

UNIVERSITI TEKNOLOGI MARA

**BIOLOGICAL ACTIVITY STUDIES OF SOME
RUBIACEOUS PLANTS
(SUBFAMILY RUBIOIDEAE)
AND PHYTOCHEMICAL INVESTIGATION
OF *HEDYOTIS PHILIPPINENSIS***

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Candidate's Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

In the event that my thesis be found to violate the conditions mentioned above, I voluntarily waive the right of conferment of my degree and agree to be subjected to the disciplinary rules and regulations of Universiti Teknologi MARA.

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ABSTRACT

In this study, the biological activities of 13 Rubioideae plants were investigated with the aim of discovering its bioactive constituents. The assays employed were ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods for antioxidant potential as well as Griess assay for the measurement of nitric oxide (NO) inhibition in lipopolysaccharide (LPS) and interferon- γ (IFN- γ)-treated RAW 264.7 cells. In the *in vitro* antioxidant assays including FTC, TBA and DPPH, all the tested plants exert strong antioxidant potential. *Hedyotis philippinensis* (leaves and stems), *Spermacoce exilis* and *Spermacoce latifolia* showed potent inhibitory activity on NO production in LPS and IFN- γ -induced RAW 264.7 cells in the anti-inflammatory assay. For the second part of this study, phytochemical investigation of the leaves and stems of selected *Hedyotis* species, namely *H. philippinensis* was carried out using various chromatographic and solid phase extraction techniques to yield the major iridoid glycoside, asperuloside along with scopoletin and astragalins. Structures of the compounds were established by means of various spectroscopic techniques including nuclear magnetic resonance (NMR) and mass spectroscopy. The major compound, asperuloside was found to inhibit LPS/IFN- γ -induced NO production by 63% at a concentration of 100 μ M (45% cell viability) in a dose-dependent manner with an IC_{50} value of 75.45 ± 2.25 μ M. Towards a chemotaxonomic study of Rubiaceae plants, the detection of marker compounds of the subfamily Rubioideae was attempted by employing the isolated asperuloside as the marker. Asperuloside detection on 13 species of subfamily Rubioideae comprising six genera (*Argostemma*, *Chasalia*, *Hedyotis*, *Lasianthus*, *Rennellia* and *Spermacoce*) was carried out using thin layer chromatography (TLC). In addition, a simple liquid chromatography mass spectrometry (LC-MS) profiling method was successfully developed to detect and quantify asperuloside in the samples. The asperuloside content was found to be significantly varied among five species, ranging from 1.46-24.64 ppm with *H. philippinensis* containing the highest amount. However, the compound was undetectable in the other eight species. In conclusion, this study has found that Rubioideae plants possessed constituents associated with various pharmacological activities such as anti-inflammatory, antioxidant and are mostly devoid of cytotoxic effects. This study has also shown that asperuloside is a good chemotaxonomic marker of Rubiaceae plants. Phytochemical investigation on these plants would likely reveal more active principles which could be potentially important markers.

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