EFFECT OF COMPOST FORMULATION ON THE ASIATICOSIDE CONTENT OF PEGAGA

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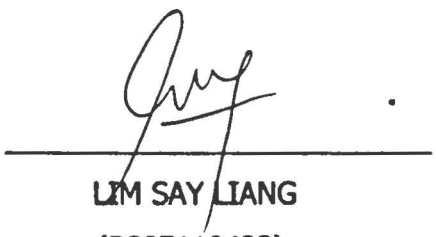

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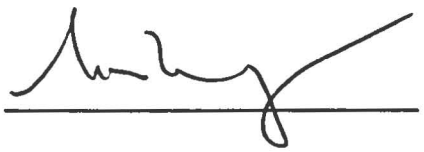

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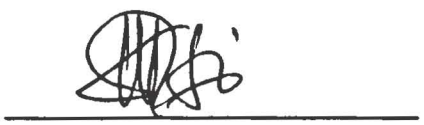

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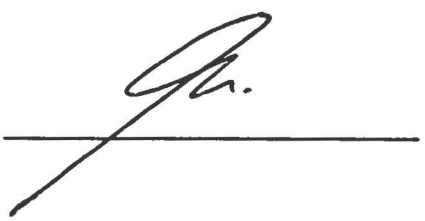

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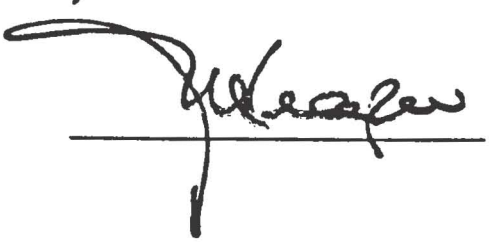

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ABSTRACT

C. asiatica cultivated using five types of composts namely (1) compost A; (2) compost B; (3) compost C; (4) commercial Biomart ; (5) control were extracted and asiaticoside content in the crude extract was analyzed. The highest crude extract yield was control with percentage yield of 42.80%. The identification and quantification analysis of asiaticoside was carried out using RP-HPLC. The RP-HPLC analysis of standard asiaticoside showed the retention time ranged from 9.798 min to 9.806 min. The asiaticoside content in the sample ranged from 3.57 mg/g to 10.55 mg/g, in which the samples cultivated using commercial biosmart had the highest content while the sample cultivated using compost A had the lowest content. This method can be used to investigate the asiaticoside content in various sample of *C. asiatica*. The asiaticoside content has significant moderate correlation with the type of compost used ($r= 0.692$, $p<0.05$). This has led to a better way for *C. asiatica* cultivation to enrich the asiaticoside content for pharmaceutical uses.

KESAN PENGARUH FORMULASI KOMPOS TERHADAP KANDUNGAN ASIATIKOSIDA DALAM TUMBUHAN PEGAGA

ABSTRAK

C. asiatica atau pegaga dikultur dengan menggunakan empat jenis kompos yang berlainan iaitu (1) kompos A; (2) kompos B, (3) kompos C; (4) Baja komersial biosmart; dan Kawalan. Semua sampel dikaji atas kandungan asiatikosida dalam bentuk ekstrak masing-masing. Peratusan ekstrak sampel yang tertinggi adalah 42.80%. Pengenalpasti dan pegiraan kandungan asiatikosida telah dikaji dengan mengunakan peralatan RP-HPLC. Julat masa retensi asiaticosida adalah dari 9.798 min hingga 9.806 min. Julat kandungan asiatikosida dalam sampel adalah dari 3.57 mg/g hingga 10.55 mg/g, pegaga yang dikultur dengan menggunakan baja komersial biosmart menunjukan kandungan asiatikosida yang tertinggi dan pegaga yang dikultur dengan kompos A menunjukkan kandungan yang terendah. Langkah ini adalah sesuai dalam menentukan kandungan asiatikosida dalam pelbagai sampel pegaga. Terdapat kesinambungan jitu kolerasi sederhana antara kandungan asiatikosida pada sampel dan kompos yang telah digunakan pada aras kesinambungan jitu ($r=0.692$, $p<0.05$). Melalui kajian ini, langkah untuk mengulturkan pegaga yang mempunyai kandungan asiatikosida yang tinggi untuk kegunaan ubat-ubatan diperkenalkan.

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LIST OF SYMBOLS, ABBREVIATIONS AND UNITS

°C	Degree Celcius
%	Percentage
g	Gram
mg	Miligram
mL	Mililiter
µL	Microliter
µg	Microgram
mg/mL	Miligram per mililiter
mg/g	Miligram per gram
UV-Vis	Ultraviolet Visible
ACN	Acetonitrile
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
DAD	Diode auto detector
rpm	Revolution per minute
ANOVA	Analysis of variance
nm	nanometer
v/v	Volume by volume

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Centella asiatica (*syn Hydrocotyle asiatica*) is known as pegaga locally which is weakly scented herb from the family *Mackinlayaceae* (Verma *et al.,* 1999). It can be found in Australia, India, Pacific Islands, New Guinea, North Iran, Malaysia, Indonesia, Thailand and other Asia countries. *C. asiatica* (L.) urban is a well known traditional herbal medicine already used in Asia countries for hundred years. It has been used in traditional medicine for the treatment of leprosy, varicose veins, ulcers, lupus, certain eczemas, and mental retardation since prehistoric times (Sharma *et al.,* 1985; Kartnig *et al.*, 1988). Along with the use of *C. asiatica* in medicine, the plant is also finding acceptance as vegetables, drinks, soap and cosmetics. In Malaysia, although this herb is commonly eaten fresh as a vegetable (salad), especially among the Malay communities, it is also said to have beneficial effects in improving memory and in treating mental fatigue, anxiety, and eczema (Goh *et al.,* 1995).

C. asiatica contains many constituents mainly such as asiatic acid, succinic acid, madecassic acid, terminolic acid, vanillic acid, asiaticoside, asiaticoside-B, asiaticodiglycoside (Matsuda *et al.,* 2001; Sahu *et al.,* 1989). The main triterpenes in *C. asiatica* are asiaticoside and madecoside (Randriamampionona *et al.*, 2007). Asiaticoside, a trisaccharide triterpene has been identified as the most active compound in the pegaga associated with healing effect of wounds and duodenal ulcers and possesses immunomodulatory properties (Plohmann *et al.*, 1994).

Asiaticoside is also used as antimicrobial agent (WHO, 1998) and antioxidant (Shukla *et al.*, 1999) and accounts for plant defenses, herbivores diseases and environmental pressures. As a result, bioactive natural products such as asiaticoside are investigated in a greater sense of urgency due to enormously importance as specialty chemicals which can be used as biological or pharmacological ingredients and nutraceuticals for the production of drugs (Henkel *et al.*, 1999). This is due to rapid expanding human population and its subsequent demand for healthy lifestyle.

Typically, asiaticoside in pegaga is present in low concentration. Compost and inorganic fertilizers are usually used to grow organic pegaga in order to boost the synthesis of bioactive constituent's concentration for commercial purposes. Compost is black soil originated from organic material that is being decomposed through aerobic decomposition. Compost is a mean for improving soils which contain high amount of phosphorus (P) and nitrogen (N) content. Compost can affect pegaga triterpenes emission since N and P, which are supplied via compost amendment (Larcheveque *et al.,* 2009) and compost induce changes in soil chemical composition.

Pegaga are collected all year long, considerable differences of the triterpenoid contents in pegaga are observed according to geographic regions, phenotype and genotype (Randriamampionona *et al.*, 2007; Jacinda *et al.*, 2008). This will also helps local population to determine the best cultivating and harvesting conditions. In order to finding a better measure to cultivate *C. asiatica,* a fast and accurate analytical procedure is required for estimating the asiaticoside content of *C. asiatica*. In this study, the effect of compost on asiaticoside will be studied by quantifying the amount of asiaticoside presents in organic pegaga samples using a rapid and simple reversed –phase high performance liquid chromatographic method which has been developed (Verma *et al.*, 1999). The relationship between compost and asiaticoside will be evaluated from correlation between asiaticoside concentration and different type of compost used to cultivate organic pegaga.

1.2 Research Objectives

The objectives of this project are:

a. to identify and quantify asiaticoside in *C. asiatica* extracts.
b. to correlate the asiaticoside content with different types of compost used in cultivation of the organic pegaga.

1.3 Scope of study

This study mainly focuses on the extraction and determination of asiaticoside quantitatively in various samples of *C. asiatica* planted using five types of composts namely (1) compost A; (2) compost B; (3) compost C; (4) commercial Biomart and (5) Control. The identification and quantification analysis of asiaticoside was carried out using Reversed-Phase High Performance Liquid Chromatography (Verma *et al.,* 1999).

CHAPTER 2

LITERATURE REVIEW

2.1 *Centella asiatica*

Centella asiatica is a small herbaceous annual plant of the family *Mackinlayaceae*, native to Australia, Pacific Islands, India, New Guinea, Northern Iran, China, Malaysia, Indonesia, Thailand and other Asia countries. The common names of this plant includes Gotu Kola, Asiatic Pennywort, Antanan, Pegaga, Kula Kud and Brahmi. Other names of *C. asiatica* include 'Luci Gong Gen' or 'Tung Chain' in China, 'Vallarai' for tamil nadu in India and 'Daun Kaki Kuda' in Indonesia (Perry, 1980; Goh *et al.*, 1995). It is used as a medicinal herb in Ayurvedic medicine and traditional Chinese medicine (Rastogi *et al.*, 1960). The leaves of *C. asiatica* are shown in Figure 2.1.

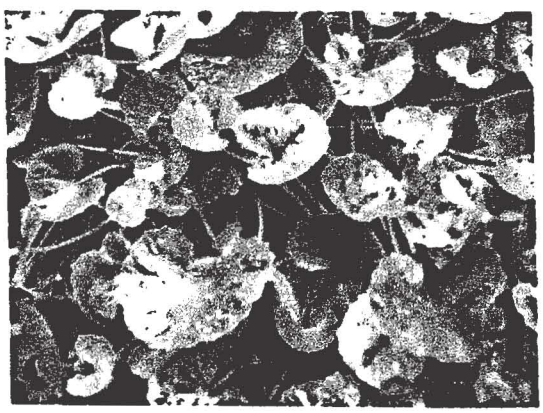


Figure 2.1 Leaves of *C. asiatica*.

2.1.2 Taxonomy

C. asiatica is a living organism which belongs to kingdom of plantae. Its' botanical synonyms is *Hydrocotyle asiatica L.* The classification of *C. asiatica* is shown at table 2.1.

Table 2.1 Classification of *Centella asiatica* (Singh *et al.*,1968)

Classification	
Kingdom:	Plantae
Order :	Apiales
Family :	Mackinlayaceae
Genus :	*Centella*
Species :	*C. asiatica*
Local names:	Pegaga
Botanical synonyms	*Hydrocotyle asiatica* L.

2.1.3 Botanical description

Pegaga stems are slender, creeping stolons, green to reddish green in color, interconnecting one plant to another. It has long-stalked, green, reniform leaves with rounded apices which smooth texture with palmately netted veins. The leaves are borne on pericladial petiols, around 20 cm. The rootstock consists of rhizomes, growing vertically down. They are creamish in colour and covered with root hairs (Singh *et al.*, 1968).

Pegaga flowers are pinkish to red in colour, born in small, rounded bunches (umbels) near the surface of the soil. Each flower is partly enclosed in two green bracts. The hermaphrodite flowers are small in size (less than 3 mm), with 5 or 6 corolla lobes per flower. Each flower bears five stamens and two styles. The fruits are densely reticulate, distinguishing pegaga from species of Hydrocotyle which have smooth, ribbed or warty fruits. The pegaga matures in three months and the whole plants, including the roots, is harvested manually (Daniel, 2005).

2.1.4 Habitat of Pegaga

Pegaga usually grows along ditches and in low wet areas. It is aquatic which is especially sensitive to pollutants in water. *C. asiatica* prefers a moist to wet soil in sun or partial shade. It also grows on walls in the wild and tolerates dried conditions. The species is not hardy in the colder areas of the country; it tolerates temperatures down to between -5 ^{0}C and -10 ^{0}C. It grows and spreads very well outdoors during summer in most part of country and is very easy to increase by division. *C. asiatica* can therefore be grown as a summer crop with division being taken during the growing season and overwintered in a green house (Daniel, 2005).

2.1.5 Distribution of Pegaga

Centella asiatica is found throughout tropical and subtropical regions of India up to an altitude of 600 m. The plant is reported to occur also at higher altitudes of 1550m in Sikkim and 1200 m in Mount Abu. It grows widely in regions of East India, China, Japan and Australia, Sri Lanka, South Africa (Rastogi *et al.,* 1960).

2.1.6 Medicinal Applications

C. asiatica has been used as medicine since prehistoric time (Kartnig *et al.,* 1988). It is used to treat dermal disorder, venous insufficiency and microangiopathy. It has been reported that *C. asiatica* can increase collagen synthesis in vitro and extracellular matrix accumulation in vivo, which can enhance tensile strength in wound tissue and can facilitate the wound healing process (Zhao *et al.,* 2002; Zainol *et al.,* 2003; Cheng *et al.,* 2004; Babu *et al.,* 1995).

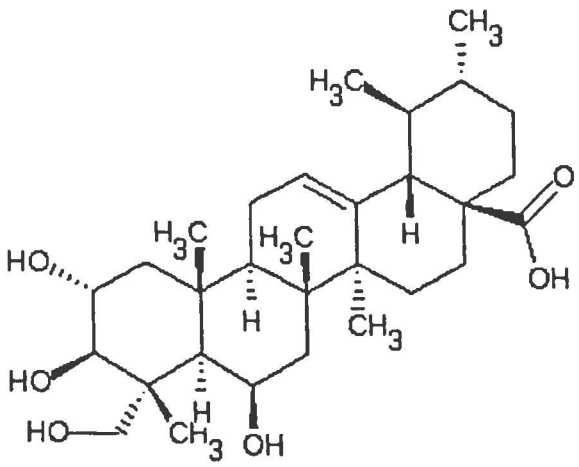
Pegaga based products can found in the form of powder, infusions, soluble and extract of fresh and dried plant, in both conventional and homeopathic preparation. It is also prepared in the form of ointments and creams (Brinkhaus *et al.,* 2000). Madecassol (asiaticoside) in tablet, ointment and powdered form was used as anti-inflammatory (Chen *et al.*, 1999) and autoimmune (Guseva *et al.*, 1998). In terms of cosmetic application, it is used to promote skin regeneration and stimulate

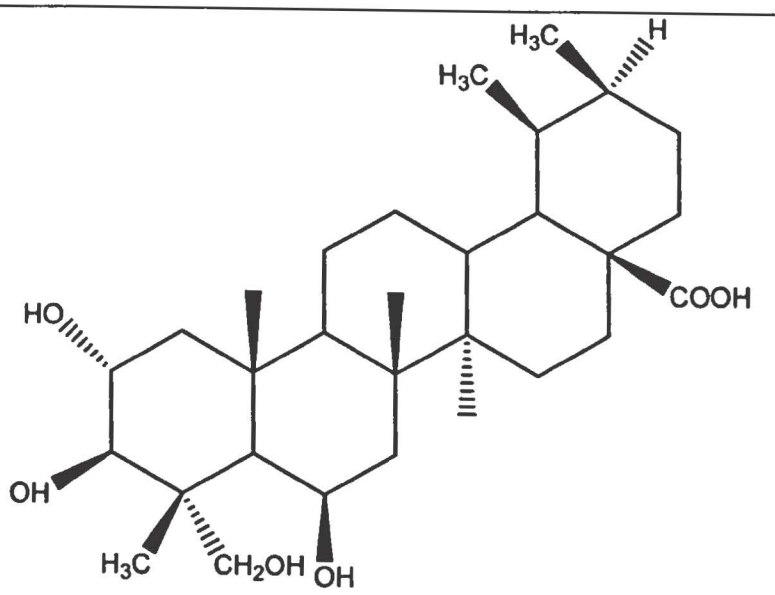
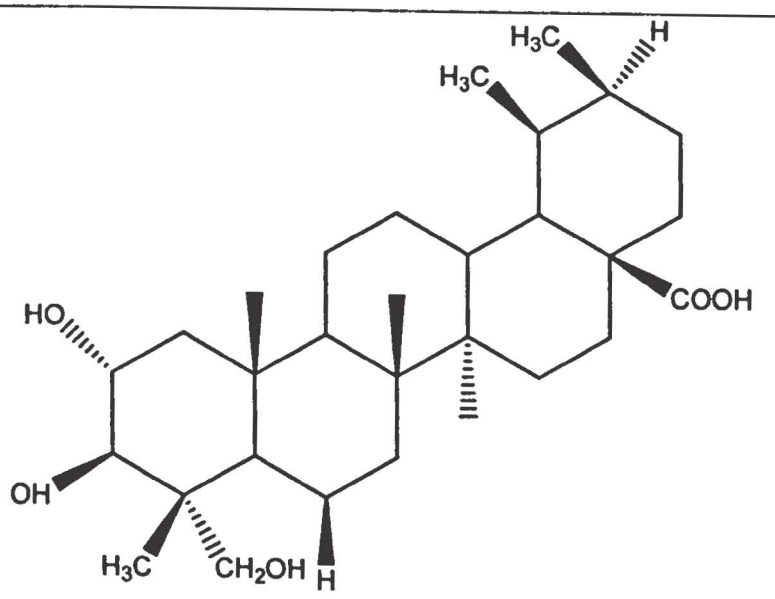
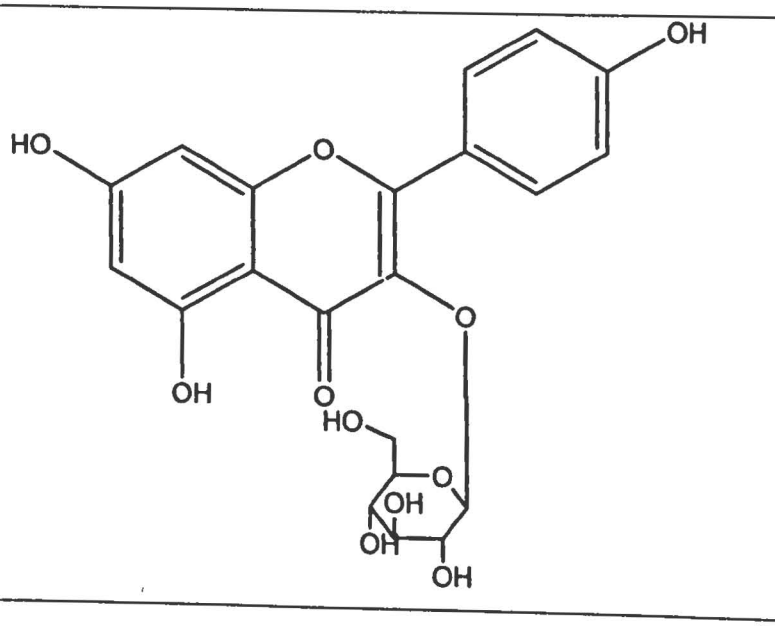
biosynthesis of collagen through the formation of lipids and proteins. Pegaga extract is reported to be effective on scar treatment (Faridah, 1998; Brinkhaus *et al.*, 2000).

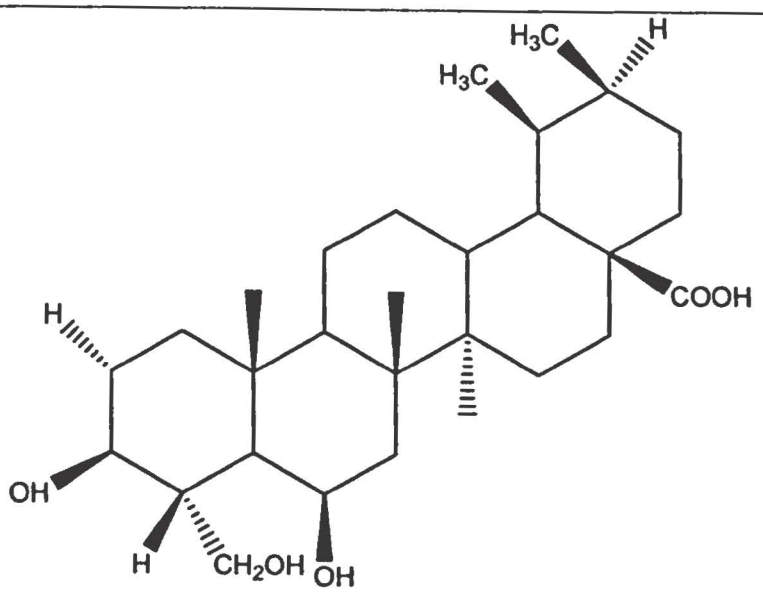
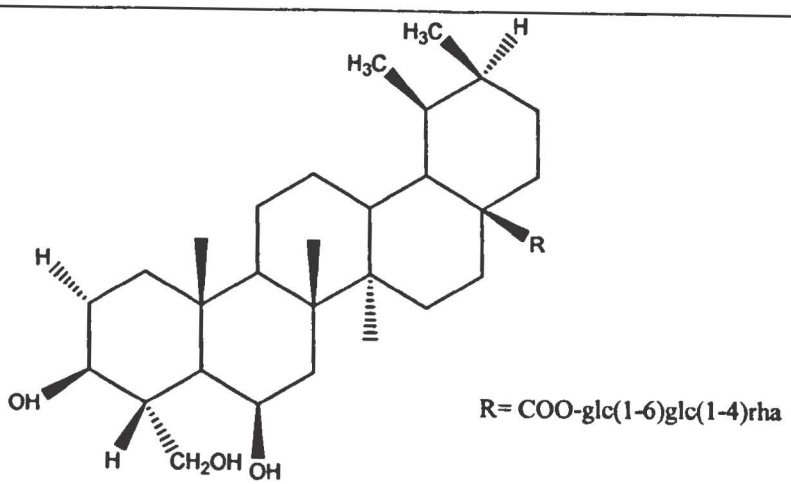
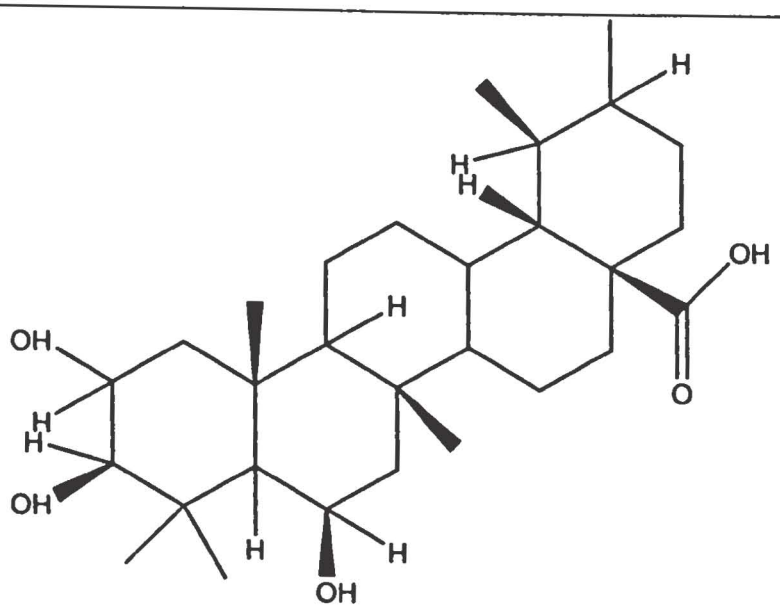
2.2 Bioactive Constituents in Pegaga

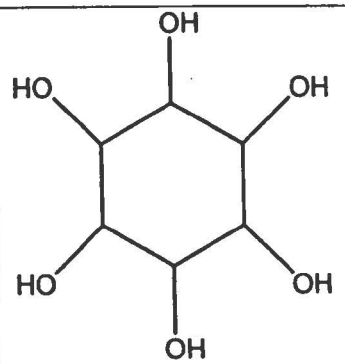
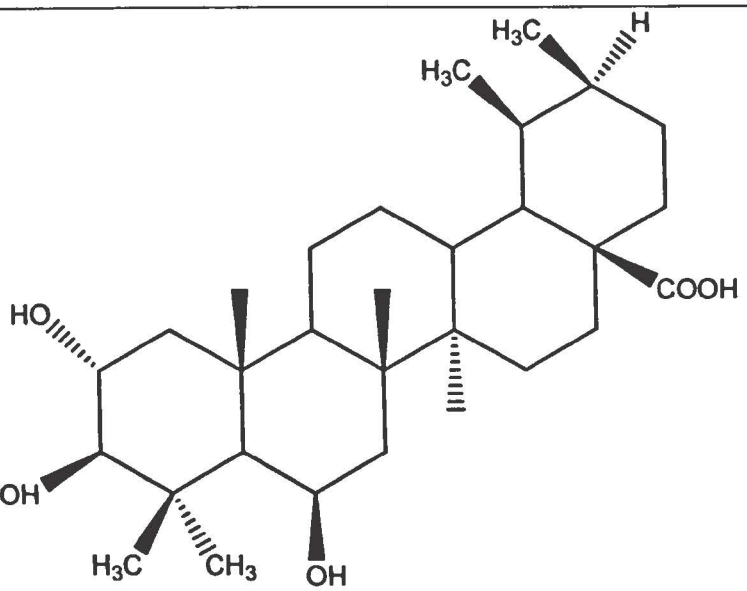
The chemical constituents of pegaga are classified into main groups including essential oil, flavone derivatives, triterpenic steroids, triterpenic acids and triterpenic acid sugar ester or saponin (Brainkhaus *et al.*, 2000). Pegaga also contains various important constituents for clinical and pharmaceutical uses (Bonte *et.al.*, 1994). Chemicals that were previously investigated from pegaga are brahmic acid, brahminoside, brahmoside, centellic acid, centelloside, hydrocotyline, 3-glucosylkaempferol, 3-glucosyl-quercetin, indocentelloside, isobrahmic acid, isothankunic acid, isothankuniside, madasiatic acid, madecassol, meso-inositol, oxyasiaticoside, thankunic acid, vallerine; alkaloid, fatty acids, flavonols, polyphenols, saponins, sterols, sugars, tannins, terpenoids, triterpenes (Goh *et al.*, 1995). Asiatic acid, asiaticoside, madecossoside and madecassic acid are the biologically active constituents in pegaga (Indu Bala & Ng, 1999).

Table 2.2 Bioactive constituents structure of *C. asiatica*

Compounds	Structure
Brahmic acid	

Brahminoside	
Asiatic acid	
3-glucosylkaempferol	

Isothankunic acid	
Isothankuniside	 R= COO-glc(1-6)glc(1-4)rha
Madasiatic acid	

Meso-inositol	
Madasiatic acid	

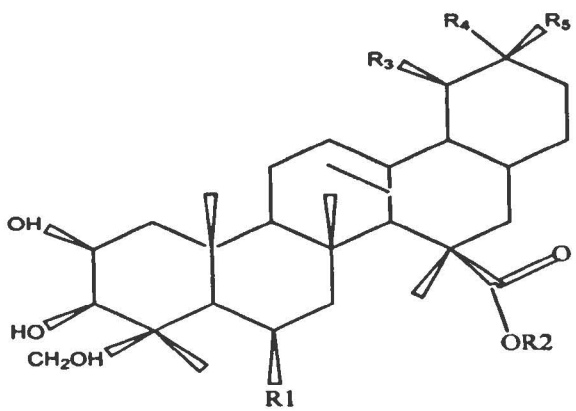
2.2.1 Triterpene Glycosides

Compost is used to stimulate the synthesis of bioactive constituents such as triterpenes glycosides in pegaga. Pegaga contains of Asiaticoside, Medacosside, Asiatic acid and Madecassic acid. The bioactive constituent of therapeutic interest in pegaga is pentacyclic triterpenoid group known as asiaticoside (De Lucia *et al.,* 1997) or saponin containing triterpene acids. The most important three sugar esters are asiatic acid, madecassic acid and the three type of asiaticosides, asiaticoside, asiaticoside A and asiaticoside B (Singh & Rastogi *et al.,* 1969; Brinkhaus *et al.,* 2000). Pegaga contains not less than 2% triterpene ester glycosides, asiaticoside and madecassoside (Kartnig *et al.,* 1988). Asiaticoside and asiatic acid were also reported to be found naturally in *Schefflera octophylla* (Sung *et al.,* 1992).

2.2.2 Chemical structure of Triterpene Glycosides

Glycosides are compounds containing a carbohydrate and non-carbohydrate residue in the same molecule. The chemical structure of each tritrepene glycoside is shown in Figure 2.2. An acetal linkage at carbon atom 1 to a non-carbohydrate residue or aglycone attaches the carbohydrate residue. In terms of chemical structure, the aglycone was classified into several group including saponin, flavonol, phenol, tannins and lactone group.

Saponin glycosides are divided into 2 types according to chemical structure of aglycone. The acid saponins possess triterpenoid structures as shown in figure 2.3. Madecassic acid and asiatic acid are classified under miscellaneous triterpenoids, whereas asiaticoside fall in a group of triglycoside (Jeffery *et al.*, 1999).



Saponins	R1	R2	R3	R4	R5
Asiatic acid	-H	-H	$-CH_3$	$-CH_3$	H
Asiaticoside	-H	-β-D-glc(6-1)-β-D-glc-(4-1)—L-rha	$-CH_3$	$-CH_3$	H
Madecassic acid	-OH	-H	$-CH_3$	$-CH_3$	H
Madecassoside	-OH	-β-D-glc(6-1)-β-D-glc-(4-1)—L-rha	$-CH_3$	$-CH_3$	H

Figure 2.2 Chemical structure as detail of Triterpenes glycosides (Brinkhaus *et al.*, 2000).

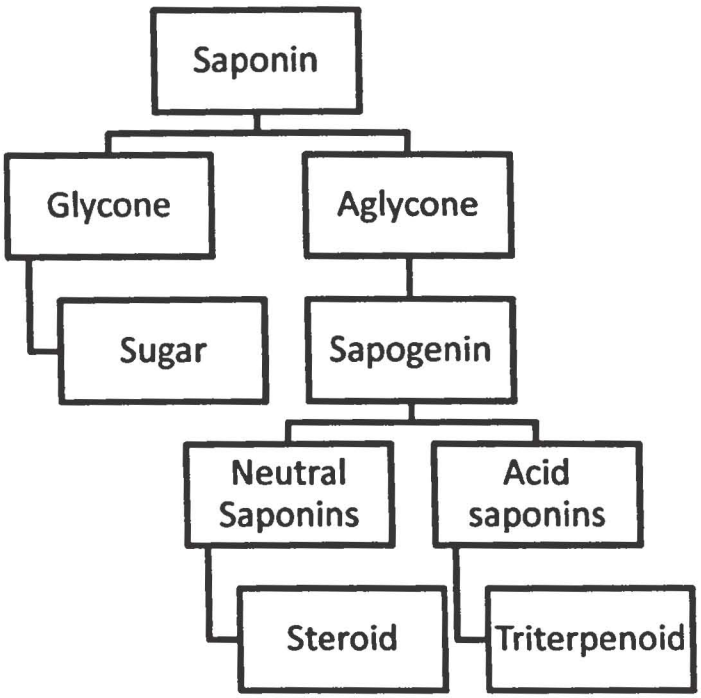


Figure 2.3 The group of saponin glycosides (Duke,1992).

2.2.3 Asiaticoside

Asiaticoside is an ester glycoside of trisaccharide triterpene. Asiatic acid is the most active compound in pegaga. Molecular formulae of asiaticoside is $C_{48}H_{76}O_{19}$ and it melting point is in the range of 230 ^{0}C to 233 ^{0}C. The chemical structure is shown in Figure 2.4.

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