

UNIVERSITI TEKNOLOGI MARA

**INCORPORATION OF SOME MALAYSIAN
HERBAL AQUEOUS EXTRACTS AS AN
ANTIOXIDANT AND ANTIMICROBIAL IN CAKES**

IZZREEN ISHAK

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

Faculty of Applied Science

Feb 2010

ABSTRACT

This research consists of two parts. The first part is to study the antioxidative and antimicrobial properties of selected Malaysian herbal plants aqueous extracts namely curry leaves (*Murraya koenigii*), *kesum* (*Polygonum minus*) leaves and *tenggek burung* (*Melicope lunu-ankenda*) leaves and also to screen for phytochemical compounds present in the plants. The selected local herbs were investigated for their antioxidative activities (AOA) employing various established *in vitro* assays such as total phenolic content (TPC), ferric-reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging ability and β -carotene bleaching assays. Oxidative stability using accelerated study in the fat system was conducted using Rancimat method. The identification of flavonoids in plant extracts also was carried out by using High Performance Liquid Chromatography (HPLC). From the study, *tenggek burung* leaves extract had the highest TPC than other extracts. It also possessed higher reducing power than *kesum* leaves and curry leaves extract at 200 ppm concentration. *Tenggek burung* leaves extract exhibited strong efficiency and showed over 90 % scavenging effect of DPPH at a concentration of 200 $\mu\text{g/ml}$ and much higher than BHA/BHT and other plant extracts. The inhibition of lipid peroxidation of BHA/BHT by using β -carotene bleaching assay showed significantly higher than all plant extracts tested. In accelerated study using Rancimat method, addition of *tenggek burung* leaves and *kesum* leaves extracts exerted potent antioxidant effects on retarding fat oxidation which was comparable to BHA/BHT combination. According to all results obtained, the antioxidant activity increased proportionally with phenolic content. Correlations between phenolic content and antioxidant activity were investigated. The identification of flavonoids were also determined by using HPLC. Three types of flavonoids were found in curry leaves extract (catechin, epicatechin and apigenin), four were found in *kesum* extract (catechin, epicatechin, rutin and quercetin) and four were found in *tenggek burung* leaves extract (catechin, epicatechin, rutin and apigenin). Antimicrobial test using disc diffusion method was also conducted. *Tenggek burung* leaves appeared to display a stronger antimicrobial effect compared to other plant extracts. Toxicity test using brine shrimp lethality method showed that all local plant extracts were non-toxic ($\text{LC}_{50} > 1000$ ppm). The screening for phytochemicals on plant extracts revealed the presence of flavonoids, saponins, steroids, polyphenols and tannins. The second part of the study is to observe the effect of Malaysian herbal extracts stored at room temperature for 15 days. The six formulations consists of control sample (cake without addition of antioxidant) (F1), cake added with curry leaves extract (F2), cake incorporated with *kesum* leaves extract (F3), cake added with *tenggek burung* leaves extract (F4), cake incorporated with ascorbic acid (F5) and cake added with BHA/BHT (F6). Results for oxidation tests on the cakes using Peroxide Value (PV) and Thiobarbituric Acid (TBA) showed that formulations with Malaysian herbal extracts were lower ($p < 0.05$) than the control. In yeast and mould count, addition of plant extracts, ascorbic acid and BHA/BHT were not able to delay the growth of yeast and mould in cakes. The present of plant extracts in the formulations were not detected by the consumers.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the help of countless individuals. First of all, I want to express my appreciation to my dear supervisor Assoc. Prof. Dr. Noriham Abdullah for her guidance and supervision to make this project possible and successful.

I would like to thank En. Ahmad Kambali, the Senior Laboratory Assistant of Prograduate Laboratories, UiTM and Laboratory staff of the Department of Food Technology especially Pn. Nora, Pn. Siti, Cik Hariyah, Cik Shuhada, En. Osman and En. Fadzli for their kindness and co-operation.

My sincere thanks also goes to my lecturers, laboratory assistant and technical staff of the Faculty of Applied Sciences, Universiti Teknologi MARA, who are directly or indirectly involved in completing this project.

Above all, a very special thanks goes to my loving parents, whole family and friends for their concern, understanding and encouragement during the preparation of this project paper.

I would like to extend my thanks to the E-SCIENCE (PROJECT CODE: 03-01-01-SF0032) under Ministry of Science, Technology and Innovation (MOSTI) and Universiti Teknologi MARA for their financial supports.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
 CHAPTER	
1 INTRODUCTION	1
 2 LITERATURE REVIEW	 5
2.1 History of antioxidants	5
2.2 Antioxidant	5
2.3 Food antioxidants	6
2.3.1 Classification of food antioxidants	9
2.3.1.1 Primary antioxidants	9
2.3.1.2 Synergistic antioxidants	10
2.3.1.3 Secondary antioxidants	10
2.3.1.4 Miscellaneous antioxidants	10
2.4 Synthetic antioxidants	11
2.4.1 Butylated hydroxyanisole (BHA)	11
2.4.2 Butylated hydroxytoluene (BHT)	12
2.4.3 Propyl gallate	13
2.4.4 Tertiary-Butylhydroquinone (TBHQ)	13
2.5 Natural antioxidants	14
2.5.1 Vitamin E	17

CHAPTER 1

INTRODUCTION

Cake manufacturers face a major problem of lipid oxidation and mould growth which limits the shelf-life of their products. The use of antioxidants and preservatives can reduce this problem. Antioxidants are widely used to protect oxidisable goods such as cosmetics, pharmaceuticals, processed food or plastics from damage caused by reactive oxygen species. They play a major role in the food industry, to minimize rancidity, to delay the emergence of potentially toxic oxidative products, to protect and to stabilise colours, aroma and nutritional quality and to increase shelf life of food products (Brand-Williams *et al.*, 1995; Fukumoto & Mazza, 2000; Nair, 1999).

Consumers are increasingly avoiding foods prepared with preservatives of chemical origin and natural alternatives are therefore needed to achieve sufficiently long shelf-life of foods and a high degree of safety with respect to food-borne pathogenic microorganisms (Rauha *et al.*, 2000; Deba *et al.*, 2008). Reducing or eliminating chemically synthesised additives from foods is a current demand worldwide. Moreover, microorganisms are associated with food spoilage causing economical losses (Rocourt *et al.*, 2003). Herbs and spices are known for their antimicrobial and antioxidative properties. Due to an increasing demand for natural food additives, plants, herbs and spices have emerged as popular ingredients and have a tendency of replacing synthetic antimicrobial and antioxidant agents (Mayachiew & Devahastin, 2008). Consumer demand for less use of synthetic preservatives has led to research and use of “naturally derived” antimicrobials (Kotzekidou *et al.*, 2008).