UNIVERSITI TEKNOLOGI MARA

BIOENGINEERING OF *Tacca integrifolia* FOR PRODUCTION OF SECONDARY METABOLITES WITH ANTIPROLIFERATIVE PROPERTIES

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Pharmacy

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AUTHOR'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 results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Tacca integrifolia or locally known as janggut Adam is a wild plant species which often used to treat gastric ulcer, hypertension, antiproliferative, haemorrhoids, heart failure and kidney disease. Since its geographical distribution is limited, with its poor germination and short-term seed viability it has raised the issues of short supply and high demand of this plant. Therefore, this study was aimed to develop an *in vitro* propagation system for *T. integrifolia* from *in vitro* seedlings to produce secondary metabolites with anti-proliferative properties. *T. integrifolia* was previously identified as a potent antiproliferative resource, but scientific information of the metabolomic and pathway study on *in vitro* plant culture of *T. integrifolia* is still inadequate and limited to wild plant. Murashige and Skoog (MS) basal medium were used for the growth of seedlings, whilst shoots from the *in vitro* germinated seedlings were excised and cultured on MS medium containing different PGRs. The optimum numbers of roots, shoots and callus were observed, measured, and analysed statistically after 12 weeks. The metabolites produced from an optimum *in vitro* culture treatments and wild plant group were profiled using LC/MS Q-TOF. Phytochemical screening, antioxidant activity, cytotoxic tests and metabolites profiling of the respective extracts were carried out in this study. The antiproliferative effects of the crude methanolic extracts of 11 *in vitro* and wild plant of *T. integrifolia* were tested for cytotoxic activity against HepG2 cancer cell lines and Chang normal cell lines. The secondary metabolites from plants were extracted using methanol and analysed using LC/MS Q-TOF platform. Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA) and Hierarchical Clustering Analysis (HCA) were used to determine differentially expressed metabolites from *T. integrifolia in vitro* culture and wild plant groups. *T. integrifolia* were successfully grown using tissue culture techniques. Phytochemical screening confirmed the presence of tannins, triterpenoids, flavonoids, saponins, anthraquinone glycosides, phenols and steroids in the extract of *T. integrifolia in vitro* plantlet and wild plant extracts. *T. integrifolia* was rich in high levels of total phenolic and terpenoid compounds. The secondary metabolites profiled include Taccalonolide A, Taccalonolide AA, Betulinic acid, Chlorogenic acid, Dioscin, Diosgenin and Withanolides. These metabolites have been reported to possess several biological activities including antiproliferative activity. The *in vitro* root extract significantly showed the highest selectivity for its cytotoxic effects in HepG2 compared to normal Chang cell lines. Linoleic acid metabolism was the most prominently perturbated pathway determined in the *in vitro* root extracts of *T. integrifolia* with the highest selectivity index towards HepG2 cells. This study has successfully grown *T. integrifolia* using an *in vitro* plant culture green technology to produce valuable source of active metabolites with novel selective anti-proliferative properties towards HepG2 cell lines. The findings may suggest that manipulating linoleic acid metabolism of *T. integrifolia* would allow production of secondary metabolites with selective anti-proliferative activities. Thus, allows the manipulation of the metabolism pathways for production of desired secondary metabolites.

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