ABSTRACT

Boesenbergia stenophylla is a critically endangered ginger native to the highlands of Borneo and has been traditionally used as herbal medicine. Nevertheless, there is a scarcity of information regarding the specific components of ginger and the techniques used to extract and preserve the bioactive compounds of *B. stenophylla* rhizome and leaf parts. Thus, the objectives of this study were to identify the chemical constituents of ethyl acetate, ethanolic and methanolic leaf and rhizome extracts via Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS), while evaluating their antibacterial activities. In this study, soxhlet extraction with ethyl acetate, ethanol and methanol were utilized to produce crude extract from rhizomes and leaves of *B. stenophylla*. Then, the identities of phytochemicals in the crude extracts were then identified using GC-FID and GC-MS. The crude extracts were diluted and tested for antibacterial activities against selected waterborne bacteria, namely *Bacillus* sp., *Staphylococcus* sp., *Citrobacter* sp., *Enterobacter* sp. and *Klebsiella* sp through disc-diffusion and colorimetric broth microdilution assays. From the yield extraction, the ethanolic rhizome extract has the most yield (5.817 ± 0.613 mm), followed by methanolic rhizome extract (5.329 ± 0.536 mm) and ethanolic leaf crude extract (3.387 ± 0.774 mm). Based on the GC-FID and GC-MS analysis, 72 out of 110 phytochemicals were identified for various biological effects including potential antibacterial effects in the crude extracts. From the disc-diffusion assay, ethanolic leaf crude extracts showed the largest inhibition zone against *Enterobacter* sp. and *Citrobacter* sp. with average diameters of 21.6 ± 0.3 mm and 20.0 ± 0.6 mm, respectively. This is followed by methanolic rhizome crude extract against *Bacillus* sp. (16.3 ± 0.8 mm). These crude extracts were tested in the colorimetric broth microdilution assay where the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethyl acetate rhizome extract against *Bacillus* sp. were identified as low as 0.781 µg/µL. The MIC/MBC ratio of ethanolic and methanolic *B. stenophylla* extracts tested were found to be less than 4, which indicates the extracts can be utilized as bactericidal agents. However, the ethyl acetate crude extracts, except for rhizome extracts against *Bacillus* sp. and *Klebsiella* sp. as well as leaves extract against *Klebsiella* sp. showed MIC/MBC ratio same or greater than 4, suggesting the potential to be utilized as bacteriostatic agents. From this study, the crude extracts of *B. stenophylla*, especially ethanolic and methanolic rhizome and leaves displayed the highest extraction to treat the selected waterborne bacteria. Thus, these mentioned crude extracts should be intensively studied by purification and identification of the fractions or molecules that had potential to be used as the antibacterial agents to treat the waterborne bacteria.

Keywords: Antibacterial, *Boesenbergia stenophylla*, Gas-chromatography mass spectrometry, jerangau merah,

Aktiviti antibakteria Boesenbergia stenophylla (Jerangau Merah) terhadap air bawaan bakteria terpilih

ABSTRAK

Boesenbergia stenophylla ialah halia liar terancam yang berasal dari tanah tinggi Borneo dan digunakan sebagai ubat tradisional. Walau bagaimanapun, terdapat kekurangan maklumat mengenai komponen spe*sifik pada sebahagian daripada halia dan teknik yang digunakan untuk mengekstrak serta memelihara sebatian bioaktif B. stenophylla bahagian rizom dan daun. Oleh itu, maklamat kajian ini adalah untuk mengenalpasti konstituen kimia di dalam daun dan rizom esktrak etil asetat, etanol dan metanol menggunakan Gas Kromatografi-Pengesan Pengionan Nyala (GC-FID) dan Gas Kromatografi-*Spekterometri *Massa (GC-MS), sambil menilaikan aktiviti antibakteria. Dalam kajian ini, teknik pengekstrakan soxhlet telah digunakan untuk menghasilkan ekstrak mentah daripada daun dan rizom B. stenophylla. Seterusnya, identiti fitokimia dalam estrak mentah telah dikenalpasti menggunakan GC-FID dan GC-MS. Ekstrak mentah tersebut dicair dan diuji kesannya terhadap Bacillus* sp.*, Staphylococcus* sp.*, Citrobacter* sp.*, Enterobacter* sp. *dan Klebsiella* sp. *melalui asai peresapan cakera dan mikro-pencairan kaldu. Daripada hasil ekstrak, esktrak etanol rizom mempunyai hasil tertinggi (5.817 ± 0.613mm), diikuti dengan esktrak metanol rizom (5.329 ± 0.536mm) dan esktrak etanol daun (5.329 ± 0.536mm) and ethanolic leaf extract (3.387 ± 0.774mm). Berdasarkan analisis GC-FID dan GC-MS, 72 daripada 110 fitokimia telah dikenapasti untuk pelbagai kesan biologi, termasuk kesan antibakteria yang berpotensi dalam ekstrak mentah. Daripada asai peresapan cakera, ekstrak mentah etanol daun menunjukkan zon perencatan terbesar terhadap Enterobacter* sp. *dan Citrobacter* sp. *dengan purata diameter 21.6 ± 0.3 mm dan 20.0 ± 0.6 mm. Ini diikuti estrak mentah metanol rizom terhadap Bacillus* sp. *(16.3 ± 0.8 mm). Ekstrak-ekstrak mentah* *ini telah dikaji menggunakan asai mikro-pencairan kaldu di mana kepekatan perencatan minimum (MIC) dan kepekatan bakterisidal minimum (MBC) ekstrak etil asetat rizom terhadap* Bacillus sp. *telah dikenalpasti serendah 0.781 μg/μL. Nisbah MIC/MBC ekstrak etanol dan metanol B. stenophylla didapati kurang daripada 4, menunjukkan bahawa ekstrak-ekstrak tersebut boleh digunakan sebagai agen pembunuhan bakteria. Walaubagaimanapun, ekstrak etil asetat, kecuali ekstrak rizom terhadap* Bacillus sp. *dan* Klebsiella sp. *serta ekstrak daun terhadap* Klebsiella sp. *menunjukkan nisbah MIC/MBC yang sama atau lebih besar daripada 4, menunjukkan potensi sebagai agen-agen bakteriostatik. Oleh itu, daripada kajian ini, ekstrak mentah B. stenophylla terutamanya etanol dan metanol rizom serta daun perlu dikaji secara intensif menggunakan proses penulenan dan pengenalpastian molekul-molekul yang berpotensi diaplikasikan sebagai agen antibakteria untuk merawat bakteria bawaan air.*

Kata kunci: *Antibakteria,* Boesenbergia stenophylla, *Gas kromatografi*-spekterometri *massa, jerangau merah*