UNIVERSITI TEKNOLOGI MARA

DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF KAEMPFERIA GALANGA (CEKUR) IN AN FE-INDUCED LIPID PEROXIDATION SYSTEM

MUHAMAD FAIZ OTHMAN

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

Faculty of Pharmacy

October 2006

ACKNOWLEDGEMENT

In the name of Allah, the Compassionate, and the Merciful. Alhamdulillah, with His will and guidance, this research project and thesis is completed.

I wish to express my deepest gratitude to my supervisor Professor Dr. Aishah Adam for her generous assistance in helping me to complete this thesis. Special thanks to my parents for their support either morally or financially. Names like Dr. Mizaton bt Hazizul Hasan and Siti Sarah bt Wahab should never be forgotten. Their help has made the journey in completing this project even more exciting and successful.

My sincere appreciation is also extended to all the lecturers and the technical staff of the Faculty of Pharmacy, UiTM for their support and assistance. To all my friends, thanks for your understanding, support and encouragement in completing this research project.

TABLE OF CONTENTS

IIILE PAGE	
APPROVAL SHEET	
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iv
LIST OF ABBREVIATIONS	vi
ABSTRACT	ix
CHAPTER 1 INTRODUCTION 1.1 Introduction	1
CHAPTER 2 LITERATURE REVIEW 2.1 Reactive oxygen species 2.1.1 Sources and types 2.1.2 The importance of free radicals 2.1.3 Formation of ROS 2.1.4 Physiological effects 2.2 Oxidative stress 2.2.1 Lipid peroxidation 2.3 Antioxidants 2.3.1 Definition and roles of antioxidant 2.3.2 Types of antioxidants 2.4 Phenolic substances 2.4.1 Effects of phenolics substances 2.5 Kaempferia galanga (Cekur) 2.5.1 Medicinal properties	11 12 12 13 14 16 16 18
CHAPTER 3 MATERIALS AND METHODS 3.1 Chemicals and apparatus 3.2 Ethyl acetate extract of Kaempferia galanga 3.3 Preparation of rat liver microsomes 3.3.1 Determination of microsomal protein concentration 3.3.1.1 Preparation or reagents 3.3.1.2 Quantification of microsomal protein concentration 3.4 Establishment of the iron-induced lipid peroxidation system using Fe/NADPH and liver microsomes. 3.4.1 Preparation of 0.25 mg/ml microsomes (final concentration) in 0.05 M Tris-HCl 26	200 200 201 23 25 25 25 25

ABSTRACT

Antioxidants play a major role in terminating the lipid peroxidation process and in scavenging free radicals. Phenolic compounds, which can be easily found in the plant kingdom, have been proven by many researchers to be potent antioxidants. A popular Malay herb, Kaempferia galanga or locally known as cekur, was reported to contain quite a lot of phenolic compounds. This study thus was carried out with the aim of determining the antioxidant activity of Kaempferia galanga using the Fe2+/NADPHinduced lipid peroxidation microsomal system. The ethyl acetate extract of Kaempferia galanga was prepared prior to this study by a Msc. candidate. For the induction of lipid peroxidation in microsomes, iron was utilized as the free radical generator in the presence of NADPH. In this study, the optimum concentration of iron for induction of lipid peroxidation was determined. The results showed that the optimal iron concentration to initiate lipid peroxidation was 15 µM. The antioxidant activity of Kaempferia galanga ethyl acetate extract was measured using this established Fe²⁺/NADPH system. Lipid peroxidation was monitored by the thiobarbituric acid reactive substance (TBARS) method. Trolox and quercetin were use as positive control as both compounds are potent antioxidant. The results showed that Kaempferia galanga ethyl acetate extract possess antioxidant activity although not as potent as the controls (trolox and quercetin). The concentration which inhibits lipid peroxidation by 50% (inhibiting concentration; IC₅₀) for the rhizome extract of Kaempferia galanga was 0.03 mg/ml while for leaf extract, it was 0.014 mg/ml. The IC₅₀ for trolox was 0.003 mg/ml The IC₅₀ for quercetin could not be determined as it has very potent antioxidant activity even at very low concentrations in this system. In summary, the results obtained showed that the ethyl acetate extract of Kaempferia galanga (both rhizome and leaf) possessed antioxidant in vitro activity as both produced inhibition of lipid peroxidation although their magnitude of activity as not high as trolox and quercetin. Potency was half that of trolox for the leaf extract and a magnitude lower for the rhizome extract based upon a comparison of the IC₅₀ values.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Antioxidants represent a first line body defense against oxidative stress produced by the generation of free radicals and reactive oxygen species (ROS) (Perry et al, 1980). They act to terminate the free radical chain reactions and suppress the formation of ROS. Under normal circumstances, the ROS generated are detoxified by antioxidants that are present in the body leading to establishment of equilibrium between the ROS generation and antioxidants capacity. However, overproduction of ROS or inadequate antioxidant defense may tip this equilibrium to favor a deleterious condition known as oxidative stress (Marks & Liebermann, 2005).

The formation of ROS is enhanced in the presence of iron (Fe) and other transient metals that participate in the Fenton reaction leading to the formation of highly reactive hydroxyl radicals. These and other ROS may initiate lipid peroxidation directly or via oxygen-anion complex formation and various other Fe redox states (Tien et al., 1981). Increased levels of ROS can initiate lipid peroxidation (LP), damage proteins and deoxyribonucleic acid (DNA), thereby causing acute tissue damage or the development of various diseases (Ozgova et al., 2003).