

ABSTRACT

The significant rise of new diseases and development of resistant pathogenic microorganisms have resulted in the increasing demand for bioactive compounds that can be used for the production of various pharmaceuticals. One of the sustainable sources of bioactive compounds is endophytic fungi which are known to have the ability to produce myriads of bioactive compounds with varying beneficial biological properties. Thus, this research was conducted to study the bioactive compounds and biological properties of endophytic fungi isolated from *Gynura procumbens* (*Sambung Nyawa*). A total of 12 over 45 isolated endophytic fungi were successfully identified. They were *Diaporthe hongkongensis* SN1, *Phomopsis* sp. SN2, *Colletotrichum truncatum* SN3, *Mycoleptodiscus indicus* SN4, *Diaporthe longicolla* SN5, *Macrophomina phaseolina* SN6, *Beltraniella portoricensis* SN7, *Colletotrichum asianum* SN8, *Colletotrichum brevisporum* SN9, *Fusarium incarnatum* SN10, *Colletotrichum gloeosporioides* SN11, and *Pestalotiopsis* sp. SN12. These isolates were tested for the production of extracellular enzymes such as amylase, protease, cellulase, lipase, and laccase. Among the isolates, only isolate SN9 was detected to produce all extracellular enzyme. Then, these isolates undergone fermentation process and were extracted using ethyl acetate and methanol. Folin-Ciocalteu and aluminium chloride methods were used to quantify the total phenolic content (TPC) and total flavonoid content (TFC) of ethyl acetate and methanolic fungal extracts, respectively. The highest TPC value for ethyl acetate and methanolic extracts were exhibited by isolate SN11 (87.0 ± 14.8 mg GAE/g) and isolate SN4 (35.0 ± 5.9 mg GAE/g) respectively. Meanwhile, the highest TFC value for ethyl acetate and methanolic extracts were shown by isolate SN6 (122.8 ± 11.0 mg QCE/g) and isolate SN4 (60.4 ± 9.5 mg QCE/g), respectively. The DPPH free radical scavenging assay was used to investigate the antioxidant properties of the extracts.

The ethyl acetate extract of isolate SN6 showed high antioxidant activity, with an inhibition percentage of $86.60 \pm 3.20\%$ and an IC₅₀ value of $104.25 \pm 18.51 \mu\text{g/mL}$. In contrast, the highest scavenging activity percentage of methanolic extract was shown by isolate SN4 ($49.95 \pm 11.58\%$). In addition, ferric reducing antioxidant power (FRAP) assay was performed to confirm the antioxidant capacity of the fungal extracts. The highest FRAP value for ethyl acetate and methanolic extract were observed from isolate SN6 ($239.9 \pm 66.2 \text{ mg Fe (II)/g}$) and isolate SN4 ($44.7 \pm 7.7 \text{ mg Fe (II)/g}$), respectively. The positive control used for DPPH and FRAP assays was 1 mg/mL ascorbic acid, while the negative control used was methanol. Some of the fungal extracts also exhibited inhibitory activity against pathogenic microorganisms such as *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* with minimum inhibitory concentration and minimum bactericidal concentration of 2500 – 5000 $\mu\text{g/mL}$ and 5000 $\mu\text{g/mL}$, respectively. The positive and negative control used were 1 mg/mL tetracycline and 1% DMSO, respectively. Besides that, gas chromatography mass spectrometry analysis was conducted to identify the bioactive compounds present in the fungal extracts. Various bioactive compounds such as heptadecane, 2,6,10,15-tetramethyl- (17.82%), 9-Octadecenoic acid (Z)-, methyl ester (11.18%), hexadecane (10.40%), pentadecanoic acid, 14-methyl-, methyl ester (9.87%), oleic acid (9.24%), 2-Coumaranone (8.18%), and many other compounds were identified. The findings of this study clearly indicated that the endophytic fungi isolated from the leaves of *G. procumbens* possesses the ability to exhibit antioxidant and antibacterial activities besides capable of producing several beneficial bioactive compounds. These findings can contribute greatly to the pharmaceutical and medicinal industries in order to improve the effort to curb the rise of current maladies.

Keywords: Endophytic fungi, *Gynura procumbens*, antioxidant activity, antibacterial activity, bioactive compounds

Pencirian Sifat Biologi dan Sebatian Bioaktif Kulat Endofit yang Diasingkan dari Gynura Procumbens (Sambung Nyawa)

ABSTRAK

Peningkatan penyakit baru yang ketara dan perkembangan mikroorganisma patogen yang rentan akan antibiotik telah menyebabkan peningkatan permintaan untuk sebatian bioaktif yang dapat digunakan untuk penghasilan pelbagai farmaseutikal. Salah satu sumber sebatian bioaktif yang mampan adalah kulat endofit yang diketahui mempunyai keupayaan untuk menghasilkan pelbagai sebatian bioaktif dengan pelbagai sifat biologi bermanfaat. Oleh itu, penyelidikan ini dilakukan untuk mengkaji sebatian bioaktif dan sifat biologi kulat endofit yang diasingkan dari Gynura procumbens (Sambung Nyawa). Sebanyak 12 daripada 45 kulat endofit yang diasingkan telah berjaya dikenal pasti. Mereka adalah Diaporthe hongkongensis SN1, Phomopsis sp. SN2, Colletotrichum truncatum SN3, Mycoleptodiscus indicus SN4, Diaporthe longicolla SN5, Macrophomina phaseolina SN6, Beltraniella portoricensis SN7, Colletotrichum asianum SN8, Colletotrichum brevisporum SN9, Fusarium incarnatum SN10, Colletotrichum gloeosporioides SN11, dan Pestalotiopsis sp. SN12. Isolat ini diperiksa dari segi penghasilan enzim ekstraselular seperti amilase, protease, selulase, lipase, dan laccase. Namun, hanya isolat SN9 yang dapat menghasilkan semua enzim ekstraselular. Selepas itu, isolat ini diekstraksi dengan etil asetat dan metanol. Jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) ekstrak kulat metanolik ditentukan dengan menggunakan kaedah Folin-Ciocalteu dan aluminium klorida. Nilai TPC tertinggi untuk ekstrak etil asetat dan metanolik ditunjukkan oleh isolat SN11 (87.0 ± 14.8 mg GAE/g) dan isolat SN4 (35.0 ± 5.9 mg GAE/g). Nilai TFC tertinggi untuk ekstrak etil asetat dan metanolik pula ditunjukkan oleh isolat SN6 (122.8 ± 11.0 mg QCE/g) dan isolat SN4 (60.4 ± 9.5 mg QCE/g). Analisis aktiviti antioksidan dilakukan dengan

menggunakan pengujian pembersihan radikal bebas DPPH. Aktiviti antioksidan tinggi dengan peratusan $86.60 \pm 3.20\%$ dan nilai IC_{50} $104.25 \pm 18.51 \mu\text{g/mL}$ ditunjukkan oleh ekstrak etil asetat isolat SN6. Sebaliknya, peratusan aktiviti antioksidan ekstrak metanol yang paling tinggi ditunjukkan oleh isolat SN4 ($49.95 \pm 11.58\%$). Selain itu, pengujian antioksidan menggunakan analisis FRAP juga dilakukan dan nilai FRAP tertinggi untuk etil asetat dan ekstrak metanol ditunjukkan oleh isolat SN6 ($239.9 \pm 66.2 \text{ mg Fe (II)/g}$) dan isolat SN4 ($44.7 \pm 7.7 \text{ mg Fe (II)/g}$). Kawalan positif yang digunakan untuk ujian DPPH dan FRAP ialah 1 mg/mL asid askorbik, manakala kawalan negatif yang digunakan ialah metanol. Di samping itu, beberapa ekstrak kulat juga menunjukkan adanya aktiviti antibakteria terhadap mikroorganisma patogen seperti Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli dan Salmonella typhi dengan nilai kepekatan perencatan minimum dan nilai kepekatan bakterisidal minima $2500 - 5000 \mu\text{g/mL}$ dan $5000 \mu\text{g/mL}$. Kawalan positif dan negatif yang digunakan ialah 1 mg/mL tetrasiklin dan 1% DMSO. Sebaliknya, analisis spektrometri massa kromatografi gas juga dilakukan untuk mengenal pasti sebatian bioaktif yang terdapat dalam ekstrak kulat. Pelbagai sebatian bioaktif seperti heptadecane, 2,6,10,15-tetramethyl- (17.82%), 9-Octadecenoic acid (Z) -, metil ester (11.18%), heksadecane (10.40%), asid pentadecanoic, 14-metil-, metil ester (9.87%), asid oleik (9.24%), 2-Coumaranone (8.18%), dan sebatian lain telah dikenal pasti. Penemuan kajian ini dengan jelas menunjukkan bahawa kulat endofit yang diasingkan dari daun G. procumbens memiliki kemampuan untuk menunjukkan aktiviti antioksidan dan antibakteria selain mampu menghasilkan sebatian bioaktif yang bermanfaat. Penemuan ini boleh memberi sumbangan besar kepada industri farmaseutikal dan perubatan untuk meningkatkan usaha membendung peningkatan penyakit semasa.

Kata kunci: Kulat endofit, Gynura procumbens, aktiviti antioksidan, aktiviti antibakteria, sebatian bioaktif