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OPTIMIZATION OF ANTIOXIDANTS IN Curcuma zedoaria (CHRISTM.)ROSCOE LEAVES AND THEIR FUNCTIONAL COSMETIC PROPERTIES

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By

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Chairman : Associate Professor Siti Salwa Abd Gani, PhD
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Medicinal plants especially leaves are an ample source of numerous pharmacologically active molecules. Therefore, continuous exploration from the leaves of *Curcuma zedoaria* has been extensively probe for its therapeutic value. In this study, the fourier transform infrared (FTIR) spectroscopy analysis was employed and it is found that the compounds present in the zedoary leaves samples are carboxylic acid, carbonyl, benzene and phenol. The levels of mineral content in zedoaria leaves were divided into two groups which are essential heavy metals; Mn (328.30 ppm), Fe (128.35 ppm), Cu (4.21 ppm), Ni (1.16 ppm), Cr (0.90 ppm) and non-essential heavy metals; (Pb) and (Cd) recorded to have 0.17 ppm and 0.03 ppm respectively which considered under permissible range for consumable medicinal plants. The extraction of antioxidants compounds from zedoary leaves were obtained by using response surface methodology (RSM). This method is used to optimize and simultaneously investigate the influence of condition variables such as temperature, time and solvent concentration towards highest total antioxidants assay. Result shows that at the optimized condition for high total antioxidants activity was found in zedoary leaves at 75^{o}C, 92 mins and 90:10 v/v% ethanol concentrations exhibited (DPPH-radical scavenging (85.76%), β-carotene bleaching test (81.35%), ferric reducing antioxidant power assay (FRAP) (5.08 mM Fe^{2+} g/DW), total phenolic (125.75 mg/g GAE) and total flavonoid content (6.12 mg QE/g of extract). The optimized zedoary leaves were further explored for its functional cosmetics towards sun protection factor (SPF), anti-microbial test, anti-tyrosinase inhibition test, total carotenoids content, total ascorbic acid and total anthocyanin content. The results revealed that SPF value is 29.94 and fall into region UVC and UVB, the inhibition zone activity for bacteria *Bacillus Sublis* is 7.0 mm at concentration 100 mg mL^{-1}, tyrosinase inhibition recorded to have 31% and total carotenoids content, ascorbic acid including anthocyanins content is 8.36 g BET/100 g, 1.2577 mg/100 g and 4.25 mg/100 g respectively. To conclude, there could be clear potential for the utilization of *Curcuma zedoaria* leaves as a good source of antioxidants for diverse cosmeceutical, medicinal and food additives applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN ANTIOKSIDA DALAM DAUN KUNYIT PUTIH *Curcuma zedoaria* (CHRISTM.) ROSCOE DAN FUNGSI SIFAT-SIFAT KOSMETIK

Oleh

NUR FAUWIZAH BINTI AZAHAR

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Pengerusi : Professor Madya Siti Salwa Abd Gani, PhD
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Tumbuhan perubatan terutamanya dari daun adalah kaya dengan sumber molekul aktif farmakologi. Oleh itu, penerokaan berterusan dari daun kunyit putih telah disiasat secara meluas untuk nilai terapeutiknya. Dalam kajian ini, analisa FTIR menunjukkan sebatian yang hadir dalam daun kunyit putih ini adalah asid karbolik, karbonil, benzena dan fenol. Tahap kandungan mineral dalam daun zedoaria ini dibahagikan kepada dua kumpulan iaitu logam berat penting; Mn (328.30 ppm), Fe (128.35 ppm), Cu (4.21 ppm), Ni (1.16 ppm), Cr (0.90 ppm) dan logam berat tidak penting (Pb) dan (Cd) direkodkan masing-masing mempunyai berat 0.17 ppm dan 0.03 ppm iaitu di bawah julat yang dibenarkan untuk pengambilan tumbuhan perubatan. Kaedah gerak balas permukaan (RSM) telah digunakan untuk pengesktrakan antioksidan dari daun kunyit putih. Kaedah ini digunakan untuk pengoptimaan dan penyiasatan secara serentak oleh pengaruh tiga pemboleh ubah iaitu suhu, masa dan kepekatan pelarut ke arah jumlah antiokida yang tinggi. Data menunjukkan bahawa keadaan pengoptimuman untuk jumlah antioksidan yang tinggi dari daun kunyit putih adalah 75^{o}C, 92 min dan 90:10 v/v% kepekatan etanol mempaparkan (DPPH-perangkap radikal (85.76%), ujian pelunturan β- karotena (81.35%), pengurangan kuasa antioksida ferik (FRAP) (5.08 mM Fe^{2+} g/DW), jumlah fenolik (125.75 mg/g GAE) dan jumlah kandungan flavonoid (6.12 mg QE/g). Penerokaan daun kunyit putih yang telah dioptimumkan diteruskan untuk fungsi kosmetik iaitu faktor perlindungan matahari (SPF), ujian anti-mikrob, ujian perencatan anti-tyrosinase, kandungan karotenoid, asid askorbik dan kandungan antosianin. Hasil kajian menunjukkan bahawa nilai SPF adalah 29.94 dan berada di kawasan UVC dan UVB, aktiviti zon perencatan untuk bakteria *Bacillus Sublis* adalah 7.0 mm pada kepekatan 100 mg mL^{-1} , data peratusan perencatan anti-tyrosinase sebanyak 31% dan jumlah kandungan karotenoid , asid askorbik termasuk antosianin masing-masing mengandungi 8.36 g BET/100 g, 1.2577 mg/100 g dan 4.25 mg/100 g secara turutan. Secara kesimpulan, daun kunyit putih mempunyai potensi yang jelas sebagai sumber antioksidan yang baik untuk diaplikasikan dalam pelbagai kosmeseutikal, ubat-ubatan dan makanan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

AA	Ascorbic acid
Anova	Analysis of variance
AOAC	Association of Analytical Communities
Ar	Arsenic
BCB	B-carotene bleaching
CCD	Central composite design
Cd	Cadmium
Cr	Chromium
Cu	Copper
cm	Centimetre
DPPH	1,1-diphenyl-2-picrylhdrazyl
Fe	Iron
FRAP	Ferric ion Reducing Antioxidant Power Assay
FTIR	Fourier Transform Infrared
GCMS	Gas Chromatography –Mass Spectroscopy
mg/L	Milligram per litre
Mn	Manganese
min	Minute
MPAES	Microwave plasma- atomic emission spectroscopy
mL	Mililiter
Ni	Nickel
nm	Nanometer
Pb	Lead
RSM	Response Surface Methodology
SPF	Sun Protection Factor
TFC	Total flavonoid content
TPC	Total phenolic content
µg/L	Microgram per litre
UV-Vis	Ultra Violet –Visible Infrared Spectroscopy
Zn	Zinc

CHAPTER I

INTRODUCTION

1.1 Background

Plants are substantial source of natural antioxidants. Active constituents present in natural antioxidants such as phenolic, carotenoids, flavonoids, folic acid, benzoic acid, and tocopherol are secondary metabolites of the plants which can provide various potential treatment and prevention of cancer, cardiovascular diseases, and neurodegerative diseases (Ghasemzadeh et al., 2010; Karabegović et al., 2014). Antioxidant is a highly reactive compound help to delay or inhibit the initiation and propagation of oxidizing chain reactions involving uncontrollable metabolic processes from reactive oxygen species (ROS) and free radical species (Chanda and Nagani, 2010). Practically, radical damage can be treated by both synthetic and natural antioxidants. However, the use of artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are suspected to increase the risk of cancer and liver damage in humans (Hue et al., 2012).

Recently, it has been investigated that countless by-products from the processing plants like leaves, barks, fruit pods, shells and even pulp contain numerous biologically active compounds. It was reported to contain high level of phytochemicals sources such as antioxidants, antimutagens and anticarcinogenic. For instance, the leaves from tea, lemon and papaya are explored and found to contain high level of phytochemicals constituents which resulted in the development of natural antioxidant formulations for cosmetic and medicinal applications.

Fortunately, Malaysia is one of world's richest tropical rainforest that can provide useful primary medicinal plants and herbs. In recent times, Zingiberaceae from the largest family of Zingiberales is getting recognition from scientists all over the world for their potential benefit to living organisms (Das and Rahman, 2012). One of the genus of Zingiberaceae families that has higher potential is *Curcuma zedoaria*, which is popularly known as white turmeric, zedoaria or gajutsu (Lobo et al., 2009). Despite numerous studies on *Curcuma zedoaria* such as rhizomes and roots which cater enormous functional components, however very limited scientific information on antioxidant properties conducted on *Curcuma zedoaria* leaves for its therapeutic potential. In this study, the antioxidant activity and functional cosmetic properties of *Curcuma zedoaria* leaves were investigated and explored.

1.2 Objectives

The general objective of this study was to investigate the phytochemical of *Curcuma zedoaria* leaves as potential for antioxidant activity and its cosmetic functional properties.
The specific objectives are as follow;

i. To identify the phytochemicals constituents present in *Curcuma zedoaria* leaves via FTIR and mineral content.
ii. To investigate the five different antioxidants assay (DPPH-radical scavenging, β-carotene, Ferric reducing antioxidant power assay (FRAP), total phenolic and total flavonoid content) present in *Curcuma zedoaria* leaves and to optimize an antioxidants activity using Response Surface Methodology (RSM)
iii. To determine the functional cosmetic properties *Curcuma zedoaria* leaves extract with respect to the sun protection factor (SPF), anti-microbial test, anti-tyrosinase inhibition test, total carotenoids, ascorbic acid and anthocyanin content.

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