EVALUATION OF *Acalypha indica* EXTRACTS FOR ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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DEDICATION

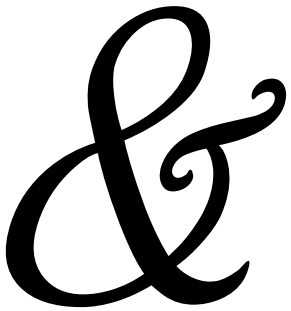
To

ADABI

As the main sponsor of UTM-ADABI Cat Food project

MINISTRY OF HIGHER EDUCATION MALAYSIA and MARA

for providing scholarship and financial support for my study



FAMILY and FRIENDS

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ABSTRACT

For centuries, medicinal plants are being used as remedy for various ailments throughout the globe. The study was conducted emphasizing on the antibacterial and antioxidant activities of several *Acalypha indica* extracts. The plant was divided to leaves and stem, whole plant and roots and extracted with hexane, methanol and ethanol by successive method. Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging assay and found to be highest in the ethanolic root extract with IC_{50} of 206 µg/ml. The antibacterial activity screening of different extracts was conducted by using disc diffusion, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*. Hexane extracts from leaves and stem, whole plant and roots showed promising results against *Enterococcus faecalis* with high inhibition zone at 10 to 12 mm as compared to standard antibiotics, 6 to 10 mm. All extracts showed antibacterial activity with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in the range from 60 to 15 mg/ml. This study concludes that *A. indica* explicit antioxidant and antibacterial activities may be potential for pharmaceuticals, cosmeceuticals, nutraceuticals, medical and food industry.

ABSTRAK

Selama berabad, herba telah digunakan sebagai penawar pelbagai penyakit di seluruh dunia.Kajian ini dijalankan dengan menekankan aktiviti antioksidan dan anti-mikrob oleh beberapa ekstrak *Acalypha indica*. Tumbuhan tersebut dibahagikan kepada beberapa bahagian iaitu batang dan daun, keseluruhan pokok dan akar. Aktiviti antioksidan diukur menggunakan kaedah pemerangkapan radikal bebas 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan menunjukkan ekstrak akar menggunakan ethanol mempunyai aktiviti antioksidan tertinggi dengan IC_{50} 206 ug/ml. Penilaian aktiviti antimikrobial oleh pelbagai ekstrak dijalankan dengan cakera resapan, kepekatan perencatan minimum (MIC) dan kepekatan bakterisidal minimum (MBC) merencat pertumbuhan *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* dan *Enterococcus faecalis*. Ekstrak menggunakan pelarut heksana menunjukkan hasil yang memberansangkan menentang *Enterococcus faecalis* dengan zon perencatan yang tinggi (10 hingga 12 mm) berbanding antibiotik standard (6 hingga 10 mm. Semua ekstrak menunjukkan nilai kepekatan perencatan minimum (MIC) dan kepekatan bakterisidal minimum (MBC) sekitar 60 hingga 15 mg/ml. Kesimpulannya, *A. indica* mempamerkan potensi aktiviti antioksidan dan anti-mikrob dan sesuai untuk penggunaan dalam industri farmaseutikal, nutraseutikal, kosmeseutikal, perubatan dan makanan.

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LIST OF ABBREVIATIONS

B.C.	-	Before Centuries
DMSO	-	Dimethylsulfoxide
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
g	-	Gram
h	-	Hour
IC_{50}	-	50% inhibitory concentration
nm	-	nanometre
m	-	Metre
MBC	-	Minimum Bactericidal Concentration
mg	-	Milligram
MIC	-	Minimum Inhibitory Concentration
mL	-	Millilitre
mm	-	Millimetre
NA	-	Nutrient agar
NB	-	Nutrient broth
rpm	-	Round per minute
%	-	Percentage
µg	-	Microgram
µL	-	Microliter
˚C	-	Degree Celcius

CHAPTER 1

INTRODUCTION

1.1 General Introduction

For thousand years, medicinal plants have been used as traditional remedies to treat numerous human illnesses in many different parts of the world. In the developing world, particularly in rural areas, herbal remedies continue to be an essential source of medicine. Scientifically, medicinal plants have been demonstrated as a numerous source of biologically active compounds, majority of them have already been formulated into beneficial therapeutic substances.

Acalypha indica belongs to *Euphorbiaceae*, a large family of flowering plants. Majority of the species is distributed in the Indo-Malayan region and tropical America (Charles *et al.*, 2007) is an annual herb, about 80 cm high, a wild plant and commonly found in waste places or fields. It is locally known as “kucing galak” or “rumput lis-lis”, “kuppaimeni” in India and “t’ie han tsai” in China (Kirtikar & Basu, 1975). For a long time, *A. indica* has been used as traditional medicines of various countries and they are also reported to possess diuretic, purgative and anthelmintic properties, and also being used for bronchitis, asthma, pneumonia, scabies and other cutaneous diseases (Kirtikar & Basu, 1999).

Solvents selection have vital role in absorbing various bioactive compounds from plants. Polar solvent able to dissolve hydrophilic compounds, semi polar solvent able to dissolve both hydrophilic and lipophilic compounds while non-polar solvent absorbs lipophilic compounds. The usage of different solvents increase the possibilities to dissolve various different compounds (Charmi *et al*., 2011) which in turn will affect the bioactivities such as antioxidant and antibacterial activities.

This experiment was conducted to evaluate the antioxidant activity of *A. indica* extracts using three different solvent by successive method. Next, the leaves and stem, roots and whole plant extract were screened on antioxidant and antibacterial properties to support the conventional therapeutic claim and to provide base line data for the scientific communities to conduct further study.

1.2 Problem Statement

For decades, *A. indica* has been used as traditional remedies to treat various ailments such as skin diseases. However, there are still limited publications on the plants' biological activities such as antioxidant and antibacterial activity of *A. indica,* which might contribute to its various medicinal properties.

Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often used in the food industries to prevent or inhibit the oxidative deterioration in foods. However, there are a few health and safety concerns in use of synthetic antioxidants due to their potential toxicity and carcinogenic (Izreen & Noriham, 2011). Thus, there is increasing importance to search the natural antioxidants from plants as an alternative to synthetic antioxidant. Besides, the knowledge on the potential of antioxidant properties of *A. indica* are still not well understood. Therefore, it is worthwhile to study the antioxidant activity of *A. indica* based on its medicinal benefits.

There have been an increase in bacterial resistance to antibacterial agents which in turn cause difficulties to treat infectious diseases (Prashanth, Asha & Amit, 2001). Thus, there is a need to search for new antibacterial agent specifically from plants as an alternative to synthetic antibiotics. This prompted the evaluation of *A. indica* as source of potential natural antibacterial agent by testing against human pathogens such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*.

1.3 Research Objectives

1.3.1 To extract the leaves and stem, roots and whole plant of *A. indica* using various solvents by successive method.

1.3.2 To evaluate the antioxidant activity of the crude extracts.

1.3.3 To screen the antibacterial activities of the crude extracts.

1.4 Research Scope

This research focuses on the study of the *A. indica* various extracts, its antioxidant activities and antibacterial activities. The fresh *A. indica* was extracted by using soxhlet extractor with hexane, methanol and ethanol. The biological activities such as antioxidant and antibacterial will be carried out on the crude extracts. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were conducted to assess the antioxidant activity. The antibacterial activity were evaluated by disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

1.5 Significance of Research

Even though the research of antibacterial agents towards important bacterial pathogens have increased throughout the world, it is well known that the number of new antibacterial agents being brought to the market has undergone a steady decline in the past several decades. Since antiquity, human has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various diseases (Rios & Recio, 2005). The antibacterial compounds from plants may inhibit bacterial growth by different mechanisms than antibiotics and may have a significant clinical value in treatment of resistant microbial strains (Eloff, 1998). This study contributing the overview of *A. indica* on antibacterial activities against common pathogenic bacteria.

Worldwide, there has been growing trend and interest in plants' natural antioxidants as natural additives in food and cosmetics. Plants are one of the mostimportant targets to search for natural antioxidants as it is known safer compared to synthetic antioxidants (Yanislieva *et al*., 2006). This study will contribute towards the growing database of knowledge on herbal medicines and help to advocate the safe and effective use of *A. indica* as traditional herbal remedies.

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