UNIVERSITI TEKNOLOGI MARA

EVALUATION OF THE HONEY PROPERTIES AND MOLECULAR MECHANISM OF CELL DEATH INDUCES BY ACACIA HONEY IN MCF-7 CELL LINES

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PhD

September 2021

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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Date	:	September 2021

ABSTRACT

Honey's increasing popularity has prompted medical researchers to study its therapeutic benefits. Honey has been shown to trigger cell death in cancer cells, directly modulate tumour progression. Polyphenols in honey have a significant role in this healing action. Despite its health benefits, the authenticity of honey remains the main challenge for consumers as intake of highly dominant fake honey cause more health issues. The purity and polyphenol content of Malaysian acacia honey were assessed in this study. Methods compiled and harmonised by the International Honey Commission were employed to determine purity per the Codex Alimentarius standard while two spectrophotometric techniques followed with polyphenols identification using HPLC assessed the polyphenol content. The potential of acacia honey to induce cell death was also studied *in vitro* using a two-pronged approach: detecting biochemical indicators of cell death and examining cell death morphologies. The assessment utilised human breast adenocarcinoma (MCF-7) and mammary gland epithelial (MCF 10A) with tamoxifen as a positive control. In terms of purity, higher moisture content associated with low marketability and shelf-life was observed while enzyme activity, HMF content and sugar profile fulfil the Codex Alimentarius standard. The polyphenol content assessment showed non-correlation between spectrophotometric and HPLC techniques where only two compounds were identified, quercetin and *trans*-cinnamic acid. Although optimisation may enhance identification, premature harvest which is common in Asian countries is suspected as outlined in a recent study considering higher moisture content was recorded. The inhibition assessment discovered cell death inducement in MCF-7 and MCF 10A cells with IC_{50} values of 5.5% and 8% respectively. The Annexin V-FITC assay revealed honey induces apoptosis-cell death after 48 hours with the highest apoptosis induction (36.04±9.61%) observed after 72 hours in the IC_{75} treatment group. The assay also validates the selectivity of acacia honey towards MCF 10A in inhibition assessment. Due to the short treatment period (16 hours), western blotting was unable to produce comprehensive, significant data. The Bcl-2 was found upregulated in MCF 10A treated with IC_{75} of acacia honey, suggesting the selectivity was achieved by suppressing apoptosis-cell death. However, the upregulation of caspase 8 in MCF-7 treated with IC_{25} acacia honey did not translate into apoptosis in annexin V-FITC assay. Caspase 8's capacity to trigger apoptosis via MOMP may be compensated by autophagy's mitophagy mechanism. Afterwards, CLSM and TEM were used to evaluate morphology. CLSM confirms the selectivity of acacia honey towards MCF 10A, whereas it exhibits apoptotic-like cell blebbing morphology against MCF-7 at IC_{50}. However, the outcome contradicts earlier findings, indicating additional biological reactions produced the single event. TEM investigate ultrastructural changes associated with cell death. Following 24 hours of acacia honey treatment, MCF-7 cells had extensive vacuoles in the cytoplasm, indicating autophagy-cell death. Finally, this study shows the impact of early harvest on honey quality and polyphenol content. The non-correlation between spectrophotometric and HPLC techniques justify the proper method selection for compound identification. The treatment of acacia honey significantly promotes apoptosis-cell death after 48 hours while autophagy-mediated cell death may inhibit the MOMP effect induced through caspase 8 activation post 24 hours treatment.

ACKNOWLEDGEMENT

Throughout this journey, many hurdles have been faced, and I am deeply grateful to Allah the Almighty for His mercy, blessing and guidance. This path has taught me lots of life lessons that would forever shape my point of view, for that, Alhamdulillah.

I like to acknowledge my supervisors who patiently guide me through this struggle. I am greatly indebted to their knowledge, effort and words of encouragement in guiding me through. Thank you Dr Zolkapli Eshak, Associate Professor Dr Wan Iryani Wan Ismail, Dr Salfarina Ramli and Associate Professor Dr Mizaton Hazizul Hasan.

I also want to acknowledge my examiners for guiding me through the finish line. I extend my gratitude to the Faculty of Pharmacy, UiTM, Academic staff and non-academic staff for their assistance.

My everlasting gratitude to my parent and in-laws who always there lending a rope every time I slipped. Thank you for believing in me and I pray that Allah will reward that kindness in this life and hereafter.

I dedicated this thesis to my dear wife, Nur Ayuni binti Roslam who have sacrificed along this path. Without your companion, I would not be able to be here. To my kids, Muádz and Fahim, I hope one fine day you will understand why I spent most of my days in the lab instead of watching both of you growing up.

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