

ANTIOXIDANT LEVEL OF TRADITIONAL
CHINESE MEDICINE HERBS FORMULATION

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Antioxidant Level of Traditional Chinese Medicine Herbs
Formulation

By

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A research project submitted to the Faculty of Veterinary
Medicine, Universiti Malaysia Kelantan in partial fulfilment of
the requirements for the degree of Doctor of Veterinary Medicine

Faculty of Veterinary Medicine
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FORMULATION

ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Oxidative stress caused by the accumulation of free radicals can lead to various detrimental effects on the physiological aspects of the body. The efficacy of traditional Chinese medicine (TCM) as an antioxidant in animals is essential to be studied to reduce or prevent the accumulation of free radicals. Hence, this study is conducted to analyse the antioxidant level of TCM herbs formulation. A TCM polyherbal formulation consisting of Hawthorn fruit, cassia seed, orange peel, cinnamon, and rhizomes from *Alisma orientale* were used in this study. Based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity results, the TCM herbal formulation has higher antioxidant level compared to reference ascorbic acid at similar concentration, which was 81.69%. Thus, the TCM herbal formulation demonstrates considerable potential as a potent antioxidant for animals by minimising the accumulation of free radicals in animals.

Keywords: Traditional Chinese Medicine, antioxidant level

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TAHAP ANTIOKSIDA FORMULASI HERBA UBATAN TRADISIONAL

CINA

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Tekanan oksidatif yang disebabkan oleh pengumpulan radikal bebas boleh membawa kepada pelbagai kesan buruk ke atas aspek fisiologikal tubuh badan. Oleh yang demikian, keberkesanan ubatan tradisional Cina sebagai antioksidan di dalam haiwan adalah penting untuk dikaji untuk mengurangkan atau mencegah pengumpulan radikal bebas tersebut. Justeru, kajian ini dijalankan untuk menganalisis tahap antioksidan formulasi herba ubatan tradisional Cina. Formulasi herba ubatan tradisional Cina yang terdiri daripada buah Hawthorn, biji cassia, kulit kering oren, kayu manis, dan rizom daripada *Alisma orientale* telah digunakan dalam kajian ini. Berdasarkan keputusan aktiviti penghapusan 2,2-diphenyl-1-picrylhydrazyl (DPPH), formulasi tersebut mempunyai tahap antioksidan yang lebih tinggi berbanding asid askorbik rujukan pada kepekatan yang hampir sama, iaitu 81.69%. Oleh itu, formulasi herba ubatan tradisional Cina menunjukkan potensi yang besar sebagai antioksidan yang kuat untuk digunakan dalam haiwan dengan mengurangkan pengumpulan radikal bebas dalam haiwan.

Kata kunci: Ubatan tradisional Cina, level antioksidan

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TABLE OF CONTENTS

	PAGE
ORIGINAL LITERARY WORK DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
CHAPTER 1 INTRODUCTION	
1.1 Introduction	1
1.2 Research Problem	2
1.3 Research Question	2
1.4 Research Hypothesis	2
1.5 Research Objectives	2
1.6 Significance of the Study	2
1.7 Scope of the Study	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Traditional Chinese Medicine	4
2.1.1 <i>Crataegus pinnatifida</i>	4
2.1.2 <i>Cassia obtusifolia</i> Leguminosae	5
2.1.3 Orange peel	6
2.1.4 <i>Cinnamomum</i>	7
2.1.5 <i>Alisma orientale</i>	7
2.2 DPPH scavenging activity	8
CHAPTER 3 RESEARCH METHODOLOGY	

3.1	Sample Collection	9
3.2	Sample Preparation	9
3.3	Preparation of the DPPH solution	9
3.4	DPPH free radical scavenging activity assay procedure	9
3.5	Standard Curve Preparation	9
3.6	Antioxidant Determination	9
3.7	Data Analysis	10
CHAPTER 4 RESULTS		
4.1	Standard Curve (Ascorbic Acid)	11
4.2	Antioxidant Value of Sample	12
CHAPTER 5 DISCUSSION		13
CHAPTER 6 CONCLUSION		14
REFERENCES		15

LIST OF FIGURES

NO.		PAGE
2.1	Dried Hawthorn fruit	5
2.2	Dried cassia seed	6
2.3	Dried orange peel	6
2.4	Dried cinnamon bark	7
2.5	Cut section of <i>A. orientale rhizome</i>	8
4.1	Standard curve of absorbance against ascorbic acid	12

LIST OF ABBREVIATIONS

°C	Degree Celsius
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ROS	Reactive oxygen species
TCM	Traditional Chinese Medicine
IC ₅₀	Half Maximal Inhibitory Concentration

LIST OF SYMBOLS

%	Percentage
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Reactive oxygen species (ROS) are free radicals that include hydroxyl, superoxide, nitric oxide, nitrogen dioxide, peroxy, and lipid peroxy, and are produced as a by-product of the normal cellular metabolism for energy production. At low concentration, ROS plays an important role in the maturation process of cell structures as well as in the host defence system (Pham-Huy et al., 2008). However, when there is excessive accumulation of ROS which can also be caused by external factors such as pesticides, pollutants and food additives, this leads to an imbalance between the production of free radicals and their neutralization by antioxidant defences. This results in oxidative stress which causes damage to the cell membranes, proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA). Furthermore, oxidative stress has a role in the progression of chronic and degenerative diseases, including cardiovascular, pulmonary and neuropathy diseases. To counteract this problem, antioxidants are produced to help reduce and prevent oxidative stress damage due to their ability to donate electrons and thus neutralizes the formation of radicals (Lalhminghui et al., 2018). Various traditional Chinese medicinal (TCM) herbs are reported to have high antioxidant activity and their protective effect are partly attributed to the antioxidant's vitamin C, vitamin E, polyphenols, carotenoids, and other active ingredients in plant-based foods (Wang & Dai, 2012). In this study, a combination of hawthorn fruit, cassia seed, orange peel, cinnamon, and rhizome from *Alisma orientale* for the TCM formulation will be used to analyse the antioxidant

activity of the TCM formulation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay.

1.2 Problem Statement

Oxidative stress caused by the accumulation of free radicals can lead to various detrimental effects on the physiological aspects of the body. Hence, the efficacy of TCM as an antioxidant in animals is to be studied to reduce or prevent the accumulation of free radicals.

1.3 Research Questions

What is the antioxidant level of TCM herbs?

1.4 Research hypotheses

There is a significant antioxidant level in TCM herbs.

1.5 Research Objectives

To analyse the antioxidant level of TCM herbs formulation.

1.6 Significance of the Study

The analysis of antioxidant level from the TCM herb formulation can contribute to the information of valuable reference on reducing the oxidative stress in animals due to accumulation of free radicals as an alternative natural based treatment, potentially providing new insights into preventative and therapeutic measures for oxidative stress-related conditions in animals.

1.7 Scope of the Study

This study aims to analyse the antioxidant levels of TCM herbs formulation, which encompasses sampling preparation, analysis and data interpretation obtained through DPPH antioxidant assay. In this study, TCM herbal formulation which consists of hawthorn fruit, cassia seed, orange peel, cinnamon, and rhizome from *A. orientale* were analysed for its antioxidant properties to be used in veterinary practices.

CHAPTER 2

LITERATURE REVIEW

2.1 Traditional Chinese Medicine

The traditional Chinese medicine (TCM) herbs have been in the food and medicine industry for over two millennia with more than 11,000 officinal plants used as TCM (Xu et al., 2019). They possess four natures (cold, hot, warm, and cool) and five tastes (pungent, sweet, sour, bitter, and salty), with natures based on the properties of treating cold or heat diseases. These natures are primarily summarized from the reaction of the body after taking the Chinese herbs (Yang et al., 2017). Epidemiological studies have shown that many natural antioxidant compounds possess varying degrees of anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities (Muanda et al., 2013). In addition, according to Larson (1988), numerous TCM herbs contain high antioxidant components such as vitamin E, flavonoids, phenolic acids, carotenoids, ascorbic acid, and glutathione. This property has earned them the growing popularity among the western practitioners. However, the safety and efficacy issues of TCM remain a major challenge in China, as it is more often used for chronic conditions where it addresses diseases from the root cause, causing a longer period for recovery and harder for evaluation of long-term results (Xu et al., 2008).

2.1.1 *Crataegus pinnatifida*

C. pinnatifida also known as hawthorn, is a member of the *Rosaceae* family and widely distributed throughout North China, Europe, and North America (Li et al., 2022). The dried hawthorn fruits commonly known as “Shanza” (Figure 2.1), hold a prominent

place as both a food source and a medicinal herb in China as the treatment for circulatory system disorder, including myocardial infarction, hypertension and atherosclerosis (Chen et al., 2013). In recent years, hawthorn fruits have gained global recognition as a significant medicinal and economical crop due to its varied and remarkable health benefits (Li et al., 2022).



Figure 2.1: Dried Hawthorn fruit

2.1.2 *Cassia obtusifolia* Leguminosae

C. obtusifolia (Sickle pod) is a widely recognised traditional Chinese medicinal plant within the Cassia genus of the Leguminosae family and is primarily distributed in China, Korea, India, and the western tropical regions (Ali et al., 2021). The cassia semen, known as “Juemingzi” in Chinese, is the dried ripe seed of *C. obtusifolia* Leguminosae (Figure 2.2). The seeds have been used widely in TCM to treat lacrimation, headaches, dizziness, diabetic hyperlipidaemia and diabetic constipation (Wang et al., 2020).



Figure 2.2: Dried cassia seed

2.1.3 Orange peel

Orange, which is a citrus fruit, belongs to the *Rutaceae* family and grows in tropical and subtropical regions (Saleem et al., 2023). In TCM, dried orange peel (Figure 2.3), known as “Chen Pi” in Mandarin, is a commonly used herb with various therapeutic properties including anti-cancer, anti-inflammatory, anti-obesity, immune-modulating, anti-osteoclastogenic, anti-viral and neuroprotective effects. (Sakile et al., 2023). Furthermore, according to Singh (2020), the peels from the oranges are the major source of antioxidant components, namely phenols and flavonoids.



Figure 2.3: Dried orange peel

2.1.4 *Cinnamomum*

Cinnamon is a spice made from the inner bark of trees known as *Cinnamomum*, a member of the Lauraceae family (Figure 2.4). It is mainly distributed in the tropical and subtropical Asia, Australia, Pacific islands, and other regions (Wang et al., 2020). Studies have shown that cinnamon supplementation can increase the antioxidant levels in blood while reducing the levels of inflammatory markers (Zhu et al., 2020). Additionally, according to Wang et al. (2020), cinnamon has been studied to have a wide range of pharmacological properties including analgesic, antitumor, anti-diabetic, anti-obesity, immunoregulatory, cardiovascular protective, as well as neuroprotective effects.



Figure 2.4: Dried cinnamon bark

2.1.5 *Alisma orientale*

A. orientale is a popular TCM herb native to a few Asian countries, including China, Korea, and Japan (Shu et al., 2016). Its rhizome, *Alismatis rhizoma* (Figure 2.5), also known as “Ze Xie” in Chinese, has been used in the aforementioned countries to treat various conditions including oliguria, edema, diarrhoea and dizziness due to its excellent pharmacological and biological effects, including diuretic, anti-urolithiatic, antinephritic, anti-atherosclerotic, immunomodulatory, and hepatoprotective activities (Shu et al., 2016).



Figure 2.5: Cut section of *Alismatis rhizoma*

2.2 DPPH radical scavenging activity

The DPPH radical scavenging assay is the most commonly used antioxidant assay for plant extract as it provides a rapid and simple way to evaluate the antioxidant by spectrophotometry and measured at 517 nm wavelength (Huang et al., 2005). It assesses the capacity of an antioxidant compound to donate hydrogen or scavenge free radical (Benkhaira et al., 2021). When an antioxidant agent is present, it will react with the stable free radical DPPH and reduce it to DPPH-H, turning the violet DPPH into a yellow hue (Baliyan et al., 2022). The degree of discoloration will be the indicator for the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

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RESEARCH METHODOLOGY

3.1 Sample

3.1.1 Sample collection

The Traditional Chinese Medicine (TCM) herbs were purchased at a local Chinese herbal shop.

3.1.2 Sample preparation

A total of 15 g of the samples were washed to remove any unwanted debris and a portion of the herbs were boiled in a 1 litre of water at 100°C for 20 minutes to produce an aqueous extract.

3.2 Antioxidant Assay

3.2.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution preparation

About 7.89 mg of DPPH (EMD Millipore 300267-50MG) were dissolved in 100 ml of 99.5% ethanol, to give a DPPH solution. It was kept in a dark area prior to usage.

3.2.2 Ascorbic Acid Standard Curve Preparation

Ascorbic acid was bought from Shopee and was used as the reference standard in the assay. Stock ascorbic acid with initial concentration at 100 mg/ml was diluted into 1 to 8 µg/ml with distilled water.

3.2.3 Antioxidant determination

3.2.3.1 DPPH Free Radical Scavenging Activity Assay

A total of 50 μl of TCM aqueous extract was pipetted into a 96-well plate and 50 μl DPPH solution was added into each well. Same steps were repeated for the different concentrations of the reference ascorbic acid. Control sample was prepared with the same volume without the addition of the extract nor the reference ascorbic acid. The plate was incubated for 30 minutes in the dark at room temperature. After incubation, the absorbance was read at 517 nm using a plate reader (BMG LABTECH's FLUOstar Omega).

3.2.4 Data analysis

The DPPH free radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH radical-scavenging} = \frac{[(\text{Absorbance of Control} - \text{Absorbance of test Sample}) / (\text{Absorbance of Control})] \times 100}{(3.1)}$$

Using Microsoft Excel, all the results were tabulated and plotted against respective concentrations to obtain antioxidant content of the sample, by referring to the ascorbic acid standard curve.

CHAPTER 4

RESULTS

4.1 Standard Curve (Ascorbic Acid)

Table 4.1 displays absorbance obtained for the standard curve prepared for ascorbic acid. As the concentration of ascorbic acid increases, the absorbance decreases.

Table 4.1 Absorbance of ascorbic acid at different concentration at 517 nm

Concentration ($\mu\text{g/ml}$)	Absorbance
0	0.2075
1	0.1793
2	0.1483
3	0.1226
4	0.0995
5	0.0744
6	0.0584
7	0.0498
8	0.0483

From the absorbance obtained, the percentage of DPPH radical-scavenging activity for each of the concentrations were calculated (Table 4.2), and the result was plotted to establish a standard curve (Figure 4.1).

Table 4.2 Percentage of DPPH radical-scavenging activity of ascorbic acid at different concentration

Concentration ($\mu\text{g/ml}$)	DPPH radical-scavenging activity (%)
0	0
1	13.61
2	28.55
3	40.90
4	52.05
5	64.16
6	71.87
7	76.02
8	76.75

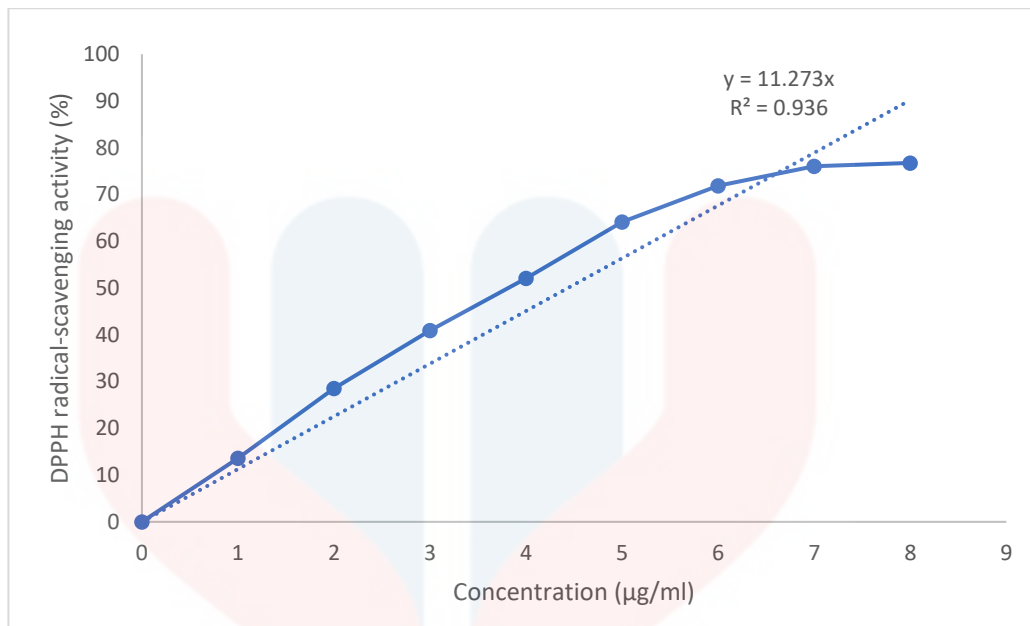


Figure 4.1: Standard curve of absorbance against ascorbic acid

4.2 Antioxidant Value of Sample

The absorbance value of the 15 g sample is 0.038 at 517 nm. Based on the calculation, the % DPPH radical-scavenging of TCM herbal formulation sample solution was 81.69% and the concentration of the antioxidant is equivalent to 7.25 µg/ml of ascorbic acid.

CHAPTER 5

DISCUSSION

The free radical scavenging potential of the traditional Chinese Medicine (TCM) herbal formulation was analysed using the DPPH method, with ascorbic acid as the reference value. The analysis involved measuring the degree of discolouration or the absorbency using a plate reader read at 517 nm and the manual calculation of the percentage of DPPH radical scavenging activity.

Based on the results obtained, the 15 g TCM herbal formulation has the antioxidant properties of 0.038 absorbency at 517 nm and showed a significant antioxidant level (81.69%) at the concentration of 7.25 µg/ml by referring to ascorbic acid. This indicates that the TCM herbal formulation contained strong antioxidant agent that can scavenge the stable free radical DPPH, converting it to DPPH-H and causing the violet DPPH to change into a yellow hue (Baliyan et al., 2022).

Therefore, the herbs formulation has the potential to become a potent antioxidant in animals due to its effectiveness in scavenging and antioxidant activity, due to the combinations of various herbal ingredients in the formulation which can synergistically enhance the total antioxidant capacity.

CHAPTER 6

CONCLUSION

In conclusion, traditional Chinese medicine (TCM) herbal formulation consisting of Hawthorn fruit, cassia seed, orange peel, cinnamon, and rhizomes from *Alisma orientale* expresses synergistic enhancement through its high antioxidant activity which is 81.69% at 7.25 µg/ml as compared to ascorbic acid standard. This indicates its potential as a significant therapeutic agent that can be incorporated in a tea bag to drink as a tea in the prevention or slowing the progress of oxidative stress-related damage to cells and tissues, which could lead to various detrimental effects on the physiological aspects of the body. Last but not least, the suggestion for future study is to have few different weights of sample to see different pattern of antioxidant activity in order to carry out statistical analysis.

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