In evaluate the anti-tuberculosis potential of metabolites

Business, Management

Inview of urgent need for new tuberculosis (TB) drugs, which were effective, cheaper and readily available from environment, thus research was carried outto evaluate the anti-tuberculosis potential of metabolites from mangroveactinobacteria against surrogate TB organisms (M. smegmatis. M. fortuitum andM.

kansasii) from Pulau Betong, Penang. 31 strains of actinomycetes were isolated and tested against surrogate TBorganisms in primary screening by using cross streak method. In secondaryscreening, crude extract was produced through fermentation method, extracted byorganic solvent extraction method, and tested against surrogate TB organisms byagar well diffusion method. The minimum inhibitory concentration (MICs) of thecrude extract against surrogate TB organisms were within 200 – 3200 ? g/mL. M. kansasii showedthe highest activity against crude extract with MIC of 200 ? g/mL. The findings of this study revealed that mangrove actinomycetes appeared tohave immerse potential source of antitubercular compounds worthy of furtherinvestigation. CHAPTER2: INTRODUCTIONTuberculosis(TB) which caused by Mycobacteriumtuberculosis was a highly prevalent infectious disease that resulted inmorbidity and mortality in human with almost one-third of global populationbelieved to be infected (Bratati & Ganguly, 2013; Manikkam, Venugopal, Subramaniam, Ramasamy, & Kumar, 2014; Sanei Taheri et al.

, 2015). TB still remains as a major causedof global public health problem (Sharma & Mohan, 2013). Based on Centersfor Disease Control and

Prevention (CDC), in 2016, 10. 4 million people aroundthe world became sick with TB disease and 1.

7 million TB-related deathsworldwide. TB was a leading killer of people who were HIV infected as the virusweakens a person’s immune system against TB germs. Theepidemiology of TB had become more serious due to the emergence of drugresistance among M. tuberculosis isolates and long-term therapy usingcombination of drugs for its treatment (Manikkam et al., 2014).

Hence, new antituberculardrugs to fight against drug resistant M. tuberculosis strains were urgentlyneeded (Ginsberg, 2010). Less sideeffects and improved pharmacokinetic properties were expected from new anti-TBdrugs with extensive and potent activity against drug resistant strains andability to reduce the total duration of treatment (De Souza, 2013). Actinomycetes werecommon soil inhabitant with excellent producers of novel antimicrobial agentswhich had the ability to produce exuberant secondary metabolites withbiological significance (Abd-Elnaby, Abo-Elala, Abdel-Raouf, Abd-elwahab, & Hamed, 2016; Rajan & Kannabiran, 2014; Xu, Ye, Han, Deng, & Hong, 2014). Out of 50% of the total microbialbioactive metabolites were reported from the members of actinomycetes (Bibb, 2013). In addition, the discovery ofstreptomycin shown that the first antibiotic used for antitubercular therapywas from Streptomyces griseus, and numerous antitubercular antibioticssuch as kanamycin and rifampicin were produced from actinomycetes ofterrestrial origin.

However, the survey of Streptomycesand other common terrestrial actinomycetes were nearly exhausted. Recently, isolation ofknown actinomycetes and antibiotic were frequently reported due to the explorationof actinomycetes from routine ecosystems but bioprospecting of less exploredecosystem such as mangrove had been proved consist a high number of bioactivecompound from novel bioactive actinomycetes (Bibb, 2013) including antitubercularmetabolites (Wang et al., 2013). CHAPTER 3: LITERATURE REVIEW3. 1TUBERCULOSIS3. 1. 1 Overview Tuberculosis(TB) was an infectious respiratory disease which was one of the oldest knownhuman diseases that was still becoming the major causes of mortality caused by M.

tuberculosis (Smith, 2003). This causal pathogen was spreadthrough airborne droplets and easily infected individuals based on the frequencyof contact with infected person, living in crowd population or unhygienic environments, and being an immunocompromised person. Therewere two types of TB disease that occurred in two different sites which are at pulmonaryand extrapulmonary organs. TB disease that infected at the pulmonary site knownas Pulmonary TB (PTB) refers to TB disease that affected the lungs with commonsigns such as cough, chest radiograph abnormality and may be infectious. While, TB disease that occurred in other parts of the body such as the brain, kidneys, larynx, lymph node, bones, or pleura known as Extrapulmonary TB (EPTB).

However, in human immunodeficiency virus (HIV)-infected person, both EPTB and PTB diseasescan occurred. As long as the person was not having PTB,

EPTB was not infectious. Centers for Disease Control and Prevention (CDC) stated that EPTB can alsooccurred in the oral cavity, or involved an open lesion with high concentrationof organisms. MiliaryTB, a rare type of TB was a widespread lymphohematogenous dissemination of M. tuberculosis, which was a lethal disease if not treated early (Ray, Talukdar, Kundu, Khanra, & Sonthalia, 2013). Tuberculousmeningitis was another form of TB disease that occurred when the tissues aroundthe brain and spinal cord were infected with TB.

3. 1. 2 Current Statistics ofTuberculosis InfectionReportedTuberculosis in the United States, 2016. Atlanta, by USDepartment of Health and Human Services, Centers for Disease Control andPrevention, CDC stated that a total of 9, 272 TB cases (a rate of 2. 9 cases per100, 000 persons) are reported in the United States in 2016. This is a decreasefrom the number of cases reported in 2015 and the lowest case count on recordin the United States. The case rate of 2. 9 per 100, 000 persons is a 3.

6%decrease from 2015. Even though the United States continues to make slowprogress, current strategies are not enough to reach the goal of TB eliminationin this century. Accordingto World Health Organization, 2015, about 9. 6 million new cases of TB wererecorded and 1.

5 million patients died, in which, 1. 1 million and 0. 4 millionwere HIV-negative and HIV-positive patients, respectively in 2014. Formultidrug-resistant TB (MDR-TB) cases, 480 000 cases reported, and 190 000 peopleswere estimated died from MDR-TB. India, Indonesia and China

accounted thelargest number of cases of the global total with 23 %, 10 %, and 10 %, respectively. 3. 1. 3 Current Treatment of TuberculosisTuberculosisdisease was when the TB bacteria became actively multiplying in the body and disruptedthe immune system function to stop the bacteria from continuously growing.

It was very important that people who had infectedwith TB disease to be treated, finished the medicine, and took the drugsexactly as prescribed, otherwise they became sick again and more crucial if theTB bacteria started to develop resistant against those drugs which will beharder and more expensive to be treated. Bytaking several drugs for 6 to 9 months, probability of the TB disease to betreated was high. There were 10 drugs currently approved by the U. S.

Foodand Drug Administration (FDA) for treating TB. Isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) were the approved drugs that acted asthe first-line antitubercular agents that form the core of treatment regimens. 3. 1. 4 Drug- Resistant Tuberculosis Incidenceof TB becomes worst with the emergence of multi-drug resistant (MDR) andextensively-drug resistant (XDR) strains of M. tuberculosis worldwidemake the incidence of TB became worst as the M. tuberculosis started to develop resistance againstboth the first-line and second-line anti-TB drugs (Guptaet al., 2010; Singh, 2007).

Strains that resistant to at least oneof the first-line drugs of isoniazid or rifampicin were known as MDR strains, whilethe strains that resistant to both

isoniazid and rifampicin, fluoroquinoloneand to at least one of the three injectable second-line drugs such as amikacin, capreomycin or kanamycin were known as XDR strains. (Galagan, 2014). The drug-resistant TB was very complicated to treat andcure, and may resulted into inappropriate management that can led to life-threatening.

3. 2 ACTINOMYCETES AS A SOURCES OFANTI-TUBERCULAR AGENTS3. 2. 1Properties of ActinomycetesActinomyceteswere Gram positive filamentous bacteria consisted of high guanosine-cytosine(GC) and classified to the phylum Actinobacteria (M Goodfellow & Williams, 1983). Mostactinomycetes are slow-growing bacteria and they form well-developed radialmycelium, which can be divided into aerial mycelium and substrate mycelium onisolation plates based on morphology and function (Chen, 2015; M Goodfellow & Williams, 1983). The mycelium maybreak apart to form rod or coccoid shaped forms in some species.

Many generaalso form spores where the sporangia, or spore cases, may be found on aerialhyphae, on the colony surface, or free within the environment. Actinomyceteswere the intermediate group between bacteria and fungi due to the present of afilamentous degree of organization like filamentous fungi (Chen, 2015). Their hyphae weresimilar to fungi, and their widths of the mycelium are quite similar to thewidth of bacterial cell. However, actinomycetes had been classified as bacteriasince they did not have chitin and cellulose in the cell walls which were oftenpresent in fungi. 3. 2.

2 Antitubercular Potential Actinomyceteshold the important position in production of bioactive metabolites which were responsiblefor the production of almost half of the discovered bioactive secondarymetabolites (Abd-Elnaby et al., 2016; Bérdy, 2005; Rajan , 2014; Xu et al., 2014).

More than 10, 000 antibiotics hadbeen isolated from actinomycetes until today (Kekuda, Shobha, & Onkarappa, 2010). Furthercharacterization of these actinomycetes had led to isolation and identificationof novel bioactive compounds with significant therapeutic potential. So far, only3 % of all antibacterial agents had been reported synthesized by Streptomyces (Watve, Tickoo, Jog, & Bhole, 2001). Actinomycetesregained its position as the center of antibiotic interest due to the inconcomitant of the information and emerging problem of multi drug resistance (MDR) and new pathogens developmentwhich inactivated the antibiotics and rendered an urgent need for newantibiotics that would target the emerging multidrug resistance. CHAPTER 4: DISCUSSIONSedimentsamples collected from Pulau Betong, Penang mangrove forest were divided intotwo parts as wet samples and dry samples.

Wet samples were directly diluted byusing serial dilution method while dry samples were allowed to air-dry at roomtemperature for a week before serially diluted. Results showed the number ofisolates from the dry samples were higher than wet samples. All31 isolates of actinomycetes were screened for their bioactive compoundproduction ability and 13 isolates was showed wide range of zone of inhibitionagainst surrogate TB organisms in the primary screening.

Primary screeningmethods like cross streak or cross spot method were not suitable for highlybiohazardous organism like M. tuberculosis thus surrogate TB organisms were used. During fermentation process, more yield ofcrude extract was produced through solid state fermentation method compared tosubmerged state fermentation method when extracted with methanol and ethylacetate solvents. The production of higher crude extract in solid statefermentation was due to the lack of water and completely miscible in organicsolvent (ethyl acetate and methanol) with the fermented biomass. These resultswere similar as previous reports (El-Naggar, El-Assar, & Abdul-Gawad, 2009; Mahdi, Termeh, Ehsan, Bahman, & Elnaz, 2012). The low yield production resulted fromthe submerged state fermentation method was due to the use of water immisciblesolvent such as ethyl acetate during extraction. TheMIC of the crude extracted from the actinomycetes were determined by using TEMAmethod against the surrogate TB organisms by observing the color change of MTTtetrazolium from yellow to purple due to the oxidation of the reagent. Theviable cells with active metabolism converted MTT into a purple color formazanproduct.

The color remained unchanged when the cells were died as they were unableto convert MTT into formazan, thus, color formation serves as a marker for theviable cells. In this study, the crude extract showed a wide range of activitytowards the test organisms with MIC values in the range of 200 – 3200 ? g/mL. The highest activity was shown by crude extract against M. kansasii withMIC of 200 ? g/mL. Therefore, the crude extract could be

potent sources ofantitubercular drug production, which will lead to the development of noveldrugs for the tuberculosis treatments.

Regardingthe tested surrogate TB organisms strain, the results showed that theslow-growing M. kansasii was more susceptible than the fast-growing M. fortuitum and M. smegmatis. These results indicated that thecellular growth rate could influenced the inhibition whereby the slow growerhad a longer doubling time compared to the fast grower. Fast grower organisms hada weaker defense ability, thus when the cells were inoculated into a newmedium, they may be lacked in essential enzymes or other components involved indefense mechanism, so as they took time to synthesize these components, theywere more susceptible to chemical attack. Besides that, the ultra-structure ofthese organisms may differ from each other especially on their unique cellwalls, which could had contributed to their defense ability.

A previous studyhad reported that cell wall thickness was significantly different betweenresistant and non-resistant strains of Mycobacterium (Velayati et al., 2009). CHAPTER 5: CONCLUSIONThis study concludedthat actinomycetes isolated from mangrove sediment were priceless naturalresources, having immerse antitubercular potential to treat tuberculosis asthey exhibited inhibitory activity against test Mycobacterium species, M. smegmatis, M.

fortuitum and M. kansasii with MICs of 200 -3200 ? g/mL. The highest activity was exhibited by crude extract against M. kansasii with MIC of 200 ? g/mL. In

general, crude extract were complexmixture of compound. However, in this study, only the crude extracts wereevaluated and found to be active against surrogate TB organisms, so additionalresearch like bioassay guided fractionation and characterization were needed tovalidate whether a single useful compound can be found, and it was also neededto determine the MIC and meaningful toxicity and specificity studies.