutic scenario and address both disease and side effects. Since no anti-TB drugs have been introduced in past 30 years, hope is built on plant based natural products due to their chemical diversity and important role as natural derived prodrugs. Mother Nature ensures a stock of medicinal herbs to become able to treat diseases. Herbs are undying gold gift chance for the treatment of many diseases in human beings since time immemorial. As a main framework of traditional health care systems, medicinal herbs have got produced a myriad impact to be able to preserve the human fitness and health. Owing to the improving tendency of human to plants derived prodrugs. Medicinal herbs are reservoirs for innovative chemical entities and offer a promising line for research. Hitherto, being efficient, simple, nontoxic, eco-friendly, cheap, fast, and safer as compared with standard treatments. According to an estimate of WHO, about 80% population rely on traditional medication for human health care. The use of herbs and phytochemicals is of great significance for the treatment of various ailments including TB. The efficacy of unique herbs as anti-Mb and anti-TB agent exemplifies progress in the search for perfect medicines. The effectiveness of herbs for the management of TB has been discussed by many researchers. This review was designed to document comprehensive details on different anti-Mb and anti-TB herbs.

Flavonoids phytocompounds are extensively utilized for their biological potentials (Mossa et al., 2015; Ibrahim et al., 2016; Salam et al., 2016; Abou Baker 2020; Abou Baker and Rady 2020; Abou Baker et al., 2020a; Abou Baker et al., 2020b; Ibrahim et al., 2020). These phytocompounds, act as antimicrobial by inhibiting the synthesis of microbial cell wall constituents (Ng et al., 2019), also play a crucial role in increasing antibiotic action against resistant bacteria (Górniak et al., 2019; Khameneh et al., 2019). Moreover, these phytocompounds shown superior EPI activity against bacteria and promising activity in reducing the side effects of anti-TB drugs such as hepatotoxicity (Fig. 2). Numerous studies support their antioxidant and hepatoprotective actions (El Gengaihi et al., 2016a,b).

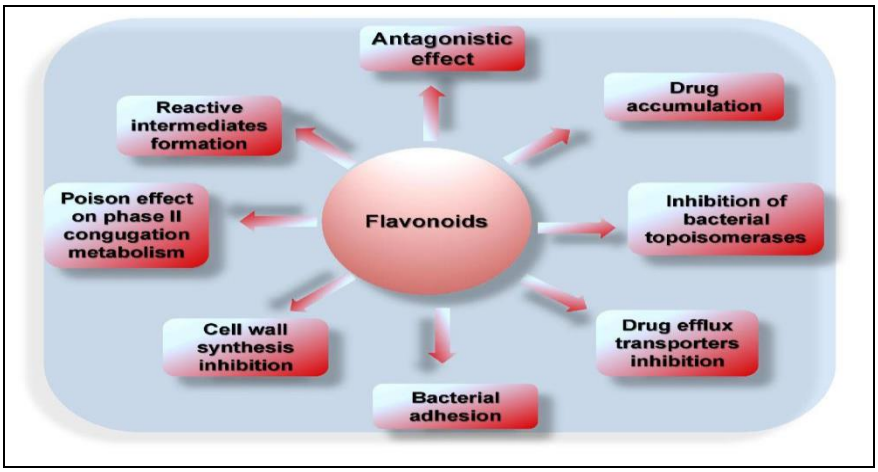

Fig. 2 : Mode of action of flavonoids on *M. tuberculosis*.

Saritha et al. (2015) reported mechanisms accountable for antimicrobial action of phytocompound, which includes disruption of bacterial cell membrane, permeabilization and leakage of cellular contents. Thus, comparable to synthetic drug, phytocompound also interfere with various cellular mechanisms to inhibit bacterial growth.

Allicin, andrographolide, coumarin, vasicine acetate, and glabridin are some examples of phytochemicals studied in recent years for their antimycobacterial actions.

Sharma et al. (2010) reported that piperine can inhibit a putative multidrug efflux pump (Rv1258c) of MB by binding with amyloid proteins and competes with lipopolysaccharides for binding with TLR4/MD-2 leading to disruption of bacterial colonization, this proficiency can result in a great inhibition of inflammation throughout the infection.

Many terpenoid displayed antimycobacterial action, such as (+)- Totarol, parthenolide, sandaracopimaric acid, Agelasine F, elisapterosin B, 1,10-epoxycostunolide, santamarine, reynosin, alantolactone, costunolide, puupehenone, elatol, deschloroelatol, debromolaurinterol, allolaurinterol, what's more, aureol. This antimycobacterial action is due to the high lipophilic properties of terpene which encourage their penetration into the*MB* cell wall (Copp, 2003).

Plants are proven to prevent multidrug efflux system of microbes as well as many health wellness benefits as hepatocurative which can restore liver function, antioxidant enzymes activity and histology of hepatic cells against anti-TB medications. Table 1 summarizes list of plant traditionally used as anti-TB treatment, an attempt has been made to highlight the promising plant species for further investigation as leads for drug development.

Table 1 : Antifungal activity of LGO and citral microemulsions formulated using single surfactants against some plant pathogenic fungi.

Plant	Part	Activity	References
Chenopodiumambrosioides	Acetone and essential oil	0.5 mg/mL	Lall and Meyen (1999)
Bidenspilosa	Ethanol	100 µg/mL	
Menthapiperita L.	Methanol	>500 µg/mL	
Struthanthusmarginatus	Hexane	Terpenes steroids 12.5 µg/mL	Leitão et al., 2013
Cryptocaryalatifolia, Eucleanatalensis, Helichrysummelanacme, Nidorellaanomala and Thymus vulgaris	Acetone as well as water extracts	inhibited MB 0.5 mg/ml	Lall, and Meyer, 1999
Sarraceniapurpurea,	Methanol	triterpenesbetulinaldehyde, β-sitosterol, betulinic acid, and ursolic acid	Morrison et al., 2016
Solanumtorvum		156.3 µg/mL	Nguta et al., 2016
Azadirachtaindica	Stem bark	Not detected	Nguta et al., 2015
Hygrophilaauriculata	Whole plant		
Chenopodiumambrosioides	Leaves		
Coixlacryma-jobi	Glumes		
Solanumtorvum	Unripe fruits and leaves		
Bidenspilosa	Whole plant		
Phyllanthusfraternus	Leaves		
Dissotisrotundifolia	Leaves		
Cymbopogongiganteus	Leaves		
Cyperus articulates	Cyperaceae Roots		
Allium sativum	Bulbs	Not detected	Faleyimu et al., 2009; Greene et al., 2010
Zingiberofficinale Curcuma longa	Rhizome	Not detected	Ogudo et al., 2014
Allium cepa	Bulbs Leaves	Not detected	(Ogbole and Ajaiyeoba, 2010; Lawal et al., 2014; Ngutaetl al., 2015)
Aloe vera	Leaves	aqueous extract Organic extract	
Cocosnucifera	Coconut water, husk	Not detected	
Enantiachlorantha	Bark methanol extract	250 µg/ml	Nkenfou et al., 2015
Cissuspetiolata	Stalk methanol extract	250 µg/mL	
Beilschmiediaobscura	Roots ethyl acetate extract	31.25 µg/mL	
Urerarepens	Stalk MeOH	62.5 µg/mL	
Acanthus montanus	Stalk Hexane	62.5 µg/mL	

Garciniapreussei	Roots Hexane/ethyl acetate	125 µg/mL	
Thymus satureioides	aerial parts essential oils	0.062% to 0.015% (v/v)	Chraibi et al., 2016
Menthapulegium		0.125 to 0.031% (v/v)	
Euphorbia paralias	Quercetin 3-O-glucoside	Methanol is the most active with no toxicity inhibits the glutamine synthetase enzyme	Safwat et al., 2018
Lippiascaberrima	Essential oil	MIC of 125 µg/mL	Reid et al., 2020
Maeruaedulisand Securidacalongepedunculata	n-Hexane extracts	31.2µg/ml	Luo et al., 2011
Tabernaemontanaelegans	ethyl acetate extract	15.6 µg/ml	
Zanthoxylumcapense	dichloromethane extract	31.2 µg/ml	
Acacia seyal	ethanol bark extracts	0.78 mg/ml	Eldeen, I.M.S. and Van Staden, J., 2008
Erythrinalatissima	ethanol Bark extracts	0.39mg/ml	
Combretumhartmannianum	Ethanol extract of leaf and bark	0.19 and 1.56mg/ml	
Ziziphusspina-christi	ethanol bark extracts	0.39 mg/ml	
Kigeliaafricana	Ethanol bark)	1.56	
Heracleum maximum	Methanolicextrac of roots	furanocoumarins and diynes	Johnson et al., 2017
Heteropyxisnatalensis	Acetone extract of bark, leaves , roots	0.08 mg/mL.	Dzoyem et al., 2016
Rhynchosiaprecatoria	*n*-hexane root extract	15.6 µg/mL	Coronado-Aceves et al., 2016
Euphorbia albomarginata	EtOAc shoot extract	250 µg/mL	
Helianthus annuus	*n*-hexane stem extract	250 µg/mL	
Thymus satureioides	Essential oil from areal parts	borneol and carvacrol	Chraibi et al., 2016
Menthapulegium		R(+)-pulegone	
Khayasenegalensis	bark and leaf chloroform extract	Hydrophobic compounds	Abuzeid et al., 2014
Rosmarinusofficinalis	The leaf extract hydrophobic fractions		
Eucalyptus spp., Warburgiasalutaris Ocimum suave ZanthoxylumchalybeumMomordicafoetida Perseaamericana Acacia hockii	decoctions and infusions	Not detected	Tabuti et al., 2010
Cola acumminata	fruit	Not detected	Ogbole and Ajaiyeoba, 2010.
Garcinia kola	leaf		
Vitallariaparodoxa	oil		
Costusafer	stem		
Pycnanthusangolensis	stem bark		
Aframomummelegueta	fruit		
Abrusprecatorius Ficussur, Pentanisiaprunelloides Terminaliaphanerophlebia	Whole plant Pet- ether, dichloromethane, 80% ethanol and water	Inhibit COX-2	Madikizela et al., 2014
Cryptolepissanguinolenta	Root	Treatment TB symptoms	Orodho et al., 2014; Bunalema et al., 2010
Garcinia kola	Leaf decoction	Treatment f TB	Ogbole and Ajaiyeoba, 2010
Crinum jagus	Methanolic extract of bulb	1.0 mg/ml	Akintola et al., 2013
Terminaliachebula	Chebulinic acid	Inhibit quinolone resistant mutants of *M. tuberculosis* DNA gyrase effectively	Patel et al., 2015

Conclusion

This review discusses in details the virulence factors of M. tuberculosis such as the cell wall, macrophage invasion, and viral proteins too control spread of TB over world. By increasing the resistance of this bacterium against antibiotics causing the mortality among people, there is an urgent need to discuss the better therapeutic scenario using phytocompounds and metallic nanoparticles. Physicians and researchers must continue to combat TB for complete eradication of TB.

Declaration of Competing Interest

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