UNIVERSITI TEKNOLOGI MARA

ACETYLCHOLINESTERASE, OXIDATIVE STRESS, AND INFLAMMATION INHIBITORY POTENTIALS OF RAW-EXTRACT *Centella asiatica* (RECA) ON CELL LINES AND BRAIN TISSUE HOMOGENATES OF SPRAGUE DAWLEY RATS

ZETTY ZULIKHA BINTI HAFIZ

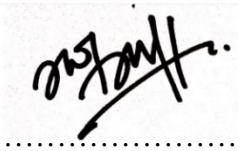
PhD

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.

Name of Student	:	Zetty Zulikha binti Hafiz
Student I.D. No.	:	2014582695
Programme	:	Doctor of Philosophy – AS950
Faculty	:	Applied Sciences
Thesis Title	:	Acetylcholinesterase, Oxidative Stress, and Inflammation Inhibitory Potentials of Raw-Extract Centella asiatica (RECA) on Cell Lines and Brain Tissue Homogenates of Sprague Dawley Rats
Signature of Student	:	 ……..
Date	:	September 2021

ABSTRACT

The present study was performed to investigate the effect of ethanolic extract of *C.asiatica*, designated as raw-extract of *C.asiatica* (RECA) on reducing the acetylcholinesterase (AChE), oxidative stress, and inflammation activities *via* both *in vitro* (SH-SY5Y and RAW 264.7 cells) and *in vivo* (Sprague Dawley rats). Selected major triterpenes of RECA were identified and quantified using high-performance liquid chromatography (HPLC), which further reveals that RECA contains significantly high proportion of glycosides than the aglycones with madecassoside is the highest component, followed by asiaticoside. RECA showed no toxicity effect at the concentrations tested since its IC_{50} could not be determined in concentrations ranging from 3.91 μg/mL to 1000 μg/mL, at 24 h and 48 h incubation times. Treatment of RECA on SH-SY5Y cells significantly reduced the AChE activity in a concentration-dependent manner with an IC_{50} value of 31.09 ± 10.07 μg/ml. The antioxidant and anti-inflammatory activities of RECA were also evaluated *in vitro*, by using lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The production of reactive oxygen species (ROS) and reduced glutathione (GSH) levels were evaluated to measure oxidative stress *in vitro*. Meanwhile, the production of nitrite, prostaglandin E_2 (PGE_2), and tumor necrosis factor alpha (TNF-α) levels were quantified as inflammatory parameters. Results showed that treatment with RECA significantly suppressed the level of oxidative stress and pro-inflammatory cytokine/mediators released in concentration-dependent manner. Among all, RECA displayed remarkable inhibition against nitrite with IC_{50} value of 5.205 ± 3.13 μg/mL. Interestingly, these patterns of inhibition were consistent as observed in the LPS-induced neuroinflammation Sprague Dawley rats' model, in both pre- and post-treatment of RECA. AChE activity, marker of oxidative stress (GSH) and pro-inflammatory cytokine/mediator (PGE_2 and TNF-α) were studied by using rats' brain. It was found that inhibitory effects of RECA in both different time of oral administration (pre- and post-treatment) on LPS-induced rats' model successfully suppressed the AChE and inflammation activities, while the GSH level was up regulated, with RECA at highest dosage (350 mg/kg) producing the most profound effects. The AChE activity was also investigated in salt-soluble (SS) and detergent-soluble (DS) fractions of cerebral cortex, hippocampus, and cerebellum, which consists of predominantly G1 an G4 molecular isoforms of AChE respectively. In general, RECA preferentially inhibited the G4 form AChE in cerebral cortex and cerebellum, whereas G1 form AChE in hippocampus. The fractionation of RECA was accomplished by using vacuum liquid chromatography (VLC). Ten fractions were obtained and pooled based on the same solvent used for elution. Its selected major triterpenes contents were quantified using HPLC. Combined fractions (F1C-F5C) at concentration 200 μg/ml were subjected to AChE assay and F5C was found as the most active fraction in inhibiting AChE activity. Subsequently, the antioxidant and anti-inflammatory activities of F5C *in vitro* were further evaluated. Results showed that F5C significantly suppressed the level of oxidative stress and pro-inflammatory cytokine/mediators released in concentration-dependent manner. Herein, findings from present study strongly suggest that RECA may offer therapeutic potential for the treatment of Alzheimer's disease through inhibiting the AChE, oxidative stress, and inflammatory activities.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful.

Alhamdulillah. First and foremost, my gratitude goes to Allah for granting me the capability and strength to complete this research successfully. All the achievements and blessings in my life are due to Him. Without Him, I am nothing.

My heartfelt appreciation to my wonderful supervisor, Prof. Dr. Mohd Ilham Adenan, for his excellent supervision, tremendous guidance, warm encouragement, and most importantly supporting me all the way till the completion of this work. You are truly an inspiration for me. My appreciation also goes to my co-supervisor, Dr. Richard Muhammad Johari James, for the consultation, advice, and knowledges regarding my research project. Thank you for helping me proofread my research articles and thesis.

I wish to extend my appreciation to the staff members in Faculty of Applied Sciences for the cooperation and kind helps since the day I registered as PhD student, Atta-ur-Rahman Institute of Natural Products (AuRINs) for the facilities it provided for my research work and staff members in Integrative Pharmacogenomics Institute (iPROMISE), tissue culture lab and LAFAM for providing valuable assistance whenever I am in any difficulties. Special thanks to Shahidah Mohd Mukhtar, Rasdin Ridwan and Nurliyana Abdul Mutalib for valuable assistance and guidance regarding the *in vivo* studies and HPLC analyses.

Besides that, I would like to extent my gratitude to Prof. Ilham`s research team, Ms. Zuriati Zahari and Mrs. Nor Atiqah Jusril for sharing a lot of ideas, constructive criticisms, and invaluable assistance. Not forgetting to all my graduate and best friends, particularly I would like to mention Khurratu Ainie`, who always been there for me by giving moral supports and Duas when I almost lost faith in myself. Their companionship has helped me through many dark days and their words of encouragement have managed to instil in me the will to go on. Thanks for the friendship and memories.

Most of all, my deepest gratitude goes to my beloved family-*ibu, ayah, and Zareez*-whom I dedicate this thesis to, for their tremendous endless love, wholehearted supports, and prayers especially when times are tough. I love these incredible people so much and they are my pillars of strength. I would not have made it this far without them.

This acknowledgement also goes to the Ministry of Agriculture (MOA) for the research grants of 100-RMI/MOA 16/6/2(5/2015) and MyBrain15 scholarship scheme for funding me to complete my studies.

Last but not least, to those who directly and indirectly contributed to this research, your kindness means a lot to me. Every assistance, big and small have helped me get to where I am today. Thank you very much. May Allah gives you all the best in return. Amin.

TABLE OF CONTENTS