

CHEMICAL CONSTITUENTS ISOLATED
FROM THE ETHYL ACETATE EXTRACT OF
THE ROOTS OF *GARCINIA ATROVIRIDIS*

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Science

Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

SEPTEMBER 2020

ACKNOWLEDGEMENTS

I would like to express my gratitude towards my supervisor, Dr. Normaiza binti Zamri for her continuous support, guidance and insightful advice throughout the time of the research and writing of this thesis. Her words of encouragement and her response towards my questions gave me confidence to finish this research.

I also appreciate the supervision that I received from my co-supervisor, Dr. Mohd Fadhlizil Fasihi bin Mohd Aluwi. I am also thankful towards Dr. Seema Zareen and Dr. Muhammad Nadeem Akhtar. I am grateful to the science officers and laboratory assistants of Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang for the help and technical assistance that they offered, in addition of providing good working environment. Special thanks to Institute of Postgraduate Studies and faculty's administration staff for their accommodating services. To my fellow friends, I thank them for their companionship when I needed it.

Deepest acknowledgement goes to my loving family especially my parents, Mr. Ahmad Roslan bin Ismail and Mdm. Noor Hashimah binti Mohd Sidek for their understanding and reassurance. This accomplishment would not have been possible without them. Thank you.

ABSTRAK

Kajian awal menunjukkan bahawa bahagian akar pokok *Garcinia atroviridis* (asam gelugur) mempunyai aktiviti antibakteria dan antioksidan yang tinggi, berkemungkinan disumbangkan oleh pelbagai bioaktif metabolit sekunder. Sebatian daripada ekstrak etil asetat akan diasingkan dengan lebih lanjut memandangkan pelarut etil asetat sangat efektif dalam mengasingkan kumpulan fenolik. Oleh itu, kajian ini dijalankan untuk mengasingkan, menulenkan, mengenalpasti sebatian fenolik, dan menilai sifat-sifat biologi bagi ekstrak etil asetat dan sebatian yang diasingkan. Sebagai tambahan, kajian biologi awal terhadap pelbagai ekstrak daripada akar *G. atroviridis* turut dijalankan. Akar *G. atroviridis* di dapati dari Maran, Pahang, telah diekstrak menggunakan pelarut yang mempunyai kekutuban yang berbeza. Kajian biologi awal iaitu antibakteria dan antioksidan telah dijalankan terhadap ekstrak etil asetat. Ekstrak etil asetat telah dipilih kerana penyelidikan terdahulu telah berjaya mengasingkan sebatian fenolik yang mempunyai pelbagai aktiviti biologi. Ekstrak ini menunjukkan aktiviti antibakteria terhadap bakteria Gram-positif iaitu *Bacillus cereus* dan *Staphylococcus aureus* melalui kaedah penyebaran cakera. Ekstrak ini juga menunjukkan jumlah kandungan fenolik (TPC) yang signifikan melalui kaedah *Folin-Ciocalteu* (FC), dengan nilai 568.6 ± 2.724 mg GAE/g. Seterusnya, aktiviti antioksidan ekstrak etil asetat telah di uji dengan lebih lanjut melalui kaedah α , α -diphenyl- β -picrylhydrazyl (DPPH) dan *ferric reducing antioxidant power* (FRAP) yang menunjukkan aktiviti antioksidan yang memuaskan dengan nilai IC_{50} 51.7 ± 2.180 μ g/mL (DPPH) dan 294.0 ± 20.173 mg AAE/g (FRAP). Memandangkan ekstrak etil asetat menunjukkan aktiviti antibakteria dan antioksidan yang bagus, pengasingan sebatian fenolik melalui teknik *silica gel column chromatography* (Si gel CC) dan *preparative thin layer chromatography* (PTLC) telah dijalankan. Sebanyak lima sebatian telah berjaya diasingkan iaitu, *atrovirisidone* (**7**), *morelloflavone* (**12**), *GB1a* (**18**), *1,3,5-trihydroxy-2-methoxyxantone* (**29**), termasuk satu sebatian baru iaitu *volkensiflavone* (**33**), dimana sebatian ini berjaya diasingkan daripada pokok *G. atroviridis* buat pertama kali. Pelbagai teknik spektroskopi digunakan untuk mengenalpasti struktur sebatian tersebut termasuklah melalui teknik *mass spectrometer* (MS), *ultraviolet-visible* (UV-Vis), *fourier-transform infrared* (FTIR), dan *1D-nuclear magnetic resonance* (NMR). Seterusnya, kajian biologi dilakukan terhadap sebatian yang telah diasingkan. Bagi aktiviti antibakteria, *GB1a* (**18**) dan *volkensiflavone* (**33**) menunjukkan perencatan terhadap *S. aureus*. Sementara itu, *morelloflavone* (**12**) menunjukkan aktiviti DPPH tertinggi dengan nilai IC_{50} 20.3 ± 1.667 μ g/mL, setanding dengan asid askorbik (AA) dengan nilai IC_{50} 13.2 ± 0.021 μ g/mL. Secara umum, semua sebatian menunjukkan aktiviti FRAP yang memuaskan dengan aktiviti tertinggi ditunjukkan oleh *1,3,5-trihydroxy-2-methoxyxantone* (**29**), 1643.3 ± 44623 mg AAE/g. Di samping itu, kajian biologi awal yang dilakukan terhadap ekstrak heksana, diklorometana, butanol dan metanol yang diekstrak daripada akar *G. atroviridis* mendedahkan bahawa ekstrak butanol mempunyai nilai farmaseutikal yang berpotensi tinggi kerana ekstrak ini menunjukkan aktiviti antibakteria dan antioksidan yang paling tinggi. Selain itu, hubungan antara ekstrak akar *G. atroviridis* dan aktiviti antioksidan menunjukkan korelasi positif yang kuat antara TPC dengan DPPH ($r = 0.8760$) dan FRAP ($r = 0.9385$), menunjukkan peranan sebatian fenolik dalam aktiviti antioksidan. Kesimpulannya, aktiviti antibakteria dan antioksidan oleh akar *G. atroviridis* berkemungkinan disumbangkan oleh kehadiran sebatian fenolik.

ABSTRACT

Several preliminary studies on the roots of *Garcinia atroviridis* have reported on their antibacterial and antioxidant properties, which might be contributed by various bioactive secondary metabolites. Chemical compounds in the roots of *G. atroviridis* were isolated from ethyl acetate extract as the solvent was reportedly efficient for extraction of phenolic compounds with intermediate polarity. Thus, this study aims to isolate, purify, elucidate the phenolic compounds, and evaluate the biological properties of ethyl acetate extract and its isolated compounds. In addition, preliminary investigation on biological activity of other extracts extracted with different polarities of solvent was also conducted. The roots of *G. atroviridis* collected from Maran, Pahang were subjected to solvent extraction with different polarities of solvents. Preliminary biological screening was done on ethyl acetate extract to investigate the antibacterial and antioxidant activities. This extract was chosen because, the chemical investigation on this extract leads to the isolation of various phenolic compounds with various bioactivities as proven in previous studies. Ethyl acetate extract showed antibacterial activity against Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, in disc diffusion experiment. Significant total phenolic content (TPC) was identified in ethyl acetate extract using Folin-Ciocalteu (FC) reagent method, with a value of 568.6 ± 25.724 mg GAE/g. Upon further investigation with α , α -diphenyl- β -picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, ethyl acetate extract showed moderate antioxidant activities with IC_{50} of 51.7 ± 2.180 μ g/mL and 294.0 ± 20.173 mg AAE/g, respectively. Based on antibacterial and antioxidant activities exhibited by ethyl acetate extract, further chemical investigation was carried out to isolate the phenolic compounds in the extract using silica gel column chromatography (Si gel CC) and preparative thin layer chromatography (PTLC). Five compounds were isolated namely atroviridone (**7**), morelloflavone (**12**), GB1a (**18**), 1,3,5-trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**). Volkensiflavone (**33**) was reported for the first time in *G. atroviridis*. Several spectroscopy techniques were used to elucidate the structure of isolated compounds, such as mass spectrometer (MS), ultraviolet-visible (UV-Vis), fourier-transform infrared (FTIR), and 1D- nuclear magnetic resonance (NMR) spectroscopy. Biological assays were conducted to all isolated compounds except atroviridone (**7**) due to limited yield. GB1a (**18**) and volkensiflavone (**33**) inhibited the growth of *S. aureus* as observed in antibacterial test. Morelloflavone (**12**) showed the highest DPPH scavenging activity with IC_{50} of 20.3 ± 1.667 μ g/mL which was comparable to ascorbic acid (AA) with IC_{50} of 13.2 ± 0.021 μ g/mL. In general, all compounds showed significant reducing power, of which 1,3,5-trihydroxy-2-methoxyxantone (**29**) demonstrated the highest FRAP activity with a value of 1643.3 ± 44.623 mg AAE/g. In addition, preliminary biological screening of different solvent extracts from the roots of *G. atroviridis* revealed the pharmaceutical potential of butanol extract, which showed the highest antibacterial and antioxidant activities. Correlation between phenolic content in root extracts of *G. atroviridis* and antioxidant activity was evaluated. Strong positive correlation between TPC and the measured capacity in DPPH ($r = 0.8760$) and FRAP ($r = 0.9385$) assays signified the role of phenolic compounds in antioxidant activity in root extract of *G. atroviridis*. In conclusion, antibacterial and antioxidant activities in root extract of *G. atroviridis* may be attributable to the presence of phenolic compounds.

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