UNIVERSITI TEKNOLOGI MARA

PURIFICATION, STEREOSTRUCTURE, α-GLUCOSIDASE INHIBITION, KINETICS, AND MOLECULAR DOCKING STUDIES OF PTEROPODINE AND ISOPTEROPODINE FROM *UNCARIA LONGIFLORA*

NURUL AINA SUZLIN BINTI SULAIMAN

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ABSTRACT

U. longiflora var. *pteropoda* locally known as "gegambir hitam" is traditionally used to treat rheumatism and framboesia. The stem methanolic extract of the plant has been reported to possess strong antidiabetic potential evaluated through α-glucosidase enzymatic activity. Two major compounds found in the stem and leaves of the plant are the C-7 diastereomeric alkaloids, pteropodine, and isopteropodine which could contribute to the plant's α-glucosidase inhibitor. However, whether these compounds could contribute to the α-glucosidase inhibitory potential of the plant remains to be investigated. This work aimed to purify and study the stereostructure, molecular docking, enzyme inhibition, and kinetics of the alkaloids through recycling high-performance liquid chromatography (R-HPLC), vibrational circular dichroism (VCD) spectroscopy, AutoDock software, *in vitro* α-glucosidase inhibitory activity, and Michaelis-Menten kinetics, respectively. The separation of pteropodine and isopteropodine in R-HPLC was completed within two cycles of 80 minutes where the former was eluted at minute 64^{th}, while the latter was at the 77^{th} minute. The stereostructure characteristic at the respective C-7*R* and C-7*S* diastereomerism were unambiguously distinguished through their opposite VCD Cotton Effects appearing at regions *ca*. 1700 and 1100 cm^{-1}. The docking analysis reveals pteropodine displayed slightly better binding energy than isopteropodine but both weaker than the standard drug, acarbose with minima values of -9.18, -9.00, and -13.83 kcal/mol, respectively. The alkaloids satisfied Lipinski's rule of five and ADMET properties and were further studied *in vitro* for α-glucosidase inhibitory activity. The docking data showed that isopteropodine has better α-glucosidase inhibitory potential compared to pteropodine with IC_{50} value of 279.16 $\pm$ 5.37 μM and 615.31 $\pm$ 7.65 μM, respectively. This contradicts with the molecular docking results of the compounds. However, a kinetics study reveals both alkaloids showed a different type of inhibitory where pteropodine was competitive whereas isopteropodine was non-competitive justifying their mechanism of action. This finding is supported by the molecular docking result where pteropodine shows its competitiveness through binding at the active site of the α-glucosidase enzyme (Asp349 and Glu276 residues), while isopteropodine reveals its non-competitiveness through binding interaction with the allosteric sites of the enzyme (His279, Asn241, Ser308, and Glu304 residues). Both compounds also showed different molecular electrostatic potentials where pteropodine has a localised electron cloud, thus supporting a stronger and shorter binding distance in the docking study compared to isopteropodine. Although the two alkaloids are not as potent as acarbose to inhibit α-glucosidase enzyme, this work suggests that pteropodine and isopteropodine contributed synergically to the α-glucosidase inhibitory activity of *U. longiflora*. However, the contribution of the other plant's chemical constituents should also be evaluated to ensure whether the activity is due to singularism or synergism. This will allow further exploration on the antidiabetic potential of the plant by using different approach. This research findings could also encourage more research on *U.longiflora* which may lead to the discovery of a natural antidiabetic and other metabolic diseases agents.

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CHAPTER ONE
INTRODUCTION

1.1 Background

Malaysia has a living heritage of a vast diversity of plant species, out of which 1300 have been reported to have therapeutic properties including the *Uncaria* genus (Burkill, 1935). *Uncaria* is a genus that belongs to the family Rubiaceae mainly distributed across South America, Africa, and Southeast Asia. Of the 34 reported species worldwide, 14 are available in peninsular Malaysia and the most common representative species are *U. gambir*, *U. acida*, and *U. longiflora*. The Malaysian *Unçaria* is locally known as 'Gambir', 'akar kait-kait' or 'akar hitam', is claimed to be used as a pain reliever, clean wounds and ulcers, and to treat inflammations of the intestines and rheumatism (Risdale, 1987). To date, over 200 compounds have been isolated and identified from the *Uncaria* genus and the compounds most prominent are alkaloids particularly the chiral pentacyclic oxindole alkaloids (POAs) (Qin, et al., 2021). Countless biological studies have been done on this class of compound which includes anti-inflammatory, antispasmodic, antiarrhythmic, anticonvulsant, antioxidant, antiviral, antithrombotic, cytotoxicity, DNA damaging, ion channel blocker, antiulcer, dementia, central nervous system effect (CNS), glutamate response modulation and many more.

Hereto, approximately 25 POAs have been reported and 12 of them were from the alkaloid-rich species *U. longiflora* var. *pteropoda* (Ahmad & Salim, 2015). Interestingly, *U. longiflora var. pteropoda* has also been reported to possess strong antidiabetic potential evaluated through α-glucosidase enzymatic activity (Ahmad et al., 2011). However, there has been no report on which compounds contribute to the antidiabetic potential of the plant. The two major compounds found in the stem and leaves of the plant are the stereostructure alkaloids, pteropodine and isopteropodine. A study by Ahmad et al. (2011) reported that the stem of *U. longiflora* has higher α-glucosidase enzyme percent inhibition compared to the leaves of the plant which is 99.1% and 48.9% respectively. Previous studies have reported that pteropodine and isopteropodine are executing mainly distinct biological activities which are probably