TOTAL PHENOLIC CONTENT OF *POLYGONUM MINUS, CENTELLA ASIATICA, COSMOS CAUDATUS* AND *ALLIUM SATIVUM*

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TOTAL PHENOLIC CONTENT OF *Polygonum minus, Centella asiatica, Cosmos caudatus* and *Allium sativum*

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ABSTRACT

Polygonum minus, *Centella asiatica*, *Cosmos caudatus* and *Allium sativum* are herbaceous plants which can be found in the region of Malaysia, Vietnam, Thailand and Indonesia. These plants are consumed as 'ulam' among Malaysians for health benefits. These herbs are known to have antioxidant properties due to its phenolic content. The purpose of this study is to determine the percentage of extracted yield and total phenolic content (TPC) of individual as well as the mixed extracts (in 1:1 ratio) of *P. minus, C. asiatica, C. caudatus and A. sativum.* The herbs were extracted by using juice extractor without any solvent. Then, TPC was determined using Folin-Ciocalteu colorimetric method. Results showed that the *C. caudatus* had the highest yield extraction (24%), *C. asiatica* (22.5%), *A. sativum* (12%) and *P. minus* (8.5%). Among these herbs, *P. minus* had the highest total phenolic (1388.19 mg TAE/ 100g extract) content followed by *A. sativum* (1177.87 mg TAE/ 100g extract), *C. caudatus* (323.59 mg TAE/ 100g extract) and *C. asiatica* (150.01 mg TAE/ 100g extract). The mixed extracts of *P. minus+A. sativum* had the highest total phenolic content (1703.59 ± 152.21 mg TAE/ 100g extract) while mixed extracts of *C. asiatica+C. Caudatus* had the lowest total phenolic content (218.35 ± 18.36 01 mg TAE/ 100g extract). Interestingly some of the mixture showed the synergistic effects compared individual extract alone.

ABSTRAK

Polygonum minus, Centella asiatica, Cosmos caudatus dan *Allium sativum* merupakan herba yang boleh didapati secara meluas di Malaysia, Vietnam, Thailand dan Indonesia. Ianya diambil sebagai 'ulam' di kalangan rakyat Malaysia dan memberi manfaat kesihatan. Herba-herba ini dilaporkan mengandungi antioksida kerana kandungan fenoliknya. Tujuan kajian ini adalah untuk menentukan peratusan hasil ekstrak dan kandungan jumlah fenol (TPC) daripada individu dan juga ekstrak campuran (dalam nisbah 1: 1) *P. minus, C. asiatica, C. caudatus* dan *A. sativum* telah diekstrak dengan menggunakan pemerah jus tanpa sebarang pelarut. Kemudian, TPC telah ditentukan dengan menggunakan kaedah perwarnaan Folin-Ciocalteu. Keputusan menunjukkan *C. caudatus* mempunyai pengekstrakan hasil tertinggi (24%), *C. asiatica* (22.5%), *A. sativum* (12%) dan *P. minus* (8.5%). Antara herba ini, *P. minus* mempunyai jumlah kandungan fenolik tertinggi (1388,19 mg GAE / ekstrak 100g) diikuti dengan *A. sativum* (1177,87 mg TAE / ekstrak 100g), *C. caudatus* (323,59 mg TAE / ekstrak 100g) dan *C. asiatica* (150,01 mg TAE / ekstrak 100g). Campuran ekstrak *P. minus* + *A. sativum* mempunyai jumlah kandungan fenolik tertinggi (1703,59 ± 152,21 mg TAE / ekstrak 100g) manakala ekstrak campuran *C. asiatica* + *C. Caudatus* mempunyai jumlah kandungan fenolik yang paling rendah (218,35 ± 18,36 01 mg TAE / ekstrak 100g). Menariknya beberapa campuran menunjukkan kesan sinergi berbanding ekstrak individu semata-mata.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	-	Analysis of variance
BHA	-	Butylated hydroxyanisole
BHT	-	Butylated hydroxytoluene
DPPH	-	2, 2-diphenyl-1-picrylhydrazyl
et al.,	-	and Others
F-C	-	Folin Ciocalteu
FRAP	-	Ferric Reducing Antioxidant Power Assay
GAE	-	Gallic acid equivalents
g	-	Gram
h	-	hour
mg	-	Milligram
min	-	Minute
ml	-	Millilitre
mM	-	Millimolar
n	-	Sampel size
nm	-	nanometre
ORAC	-	Oxygen Radical Absorbance Capacity
R^2	-	coefficient of determination
ROS	-	Reactive oxygen species
SPSS	-	Statistical Package for the Social Sciences
STDEV	-	Standard deviation
TPC	-	Total Phenolic Content
v	-	Volume
v/v	-	volume per volume
w	-	Weight
w/v	-	weight per volume

%	-	Percentage
<	-	Less than
>	-	More than
°C	-	Degree Celsius
µg	-	Microgram
µl	-	Microlitre
µM	-	Micromolar

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CHAPTER 1

INTRODUCTION

1.1 Research Background

'Ulam' is a local name for traditional vegetable among the Malays in Malaysia. 'Ulam' is essentially vegetable eaten raw (Norhanom *et al.,* 1999; Ismail, 2000). About 120 species of 'ulam' has been found and comprised of several families from herbs to trees (Mansor, 1988). The leaves part of 'Ulam' is normally eaten fresh as salad with rice among the local people, especially the Malays and indigenous communities and has become an important part of the food intake (Bautista *et al.,* 1988; Mansor 1988; Norhanom *et al.,* 1999). Nutritional studies have found that many 'ulam' are rich in carbohydrates, proteins, minerals and vitamin which give a benefit of healthy (Ismail, 2000; Abas *et al.*, 2006; Fatimah *et al*., 2012). Some 'ulam' have high potential in medicinal properties such as induction of uterine contractions, blood cleansing, prevention or cure of ailments such as diabetes, high blood pressure, cardiovascular disease, arthritis, fever and coughs and also used as an anti-ageing agent (Abas *et al.,* 2006).

Plants have potential medicinal properties because of the presence of bioactive components (Kris-Etherton *et al.,* 2002). There are many types of bioactive components (Bernhoft, 2010), one of the bioactive components which will

be the focus of this study is the phenolic compounds. Phenolic compounds can be found in edible and inedible plants and was reported to have various biological functions including antioxidants (Wojydylo *et al.*, 2007). According to Zobel (1997), phenolic compound in plants may act as phytoalexins, antifeedants, attractants for pollinators, and contributors to plant pigmentation, antioxidants and protective towards parasites, wounding, air pollution and exposure to extreme temperatures.

Other than that, phenolic compounds also contribute as antioxidant by scavenging the superoxide anion, hydroxy radical and peroxy radical by inhibiting lipid peroxidation in the biological system (Izunya *et al.,* 2010). In other words, antioxidants have the capability to donate electrons to unstable free radicals and prevent free radicals to snatch electrons from unsuspecting nearby cells and prevent cell from damage (Kumar *et al.,* 2006).

Singh *et al.* (2012) reported that population in developing countries are interested to use synthetic drugs than a traditional medicine though some treatments used synthetic drugs has produced side effects. Thus, recently researchers tried to find out safe alternative for the treatment by using the plants and herbs which potentially can cure a disease.

Four medicinal herbs were selected in this study *(Polygonum minus*, *Centella asiatica*, *Cosmos caudatus* and *Allium sativum*) considering these herbs contain phenolic compounds and medicinal properties (Abas *et al.,* 2006; Kris-Etherton *et al.,* 2002). The presence of phenolic compounds in medicinal plants are the one that responsible for the antioxidant and anti-inflammatory activities, by allowing these herbs to be used, it has the potential to replace synthetic drugs. Medicinal herbs can be considered as clinically effective and a safer alternative to synthetic antibiotics or antioxidants (Solanki, 2010).

1.2 Problem Statement

Extraction yields of total phenolic compounds and recovery of antioxidant compounds from plant materials are typically dependent on different extraction methods. Besides that, the difference in polarity of extraction solvents might influence the solubility of chemical constituents in a sample and its extraction yield. Therefore, the selection of an appropriate method is the most relevant steps in order to determine the total phenolic content.

Bioactive compounds in fruits and vegetables act as antioxidants, antimutagenic and anticarcinogenic inhibitory activities and gave a potential for health benefits (Hodek *et al.,* 2002; Wojdylo *et al.,* 2007; Doughari *et al.,* 2007). One of the bioactives compounds which has potential to benefit health are phenolic compounds. Phenolic compounds are secondary metabolites that are synthesized by plants during normal development (Harborne, 1982) and in response to stress conditions such as infection, wounding, and UV radiation, among others (Beckman, 2000; Nicholson and Hammerschmidt, 1992).

According to Harbone, (1982 and 1984), phenolic compounds are omnipresent in plants and can be essential in a human diet due to antioxidant properties. These compounds have one or more hydroxyl groups, which are bonded directly to an aromatic ring bearing (Balasundram *et al*., 2006). Phenolic structures also is a simple molecule based on the number of phenol units in the molecule (Balasundram *et al*., 2006).

The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim *et al.,* 2002). Plants, especially herbs, have antioxidant properties and potentially promote good health by lowering the risk of cancer, hypertension and heart disease (Wolfe and Liu, 2003; Valko *et al.,* 2007).

Consequently, studies involving antioxidant properties of plant extracts have increased, a testament to growing interest among reseachers towards medicinal plant research. Several reports have shown out that *P. minus, C. asiatica, C.caudatus and A. sativum* promote high level of free radical scavenging activity individually by using different extracting solvent (Norazlina *et al.,* 2013; Faujan *et al.,* 2007; Suhaila *et al.,* 2011) but no reports about phenolic content using pure extract except for *P. Minus* (Maizura *et al.,* 2011).

1.3 Objectives

i) To determine the percentage of pure yield extract of *P. minus, C. asiatica, C. caudatus and A. sativum.*
ii) To determine Total Phenolic Content (TPC) individual as well as the mixed extracts (in 1:1 ratio) of *P. minus, C. asiatica, C. caudatus and A. sativum* using Folin-Ciocalteu method.
iii) To determine the interaction effecst of mixture extracts versus individual extracts.

1.4 Scope of study

In this study, *P. minus, C. asiatica, C.caudatus and A. sativum* were extracted by using a juice extractor without the addition of water. These herbs were determined for moisture content, percent yield and the extracts were analyzed for total phenolic content (TPC). Samples in different mixtures of herbs were prepared before being analyzed. Lastly, statistical assessment was performed using IBM SPSS 16 statistic software.

1.5 Significance of study

P. minus, C. asiatica, C.caudatus and *A. sativum* were reported to have high total phenolic content (TPC) especially in *P. minus* and *A. sativum* using different solvents in extraction (Norazlina *et al.,* 2013; Maizura *et al.,* 2011; Azlim Almey *et al.,* 2010; Faujan *et al.,* 2007), but there are no reports about the synergism related to the use a combined herbs. Accordingly, in the present study, the phenolic content of individual and combined herbs were evaluated through Folin-Ciocalteu method. Furthermore, this is the first study reporting phenolic content of a combination of fresh pure extract (without the addition of any solvent) of *P. minus, C. asiatica, C. caudatus and A. sativum*.

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