EFFECTS OF CARBON/NITROGEN COMPOST RATIO ON ASIATICOSIDE AND MADECASSOSIDE CONTENT AND MORPHOLOGICAL TRAITS OF PEGAGA (*Centella asiatica*)

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FACULTY OF SCIENCE AND NATURAL RESOURCES
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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

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2014

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

22 September 2014

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ABSTRACT

Centella asiatica (C. asiatica) L. Urban (Umbelliferae) or Pegaga is an important herbal plants used in traditional medicine, modern herbal preparation and in the drug development for host of diseases. In this study, the yield of phytochemicals in *C. asiatica* namely madecassoside and asiaticoside and its' morphological traits were studied in relation to their compost carbon/nitrogen (C/N) ratio. The preparation and characterisation of three types of compost namely (1) compost A; (2) compost B; (3) compost C; and another commercial compost Biosmart and control were undertaken. The *C. asiatica* kampong variety with cordate leaves and crenate margin was used. The leaf and petiole of *C. asiatica* were harvested in a monthly interval, and five monthly reading was recorded for statistical analysis and for the extraction and determination of asiaticoside and madecassoside using Reverse Phase Liquid Chromatography (RP-HPLC). Results show that the four types of compost and control have C/N value ranging from 7.15 to 13.43. A good compost quality has a C/N value between 12 to 15. *C. asiatica* asiaticoside content range from 1.80 mg/g (0.18%) to 35.32 mg/g (3.53%) with *C. asiatica* planted with Biosmart yield the highest asiaticoside content of 35.32 mg/g (3.53%) in August, three months after planted as compared to control (23.21 mg/g or 2.31%). The *C. asiatica* madecassoside content range from 1.84 mg/g (0.18%) to 22.59 mg/g (2.26%) with *C. asiatica* planted with commercial Biosmart having the highest madecassoside content of 22.59 mg/g (2.26%) in August compared to negative control of 16.72 mg/g (1.67%). In this study, it is found that C/N had significant effect to the morphological trait (length of petiole, size of leaves and number of progenies) and the asiaticoside and madecassoside content using Pearson Correlation Coefficient (P=+1 or -1) . Their C/N value is steadily low (8.92 to 10.13) compared to the standard of a good compost (C/N 12 to 15) throughout the cultivation.

ABSTRAK

KESAN NISBAH KOMPOS CARBON/NITROGEN TERHADAP KANDUNGAN ASIATICOSIDE DAN MADECASSOSIDE SERTA CIRI-CIRI MORFOLOGI DALAM PEGAGA(Centella asiatica)

Centella asiatica (C. asiatica) L. Urban (Umbelliferae) atau Pegaga merupakan herba yang penting dalam ubatan tradisional, persediaan herba moden dan dalam pembangunan dadah untuk pelbagai penyakit. Dalam kajian ini, penghasilan fitokimia dalam C. asiatica seperti madecassoside, asiaticoside dan ciri-ciri morfologinya telah dikaji hubung kaitnya dengan nisbah karbon/nitrogen (C/N) dalam kompos. Penyediaan dan pencirian secara kimia tiga jenis kompos yakni (1) kompos A; (2) kompos B; (3) kompos C; dan kompos komersil Biosmart serta kontrol telah dilaksanakan. C. asiatica variasi kampong dengan daun kordat dan tepinya beralur telah digunakan. Daun dan petiole C. asiatica telah dituai selang sebulan, dan bacaan untuk lima bulan telah direkodkan untuk analis statistik dan juga untuk penentuan hasilan madecassoside, asiaticoside menggunakan Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Keputusan menunjukkan bahawa empat jenis kompos dan satu kontrol negatif mempunyai nilai C/N dalam julat 7.15 -13.43. Kualiti kompos yang baik mempunyai nilai C/N antara 12 -15. Keputusan menunjukkan kandungan asiaticoside sampel C. asiatica adalah dalam julat 1.80 mg/g (0.18%) kepada 35.32 mg/g (3.53%) dengan C. asiatica ditanam dengan Biosmart memberikan hasil yang tinggi sebanyak 35.32 mg/g (3.53%) pada bulan Ogos, tiga bulan selepas ditanam berbanding dengan control (23.21mg/g atau 2.31%). Kandungan madecassoside yang didapati adalah dalam julat 1.84 mg/g (0.18%) kepada 22.59 mg/g (2.26%) dengan C. asiatica ditanam dengan Biosmart mempunyai kandungan madecassoside yang tertinggi sebanyak 22.59 mg/g (2.26%) dalam bulan Ogos berbanding dengan kontrol 16.72 mg/g (1.67%). Dalam kajian ini, didapati C/N mempunyai kesan signifikan terhadap perkembangan morfologi (panjang petiole dan daun, bilangan anak) dan kandungan madecassoside dan asiaticoside menggunakan korelasi Pearson Correlation coefficient (P=+1 atau P=-1). Nilai C/N kekal rendah (8.92 hingga 10.13) berbanding dengan piawai kompos yang baik (C/N 12 to 15) sepanjang penanaman.

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LIST OF SYMBOLS, ABBREVIATION AND UNITS

IDS(SABAH)	-	Institute for Development Studies (Sabah)
MOSTI	-	Ministry of Science Technology and Innovation
Demoplot	-	UNDP/SGP-IDS plot at Kimanis, Papar,Sabah
g	-	Gram
Mg	-	Miligram
Ml	-	Mililiter
µL	-	Microliter
µg	-	Microgram
mg/mL	-	Miligram per mililiter
Mg/g	-	Miligram per gram
UV-Vis	-	Ultrviolet Visible
CAN	-	Acetonitrile
RP-HPLC	-	Reversed Phase High Performance Liquid
DAD	-	Chromatography Diode auto detector
rpm	-	Revolution per minute
ANOVA	-	Analysis of variance
Nm	-	nanometer
v/v	-	Volume by volume
P	-	Pearson Correlation Coefficient
>	-	More than
≠	-	Not equivalent to

CHAPTER 1

INTRODUCTION

1.1 Background

Centella asiatica (C. asiatica) L.Urban (Umbelliferae) or Pegaga is an important herbal plant widely cultivated and collected as a vegetable 'salad' or spice in China, Southeast Asia, India, Sri Lanka, Africa, and Oceanic countries.

In addition to neuroprotective effect of *C. asiatica*, it has been reported to own a wide range of biological activities desired for human health such as wound healing, anti-inflammatory, antipsoriatic, antiulcer, hepatoprotective, anticonvulsant, sedative, immunostimulant, cardioprotective, antidiabetic, cytotoxic and antitumor, antiviral, antibacterial, insecticidal, antifungal, antioxidant, and for lepra and venous deficiency treatments (Orhan, 2012). *C. asiatica* is a local plant in Sabah traditionally being used by the Sabah herbal practitioner as herbal remedy for diuretic, diarrhoea, hypertension and sexual diseases (Johari *etal.*, 1998).

The extracts of *C. asiatica* had been evaluated for several biological properties. These properties include antioxidant activity (Jayashree *et al.,*2003 and Gnanaprasagam *et al.,*2004); and anti-proliferative effect(Yoshida *et al.,*2005). Pure triterpenoids of *C. asiatica* had also been reported to cause alteration in gene expression in human fibroblast (Coldren *etal.,*2003;Lu *et al.,*2004). Recently asiaticoside had been shown to induce type I collagen in human fibroblast (Lee *etal.,*2006). Active constituents of *C. asiatica* are components of many drugs and cosmetic preparations worldwide in the field of skin care. Madecassol® and Blastoestimulina® are the most known pharmaceutical products that contain *C. asiatica* constituents as active ingredients (Randriamampionona *etal.,* 2007).

The main principle components of *C. asiatica* are two glycosides namely asiaticoside and madecassoside. There are however, significant differences in active

constituents observed between samples of *C. asiatica* originating from different countries such as Nepal and Madagascar. The highest asiaticoside content of 8.14 per cent (8.14±0.05) was measured in samples collected from wild in Gorkha, Nepal (84º38.74´E Long: 28º01.39´N Lat) at 600 metres above sea level (Devkota *et al.*,2010).Secondly, 6.42 per cent was measured in samples collected in Mangoro region, Madagascar(Randriamampionona *et al.,* 2007).The third highest of asiaticoside(1.70±0.02 %) and madecassoside (5.67±0.08 %) contents were detected among sixty accessions of *C.asiatica* in South India and the Andaman island (Thomas *et al.*, 2010). In Malaysia, the second accession (leaves 7.85cm) of *C. asiatica* from Pontian, Johor yielded an asiaticoside content of 2.56µg/mL and madecassoside content of 5.30µg/mL. These phytochemicals are only detected in the leaves and madecassoside in roots (1.57µg/mL), but none on the petiole (Zainol *et al.,*2008). In comparison, using the same methods of extraction, the glasshouse-grown F-line Malaysian origin yielded an asiaticoside content of 0.79±0.03 (%) and madecassoside content of 0.97±0.06 (%) (Aziz *et al.,* 2007).

Malaysian imports about 30 per cent of dried herbs including *C. asiatica* from the Madagascar origin for the herbal formulation and further commercial processing in the Malaysian herbs factory (Johari *et al.,*1998). In Malaysia, *C. asiatica* is collected all year long for further processing and extraction of their phytochemical constituents. The phytochemicals were extracted without taking into consideration the cultivation period and the best harvesting time for *C. asiatica*. This could be one of the factors that result in low concentration of asiaticoside and madecassoside content in *C. asiatica* (Zainol *et al.*,2008; Aziz *et al.,* 2007). The concentration of active compound is very much needed in time series data to help farmers to determine the best cultivation and harvesting conditions.

Composts are considered as organic soils improvement (Gomez, 1998). Traditionally, composts are made of plant and animal waste material decomposed by many agents such as bacteria and actinomycetes. Composts provide plants with good nutrient especially Phosphorus(P) and Nitrogen(N) for its physical growth especially the length of petiole, leaf sizes and its lushness. Composts added to soil not only provide a media for plant growth but also affect the production of

phytochemical constituent in plants (Barker *et al.*, 2007). The *C. asiatica* triterpenes production can be affected by compost since N and P is supplied via the amendment and inducement changes in soil chemical composition in compost (Ormeno*etal.,*2009).

Carbon to Nitrogen ratio (C/N) is an important component in compost quality. Carbon is an energy-producing factor while nitrogen builds tissue. In the photobioreactors (PBRs), the carbon to nitrogen ratio in the feed is a critical parameter that significantly influences microbial growth and hydrogen production (Androga *et al.,* 2011). Raw organic matter has a higher carbon-nitrogen ratio than humus or of the average soil. The average carbon-nitrogen ratio of the bodies of bacteria and fungi falls between4:1 to 10:1. The ratio of C/N 17:1 will increase the nitrogen store of the soil. If the C/N ratio is too high (excess carbon), decomposition slows down. If the C/N ratio is low (excess nitrogen) the compost became a stinky pile(Crow and Miller, 2000).A good compost should have a C/N ratio of 12 to 15, (C=12 to 15 and N=1) for stable organic matter. Humus has a low carbon-nitrogen ratio (Barry, Cogger and Sullivan, 2002).

In this study, the effect of compost C/N ratio on asiaticoside and madecassoside in time series were studied by quantifying the amount of these two compounds present in organic *C. asiatica* samples using a rapid and simple reverse-phase high performance liquid chromatographic method (Verma *et al.,* 1999).The relationship and correlation between compost C/N ratio, asiatico side and made cassoside concentration, plant part(leaf and petiole), different type of compost used to cultivate organic *C. asiatica* and also time of growth were evaluated. C/N ratio is one of the compost important element to indicate the type of material and ease of decomposition; hard woody materials with a high C/N ratio being more resilient than soft leafy materials with a low C/N ratio.

The field of the study was conducted in Demoplot project area in Kimanis Papar, Sabah, Malaysia. The five acres project site is a test-field for ex-situ herbal cultivation. There were 200 species of herbs being domesticated at that area. The species of *C. asiatica* with *C.asiatica* Kampung variety was chosen to be

investigated in this study. This *C.asiatica* was cultivated in a bog in a nursery in Sabah, Malaysia with climate and geographic attribute under controlled conditions.

1.2 Research Objectives

The objectives of this study are as follows:

a. To prepare and characterize the ratio of Carbon to Nitrogen of three types of compost;

b. To cultivate *C. asiatica* herbs using the compost;

c. To quantify and compare the asiaticoside and madecassoside and its' morphological traits in the *C. asiatica* cultivated; and

d. To determine the best harvesting time for *C. asiatica.*

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Pegaga or *Centella asiatica* L.Urban(Umbelliferae) or *C. Asiatica* is described as a small, slender, creeping herb and has long-stalked, green reniform leaves with rounded apices which have smooth texture with palmately netted veins (Bala and Ng,2000). The systematic classification(taxonomy) of *C. asiatica* are shown in Table 2.1 and the names and synonyms in Table 2.2 Brinkhaus(*et al.*,2000).

Table 2.1: Systematic Classification (Taxonomy) of *C. asiatica*

Classification	**Name**
Kingdom	Eukaryota
Subkingdom	Embryophyta
Division	Spermatophyta
Subdivision	Angiospermae
Class	Dicotyledoneae
Subclass	Rosidae
Superorder	Aralianae
Order	Araliales(Umbelliflorae)
Family	Apiaceae or Umbelliferae
Subfamily	Hydrocotyle
Genus	Centella
Species	*Centellaasiatica*

Source : Brinkhaus *et al.* (2000)

Presently, in Malaysia, there are three types of *C. asiatica* subspecies, the *C. asiatica* salad (Figure 2.1), *C. asiatica* Keriting or Nyonya (Figure 2.2) and *C. asiatica* kampong (biasa) (Figure 2.3) or *C. asiatica* ubi. Among the three types,

the *C. asiatica* kampong has been used largely by the traditional healer and also as salad or ulam by the community(Bala and Ng,2000).

Table 2.2: Names and Synonyms

Synonyms	Hydrocotyleasiatica L., Hydrocotylelunata Lam., CentellacoriaceaNannfd.,Centellacordifolia,(Hooker fil.) Nannfd. CentelladuseniiNannfd.,Centellafloridiana (C.et R.) Nannfd.,Centellarepanda(Pers.) Small., Centellatriflora (R.et P.)Nannfd.,Centellauniflora (Col.) Nannfd.
Chinese name	Luei Gong Gen, Tungchian
English name	Indian Pennywort
French name	Hydrocotyleasiatiquenabel
German name	Asiatischerwasser
Indonesian names Sumatra Jawa Sulawesi Bali Flores	 Kaki kuda Kaki kuda,C. asiatican, Antanangede, Gagan-gagan, Gang-gagan, Kerokbatok, Panegowan, Rendeng, Calinganrambat, Kos tekosan Pagaga, Tungke-tungke Papaiduh, Pepiduh, Piduh Puhe beta, Kaki kuta,Tetekaro,Tetekadho
Italian name	Indrocotile
Japanese name	Tsubo-kusa
Mauritius	Bavilacqua
Spanish name	Blasteostimulina (asiaticoside)

Source : Brinkhaus *et al.*(2000)

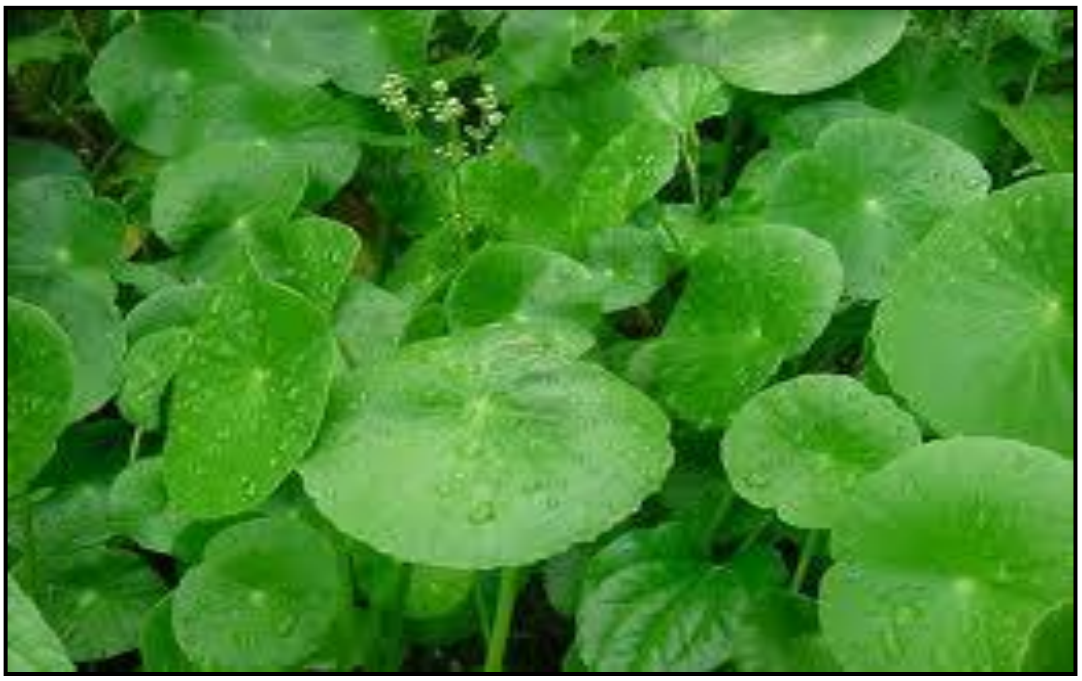

Figure 2.1: *C. asiatica* salad. Waxy surface.

Figure 2.2: *C. asiatica* Keriting. Lobed leaves.

Figure 2.3: *C. asiatica* Kampung. Coarse Surface and Crenate Margin.

C. asiatica's main phytochemical constituents with commercial potential include asiatic acid, asiaticosides, madecassic acid and madecassoside. The structures of these compounds are shown in Figure 2.4.