ANTIMICROBIAL AND PHARMACOLOGICAL EFFECTS OF LAMIACEAE PLANT LEAF EXTRACTS IN RELATION TO ITS PHENOLIC AND ANTIOXIDANT ACTIVITIES

BY

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ABSTRACT

Leaves of *Coleus blumei* (two species), *Coleus amboinicus* (two species), *Coleus aromaticus* and *Pagostemon cablin* from *Lamiaceae* family were collected from different localities, freeze dried and extracted with aqueous methanol. The biological activity in vitro, especially in relation to total phenolic & flavonoid contents, antioxidant and antimicrobial activities were studied. Total phenolic content was determined according to the Folin-Ciocalteu method whilist in antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The antimicrobial activity of the extract was determined by making use of macro dilution and disc diffusion methods on two gram positive bacteria, two gram negative bacteria and on fungus. Furthermore, the toxicity was also assessed for the extracts by performing acute toxicity test. The phenolic content among the six Lamiaceae leaves extract showed significant difference ($p<0.05$) in result ranging from 55.21 - 95.17 mg GAE/g of dried samples. *Pogostemon cablin* (PCM) had the highest content of phenolic followed by *C. blumei* (CBPM). There were significant differences ($p<0.05$) of IC_{50} value of six *Lamiaceae* leaves extract ranging from 10.5 – 34.1 µg/ml. Among the species studies, *Coleus amboinicus* – Malaysia (CALM) and *Pogostemon cablin* (PCM) showed high antioxidant activity compared to the other leaf extracts. All leaf extracts showed activity at least against one strain of bacteria and result showed significant difference ($p<0.05$) between activities on the five microorganism. On the contrary, all of the leaf extracts were not effective against *C. albicans*. The minimum inhibitory concentration (MIC) of all leaf extracts ranged from 1.0–2.0 mg/ml in inhibiting the growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *B.subtilis*. The acute toxicity test using *C. Blumei* leaf extract showed that there was no mortality of animals experimented recorded even at the highest dose level of 5000 mg/kg body weight and observed for the 24 hours after administration. This shows that *Coleus blumei* plant extract have no toxic effect in mice. This study shows that the extracts can be used as antioxidant and antimicrobial agent without having the toxic effect.

ملخص البحث

لقد تمّ جمع أوراق النباتات التالية من مناطق مختلفة: *Coleus blumei*، *Coleus amboinicus* ، *Coleus aromaticus*، *Pagostemon cablin* وهي من عائلة نباتية تُدعى: *Lamiaceae*، ومن ثم تجميدها وتجفيفها مع استخراج الميثانول المائي. وقد تمّ دراسة النشاط البيولوجي في المختبر ، وخاصةً بالنسبة لمجموع محتويات الفينول، والفلافونويد، والأنشطة المضادة للأكسدة، والمضادة للجراثيم. حُدِدَ مجموع محتوى الفينول وفقاً لنظرية *فولين كوليكاتو* بينما تم تقييم نشاط مضادات الأكسدة باستخدام نظرية الـ *2,2-diphenyl-1-picrylhydrazyl* (DPPH)، وقد حُدِدَ نشاط مضادات الميكروبات المستخرجة من أوراق النباتات باستخدام نظرية ماركو التي تقتضي اضافة الماء لتخفيف كثافة السائل، ونظرية نشر القرص على جرامين من البكتيريا الإيجابية، و جرامين من البكتريا السالبة، وعدد واحد من الفطريات، وعلاوة على ذلك، فقد تم أيضاً تقييم التسمم من المادة المستخلصة بواسطة اختبار قياس مقدار التسمم.وقد ظهرت فروق كبيرة ($p<0.05$) في محتوى الفينول في ست أوراق الـ *Lamiaceae* المستخلصة، وكانت النتيجة متراوحة بين 55.21 - 95.17 *mg GAE/g* في العينات المجففة، وقد احتوت ورقة الـ*Pogostemon cablin* على أعلى محتوى من الفينول تليها ورقة الـ*C. blumei* وقد أظهرت الدراسة فروقاً ذات دلالة إحصائية بين أوراق الـ *Lamiaceae* الست تتراوح بين 10.5 -- 34.1 ميكروغرام / مل. ومن بين النباتات التي تمت دراستها فقد أظهرت أوراق النباتات التالية: *Coleus amboinicus (CALM) and Pogostemon cablin (PCM)* ارتفاعاً في النشاط المضادّ للأكسدة مقارنةً مع غيرها من الأوراق. وأظهرت جميع الأوراق نشاطاً واحداً على الأقل ضدّ سلالة من البكتيريا. وأظهرت النتيجة اختلافاً كبيراً ($p<0.05$) بين الأنشطة على الكائنات الحية الدقيقة، على العكس من ذلك، كانت كل مستخلصات الأوراق ليست فعالة ضدّ الـ*C. albicans* وقد كان التركيز المثبط (*MIC*) في كل الأوراق متراوحاً بين 1.0-2.0 ملغ / مل في تثبيط نمو الـ*S. aureus, E. coli, P. aeruginosa and B.subtilis* . وقد أظهرت نتائج تجارب اختبار السمية الحادّةِ باستخدام أوراق الـ*C. Blumei* عدم وجود وفيات في الحيوانات حتى على مستوى عالٍ من الجرعات قد يصل إلى 5000 ملغم / كغم من وزن الجسم. وقد لُوحِظَ ذلك بعد 24 ساعة من الإجراء. وهذا يدل على عدم وجود أي أثر سامٍ لنبات الـ*Coleus blumei* على الفئران. وتبين هذه الدراسة أنه يمكن استخدام مضادات الأكسدة ومضادات الميكروبات دون ان يكون لها تأثير سَامّ.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Health Science (Nutrition Sciences)

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Azhary Yahaya

Signature ..... Date 30/9/2010

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LIST OF ABBREVIATIONS

A1C13.6H20	Aluminium chloride hexahydrate
CVD	Cardiovascular Disease
DNA	Dinucleotide Acid
DPPH	1,1 -diphenyl-2-picry Ihydrazyl radicals
DW	Dried weight
et al.	(et alia); and others
etc	(et cetera): and so forth
e.g.	For example
g	gram
H2O2	Hydrogen peroxide
H2O	Water
i.e.	Included example
MeOH	Methanol
mg	milligram
min	minute
ml	milliliter
mm	millimeter
nm	nanometer
OH.	Hydroxyl radical
O2	Oxygen molecule
O2	Superoxide anion
ppm	part per million
ROI	Reactive oxygen intermediates
ROO.	Peroxide radical
r	Symbol for correlation in graph
ug	microgram
um	micrometer

CHAPTER 1

INTRODUCTION AND OBJECTIVE

1.1 GENERAL INTRODUCTION

In Malaysia traditional medicines are still in use and are practised in the community for treatment of disease and maintaining health. There are more than 14,500 species of flowering plants and approximately 1,200 of these plants are reported to have medicinal properties (Koshy et al., 2009). However, currently only 200 species are used in the preparation of various traditional medicines. Well known Malaysian medicinal plants include Kacip Fatimah (*Labisia pothoina*), Tongkat ali (*Eurycoma longifolia*), Misai kucing (*Orthosiphon stamineus*), Pegaga (*Centella asiatica*) and Mengkudu (*Morinda citrifolia*) are used to produce herbal products in various forms such as pills, powders, syrups, tea bags, tablets and tonics. These herbs are available in Malaysian markets and can be obtained at reasonable price.

According to WHO (2005) the use of traditional medicine was rapidly increasing. In Africa up to 80% of its population using traditional medicine to help meet their health care needs. In Asia and Latin America, the communities use traditional medicine due to the history and cultural believe. The use of Complementary and Alternative Medicine is also becoming more popular, in Australia (48%), Canada (70%), USA (32%), Belgium (38%) and in France (75%).

Coleus blumei, a herb has not yet been studied for its potential of medicinal values. This herb can be found in several parts of South East Asia including Malaysia, Indonesia and Vietnam. Currently, this plant is only used as an ornamental

plant because of having attractive leaf shape and colour. This plant is also used in some medicinal preparation by certain communities in Indonesia and Malaysia. The leaves are used to treat wound, reduce cough and to enhance production breast milk by lactating mothers (Damanik et al., 2006).

Plants from the *Lamiaceae* family are most widely used medicinal plants compared to other plants families. A study by Gulhan et al., (2003) showed that members from this family contain high phenol, antioxidant agent and antimicrobial agent. As a members of the mint plant family, coleuses are close relatives to peppermint, spearmint, salvia, basil, thyme, oregano, and swedish (Consolacion et al., 2001).

1.2 MEDICINAL PLANTS POTENTIAL

The search of new medicinal plant or herbs is still underway. Studies have been conducted in different countries to understand their properties, safety and efficiency for natural therapies. According to WHO (2005), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries are still using traditional medicine and the demand for plant based pharmaceutical is increasing.

The presence of phenolic compounds in plants is important for normal growth of plant and defence against infection and injury. Also, phenolics may have an important effect on the oxidative stability and microbial safety in injured plants (Gordana et al., 2007). Antioxidant agent play important roles in human health include preventing oxidative damages and reduce the risks of chronic disease (Demiray et al., 2009; Dimitrios, 2006; Muktar et al., 1994; Stanner et al., 2004). The balance

between an individual's intake of antioxidants and exposure to free radicals may literally be the balance between life and death (Holford, 1997).

Plant extract contain many phenolic compounds, including flavonoids, have attracted considerable attention because its antioxidant activity was more powerful than vitamins, C, E and β-carotene (Gazzani et al., 1998; Vinson et al., 1998). Recently, studies on tea provided evidence that green tea catechins, in addition to their antioxidative properties also affect the molecular mechanisms involved in angiogenesis, extra cellular matrix degradation, regulation of cell death and multidrug resistance (Demeule et al., 2000). One of the best approaches for discovering new antioxidants is the screening of plant extracts. The use of plants and herbs as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (Charalampos et al., 2008; Sari et al., 2007; Souri et al., 2008).

Scientists have discovered range of antimicrobial agents synthetically to overcome variety of diseases. Most of synthetic antimicrobial have extensive use with lack of awareness has been cause side effects. The resistant of microorganisms to antibiotic has increased, due to the frequent and unwise use of antibiotics. This is a problem in hospital environments and can lead to the spread of resistant strains in communities. Modern pharmaceutical industry continues to search for new antimicrobial agents to which bacteria have developed resistance. The use of plants compounds for pharmaceutical purposes has gradually increased. Therefore, such plants should be investigated to understand their properties, safety and effiency, resulted from the frequent and unwise use of antibiotics, is a problem in hospital environments and can lead to the spread of resistant strains to communities. The most

common bacterial pathogens causing nosocomial infections are *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Lin et al., 2004).

Many plants have been used because of their antimicrobial traits, which are due to active substances compounds synthesized in the secondary metabolism of the plant, for example, the phenolic compounds which are part of the essential oils (Gislene et al., 2000). *Lamiaceae* is one of the family plant show positive impact as antimicrobial agent. Many plant from this family has been used such as *Coleus forkohlii*, *C. amboinicus, C. aromaticus, C. blumei* and others. The leaves of these plants are traditionally used for treatment of severe bronchitis, asthma, diarrhea, epilepsy, renal and vesile calculi and fever (Kumar et al., 2007).

The *Coleus species* are used in the Asian traditional medicine to treat angina (chest pain), asthma, bronchitis, epilepsy, insomnia, painful urination, skin rashes and a wide range of digestive problems. The studies have been reported that a number of phenolic components and phenolic content are fairly higher. Therefore capacity to act as antioxidants and antimicrobial also shows positive effects.

1.3 SIGNIFICANCE OF STUDY

Natural products consumed as a food or used as an alternative medicine causes no side effects in the individuals. Therefore, there is a growing interest in preventive health care and efforts are made to accomplish this through the extraction of plants materials and evaluation for medicinal uses. The use of plant extract as an alternative medicine plant still increases (Demiray et al., 2009). There is great potential for the *Lamiaceae* use as alternative medicine, food industry and cosmetics.

Therefore, in this study few species of the *Lamiaceae* family which are traditionally used but have not been studied, will be asses for the assessement of phenolic and flavonoid contents, antioxidant activity, antimicrobial activity and toxicity etc. These are considered to be important for the evaluation of plant extract to be used as alternative medicine, food or cosmetic.

1.4 RESEARCH HYPOTHESES

1) Selected *Lamiaceae* plant leaves extract contain higher total phenolic compound and flavonoids.
2) Selected *Lamiaceae* plant leaves extract has high antioxidant activity.
3) There is significant antimicrobial activity among *Lamiaceae* leaf extracts against selected microorganism (Gram-positive bacteria, Gram-negative bacteria and fungus).

1.5 RESEARCH OBJECTIVES

The aim of this research is to evaluate the potential of *Coleus blumei* (ati-ati leaves) extracts as antioxidant agent and compare to other *Lamiaceae* species (*Coleus amboinicus, Coleus aromaticus* and *Pagostemon cablin)* on their total phenolic, flavonoid contents, antioxidant activities and antimicrobial activity against standard microorganism strain. The toxicity of the *Coleus blumei* extract was determined to ensure that the extract is safe for human consumption.

Objective :

1. To determine the total phenolic and flavonoid contents of the leaf extracts of selected *Lamiaceae* leaf extracts.
2. To determine antioxidative activity of selected *Lamiaceae* leaf extracts.
3. To determine antimicrobial activity of selected *Lamiaceae* leaf extracts against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans.*
4. To assess the toxicity of *Coleus blumei* (ati-ati leaves) extracts in experimental mice.
5. To compare the phenolic contents, antioxidant activities and antimicrobial activity between *Coleus blumei* (ati-ati leaves) extract with other *Lamiaceae* species.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION TO *LAMIACEAE*

The *Lamiaceae* (syn. *Labiatae*) family is one of the largest plant family shrubs and herbs, found all over the world. The *Lamiaceae* is also called 'mint family' includes herbs (eg: Basil, Mint, Sage and Thyme), ornamental plants (*Coleus, Leonotis*) and weeds (eg: Henbit, Ground Ivy and Self-Heal). Its consist of more than 200 genus and 3500 species. Some common genera of *Lamiaceae* are given in Appendix 1.

Many species in the *Lamiaceae* have a long history of use in culinary spices and folklore medicine, including lavender, mint, oregano, basil and thyme. The use of medicinal plants in the treatment of diseases from *Lamiaceae* species in Malaysia are compiled in the

Table 2.1. The importance of many family members plants to the culinary and essential oil industries has been commercially explored for more than 75 years (Ghalem et al., 2008; Lawrence, 1992).

Table 2.1
Some Medicinal plants from *Lamiaceae* species in Malaysia

Species	Local Name	Claimed Properties
Hyptis breyipes	Sawi enggang, sawi hutan, ati-ati puteh	Cuts, worms
Ocimum sanctum	Selasih	Diuretic, anti-pyretic
Orthosiphon aristatus	Misai kucing	Reduces blood presure, anti-diabetic
Pogostemon cablin	Pokok nilam	Boils, headaches

2.1.1 Botanical Descriptions

The *Lamiaceae* is one of the most readily recognized families of flowering plants. Members of the family *Lamiaceae* are herbaceous, less often shrubs or rarely tree with stems and branches usually four-angled. The leaves are simple and not divided, in pairs up the stem, each pair at right angles to the last, and they are frequently hairy or with scent glands and emitting minty smell when crushed. Their flowers have two lips, one more protruding than the other (labia is the Latin for 'lip') give this plant family its original name of *Labiatae*. Generally, the upper lip has two lobes and forms a hood over the lower lip, and the lower lip consists of three lobes which form a landing platform for pollinating insects.

2.1.2 Uses of Lamiaceae plants

Members of the family *Lamiaceae* possess great pharmacological and commercial values. Many of the species are used in traditional and modern medicine. There are different uses of the family members in traditional ways in different parts of the world. Members of the family can group to their uses into three main categories;

1) medicinal; 2) ornamental and 3) aromatic plants which are used as culinary herbs, vegetables and in the perfume industry (Farzaneh et al., 2005).

2.1.2.1 Medicinal

Orthosiphon Stamineus Benth also known Misai kucing from *Lamiaceae* has been used as a medicinal herbs for many centries in South East Asia (Malaysia, Indonesia and Thailand) for treating kidney ailments and bladder related diseases (Sahib et al., 2009). In Iran, 18% of the species are used for medicinal purposes. Leaves are the

most widely used plant parts (Farzaneh et al., 2005). Essential oils of various members possess biological activities. Hori (1999) investigated the essential oils of ten *Lamiaceae* plants and found that it possesses the inhibitory and insecticidal activities.

2.1.2.2 Culinary herbs

Lamiaceae also known for their culinary properties like oregano, rosemary, sage and thyme used as seasonings in the Mediterranean region, and especially oregano. United State Food and Drug Administration give Generally Recognized as Safe (GRAS) status to Oregano, rosemary, sage and thyme. Therefore, these are generally recognized as safe for human consumption without limitations on intake (U.S. Food and Drug Administration, 2006).

Lamiaceae are best known for their essential oils common to many members of the family. Many biologically active essential oils have been isolated from various members of this family (Farzaneh et al., 2005). The essential oils of Lamiaceous plant from mint like menthol, is being used in a variety of food and medicinal products.

2.1.2.3 Ornamental

Many species of the *Lamiaceae* are attractive to grow in the garden, for example, the genus *Coleus*. *Coleus* is grown as an ornamental in various parts of the world. These plants are natives of Indonesia and Africa. *Coleus blumei*, which has nettle-like, bronze-coloured leaves. The colours of their foliage range through yellow, red, crimson, and pink (Chung et al., 2008).

2.2 SELECTION OF PLANTS

There were four standard approaches available for selecting plants: 1) random selection followed by chemical screening 2) random selection followed by antimicrobial assays 3) follow-up of antimicrobial activity reports and 4) follow-up of ethno-medical or traditional uses of plants against infectious diseases (Fabricant et al., 2001).

In the present study the plants were selected based on their use in traditional medicine, ethnobotinical use, their disease resistance and on personal observation. The ethno-medical approach, oral or written information on the medicinal use of a plant forms the basis for selection and focused evaluation (Clardy et al., 2004; Paul et al., 2006). The selection of plant leaves base on traditional uses of plants and followed up with phenol and flavonoid content and antioxidant and antimicrobial activity.

The following six members of *Lamiaceae* were investigated in this study. They are; two species of *Coleus blumei,* two species of *C. amboinicus, C. aromaticus* and *Pagostemon cablin* and the classification of the plants kingdom in Figure 2.1 (USDA, 2008).